

Microfluidic photomechanic infrared receptors in a pyrophilous flat bug

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Abstract Infrared (IR) receptors are so far known only in boid and crotalid snakes and in three genera of pyrophilous beetles that seek out forest fires. Pyrophilous insects can also be found in other orders, however, so it can be hypothesised that IR receptors also occur in some of these species. We investigated the pyrophilous Australian flat bug *Aradus albicornis* and found a small number of dome-shaped sensilla (diameter 13 μm) on the prothorax, which have previously not been described. Ultrastructural investigations revealed that the sensilla are characterised by a fluid-filled inner compartment enclosed in a round cuticular shell. The cuticular apparatus is innervated by the dendrite of a ciliary mechanoreceptor, which is fluidically coupled to the inner compartment. Electrophysiological recordings demonstrated that the sensilla respond to brief warming by red laser light or to broadband IR radiation. Depending on the radiation intensity (4.4–549 mW/cm^2 tested, threshold measured as 11.3 mW/cm^2), first spike latencies varied between 3.4 and 7.5 ms. Thus, our findings demonstrate that *A. albicornis* most probably possesses photomechanic IR sensilla resembling the metathoracic IR sensilla of buprestid beetles of the genus *Melanophila*. In the *Melanophila* sensillum, IR radiation causes thermal expansion of a fluid, which rapidly deforms the dendritic membrane of a mechanosensory cell. The existence of photomechanic IR receptors in both beetles and bugs demonstrates a remarkable convergent evolution towards this particular biophysical transduction mechanism and suggests that it provides selective advantages over other possible solutions.

Keywords Infrared receptor · Mechanoreceptor · Sensory ecology · Pyrophilous insect · Aradidae

Introduction

About 50 species of insects, which have been termed pyrophilous, can be found more frequently after forest fires on the burnt areas than on adjacent unburnt land (Wikars 1997). Pyrophilous behaviour can be adaptive because the burnt trees can be used as breeding material, nutrient sources like fast-growing post-fire fungi are easy to exploit, and competition is reduced. The microclimate is modified because the lack of the leafy canopy means that solar radiation heats up the darkened soil which results in higher ambient temperatures (Wikars 1992; Esseen et al. 1997; Wikars 1997). Pyrophilous insects can be found amongst the Diptera, Lepidoptera, Coleoptera and Heteroptera (Wikars 1997). In Heteroptera, pyrophilous species are known within the flat bugs (Aradidae) (Wyniger et al. 2002; Hjältén et al. 2006). They colonise burnt trees and presumably feed on fast-growing post-fire fungi (Heliövaara and Väisänen 1983; Coulianos 1989; Wikars 1992).

About 50% of the pyrophilous species described so far appear while a fire is still burning and invade the freshly burnt area (Wikars 1997), suggesting that they are attracted by fire-specific stimuli like smoke and infrared (IR) radiation (Evans 1971; Wikars 1992). Examples are beetles of the genus *Melanophila* (VanDyke 1926; Linsley 1943; Apel 1989), *Merimna* (Poulton 1915; Schmitz et al. 2000; Schmitz and Schmitz 2002) (Buprestidae) and *Acanthocnemus nigricans* (Acanthocnemidae) (Schmitz et al. 2002; Kreiss et al. 2005). In flat bugs (Heteroptera, Aradidae), *Aradus lugubris*, *Aradus signaticornis*, (both inhabiting North America and Europe), *Aradus crenaticollis*, *Aradus*

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laeviusculus and *Aradus anisotomus* (inhabiting Europe) are attracted by open fire, hot ash or smoke (Wikars 1997).

Flat bugs of the genus *Aradus* also occur in Australia, but none have previously been described as pyrophilous, and there have been no reports of putative fire-detecting receptors in any pyrophilous flat bug. However, our investigations on freshly burnt areas in Western Australia in the years 2005–2007 revealed a pronounced pyrophilous behaviour of *Aradus albicornis*.

To search for the underlying receptors seemed promising because specialised smoke (Schütz et al. 1999) and IR sensors (Evans 1964; Schmitz et al. 1997) occur in pyrophilous beetles. It is reasonable to suppose that smoke sensors are located amongst the olfactory sensilla on the antennae, whereas all known IR receptors in insects are located on the thorax or abdomen (Evans 1966; Schmitz et al. 2001; Schmitz et al. 2002). We therefore investigated the ventrolateral regions of the thorax and the abdomen of *A. albicornis* for putative IR receptors.

Material and methods

Animals Adult *A. albicornis* were caught on burnt areas in January 2007 in Western Australia, 2 to 4 weeks after fires in Eucalypt forests. Bugs were kept alive in small containers on burnt logs infested with post-fire fungi.

Scanning electron microscopy Bugs fixed in 70% ethanol were cleaned by sonication in a mixture of chloroform and ethanol (2:1) for 2 min. After drying in air, specimens were glued onto holders with carbon glue (Leit-C, Fa. Neubaer), sputtered with gold and examined in a LEO 440i (Leica, Bensheim, Germany) scanning electron microscope (SEM).

Light and transmission electron microscopy Pieces of cuticle excised from the propleural regions of the prothorax were fixed with 3% glutaraldehyde, post-fixed in 1.5% OsO₄ and embedded in Epon. Semithin and ultrathin sections were taken from ten IR sensilla. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with a Zeiss EM 109 transmission electron microscope. To demonstrate the different types of cuticle, semithin sections were stained according to a Mallory trichrome protocol for epoxy sections. For more details, see Weyda (1982).

Electrophysiology Electrophysiological experiments were performed on a total of 22 *A. albicornis*. The sensillum-bearing areas of the propleurum were probed for neural activity with electrolytically sharpened tungsten electrodes. Distinct spikes were recorded extracellularly from ten bugs.

A rod-shaped heating element (1.5 mm in diameter, length of hot segment 10 mm, 170°C surface temperature) and an unfocused red light cw-laser ($\lambda=632.8$ nm, average output power: 5.4 mW, spot size 4 mm diameter) were used for stimulation. The laser beam was attenuated by grey neutral-density filters (applied intensities 4.4–549 mW/cm²). Radiation emitted from the sources was set by a computer-controlled motor shutter allowing exposure times of 20–100 ms and pause durations between exposures of 3,500 ms. Data capture and analyses were carried out with a CED Micro 1401 and with Spike2 software (Cambridge Electronic Design, Cambridge, UK).

Results

The bodies of flat bugs are densely covered with hair mechanoreceptors (sensilla trichodea). In *A. albicornis* (see http://agspsrv34.agric.wa.gov.au/ento/images/A_albicornis.jpg for a total view), these cuticular mechanoreceptors exhibit bollard-like bases, from which a short peg arises laterally (Fig. 1a,b). On the lateral regions of the prothorax (the propleurae), we found hitherto unknown sensilla which are interspersed between the hair mechanoreceptors in both sexes (see arrows in Fig. 1a). These sensilla are characterised by a dome-shaped elevation and a small apical aperture (Fig. 1b). The upper hemispheres have a diameter of about 13 μ m. The dome-shaped sensilla were only found on the trapezoidal regions of the propleurum, which lie behind the swelling of the first (prothoracic) leg base. These areas are developed as thin wing-like duplications of the body wall. In the wingless nymphs of *A. albicornis*, the propleurae are much smaller than in adults and do not develop a posterior duplication. They appear not to possess dome-shaped sensilla. Additionally, we investigated two European non-pyrophilous flat bug species, *A. cinnamomeus* and *A. depressus*, which also appear to lack dome-shaped sensilla on the propleurae. Mallory trichrome staining showed that the cuticle of the propleural duplications in *A. albicornis* mainly consists of sclerotised exocuticle. In some sections, however, a slight reddish colour of the inner cuticular layer indicated less sclerotised mesocuticle.

An eccentric section through an IR sensillum revealed an internal cuticular sphere in the upper curvature of the sensillum (see arrow in Fig. 1c). Serial sections revealed that the sphere (diameter of about 10 μ m) has an exocuticular lamellated outer shell and an inner core. The upper part of the core consists of amorphous cuticle (Fig. 1d,e). Mallory trichrome staining suggested that the cuticle of the core partly consists of mesocuticle. In the majority of the sections, however, the propleural cuticle did

not stain, or stained light yellow, indicating exocuticle (Weyda 1982; Schmitz et al. 2007). In the equatorial plane of the core, the cuticle becomes more fissured and finally disappears (Fig. 1d,e). The numerous clefts in-between are filled with a granular electron-dense material. The lower part of the core contains a torus-like fluid-filled space (Fig. 1d–f).

Sections through the centre of the sensillum revealed that the apical aperture continues into a small outer canal. This canal runs about 3 μm into the sphere, thereby penetrating the lamellated shell (Fig. 1d). Thus, the internal blind end of the outer canal consists of the same cuticular material

that fills out the upper half of the core. The tip of a single cylindrical dendrite enters the sphere from the inside and terminates in the cuticle of the core about 700 nm from the blind end of the outer canal (Fig. 1d,f). The dendrite passes through an inner canal which penetrates the sphere from the inside. The outermost tip of the dendrite, which contains a well-developed tubular body, is surrounded by a porous layer consisting of the fragmented cuticular material of the core and the granular electron-dense material. The nanocavities inside this layer presumably allow a fluidic communication between the membrane of the dendrite and the aqueous fluid inside the fluid-filled space (Fig. 1f);

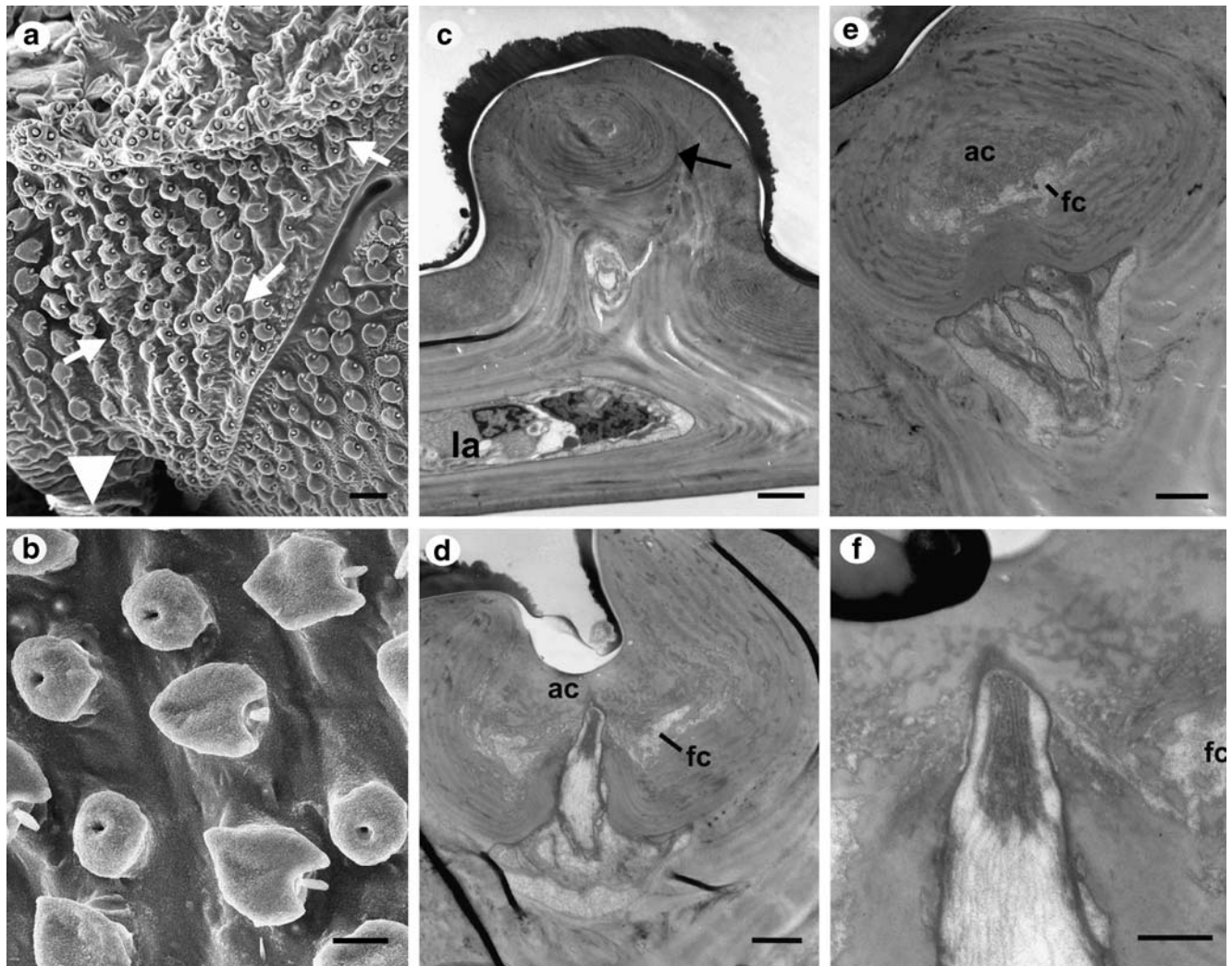


Fig. 1 **a** Propleural region of *A. albicornis*. The head is located to the left; arrowhead points downwards towards the coxa of the prothoracic leg. Arrows indicate 3 of 11 IR sensilla (bar 30 μm) among many hair mechanoreceptors. **b** Four IR sensilla (with apical pores) amongst hair mechanoreceptors (bar 10 μm). **c** Section through an IR sensillum on the propleural duplication. Arrow points to the lamellated sphere. Somata of sensory and enveloping cells are confined to small lacunae (la). The line of black material that appears above the sensillum most probably represents the outermost epicuticular layer (bar 2 μm). **d** Section through the center of the sphere. The tip of the mechanore-

ceptor containing a tubular body is anchored in the amorphous cuticle (ac) of the core and is surrounded by a spongy material inside a torus-like fluid-filled compartment (fc, bar 1 μm). **e** Section with the same orientation as in **d**, about 2 μm from the dendrite. Inside the lamellated shell, a round core region is visible. In its distal part, the core consists of amorphous cuticle (ac), whereas a fluid-filled compartment (fc) can be found in the proximal part (bar 1 μm). **f** Detail of dendritic coupling to the cuticular apparatus. The nanocavities inside the spongy layer may permit transmission of pressure from the fluid-filled compartment (fc) to the dendritic membrane (bar 0.5 μm)

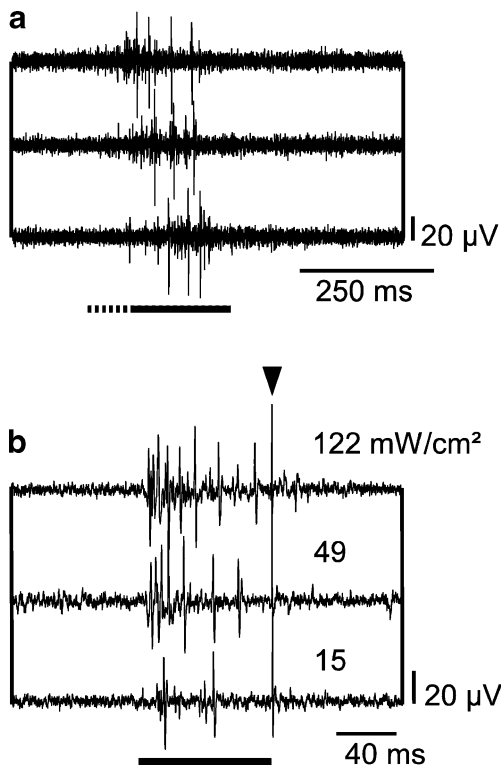


Fig. 2 Extracellular recordings from the ventral propleurum close to the IR sensilla. **a** A heated rod moving along the propleurum (at a distance of 1 cm) elicited spikes of several amplitudes. Without stimulation, no ongoing activity was detectable. Bar: approximate duration of movement; the dashed segment of the bar symbolises the variable onset of movements. **b** Warming the propleurum with red laser light also evoked discharges of spikes. Note the increasing spike count and decreasing first spike-latencies with increasing intensities. Bar: duration of irradiation; arrowhead: artefact caused by closure of the motor shutter

thus, it most probably establishes a microfluidic compartment inside the sphere. The dendrite runs through the internal canal towards a lacuna, which contains the somata of the sensory and the enveloping cells (Fig. 1c). This structural organisation is summarised in Fig. 3b.

Extracellular recordings showed that a warm heating element moving alongside the propleurum at a distance of 1 cm elicited bursts of spikes (Fig. 2a). Identical movements with the cold heating element did not elicit any spikes. Responses were also absent when the recording electrode was inserted into adjacent mesothoracic cuticle devoid of dome-shaped sensilla (not shown). Hence, IR radiation and warm air from the heating element are candidates for the adequate stimulus for the dome-shaped sensilla. The receptors responded to radiation pulses from a red light laser with phasic-tonic discharges, the spike counts of which increased with increasing radiation intensity (Fig. 2b). The threshold for the generation of one spike was 11.3 mW/cm^2 in one recording. Responses were tested up to 549 mW/cm^2 but saturated beyond 122.4 mW/cm^2 . First spike latencies varied between 3.4 and 7.5 ms when stimulation intensity was 549 mW/cm^2 ($N=10$, mean latency $4.9 \pm 1.3 \text{ ms}$). Stimuli other than thermal (mechanical, visual, acoustical) failed to elicit responses.

Discussion

We have described for the first time dome-shaped sensilla on the propleurae of *A. albicornis*. A function in chemo- or thermo/hygroreception can be ruled out because the cuticular apparatus is innervated by a ciliary mechanore-

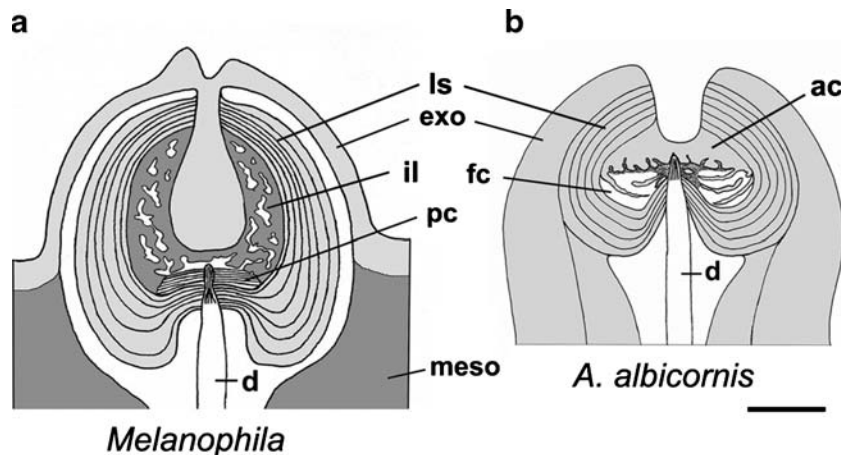


Fig. 3 Schematic drawing of an IR sensillum in *Melanophila* (**a**) and *A. albicornis* (**b**). Bar: $3 \mu\text{m}$; **a** redrawn from Schmitz et al. 2007. Common components: (1) a sphere with a lamellated exocuticular shell (*ls*) reinforced by layers of chitin fibres. (2) An inner microfluidic compartment. Pressure generated within the compartment is conveyed by a fluid to the dendritic tip (*d*) of a mechanosensory cell. *exo*: exocuticle; *meso*: mesocuticle. Structural differences: In *Melanophila*, the microfluidic compartment consists of a porous mesocutic-

ular layer (*il*) and a inner pressure chamber (*pc*) containing the tip of the mechanoreceptor dendrite (*d*). The center of the sphere is filled out by a pear-shaped exocuticular plug. In contrast, the microfluidic component in *A. albicornis* consists of a torus-like fluid-filled cavity (*fc*) surrounding the dendrite tip. The tip of the dendrite is anchored in the amorphous cuticle (*ac*) of the inner core of the sphere and surrounded by a spongy material

ceptor clearly identifiable by its tubular body. Therefore, we speculate that the dome-shaped sensilla have evolved from cuticular hair mechanoreceptors. According to this evolutionary scenario, the electron-dense material surrounding the dendritic tip could represent the relics of the former joint membrane of the hair shaft articulation now stabilising the dendritic tip inside the sphere. Because no moveable or deformable parts of the cuticular apparatus can be found, the mechanical deformation of the dendritic tip must be accomplished differently. A comparison with *Melanophila* photomechanic IR sensilla can help to interpret the structure and probable function of the *Aradus* sensilla. In *Melanophila* IR sensilla, thermal expansion of IR-absorbing biopolymers of the cuticular apparatus and, in particular, of an aqueous fluid inside a sphere causes deformation of the dendritic membrane of a mechanoreceptive cell (Vondran et al. 1995; Schmitz et al. 1997; Schmitz and Bleckmann 1998; Schmitz et al. 2007). The two important components of a photomechanic sensillum, the exocuticular lamellated shell of an internal sphere and an inner microfluidic compartment, can also be identified in the dome-shaped sensilla found in *A. albicornis* (see Fig. 3).

Warming of the *A. albicornis* sensilla by broadband IR radiation or by a red laser evoked a fast phasic response. As in *Melanophila* IR sensilla, spiking response latencies were only a few milliseconds. We therefore propose that the same mode of photomechanical transduction is effective in both *Melanophila* and in *A. albicornis* IR sensilla. According to this mechanism of IR reception, the hard outer shell of the sphere – most probably having a low coefficient of thermal expansion – restricts the thermal expansion of the inner softer cuticle and, especially, of the fluid it contains. This causes an internal increase in pressure which is transferred immediately by the fluid to the tip of the dendrite. A membrane deformation of less than 1 nm can be sufficient to induce the opening of stretch-activated ion-channels in mechanoreceptors in insects and vertebrates (Thurm et al. 1983; Hudspeth 1989). *Aradus albicornis*, which shows a strong pyrophilous behaviour, may use its IR receptors for the detection of forest fires. When the bug flies over a freshly burnt area, the IR receptors may help to distinguish between hot and cool spots, enabling the insect to find safe places for landing.

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