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A Functional Visual System in the Cave Beetle *Ptomaphagus hirtus*

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Abstract

Cave species exhibit a suite of dramatic differences in comparison to their surface living relatives, commonly referred to as troglomorphy (Christiansen 2005). One hallmark feature of troglomorphy is the severe reduction or complete loss of eyes and functional vision. The two most abundant cave beetle species in Mammoth cave, the 2-3 mm small carrion beetle *Ptomaphagus hirtus* (Packard 1888; Peck 1973; Peck 1975; Tellkampf 1844) and the 6-8 mm long predatory ground beetle *Neaphaenops tellkampfii* are good examples of this (Barr 1979). *P. hirtus* tends to hide in crevices and usually goes unnoted to the regular visitor of Mammoth cave. *N. tellkampfii* by contrast is very active and therefore noticed by most attentive visitors of Mammoth cave as the fast moving insect crossing their path. Neither of these two beetles possesses compound eyes typical for diurnal insects (Barr 1962). In *N. tellkampfii*, external eyes as well as the related regions in the brain are completely absent (Ghaffar et al. 1984). Compound eyes are also missing in *P. hirtus*. However, small lens structures can be noted in the lateral head (Figure 1). *P. hirtus* is also flightless because of the complete reduction of the hind wings (Peck 1973). In his comprehensive study of North American cave animals, Alpheus Spring Packard (1888) studied the anatomy of the lens-like structures in *P. hirtus*. He concluded that P. hirtus was blind based on his failure to find an optic nerve connecting from cells underneath the lens structure to the brain.

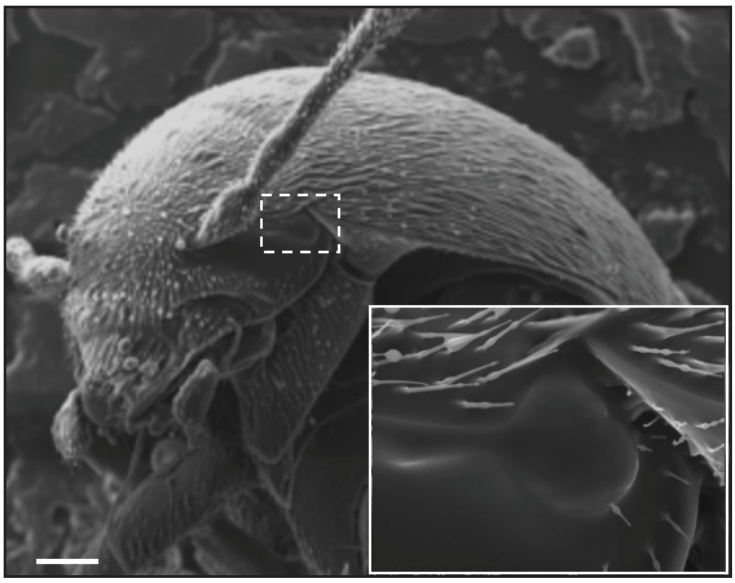

Figure 1: Head and lens morphology in *P. hirtus*. Scanning electron microscopy image view of the *P. hirtus* adult head. Inset: High power view of the lateral lens, outlined by hatched box in the overview image. Scale bar: 100 μm.

A series of experiments in the 70ies established that *P. hirtus* can be easily cultured in the laboratory (Peck 1975). We therefore chose *P. hirtus* for studying the genetic mechanisms of cave adaptation, complementing similar studies in the Mexican cavefish *Astyanax mexicanus* (Jeffery 2005). As a first step into this endeavor, we readdressed the question of vision in *P. hirtus*. Key rationale for suspecting the conservation of a small but functional visual system in *P. hirtus* came from genetic studies in other insects. These have shown that the formation of lens forming cells is absolutely dependent on the preceding differentiation of light-

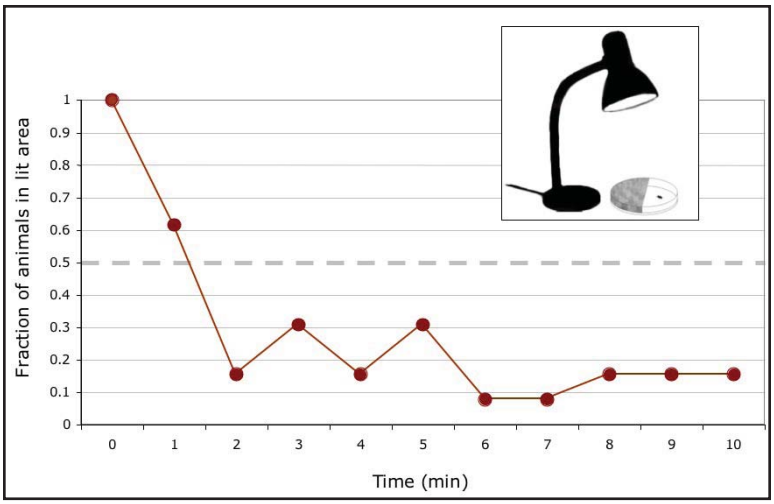

Figure 2: Quantitative analysis of *P. hirtus*' response to exposure to white light. Inset shows test setup. X-axis indicates time points of single observations. Y-axis represents the fraction of test animals present in the lit area of the test arena. Each data point represents the proportion from 13 tested animals. Dashed line indicates neutral expectation. Modified from Friedrich et al. 2011.

sensitive cells (photoreceptors) (Buschbeck and Friedrich 2008). The presence of presumptive lenses in *P. hirtus* therefore suggested the presence of photoreceptive cells in contrast to Packard's earlier conclusions.

We first took a genetic approach test for light-sensitive cells in the adult *P. hirtus* head, employing a new high throughput sequencing method (Nagalakshmi et al. 2008) to probe for the activity of light perception related genes in this species. In the course of this analysis, we characterized the transcript sequences of over 5000 different genes in *P. hirtus*. In the next step, we used bioinformatics methods to search these transcripts for sequences corresponding to genes that are specifically known for their visual function in the widely used model insect *Drosophila melanogaster* (Wang and Montell 2007). This approach revealed the expression of all genes in *P. hirtus* that are currently known to be essential for light perception in insects (Friedrich M. et al. 2011).

As these results strongly indicated the preservation of vision in *P. hirtus*, we subjected laboratory animals to a classic light-dark choice test (Figure 2). These tests revealed a pronounced avoidance of light (negative phototaxis) by the adult animals (Friedrich M. et al. 2011). After only two minutes observation time, on average at least 70% of the tested animals had withdrawn into the shaded area in these experiments. Thus taken together, both genetic and behavioral data produced unambiguous evidence of a functional visual system in *P. hirtus*. In ongoing studies, we are exploring the anatomy and adaptive function of vision in this inconspicuous cave dweller, now to be considered in microphthalmic (small-eyed).

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