INSECTS: From Behaviour to Physiology and Biodiversity

Andrej Čokl and Meta Virant-Doberlet

Professor Dr Andrej Čokl

Dr Meta Virant-Doberlet

Life on Earth depends on insects, which are present in almost all habitats: in the air, water and on land. The number of insect species is unknown, but several million is probably the most realistic estimate. Insects were the first animals to colonize Pacific islands following nuclear weapons experiments; plant reproduction and fruit production depend on pollination by insects; they destroy at least one third of all the food produced by humans; insects are important elements in food chains; and finally, insects are the vectors for many serious human, animal and plant diseases. Research into insect life is the subject of entomology, one of the central branches of biology.

The great diversity of insect species, their rapid reproduction, the incredible variety of mechanisms which they have evolved for coping with challenges in their environment, and rapidly expanding knowledge as a result of new technologies are key reasons for the specializations of different groups of entomologists. Basic research on insect life provides opportunities for fast and direct application in agriculture, medicine, environmental protection and the development of new technologies based on the biological mechanisms which insects have evolved. Heightened public attention and expectations of obtaining straightforward answers to questions crucial for everyday life compel specialized groups in the field to co-operate intensively in order to exchange knowledge, technology and information. The Department of Entomology at the National Institute of Biology comprises a group of researchers whose investigations of insect behaviour, communication and neurobiology are recognized internationally.

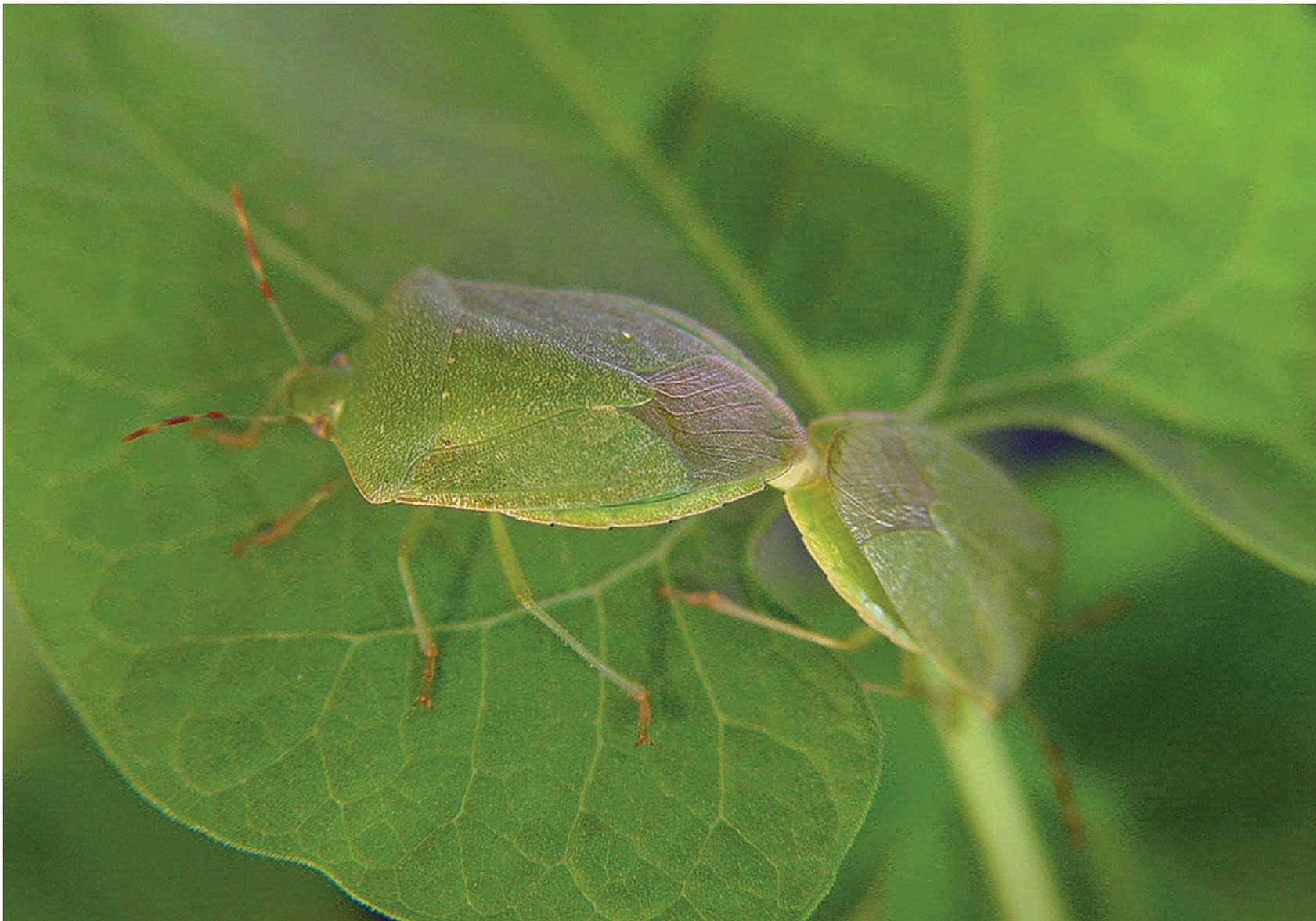
Proximate and ultimate levels of analysis of behaviour offer an opportunity to discriminate between mechanistic and evolutionary explanations. The proximate level is focused on genetic-developmental and sensory-motor mechanisms, while ultimate causes focus on the historical pathways leading to behaviours and on the past effects of natural selection in shaping current behaviour. On this theoretical basis the research programme of the Department of Entomology is directed towards different topics regarding communication processes in insects. Communication plays a central role in animal societies, and the study of signals identifies the types and amounts of information animals convey to each other. The results of animal communication research can be used as tools for the elucidation of general evolutionary principles and, finally, they provide ideas for various practical applications. The research programme at the Department of Entomology is concerned mainly with vibrational communication, which is prevalent in insects within species, families or phylogenetic distributions. Communication may be defined most simply as the exchange of information between a transmitter and a receiver by signals transmitted via various media.

The biophysical properties of the medium (air, water or land) change the quality of the signals transmitted. The transmitter and receiver have to communicate with signals which preserve the information despite its transformation during transmission. The study of the biophysical properties of transmission media assists us in understanding the evolution of signal production and reception structures. Plants are the most common substrates for vibrational communication in insects, and the study of their mechanical properties is at the centre of scientific interest of the group. Low-intensity signals emitted by different insects are recorded

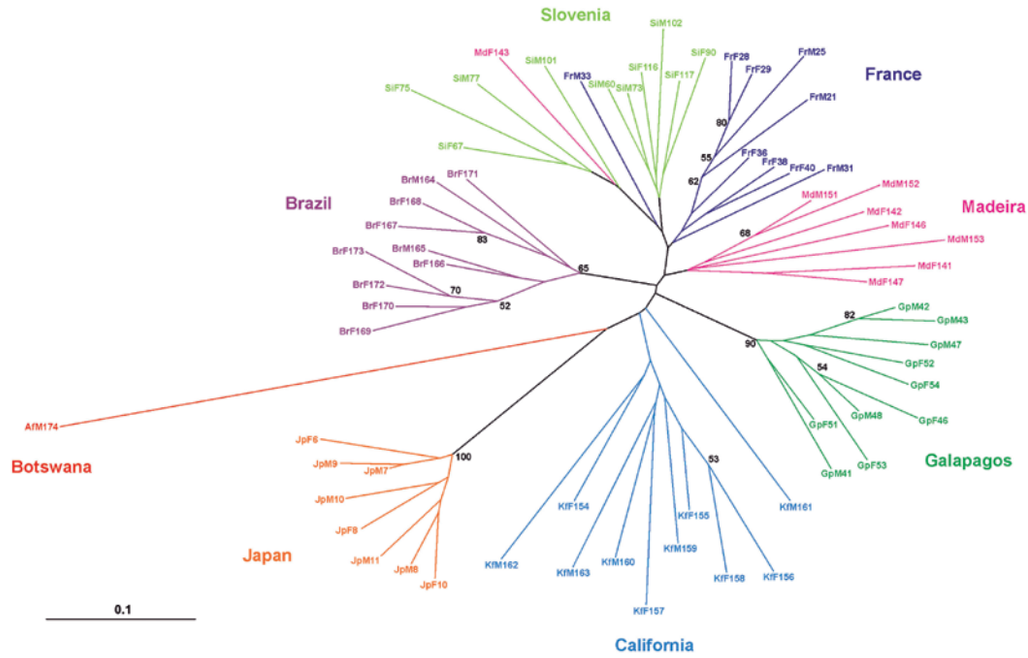
by laser vibrometers in different plant segments and at different distances from their sources; changes in their temporal and spectral characteristics are correlated to obtain explanations about the information which enables an insect to respond to basic problems such as where to go to meet a mate, who is calling, warning or courting, how far away they are, whether the transmitter is a threat, whether the insect has entered another's territory, etc. Knowledge obtained through basic research constitutes the scientific background of applied projects in which researchers in the group, along with colleagues from the University of Ljubljana, apply laser technology to monitor the presence of wood-boring insects in religious and artistic objects, and in testing the resonant properties of the differently treated woods used to make musical instruments.

Signals used for communication are species-specific; they enable discreteness and thus isolate species and prevent hybridization. Although species are rarely recognized and identified by direct studies of reproductive behaviour, this is actually the ultimate determinant for biological species. The vibratory signals emitted by different species are recorded and systematically investigated relative to their temporal and spectral characteristics. Comparative studies enable an understanding of the languages and dialects of different animal groups. These studies are upgraded by investigations of the informational value of various signal parameters for the recognition and localization of sources. The outcome of long-term research is the scientific basis for the optimization of biological control techniques such as pheromone traps, which attract mates by species-specific signals. The latter are useful taxonomic tools for the recognition of species in environments where individuals cannot be accessed. The study of signals has accelerated due to the necessity of enhanced research of biodiversity, the geographical origins of invasive species, and of plant-insect interactions in the context of global warming. The recent results of the Department of Entomology on the diversity of signals between eight geographically separated populations of the globally invasive pest species *Nezara viridula*, together with molecular analyses, have demonstrated among other things that the species originates in Africa and has revealed African, Japanese (Asian) and divergent European-American lineages. A significant positive correlation between geographical isolation and genetic divergence on the one hand, and on the other, no correlation between either geographical or genetic distance and differentiation of populations by song, indicates that evolution has enforced a stable structure for the information carried by vibratory signals.

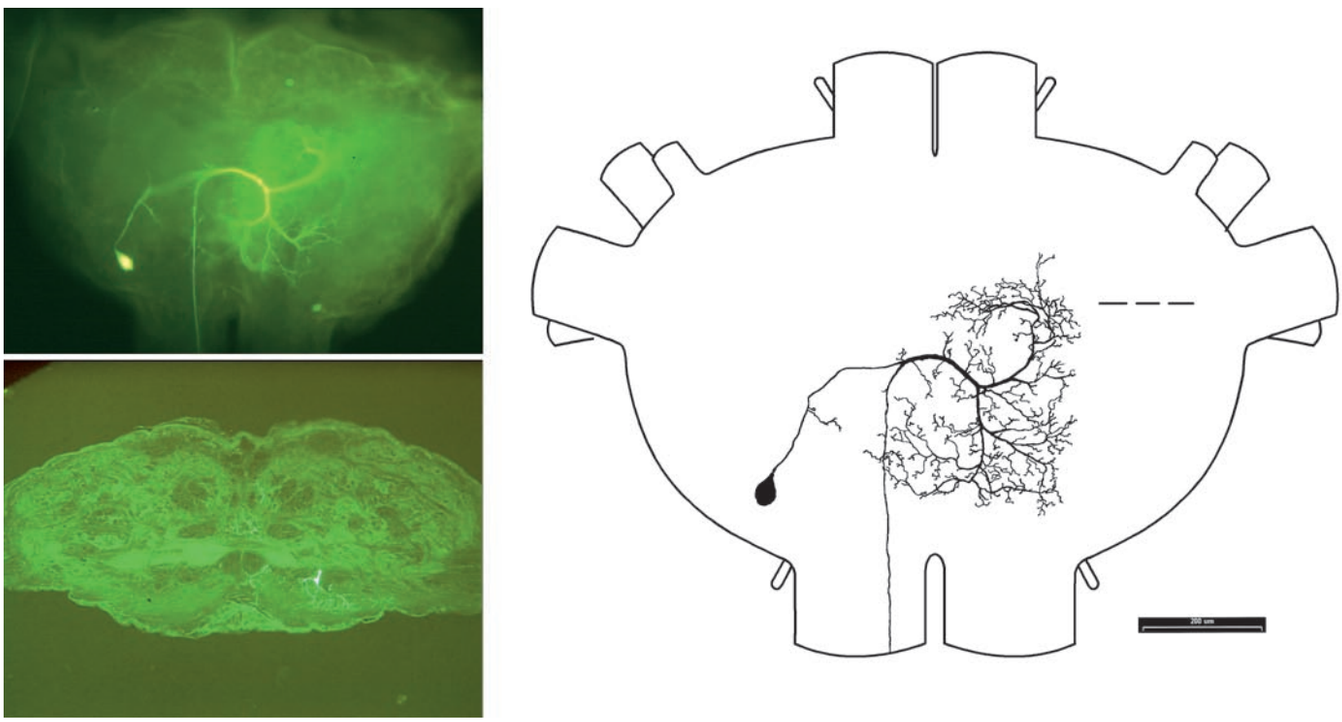
The anatomy and function of receptor organs and the underlying sensory element of the neuronal system pathway depend significantly on signal modality and on the influence of the medium on transmitted signal amplitude, time and frequency characteristics. New methods developed for anatomical and morphological studies demand re-investigation of sensory organs in order to clarify in detail the role of supporting structures in signal transduction, and to



Southern green stink bug *Nezara viridula*. (Photo by Meta Virant-Doberlet)



Neighbour-joining tree based on individual RAPD (Random Amplified Polymorphic DNA) profiles of 66 *Nezara viridula* specimens sampled from eight allopatric locations. Bootstrap values (1,000 replications) above 50% are given at the nodes. (From: Petra Pavlovčič, PhD Thesis, University of Ljubljana, 2005).



The morphology of a vibration-sensitive interneuron located in the prothoracic ganglion of the cave cricket *Troglophilus neglectus* (plan view, scale 200 μm). The neuron responds to the vibrations of the front legs of the animal. An image of a transverse section of the preparation is taken at the level indicated by a dashed line. (Courtesy of Nataša Stritih).

ethical or technical reasons do not allow a direct experimental approach. The comparative neuroanatomy and neurophysiology of specific neuronal networks help in our understanding of the evolution of the central nervous system as the basis for any behaviour.

An important step forward has been taken with the investigation of ontogenetic development in fruit flies which began recently with the transfer of knowledge and technology from Queensland University in Brisbane (Australia) to the laboratories of the Department of Entomology. Under optical control, an identified neuronal cell in a living embryo is destroyed by a laser beam and the specific behavioural pattern during ontogenetic development of the specimen is monitored. Since the history of the destroyed part of the central nervous system is known, one can determine the neuronal basis of the ontogenetic development of the behaviour pattern under investigation. Studies of the ontogenetic development of symmetrical posture and the role of sensory neurons in fruit flies as understand the process of the transformation of mechanical to electrical energy at the receptor membrane. The experimentally confirmed relevance of various signal parameters enables the study of the functional properties of specific neuronal networks at the single cell level. The intracellular recording of electrical activity of stimulated nerve cells and their simultaneous staining with highly selective dyes provide an opportunity to study connectivity within the network together with input/output relations. Neurobiological investigations of invertebrates are important as model studies for understanding the functioning of the central nervous system in vertebrates when the most powerful insect model open up opportunities for investigation of movement control and its development at the single cell and molecular genetic levels.

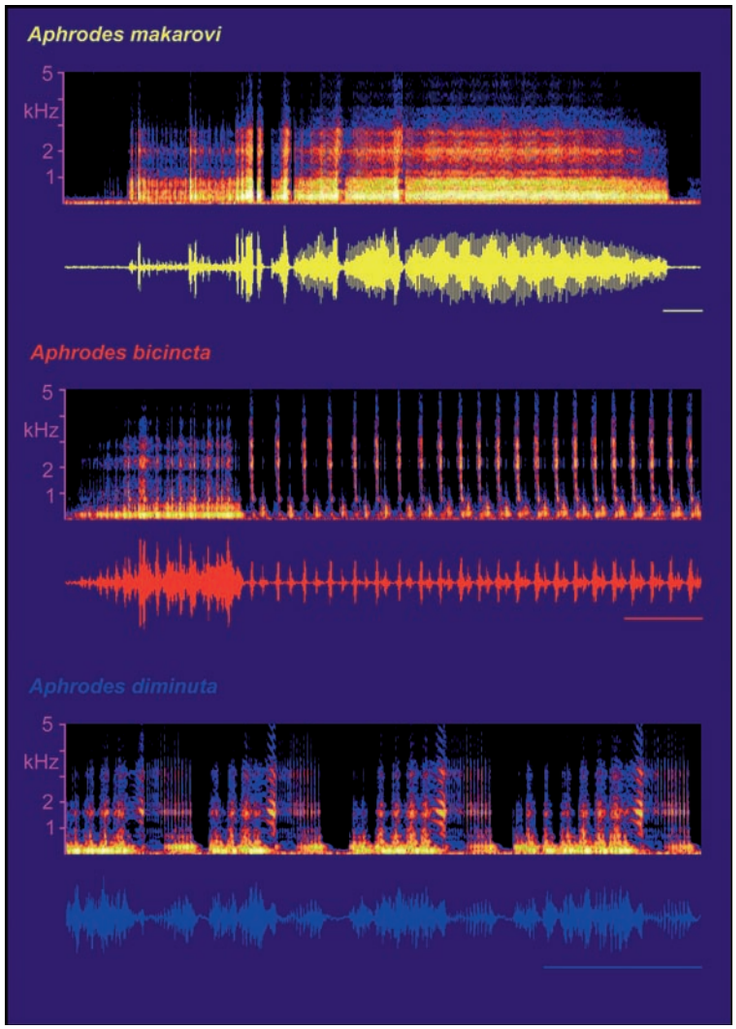
Bees constitute one of the most popular insect species and have various beneficial impacts on human life. In recent decades bee-keeping has faced various

problems which have endangered it worldwide. The use of the insecticide imidacloprid in the last decade was probably the most serious problem to have divided the scientific community, although many other questions deserve attention. To reinforce investigations on bees in Slovenia the Department of Entomology began studying the influence of diseases on behaviour and the immune response of foraging bees (*Apis mellifera)*. This project has connected the Department with outstanding research institutions in this field in Germany.

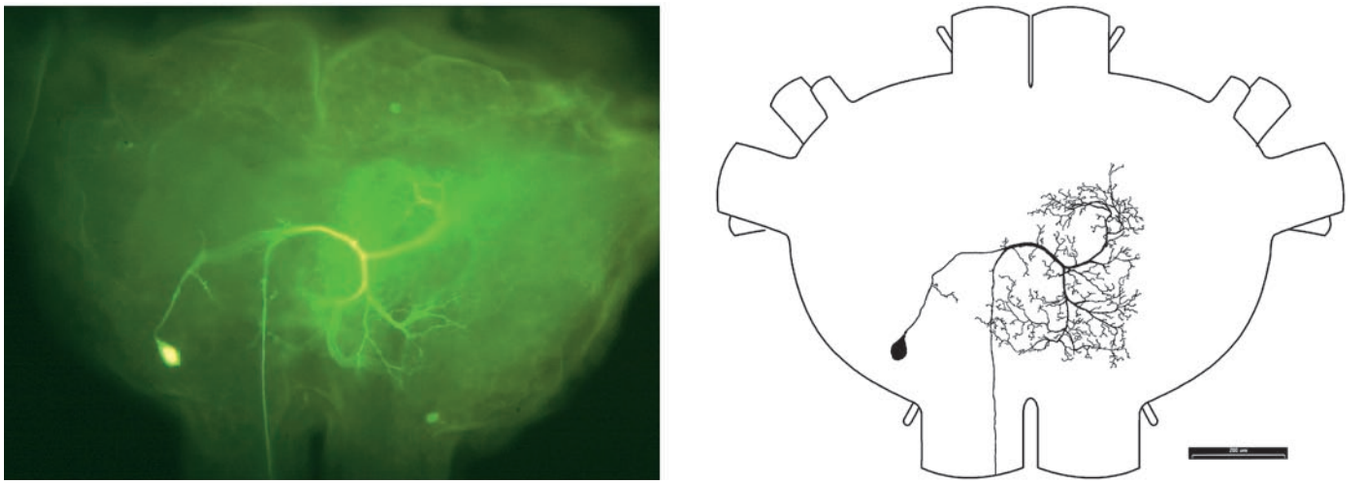
With several million species, insects have evolved such a wide variety of responses and adaptations that any group of entomologists must restrict itself to only a limited number of groups or species. Thus it is important to have close connections with other groups in order to incorporate knowledge within a broader framework. The animals used in research in the Department of Entomology are those which are economically important: pollinators, such as bees and bumblebees; pests, such as stink bugs, leafhoppers and plant hoppers; species studied as models for adaptations to specific environmental pressures, such as cave crickets; invasive species; and insects which are used as models in neurobiology or genetic research, such as cockroaches and fruit flies.

In the last ten years the Department of Entomology has collaborated on various projects in Slovenia, mainly with the Agricultural Institute of Slovenia and the Biotechnical Faculty of the University of Ljubljana. There has been international collaboration on different kinds of projects, including the EU's 6th Framework Programme. Our main international partners are Karl-Franzens University of Graz (Austria); University of Pisa (Italy); University of Würzburg (Germany); University of Göttingen (Germany); Aristotel University of Thessaloniki (Greece); INRA Versailles (France); University of Madeira (Portugal); University of Sussex (UK); Cardiff University, Wales (UK); The National Museums & Galleries of Wales, Cardiff (UK); University of California, Riverside (USA); EMBRAPA (Brazil); Chinese Academy of Science, Beijing (China); and Queensland University, Brisbane (Australia). Scientists in the group are involved in education at various levels at the University of Ljubljana and Nova Gorica Polytechnic.

The results of the group's scientific work have been presented in articles published in the leading scientific journals in the field, such as *The Annual Review of Entomology*, *Journal of Experimental Biology*, *Physiological Entomology* and *Journal of Comparative Physiology*. Researchers in the group are also members of the editorial boards of esteemed international journals. In 2006 two chapters published by Taylor & Francis in the book *Insect Sounds and Communication: Physiology, Behaviour, Ecology, and Evolution*' reviewed part of the scientific output of the group. The group's work has been presented in the BBC TV series *Talking with Animals*, and reviewed in *New Scientist* and the BBC's *Wildlife* magazine. Researchers at the Department of Entomology are members of the Entomological Society of America, the Royal Society of Entomology, London, and the Entomological Society of Brazil.



Vibrational signals are the best characteristics for identifying different species of the leafhopper genus *Aphrodes*. Sonograms (above) and oscillograms (below) of the male call songs for three species are shown. Scale bars indicate 1s.



The morphology of a vibration-sensitive interneuron located in the prothoracic ganglion of the cave cricket Troglophilus neglectus (plan view, scale 200 µm). The neuron responds to the vibrations of the front legs of the animal.