Leg chordotonal organs and campaniform sensilla in Chrysoperla STEINMANN 1964 (Neuroptera): structure and function

D. DeVetak, M.A. Pabst & S. Lipovšek Delakorda

Abstract: In green lacewings of the genus Chrysoperla STEINMANN 1964, recognition of sexual partner relies on courtship songs produced by species-specific volleys of abdominal vibration. Vibration signals are detected by subgenual organs and, in short-distance communication, possibly also by some other leg mechanoreceptors. In legs of green lacewings, campaniform sensilla and four chordotonal organs are known by now. Gross morphology, ultrastructure and physiological properties of the leg mechanoreceptors and their possible role as vibratory detectors are reviewed. Green lacewings are able to detect substrate vibration at sensitivities sufficient to tell of the proximity of mates, competitors, or predators.

Key words: chordotonal organs, subgenual organ, femoral chordotonal organ, campaniform sensilla, serotonin, ultrastructure, electrophysiology, Chrysoperla.

Introduction

In insects, different sensory organs from the legs provide information about leg movements or presence of mechanical signals from their surroundings. Mechanical signals (sound, vibration) are important in conspecific communication or are used as cues for prey recognition or localization, or in potential predator detection.

Communication with substrate-borne signals during mating is used in many insect orders (for reviews see Gogala 1985, Cokl & Virant-Doberlet 2003). In courtship behaviour of many neuteroid insects, vibrational communication plays an essential role (DeVetak 1998). The most extensively studied neuropterans are green lacewings (Chrysopidae). Sexually receptive green lacewings of the genus Chrysoperla STEINMANN 1964 produce low-frequency, substrate-borne signals by vibrating their abdomens (Henry 1979, 1980, Henry et al. 1996, 1999, 2002, 2003). Songs are quite elaborate and are part of specific mate recognition systems. Mating will not occur under natural conditions unless the conspecifics engage in a prolonged and accurate duet (Wells & Henry 1992). In green lacewings, mating signals are reliable indicators of species identity over wide geographical ranges (Henry et al. 1999, 2003). Vibratory signals are transmitted through the legs of the neuropteran to substrates that are easily displaced, like leaves or plant stems, and are picked by subgenual organs in the tibiae of the legs of potential mates (DeVetak & Amon 1997). In insects, subgenual organs are the most sensitive vibration receptors known (Schnorbus 1971, Dambach 1989, Cokl & Virant-Doberlet 1997, Kilpinen & Storm 1997). However, less sensitive leg mechanoreceptors are other chordotonal organs and the campaniform sensilla.

In this paper, we review gross morphology, ultrastructure and functional properties of the leg mechanoreceptors in the green lacewing genus Chrysoperla.

Material and methods

Adult green lacewings Chrysoperla carnea (Stephens 1836) and Chrysoperla lucasina (Lacroix 1912) were collected around Maribor, Slovenia.

Morphological and histological investigations

Lacewing leg mechanoreceptors have been investigated by light and electron microscopy. For details of the methods, see DeVetak & Pabst (1994), Lipovšek et al. (1999). Serotonin-immunoreactivity was studied by immunofluorescent staining (Lipovšek et al. 2003).

Stimulation and electrophysiological recordings

Measurements of the sensory cells’ activity were conducted with standard electrophysiological methods. For details of the methods, see DeVetak & Amon (1997).

1This paper is dedicated to Univ.-Prof. Dr. Horst Aspöck on the occasion of his 65th birthday (21 July 2004).
Vibratory signals of frequencies between 50 and 5.000 Hz were synthesized using a sine wave oscillator (Bistim R1) and attenuator Bruel & Kjaer 2706. The motion of a minivibrator B & K 4810 served as a source of vibration. A lacewing was fixed with the dorsal part of the thorax, and prepared so that the legs were free and the animal could grasp the minivibrator surface. The signals were sine waves lasting 100 ms, with rise and decay times of 10 ms. Starting at a subthreshold intensity, each stimulus was presented at least five times, at a rate one per second. The response of the chordotonal organs was elicited only when the limb was in contact with the substrate. The signals were calibrated using accelerometer B & K 4381, measuring amplifier B & K 2525 and vibrational calibrator B & K 4293.

To record summed activity from the leg nerve, two electrolytically sharpened tungsten electrodes were implanted with micromanipulators. For most experiments one electrode was inserted in the coxa of the investigated leg and the other was implanted into the abdomen. The recorded and amplified signals from the leg nerve were then displayed on an oscilloscope and stored on PC or with a tape recorder for later analysis.

Excluding certain mechanoreceptors

A part of the leg containing certain receptor organ was damaged by means of local thermocauterization, using a 0.5 mm diameter iron needle with a sharpened tip.

Results

1. Structure of the leg chordotonal organs

In each leg of both species (Chrysoperla carnea and Chrysoperla lucasina) there are four chordotonal organs: the tarso-pretarsal organ, tibial distal chordotonal organ, subgenual organ and femoral chordotonal organ. The position of the leg mechanoreceptors is shown in Fig. 1.

Structural unit of all chordotonal organs so far investigated is a scolopidium (FIELD & MATHESON 1998). The scolopidium in the legs of Chrysoperla is composed of one or two bipolar sensory cells and a few enveloping cells (PABST & DEVETAK 1992, DEVETAK & PABST 1994, LIPOVŠEK et al. 1999). In the tarso-pretarsal organ and femoral chordotonal organ scolopidia are arranged in groups, so called scoloparia. Each scoloparium is composed of a different number of scolopidia, from 3 to 20.

1.1. Tarso-pretarsal organ

The tarso-pretarsal organs monitor the movement and position of the tarsal joints during walking on the substrate. This receptor organ is located in the fifth tarsal segment and in the pretarsus of each leg (Fig. 2). The tarso-pretarsal organ of Chrysoperla carnea is composed of two string-like scoloparia, the anterior and posterior,
running parallel to the tarsal long axis (DEVETAK et al. 1996). The number of scolopidia in the receptor varies from six to seven; in each scoloparium there are approximately three scolopidia. The caps and sensory cell bodies are located in the fifth tarsal segment; the thin, string-like ligament of each scoloparium runs from the caps to the claw base. The ligament is composed of attachment (=cap) cells. The ligament of the anterior scoloparium is in contact with the anterior claw, and that of the posterior scoloparium with the posterior claw. Both scoloparia run parallel to the tendon of musculus flexor praetarsi.

1.2. Tibial distal chordotonal organ

This proprioceptor which controls the movements of the tarsus in relation to the tibia is located in the very distal part of each tibia. The tibial distal chordotonal organ (Fig. 1) is a string-like receptor running between the trachea and the dorsal tibial wall. The thin ligament composed of attachment cells runs from the membrane of dorsal part of the tibio-tarsal joint. The number of scolopidia is unknown. Further study of functional morphology of this organ in Chrysoperla is needed to evaluate its role as proprioceptor.

1.3. Subgenual organ

The subgenual organs are the best investigated leg chordotonal organs of green lacewings (PABST & DEVETAK 1992, DEVETAK & PABST 1994). The subgenual organ in Chrysoperla carnea and Chrysoperla lucasina lies in the proximal part of the tibia and is composed of only three scolopidia (Fig. 1, 3). Distal to the sensory cell bodies is a velum, a lens-like part of the subgenual organ (Fig. 4), dividing the blood canal in two separated parts. The velum extends from the tibial dorsal wall to the leg trachea and is an attachment point of the scolopidia (Fig. 1, 4). The thin string-like main parts of three scolopidia extend from the centre of the velum to the dorsal leg wall. The velum is coated with a thin extracellular material which attaches the velum to the leg wall and to the trachea. The elasticity of the extracellular material is very probably important in the subgenual organ's function (DEVETAK & PABST 1994).

Each scolopidium of the green lacewing (Chrysoperla carnea) subgenual organ is composed of one bipolar sensory cell, one scolopale cell, one attachment (=cap) cell and one glial cell. The distal part of the sensory cell's dendrite, the so-called ciliary segment, contains the structure of a modified cilium with the axoneme of the "9x2+0" type. The ciliary root, a unique rod-like cross-banded structure, branches in the cell body of the sensory cell to form ciliary rootlets.

The sensory cell is in contact with other cell types. The scolopale cell contains an electron-dense intracellular material, the scolopale (Fig. 5), and is wrapped around the dendrite and the cell body of the sensory cell. The scolopale cell also secretes an electron-dense extracellular material, the cap. The tip of the cilium penetrates the cap.

Desmosomes are very common in the subgenual organ. In the mesaxon of the scolopale cell, septate junctions and spot desmosomes occur. Gap junctions were found between two scolopale cells (DEVETAK & PABST 1994).
dilation at the end of the cap (>). Abbreviation: A, attachment cell. Scale bar 1 um.

especially dense in the middle part. Two cilia terminate separately in a terminal
scoloparium (DS) in the proximal part of the femoral chordotonal organ of
containing dark granula on the periphery. Scale bar 1 um.

The attachment cell. Each scolopidium is embedded in extracellular material
through an extracellular space, surrounded by the scolopale cell (S) and the
attachment cell (A). Septate junctions (>) are seen between the scolopale cell and
the extracellular structures of the scolopale cell (S). Scale bar 1 um.

Fig. 6: Cross-section of the proximal scoloparium (PS) and a part of the distal
scoloparium (DS) in the proximal part of the femoral chordotonal organ of
Chrysoperla carnea. Each scolopidium of the femoral chordotonal organ contains
two sensory cells. Two scolopidia are cut at the level of sensory cell body (SC), four
scolopidia are cut at the ciliary root level (>), one at the level of sensory cilia (>),
and two at the level of attachment cells (AC). Scale bar 1 um.

Fig. 7: Cross-section of the scolopidium of the subgenual organ of
Chrysoperla carnea. The distal region of the dendrite is the sensory cilium (>),
which has a (9x2+0) structure and a diameter of about 200 nm. Sensory cilia pass
through an extracellular or scolopale space, surrounded by the scolopale cell (S) and
the attachment cell (A). Septate junctions (>) are seen between the scolopale cell and
the attachment cell. Each scolopidium is embedded in extracellular material
containing dark granula on the periphery. Scale bar 1 um.

Fig. 8: Longitudinal section of the scolopidia of the femoral chordotonal organ of
Chrysoperla carnea. The extracellular space (E) contains granular material, which is
especially dense in the middle part. Two cilia terminate separately in a terminal
dilation at the end of the cap (>). Abbreviation: A, attachment cell. Scale bar 1 um.

The cell bodies of all three attachment cells form the
velum. From the cell body of each attachment cell a thin
process runs to the cap, forming the main part of the
string-like structure of each scolopidium.

1.4. Femoral chordotonal organ

The femoral chordotonal organ in Chrysoperla carnea (Fig. 1) is situated in the distal dorsal part of the femur and consists of two scoloparia, the distal scoloparium (17-20 scolopidia) and the proximal one (6 scolopidia) (LIPOVŠEK et al. 1999, LIPOVŠEK et al. 2003). Each scolopidium of the femoral chordotonal organ contains three
different types of enveloping cells (scolopale, attachment
and glial cell) and – unlike subgenual scolopidia – two sensory cells. The sensory cells of different scolopidia do
not lie at the same level of the femoral chordotonal organ
(LIPOVŠEK et al. 1999). The sensory cells of the proximal
scoloparium are located in the proximal part of the scoloparium, and not at the same level as distal one. The
sensory cells of the distal scoloparium were found on its
whole length. Both scoloparia are fused distally and at-
tached to the apodeme. The axons of the sensory cells in
the femoral chordotonal organ are joined to the tracheal
nerve, which is joined to one of the major leg nerves in
the region of the trochanter and forms the nerve N5 (FIELD & PFLUGER 1989, MATHESON & FIELD 1990).

Each scolopidium has two sensory cells (LIPOVŠEK et
al. 1999). In the cell body of the sensory cell (Fig. 6), a
large ovoid nucleus, many mitochondria, granular ER,
Golgi apparatus, microtubules and ciliary roots are pres-
ent. The dendrite is connected at its proximal part to
the surrounding scolopale cell by septate junctions, and
more distally by desmosomes. The distal region of the
dendrite is the sensory cilium (Fig. 7) which has a „9x2+0“ structure and a diameter of about 200 nm. The cilia pass
through an extracellular or scolopale space, surrounded
by the scolopale rods. Both cilia terminate separately in
the cap (Fig. 8). In different sensory cells three types of
ciliary root were found.

The three types of enveloping cells isolate two sen-
scory cells of the scolopidium. The scolopale cell
envelops the dendritic inner segments and encloses both cilia in
the extracellular space. In its middle part it forms the
labyrinth, a complex system of cell processes. At the
distal part of the scolopale cell, where it is overlapped by
the attachment cell, these two cells are connected by
septate junctions (Fig. 7). The attachment cells (Fig. 6,
7, 8) are densely packed with microtubules. At their distal
part, the attachment cells are connected to the cutic-
ular apodeme. Glial cells are wrapped around sensory cell
bodies and around axons. These processes are connected
with each other by belt desmosomes.

2. Distribution
of the campaniform sensilla

Campaniform sensilla are situated in all leg segments
of Chrysoperla carnea (Fig. 9). Along the limbs they occur
singly, but in the joints or close to them (on the
trochanter of the pro- and mesothoracic leg and on the
proximal end of the metathoracic femur) they are
arranged in groups of sensilla (Fig. 10). The campaniform
sensilla are usually ovoid in shape and the orientation of
their cuticular cap long axis is in the majority of the sens-
illa parallel to the leg's long axis (Fig. 11). Some of the
campaniform sensilla arranged in a group around the
trochanter are oriented perpendicular to the leg's long
axis. In the pro- and mesothoracic leg there are ca. 50
sensilla in each limb, and in metathoracic leg there are ca. 60 campaniform sensilla. Dimensions of the campaniform sensilla vary from 3 x 5 μm (in the trochanter) to 13 x 16 μm (in the femur of the metathoracic leg).

3. Electrophysiology of the leg mechanoreceptors

3.1. Electrophysiology of the leg chordotonal organs

The summed responses from the leg nerve fibres of different legs of *Chrysoperla carnea* did not differ much (DEVETAK & AMON 1997). The most sensitive were the chordotonal organs of the metathoracic legs. In the prothoracic and mesothoracic legs the threshold for acceleration was near 0.1 m/s² from 50 to 300 Hz, from 400 to 2000 Hz below 0.1 m/s². In terms of displacement, sensitivity is low up to about 300-500 Hz (depending on the leg examined). The threshold for displacement was at 1500-2000 Hz near 0.2 nm (DEVETAK & AMON 1997).

The candidates for substrate vibration-detectors were chordotonal organs and the campaniform sensilla. Because of the low threshold value, it was assumed that the campaniform sensilla were not the actual receptors (Fig. 12, 15). After ablation of the tarso-pretarsal organ and the tibial distal chordotonal organ, it was demonstrated that these two chordotonal organs were not sensitive to substrate vibration (DEVETAK & AMON 1997). In each leg of green lacewings (in *Chrysoperla carnea, Ch. lucasi*na and other species of the genus) there are two other mechanoreceptors, namely the femoral chordotonal organ and the subgenual organ.

The femoral chordotonal organ is less sensitive (Fig. 12), following constant acceleration in a wide frequency range (up to about 1 kHz). This organ monitors leg movements in the tibio-femoral joint but according to its threshold sensitivity probably also detects vibratory sig-
The subgenual organ is the most sensitive vibration receptor (Devetak & Amon 1997, Devetak 1998). The threshold values for the subgenual sensory cells occurred at the frequency range 1500-2000 Hz at a displacement near 0.2 nm and acceleration near 0.02 m/s² (Fig. 13). Even at low frequencies (50-300 Hz), in terms of displacement, sensitivity of the subgenual organ is still relatively high, with a displacement threshold below 1 μm (peak value).

The subgenual organ is composed of three sensory cells. Two different cell types can be distinguished electrophysiologically (Fig. 13, 14). Rapidly adapting sensory cell is less sensitive and responds transiently only at the onset of the stimulus (Fig. 13B, 14B). Slowly adapting sensory cell (Fig. 13A, 14A) is another type of subgenual sensory cells and stimulus duration may also be signalled by the persistent response of this cell.

Both green lacewing mechanoreceptors – the subgenual organ and the femoral chordotonal organ are sensitive in a broad frequency range (50 Hz-3 kHz) which is believed to comprise all frequencies of biological significance.

### 3.2. Electrophysiology of the campaniform sensilla

Action potentials recorded from the main leg nerve (N5) occurred as a response to relative movements of the metathoracic femur under certain conditions (for details see Fig. 15). This recording was interpreted as a possible activity of the group of campaniform sensilla in the proximal end of the femur. Experiments with excluding certain leg receptor types demonstrated that the leg campaniform sensilla are less sensitive to substrate vibration (Fig. 12).

### 4. Neuroactive substances in the sensory cells of the femoral chordotonal organ

Based on information from a previous study on locusts (Lutz & Tyrer 1988), serotonin seemed to be a potential candidate for neuroactive substance in mechanosensory cells in the femoral chordotonal organ in Chrysoperla carnea and therefore was tested first (Lipovšek et al. 2003). The femoral chordotonal organ was also screened for the presence of acetylcholine and histamine, because these neuroactive substances were found to be present in different receptors in different insects (Lutz & Tyrer 1988, Buchner et al. 1993).

Fig. 13: Responses of two sensory cell-types of the subgenual organ in the mesothoracic leg of Chrysoperla carnea to vibration signals with different intensities. A: Response of the slowly adapting sensory cell (phasic-tonic type) to 1500 Hz. B: Response of the rapidly adapting sensory cell (phasic type) to 1000 Hz. Bar represents stimulus with duration of 100 milliseconds.

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The location of serotonin-immunoreactive sensory cells was studied by immunofluorescent staining (LIPOVŠEK et al. 2003). The results of light microscopical immunohistochemistry showed immunoreaction with an antiserum against serotonin in the proximal and the middle part of the femoral chordotonal organ (LIPOVŠEK et al. 2003). In the femoral chordotonal organ about 16 serotonin-immunoreactive sensory cells were found. In the proximal scoloparium, all 12 sensory cells seem to be serotonin-immunoreactive. In the distal scoloparium, only four of 40 sensory cells showed immunostaining. Serotonin-immunoreactivity was additionally seen in some axons of the femoral chordotonal organ, in the nerve N5 and in the ganglion, in which the nerve N5 enters.

The location of histamine antibodies was tested with fluorescence labelling on cryosections for confocal laser scanning microscopy (LIPOVŠEK et al. 2003). All sections of the femoral chordotonal organ, treated with an antiserum against histamine showed no staining. Additionally, the tissue was screened for the presence of acetylcholine. All sections, treated for the presence of acetylcholine, showed no staining in immunoreactions.

Discussion

All chordotonal organs that have been investigated electrophysiologically have been shown to be mechanotransducers. An organ of this type may function as a proprioceptor – a detector of movements in intersegmental joints (in legs: tarsal-pretarsal organ, tibial distal chordotonal organ, femoral chordotonal organ), a substrate vibration receptor (subgenual organ, tarso-pretarsal organ in Notonecta), a detector of air-borne sounds (sympathial organ), or a sensor of antennal movements (Johnston's organ) (for review see FIELD & MATHESON 1998).

In all Neuropterid families so far investigated, at least subgenual organs occur (DEBAISIEUX 1934, 1938, DEVETAK 1998). In the legs of green lacewings (Chrysoperla) four chordotonal organs are present, thereof three (tarsal-pretarsal organ, tibial distal chordotonal organ, femoral chordotonal organ) are involved in transmitting sensory information to the central nervous system during locomotory activity.

Tarso-pretarsal organ of Chrysoperla camea is not sensitive for substrate vibration (DEVETAK & AMON 1997) and controls the movements of the pretarsus in relation to tarsal segments. The role of tarso-pretarsal organ as a joint angle receptor was demonstrated in the cockroach Periplaneta (LARSEN et al. 1997).

Subgenual organ is the most sensitive leg chordotonal organ gathering information from insect's environment that is of particular biological importance to the animal (e.g. signals of predators and potential mates). It

Fig. 14: Responses of two sensory cell-types of the subgenual organ in the mesothoracic leg of Chrysoperla cornea to prolonged stimulation. A: Response of the slowly adapting sensory cell (phasic-tonic type) to 1500 Hz and 0.1 m/s² (n=6 measurements). B: Response of the rapidly adapting sensory cell (phasic type) to 2000 Hz and 0.8 m/s² (n=18 measurements). A single measurement is shown in the insert (the upper trace); start of prolonged stimulus is marked with an arrow. Scale bar in the upper trace: 1 second. Mean values (± standard deviation) are shown in both graphs.

Subgenual organ is the most sensitive leg chordotonal organ gathering information from insect's environment that is of particular biological importance to the animal (e.g. signals of predators and potential mates). It
is the most important receptor for detecting vibrational mating signals of green lacewings. At least two sensory cell types are present in the subgenual organs. Less sensitive, rapidly adapting sensory cell operates only at the onset of the stimulus and is important to inform an insect about the start of a long-lasting signal. Slowly adapting sensory cell, with a much higher sensitivity, responds to the persistent signal. What function might adaptation serve? It is perhaps less important for an insect to be continually reminded of „constant“ stimuli, but it is necessary to be aware of stimuli that change.

The both cell types, rapidly and slowly adapting sensory cells could be important to inform the insect about the presence of a conspecific potential mate. Mating calls of European green lacewings are low frequency signals, regularly not exceeding 120 Hz (HENRY et al. 1996, 1999, 2002, 2003). Why are subgenual organs sensitive in a broad frequency range? Perhaps these receptors also receive high frequency signals from potential predators (DEVETAK & AMON 1997).

Green lacewings' femoral chordotonal organ is – like in locusts (FIELD & PFÜGER 1989) – a bimodal receptor, important in monitoring leg movements and detecting substrate vibrations. Our results suggest an essential role of serotonin as a neuroactive substance in the femoral chordotonal organ (LIPOVŠEK et al. 2003). Specialization in employed neurotransmitters is present in sensory cells of the femoral chordotonal organ, therefore the function of the femoral chordotonal organ in the green lacewing seems to be very complex.

The leg campaniform sensilla are situated in parts of the green lacewings' cuticle, where bending and other stress exerted on the exoskeleton can lead to deformation of their cuticular cap as was already known for other insects (SPINOLA & CHAPMAN 1970, HUSTERT et al. 1981, ZILL et al. 1981). Campaniform sensilla, therefore, may monitor external forces acting on the skeleton, or relative movements of leg or body segments and other cuticular subunits. The specific activity of a single campaniform sensillum depends upon the orientation of its cuticular cap (ZILL & MORAN 1981, ZILL et al. 1981). The hypothesis for the function of campaniform sensilla of insect legs is that they measure forces exerted on the leg segments by body position and active leg movements (HUSTERT et al. 1981) and (in orthopterans) also substrate vibration in standing insects (KÖHNE 1978).

Six legs of green lacewings represent six channels with different inputs – chordotonal organs, campaniform sensilla, hair receptors and other mechanosensory structures – sending information to integrate in the central nervous system. In the future, we have to combine laboratory and field work for better understanding of the role of the leg mechanosensory system in green lacewing communication.

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References


Addresses of the authors:
Prof. Dr. Dušan DEVETAK
Department of Biology, University of Maribor
Koroška 160
SI-2000 Maribor, Slovenia

Prof. Dr. Maria Anna PABST
Institut für Histologie und Embryologie
Universität Graz
Harrachgasse 21
A-8010 Graz, Austria

Dr. Saška LIPOVŠEK DELAKORDA
Department of Biology, University of Maribor
Koroška 160
SI-2000 Maribor, Slovenia
E-Mail: dusan.devetak@uni-mb.si
ZOBODAT - www.zobodat.at

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