ABSTRACTS OF

TALKS AND POSTERS

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Contents

Welcome to Berkeley!

We hope you will have a rewarding meeting. Many of us have worked towards making this meeting both enjoyable and productive. We hope you will enjoy it.

Acknowledgements

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Fossils *versus* Molecules and Cladistics: Controversies over the Hemiptera Phylogeny

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Ten years ago grouping Heteroptera (+Coleorrhyncha) together with Auchenorrhyncha as Euhemiptera, their sister relationships with Sternorrhyncha, and therefore paraphyly of Homoptera have been inferred from the 18S rDNA (Campbell et al. 1994, 1995; Sorensen et al. 1995; von Dohlen and Moran 1995). There is nothing new under the moon: morphological synapomorphies of Heteropterodea and Auchenorrhyncha have been listed by Emeljanov (1987), their common ancestry has been traced by Popov (1980), and they have been united as Hemelytrata (=Euhemiptera) and opposed to Hymenelytrata (sternorrhynchans and thrips) within Hemiptera by Fallen (1829). So the only novelty was a cladistic claim to abandon paraphyletic Homoptera. However, paraphyletic taxa are inherent in the Linnean classification (Brummitt 2003), so taxonomists and paleontologists will continue to use such concepts as Reptilia, Blattodea or Homoptera. When tracing phylogeny through the fossil record we arrange taxonomic clusters in branched chains of ancestry, rank these clusters according to the hierarchy of hiatuses between them, and accept all non-polyphyletic taxa as natural (Rasnitsyn 1996); holophyly and paraphyly are merely two stages in evolution of a taxon: all paraphyletic taxa had once been holophyletic, and all holophyletic taxa are potentially paraphyletic. Even taxa that do not have any diagnostic characters in common may fall into the same cluster if we find all the intermediate steps between them: 'The characters do not make the genus; but the genus gives the characters' (Linnaeus 1751).

For the basal branching of Hemiptera, molecular cladograms differ from morphology- and fossil-based phylogeny (Shcherbakov and Popov 2002). The lineage Psocida (Permopsocina)→Lophioneurina→Thysanoptera is traceable back along with the Hemiptera lineage into the earliest Permian (285 Myr ago), both descending from Paleozoic Hypoperlina. Fossils indicate that Thysanoptera and Hemiptera developed sucking mouthparts in parallel, contrary to their grouping as Condylognatha based on putative synapomorphies (Börner 1904) and 18S rDNA (Johnson et al. 2004).

The Psyllina lineage (Psyllomorpha and their offshoot Aleyrodomorpha, known since the Jurassic – Shcherbakov 2000) and Aphidina lineage (extinct Pincombeomorpha and their descendants Aphidomorpha and Coccomorpha, both since the Triassic) separated before the mid-Permian (270 Myr) from Permian Paleorrhyncha (=Archescytinina), the stem hemipterans ancestral also to Hemelytrata and showing apomorphies of neither major lineage of the order. Extant Sternorrhyncha are diphyletic, but the transition from Paleorrhyncha to Hemelytrata is not as gradual as those from Paleorrhyncha to Psyllomorpha and to Pincombeomorpha, so one may include Paleorrhyncha in Sternorrhyncha s.l., which thus become paraphyletic with respect to Hemelytrata (Popov 1980). On the contrary, the 18S rDNA points to holophyletic Sternorrhyncha, usually with the topology Psyllomorpha+(Aleyrodomorpha+Aphidina) (Campbell et al. 1994, Aleshin et al. 1995), or a trichotomy in a later analysis (Ouvrard et al. 2000). However, not a single sound morphological synapomorphy of whiteflies with Aphidina has been found, whereas the opposition of Aphidina to Psyllina is well substantiated (Börner 1904, Schlee 1969). Psyllids show deviations from normal 18S rDNA sequence in places of insertions characteristic of the remaining Sternorrhyncha, hinting that these insertions had once been present, but later became lost (Aleshin et al. 1995). Long branch attraction (see e.g. Maddison 2004) may explain association of unusually long 18S rDNA sequences of whiteflies and Aphidina (Shcherbakov 2000).

The basic divergence of Hemelytrata is between Fulgoromorpha and Cicadomorpha (s.l.). Fulgoroidea are known since the mid-Late Permian (260 Myr), and their presumed ancestors, Coleoscytoidea, appeared before the mid-Permian (270 Myr), concurrent with the earliest Cicadomorpha (Prosboloidea: Prosbolopseidae and Ingruidae). Cicadomorphans diversified rapidly during the Late Permian. Hylicelloidea, the common ancestors of the three living superfamilies, evolved from Prosboloidea by the Middle Triassic (240 Myr). Coleorrhyncha, traceable back via Progonocimicidae to the latest Permian (255 Myr), are derivable from Ingruidae; this lineage evolved in parallel to true bugs, acquiring some superficial similarities but retaining basic differences (Popov and Shcherbakov 1991, 1996). Heteroptera, appearing much later, in the Middle Triassic, share the costal fracture and forewing-thoracic coupling device with, and doubtless descended from, a superfamily of primitive Cicadomorpha, Scytinopteroidea, which, like Coleorrhyncha, is derivable from Ingruidae. Ingruids form a base of the Heteropterodea clade, but are deep within the Prosboloidea grade, remaining typical primitive Cicadomorpha and showing no heteropterodean traits, except for a presumed shift of the forewing coupling lobe onto the stem of the Y-vein. Fossils show Cicadomorpha s.l. and Auchenorrhyncha paraphyletic with respect to Coleorrhyncha and Heteroptera (Popov and Shcherbakov 1991, Shcherbakov 1996).

Fulgoromorpha have been united with Heteropterodea as Neohemiptera (making Auchenorrhyncha paraphyletic) based on the 18S rDNA (Sorensen et al. 1995), the same having been suggested earlier on account of anatomical evidence (Goodchild 1966). Support of this grouping became equivocal when Coleorrhyncha were included in 18S rDNA analyses (Campbell et al. 1995, Ouvrard et al. 2000), and subsequent studies suggested a closer relationship of Heteropterodea to Cicadomorpha (Bourgoin and Campbell 2002, Johnson et al. 2004). Putative morphological

synapomorphies of Neohemiptera are either symplesiomorphies, or synapomorphies of Hemelytrata (Y-vein in clavus, transformed to imitate two free veins in extant Cicadomorpha s.str.), or homoplasies not shared by Coleorrhyncha. Mesonotal carinae of Fulgoroidea and some Progonocimicidae and Corixidae represent one more symplesiomorphic trait in 'Neohemiptera'.

Virtually all Triassic Heteroptera are Nepomorpha; these shore or water dwellers are the most hopper-like among bugs (including shorter antennae); other infraorders appeared by the earliest Jurassic (200 Myr), except for enicocephalids known since the Cretaceous (120 Myr). Primitive nepomorphans and leptopodoids were united with homopterans as Hypostomophora and opposed to remaining bugs (Prostomophora) by Spinola (1850). Contrary to cladistic analyses of the morphology (Schuh 1979) and 18S rDNA (Wheeler et al. 1993) showing Enicocephalomorpha as the most basal branch, fossils help us to reconsider character polarity and point to nepomorphans as the most primitive bugs (Handlirsch 1906–1908), to other bug lineages as their descendants, and to enicocephalids as derivatives of Dipsocoromorpha.

Topologies of molecular trees, especially basal branching, are sensitive to choice of outgroup, taxon sampling, alignment parameters, and method of tree reconstruction (Jenner 2004). Using neighbor-joining analysis of 18S rDNA sequences from GenBank, aligned with ClustalW, we obtained trees looking quite reasonable from the paleontological point of view in one or more aspects, such as Aphidina opposed to Psyllina, Coleorrhyncha grouped with Cicadomorpha, Nepomorpha basal to other bugs, etc. Demise of 'Neohemiptera' with molecular methods make us optimistic about remaining controversies, stemming from earlier 18S rDNA analyses of few species. Indeed, comprehensive rDNA analyses of broader sets of taxa agree with fossils and morphology in: (1) placing Cixiidae and/or other cixiid-like families as the most basal branch, and Tettigometridae near the tip, of Fulgoroidea (Shcherbakov 1996, Bourgoin et al. 1997, Yeh et al. 1998); (2) grouping Cicadoidea with Cercopoidea, Clastopteridae with Machaerotidae, and placing Myerslopiidae at the base of Membracoidea (Shcherbakov 1981; Emeljanov 1987; Hamilton 1999, 2001; Cryan 2004); (3) showing Cicadellidae paraphyletic with respect to Membracidae+Aetalionidae, and Stegaspidinae as basal among treehoppers (Hamilton 1971, 1983; Shcherbakov 1992; Dietrich et al. 2001; Cryan et al. 2004); (4) showing archaeococcids paraphyletic with respect to neococcids (Koteja 1996, Cook et al. 2002).

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Acoustic Signals, Species and Speciation in Auchenorrhyncha: A Historical Review

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Males of Cicadidae have been known since classical times to produce loud noises. These are usually species specific and function in mate finding and courtship. It is little more than fifty years since we first recognised that all other families of Auchenorrhyncha have the potential to produce sounds, albeit of very low intensity, by a diversity of tymbal-like mechanisms (Ossiannilsson, 1949). Frej Ossiannilsson had the simplest of technologies available both to record and analyse any low intensity sounds that he heard, but he did demonstrate that all species studied have an apparent mechanism to produce acoustic signals, and he heard and recorded many. We now know that smaller hoppers communicate primarily by transmitting acoustic signals through the substrate on which they live – generally parts of living green plants (Claridge, 1985). Recent digital systems both of recording and analysis are revolutionising acoustic studies on these insects.

Some form of the biological species concept (Mayr, 1942) is most useful for analysing diversity in most groups of insects. Such species are characterised by more or less complete reproductive isolation between them, maintained by species isolating barriers (Coyn and Orr, 2004). Of these, pre-mating barriers are of the greatest evolutionary significance and are more or less equivalent to the specific mate recognition systems of Paterson (1985). In all known groups of Auchenorrhncha these are characterised by signals that are primarily acoustic and therefore relatively easy to record and to manipulate. Also it is then possible to play back prerecorded signals in experimental procedures. The classic studies by Alexander and Moore (1962) on the periodical cicadas of USA, *Magicicada*, first showed the presence of more biological species than had been previously recognised of those well studied insects and play-back experiments demonstrated the isolating functions of specific male calls. Many other cicada species have since been recognised mainly on a basis of song differences. Since the discovery that substrate transmitted vibratory signals are produced by other Auchenorrhyncha, similar increased diversity of species has been shown in most families. Also play-back experiments have shown the isolating function of acoustic signals in some well studied examples (Claridge, 1985).

A major revolution in thinking about species and speciation over the past twenty years has been a result of the development of molecular genetic technologies. For example it is now possible to study gene flow in natural populations at very fine levels. Such studies clearly show that many distinct sympatric biological species do show low levels of genetic exchange without loosing the integrity by which we recognise them. Mallet (1995), with some other geneticists, has suggested that this level of gene flow is incompatible with the biological species concept and therefore has advocated yet another new species concept – the genotypic cluster concept. What the new data really show is the significance of natural selection in maintaining species characters despite low levels of hybridisation and thus gene flow. Reproductive isolation need not be absolute in order to maintain co-existing biological species.

Theories of allopatric speciation, which require at least some period of spatial or geographic isolation of incipient species populations, have dominated evolutionary thinking since mid 20thC. Contrary ideas of sympatric speciation where there is no requirement for such spatial isolation (Bush, 1975) are equally old and now widely supported. The relatively new realization that natural selection may maintain distinct species in the face of gene flow may resolve these old controversies? There is strong evidence of both apparent sympatric host race formation (Wood, 1993) and also of geographical variation in pre-mating acoustic isolating barriers (Claridge, 1993) among Auchenorrhyncha. These insects provide excellent material to pursue these ideas.

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An Ancient Symbiont of Auchenorrhyncha from the Bacterial Phylum *Bacteriodetes*

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Several insect groups have obligate, vertically transmitted bacterial symbionts that provision hosts with nutrients limiting in the diet (Buchner 1965). Some of these bacteria have been shown to descend from ancient infections of ancestral hosts. Many groups within the Auchenorrhyncha including representatives of Cicadidae, several subfamilies of Cicadellidae, Membracidae, Cercopoidea, and Fulgoroidea are hosts to a distinct clade of bacterial symbionts, which inhabit specialized bacteriomes of their hosts (Moran et al., in press). This newly described symbiont lineage belongs to the phylum Bacteroidetes. It corresponds to the "a-symbiont" of Mueller (1962), Buchner (1965) and other authors, although some confusion is associated with that designation in the literature. Among previously studied bacteria, this new taxon is most closely related to the genus *Blattabacterium* consisting of symbionts of cockroaches. Analyses of 16S rRNA genes indicate that the phylogeny of the Bacteroidetes symbiont is completely congruent with the phylogeny of insect hosts as currently resolved, based on published studies. Certain groups, such as Delphacidae and Flatidae and some leafhoppers, lack the symbiont, probably due to secondary loss, as first proposed by Mueller (1962). Two species of Peloridiidae (Coleorrhyncha) were found to lack this symbiont taxon, although other bacterial sequences, probably representing symbionts, were obtained.

The distribution and phylogenetics of this symbiont taxon are most readily interpreted as supporting the ancient acquisition of a symbiont by a shared ancestor of these insects. Thus, the results are consistent with a monophyletic Auchenorrhyncha. Alternative interpretations of current evidence are that some or all Fulgoroidea acquired a closely related symbiont independently or that a single colonization occurred in the ancestor of all Hemiptera followed by loss of the symbiont in the hemipteran sister group of Fulgoroidea. As visualized in a species of spittlebug (Cercopidae), the symbionts have extraordinarily large cells with elongate shape, often more than 50 microns in length; *in situ* hybridizations verified that these correspond to the phylum Bacteroidetes. All of the host insects also harbor at least one additional obligate symbiont, the phylogenetic affiliation of which varies among insect host groups. For example, in most Cicadellinae the second symbiont is *Baumannia cicadellicola*, a member of the *Gammaproteobacteria* (Moran et al. 2003).

Limited genomic sequencing of this symbiont from *Homalodisca coagulata* indicate that it has a small genome and retains a number of pathways for the biosynthesis of essential amino acids (N. A. Moran, DongYing Wu, J. Eisen, unpublished data). Thus, this symbiont is mostly likely involved in host nutrition and may have been an important element in the evolution of the sap-feeding lifestyles of auchenorrhynchan groups.

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Why Do We Study Auchenorrhynchan Feeding Processes?

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The Symposium on Feeding Processes and Their Role in Hopper-Plant-Microbe Interactions will highlight the multi-facetted role of feeding in the life of Auchenorrhynchans, a topic that is a bridge across many disciplines in the study of these insects. The speakers in this Symposium will provide their diverse discipline's insights, both empirical and theoretical, from many points of view, into the many aspects of feeding. The Symposium will emphasize holistic blends of modern techniques, often presented by interdisciplinary teams of researchers in the field. It also will emphasize interactions between Auchenorrhynchan feeding and other organisms such as microbes (both vectored and non-vectored) and plants. This preview of the Symposium will give an overview of Auchenorrhynchan feeding, provide some additional information that will not be covered in the Symposium, explain why these studies are important, and introduce the major topics and speakers.

The very earliest natural history studies of Auchenorrhyncha noted their highly specialized, piercing-sucking mouth parts. It has always been recognized that understanding the anatomy, or "plumbing", of the feeding structures informs our understanding their function. It wasn't until the late 1800's that detailed, empirical studies began, culminating eventually in the major works of (Snodgrass 1935) and (Goodchild 1966). In the Symposium's first paper, Wayadande and Ammar will review modern work on the anatomy of the alimentary canal and salivary glands, discussing how understanding functional anatomy is crucial for explaining the mechanisms of pathogen transmission by vectors. This preview talk will complement their talk by providing some extra information on the sensory systems that control feeding.

The insect arrives at the plant armed with an array of anatomical devices to penetrate the plant tissues and propel the tips of the stylets into position to ingest from only certain cell types within the plant. This selection is highly specialized within the Auchenorrhyncha, uniquely among all animals on Earth. Depending upon the taxonomic family or subfamily, different insects normally prefer to ingest from either vascular tissues (usually phloem or xylem, seldom both) or parenchyma/mesophyll tissues. They do so utilizing different strategies or sub-strategies (tactics) of feeding behavior that also differ by taxonomic group (Miles 1972, Backus et al. 2005). This preview talk will introduce the feeding strategies and stylet penetration tactics as well as the Auchenorrhynchan taxa that use each. Three presentations will elaborate on these very specialized stylet penetration behaviors. Bextine and Walker will provide an overview of stylet penetration by sheath-feeding leafhoppers, and how such details can aid in management of glassy-winged sharpshooter. Reynaud will discuss sheath-feeding planthoppers. Ranger will discuss cell rupturing leafhoppers and the behaviors that cause their direct feeding damage. These wide-ranging talks will also examine methods for how we study stylet penetration, examples of how such intricate feeding behaviors facilitate exploitation of the host plant, ways the host plant can resist that exploitation, and also how feeding behavior controls transmission of plant pathogens by vectors. One of the most rigorous and informative methods used to study feeding is the electrical penetration graph (EPG). All of the speakers in this section will discuss findings from EPG studies, as well as other types of studies. My introductory talk for the afternoon session will be a quick overview of the principles and applications of EPG, and the talk by Reynaud in the Symposium will describe some of the latest methods used for automated, computerized analysis of EPG waveforms.

Feeding behavior allows the insect to consume (sometimes very large) quantities of fluid from its host plants. Coudron, Hunter and Labavitch will blend their expertises in insect nutritional biochemistry and molecular genetics as well as plant biochemistry for their presentation. They will discuss both existing results and testable theories about the interplay among feeding (including extra-oral digestion by saliva), midgut digestion, and nutrition. Finally, Mizell (an entomologist) and Anderson (a plant chemist) present an integrated picture of the complex interactions among vector feeding and nutrition, plant chemistry, and natural enemies, using the glassy-winged sharpshooter as a model system.

Finally, we will bridge from this Symposium on the functions of feeding across to Chris Dietrich's symposium on the evolution and phylogeny of Auchenorrhyncha, by examining fossil evidence for piercing-sucking feeding. This preview

talk will summarize the elegant work of Conrad Labandeira (who was unable to attend the Congress), on how fossilized feeding tracks, salivary sheaths, and insect mouthparts suggest the feeding behavior and physiology of ancient Auchenorrhynchan ancestors and ecological analogues.

To answer the question posed in the title, we study Auchenorrhynchan feeding because it is fascinating. The details are unique in the Animal Kingdom, it has been mysterious for many years until the advent of improved technology, and knowledge of these details leads to better understanding of all aspects of these insects' lives and interactions with other organisms. Ultimately, sustainable means of managing populations of agricultural pest species will depend in part on such knowledge.

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Symposium – Auchenorrhychan Feeding Processes

Background on Electrical Penetration Graph (EPG) Monitoring in the Study of Auchenorrhynchan Feeding Processes

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Studies of Auchenorrhynchan feeding processes have been carried out for many years, using a variety of methods. The earliest studies, in the late 1900's, relied heavily on light microscopy of hopper-probed plant tissues to indirectly infer stylet pathways and sheath termini by examining the intact salivary sheaths. Even the oldest of these studies often have visually beautiful pictures of plant tissues and sheaths. However, salivary sheaths alone provide only a snapshot view of feeding, taken after the events have been completed. Until the invention of electrical penetration graph (EPG) technology in 1964 (McLean and Kinsey 1964), there was no rigorously quantifiable means of studying the intricate details of stylet penetration in real time. Various rapid stylet activities, cell types penetrated, fluids ingested, valve and diaphragm movements, can all be visualized in real time by using this technique.

This presentation is the Introduction to the Symposium on Feeding Processes and Their Role in Hopper-Plant-Microbe Interactions. The purpose of this talk is to provide basic information on the principles and applications of EPG. This will provide sufficient background for the audience to understand and appreciate the results to be presented in several of the papers in the Symposium.

The basic principle of EPG is simple. An output wire from the monitor into the soil of a potted plant electrifies the plant with a low-voltage AC or DC signal. A very thin, gold wire is glued to the dorsum of a test insect using silver conductive paint. The tethered insect is then connected to the input of the monitor, and placed on the electrified test plant. When the insects inserts it stylets into the plant (to begin stylet penetration, also called probing), the circuit is closed and current flows through the stylets, the insect and into the monitor. The voltage of that signal is then measured across the input resistor of the monitor, which provides an accurate model of the voltage of the plant-insect interface. Feeding behaviors of the insect cause variable resistance to the applied signal. This changes the constant applied signal into a variable voltage that, when collected over time, produces a waveform. In addition to resistance, some waveforms (depending upon how high the input resistor value is) can also be generated by biopotentials within the insect or plant. These include plant cell membrane breakages and streaming potentials caused by charge separation of fluid flowing rapidly through narrow tubes like stylets. Each insect species has a unique set of waveforms that represent its stylet penetration behavior. After a series of correlation experiments have been performed to define the waveforms, EPG can be used to visualize stylet penetration in real time, at the instant it is occurring.

After initial invention and introduction by McLean and Kinsey (1964) of the original, AC version of the monitor, many years were spent improving the technology. Significant improvements were made by: 1) Tjallingii (1978) who developed the detection of biopotentials and invented a DC version still in very popular use today, 2) Backus and Bennett (1992) who devised a more modern and noise-free AC device, and very recently, 3) Backus, Bennett and Tjallingii (ms. in prep.) who have developed the first universal AC-DC monitor with switchable input resistors, to better match the inherent resistance of each insect species recorded, and thus provide a more optimum blend of resistance signals and biopotentials in the waveforms.

EPG has been extensively used for aphids in the 40 years since its invention, and has now allowed identification of the finest details of, for example, the mechanisms of pathogen transmission by these insects. Application of EPG has been slower for Auchenorrhyncha, with which it was first used in 1970 (Crane 1970) and published in 1978 (Kawabe and McLean 1978). Unlike aphids, whose behavior is quite stereotypical and almost unvarying from species to species, Auchenorrhynchan feeding and waveforms vary significantly among taxa. This has necessitated that the time-consuming correlation process be performed for each new species. It is hoped that this process can now be hastened with the advent of the new AC-DC monitor, and that fascinating details of hopper stylet penetration will soon be revealed.

The depth and rigor of analysis of feeding behavior that EPG allows has many applications to the topics of the Symposium. Several of the papers of the afternoon will explore these applications, and others, in depth.

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Functional Anatomy of the Alimentary Canal and Salivary Glands in Leafhoppers and Planthoppers and Their Role in Pathogen Transmission

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For successful insect transmission of circulative and propagative plant viruses or mollicutes to occur, these microbes must overcome several barriers in their vectors, most notably midgut and salivary gland infection and/or escape barriers (Ammar, 1994). Stylets, foregut (precibarium, cibarium), alimentary canal and salivary gland functional anatomy is described for leafhopper and planthopper vectors (Ammar, 1985; Backus, 1985; Wayadande et al., 1997). Examples are given for the use of transmission and scanning electron microscopy, confocal laser scanning microscopy, immunolabeling and other techniques, in studying the routes, transmission barriers, and accumulation or multiplication of some circulative/ propagative plant viruses (e.g. maize streak Geminivirus and maize mosaic Rhabdovirus) and mollicutes (e.g. *Spiroplasma kunkelii* and *S. citri*) in their leafhopper or planthopper vectors (Ammar and Nault, 2002; Ammar and Hogenhout, 2005; Kwon et al., 1999). Recent studies also demonstrated the retention sites of semipersistent and non-persistent viruses in their leafhopper or aphid vectors, respectively, and the role of the helper component proteins in binding these viruses to the cuticular lining of the foregut and/or the food canal in the maxillary stylets. Maize chlorotic dwarf virus, transmitted by leafhoppers, and several potyviruses transmitted by aphids are examples of these two groups of viruses.

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The Role of Leafhopper Feeding in Vector-Microbe-Plant Interactions: Manipulating the System for Pathogen Management

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The first part of the presentation, by Walker:

Feeding behavior plays a large role in the transmission of microbes by vector insects from host plant to host plant. Understanding the intricacies of feeding behavior provides insights that may lead to development of better control tactics of insect-transmitted pathogens by use of plant genotypes or agrochemicals that interfere with components of feeding behavior that are critical to the transmission of the pathogen. In the first part of this presentation, our current state of knowledge of feeding behavior of leafhoppers is reviewed. The most important advances in our understanding of leafhopper feeding behavior have been made using the electrical penetration graph (EPG) technique, and these types of studies will be emphasized. While all leafhoppers are classified as piercing-sucking feeders, there is great variation in feeding behaviors among leafhopper species, and even within species. Some are primarily phloem-feeders, some primarily xylem-feeders, and others primarily mesophyll-feeders. Some, like *Empoasca* species, have a repertoire of various feeding behaviors that they can vary depending on circumstances. Also, in the first part of this presentation, the role of feeding behavior in acquisition and inoculation of plant pathogens will be very briefly reviewed. Most of this work has been done with Sternorrhyncha, but lessons learned there are very applicable to transmission of plant pathogens by Auchenorrhyncha.

The second part of this presentation, by Bextine:

The development of molecular techniques that allow for detection of as few as one cell have allowed us to understand the movement of microbes on a cell for cell basis. Combining these detection capabilities with traditional feeding behavior studies has led to more accurate diagnosis of pathogen movement. The glassy-winged sharpshooter (*Homalodisca coagulata*)/*Xylella fastidiosa* (*Xf*) model system has been studied to describe transmission events in a quantitative fashion. Movement of *Xf* from one plant to another depends on the transmission of the bacterium from an infected host to an uninfected host by the insect vector. For transmission to occur, two major events have to occur, acquisition and inoculation. In these studies we determined behaviors and timed events that are associated with successful movement of the bacterium. Positive correlations were detected between acquisition events and total ingestion time or acquisition access period (AAP) length, but not increased number of probes. On the other end of the disease cycle, positive correlations were detected between inoculation of *Xf* and number of probes or inoculation access period (IAP) length, but not increased total ingestion time. Understanding these associations will allow epidemiology studies of inoculative insects to be more accurate and help develop a means of reducing the efficiency with which the pathogen is spread from an infected plant to a non-infected one.

Planthopper (Hemiptera, Fulgoromorpha) Feeding Behavior: History and Current Prospects with EPG

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Feeding behavior is fundamental in the process of phytophagous insect diversification (Caillaud & Via , 2000). The feeding behavior of phytophagous insects is responsible for both direct damage and the transmission of pathogens. Planthoppers are a biogeographically diverse, monophyletic clade with about 12,000 described species grouped into 18 families (O'Brien, L.B. 2002; Stroinski A& Szewedo J., 2002). About 30 species of planthoppers are reported as vectosr of plant pathogens, in such familes as Delphacidae, which transmit viruses (Nault & Ammar, 1989), or Cixiidae, which transmit phytoplasmas (Purcell, 1985). Transmission of circulative and non-circulative pathogens depends on stylet localization and feeding activities. Electrical Penetration Graph (EPG) is a very powerful method to study stylet penetration behavior of hemipterous insects (Van Helden & Tjallingii , 2000). Within the planthopper clade, 5 species of Delphacidae, all in the tribe Delphacini, have been EPG-recorded.

History of planthopper EPG recording:

Chang (1978) was the first to record a planthopper by EPG: i.e. the sugarcane planthopper *Perkinsiella saccharicida* Kirkaldy, with the very first AC system. Three major EPG phases were identified and correlated respectively with mesophyll, xylem and phloem tissue localizations via salivary sheath histology. Kimmins (1989) identified six different EPG phases from *Nilaparvata lugens* Stal on rice using the DC system (Tjallingii, 1985). Two EPG phases were correlated with phloem and xylem ingestion via analysis of honeydew production. Kimmins (1989) argued that planthopper EPG waveforms were different from those of aphids because the salivary sheaths of planthoppers were intracellular. Spiller (1989) observed by TEM that the maxillary stylets of *N. lugens* enter the sieve element accompanied by sheath saliva, unlike what has been observed with aphids, where the salivary sheath stops at the sieve element cell wall and only the stylets protrude into the cell.

We have studied the feeding behavior of maize planthopper, *Peregrinus maidis* (Asmead), taking advantage of the new possibilities to acquire EPG data via a microcomputer (Buduca & *al*, 1996). Statistical analysis of digital data showed that some physical parameters could be distinguished, especially three major EPG phases. Spectral analysis allowed us to show major frequencies related to muscular activities. The knowledge of planthopper feeding behavior revealed by EPG was used to study plant resistance. Indeed, EPG analysis showed that the Delphacini are essentially phloem feeders, and that the major effect of plant resistance is a reduced duration of phloem phase (Chang & Ota, 1978; Kimmins, 1989).

Current prospect of planthopper EPG recording:

The possibilities for spectral and time-frequency analysis of digital data were used to more rapidly differentiate ingestion from salivation activities in sieve tubes for *P. maidis*, vector of *Maize mosaic virus* and *Maize stripe virus*, both circulative and propagative viruses. Indeed, ingestion and watery salivation are essential aspects of the feeding behavior of piercing-sucking insects, respectively correlated with acquisition and inoculation of circulative viruses (Prado and Tjallingii, 1994). Based on our time-frequency analysis and the observation by transmission electron microscopy of the fine structure of stylet pathways, passive ingestion and salivation were differentiated. Morever, temporal and spectral analysis of digital data from EPG recording produced many physical signal parameters. We chose the most significant ones to separate EPG phases, and with them developed software (termed 'EPG-Soft') for automated recognition of EPG recording (Reynaud & *al.*, 2003). An example will be presented of the use of our software for the analysis of data for development of host plant resistance. Indeed, in some maize inbreds MStV and MMV disease incidences varied in relation to the cumulative number of inoculative planthoppers, and revealed a non-specific virus resistance in maize (Dintinger & *al.*, 2005). EPG analysis by EPG-Soft showed that cumulative time in phloem mostly explained the resistance-susceptibility status of these maize inbreds. These results are further discussed in a broad perspective in relation to study of planthoppers and other hemipterans with EPG.

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Cell-Rupture Feeding by *Empoasca* spp.: How It Causes Hopperburn and Plants Defend Against It

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Hopperburn is a noninfectious plant disease attributed to the direct feeding damage caused by *Empoasca* spp. leafhoppers (Cicadellidae: Typhlocybinae) (Backus et al. 2005). Symptoms include leaf chlorosis, stunting of stems, and wilting of terminals and leaves. Mechanical damage by the lacerating stylets is likely the initial trigger of the hopperburn cascade (Ecale and Backus 1999). Cell rupturing is the characteristic feeding strategy employed by all *Empoasca* spp. (Backus et al. 2005). Electrical penetration graph monitoring determined three different stylet penetration tactics comprise the cell rupturing strategy, namely, lacerate-and-sip, lacerate-and-flush, and lance-and-ingest. While all *Empoasca* spp. possess the same repertoire, variations of the tactics and the tissues in which they are performed influence whether or not the hopperburn cascade will be initiated. *Empoasca* salivary components amplify the severity of the wound response first initiated by mechanical damage. Thus, initiation of the cascade of plant physiological events that cause hopperburn is termed a saliva-enhanced wound response (Ecale and Backus 1995, 1999). Insects employing a lacerate-and-flush style of feeding secrete large amounts of watery saliva that readily diffuses from the site of secretion, thereby exposing cells within a localized region to the salivary components. Watery saliva from phytophagous Hemiptera contain hydrolyzing and cell wall degrading enzymes, such as amylase, cellulases, hydrolases, and proteases. The combination of mechanical damage and salivary stimuli results in localized necrosis within probed regions, enlargement and proliferation of phloem parenchyma and vascular cambial cells. Vascular bundles become disorganized, collapsed, and constricted, resulting in an accumulation of photoassimilates in the leaves (Ecale and Backus 1995, 1999). Overall, the vascular constriction that ultimately occurs as part of the saliva-enhanced wound response has considerable consequences on whole-plant physiology, such as reduced phloem and xylem translocation, photosynthesis, and nonstructural carbohydrates.

Plants can successfully defend themselves against hopperburn by altering the repertoire of *Empoasca* feeding tactics (Serrano and Backus 1998, Serrano et al. 2000). Elevated healing/compensatory responses to mechanical and salivary factors can also minimize hopperburn symptoms. An additional well-studied example of a plant defending itself against hopperburn involves alfalfa, *Medicago sativa* L., and the hopperburning potato leafhopper, *Empoasca fabae* (Harris) (Ranger et al. 2001a,b, 2002, 2004a,b, 2005a,b, Shockley et al. 2001). Glandular trichomes found mainly on the stem and petiole surface secrete nonvolatile fatty acid amides that act as behavioral deterrents to settling by the potato leafhopper (Ranger et al. 2004a,b, 2005a). The glandular trichomes provide an effective barrier to colonization by the potato leafhopper and provide a crucial source of resistance against hopperburn.

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Finding the Continuum between Nutritional Needs and Feeding Processes

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As part of nutrient acquisition, insects respond to quality, relative frequencies and distributions of potential food resources in their environment. The acquisition process is comprised of a continuum of events, starting with a nutritional need and ending with nutrient intake. The recurring necessity of nutrient acquisition enables microbes to capitalize on this series of events to enhance their interactions with plants. The objectives of this presentation are to: 1) convince the audience of the plasticity in the digestive physiology of insects and, 2) pose the possibility that, together with similar malleability in feeding processes, this plasticity affords insects adaptability beyond our initial perception about the array of possibilities that impact the interactions of pathogens with plants and their insect vectors. At the very least, understanding this plasticity will stimulate an evaluation of how we approach research and design experiments.

Carnivores, herbivores and omnivores all face heterogeneous nutritional environments. Consequently, they could benefit from possessing the ability to manipulate their nutrient intake in order to redress nutritional imbalances (Mayntz et al., 2005). Theoretically, this manipulation could be expressed through foraging, food selection, feeding behavior, and/or digestive mechanisms. Evidence demonstrating that alterations in digestive processes occur in response to dietary changes will be provided in this presentation. Arguments will be made that those changes are a compensatory response to differences in the nutritional quality among diets and that those changes can have a profound impact on plant responses to herbivory.

One way to demonstrate the plasticity involved in nutrient-retrieval is to observe gene and biochemical changes that take place when the quality of diet is altered. When a living organism is presented with a challenge such as a suboptimal diet, that organism compensates for the reduced nutritional quality, in order to stabilize activities critical to development and thereby minimize the impact of the suboptimal nutrition. Those compensatory processes involve regulation of nutrient signaling, metabolism, or developmental rates. These processes enable the organism to bring into balance key physiological and biochemical events that otherwise would have been diminished had compensation not occurred.

Typically, compensatory processes are not intuitively known, but they likely are the result of changes in gene and protein expressions. Genomic and proteomic technologies (which measure alterations in global gene expression patterns among living organisms) are particularly well-suited for finding those gene and protein events associated with compensation responses (Tittiger, 2004). These technologies allow for the comparison of all expressed genes and proteins between treatments and developmental stages, so as to find where differences occur rather than comparing one gene or one protein wherein an expected difference is anticipated. Coordinated changes in expression of genes or proteins with identified functions will identify pathways associated with the compensatory events.

For their entry into this challenge, Coudron et al. are using genomics and proteomics to analyze biological processes associated with nutrition, to identify differentially expressed genes in prey- and diet-fed insects. For example, differentially expressed genes in immature pentatomids, fed either natural prey or an artificial diet (Coudron and Kim, 2004), were analyzed using a *Drosophila*-based cDNA microarray. Of the ca. 6000 genes surveyed in the cDNA microarray, 47 (0.7%) of the genes displayed a greater than 1.5-fold difference in expression. *Drosophila* cDNA microarrays were also used to compare gene expression between diet-fed adult females that manifested low or high fecundity. Five genes associated with decreased fecundity and one gene associated with increased fecundity were identified. Suppressive subtractive hydridization methodology was used to successfully identify additional differences in the gene expression patterns between the immatures fed on artificial diet and prey. Those differences are proving to be valuable to our understanding of an insect's response to suboptimal diets. A comparison of the salivary proteins from four hemipteran insect species (including two *Empoasca* spp. leafhoppers) was conducted in their laboratory (Habibi et al., 2001), to determine the effect of past dietary history on protein composition and quantity. A number of proteins and the percentage composition of certain proteins in the saliva changed depending on the past dietary history. This is evidence that insects can produce a variable 'digestive cocktail' dependent upon the chemistry of their food. In that manner, the 'digestive cocktail' is a coded message containing the insect's response to the contents of the food source. Also, they have successfully used a new technique, which measures the fluorescence of peptides, to determine baseline proteolytic activities associated with salivary glands and midgut tissues in *Lygus hesperus*. Protease class specificities against casein were found to vary considerably with changes in pH and between salivary glands and midgut homogenates.

Employing a novel approach, Hunter et al., are constructing expressed sequence tags (EST) libraries from salivary glands and midgut tissues of the glassy-winged sharpshooter, *Homalodisca coagulata* (Say), as a way to identify specific genes within the insect. The value of this approach is that the information provides the full array of genes potentially available to the insect. This allows expression levels to be compared when the insect is subjected to different sensory cues. The EST libraries have already resulted in the putative identification of over 800 genes, consisting of regulatory genes for metabolic functions, defensive genes (e.g., catalase, cytochrome P450, glutaredoxin and defensin A & C, and digestive enzymes (e.g., serine protease, proline oxidase, chitinase and lipase). These findings also support the concept that insects, including Auchenorrhynchans, have the adaptability to alter their responses to food quality and other stimuli.

Labavitch et al., have studied insect salivary digestive activity in order to understand the impact of insect herbivory on plants. They have focused their attention on the salivary polygalacturonase (PG) of *L. hesperus*. PG is an enzyme that digests the polymer backbones of cell wall pectic polysaccharides. PG has been identified as a virulence factor for some fungal pathogens of plants (Ten Have et al., 1998). Their studies have shown that the Lygus bug PG is an important contributor to the damage caused when the insect feeds on alfalfa and cotton. The identification of a key role for PG in insect-caused plant damage was demonstrated with the use of a micro-injector that allows placement of minute quantities of enzyme preparation into the plant tissue targets generally visited by the lygus bug (Shackel et al., 2005). The exciting aspect of these findings from the crop plant protection perspective is their finding that plant proteins that inhibit PG action (PG-inhibiting proteins, PGIPs) inhibit the salivary PG of lygus bug and other insects. PGIPs contribute to plant defenses against some pathogens (Powell et al., 2000). This raises the possibility that PGIPs might also mitigate the damage caused by insect herbivory when PG is present. Similar studies are underway for *H. coagulata*.

The useable products from this research will be: 1) genetic probes to measure transcript expression during feeding, 2) antibodies to probe salivary and midgut proteins in insects and plant tissues before, during and after feeding, 3) cloned proteins for further biochemical and fitness studies, and 4) with this knowledge, the ability to design further experiments to evaluate gene functions in protein production necessary for insect feeding and digestion. Also, genes and protein, necessary for insects to successfully feed upon and digest food, may become prime targets to manipulate pathogen vectoring processes.

Clearly our vista expands as we take into consideration that, through changes in gene expression, insects can access a dynamic cocktail of offensive substances and that plants respond with defensive substances to minimize and counteract

the insect. All the while, we as researchers make incremental observations about this dynamic continuum, in an attempt to understand and appreciate the full interaction.

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Impact of Plant Xylem Chemistry on Leafhopper Vectors of Diseases Caused by *Xylella fastidiosa*

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Xylophagous leafhoppers are vectors for numerous economically-important diseases such as Pierce's disease (PD) of grapevines, citrus variegated chlorosis, plum leaf scald, phony peach disease and leaf scorch of almond and many other tree and landscape species. *Xylella fastidiosa* (Xf) is the causal agent for these xylem-limited diseases. *Homalodisca coagulata*, also known as the glassy-winged sharpshooter (GWSS), is endemic to the southeastern United States where it is the most important vector for Xf diseases. Adult GWSS may feed on the xylem fluid of hundreds of plant species; however, successful nymphal developmental occurs on only a subset of the adult hosts. The distribution of this highly polyphagous leafhopper is in part due to host plant nutritional quality. Xylem fluid is 95 to 99% water with an osmolarity of 10 to 40 mM. The major constituents are amino acids, organic acids and sugars; inorganic ions comprise most of the remaining osmolarity.

Characteristics of GWSS that enable it to subsist on such a nutrient-poor diet include: 1) high feeding rates (10 to 100x body weight per day); 2) >99% assimilation efficiency of ingested organic compounds; 3) ammonotelism, and; 4) the seasonal and diurnal selection of host plants with superior nutrient content. Other xylophagous leafhoppers such as *H. insolita* and *Cuerna costalis* also share the above adaptations. Feeding rate of GWSS on most host plants is highest during midday, at a time when plant nutrient content and xylem tensions are also at their maximum. The majority of organic nitrogen transported in xylem fluid of many woody plants is in the form of the amides (asparagine and/or glutamine). Host selection and feeding of GWSS has been correlated with the amides or the ratio of the amides to total amino acids. In *Vitis* genotypes, glutamine accounts for 65 to 90% of the total amino acids in xylem fluid, and insect abundance and feeding have been correlated to the ratio of glutamine to proline concentrations.

Nymphs, particularly the early instars, develop more successfully on xylem fluid with low amide concentrations and proportionally higher concentrations of many of the more dilute amino acids that are deemed 'essential' for insect development. We have established that adult GWSS can efficiently use nitrogen and carbon from high amide concentrations, whereas young developing nymphs cannot. Female GWSS can consume more nutrients and produce more eggs on high amide diets, yet oviposition on these same hosts may result in up to 100% mortality from malnutrition of developing nymphs.

Malnutrition of nymphs and parasitoid-mediated reductions in oviposition success are key mortality factors for GWSS, with potential for manipulation to suppress vector populations and Xf diseases. Many of the important interactions, their critical timing, and identification of the host plants in mediating them remain to be elucidated. We do not fully understand the cues involved or the behavioral decisions female GWSS make in response to host plants during feeding and oviposition. These decisions involve movement and directly determine egg parasitism, oviposition success and nutrition of young nymphs and are critical to GWSS survival and fitness. We have developed a conceptual model of the critical choices facing GWSS females, their implications to leafhopper fitness and the potential environmental cues and response behaviors involved at each juncture in the processes. This heuristic device will be discussed.

Via host plant selection, feeding and the accompanying behaviors, xylem fluid chemistry plays a pivotal role by affecting egg quantity and distribution and ultimately the success of leafhopper (and GWSS) eggs through parasitism. For example, GWSS that deposit a high number of eggs on a high quality nymph host may potentially increase survival of nymphs, but also increase the rate of egg parasitism. Conversely, GWSS that scatter eggs across a number of lower quality hosts in time and space may potentially decrease egg parasitism but result in increased mortality of nymphs from malnutrition. Thus, opposing selection pressures may affect the feeding-oviposition behavior of GWSS. Necessary movements between feeding and ovipositional hosts or during feeding and oviposition may result in increased exposure of GWSS life stages to predation, or inability to locate either suitable ovipositional or feeding hosts.

To illustrate the conflicting requirements, in Florida, crape myrtle, *Lagerstroemia indica* L., appears to be the most frequently used mid-summer host of adult GWSS (highest abundances and consumption rates), yet GWSS oviposition is relatively low on this host. Adjacent hedges of *Euonymus japonica* have the highest concentrations of GWSS eggs that we have recorded, yet adults confined on this host feed very little. The ideal scenario for sustained GWSS population growth appears to be the close proximity of high quality GWSS ovipositional/developmental hosts to adult feeding hosts. These two conflicting requirements for population growth of GWSS (quality adult feeding hosts and ovipositional and/or developmental hosts) make it imperative to study host species and assemblages of host plants for their relative value and impact on GWSS. Such data may lead to pivotal decisions regarding removal or addition of key plant species, developing tactics such as trap crops (addition of plants to a landscape), cropping systems, crop/landscape systems or deployment of resistant hosts that suppress GWSS and/or Xf populations. Basic understanding of insect nutrition and behavior at the tissue, plant, and habitat levels, provide the critical information necessary to initiate a synthesis that will lead to practical applications for manipulation and suppression of vector populations and ultimately to disease suppression.

SYMPOSIUM - PHYLOGENY AND EVOLUTION OF AUCHENORRHYNCHA

Re-Examining the Phylogeny of Auchenorrhyncha (Insecta: Hemiptera)

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The phylogeny of Auchenorrhyncha and its included lineages, has been the subject of some controversy in the systematic literature. Traditionally treated as monophyletic, Auchenorrhyncha includes the lineages Fulgoromorpha (planthoppers) and Cicadomorpha (cicadas, leafhoppers, treehoppers, froghoppers, and spittlebugs). In 1995, however, the monophyly of Auchenorrhyncha was debated in a series of publications based on phylogenetic analyses of partial 18S rDNA nucleotide sequences (von Dohlen & Moran, 1995; Campbell et al., 1995; Sorensen et al., 1995); it seemed that molecular evidence showed Auchenorrhyncha to be paraphyletic (Fig. 1). Although new morphological evidence (primarily, features of the forewing) supporting the monophyly of Auchenorrhyncha has been discovered recently (Yoshizawa & Saigusa, 2001; D'Urso, 2002), no new molecular evidence has been generated to examine this question.

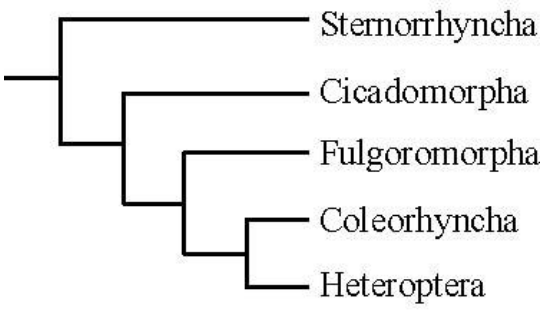

Fig. 1: Phylogeny of Heniptera showing Auchenorrhyncha as paraphyletic

Cicadomorpha, one of the major auchenorrhynchan lineages, comprises the superfamilies Cicadoidea (cicadas), Cercopoidea (spittlebugs and froghoppers), and Membracoidea (leafhoppers and treehoppers). Figure 2 illustrates alternative phylogenetic hypotheses that have been proposed for relationships within Cicadomorpha, whether Cicadoidea is sister to Cercopoidea + Membracoidea (Fig. 2A; Hamilton, 1981; Sorensen *et al*., 1995; von Dohlen & Moran, 1995), Membracoidea is sister to Cicadoidea + Cercopoidea (Fig. 2B; Boulard, 1988; Campbell *et al*., 1995; Ouvrard *et al*., 2000; Bourgoin & Campbell, 2002; Dietrich, 2002), or Cercopoidea is sister to Cicadoidea + Membracoidea (Fig. 2C; Evans, 1963; Hamilton 1996, 1999). Earlier attempts to reconstruct relationships among these three monophyletic lineages using either morphological or molecular data suffered from insufficient sampling (taxonomic and data) and problematic tree rooting, leading to the discordant results depicted in Fig. 2. To address this controversy, I recently published the results of a phylogenetic analysis of Cicadomorpha based on DNA nucleotide sequence data from three genetic loci (Cryan, 2005); analyses of the combination of data sets support the major relationships within Cicadomorpha as (Membracoidea, (Cicadoidea, Cercopoidea)). Internal relationships recovered within each superfamily shows evidence for: 1) the placement of Myerslopiidae as the sister group of the remaining Membracoidea; 2) the paraphyly of Cicadellidae; 3) the sister group relationship between Machaerotidae and Clastopteridae; 4) the monophyly of Cercopidae; 5) the diversification of Epipygidae from within the possibly paraphyletic Aphrophoridae.

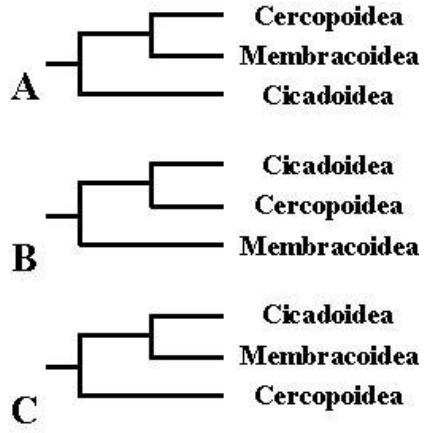

Fig. 2: Alternative phylogenies for Cicadomorpha

Fulgoromorpha, the other major lineage within Auchenorrhyncha, comprises approximately 20 planthopper families. Julie Urban will discuss the contentious phylogeny of this group in her presentation at this symposium.

The first goal of this presentation is to address the topic of auchenorrhynchan monophyly using evidence from both morphological data and DNA nucleotide sequence data. I will discuss the results from analyses of a preliminary data set comprising nearly complete sequences of 18S rDNA generated from exemplar taxa representing the major lineages of Hemiptera. These results demonstrate molecular evidence, from the same gene used in the 1995 papers, supporting the monophyly of Auchenorrhyncha. The second goal of this presentation is to discuss the phylogeny of Cicadomorpha and included superfamilies.

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Fulgoroid Phylogeny: Using Combined Molecular Datasets to Unravel Planthopper Evolution (Insecta, Hemiptera, Fulgoroidea)

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The planthopper superfamily Fulgoroidea comprises more than 12,000 described species in 20 families. While Fulgoroidea is monophyletic (Asche, 1987; Bourgoin et al., 1997), the relationships among the fulgoroid families remain largely contentious. A number of hypotheses of planthopper phylogeny have been proposed based on morphological evidence (Muir, 1923, 1930; Asche, 1987; Emeljanov, 1990; Bourgoin, 1993). Although there is congruence concerning relationships among some planthopper families (e.g., the monophyly of Delphacidae + Cixiidae, and of Kinnaridae + Meenoplidae), the results of these studies are otherwise incongruous with respect to relationships among all 20 families.

More recently, DNA nucleotide sequence data have been employed to attempt to elucidate planthopper phylogeny (Bourgoin et al., 1997; Yeh et al., 1998; Yeh & Yang, 2001). These studies concluded with several points of concordance, suggesting the overall utility of molecular data for resolving fulgoroid phylogeny. However, many of their conclusions were discordant, both with each other and with the previous morphological studies, leaving many questions regarding the phylogeny of Fulgoroidea unanswered.

In an attempt to better reconstruct fulgoroid phylogeny, I will discuss results of analyses including approximately 60 taxa in 17 planthopper families based on DNA nucleotide sequence data from three genetic loci. These results will be interpreted in light of the phylogenetic trends in ovipositor structure proposed by Asche (1987) and Bourgoin (1993), as well as what is known concerning host plant associations (based on Wilson et al., 1994).

Additionally, I will present results of an ongoing phylogenetic investigation of the planthopper family Fulgoridae. Although fulgorid planthoppers have received attention based on their often bizarre morphology and wax production (O'Brien, 2002), no phylogenetic hypothesis has yet been proposed for this group. My analyses, to date, include approximately 50 taxa in 35 genera based on DNA nucleotide sequence data from three genetic loci.

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Froghoppers -Diversity and Classification (Hemiptera: Auchenorrhyncha: Cercopoidea)

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Morphological criteria combined with life-history information supports the recognition of three families of froghoppers (Cercopoidea): Cercopidae, Clastopteridae (with antennae concealed in deep antennal pits, like those of Xestocephaline leafhoppers), and a rare Neotropical family Epipygidae (with adults that do not feed, relying instead on copious body fat reserves). Clastopteridae are usually small insects. Their three subfamilies have various life styles, Clastopterinae with nymphs that produce "spittle masses" with tiny bubbles; Tremapterinae that produce fragile tubes of hardened spittle, and Machaerotinae that live as nymphs in hardened tubes resembling calcium deposits. Cercopidae are "true" spittlebugs, the nymphs producing large globs of foamy sap exudates that completely conceal them from parasites and predators. True spittlebugs are divided into subfamilies Cercopinae and Aphrophorinae, but with several tribes that are intermediate between these two major taxa, which also probably deserve subfamily rank. Distinctive examples of synapomorphic characters are presented. Examples of polyphyly include Lepyroniini and Philagrini; these presently encompass genera convergent in external form but quite dissimilar in male genitalic characters. There is a great need for both biological and taxonomic work throughout the superfamily. Priority is elucidation of the biology and taxonomy of the Neotropical Epipygidae, which are rarely found as adults and not at all known from immatures. Many undescribed Cercopoid genera are known throughout the world, and collaborators throughout the world are sought to complete the generic revision of the world fauna. Some very large genera (e.g., old-world *Aphrophora* and *Clovia*, plus Neotropical *Clastoptera*) and many regional faunas (e.g., in New Guinea) are in urgent need of taxonomic revision.

Phylogeny of Cicadellidae (Cicadomorpha: Membracoidea) Based on Combined Morphological and 28S rDNA Sequence Data

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The higher classification of leafhoppers (Cicadellidae), a family comprising ca. 23,000 described species, has been controversial and unstable for many decades. Evans (1947) made the first attempt at a modern natural classification that included all known genera, recognizing 17 subfamilies and 47 tribes, but his classification suffered from reliance on relatively few characters of the head and wings. Subsequently, various authors (e.g., Wagner 1951, Ross 1957, Davis 1975, Rakitov 1998, Dmitriev 2002) discovered additional characters of the thoracic sclerites, male and female genitalia, leg chaetotaxy, and nymphal morphology, and the number of tribes and subfamilies proliferated as tropical faunas of Africa and the Americas became better known (e.g., Linnavuori 1959, 1965, 1979, Linnavuori & DeLong 1977, Linnavuori & Ne'Amy 1983). Hamilton (1983), based on analysis of 16 previously neglected characters, proposed reducing the number of subfamilies and tribes to ten and 51 respectively, but subsequent authors have continued to recognize as many as 40 subfamilies and 119 tribes (Oman et al. 1990). Many of these groups are poorly characterized and doubtfully monophyletic but, so far, few attempts have been made to elucidate the phylogenetic status of cicadellid family-group taxa through explicit phylogenetic analysis.

Previous analyses using parsimony-based cladistic methods and incorporating multiple cicadellid family-group taxa incorporated either morphological (Dietrich 1999, 2004) or molecular data (Dietrich et al. 2001), but not both. Dietrich's (1999) phylogeny based on analysis of 93 morphological characters recovered two lineages, roughly corresponding to Wagner's (1951) divisions Iassides and Cicadellides, but with Ulopinae and Ledrinae placed with Iassides and Typhlocybinae derived from within Cicadellides. A subsequent analysis based on 28S rDNA sequences (Dietrich et al. 2001) was poorly resolved overall, but provided strong support for certain clades, including one comprising Deltocephalinae, Eupelicinae, Koebeliinae, Penthimiinae, and Selenocephalinae. The phylogenetic estimates resulting from these analyses were congruent in many respects, with clades well supported by morphological data also tending to be well supported by DNA sequence data.

Methods and Materials

To provide a more robust phylogenetic estimate for Cicadellidae, we analyzed an expanded dataset including a larger number of morphological characters and 28S sequence data from several tribes and subfamilies not included in the analysis of Dietrich et al. (2001). The data, with unalignable regions of the 28S sequence excluded, were analyzed both using the maximum parsimony (MP) criterion as implemented in PAUP* (Swofford 2000), with all characters assumed to be of equal weight, and Bayesian statistical phylogenetic inference using the Markov Chain Monte Carlo (MCMC)

method as implemented in MrBayes (Huelsenbeck and Ronquist 2001). The maximum parsimony analysis was performed on 300 random addition sequence replicates with TBR branch swapping. For Bayesian analysis, the data were treated as separate morphological and molecular partitions so that different evolutionary models could be applied to each partition. The analysis incorporated a substitution model with one parameter for each of the six possible substitution types, a correction for invariant sites and another for among-site rate variation using the shape parameter of a gamma distribution. The model for the morphological data also also incorporated a correction for variation in rates of change among characters. The analysis was run on a supercomputer with 4 chains for 5 million generations each, sampled every 500 generations with a burn-in of 1 million generations. Prior probabilities were flat, meaning that all possible topologies were considered equally likely.

Results and Discussion

The results obtained using MP were similar to those obtained for the 28S data alone (Dietrich et al. 2001) but better resolved overall and with generally stronger clade support. As in the previous analysis, Membracidae and Aetalionidae arose from within a clade comprising the cicadellid subfamilies Ulopinae and Megophthalminae (sensu lato), Eurymelinae and Idiocerinae were recovered as sister groups, the ledrine tribes Stenocotini and Thymbrini grouped with Tartessinae, and Cicadellinae sensu Young (Cicadellini and Proconiini) was recovered as monophyletic but the broader concept of this subfamily was not supported. Also, a large clade was recovered comprising Deltocephalinae and several other previously recognized subfamilies, some of which (Eupelicinae, Koebeliinae, Penthimiinae, Selenocephalinae) have recently been treated as synonyms of Deltocephalinae (Dietrich and Rakitov 2002, Dietrich and Dmitriev 2003).

In the Bayesian MCMC analysis (Fig. 1), clades recovered by the previous MP analysis of 28S sequence data alone with bootstrap support > 50% (Dietrich et al. 2001, Figs. 3-4) generally received posterior probability scores of 90% or higher. In addition, the analysis recovered a clade comprising Scarinae (=Gyponinae) + Iassinae with 100% posterior probability several other clades with somewhat lower probabilities.

Although analysis of combined morphological and 28S sequence data yielded a more resolved and robust estimate of leafhopper phylogeny than analysis of either dataset alone, several areas of the tree remain poorly resolved, accentuating the need for both improved taxon sampling and the addition of sequence data from other regions of the genome.

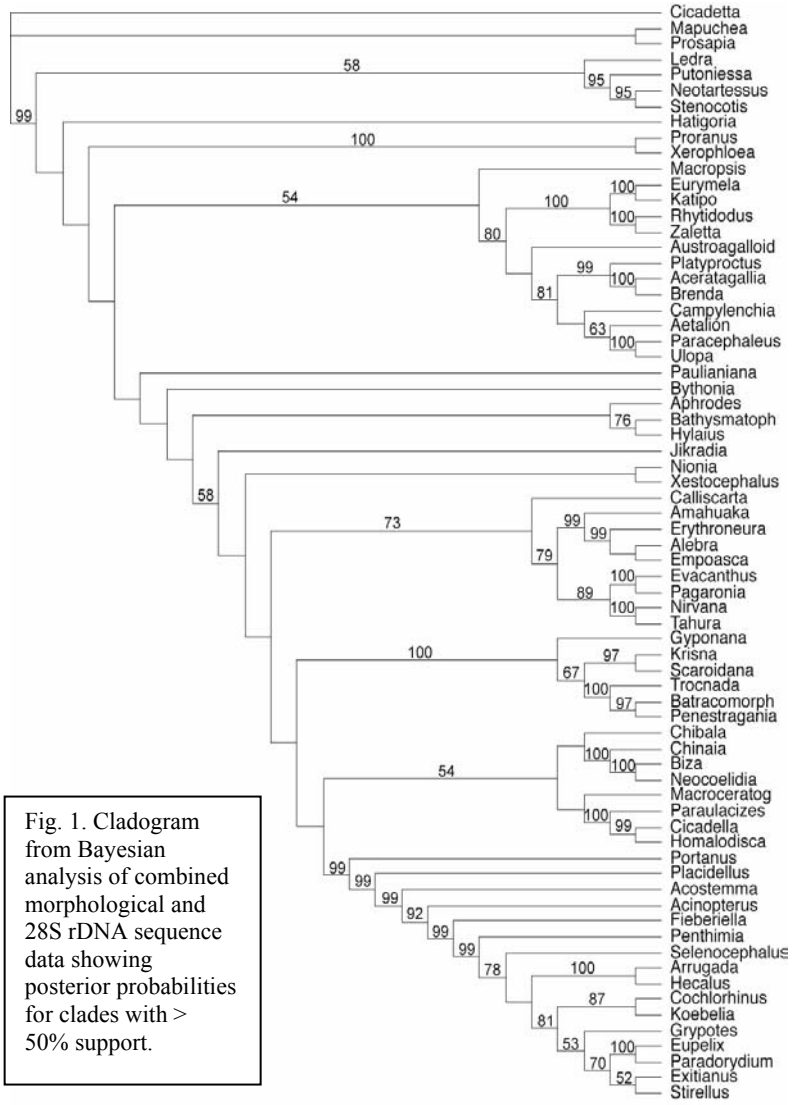

Fig. 1. Cladogram from Bayesian analysis of combined morphological and 28S rDNA sequence data showing posterior probabilities for clades with > 50% support.

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Phylogeny and Evolution of the Subfamily Orgeriinae (Homoptera, Dictyopharidae)

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The subfamily Orgeriinae is interesting for an evolutionary study because its recent representatives demonstrate successive stages of adaptation to conditions of mild to extreme aridity. The subfamily comprises 185 species in 38 genera of four tribes: Ranissini (7 genera, 43 species), Colobocini (1 genus, 1 species), Almanini (20 genera, 104 species), and Orgeriini (10 genera, 37 species). It is restricted to the arid regions of the Holarctic: the first three tribes are Palearctic, and Orgeriini are Nearctic. Some non-holarctic groups of independent origin (Lyncidinae, Strongylodematinae, Capeninae, and Risiinae) have been erroneously assigned to the subfamily in the past.

The knowledge of the taxonomy and biogeography of the group is fairly complete, except at the southern extremes of the range. Emeljanov (1969) published a taxonomic revision of Orgeriinae and analyzed their phylogeny and evolution with traditional methods in 1980. Kuznetsova (1985) clarified the karyology of the tribes Ranissini and Almanini and suggested that the karyotype of Ranissini with 2n = 26+XO had arisen from the modal for Dictyopharinae (2n = 28+XO) by the fusion of two large autosomal pairs with formation of a huge chromosomal pair. The karyotype of Almanini (2n = 24+XY) in its turn had originated from that of Rinissini by X-autosomal fusion involving the huge chromosomal pair mentioned above. Nokkala et al. (in preparation) investigated the molecular phylogeny of the same tribes and constructed a cladogram based on the 16S rDNA. The morphology- and DNA-based trees show only minor differences.

The tribal taxonomic rank of the intercontinental disjunction and the association of the subfamily with the xeric habitats indicates the Eocene age of this disjunction. The presence of a member of the advanced tribe Almanini (*Tilimontia*) on the Canary Islands, isolated since the Miocene, also attests to the at least Miocene age of this tribe.

The Palearctic branch of Orgeriinae demonstrates progressive adaptation to the more xeric and cooler environments parallel to the climatic changes during the Cenozoic: dry meadows (subtropical savannahs), tomillares, sagebrush semideserts, saltwort and other subshrub deserts, and cold steppes.

The analysis of the geographic distribution of particular genera and species reveals them being restricted to particular vegetation types within the framework of individual climatic types (as determined by the thermal regime, humidity level and dynamics, and continentality).

The Orgeriinae evolution was reconstructed based on the following assumptions: (i) New groups of generic or higher rank originate in the process of colonization of novel environments. (ii) Elements of the local fauna undergo gradual transformation. (iii) Groups evolving in particular conditions colonize all the available territories where these conditions exist and follow the fate of this area. Disappearance of such conditions results in disappearance of a corresponding group (genus, tribe) due to its extinction or transformation. (iv) In a genus with a wide range of ecological types of species, the more mesophilous representatives are the ancestral ones. An evolutionary return to more favorable conditions is unlikely.

The main source of the paleoclimatic data for the following reconstruction is the comprehensive work by Sinitsyn (1965).

(1) The origin of the tribe Ranissini (i.e., of the subfamily Orgeriinae) is associated with the formation of the initial subtropical savannah center in Central Asia (Kashgaria) after a more humid epoch of dry savannah meadows (Paleocene – Eocene). The ancestor of Ranissini is unclear (Dictyopharini or Orthopagini).

(2) The origin of the tribe Colobocini and of the common ancestor of Almanini and Orgeriini is associated with the formation of protomediterranean landscapes in the northwestern part of the arid center (territory of Kazakhstan) in the Early Eocene.

(3) A short period of partial aridization of the North-Atlantic land bridge permitted migration of the ancestral Orgeriini into Sonoran North America in the Middle Eocene. The Beringian land bridge was permanently humid up to the glacial time.

(4) With the progressive continentalization and cooling of climate in the Oligocene, the (proto)mediterranean environments shifted to the west and south-west; the ranges of Colobocini and ancestral Almanini (Almanae) shifted accordingly. The vacated eastern territory was occupied by the group Nymphorgerii, a direct descendant of Almanae. Probably, this is the first group of Orgeriinae that shifted onto semixerophilous dicot herbs, and then to xerophiles like *Artemisia*.

(5) During the Messinian Miocene crisis (drying up of the Mediterranean Sea), the group Almanae reached the Atlantic and the Canary Islands, which were connected to Africa.

(6) Also in the Miocene, due to the appearance of the Central-Asian center of maximum aridization with desert conditions, the next group, Scirtophacae, shifted to desert Chenopodiaceae and analogous semishrubs with succulent leaves.

(7) In the Pliocene, Scirtophacae expanded their range as the desert area expanded, and branched into the steppes in connection with cooling at the northern border of the arid center.

(8) At the same time, in the western (Middle-Asian) part of the arid area, Scirtophacae gave origin to a more halophilous desert group Tigrohaudae and psammophilous Orgamarellae and Ototettiges.

(9) Superaridization and progressive cooling of the Central-Asian desert region (i.e., the eastern part of the large Asian arid area) resulted in the retreat to the west (i.e., to a more acceptable Middle-Asian area) of all orgeriine groups (Ranissini, Nymphorgerii, Tigrahaudae, Orgamarellae), except some tolerant Scirtophacae (*Mesorgerius* in steppes, *Scirtophaca* in deserts).

(10) The tribe Ranissini retains the association with the climate characterized by summer rainfalls. Under the pressure of the gradual aridization, this tribe retreated from the lowland savannah meadows into low mountain elevations (Tien Shan center) and colonized the arising steppes (South Siberian center). Further cooling of the main steppe area pushed the steppe Ranissini (*Ranissus, Schizorgerius*) westward up to the Balkan region.

(11) The evolution of the North American Orgeriinae, represented by the tribe Orgeriini, proceeded from dry subtropical meadows to colonization of the chapparal (i.e., vegetation of the Mediterranean type) and diverse kinds of semidesert and desert vegetation (sagebrush, saltbush, yucca, agave) independently in several lineages.

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Revisiting body size constraints in Auchenorrhyncha: the influence of alternative phylogenetic scenarios and multiple origins of xylem-feeding

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Except for mesophyll-feeding typhlocybine leafhoppers, representatives of Auchenorrhyncha feed on vascular sap. The vast majority of species are primarily phloem feeders, while only Cicadoidea, Cercopoidea, and few leafhopper lineages (including tribes Cicadellini and Proconiini, i.e., sharpshooters) feed on xylem. Xylem fluid is more than 95% water, by far the most dilute food source encountered by herbivores (Andersen *et al*. 1992). Xylem-feeders show some behavioral and physiological specializations to maximize the utilization of xylem sap (Brodbeck *et al*. 1993). One such strategy is to have feeding rates reaching as high as 300 times their own body weight per day (Andersen *et al*. 1989), despite having to contend with a vascular system that is under very strong negative pressure.

Previous authors have suggested that the presence of a large cibarial pump in cicadomorphans may be a *sine qua non* of xylem feeding (Raven 1983). The cibarial pump is surrounded by heavily sclerotized walls providing attachment points for powerful dilator muscles (Backus 1985), which when contracted provide strong suction. Also, the dimensions of the food canal should influence the flow of fluid. Novotny & Wilson (1997) showed that the length and radius of the food canal and the volume of the cibarial pump are approximately isometrically related to body size in Auchenorrhyncha. This relationship supported their suggestion that there should be a minimum body size threshold (about 8 mm) for xylem feeders, above which the suction pressure necessary to overcome resistance, as well as its energetic cost, becomes negligible (Novotny & Wilson 1997). Based on measurements of Auchenorrhyncha body size taken from the literature, Novotny & Wilson (1997) showed that minimum body sizes in xylem-feeders tend to be larger than phloem and mesophyll feeders, but that only one xylem feeding lineage (Cercopoidea + Cicadoidea) is significantly larger than its putative phloem feeding sister group.

Previous analyses of associations between body size and feeding guilds in Auchenorrhyncha either examined only two xylem-feeding lineages (Novotny & Wilson 1997) or had no phylogenetic correction (Novotny & Basset 1999). Recent phylogenetic analyses of the Hemiptera have not yet yielded a strong consensus on relationships within Clypeorrhyncha (Cicadoidea + Cercopoidea + Membracoidea) and Cicadellidae (see Fig. 1).

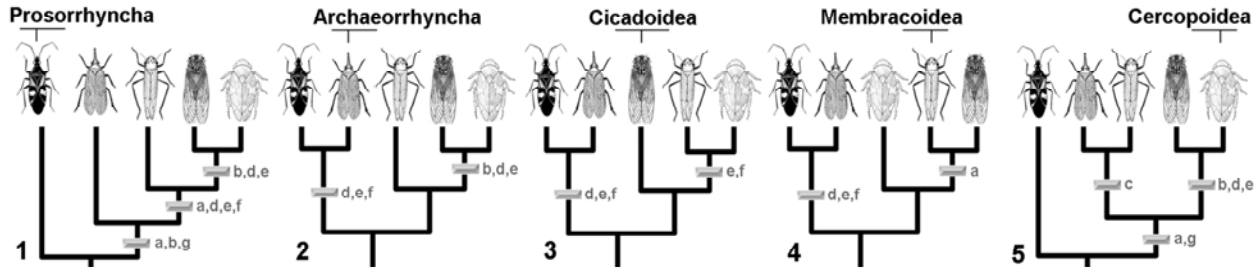

Fig. 1. Phylogenetic scenarios (numbered 1-5) of higher-level Hemiptera groupings. Possible group relationships recovered in analyses of: a, Evans 1963; b, Evans 1977; c, Emel'yanov 1987; d, Campbell *et al.* 1995; e, Sorensen *et al.* 1995; f, von Dohlen & Moran; g, Yoshizawa & Saigusa 2001.

Alternative phylogenetic scenarios suggest that xylem feeding in Auchenorrhyncha may have evolved two or more times. Also, although the feeding habits of the vast majority of leafhopper species have not been observed, previous workers have assumed that sharpshooters (Cicadellinae) are the sole primarily xylem-feeding lineage of leafhoppers. The objectives of the present study were to investigate the evolution of body size in clypeorrhynchan xylem feeders based on a phylogenetic comparative framework, and test the hypothesis that xylem feeders are larger than phloem or parenchyma feeders.

Materials and Methods

Relationships between xylem feeding and body-size in Auchenorrhyncha were assessed using Felsenstein's (1985) independent contrasts, which corrects for non-independence of data points due to phylogenetic inertia (Harvey & Pagel 1991). Phylogenies were taken from published morphological and molecular analyses or classification schemes. Supertrees combining multiple overlapping cladograms were constructed using Matrix Representation with Parsimony (MRP). Heuristic trees searches were run in PAUP* 4.0b10 under the maximum parsimony criterion, with characters treated as irreversible. Based on recent molecular evidence Sternorrhyncha was assumed to be the sister group to all other Hemiptera (Campbell *et al*. 1995, von Dohlen & Moran 1995, Sorensen *et al*. 1995). Five alternative scenarios

concerning relationships of other hemipteran lineages were analyzed to explore the effects of phylogenetic uncertainty on the results (Fig. 1). Body size data were compiled from thirty published works, emphasizing Cicadellidae, the largest family, in which most of the variation in feeding mode occurs. Heteroptera and Fulgoroidea body size ranges were compiled at the familial level, Membracidae at tribal level, and Cercopoidea, Cicadoidea, and Cicadellidae at generic level. An independent categorical binary variable, "feeding mode", was coded as (0) non-xylem feeding or (1) xylem feeding for all taxa. For leafhoppers xylem feeding, has only been observed in species belonging to Cicadellini, Mileewini, and Proconiini, but also assumed to occur in Signoretiinae, Errhomenini, and Evacanthini based on the enlarged frontal sclerite of the face. Phylogenetic contrasts for dependent continuous variables of minimum, maximum, and mean body size for xylem-feeding lineages were calculated with the aid of the computer program CAIC 2.0.0 using the Brunch algorithm and assuming branch lengths proportional to the number of terminal taxa (Grafen 1989). Contrasts were tested for statistical significance against zero using a one-tailed Wilcoxon sign-ranked test.

Results and Discussion

Preliminary analysis incorporating available phylogenetic information suggests that at least four shifts to xylem feeding occurred in Auchenorrhyncha. Three occurred within Cicadellidae (Membracoidea ancestor reconstructed always as a phloem feeder): two independent shifts in putative xylem feeders Errhomenini and Evacanthini, and another occurring in the lineage comprising all the proven xylem feeders (Cicadellini, Mileewini, and Proconiini) and Signoretiinae. A shift to mesophyll feeding in Typhlocybinae apparently occurred from a xylem-feeding ancestor.

Only contrasts for minimum body size were statistically significant ($P<0.05$) in all tested phylogenetic scenarios, implying an increase in minimum body size in xylem-feeding lineages. All contrast values were positive (0.06 to 1.16), except the comparison between Cercopoidea and Membracoidea in phylogenetic scenario 3 (-0.07). These results agree with those of Novotny & Wilson (1997), but do not support their conclusion that the body length threshold for efficient xylem feeding is 8 mm. In the present study, xylem-feeding leafhoppers show ancestral minimum sizes ranging from 3.5 - 7 mm and only in Cicadoidea (ancestral minimum size of 17.06) was the ancestral minimum body size more than 8 mm. Interestingly, the present result does show an increase in body size in xylem-feeding leafhoppers not found previously (Novotny & Wilson 1997). Their non-significant results were probably due to the lack of a robust phylogenetic hypothesis for Membracoidea, and their consequent inability to correct for phylogenetic non-independence.

This was the first attempt within the Hemiptera to study ecological parameters using a detailed method of phylogenetic correction and estimation of ancestral states (Felsenstein 1985). Due to the immense number of species in this group, it was necessary to use family-groups as terminal taxa (122 used herein), for which phylogenetic hypotheses had either not been published or remain poorly resolved. Thus the results should be interpreted with caution due to the many uncertainties regarding relationships. Further limitations include the sparse information on feeding habits of major leafhopper lineages and incomplete sampling of leafhopper higher categories in published phylogenetic estimates. Before a more precise study on the evolution of body size in Hemiptera is possible, more robust phylogenetic hypotheses on higher-level groupings and detailed information on feeding habits of many leafhopper lineages will be needed.

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Mating Signals and the Evolution of Behavioral Isolation in the *Enchenopa binotata* Species Complex of Treehoppers (Hemiptera, Membracidae)

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The *E. binotata* complex of treehoppers is among the most frequently cited examples of sympatric speciation resulting from shifts to novel host plants (Wood 1993; Coyne and Orr 2004). The nine species in the *E. binotata* complex, each of which feeds on a different host, are widely and sympatrically distributed in eastern North America (Lin and Wood 2002). Host shifts are hypothesized to initiate divergence by causing allochronic shifts in life history timing, which, in concert with high host fidelity, reduces interbreeding between populations on different hosts (Wood 1980, Wood and Guttman 1982). This assortative mating facilitates the response to divergent natural selection on performance traits (Wood 1993).

Reproductive isolation in the *E. binotata* complex may result not only from allochrony and host fidelity, but also from variation in vibrational mate attraction signals. As with many insects that live on plants (Claridge 1985; Cokl and Virant-Doberlet 2004; Cocroft and Rodríguez 2005), *E. binotata* engage in vibrational duetting during pair formation (Hunt 1994). Males produce bouts of four or more signals; each signal consists of a tone that drops in frequency (the "whine"), followed by a series of pulses (Hunt 1994; Sattman and Cocroft 2003). Males often pursue a "call-fly" searching strategy in which they move between a series of stems or plants, signaling on each one and waiting for a female response. Receptive females alternate their own response signals with those of the male, eliciting localized searching by the male.

We tested the potential role of mating signals in reproductive isolation in the *E. binotata* complex using two approaches. First, we surveyed mating signal variation among and within species in the complex. Second, we used playback experiments to assess the importance of this variation for female mate preference.

Methods

To assay variation in mating signals within the species complex, we collected individuals as late-instar nymphs and raised them to maturity in the greenhouse. Once males were mature, we recorded their signals using laser vibrometry.

To evaluate the importance of variation in male signals for female mate choice, we conducted two sets of playback experiments. Female response signals provide an assay of mate choice. We first tested the contribution of male signals to behavioral isolation by presenting females of one species (from *Viburnum*) with playbacks of recordings of male signals from six species in the complex (Rodríguez et al. 2004).

We also examined female preference functions for individual signal characteristics by vibrational playback of digitally generated signals, which are as effective as natural signals in eliciting female responses

Results

There is substantial between-species variation in male signals in the *E. binotata* complex. Signals differ in multiple characteristics, especially in frequency. In most species, variation between species is greater than geographic variation within species.

Female *E. binotata* from *Viburnum* were most likely to respond to the signals of conspecific males, with the exception of one species with similar signals. Females thus recognize potential mates on the basis of their signals, and exert choice through their decision to signal in response to the male (Rodríguez et al. 2004).

Preference curves based on individual signal characteristics varied between species in the preferred value of individual signal characteristics and in the relative importance of different signal characteristics. Male signal characteristics matched the peaks of the strongest preference (for frequency), but not for weaker preferences (whine length, signals per bout), implying stabilizing and directional selection, respectively. The close match between the peak of female preferences for frequency and mean male signal frequency indicates that there is coevolution between at least some male signal characteristics and female preferences.

Discussion

Our findings confirm the role of sexual signaling in reproductive isolation in *E. binotata*, and suggest that sexual selection could contribute to sympatric divergence.

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SYSTEMATICS SYMPOSIUM – II

Contrasting Effects of Pliocene- and Pleistocene-Age Environmental Changes on Speciation in New Zealand Cicadas

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Speciation theory emphasizes the role of divergent natural selection in population divergence. Unexpectedly, however, empirical studies only partially confirm widely assumed effects of dramatic Pleistocene climate shifts on organismal diversity (e.g. Klicka and Zink 1997, Zink *et al.* 2004). In New Zealand, two founder lineages of cicadas (Auchenorrhyncha: Cicadidae) have radiated to create at least 50 taxa during approximately the last ten million years (Arensburger *et al.* 2004a), a period characterized by dramatic landscape changes and then climate (Quaternary glacial cycles, beginning ca. 2.5-1.8 mya) (Cooper and Millener 1993). We have chosen the largest New Zealand cicada genus (*Kikihia*, now 30 taxa) to investigate the possible influences of these processes on cicada speciation. Cicadas make excellent organisms for studies of population divergence and speciation because their loud, prominent songs evolve rapidly and (usually) divergently, facilitating the discovery of cryptic taxa and the rapid collection of distributional data. The more cryptic taxa identified, the more accurate are estimates of recent speciation rates (such as late-Pleistocene divergence events) (e.g., Johnson and Cicero 2004). In addition, the low vagility caused by certain features of cicada life history (multiple-year juvenile development spent underground, and brief aboveground life; de Boer and Duffels 1996) suggests the potential for considerable phylogeographic structure.

Methods and Materials

Acoustic and genetic surveys of the genus were conducted, emphasizing the principal remaining unresolved *Kikihia* species complex (the *K. muta* grass cicadas). For the *K. muta* complex, digital recordings were used together with an mtDNA-based phylogeographic survey to identify concordant patterns of song and mtDNA divergence. For the overall molecular phylogenetic dataset, nuclear (1500 bp ef1-alpha intron and exon) and mitochondrial DNA (2150 bp COI, COII, ATPase 6, ATPase 8) sequences were obtained for 1-3 specimens of each taxon. Phylogenies were constructed using ML and partitioned Bayesian techniques. Dating analyses were conducted using the better-resolved mtDNA tree, which was converted to a time-calibrated chronogram using two methods: (1) Sanderson's (2002) penalized likelihood method, with the date of origin of Norfolk Island (location of *Kikihia convicta*) used for calibration (see Arensburger et al. 2004b), and (2) ML/Bayesian analyses conducted under the assumption of a molecular clock (which was first confirmed using a likelihood-ratio test after one unusually long branch was removed), and time-calibrated using a mtDNA evolutionary rate estimated using the method of Gilooly *et al.* (2005). Lineage-through-time plots were used to measure changes in speciation rate over time. Because speciation is a gradual process, progress toward speciation was investigated by identifying exclusively allopatric or parapatric groups of taxa and plotting the time of establishment of sympatry between such groups on the tree.

Results and Discussion

Acoustic and genetic surveys confirmed ten cryptic taxa first proposed by J. S. Dugdale and C. Fleming (unpublished notes), and added four additional forms not previously discussed. (Note that nicknames in quotes are used here to temporarily identify these undescribed taxa and that no description is implied.) Especially interesting are two new western South Island forms ("westlandicas") discovered within the *K. muta* complex; these taxa appear unrelated to true *K. muta* (based on concordant nuclear and mtDNA phylogenies) – an apparent case of parallel morphological and acoustical evolution accompanying independent radiations into grass habitats. Only one significantly diverged (ca. > 1%) mtDNA "phylogroup" (Avise 2000) cannot be characterized by a fixed song difference, and all forms distinguishable by song are also distinguishable by significant mtDNA divergence (two apparent cases may be attributable to recent interspecific hybridization/introgression).

Both the penalized-likelihood and molecular clock mtDNA trees suggest that the *Kikihia* radiation accelerated around the early Pliocene (coincident with the onset of major mountain-building) and that most *Kikihia* taxa have Quaternary origins, although diversification rates do not appear to have accelerated during the Pleistocene (Fig. 1). Population divergence may have been influenced more by directional habitat change (occurring continuously through both the Pliocene and Pleistocene Epochs) than by the predominantly oscillating Pleistocene changes (Jansson and Dynesius 2002). Comparatively localized distributions are the rule for recently diverged taxa, and few taxa originating after 2.5 my have evolved the ability to coexist in local sympatry with close relatives, suggesting that speciation has not reached its final stage for most Pleistocene-era lineages.

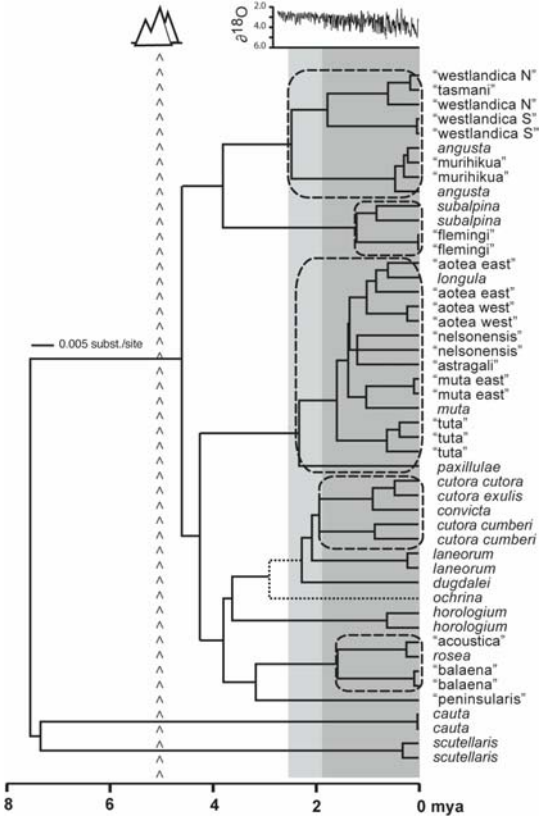

Figure 1. Mitochondrial DNA chronogram of the genus *Kikihia* showing estimated timing of cladogenesis events in relation to the onset of major mountain-building processes (ca. 5 mya) and the onset and intensification (1.8 mya) of mainly Pleistocene-age glacial cycles (climate data at top of figure is adapted from Webb and Bartlein 1992). Dashed lines outline groups of mutually allopatric or parapatric taxa. Taxa in different groups, and those not outlined, are sympatric or appear potentially so, with the exception of the "westlandica" and "murihikua" forms, which cannot coexist with members of the largest outlined group (true *K. muta* group). Location of *K. ochrina* lineage (removed for molecular clock analysis) is shown with a dotted line.

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Phylogeography of a widespread New Zealand subalpine cicada, *Maoricicada campbelli* (Hemiptera, Cicadidae)

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New Zealand, because of its wide range of habitats and rapidly changing, well-studied palaeohistory, provides an excellent opportunity to investigate the impact of earth processes on the distribution of taxa. This presentation shows a detailed phylogeographic history of a widespread, endemic New Zealand cicada, *Maoricicada campbelli* (Fig 1), that is abundant throughout much of the South Island and is also found in the central volcanic plateau of the North Island. This species has been divided into different populations that have been evolving and dispersing over the landscape for at least 2.3my, over and around mountains, valleys, rivers and plateaus (Buckley *et al.* 2001) and eventually establishing secondary contact as shown here. *M. campbelli* is the most widespread member of its genus in both habitat tolerance and altitudinal, latitudinal and longitudinal range, but like all other *Maoricicada* species is restricted to the largest two New Zealand islands. Most *Maoricicada* call from rocky scree and open ground, rather than from trees and bushes like most other cicadas. This unique habitat means that many *Maoricicada*, including *M. campbelli* in some parts of its range, may have a greater distribution during the ice ages than during the interglacials. Mitochondrial DNA sequences of 223 individuals from 70 populations of *M. campbelli* were studied using both traditional phylogenetic methods and nested clade analysis (NCA).

Fig 1. *Maoricicada campbelli* male on a thumb.

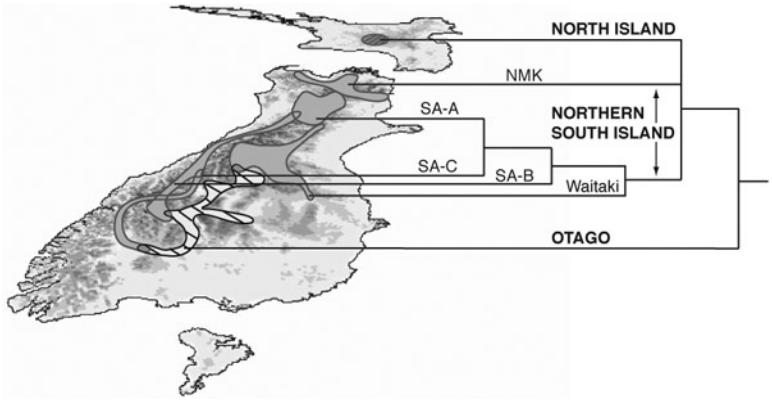

Fig 2. *Maoricicada campbelli* clade ranges over New Zealand. Phylogeny superimposed. NMK = Nelson, Marlborough and Kaikoura; SA-A = Southern Alps A.

Results and Discussion

As found in a previous study of 35 *M. campbelli* individuals (Buckley *et al.* 2001), geographic structuring was strong, with two main clades (North Island+northern South Island versus Otago) representing diverse lineages that may in fact be different species (Fig 2). Population structuring within the northern South Island clade suggested that the central South Island was mostly uninhabitable during glacial periods and demonstrated a sister-group relationship between northern and southern Southern Alps populations to the exclusion of the more central Southern Alps populations. Population histories estimated from NCA support the hypothesis that most *M. campbelli* populations were formed through dispersal rather than via fragmentation or Alpine Fault vicariance. Three areas of ring-species-like secondary contact were found between the Otago and northern South Island clades, between lineages that had been isolated for approximately 2.3my. Further study is predicted to confirm the presence of additional suspected contact zones, and will demonstrate whether the different lineages are reproductively isolated or hybridizing at these areas of secondary contact.

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Evolution of Egg-Powdering in *Cuerna* (Cicadellidae: Proconiini)

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Evolutionary reconstructions show that a trait acquired by a group of organisms is often lost multiple times among its descendants. The repeated nature of such losses provides an opportunity for testing hypotheses about the function of the trait and about relevant evolutionary mechanisms (e.g., Wiens 2001). Our study focuses on the unique maternal "egg-powdering" behavior and associated physiological and morphological traits (Rakitov 2004) that evolved and have been subsequently lost multiple times within the leafhopper tribe Proconiini. Females of the egg-powdering species produce specialized brochosomes in the Malpighian tubules, place them onto the forewings prior to oviposition, and finally scrape them off onto the plant epidermis above the freshly laid eggs with macrosetae of the anteroventral row on the hind tibiae. The function of egg-powdering is not yet completely understood but may include protecting the eggs (e.g., against egg parasitoids), creating optimal microclimatic conditions for their development, or both. Phylogenetic analyses indicated that several non-powdering proconiine genera are nested within a predominantly egg-powdering clade (Takiya, Rakitov, and Dietrich in prep.). Moreover, some genera contain both powdering and non-powdering species. One such genus, *Cuerna*, is broadly distributed in North America and found in a variety of habitats, ranging from deserts to moist prairies. The majority of the 31 currently recognized species of *Cuerna* occur in the U.S. and adjacent parts of Canada and Mexico (Nielson 1965; Hamilton 1970). Preliminary studies suggested that some of these species represent multiple independent instances and various stages of loss of egg-powdering. The goal of our ongoing research is to reconstruct the evolution of egg-powdering and obtain insights into the patterns, mechanisms, and causes of its losses in the genus.

Methods

Because the published bionomical information on the genus is scarce, we collected data on the egg-laying and host plants of *Cuerna* in a series of field trips. To date, we characterized egg-laying behaviors of 20 described and 3 undescribed species through direct observation/recording of captive females. Additionally, for each population/species, the glandular parts of the Malpighian tubules were examined in both sexes, externally or with TEM. Brochosomes produced by gravid females were examined with SEM. We also characterized the degree of modification and sexual dimorphism in the hind tibial anteroventral macrosetae. Several species not examined in nature were characterized based on museum specimens. The phylogeny was estimated using neighbor-joining, maximum parsimony, and Bayesian analyses of a DNA dataset containing 1,318 bases of partial COI and COII mitochondrial genes and the intervening tRNA, obtained for 76 populations representing ca. 24 species. A morphological study is in progress to provide additional evidence of relationships among species and update the taxonomy of the genus.

Results and Discussion

Among currently recognized species of *Cuerna*, 12 are powdering (*alba, alpina, arida, alta, balli, curvata, obtusa, sayi, septentrionalis, stitti, striata,* and *yuccae*) and 9 are non-powdering (*angusta, costalis, cuesta, fenestella, gladiola, hasbroucki, kaloostiani, obesa,* and *semibulba*). While, in most cases, the presence or absence of egg-powdering is a consistent attribute of a "good" species, in *C. unica* and *C. occidentalis*, we found populations of both types, possibly representing an early step in the evolution of a non-powdering lineage.

The basic egg-laying technique is identical in both kinds of species: multiple eggs are inserted, two at a time, under the epidermis on the adaxial side of leaves of shrubs, forbs, or grasses. This behavior is typical of the proconiine genera displaying egg-powdering (Rakitov 2004). Summarized below are differences between the powdering (left) and non-powdering (right) species of *Cuerna*:

1. In gravid females, glandular segments of the Malpighian tubules are inflated, actively secreting specialized, "egg" brochosomes.	1. In gravid females, glandular segments of the Malpighian tubules are empty, deflated; no brochosome secretion of any kind (unspecialized brochosomes produced in 1 sp.).
2. Prior to oviposition, the female places brochosomes onto the forewings.	2. No such behavior (vestiges observed in 1 sp.).
3. After laying each pair of eggs, the female transfers brochosomes onto the eggs with brushing strokes of the hind tibiae.	3. No such behavior (vestiges observed in 4 spp.); the female rests between laying subsequent egg pairs.
4. Female hind tibial anteroventral macrosetae vary from moderately to strongly elongated and curved.	4. Female hind tibial anteroventral macrosetae vary from unmodified to moderately elongated and curved.

The phylogeny derived from the mtDNA suggests 1) that egg-powdering has been lost during the evolution of *Cuerna* at least six but probably more times (if we assume that the losses of this complex trait were irreversible), and 2) that the non-powdering species displaying vestigial "powdering" behaviors are those that have closely related powdering species;

the completeness of the vestiges appears to decrease with increasing genetic divergence. These conclusions are consistent with morphological observations. However, in one area, the tree disagreed strongly with morphology, suggesting introgression of the mtDNA of *C. curvata* into one of the sampled population of *C. yuccae* through hybridization. Therefore, until verified by independent datasets, the inferred topology and distances should be interpreted with caution.

Our results suggest that the losses do not affect all powdering-related traits instantaneously. Rather, they begin with the "failure" of the secretory cells in the Malpighian tubules, which abort their normal synthetic activity instead of switching to production of the specialized brochosomes when the female becomes gravid (Rakitov 2000). The vestigialization and eventual loss of the related stereotyped behaviors and of modification of the hind tibiae follow and require a longer time for completion.

The consistent association between modification of the female tibial macrosetae and powdering provides indirect evidence of the adaptive value of this behavior in *Cuerna*. Therefore, comparing the ecologies of powdering and non-powdering species, especially those closely related, may help explain the function of this behavior and hint at the causes of its losses. Yet, so far, no obvious ecological correlates of these losses were discovered. No large-scale pattern in the geographic distribution of the two kinds of species is apparent. Moreover, species of both kinds often co-occur in the same habitats, where they are likely to be exposed to the same environmental conditions, including the same fauna of predators and parasites. No difference in the utilization of particular plants, such as having glabrous *versus* pubescent leaves, by powdering *versus* non-powdering females was noted. Further comparative studies of the biology of *Cuerna*, in the framework provided by our results, are obviously needed and should include detailed assessment of costs (such as the investment in synthesis of brochosomes and its potential trade-offs with fecundity) and benefits of powdering in the context of taxon-specific reproductive strategies.

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Phylogeography of the *Enchenopa binotata* (Say) (Hemiptera, Cicadamorpha, Membracidae) Sympatric Species Complex.

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Sympatric speciation, or speciation in the absence of geographic barriers to gene flow, is hypothesized to occur by several mechanisms. Probably the most applicably to Auchenorryncha is a mechanism involving host shifts in specialist plant feeders, who are uniquely adapted to their host plants. Host shifts may lead to assortative mating, which intern lead to population subdivision. Natural selection can then cause local adaptation in both physiological and behavior traits. As populations diverge local adaptation can lead to reduced hybrid fitness. Behavioral traits, such as host and mate recognition, would be under strong directional selection to reduce gene flow between host races. Thus reinforcement, the adaptive strengthening of prezygotic isolation, of behavioral traits eventually leads to reproductive isolation if gene flow and hybridization is deleterious.

Although, mathematical models have failed to support a hypothesis of sympatric speciation a mounting literature of empirical data suggest the possibility. One such example is the *Enchenopa binotata* (Say) complex of Membracid treehoppers. The complex contains 11 species, yet to be described, that utilize 11 distantly related host plants (with the exception of two species of *Juglans*). The complex is restricted to eastern United States and Canada and follows the distribution of host plants. Species are morphological distinct in the nymphal stage while adults differ mainly in quantitative size variation (see Wood 1993, Pratt & Wood 1992 for complete descriptions of morphology and life history of the complex).

In a phylogenetic framework sympatric species must be monophyletic sister taxa. While species level monophyly alone does not directly support a sympatric hypothesis, polyphyly does reject it. However , species level polyphyly (polyphyly in the broad sense including paraphyly) can be caused in several ways. True species level polyphyly is due to gene flow or introgression and is clear evidence for hybridization. While incomplete lineage sorting of ancestral polymorphisms will yield false polyphyly in recently diverged taxa. Only true polyphyly can reject a sympatric hypothesis in a phylogenetic framework. The sympatric speciation via host shift hypothesis predicts that each host species is reproductively isolated and forms a monophyletic lineage. The goal of this study was to establish the phylogenetic and phylogeographic relationships within and between members of the *E. binotata* complex.

A phylogeographic test of sympatric speciation was preformed on 58 individuals of the *Enchenopa binotata* complex, using both mitochondrial and nuclear genes. Both support a monophyletic *Enchenopa binotata* complex, when compared to Central American out group taxa. However, species level gene trees failed to agree on the monophyly of the 11 host species within the complex, with mtDNA gene trees supporting monophyly and the more slowly evolving nuclear gene trees supported polyphyly. Suggesting that gene flow or introgression is occurring between host species. However, the polyphyly of the nuclear gene trees is to be expected based on the low levels of polymorphism in the mitochondrial gene data, which suggest that the time since divergence is relatively short between sister taxa. To determine if nuclear gene tree species level polyphyly is simply an artifact of a recent divergence and ancestral polymorphism or rather the signature of gene flow or introgression, further genetic markers are required. Implications for sympatric speciation and future directions are discussed.

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Phylogeny of Deltocephalinae (Hemiptera: Cicadomorpha: Cicadellidae) and Related Subfamilies and the Evolution of Grass Specialization

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Deltocephalinae is currently the largest subfamily of leafhoppers, with approximately 6500 described species classified in 655 genera (Oman et al., 1990). Deltocephalinae is also among the most economically important leafhopper subfamilies, as it contains 117 of 151 reported leafhopper vectors of phytopathogenic diseases of economically important crops (Nielson, 1968). Deltocephalines feed on phloem sap from host plants spanning the angiosperms (and occasionally gymnosperms), and show varying degrees of host fidelity. But the most conspicuous host use pattern in this group is the conserved grass and/or sedge-feeding habit observed in at least 9 of the 23 tribes.

Classifications of this group have been unstable over the last 50 years because they have not been based on explicit phylogenetic analyses. Recent phylogenetic analyses including members of most subfamilies of Cicadellidae using morphological (Dietrich, 1999) and molecular (Dietrich *et al*., 2001) data indicated that Deltocephalinae as traditionally defined (*sensu* Oman *et al*., 1990) is paraphyletic and that several other subfamilies, including Penthimiinae, Selenocephalinae, Eupelicinae, Drakensbergeninae, and Koebeliinae have their closest relatives within the deltocephaline lineage.

However, taxon sampling in these recent phylogenetic analyses was limited. The purpose of the analyses presented here is to provide the first comprehensive phylogenetic hypothesis based on analyses of morphological and molecular data including representatives from all tribes in the deltocephaline lineage. In light of the phylogenies presented here, some morphological characters supporting clades on the trees are reexamined and their ability to inform higher level phylogeny and to diagnose natural groups is reassessed. Also, to provide a preliminary indication of the degree to which grass specialization is conserved, this trait is optimized onto a phylogenetic tree. The role of grass specialization in the evolution and diversification of the group is discussed and placed in a historical biogeographic context.

Materials and Methods

119 morphological characters were scored for 85 taxa, including representatives from all tribes of Deltocephalinae and related subfamilies, and 1848 base pairs of nuclear 28S rDNA were sequenced for the same set of taxa. Six outgroup taxa were included in the analyses: *Errhomus*, *Calliscarta*, *Portanus*, *Xestocephalus*, *Chinaia*, and *Aphrodes*. Parsimony, likelihood (molecular data only), and Bayesian analyses were run for separate and combined data sets. The evolution of grass specialization was mapped onto the resulting phylogeny using Bayesian ancestral states determination.

Results and Discussion

Separate analyses of morphological and molecular data sets yielded similar phylogenetic estimates. Overall, the morphological data provided lower clade support (posterior probabilities) and a less resolved topology than the molecular data alone. Analyses using combined data improved resolution and clade posterior probabilities.

The resulting phylogeny corroborates previous phylogenetic analyses of Cicadellidae and shows that members of some groups currently classified in separate subfamilies are nested well within the deltocephaline lineage. The outgroups used here rooted the tree such that Acinopterini, Fieberiellini, and Goniagnathini (Deltocephalinae), *Pachymetopius* (Stegelytrinae), and Acostemminae, all of which do not possess the typical deltocephaline male genitalia, are near the base of the tree. The remainder of the ingroup possesses the typical deltocephaline male genitalia (valve articulated to pygofer, pygofer with lateral oblique membranous cleft, subgenital plates dorsoventrally flattened and triangular, anterior arms of connective not widely divergent, styli broadly bilobed).

Other than characters of the male genitalia, some characters that proved to be useful in inferring relationships among tribes were found in the leg chaetotaxy and female genitalia (see Fig.1). The profemur provided some especially good characters, such as the development of row AV (*sensu* Rakitov, 1998), the development of the intercallary row, and the presence of additional row AM macrosetae. Other useful chaetotaxic characters were found in the metafemur, metatibia, and metatarsomere I. The female genitalia, which have historically been underutilized and are rarely illustrated in leafhopper systematics, yielded several useful characters. Among them are the presence/absence of pygofer macrosetae, the 1st valvula dorsal sculpturing pattern, the curvature of the ramus of the first valvula, and the presence/absence and shape of the teeth of the second valvulae.

One large clade was resolved which includes most of the grass-specializing tribes. This suggests that host switches from dicotyledonous hosts to grasses or sedges have occurred relatively few times in the evolutionary history of the deltocephaline lineage and that once acquired, grass-feeding is rarely lost. The extensive morphological and species-level diversity of this clade also suggest that grass feeding is very advantageous and potentially indicate a case of adaptive radiation. Once fossils have been identified for this group, phylogenetic dating may shed light on its time of origin, which can then be compared to the timing of the expansion of grasslands in the mid-Miocene (15-20 mya).

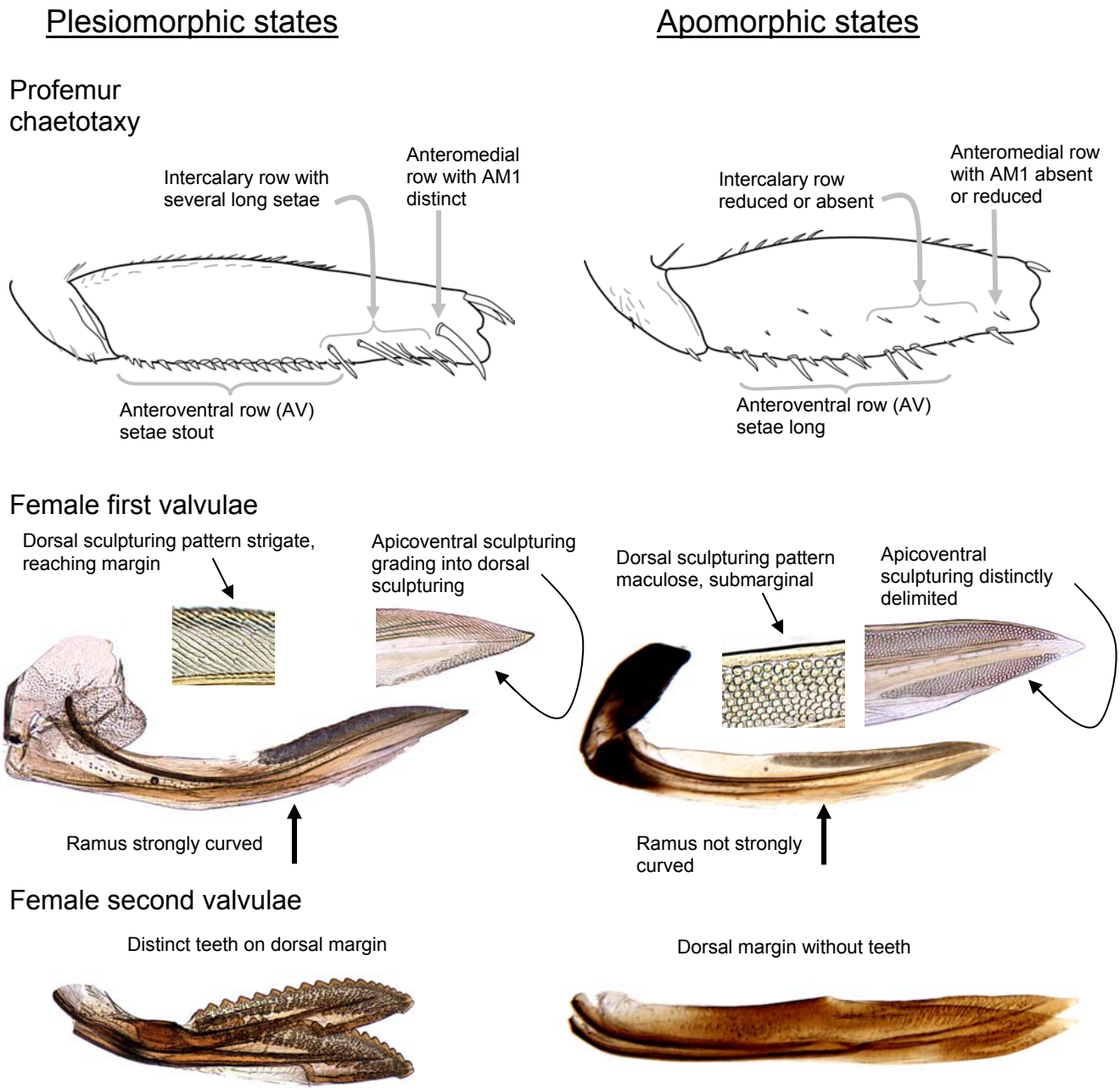

Figure 1. Some characters of the leg chaetotaxy and female genitalia that were useful in determining relationships among tribes of deltocephalinae.

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Progress in the Phylogeny of the Delphacidae using Molecular and Morphological Tools

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The principle phylogenetic hypothesis for the higher taxa of the Delphacidae is that of Asche (1985, 1990), who proposed 6 subfamilies based on a cladistic morphological analyses (Figure 1). Asche's (1985, 1990) hypothesis has a comb-like structure, with the primitive delphacids (the "Protodelphacida") combined into a paraphyletic subfamily, the Asiracinae, with two proposed tribes, the Ugyopini and the Asiracini. The advanced Delphacidae (the "Eudelphacida") consist of the subfamilies Kelisiinae, Stenocraninae, Plesiodelphacinae and Delphacinae; the latter with tribes Saccharosydnini, Tropidocephalini, and Delphacini. The very large tribe Delphacini includes 75% of delphacid species (1,569 of 2,084 species), and several previously recognized taxonomic groupings (e.g., Stirominae, Achorotilinae, Chlorioninae, Megamelinae, Alohini) dissolved by Asche (1985) because of a lack of synapomprphies. No phylogenetic hypothesis for the Delphacini has been proposed. The Vizcayinae form a "phylogenetic link" between the Protodelphacida and the Eudelphacida (Asche 1990).

Emeljanov (1996) proposed a modification of Asche's (1985, 1990) hypothesis based on features of the immatures. Emeljanov (1996) raised the Ugypini to subfamily status with three tribes (Ugypini, Neopunani, and Eodelphacini), the Asiracini to subfamily status with four paraphyletic tribes (Asiracini, Idiosystatini, Tetrasteirini, and Platysystatini), and subsumed the subfamilies of Eudelphacida plus Vizcayinae as tribes within the Delphacinae (i.e., 7 tribes, Vizcayini, Kelisiini, Stencranini, Plesiodelphacini, Saccharosydnini, Tropidocephalini, and Delphacini) with the same branching sequence proposed by Asche (1985, 1990).

Table 1. Taxon Sampling

Taxon	# genera	# species
Outgroup: Cixiidae	3	4
Delphacidae		
Ugypinae:		
Ugyopini s.s.	1	1
Neopunanini	1	1
Asiracinae:		
Asiracini s.s.	2	2
Vizcayinae	0	0
Kelisiinae	2	2
Stenocraninae	2	6
Plesiodelphacinae	1	1
Delphacinae:		
Saccharosydnini	1	1
Tropidocephalini	1	1
Delphacini	35	67
Totals	49	86

Here we present results of phylogenetic analyses using morphological and molecular data for 49 genera and 86 species, including 3 genera and 4 species of cixiids as outgroups, presented both independently and combined (Table 1). Taxonomic sampling includes all of Asche's (1985, 1990) higher taxa, except for Vizcayinae; and 35 genera and 67 species of Delphacini are included to investigate the relationships among genera of this tribe. The morphological data consists of 139 parsimony informative characters. The molecular dataset consists of 1533 bp of 18s and 1653 bp of 28s ribosomal DNA. All analyses are maximum parsimony as implemented by PAUP* ver 4.0 beta .

All of the present analyses suggest that the Protodelphacida are a monophyletic lineage, and that the Plesiodelphacidae are much more primitive than previously suspected. The morphological analysis places the Plesiodelphacidae as the first branch following the Protodelphacida, whereas the molecular and combined analyses place the Plesiodelphacidae *within* the Protodelphacida (Figure 2). The placement of Plesiodelphacidae requires further investigation. The molecular and combined analyses suggest that the Kelisiinae and Stenocraninae are sister groups. Bootstrap analyses suggest strong support for most clades, except relationships among the tribes of Delphacinae were equivocal.

Analyses for the Delphacini consistently suggest relationships among certain genera, however, relationships among clades within the Delphacini varies considerably between analyses. Although some analyses generate few trees (less than 20), bootstrap analyses suggest little support in several positions along the spine of the tree. Analyses are hampered in the current data sets by homeoplasy in the morphological dataset and insufficient variation within the sequenced portions of the 18s and 28s ribosomal genes. Additional data will be required to clarify relationships among the Delphacini.

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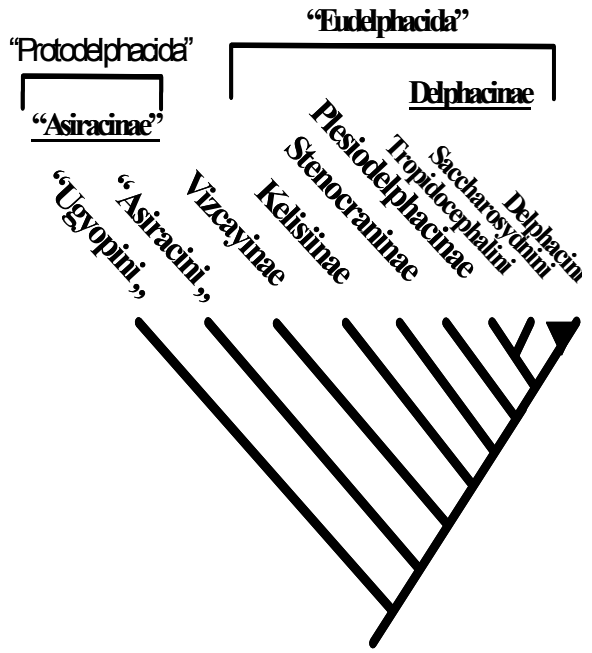

Figure 1. Phylogeny of the Delphacidae according to Asche (1985, 1990)

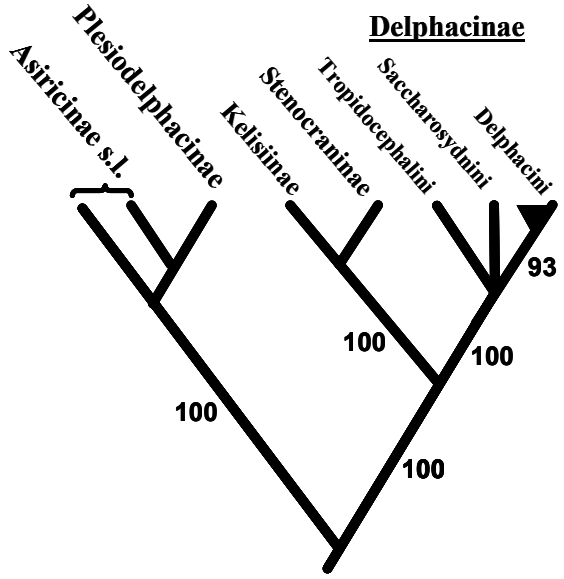

Figure 2. Phylogeny of the Delphacidae based on combined molecular and morphological analyses (1 tree, CI = 0.819, RI = 0.771). Numbers on branches are bootstrap values based on 100 replicates.

Evolutionary patterns of the Achilidae and their allies (Hemiptera: Fulgoromorpha)

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Fulgoromorpha is a group of Hemiptera that differentiated very early from the common hemipteran ancestors, i.e. Archescytinoidea, known and widely distributed during the Permian. A descendant of Archescytinidae was the earliest Fulgoromorpha — Coleoscytoidea, known from the Permian. Coleoscytoidea were small hoppers, with the frontoclypeus swollen, but not hypertrophied and long rostrum, implying they feed on the phloem or other plant tissues high in nitrogen: buds, seeds, fruits, meristems, etc. of gymnosperms. Coleoscytoidea did not survive the biotic crisis at the Permian Triassic boundary. Their descendants, Surijokocixiidae, are present from Upper Permian through the Triassic, but were a minor element of the fauna. Surijokocixiidae, extinct at end of the Triassic, had been replaced in the Jurassic by Fulgoridiidae, a highly differentiated taxon, believed to be ancestral to all extant Fulgoroidea families. Fulgoridiidae have been common in the Jurassic strata, with over 130 species described in several genera from Western Europe and China (Szwedo *et al*. 2004). Representatives of this family have a characteristic habitus and tegmen venation similar to Cixiidae, recorded since the Late Jurassic/Early Cretaceous. Fulgoridiidae had very long rostra, indicating that they were more often associated with arboreal gymnosperms, sucking from trunks and thick branches, than were extant planthoppers. Their clypeus was not hypertrophied, suggesting phloem-feeding for Fulgoridiidae. Another feature of Fulgoridiidae was distracting color patterns, with dark 'false eye' spots near the apex of tegmen or disruptive, cryptic

patterns with dark spots on tegmina. Nymphs of Fulgoridiidae are not known, but it seems reasonable to assume that they were cryptic, flattened, biscuit-like creatures with short legs, small frontoclypeus and long rostrum. They might have fed on phloem of rather thick stems or in bark cavities (Shcherbakov & Popov 2002, Bourgoin & Campbell 2002). Late Fulgoridiidae or their descendants, lacking the filter chamber of co-existing plant sucking lineages (Sternorrhyncha, Cicadomorpha) probably found fine roots and/or fungal hyphae with relatively nutritious cells that were easily attacked and that had relatively high soluble nitrogen content. Particular types of mutualistic relationships between plant roots and fungi, e.g. ectomycorrhizal, ericoid and orchid mycorrhizae, originated in the Jurassic or Cretaceous and evolved during the rapid angiosperm radiation in the Cretaceous (Brundrett 2002). Sorensen *et al.* (1995) suggested that early Fulgoromorpha initially evolved to feed on roots and fungal hyphae in subterranean/semisubterranean (duff) niches, much as many of their immatures do now. However, this supposition may be restricted only to Fulgoroidea. It is quite evident that the Jurassic/Cretaceous border and Cretaceous period were important times for origination and diversification of main lineages of extant Fulgoromorpha, but fossil data from this period are very scarce (Szwedo *et al.* 2004). Planthopper families recorded from Lower Cretaceous contain more "basal" groups: Cixiidae, Achilidae, probable Derbidae and Fulgoridae, as well as the extinct family Lalacidae, restricted in distribution to Brazil. It is noteworthy that in the Cretaceous some important features appeared: free living nymphs, adult-like and able to jump, in contrast to sessile forms from earlier periods (Shcherbakov & Popov 2002).

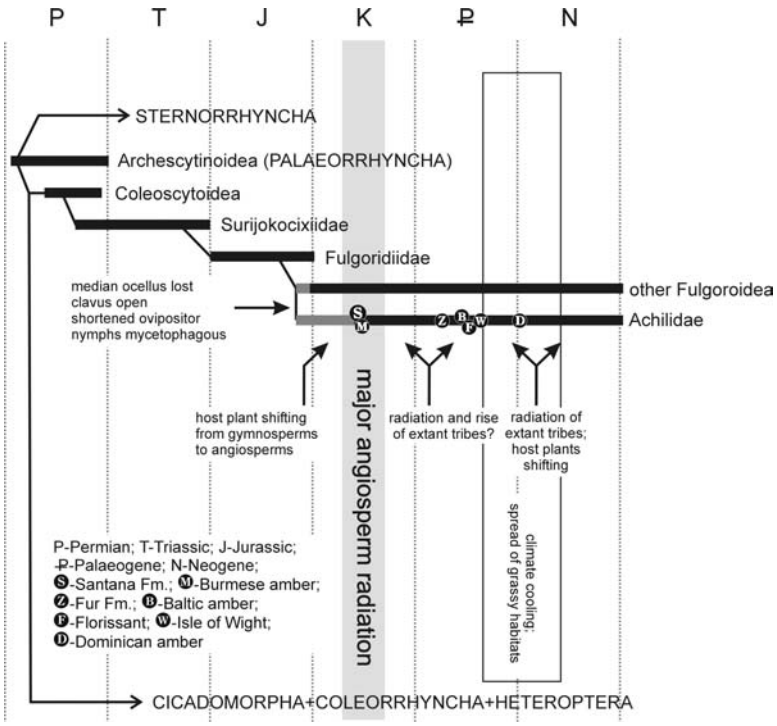

Fig. 1. Scheme of events affecting evolution of Achilidae

The family Achilidae is one of these old families, lying near the basal stock of recent Fulgoromorpha (Fig. 1), but with unresolved taxonomic problems. Numerous fossil taxa ascribed to Achilidae have been described, but some of them with limited validity and a need for revision (Szwedo *et al.* 2004). The first trace of Achilidae is known from the Lower Cretaceous strata of Santana Formation of the Araripe Plateau in North-Eastern Brazil. These fossils have more primitive venation than in extant genera, resembling that of primitive Derbidae. A very long rostrum, extending beyond the hind coxae near the apex of hind femorae, is another feature of these achilids. In Lower Cretaceous amber of Myanmar (Burma), few inclusions have been identified as Achilidae, but only a few of them are preserved in a more or less complete state that would permit further analyses. Only a single species, *Niryasaburnia burmitina* (Cockerell, 1917), is formally described. None of Lower Cretaceous Achildae can be placed among recently recognized tribes of Achilidae (Szwedo 2004). The Palaeocene record of Achilidae seems to be relatively rich both in imprints and in fossil resins. From the Uppermost Paleocene/Lower Eocene strata of Fur Formation from Denmark, a limited number of undescribed Achilidae have been reported. A number of taxa ascribed to Achilidae are known from the Middle Eocene Baltic amber. Baltic amber inclusions of Achilidae are relatively rich and represent most recently recognized tribes, as well as a tribe known only from this period and area. The little known taxon *Elidiptera regularis* Scudder, 1890 comes from Eocene deposits of Florissant, Colorado, U.S.A. Eocene/Oligocene deposits of the Gurnet Bay (Isle of Wight) in England contains *Hooleya indecisa* Cockerell, 1922, originally placed in Derbidae, but transferred to Achilidae. The recent genus *Synecdoche* L. O'Brien was identified in Oligocene/Miocene amber of Dominican Republic, dated 20–15 Mya, and a few specimens are recorded.

Although the data are scarce, some hypotheses could be proposed for the evolution of the Achilidae. It could be hypothesized that ancestral Achilidae lacked a median ocellus, had fused transverse carinae at vertex/frons border, retained a very long rostrum, evolved short pronota, with a shifted and anteriorly elevated disc, fused veins on costal margin, a few branches of longitudinal veins, with more terminals of RA and RP in marginal portion, two-branched CuA, open truncate clavus with veins Pcu+ A_1 entering apex, hind leg tarosomeres basal and middle one with subapical setae and/or platellae, and shortened ovipositor of raking type. Fossil nymphs of Achilidae are not known, but

mycetophagy was postulated for immatures, which is in concordance with raking type of ovipositor. Rotten wood seems to have been used as nymphal habitats by the common ancestor of Achilidae and Derbidae, because in these two closely related families, extant nymphs are mycetophagous and feed under bark, in cavities of rotting wood, or in litter. It appears that soil-dwelling and mycetophagous nymphs have been the source of separation of Achilidae+Derbidae lineage. The ancestral lineage of achilids may have lived in gymnosperm woods, which diversified during the Late Jurassic and Early Cretaceous. Angiosperm radiation seriously affected various groups of insects, and it is probable that some Achilidae shifted from a gymnosperm hosts to woody angiosperms, perhaps during Lower Cretaceous/Upper Cretaceous floristic events. It seems that both groups – gymnosperm-associated and angiosperm-associated Achilidae - survived the late Cretaceous events and extinctions. Some of the plant families known as hosts of Achilidae underwent adaptive radiations during the Eocene and it could be supposed that also some achilids diversified with these host plants, while others, strongly related to conifers became extinct. Terminal Eocene events and climatic cooling in the Early Oligocene (Prothero 1994) also probably strongly affected Achilidae. It seems that accelerated evolution and maybe also host-plant shifting could be related to climatic changes, as well as spread of open communities dominated by grasses and dicotylodones herbs during the Miocene. Switching to new host plants in many cases influenced the speciation rate in phytophagous insects (Farrell 1998, von Dohlen & Moran 2001), and such a situation could also have taken place within Achilidae. It seems that accelerated evolution and maybe also host-plant shifting could be related to climatic changes, as well as the spread of open communities dominated by grasses and dicotylodones herbs during the Miocene. Extant taxa of Achilidae are trophically related to rather old plant families of gymnosperms, dicotyledons, monocotyledons, a few, namely Plectoderini, within Poaceae.

Acknowledgements

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SYMPOSIUM – INVASIVE AUCHENORRHYNCHA

Why are some organisms so invasive? Parasites, predators, and pathogens

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Despite the intense interest in invasive species, it is not generally clear *a priori* which species are likely to colonize new habitats and, once established, whether an introduced species will invade by attaining far greater population growth rates, population densities, and population sizes, compared to its native range. Because of these uncertainties researchers question whether the study of biological invasions has reached the status of a "predictive science". A number of non-exclusive hypotheses/ideas have been submitted to underpin the success of invaders in their new habitat. As an introduction to the symposium, I consider four hypotheses/ideas which are relevant to invasions of leafhoppers and planthoppers as well as other organisms: (1) the *enemy release hypothesis, (2) biotic resistance hypothesis, (3) adaptation towards increased competitive ability,* and *(4) genetic variation/hybridization.* To date, there is much evidence to support the importance of release from enemies, including parasites and predators, while evolutionary-type explanations appear to be less important.

As talks in this symposium emphasize, invasive insects in California are not only taxonomically diverse, but they cause extensive ecological change in both natural and managed systems. Accordingly, the many State and Federal resources are targeted at the eradication and management of invasive species, but also towards research. There is recent progress along several avenues at making the study of invasive species more of a predictive science—climate modeling is a good example; predictive demography is another. Finally, as noted in this symposium, the impact of invasions by leafhoppers and planthoppers is compounded in notable cases by their ability to vector plant diseases. In sum, parasites, predators, and pathogens.

Invasive Auchenorrhyncha in California and an Overview of Two Major Control Programs Aimed at Them

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Provides case history examples of two invasive species; the glassy-winged sharpshooter, *Homalodisca coagulata* (Cicadellidae: Cicadellinae), and the beet leafhopper, *Circulifer tenellus* (Cicadellidae: Deltacephalinae).

Auchenorrhyncha in California that are Potential Invaders Elsewhere

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A brief summary of economic Auchenorrhyncha problems in California.

The primary economic problems caused by Auchenorryncha in California are related to Agriculture and to the ability of many Cicadellid species to transmit virus, MLO and similar plant diseases. Fortunately there are no outstanding Fulgoroidea currently known as vectors for economic diseases to crops in this state, although the potentials for this occurrence are very real. This paper will briefly summarize some of the more important plant disease-leafhopper associations such as curly top virus, Pierce's disease, corn stunt and aster yellows. Also discussed will be other non-disease economic injury due to other species of Auchenorrhyncha.

On the Move: The Potential Adventive Geographic Range of Glassy-Winged Sharpshooter, *Homalodisca coagulata*

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The invasion risk posed by the xylem feeding hemipteran, *Homalodisca coagulata* (native to the southeast U.S.A. and northeast Mexico and a recent invader of California [U.S.A], Tahiti, and Hawaii) was examined using the computer climate modeling program CLIMEX. Model predictions indicated that suitable climatic conditions for *H. coagulata* exist in almost all grape production areas of the world. Additionally, the model indicated that regions north of California will be unable to sustain populations of *H. coagulata* because of cold stress, and that irrigation of agricultural and urban areas in California's deserts has removed dry stress limitations, which when combined with a depauperate natural enemy fauna most likely facilitated successful invasion by *H. coagulata*. The model clearly indicated that Tahiti and Hawaii have climates extremely favorable for *H. coagulata* and invasion of these remote island systems has occurred as predicted.

The potential for continued spread of *H. coagulata* throughout the South Pacific is extremely worrisome. It is recommended that countries such as Australia and New Zealand that have very important agricultural industries be particularly vigilant and take proactive steps to manage incursion risk from either Hawaii or Tahiti. Further, steps need to be taken to determine if the xylem-dwelling pathogenic bacterium, *Xylella fastidiosa*, is present in recently invaded countries and those potentially at risk of invasion. *Xylella fastidiosa* is readily acquired and transmitted by *H. coagulata* and this vector-pathogen combination has demonstrated considerable destructiveness in southern California vineyards and urban areas which could be repeated in newly invaded areas if *X. fastidiosa* is present.

Invasion of Polynesia by the Glassy-winged Sharpshooter (Hemiptera: Cicadellidae): A New Threat to the Pacific

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The glassy-winged sharpshooter *Homalodisca coagulata* (Say) (Hemiptera : Cicadellidae) is a major pest of agricultural, ornamental and native plants. It was first recorded in Tahiti (French Polynesia) in 1999. It reproduced and spread rapidly in French Polynesia and is currently found in almost all islands in the Society Islands group, and was recently found in the Marquesas and in the Australs. Tahiti and Moorea are the most infested islands where *H. coagulata* populations have reached densities far exceeding those observed in California or in its native range. *Homalodisca coagulata* causes several problems in French Polynesia; high densities of feeding adults and nymphs are a social nuisance and this pest is suspected of causing impaired growth of plants. The major concern for French Polynesia is that this pest will acquire and vector the lethal plant bacterium, *Xylella fastidiosa*, which could have a disastrous impact on ornamental, agricultural and native plants. The presence of large populations of *H. coagulata* in French Polynesia presents a major threat to the agriculture and the biodiversity of South Pacific countries as this insect has clearly demonstrated high invasion potential.

A classical biocontrol program using the mymarid egg parasitoid *Gonatocerus ashmeadi* (Hymenoptera: Mymaridae) was initiated in 2004 to minimize both actual and potential problems caused by this pest. Risk assessment studies for native cicadellids were conducted before introducing this parasitoid in French Polynesia. Twenty-two cicadellid species were found in the Society Island group. Their risk of attack was assessed by using three criteria: taxonomy, morphology and egg laying behavior. From our results, none of the species has a high risk of being attacked by *G. ashmeadi*. Therefore, the French Polynesian Government authorized its release and first releases began in Tahiti May 2005. More than 6000 parasitoids have been released in three experimental sites. The first results are very promising: *G. ashmeadi* parasitizes almost all *H. coagulata* egg masses and spread fast.

Invasive strains of *Xylella fastidiosa* Increase the Importance of Indigenous and Invasive Vectors

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Xylella fastidiosa is a xylem-limited bacterium that has a broad host plant range, occurring mainly in tropical and subtropical areas of South, Central and North America. Although this bacterium colonizes many hosts as an endophyte, without causing visible damage or symptoms, some particular strains cause severe diseases in important crops, such as Pierce's disease (PD) in grapevines, citrus variegated chlorosis (CVC), coffee stem atrophy (CSA), plum leaf scald (PLS), phony peach disease and almond leaf scorch (ALS). Strains of *X. fastidiosa* can be distinguished based on pathogenicity and population behavior in certain host plants, growth on culture media, and genetic characteristics (Almeida and Purcell 2003). In Brazil and Argentina, *X. fastidiosa* became very important after a new strain was identified as the causal agent of CVC, in 1993 (Chang et al. 1993; Lee et al. 1993). CVC is now the major problem of Brazilian citriculture, causing losses of 100 million dollars per year. Recently, a citrus isolate of *X. fastidiosa* associated with a disease similar to CVC was found in Costa Rica (Aguilar et al. 2005) and could be a serious threat to citrus and other possible hosts (e.g. grape) in southern US, if introduced into that region.

Long-distance spread of *X. fastidiosa* strains to a new region might be enabled by invasive vector species, or by movements of infected nursery trees and/or plant propagative materials. Once introduced, a new strain depends on the availability of suitable invasive or indigenous vectors for local spread in the susceptible crops. Most vectors of *X. fastidiosa* are xylem-feeding leafhoppers in the subfamily Cicadellinae (sharpshooters), although spittlebugs (Cercopidae) and cicadas (Cicadidae) were also shown to transmit the bacterium to some hosts (Redak et al. 2004). In general, there is little vector specificity; the xylem-feeding habit seems to be a major requirement for transmission. This broad vector specificity means that many regions already have vectors in place to spread new strains that might invade a region. However, the epidemiological relevance of particular vectors depends on their ecological interactions with host plants and the environment. Key vectors are generally distributed on diverse ecosystems and show preference for feeding on inoculum sources and susceptible crops (Almeida et al. 2005). In southeastern Brazil, key vectors associated with CVC spread were shown to be indigenous sharpshooters, which were previously unknown by the citrus growers. Of several sharpshooters known to transmit *X. fastidiosa* to citrus, *Acrogonia citrina* Marucci & Cavichioli, *Bucephalogonia xanthophis* (Berg), *Dilobopterus costalimai* Young and *Oncometopia facialis* (Signoret) are particularly important because of their abundance on citrus trees, which is the major source of inoculum for CVC spread.

Because some indigenous sharpshooters are adapted to particular climates and habitats, different vector species might be associated with spread of *X. fastidiosa* in a given crop, in different regions. Surveys of sharpshooters associated with citrus groves in Brazil showed a distinct species composition between the northeastern (State of Bahia) and southeastern (São Paulo State) growing regions. Only one (*A. citrina*) of the key vectors in São Paulo was frequently found in citrus groves of Bahia. The two most abundant sharpshooters in the northeast are being described as new species. Therefore, surveys of indigenous sharpshooters are needed for each new region or crop threatened by *X. fastidiosa* strains, in order to identify potential vectors. These surveys require close collaboration between taxonomists and vector specialists.

Intensive trading of ornamental plants and nursery stocks between regions or countries increases the risks of introducing invasive bacterial strains and/or sharpshooters to previously unaffected areas. Berisha et al. (1998) isolated a PD strain of *X. fastidiosa* from diseased grapevines in Kosovo. It was the first confirmation of this pathogen in Europe, which could become a serious threat to viticulture if suitable bacterial strains and vectors were to become established in that region. One hypothesis to explain why Europe may not have yet been invaded by diseases caused by *X. fastidiosa* is the lack of suitable vectors that overwinter as adults and thus are capable of infecting plants early during the spring growing season (Purcell 1997). Only early season (April-May) infections of grape were able to survive the following winter in temperate areas of California (Feil et al. 2002). The spread of the glassy-winged sharpshooter [*Homalodisca coagulata* (Say)], an important vector species originally restricted to southern US and Mexico, to California in the early 1990s had dramatic impacts in increasing the spread of Pierce's disease of grape in some viticultural regions of the state. This species has since invaded Tahiti and Hawaii, showing that even distant islands are under risk of invasions promoted by the speed and volume of modern transportation. In addition, the high diversity of Cicadellinae species and the polyphagy observed in this group of vectors certainly increase the opportunities for *X. fastidiosa* to adapt to new host plants and habitats, perhaps originating new bacterial strains or increasing the prevalence of previously rare strains (Almeida et al. 2005).

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Grapevine Yellows vectors: a threat for viticultural areas worldwide

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Phytoplasma associated diseases are persistently transmitted by leafhoppers, planthoppers and psyllids. Phytoplasmas infect a huge number of plant species, both wild and cultivated. Grapevine Yellows (GYs) are among the most dangerous phytoplasma diseases worldwide, and their presence has been reported in Central and Southern Europe, Middle East, Northern Africa, South Africa, Northern and Southern America and Australia. The area where GYs have been reported is almost coincident with the area of grapevine growing. Moreover, many phytoplasmas, including some of those associated with GYs, are quarantine organisms in several countries. GYs are caused by several different phytoplasma strains (belonging to the genetic groups 16Sr-I, -III, -V, -VII, -X, -XII) but grapevine reacts with very similar symptoms to different phytoplasmas. The different GYs are transmitted by different vector species of leafhoppers (Cicadellidae) and planthoppers (Cixiidae), but for some GYs vectors are still unknown. Because plants with phytoplasma diseases cannot be satisfactorily cured, control strategies are directed towards prevention and rely on an integrated approach, combining the use of healthy propagation material, early detection and eradication of infected plants, and finally, vector control. This latter measure is always difficult because it is hard to prevent colonisation of crops by mobile insects and it is even harder to prevent phytoplasma transmission that may occur with short feeding periods. Moreover, the insecticide treatments against vector hoppers in vineyards interfere with the IPM strategies designed to control other grape pests, such as berry moths.

Two main case studies, *Scaphoideus titanus* Ball and *Hyalesthes obsoletus* Signoret, vectors of Flavescence dorée (FD) and Bois Noir (BN), respectively, are provided as examples of invasive and potentially invasive vector species.

FD (16Sr-V, elm yellows group, *Candidatus* Phytoplasma vitis) was first reported in France in the 50's by Caudwell (1957) and has been named Flavescence Dorée (= Golden Flavescence) because of the bright yellow colour induced by the disease on the leaves of white grape varieties. Later, FD was observed in Northern Italy in late 1960s, then in Corsica (France), in new areas of Northeast and Northwest Italy, in Spain and, very recently, in Switzerland, Serbia and central Italy. The Nearctic vector species, *S. titanus* (Schvester et al., 1963), was first identified in Southern France in 1960. Within the following 10 years *S. titanus* was found in Northern Italy, then in Corsica during the 70's, in Slovenia, Croatia (former Yugoslavia) and Switzerland during the 80's. During the 90's, *S. titanus* also invaded the Iberian Peninsula and was found both in Spain and Portugal. In very recent years the leafhopper has moved south and has been recorded in spots in Central, Southern Italy (across the 40th parallel) and Serbia. Studies on active movement and dispersal of *S. titanus* showed that the species flies over a very short distance, especially in the absence of grapevine plants. Therefore, the active movement capability of *S. titanus* can hardly explain the relative speed of diffusion in an area of about 3,000 Km, especially if we consider that the species, under field conditions, is monophagous on grapevine and that viticultural areas are discontinuous. Moreover, high mountains areas, like Pyrenees and Alps, represent further geographic barriers. It is then likely that marketing of nursery material, cuttings and rootstocks, spread the leafhopper vector as well as the FD phytoplasma. The egg stage of *S. titanus* overwinters in the bark of two-year old branches. In Southern France, Corsica, and Northern Italy the introduction of *S. titanus* has resulted in severe outbreaks of FD that led to great alarm among vine growers, so that eradication programs of infected plants and mandatory insecticide treatments against *S. titanus* have been carried out. In other areas more recently colonised by the vector the disease is not yet present or has not yet established noticeable epidemics, probably because *S. titanus* is still present with very low populations and in localized distribution or/and because severe phytosanitary measures and controls have hampered the diffusion of infected propagation material. If we compare the climographs of the North American regions that comprise the reported range of *S. titanus* (Barnett, 1977) with those of the areas of viticultural interest all over the world, we can see that many zones fall within the area of potential *S. titanus* spread. The exceptions are areas characterized by a dry warm climate (in Greece, Spain and Australia) or by high rainfalls (in Portugal and Australia). Thus *S. titanus* and FD have the potential to widen their current geographic distribution.

BN (16Sr-XII, stolbur group) is a GY largely spread in viticultural areas of Europe and Middle East. The disease presumably has been present for a long time but, due to the lack of molecular tools for the identification of phytoplasma strains, it has been reliably identified only in the last 15 years. The disease was first described by Caudwell *et al*. (1971) as a GY non-transmissible by *S. titanus*. Later, the phytoplasma associated with the disease was identified as belonging to the stolbur group, which also infects a number of other plant species, mainly horticultural. The vector species, *H. obsoletus* (*Cixiidae*) was identified recently by Maixner (1994). While FD has a "closed" cycle, from grapevine to

grapevine, the cycle of BN is "open". In fact *H. obsoletus* is not a grapevine feeder and develops on the roots of herbaceous hosts, mainly *Urtica* and *Convolvolus* where it can acquire the phytoplasma that can be later transmitted to grapevine during occasional feedings. Since its identification, BN has been reported with variable incidences from France, Germany, Switzerland, Hungary, Slovenia, Serbia, Croatia, Macedonia, Greece, Ukraine, Spain, Northern and Southern Italy, Israel, Palestine and Morocco. The vector *H. obsoletus*, which is a Palaearctic hopper found in Europe, the Middle East, Asia Minor and Afghanistan, is also present in the same area. The importance BN has been underestimated because the disease spreads more slowly compared with FD. Recent surveys have shown that BN is widespread over a large area, wider than that of FD. The stolbur phytoplasma is very common in weeds (e.g. *Urtica, Convolvolus, Calystegia, Ranunculus*) and in natural populations of *H. obsoletus* (more than 20% of plant samples positive for stolbur have been repeatedly reported and sometimes more than 60% of the samples were positive). Therefore, the disease now spreads faster in vineyards so that high incidences of BN (up to 30% of the plants) are now not uncommon. Moreover, other planthoppers, besides *H. obsoletus,* are potential vectors: eleven hopper species tested positive by DAS-ELISA with a stolbur-specific monoclonal antibodies in France, *Pentastiridius beieri* (Wagner) transmits stolbur to sugar beet. *Reptalus panzeri* (Löw) samples collected in Hungarian vineyard were found by PCR to be stolbur-infected, and even a leafhopper, *Goniagnathus guttulinervis* (Kirschbaum), collected in Sardinian (Italy) vineyards, tested positive for stolbur phytoplasma. If BN phytoplasma is introduced in new areas, the presence of *H. obsoletus* will be a threat to the existing viticulture. So far there is no evidence of introduction of this planthopper into new areas. *H. obsoletus* cannot be introduced with grapevine propagation material since it does not oviposit on grapevine and only herbaceous host-plants could eventually carry nymphs on their roots. Following an eventual introduction, the polyphagy and plasticity of this cixiid vector, whose geographic range covers a wide area with different climates, could enable it to invade and establish in new areas. Moreover, if other planthoppers can vector the disease, then the introduction of BN (stolbur) phytoplasma alone would be potentially dangerous because indigenous cixiid species could act as vectors.

Other GY diseases

GY diseases, associated with phytoplasmas belonging to other genomic groups-subgroups, are spreading in the same areas of FD and BN as well as in Australia, US (Virginia and New York), Chile and South Africa; particularly in US and Australia the disease can cause damages of economic importance. For these latter GY, no vector species have been identified, but at least 15 leafhopper species tested positive for the phytoplasmas associated with Australian and North American GYs. We can not exclude that some of these species could be further potentially invasive vector species.

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Myndus spp, (Fulgoromorpha, Cixiidae) public enemy number one of the coconut palm, *Cocos nucifera*

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The coconut palm (C*ocos nucifera L.*), an often crucial crop for small landholders, plays a major role in the intertropical farming system. This plant is considered in several countries as one of the nature's most useful gifts to man, a primary source of drink, food, and shelter. Every part of coconut is utilized for some human need. It is why coconut palm is called the "tree of life" or the "tree with 100 uses." Contrary to what is claimed by those marketing competing edible oils, the properties of coconut oil imparts many physiological benefits for those struggling to overcome obesity, heart disease, and other disorders. Coconut palms have become strongly associated with tropical tourism. Posters show white sandy beaches, with a clear, transparent sea ... and coconut palms. Unfortunately, the parasite pressure exerted on this crop is a threat to its sustainability and sometimes leads to its disappearance. Various pathogens have been identified that could be responsible for different diseases that have long been classed as of "unknown etiology": viroids, viruses, phytoplasmas (Mollicutes), and *Phytomonas* (Trypanosomatidae). The discovery of the role of insects as vectors of several for these disorders has been important landmarks of the end of the 20th century.

Insect vectors

The first insect vectors were discovered in West Africa in the years 1979-1982, mainly thanks to the strategy of insect introductions *en masse* in cages (Dollet, 1992). The discovery of *Recilia mica* Kramer (Homoptera, Cicadellidae, Deltocephalinae) as the vector for blast disease, which is the main nursery disease of oil palm and coconut and presumed to be caused by a phytoplasma, was the first success opening the doors for several others (Desmier De Chenon 1979). *R. mica,* vector of blast, also transmits phytoplasma to periwinkle (Dollet 1980). Then two species of *Sogatella* (Delphacidae)- *S. Kolophon* Kirkaldi and *S. cubana* Crawford were shown to be vectors for Dry bud rot, another nursery disease of coconut and oil palm, presumed to be viral (Julia 1979, Julia and Mariau ,1982). During 1980-1983 experiments provided evidence that *Myndus crudus* Van Duzee (Homoptera: Cixiidae) was the vector of the causal agent of Coconut lethal yellowing (LY) in Florida (Howard and Thomas 1980, Howard et al., 1983; 1984). LY is the most devastating disorder of coconut in the Caribbean. Besides coconut palm, LY also affects more than 35 other palm species. The disease was first reported in the Cayman Islands in 1834; by 1980 LY had killed over 7 million coconut palms in Jamaica alone. *M. crudus* is known from northern South America (Brazil), Central America; Mexico, and several islands of the Caribbean as far south as Honduras and Dominican Republic. However the southern limits of LY are the Dominican Republic and Honduras, and experimental demonstration of the LY transmission by *Myndus* was successful only in Florida.

Another *Myndus*, species –*M. taffini* – is prohibiting any new introduction of coconut germplasm in the Pacific island of Vanuatu. *M. taffini* Bonfils is the vector of the Coconut Foliar Decay Virus (CFDV), a disease killing all the introduced coconut varieties in Vanuatu (Julia, 1982, Julia et al. 1985). Only the local variety, the "Vanuatu Tall" is tolerant to the disease, caused by a small DNA virus (Randles et al. 1986, Randles and Hanold, 1989). *M. taffini* appears to be endemic to Vanuatu. An additional *Myndus* species, *M. adiopodoumeensis* Synave, is suspected as the vector for a lethal yellowing disease (LYD) in West Africa (known as Cape Saint Paul Wilt in Ghana and Kaincopé disease in Togo) (Dery et al.1996.). But its role in the propagation of the LYD has not yet been proven. In East Africa, similar LYD occurs in Kenya, Tanzania and Mozambique. Although the phytoplasma associated with LYD in Mozambique is very similar genetically to those found in LYD-affected palms in Ghana, *M. adiopodoumeensis* has not been found in Mozambique nor in Tanzania.

Hartrot, a disease of coconut (called Marchitez when affecting oil palm) has the same syndrome as Lethal Yellowing Disease in the region stretching between Peru/Brazil and Honduras through Trinidad. Phloem-restricted *Phytomonas* spp. (flagellate protozoa, Trypanosomatidae) are responsible for this syndrome (Dollet 1984, 2001). Different species of *Lincus* Stal (Hemiptera: Pentatomidae, Discocephalinae) are vectors of these trypanosomatids (Desmier De Chenon 1984, Louise et al. 1986, Dollet et al. 1993)

Discussion.

Different *Myndus* species are associated with the propagation of very severe wilt diseases of coconut. But their role was demonstrated so far only in Florida. Why ? Is the percentage of infectious insects very low? Are there distinctive taxa (sibling species, subspecies?) of what are now considered "*Myndus crudus"?* Is there another vector? Could *M. crudus* transmit CFD virus? And can *M. taffini* transmit LY? What are exactly the similarities between *Myndus* spp and *Lincus* spp, both phloem feeders on coconut palm? What are the similarities between LY Phytoplasma (prokaryote), and Hartrot *Phytomonas* (eukaryote), which both multiply in coconut sap?

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Spiroplasma-like Organisms and Other Mollicutes in the Gut Lumen of Five Leafhopper Species (Hemiptera, Cicadellidae)

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Spiroplasma-like organisms and other mollicutes were observed by transmission electron microscopy (TEM) in thin sections and in negatively stained preparations of the midgut and hindgut of five leafhopper species from laboratory reared colonies. The spiroplasma-like organisms were long, tubular shaped, bound by a single membrane, with dimensions similar to those of spiroplasmas, but they seemed to lack the marked helicity known for most spiroplasmas (Gasparich, 2002). They also possessed tip structures analogous to those of other *Spiroplasma* species (Ammar et al., 2004). The tip structures were most often attached to the midgut epithelium in close association with microvilli. These spiroplasma-like organisms, some of which appeared to be in the process of dividing, were abundantly found in the gut lumen of *Dalbulus elimatus, Endria inimica* and *Macrosteles quadrilineatus.* Shorter, mostly rod-shaped, mollicute-like organisms were found in the gut lumen of *Dalbulus maidis* and *Graminella nigrifrons*. Confocal laser scanning microscopy of whole-mount guts using the nuclear stain propidium iodide (Ammar et al., 2005) suggested that these two types of mollicutes are more commonly found in males than in females of *D. elimatus, D. maidis* and *M. quadrilineatus,* whereas they were found almost equally in both sexes in the other two leafhopper species examined.

TEM studies of thin sections indicated that none of these mollicutes invade the gut epithelium or other tissues in any of the five leafhopper species investigated. TEM studies also showed the presence of brochosomes, known to be excreted by Malpighian tubules in leafhopper species, in the esophagus of some of the leafhoppers examined. This observation suggests that the mollicutes described above may be transmitted from one individual to another by the leafhoppers probing and contaminating their mouth parts with their excretions, possibly during the anointing or grooming behavior known in some leafhopper species (Rakitov, 1996). Commensal/symbiotic mollicutes, including *Spiroplasma* and *Entomoplasma* spp., have been reported earlier in the gut lumen of some beetles and tabanid flies (Gasparich, 2002; Wedincamp et al., 1996) but apparently never in leafhoppers or planthoppers. Nonpathogenic gut bacteria are commonly found in several insect orders and are thought to play nutritional or other symbiotic roles in their insect hosts (Dillon and Dillon, 2004). Further investigation is required to elucidate the significance of the above described mollicutes in the evolution of leafhopper-borne plant-pathogenic spiroplasmas or phytoplasmas and to study their possible effects on the biology or transmission efficiency of vector leafhoppers.

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Revision of Delphacini (Hemiptera: Delphacidae: Delphacinae) present in Australia

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Abstract

Examination of delphacini holdings in Australian insect collections has revealed ten species previously not recorded from Australia. Australian records of *Dicranotropis fuscifrons* (Muir), the synonymising of *Gelastodelphax* with *Eumetopina* and the resurrection of *Hadeodelphax* have been rejected. An updated checklist of Australian Delphacini is provided. More than 120 species are represented in the collections, more than half of which appear to be new to science and many of these will require the erection of new genera to accommodate them. The fauna of Australia would still appear to be incompletely surveyed as many of these new species are represented by only one or two specimens or only by female specimens and relatively few specimens of any species have been collected in southern or inland regions.

Within-species variability in chrysanthemum yellows (CY) transmission by *Euscelidius variegatus* Kirschbaum (Cicadellidae)

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Chrysanthemum yellows (CY) is a disease of several herbaceous crops (Conti *et al*., 1998) associated with phytoplasmas of genetic group 16Sr-IB (Candidatus *Phytoplasma asteris*) and transmitted by at least three species of *Deltocephalinae* leafhoppers (Palermo *et al*., 2001). In preliminary assays, a vector species, *Macrosteles quadripunctulatus* Kirschbaum, had 100% of CY transmission efficiency, and *Euscelidius variegatus*, transmitted with lower efficiency under the same conditions. Because individual differences in transmission capability can help to identify factors involved in interactions with phytoplasmas, CY transmission patterns of *E. variegatus* were analysed in detail.

Methods and Materials

Nymphs of *E. variegatus* were allowed to acquire CY from infected *Chrysanthemum carinatum* Schousboe plants (daisy) for one week, then transferred onto oat plants for two weeks and finally isolated singly on test daisy seedlings either for one inoculation access period (IAP) of one week or for serial transfers of 3-7 days until death.

About 100 leafhoppers that acquired CY under the conditions described above were singly isolated for a 48 h IAP inside Eppendorf tubes and allowed to feed through Parafilm on an artificial feeding medium.

One month after the transmission assays, total DNA was extracted from symptomless plants and the presence of CY was analysed by Real Time Polymerase Chain Reaction (RT-PCR). Some of the leafhoppers from transmission trials were sampled and singly analysed by direct, nested and RT-PCR for the presence of CY.

The presence of BEV, a bacterium associated with *E. variegatus* and known for its effect on phytoplasma transmission (Purcell and Suslow, 1987) has been checked by PCR on healthy leafhoppers from two colonies (Torino and Udine, Italy) and on both CY transmitter and non-transmitting individuals.

Results

Two sets of time-specific transmission experiments carried out with single leafhoppers serially transferred on daisy plants until death allowed the identification of 7 insects, out of 32 tested, that did not transmit CY throughout their whole life (~78% of transmitting and ~22% of non-transmitting individuals). These non-transmitting insects survived for a minimum of 41 days to a maximum of 125 days post acquisition.

Four sets of transmission experiments carried out with single leafhoppers that fed for 7-day IAP, allowed the identification of 182 transmitting and 52 non- transmitting leafhoppers (~78% of transmitting and ~22% of non-transmitting individuals).

We detected CY in leafhopper saliva after 48 hr feeding in the feeding substrates of 35 out of 59 leafhoppers (~59% of transmitting leafhoppers).

The transmitting insects always yielded a CY-specific amplicon in PCR, but among the non-transmitters, 21 were CY-negative and 31 CY-positive. The titre of CY recorded in these last non-transmitting leafhoppers was always low, as threshold cycles in Real Time PCR assays were much higher than those of the transmitters.

The analysis of 32 *E. variegatus* adults, both exposed and non-exposed to CY-infected plants, detected the presence of a putative BEV-specific band in only one insect.

Discussion

About 20% of *E. variegatus* did not transmit CY even when allowed to acquire and inoculate under optimal conditions. The non-transmitting insects appear top be either "immune" (CY-negative) to CY or infected with a low titre (high vs. low threshold-cycles in Real Time PCR). Transmission assays on artificial feeding medium undervalued the actual proportion of infective insects; therefore a more sensitive protocol for identification of CY in insect saliva from artificial feeding medium should be developed. The inability in transmitting CY seemed not to be correlated with the presence of BEV. The pathway of CY inside the body of non-transmitters should be investigated. Crosses under controlled conditions among non- transmitting *E. variegatus* will be performed to verify if the transmission inability is an inherited character.

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Characterisation of leafhopper, *Kahaono montana* Evans, silk (Cicadellidae: Typhlocybinae: Dikraneurini)

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Silk is a fibrous proteinaceous substance produced by several arthropod animals. Some of these exhibit outstanding physical properties and have attracted considerable research interest, in particular spider silks and silkworm silk.

In 1994, Day and Fletcher [1] reported the discovery of a leafhopper associated with silk covers on leaves of *Eucalyptus* (Myrtaceae). This discovery was confirmed in 2002 by Fletcher and Kent [2]. The leafhopper, *Kahaono montana* Evans (Cicadellidae: Typhlocybinae: Dikraneurini), produces silken "tents" on gum leaves. The tents are characteristically coated by a heavy mixture of honeydew, sooty mould and brochosomes.

In this study, the mechanical strength, internal structure, chemical composition and solvent interaction of *K. montana* silk were examined using Instron tensile tester, ion etching, SEM and amino acid analysis. The results were compared with previously studied silks.

Methods and Materials

Raw silk samples were obtained from the leaves of *Eucalyptus robusta*, one of the host plants of *K. montana*. Mechanical strength experiments were performed on multiple fibres using an Instron tensile tester. The stretch force was detected at a constant stretch rate of 2 mm/min. The ion etching technique was performed using Dual Ion Miller under vacuum to expose the internal structure of the silk, at different powers (1.5 W/4 W) and durations (3-5 mins). The amino acid composition of the silk was measured with an HPLC equipped with Waters AccQ-Tag system. Acidc, basic, organic, ionic, and surfactant solutions were used to study the interaction of silk in solvents with different properties

Results and Discussion

The average tensile strength of *K. montana* silk, 280±72 MPa, is several times weaker than that of silkworm silk and spider silk. The breaking strain of *K. montana* silk, 6.9±1.6 %, was also significantly lower, while its Young's modulus, at 4.4 GPa, is in the medium range. The mechanical properties of *K. montana* silk exhibited degrees of variability, as was also observed in silkworm silk and spider silk.

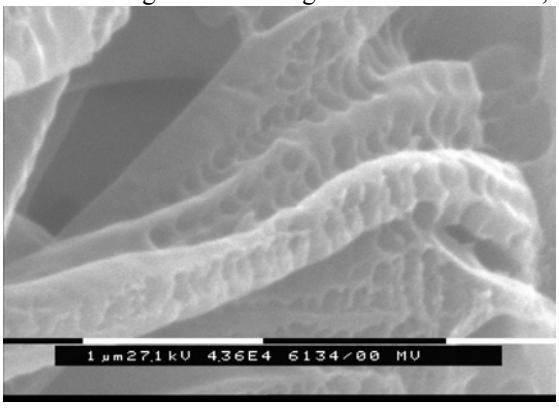

Fig 1. *K. montana* silk after ion-etching at 4 W for 5 min

As a result of ion etching, the outer part of the fibre is removed and the presumably harder interior core is exposed. Five minutes of etching reduced the fibre diameter to less than half its original size (< 0.5 µm). The core section of the fibre appears regular in structure and is longitudinal to the fibre axis. Along the core there are evenly spaced transverse bands, which are continuous, periodic, slightly parabolic along the axis, and separated by ~100 nm (Fig 1).

The amino-acid composition of *K. montana* silk samples is dominated by serine, glycine, and alanine, representing about 57% of the total amino-acid concentration. Glutamic acid, aspartic acid, leucine, proline and threonine account for the second highest amount of residues, 32%, followed by valine, isoleucine, methionine, lysine, arginine, histidine, phenylalanine and tyrosine, 11%. Other amino acids are either in trace quantities or absent from the silk fibroin.

K. montana silk is only soluble in concentrated NaOH, HCl and $NaHCO_3$. Organic solvents and surfactant solutions showed little or no effect. The solubilities of silk in various aqueous solutions suggest that the bonding force in its tertiary structure is primarily electrostatic and not hydrophobic. Changing the pH alters the degree of ionization of amino-acid side chains, which disrupts the charge distribution of the protein and hence breaks the intermolecular interactions.

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Relationships between the Erythroneurini (Cicadellidae: Typhlocybinae) of Australia and New Zealand.

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Australia has a wealth of undescribed species of Erythroneurini along with nine described species currently placed in the Northern Hemisphere genus *Zygina* Fieber, two in the primarily Oriental genus *Empoascanara* Distant and three in *Pettya* Kirkaldy (Day & Fletcher 1994, Fletcher & Larivière 2001-5). By contrast, New Zealand has five described species all currently in *Zygina* (eg *Zygina agni* Knight, Fig 1) (Knight 1976, Larivière & Fletcher 2004).

Fig 1. *Zygina agni* Knight

None of the species is congeneric with *Zygina nivea* Mulsant and Rey, the type of the genus, nor with *Zygina (Hypericiella) hyperici* (Herrich-Schäffer), the type of the subgenus.

The aedeagus of *Zygina zealandica* (Myers) has two shafts each with an apical gonopore (Figure 2) and although a second, undescribed, species in Australia has this configuration the two species are not closely related. The *honiala* group combines *Z. agni* and *Z. dumbletoni* Ghauri in NZ with a number of Australian and New Guinean species in which the aedeagus is a single tapered tube bearing apical processes (Figure 3). This group was recognised by Ghauri (1980) who also noted that the group has the aedeagal shaft forming a somewhat U-shaped structure with its dorsal apodeme. The remaining NZ species are the endemic *Z. ramsayi* Knight and *Z. toetoe* Cumber in which the manubrium is expanded ventrally and bears a long basal process (Fig 4). A number of Australian species have similar aedeagal structure but they are not closely related and cannot be recognised as a species group on the basis of aedeagal structure.

Both *Z. agni* and *Z. dumbleton* have been recognised in the Australian fauna which changes the previously recognised endemicity of the NZ Erythroneurini.

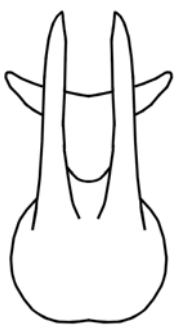

Fig 2. Aedeagus of *Z. zealandica* (Myers)

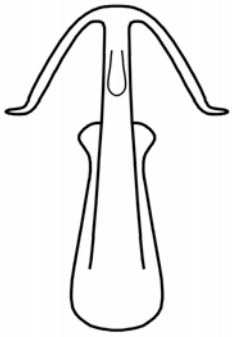

Fig 3. Aedeagus of *Z. dumbletoni* (Ghauri)

Fig 4. Aedeagus of *Z. toetoe* (Cumber)

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Biology and Host Range of 2 South American Egg Parasitoids (Hymenoptera: Mymaridae), Possible Biocontrol Agents for Glassy-Winged Sharpshooter (Say) (Hemiptera: Cicadellidae; Proconiini)

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Egg parasitoids are the most important known natural enemies of the glassy-winged sharpshooter, *Homalodisca coagulata* (Say) (GWSS), in its native range across the southeastern U.S. and northeastern Mexico, as well as for leafhoppers in general (Döbel and Denno 1993). Following the discovery of this devasting plant disease vector in California, efforts were initiated to survey existing parasitism (Hoddle et al. 2001), as well as import egg parasitoids from its native range (Morgan et al. 2000, Triapitsyn and Hoddle 2001). But parasitism is currently relatively low in eggs deposited by overwintered GWSS. Surveys in south Texas have shown that egg parasitism is generally high throughout the season, primarily by *Gonatocerus triguttatus* Girault (Hymenoptera: Mymaridae) (Jones 2002). The most important egg parasitoid elsewhere is *G. ashmeadi* Girault, followed by *G. morrilli* (Howard). Although *G. triguttatus* from Texas and Mexico have been released in California, this species is known only from the tropics and subtropics, and thus may be unlikely to possess the ability to suppress populations of the GWSS under California climatic conditions. Thus, collections of egg parasitoids from other related Proconiini were made in climate-matched areas in South America (Jones 2001). At least 10 *Gonatocerus* spp. and 3 Trichogrammatidae (Hymenoptera) species were collected from Argentina, Chile and Peru. Among those that successfully developed in GWSS eggs in U.S. quarantine, two *Gonatocerus* spp. from Argentina were selected for further screening: *G. tuberculifemur* Ogloblin and *G. metanotalis* (Ogloblin), collected primarily from egg masses of *Tapajosa rubromarginata* Signoret (Homoptera: Cicadellidae; Proconiini) on citrus. Reported here are the initial results of studies on their biology on GWSS eggs, and on possible non-target effects on other native Cicadellidae.

Materials and Methods

Parasitoid Biology. Parasitoid developmental times (d) to adult emergence from GWSS eggs, and longevity of individually-maintained adults, were recorded at 25°C and 60% RH.

Non-target Studies. Seven species of native Cicadellidae representing 3 subfamilies and 4 tribes were colonized for host range evaluation. From California: *Colladonus montanus* (Van Duzee) and *Euscelidius variegatus* (Kirschbaum) [Deltocephalinae; Athysanini]; *Macrosteles fascifrons* Stål and *M. quadrilineatus* Forbes [Deltocephalinae; Macrostelini]; and *Homalodisca liturata* Ball [Cicadellinae; Proconiini]. From Texas: *Homalodisca insolita* (Walker) and *Oncometopia* sp. [Cicadellinae; Proconiini]. GWSS were colonized from collections in Hidalgo Co., Texas.

Results

Parasitoid Biology. Development rate of *G. tuberculifemur* on GWSS eggs was 12.2d (range=8-14d) and 12.4d (range=9-17d) for males and females, respectively. Males lived 8.9d (range=4-16d), and females 7.9d (range=4-12d). *G. metanotalis* developed in 12.4d (range=11-14d) and 13.5d (range=12-17d) for males and females, respectively. Males lived 4.9d (range-3-12d), and females 4.9d (range=1-12d).

Non-target Studies. Both parasitoid species successfully attacked and emerged from *H. liturata* and *Oncometopia* sp. eggs deposited on cowpea leaves. Eggs of the other species, including *H. insolita*, were not attacked on their host plants.

Discussion

The development rates for both sexes of *G. tuberculifemur* on GWSS eggs were within the ranges of development at similar temperatures reported from its native Argentine host, *T. rubromarginata* (Virla et al. 2005). Adults reared from GWSS lived about 2 days longer than those reported from *T. rubromarginata*. Female *G. metanotalis* took about 1 day longer to develop than males of the same species and females of *G. tuberculifemur*. Longevity of both sexes of *G. metanotalis* reared on GWSS was shorter than *G. tuberculifemur* reared from GWSS and from *T. rubromarginata* eggs in Argentina. The results show that the 2 South American egg parasitoids can successfully utilize GWSS eggs as hosts

without apparent adverse affects. Furthermore, there appears to be a taxonomic limit on their potential host range. Notably, the proconiine *H. insolita*, a grass feeder, was not attacked.

Acknowledgement. This research was funded in part by USDA-APHIS and USDA-ARS.

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Taxonomic and phylogenetic revision of the Australian Cixiidae

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The family Cixiidae is one of the largest families within Fulgoromorpha and is distributed worldwide. Some cixiids are known to be vectors of phytoplasmas, which cause yellows diseases. Prior to this project 59 cixiid species distributed among 23 genera were recognised in Australia, but the group has never been comprehensively revised. Some important recent studies have reviewed cavernicolous species (Hoch & Howarth 1989a, Hoch & Howarth 1989b, Erbe & Hoch 2004) and the higher classification (Emeljanov 2000, Emeljanov 2002, Holzinger et al 2002) only.

Our studies focussed initially on the tribe Gelastocephalini (see Fig.1) which is currently known from Australia and New Caledonia. Our revision of the tribe has increased the number of genera from 7 to 27 and the number of species from 9 to 60. A cladistic analysis of morphological data has been performed to test the monophyly of the genera.

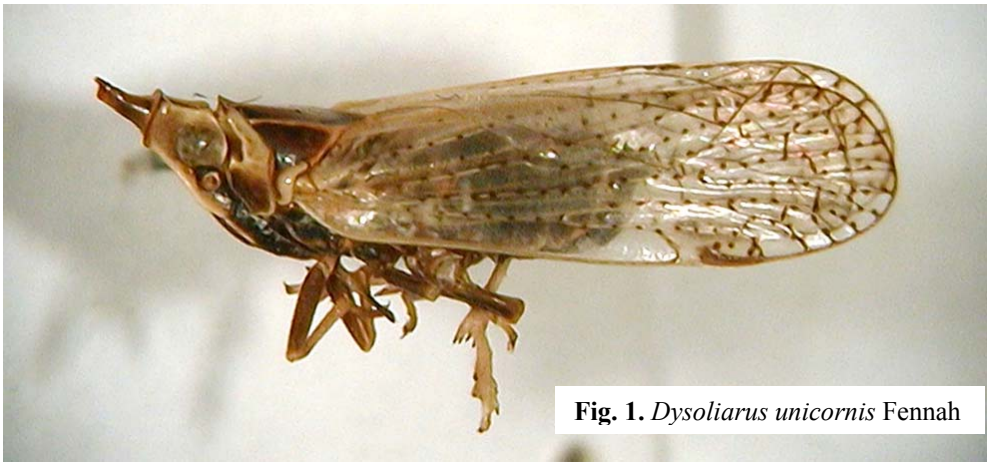

Fig. 1. *Dysoliarus unicornis* Fennah

Results from a current examination of the Australian Pentastirini (see Fig. 2) will be presented, including a three-fold increase in the number of species as opposed to an almost seven-fold increase in Gelastocephalini.

Acknowledgements
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Fig. 2. *Oliarus felis* Kirkaldy

Female genital characters in palaeartic *Kybos* (Hemiptera, Cicadellidae)

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The large genus *Empoasca* Walsh is traditionally divided into three subgenera: *Empoasca* s.str., *Kybos* Fieber and *Kyboasca* Zachvatkin. The holarctic subgenus *Kybos* comprises some 85 species, 38 of which are are palaearctic. *Kybos* species are usually monophagous on *Salix*, *Populus* and *Betula* spp. The taxonomy which is currently based mostly on male characters (genitalia, sound apodemes) is, in parts, confused, and virtually nothing is known about the phylogeny and biogeography. Studies on female genital characters in some nearctic Empoascini have been made in the past by Balduf (1934) and Cunningham & Ross (1965). They described several characters for species identification. However these characters have never been used for phyologenetic studies on the genus *Empoasca*.The present study adddresses these questions using in addition female genital structures.

Methods and Materials

For observations of the inner female genital structures with the optic microscope the abdomen of fresh or dried specimens were removed and cleared in hot 10 % KOH. After cleaning in 70 % alcohol the genitalia were stained with chlorazol black. Drawings and digital photographs of the female genitalia were made in glycerin or glycerine-gelatine.

Dry specimens used for SEM photographs where relaxed and the abdomen was removed from the body. The 7th abdominal sternite was removed to make the base of the ovispositor visible.

Results

Several species are morphologically similar and are separated usually by the shape of the aedeagus and sound producing organs (apodemes). Male genitalia have been considered the only reliable characters for species separation. However, these characters are often variable, and intermediate individuals occur. Therefore, the outer and inner female genital structures and the larval morphology is studied.

First SEM pictures (Fig. 1 and Fig. 2) of the outer female genital structures in palaeartic *Kybos* show species-specific characters. But they show similar results to the morphology of male genitalia. That means that species with only small differences in male genitalia have only small or no differences in the female genitalia as well. This is another indication to a broader species concept in *Kybos*. Some nominal species are probably artificial and should be synonymised according to the male and female genital characters.

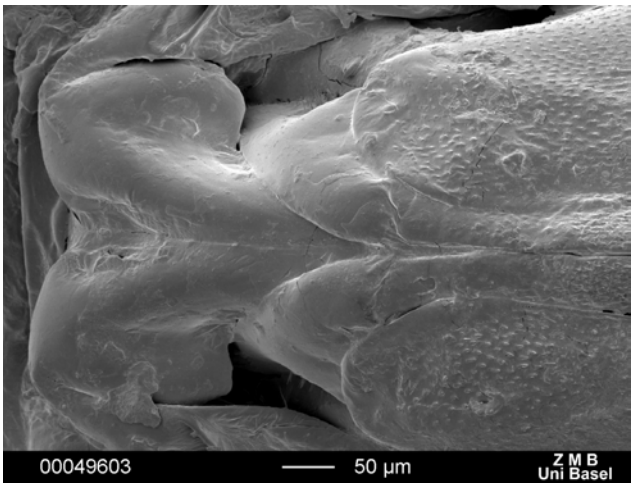

Fig 1. Ovipositor base of *Kybos populi* (Edwards)

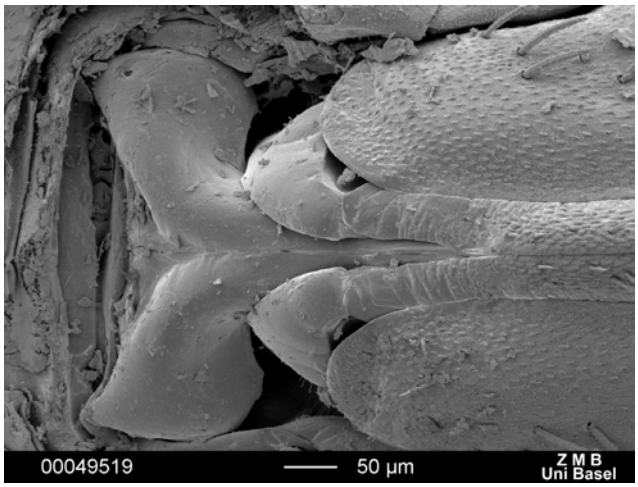

Fig 2. Ovipositor base of *Kybos strigilifer* (Oss.)

Discussion

Work in progress suggests that these additional structures help to gain a better understanding of the palaeartic *Kybos*. The preliminary results indicate that the current species concept of *Kybos* is narrow. There are some species described with only small differences in male genitalia, although later observations have shown that intermediate forms occure.

Our studies on outer and inner female genitalia show similar results. So there are no distinctive diagnostic characters for some species available and they have to be synonymised.

Results from this work are potentially also applicable to the other two subgenera, *Empoasca* s.str. and *Kyboasca*, which contain many species acting as vectors of plant diseases.

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Xylem Sap-feeding Insects as Sap Samplers to Discover Xylem-specialist Bacteria

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Although sap feeding Auchenorrhyncha in many families probe xylem vessels during feeding, xylem sap-feeding insects in the families Cercopidae, Cicadidae, and the Cicadellid subfamily Cicadellinae, and members of some other Cicadellid subfamilies specialize on ingesting xylem sap. The best evidence for this is their extraordinarily high rates of consumption (Mittler ; Raven 1984), combined with the chemical nature of their excrement (Brodbeck et al. 1993). The bacterium *Leifsonia* [*Clavibacter*] *xyli* ssp. *cynodontis* is also a xylem specialist, found only in xylem tissues within its host plants (Davis et al. 1988). Our experimental tests of several xylem sap-feeding sharpshooter leafhoppers (Cicadellinae) failed to show any transmission of *L. xyli* ssp *cynodontis* to a sensitive host plant, Bermuda grass (*Cynodon dactylon*), despite our finding that many insects acquired large numbers (up to log 5 to log 6 cultivable cells per insect) of the bacterium after feeding on plants colonized by high populations (>log 9 cells per ml xylem sap) of this bacterium for one or two days. This led to the idea that because xylem sap-feeders were capable of ingesting large quantities of xylem-inhabiting bacteria that survived within their alimentary tracts, they might be useful in surveys to detect other bacteria that live in xylem sap.

A problem in detecting such bacteria by culturing, however, is that xylem specialist bacteria studied to date usually require specialized media (Purcell and Hopkins 1996). Most searches for "endophytic" bacteria rely on general culture media for aerobic bacteria that would not be suitable, for example, for the most studied xylem specialists to date, *L. xyli* and *Xylella fastidiosa*. To develop a useful screening method of detecting xylem-inhabiting bacteria by using xylem sap-feeders as sampling devices, we used light microscopy to detect multiplication and movement of any bacteria that might multiply and move systemically within the plant. The bacterium *L. xyli* ssp *cynodontis* was selected as a model system, since its populations of live (cultivable) cells in plants and insects could easily be enumerated by dilution plating on solid culture medium. *L. xyli* ssp *cynodontis* readily infects its host plants by mechanical inoculation of cultured or plant-extracted bacteria and also reaches very high populations in plant xylem sap.

Methods

Several sharpshooter species that had fed on maize (*Zea mais*) or Bermuda grass colonized by *L. xyli* ssp *cynodontis* were macerated in the minimum volume of buffer required to liquefy the macerated leafhoppers, and this mixture was inoculated into maize and Bermuda grass plants by needle puncture of leaf veins or stems. After 30 days following inoculation, the most distal leaves of the plant were sampled. Xylem sap was extracted from surface-sterilized mid-veins of maize by centrifugation (500G) and plated on SMC medium (Davis 1991) and also examined in dark field and phase contrast microscopy, with and without Gram staining.

Results

As a proof of principle, the detection of bacteria from inoculated plants showed that it was possible to detect without culture bacteria (i) with densities within plants (or that could be concentrated by centrifugation) that were detectable by light microscopy (log 5 to log 6 per ml), (ii) moved systemically within plants (iii) within a host plant of interest . All

of these features are desirable characteristics of xylem specialist bacteria that might be developed for biological control or biotechnological uses in crop plants. Once such bacteria are detected by microscopy, they can be concentrated from xylem sap collections by centrifugation and mechanically re-inoculated into additional plants to increase the numbers of colonized plants. Culture media and methods could then be developed to attempt to culture the bacterium. This method will not apply to bacteria that do not meet the three criteria listed above or that cannot colonize plants via mechanical inoculation.

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Study on interactions between *Nilaparvata lugens* Stål (Hemiptera: Delphacidae) and mutants of IR64, a commercial cultivar of rice (*Oryza sativa* L.): a step to discover insect defense genes

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The brown planthopper (BPH), *Nilaparvata lugens* Stål is an important pest of rice in Asia. It causes severe hopper burn and susceptible rice cultivars can suffer up to 60% yield loss from its attack (Panda and Khush, 1995). Many varieties have been developed carrying different genes for *N. lugens* resistance but the resistance does not appear to be durable. *N. lugens* can quickly evolve resistance to these genes. There is thus need to identify new sources for durable resistance and transfer genes/ QTLs to develop varieties resistant to *N. lugens*.

IR64, a popular cultivated rice variety, has moderate resistance to BPH. One major gene and several QTLs contributing to resistance in IR64 has been identified (Alam and Cohen, 1998). But this variety does not tolerate high *N. lugens* infestation. Enhancing resistance of this variety will be useful for sustainable crop production. The IR64 mutant collection has been developed at IRRI through physical and chemical mutagenesis and is being used to understand defense pathways in rice (Leach et al. 2001). The mutants with higher insect resistance and good agronomic performance can be isolated and used for testing the practical effectiveness of candidate defense genes.

In order to understand interactions between *N. lugens* and host plants and to identify genes responsible for insect resistance, we initiated research on two kinds of mutants (a) gain of resistance (GOR) and (b) loss of resistance (LOR). We studied how *N. lugens* responds on mutants and IR64, and what are the protein expression differences between mutants and IR64 under stress induced conditions.

Methods and Materials

The plant material consisted of IR64, a GOR mutant and a LOR mutant. All the experiments were conducted using the *N. lugens* colony reared on a susceptible variety, Taichung Native-1 (TN1) at the IRRI greenhouse. To test if *N. lugens* shows differences in preference to settle and oviposit on test entries, a free-choice experiment was conducted. Thirty days after sowing, 3 potted plants of each entry were arranged in a circle in a replication. Six *N. lugens* females per plant were released in the centre. The females were counted on the plants 24, 48 and 72 hours after infestation. We also measured *N. lugens* performance based on honeydew production in parafilm sachets, nymphs per adult, development and survival of nymphs and adult weight on different entries. Tolerance was measured based on dry weight biomass (Dixon et al. 1990) and recovery of plants for grain yield after 15 days of stress at seedling stage. We used a randomized complete block design (RCBD) with 15 replications for all experiments.

Two dimensional gel electrophoresis was used to determine whether protein expression profiles from the leaf sheaths of resistant and susceptible lines differed in response to *N. lugens* feeding. Sampling was done at various points of *N. lugens* development on rice plants to characterize the timing for constitutive and induced expression of defense genes.

Results

N. lugens females avoided settling and egg laying on the GOR seedlings. Significantly fewer *N. lugens* were recorded on the GOR after 24, 48 and 72 hours of infestation. The *N. lugens* fecundity was also low on GOR. The amount of honeydew excreted by *N. lugens* on GOR seedlings was lower than on other entries. The number of nymphs per female recorded on GOR was significantly lower than on LOR and IR64. Development and survival of nymphs and weight of *N. lugens* adults on both GOR and LOR mutants did not show significant differences from IR64. The GOR mutant was more tolerant to *N. lugens* stress, as its tolerance index value was significantly lower than LOR and IR64. It also produced significantly higher biomass and grain yield than IR64.

Protein profiles of the two mutants and IR64 from 2D gels showed a number of both constitutive and stress-induced differences that were reproducible. One constitutively expressed protein in the GOR line had a lower molecular weight and slightly higher pH value than its original form in IR64. This indicates that some amino acids have been removed from the pure form of the protein. The differences in induced expressions of proteins were found only after emergence of second generation nymphs, i.e., 23 days after infestation with parent *N. lugens*. Three induced proteins were over-expressed in GOR and down-regulated in LOR as compared with IR64. These proteins are currently being sequenced to identify candidate genes involved with resistance and to study their role in mechanism of resistance.

Discussion

Non-preference shown by insects as well as low antibiosis and tolerance of plants to insect feeding are considered key components for durable resistance. This type of resistance will have little selection pressure on the insect population. Such mechanisms of resistance in rice may involve presence of certain toxic chemicals (Grayer et al. 1994; Zhang et al. 1999). Low content of amino acids have also been reported as a cause for *N. lugens* resistance in rice (Sogawa and Pathak, 1970). The GOR mutant exhibits resistance to *N. lugens* either due to the presence of these deterrents or a nutritional deficiency in certain amino acids for the insect's diet. This could explain avoidance by *N. lugens* to settle, oviposit and feed. GOR is also morphologically similar to IR64. The pattern of F3 segregation suggests that resistance in GOR is controlled by a single dominant gene. Thus it is a valuable elite germplasm for developing durable resistance to *N. lugens*.

From above studies, the mutants seem useful for understanding defense pathways and to find candidate genes. Mutations of genes in IR64 might have resulted in gain and loss of resistance to *N. lugens*. Sequencing of up-regulated or down-regulated proteins will help to identify candidate defense genes and understand defense pathways involved with *N. lugens* stress. Once the genes for resistance are identified, they may be used in improving the rice germplasm to protect the crop from *N. lugens* damage.

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A Systematic Study of the Macropsinae (Cicadellidae) of Australia

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The Macropsinae are known from all regions of the world (except South America, Oceania and Antarctica) (Hamilton 1980). They feed on woody trees and shrubs and frequent a wide range of habitats from arid to alpine regions (Linnavuori 1978), and some species are tended by ants (Linnavuori 1978, Viraktamath 1980). Macropsinae are morphologically distinctive with the head and pronotum often punctate or rugose (Evans 1936,1947), the pronotum declivous with transverse or oblique striations, a ventrally flattened face with ocelli located on ventral aspect, the face with oblique antennal ledges, swollen lorae**,** and a very short crown which is barely visible in dorsal aspect and a very narrow tegminal appendix.

Currently, 550 species of Macropsinae are known world-wide in 26 genera and sub-genera (Dietrich 2001). Many of these species were described from the Northern Hemisphere, with *Macropsis* Lewis and *Oncopsis* Burmeister encompassing the largest number of species. The Australasian fauna has been largely neglected. The most recent study which included the Australian Macropsinae was by Hamilton (1980) who reviewed the world Macropsini and recognised Australia as a "key" region requiring further work. Currently, Australia's fauna comprises 46 described species in 9 genera although many species were tentatively placed by Evans (1966) within the holdall genus "*Macropsis*". Few Macropsinae are known to be disease vectors or agricultural pests. Maixner *et al.* (2000) reported that *Oncopsis alni* (Schrank) was a vector of alder yellows in grapevines. This sparked an interest in the Australian Macropsinae, particularly to determine whether any of the 23 Australian "*Macropsis*" species actually belong to *Macropsis* or *Oncopsis*.

A serious problem for our study is the predominance of females amongst Evans' holotype specimens, while it is the characters of the male genitalia that are considered by Hamilton (1980) as most useful in determining generic placement and relationships. Of 25 generic characters identified so far, based largely on those used by Hamilton (1980), 13 are derived from the male genitalia, particularly the configuration of the dorsal connective shape and armature, pygophore armature, the aedeagus, and the shape of the paramere apex. External morphological characters used in generic determinations include the direction of pronotal striations, the relative dimensions of the face, the tegminal venation and the macrosetal armature on the hind tibia.

A further difficulty with working on the Australian Macropsinae is that some type specimens are missing and paratypes, or even identified non-type specimens, are unavailable for many of the species.

Most Australian Macropsinae males do not fit within the definition of *Macropsis* (*sensu*. Hamilton 1980). Some female types may share external characters attributed to the subgenus *Parapediopsis* Hamilton but this Oriental/ Australasian subgenus may itself require revision as some characters do not seem to be congeneric with other *Macropsis* subgenera.

Some Australian species display a mixture of different generic characters and some males and females of a single species may key to different genera. Revision of *Hephathus* Ribaut and *Toropsis* Hamilton is required, along with genus *Pediopsis* Burmeister which, as Hamilton (1980) indicates, holds diverse species forms and could include many Australian species. Investigation of the world fauna is needed before it will be possible to adequately reconcile generic groupings within the Australian fauna with the world Macropsinae. Further results of this study will be presented.

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Fig1. *Toropsis bella* (Evans) Holotype (female) (image source NSW DPI – http://www.agric.nsw.gov.au /Hort/ascu/leafhop/cicaspp/tbella.htm)

Umbonia crassicornis (Hemiptera: Membracidae) Mothers Provide Their Offspring With Nutritional Enhancement and Access to Preferred Ingestion Tissues

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U. crassicornis mothers make a series of extra-ovipositional slits in the natal host plant around which a single clutch of nymphs cluster. *U. crassicornis* slit-making behavior was described in detail by Wood (1974) and Shugart (2004). Females make slits after the eggs are laid (mean of 6 days following egg laying) and always a few days prior to nymphal hatching. The slits are always present and always made in a specific pattern on relatively small stems (usually less than 1.5 cm in diameter) usually near the terminal bud of the branch. After hatching, the nymphs move onto the slit complex and remain there under maternal protection until they reach sexual maturity and disperse.

Prior to this study, little was known of the feeding behavior of *U. crassicornis,* potential host plant responses to that feeding, and how the slits might affect nymphal feeding behavior, despite their appearance of being integral to nymphal survival (Wood 1974). Our objective was to determine how the slits affect the feeding and social behaviors of *U. crassicornis* nymphs. We were especially interested in how slit use changes as the nymphs get older.

Methods and Materials

A histological study was performed on a range of slit containing tissues and compared to fed-upon and control tissues. All insects were reared and their feeding tested on *Albizia julibrissin* (Mimosaceae) and all histology was performed on *A. julibrissin* stems. Feeding sites were sectioned to a thickness of 10 µm and stained with safranin and fast green stains.

Results

Our results show that young nymphs feed through the extra-ovipositional slits and that older nymphs feed independent of the slits. Nymphs feeding through the slits make incomplete salivary sheaths made up of discontiguous salivary blobs deposited along the path leading to ingestion tissues while older nymphs make more complete sheaths leading directly to the ingestion tissues. We learned that the timing of slit-making is integral to slit use by nymphs. In addition, slits widen dramatically as they age due to an increase in new cell growth in the vicinity of each slit. This widening also facilitates feeding by permitting the nymphs to place their labia directly into the slit during feeding.

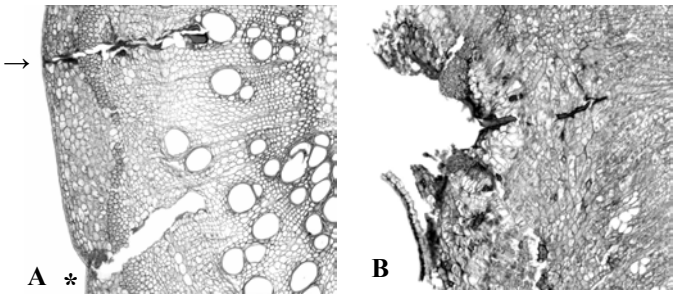

Fig 1. (A). A one-day-old slit (*) adjacent to one of the mother's salivary sheaths (→). (B). A two-week old slit with a nymphal salivary sheath beginning at the base of the slit.

Discussion

Our results demonstrate that *U. crassicornis* mothers provision their young. We found that young nymphs rely on the slits exclusively for access to feeding tissues. Also, feeding behavior in *U. crassicornis* is plastic, and changes as they mature as older nymphs are able to feed independent of slits.

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Correlating DC electrical penetration graph (EPG) waveforms with feeding behavior of beet leafhopper (Hemiptera, Cicadellidae, *Neoaliturus tenellus* (Baker) (syn. *Circulifer tenellus*)

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Beet leafhopper is the only known vector of the curly top virus in North America. In the arid and semi-arid regions of the western United States, curly top virus causes major losses in a number of crops including sugar beets, tomatoes, peppers, spinach, and beans. The feeding behavior of beet leafhopper on petioles and leaves of young sugar beet plants was studied using a DC electrical penetration graph (EPG). The main feeding behaviors of interest include initiation and termination of probing behavior, ingestion from xylem and phloem, and salivation. Correlations between EPG waveforms and specific components of feeding behavior were determined using a number of techniques. Simultaneous EPG recordings and high magnification video recordings were used to determine the EPG waveforms that correlated with initiation and termination of probing. Honeydew excretion was also observed in the simultaneous EPG/video recordings, and provided clues regarding which waveforms were correlated with ingestion since excretion of honeydew occurs as the insect in ingesting. In order to determine whether these ingestion waveforms were the result of phloem or xylem ingestion EPG waveforms were recorded from leafhoppers, and the stylets were severed by high frequency radio microcautery while the leafhopper was producing the waveform of interest. The portion of the leaves and petioles containing the severed stylets were processed for histological examination to determine the placement of the stylet tips within the plant tissue at the time the last EPG waveform was recorded. The site of ingestion during the ingestion waveforms was further determined through the use of fluorescent markers that are transported in either the phloem or xylem. Carboxy-fluorescein diacetate (CFDA) labels phloem sap fluorescent, but not xylem sap. Conversely, lucifer yellow labels xylem sap fluorescent, but not phloem sap. The leafhopper was allowed to feed on excised leaves containing one or the other of the two fluorescent markers until the waveform of interest was produced. The leafhopper was then allowed to complete this waveform, at which time it was removed from the plant, squashed on a microscope slide, and the gut was examined for the presence of the fluorescent marker using an epifluorescent microscope. After completion of the correlations between EPG waveforms and specific components of feeding behavior, EPGs can be used to examine and compare the details of feeding behavior of beet leafhopper on different plants. In the future, we plan to use EPGs to determine when and how beet leafhopper inoculates and acquires curly top virus during the feeding process. Such information can be used to aid development of resistant crops and better pesticides that inhibit the inoculation phase of feeding.

Leafhopper (Hemiptera: Cicadellidae) Systematics in South Africa

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Naudé described 71 new species in 1926 and in 1961 the Finnish researcher Linnavuori described 34 new species collected by the Lund University expedition, as well as many monographs describing species of the Ethiopian Region, however concentrating mainly on Tropical, Northern and Western Africa. Theron described many new species from the late 1960's for almost 30 years. He worked mainly in the fynbos, that is known for its high level of plant diversity, namely 7800 species and 68% endemicity, and adjacent nama karoo biomes. About 200 leafhopper species in 53 genera have been described from 247 localities. The fauna of three host plants (*Salsola esterhuyseniae* Chenopodiaceae*, Elytropappus rhinocerotis* Asteraceae*, Aspalathus linearis* Fabaceae) and one family (Restionaceae) were examined thoroughly.

The grassland biome is found within the borders of South Africa. To the north and east the savanna biome extends further into Africa. Large populations and high species diversity of leafhoppers appear to make their use in environmental issues more relevant. The taxonomic impediment of species that are grass-feeding is probably not as great as it is for dicotyledon feeding. Also the species cannot often be strictly associated with a biome or even vegetation type within the biome, possibly due to their flying ability and wide feeding habits. Perhaps however the wingless species might be more valuable. At present the only the Paralimnini are being examined. Webb & Heller (1990) listed 22 Afrotropical Paralimnini genera, of which 11 genera are associated with the savanna, four in the grassland, and some in

both or in the fynbos biome. *Elginus* Theron, 1975, with 13 new species is found in the grassland and fynbos biomes only. The genus with at least 20 new species is *Pravistylus* (Theron, 1975), and *Platentomus* Theron, 1980 with at least 5 new species, and also mainly in grassland and fynbos. At least three new genera from grasslands require description, and two new genera from savanna. Most feed on grass, but others on Restionaceae (*Restiobia* Davies, 1988) and Cyperaceae (*Teyasteles* Linnavuori, 1969, *Samuraba* Linnavuori, 1961 and *Vecaulis* (Theron, 1975)).

In Deltocephalinae genera such as *Basutoia* Linnavuori, 1961, *Bloemia* Theron, 1974 and *Tzitzikamaia* Linnavuori, 1961 (all Athysanini) require revision. Of the five tribes in the grassland biome in Deltocephalinae and in Drakensbergeninae there are about 30 brachypterous and 37 macropterous species which suggests a high degree of endemism.Grass-living species from other biomes and regions such as the Eastern Cape Province are poorly represented. Savanna is occupied by about 188 macropterous and 2 brachypterous grass-feeding species, with most also distributed throughout the grassland biome, and occasionally in the fynbos biome. Species on dicotyledons in grassland number about 160, and in savanna about 210, and are still poorly known.

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The life cycle of *Clastoptera distincta*, the Dwarf Mistletoe Spittlebug, is synchronized with monsoon climate in the Southwestern United States

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Spittlebugs (Hemiptera: Cercopoidea) parasitize the xylem sap of living plants, a nutrient source shared only with cicadas and sharpshooter leafhoppers among insects, and hemiparasites like witchweeds (*Striga* spp.) and mistletoes among plants (Parker and Riches, 1993; Press and Whittaker, 1993; Thompson, 2004). The spittlebug *Clastoptera distincta* Doering (Clastopteridae) (originally misidentified as *Clastoptera obtusa*) has been reported on two dwarf mistletoes of conifers: *Arceuthobium vaginatum* subsp. *cryptopodum* in Arizona, New Mexico and Southwestern Colorado, and *Arceuthobium abietinum* f.sp. *concoloris* in Arizona, with occasional damage to the host mistletoes (Hawkesworth and Wiens,1996, and refs. therein). These limited observations plus the original taxonomic description (Doering, 1928) comprise the only previously published information on *C. distincta*.

Methods

In July 2003 I carried out field investigations at four localities in the Southwestern United States (Table 1) to verify the presence of *C. distincta* on *Arceuthobium* mistletoes and elucidate relationships among the spittlebugs, the mistletoes, and the ultimate conifer hosts.

Results

At Laporte, Colorado spittlebugs were absent from extensive stands of *Pinus ponderosa* heavily infested with *A. vaginatum* subsp. *cryptopodum*. This is consistent with their observed absence in nearby Boulder County and in Teller County in central Colorado (K. Mooney, pers. comm.). At Rustler Park Campground in the Chiricahua Mountains of southeastern Arizona, examination of dozens of clusters of *A. vaginatum* subsp. *cryptopodum* parasitizing *P. ponderosa* revealed a single *Clastoptera* nymph, probably *C. distincta*. The Southwestern Research Station collection includes one adult *C. distincta* specimen taken at Rustler Park in August 1968, verifying the species presence at this location.

In the San Francisco Peaks north of Flagstaff, Arizona a large population of *C. distincta* nymphs occupied *A. vaginatum* subsp. *cryptopodum* infecting *P. ponderosa*. Spittle masses on the mistletoes of some of the older, lower, heavily infested pine branches were densely clustered and coalescing, with multiple nymphs per spittle. Sweeping of the mistletoes, their host pines and the surrounding open grassy understory yielded no adult spittlebugs, apparently because adults had not emerged by the date of observation. Adults emerging from sleeved spittle masses were later recovered to verify species identity and determine sex and color form (Table 2).

C. distincta spittles with nymphs were also abundant on *A. vaginatum* subsp. *cryptopodum* parasitizing *P. ponderosa* two miles northwest of Jacob Lake, Arizona. Adult *C. distincta* were never observed directly on the mistletoes or swept from the surrounding forest understory, but 26 individuals were swept from the host pines (Table 2). Among these 26 adults, 81% were female, compared to 83% (n=12) of the adults that emerged from sleeved spittle masses at San Francisco Peaks (Table 2), suggesting strongly female-biased sex ratios in both populations.

Discussion

The *C. distincta-Arceuthobium* relationship raises a conundrum. Why, from an evolutionary point of view, should spittlebug nymphs feed on the xylem sap of a mistletoe tapping a pine, when they could, as the adults evidently do, feed directly on the xylem sap of the pine itself? The answer, I suggest, lies in the southwestern monsoon climate in which *C. distincta* has evolved as a mistletoe hyperparasite.

Clastoptera distincta appears to have a single generation per year. Nymphs develop in late spring and early summer. Adults emerge in July. At all three Arizona collection sites, the nymphal period coincides with a pronounced May-June pre-monsoon drought (Table 1), usually broken by monsoon rains in early July (Higgins *et al*., 1997), before *C. distincta* adults begin to emerge. Conifers resist the effects of drought by minimizing transpiration and the flow of xylem sap during periods of water deficit. In contrast, their mistletoe parasites transpire freely during droughts to maintain water and nutrient flow, often damaging their hosts in the process (Parker and Riches, 1993; Press and Whittaker, 1993; Hawkesworth and Wiens, 1996). By hyperparasitizing mistletoes during the May-June dry season, *C. distincta* nymphs gain indirect but effective access to the conifer xylem stream, circumventing drought-induced reduction of conifer transpiration. In the wet season, it appears, the adults feed directly on the xylem sap of the ultimate conifer hosts. This hypothesis is consistent with the geographical distribution of *C. distincta*, which is present on *Arceuthobium* in monsoonal areas of Arizona, New Mexico and adjacent Southwestern Colorado, but absent from *Arceuthobium* in non-monsoonal northern Colorado (Table 1, Laporte) and in Central Colorado (Teller County) where the monsoonal May-June drought is not pronounced (*see* climate maps in Higgins *et al*., 1997).

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Table 1--Summer 2003 survey of 4 localities for *Clastoptera distincta*. In all 4 localities the mistletoe *Arceuthobium vaginatum* var. *cryptopodum* was locally common on *Pinus ponderosa*. See text for more details.

		Clastoptera distincta		Mean monthly precipitation (cm)			
Locality	Date	Nymphs	Adults	May	June	July	August
Laporte, CO	7 July	Absent	Absent	7.1	4.6	4.0	3.5
Rustler Park, AZ	16 July	Rare	Absent	0.8	2.1	10.4	10.5
San Francisco Peaks, AZ	19 July	Abundant	Absent	2.0	1.4	7.3	8.1
Jacob Lake, AZ	22 July	Abundant	Abundant	3.0	2.0	6.9	6.8

Table 2--Adult specimens of *Clastoptera distincta* collected in Arizona in Summer 2003, by sex.

Locality	Male n	Female n	Total
San Francisco Peaks	2	10	12
Jacob Lake	5	21	26
Total	7	31	38
Proportion	18.4%	81.6%	

Imaging the world's sharpshooters: Digital Imaging of the world genera of the sharpshooters (Hemiptera: Cicadellidae subfamily Cicadellinae)

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The leafhopper subfamily Cicadellinae, was monographed on a world basis by David Young (North Carolina State University, Raleigh) in 3 remarkable taxonomic volumes (1968 – 1986). The Cicadellinae contain among the very largest leafhoppers, up to 2 cm long and many are brightly coloured. They are a predominantly tropical group, especially so in the Neotropics. Around 370 genera and 2300 species were included in Young's monographic treatments to the tribes Proconiini and Cicadelliini

The objectives of the 2-year project funded by The Leverhulme Trust is to provide high quality digital images of the world's leafhoppers of the Proconiini and Cicadellini, based primarily on the species covered by Young monographs but also with the additional species and genera described since their publication.

The outputs of the project will include an interactive web-based key to allow world genera and species

BEHAVIOR POSTERS

Specific Stylet Activities by Sharpshooters Are Involved in Inoculation of *Xylella fastidiosa*

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Almost nothing was known, until the work of the Backus lab, about the stylet penetration behaviors of the glassy-winged sharpshooter (GWSS), and how they interact with populations of *Xylella fastidiosa* (*Xf*) to facilitate transmission to grapevine. Our work combines the three most successful methods of studying leafhopper feeding (i.e. histology of fed-upon plant tissues, videotaping of feeding on transparent diets, and electrical penetration graph [EPG] monitoring) to identify most details of feeding. The long-term goals of the Backus lab are to discern the role of stylet penetration behaviors in *Xf* transmission, to use this information to develop a Stylet Penetration Index for screening grape germplasm for resistance to *Xf* inoculation behaviors by the sharpshooters, and to aid in epidemiological modeling for risk assessment.

Previous studies in the Backus lab [1, 2] found that the B1, C and N waveforms (Table 1) are associated with inoculation of *Xf* into susceptible grape plants. The objective of the present study is to further develop EPG as a tool to study GWSS stylet penetration behavior, by correlating specific EPG waveforms with specific stylet activities, as viewed in transparent artificial diet.

Methods and Materials

- A wired GWSS was placed on a Parafilm-covered chamber slide containing expressed xylem sap from 'Cabernet Sauvignon' grape. A 25 mV, 1000 Hz AC signal from an AC EPG monitor (input impedance 10^6) was applied to the sap via a gold wire.
- Digital EPG and analog video images were synchronized and combined via the instruments in FIG. 1.
- The split screen video of GWSS stylet activities and EPG waveforms were visualized on a monitor and recorded on VHS media for analysis (FIG. 2).

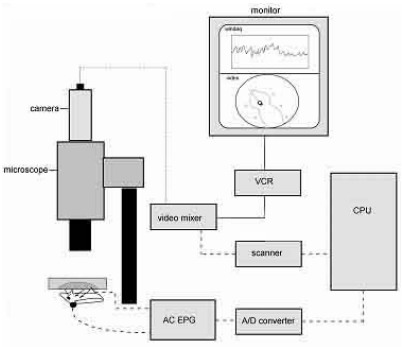

Fig. 1. Equipment configuration for split-screen video of synchronized GWSS stylet activity and EPG waveforms.

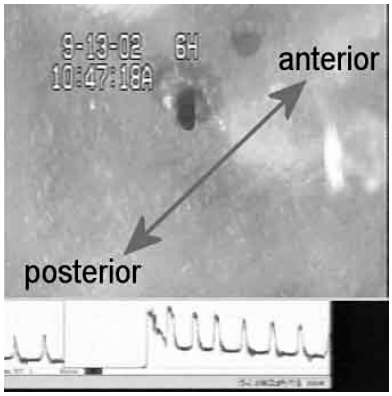

Fig. 2. Split screen view of EPG waveform and GWSS mouthparts within diet. The body of the GWSS is visible through the Parafilm, with orientation shown.

Results and Discussion

Insects' stylets could clearly be seen performing stereotypical behaviors during three pathway waveforms frequently seen on grape, i.e. A1, B2 and B1. Results for A1 are shown in the Backus & Joost poster; results for B1and B2 are summarized in Figs. 3 and 4, respectively, below. In summary, A1 represents the primary formation of the salivary sheath, B1 represents sheath salivation, stylet tip fluttering and probably tasting (Figs. 3) and B2 represents stylet sawing through the hardened sheath or tough plant material (Fig. 4). It is particularly interesting that the B1 spikelet burst is dispersed intermittently throughout other pathway waveforms, e.g. between peaks of A1, as well as in continuous durations by itself (Fig. 3) [3]. This dispersion, plus last year's research finding that B1 was the only pathway waveform associated with *Xf* inoculation, suggest that the spikelet bursts might represent precibarial valve movement, an important component of a hypothesized inoculation behavior for *Xf* [2]

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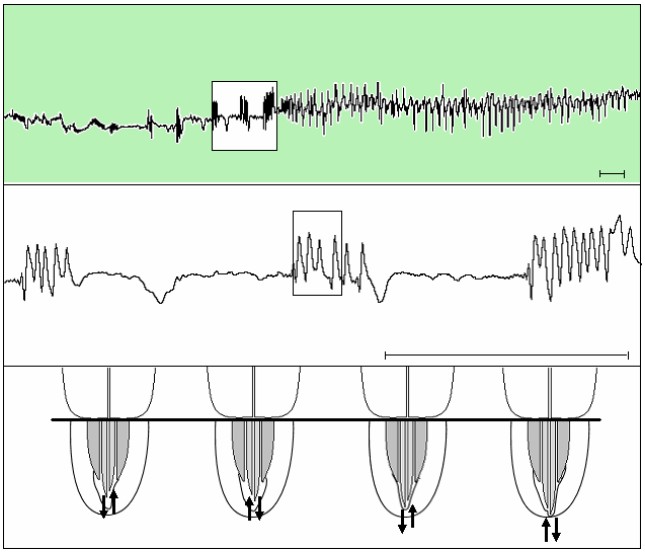

FIG 3. Correlation of B1 waveform with GWSS stylet activities in artificial diet. Top panel is a B1 waveform trace compressed 5 times. The middle panel is an uncompressed B1waveform trace that corresponds to the boxed portion of the waveform in the top panel. The bottom panel is the stylet activities that correspond to the boxed portion in the middle panel. Time marks in the lower right hand corner of the top and middle panel equal one second.

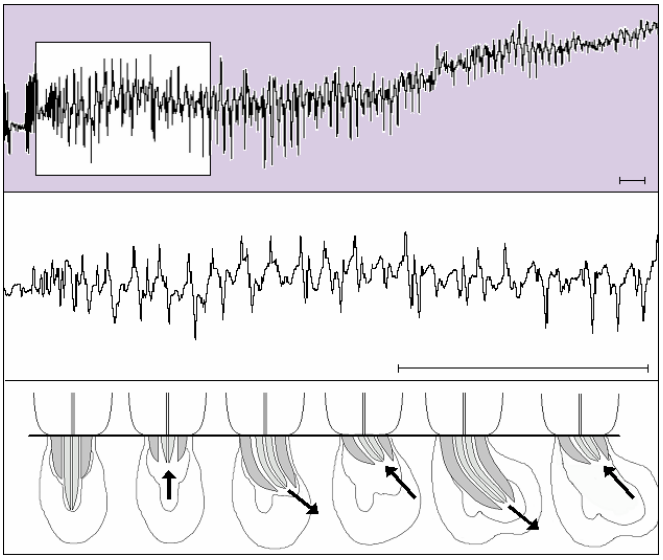

FIG 4. Correlation of B2 waveform with GWSS stylet activities in artificial diet. Top panel is a B2 waveform trace compressed 5 times. The middle panel is an uncompressed B2 waveform trace that corresponds to the boxed portion of the waveform in the top panel. The bottom panel is the stylet activities that correspond to the boxed portion in the middle panel. Time marks in the lower right hand corner of the top and middle panel equal one second.

Table 1. Current definitions of the AC EPG waveform phases, families and types of GWSS on grape.

Waveform Phase	Waveform Family	Waveform Type	Waveform Characteristics	Proposed Biological Meanings	
				Plant Tissue/Cell	Insect Activity
Pathway	A	A1	Highest amplitude, hump-like waveform at beginning of probe; usually with spike at the top	Parenchyma or mesophyll	Major salivary sheath formation; deep extension/ retraction of stylets; some watery salivation
		A2	Medium amplitude, variable slope; irregular, high frequency with occasional trenches and/or potential drops	Parenchyma or mesophyll	Lengthening and/or hardening of salivary sheath; cell membrane breakage; some watery salivation
	B	B1	Short, single- or multi-peak "spikelet bursts" (20-28 Hz) separated by flatter, wave-like sections	Parenchyma or xylem or pith	Stylet tip fluttering; possible internal muscle/valve movement; involved in inoculation
		B2	Extremely regular, stereotypical pattern of peaks (6 Hz), with distinct phrases	Parenchyma or xylem or pith	Stylet sawing through salivary sheath or tough wood; sheath branching; sheath salivation
Ingestion	C	C (to be subdivided)	Very regular, low rep. rate (3 Hz) with with distinct phrases	Parenchyma or xylem or pith	Trial (short) or sustained (long) ingestion (watery excretory droplets correlated)
Interruption	N	N (to be subdivided)	Irregular, appearing A-like at times, but interrupting continuous C; ave. dur. 16 sec.	Parenchyma or xylem or pith	Sheath or watery salivation in ingestion cell; sheath extension

Mud-Puddling (Aggregation and Feeding at Moist Ground) in Leafhoppers

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One so far unexplained fact in the biology of Cicadellidae, usually thought of as deriving their livelihood exclusively from plants, is that adults of some species have been observed in large numbers on moist ground: sand, mud, rocks, dead wood, etc. A few such published observations (reviewed in Adler 1982) indicated that (i) these gatherings consist almost exclusively of males, and (ii) the males apparently drink water from the substrate. Adler (1982) pointed out the similarity between this phenomenon and the "mud-puddling" behavior of Lepidoptera, also usually limited to males, and suggested that in both cases the behaviors may be related to nutritionally deficient diet. Puddling in Lepidoptera involves active collecting of soluble nutrients, especially sodium and proteins, from mud puddles, sweat, tears, excrements, and carrion (Pivnick & McNeil 1987; Beck et al. 1999). In at least two species, the sodium was found to be absorbed by the male accessory glands, incorporated into spermatophores, and passed on to females, apparently enhancing their fecundity (Pivnick & McNeil 1987; Smedley & Eisner 1995, 1996). Other potential benefits of sodium-collecting for males, including increased sperm motility, have been proposed but not yet tested experimentally (Molleman et al. 2004). Lepidopteran species and families generally differ in the presence/absence of puddling, preferences for particular substrates, and sex and age of puddling individuals, apparently reflecting taxon-specific differences in nutritional physiology and mating strategies (Beck et al. 1999; Boggs & Dau 2004). In contrast to Lepidoptera, ground visitation and feeding in Cicadellidae remain poorly documented and generally unexplained. Here we review the existing and report additional observations of leafhoppers on the ground, including spectacular mass gatherings in the Peruvian rainforest, and discuss possible explanations and future study directions. While applying the term "mud-puddling", established in the research on Lepidoptera, to leafhoppers, we recognize that the mechanisms involved in this behavior and its adaptive significance may differ between the two groups.

Typhlocybinae. In the temperate regions of U.S.A. (Adler 1982) and Russia, the vast majority of observed puddling leafhoppers were male typhlocybines of common, mostly arboreal genera, such as *Empoasca*, *Kybos, Erythroneura, Edwardsiana, Ribautiana, Typhlocyba, Ossiannilssonola,* and *Linnavuoriana.* One or several species together were found on roads, stream banks, sand piles, tree stumps, and rocks. Drier substrates are used when moistened by rain. Adler (1982) observed males regularly gathering on moist areas of cinder, dirt, and gravel roads but only after dusk. Interestingly, in many Typhlocybinae, the individuals attracted at night to light traps (commonly supposed to be migrants) are also exclusively males. These may be individuals in search of puddling sites or, conversely, the males observed on the ground can be migrating individuals replenishing their supplies. The only observation of large numbers of puddling typhlocybines in the tropics was made by one of us (CD) in Taiwan, where males of *Agnesiella*, *Eurhadina*, *Ribautiana*, *Typhlocyba*, *Igutettix*, *Salka*, and *Alnetoidia* gather on sand and rocks in rapidly running streams. The absence of generally omnipresent Typhlocybinae from the large puddling swarms in the Peruvian rainforest, described below, is intriguing, although a few individuals of undetermined Dikraneurini were observed on mossy rocks in a stream bed at one Peruvian locality (CD).

Cicadellinae and Phereurhininae have not been previously reported from the ground. However, at a site in southern Arizona, we collected 85 males of *Graphocephala atropunctata* (Signoret) (Cicadellinae) from a sandy stream bank in close proximity to shrubs where both sexes were collected (CD & RR). In several areas in the Peruvian lowland rainforest, we observed aggregations of thousands of males of dozens of species on silt and clay near rivers, dominated (>90%) by Cicadellini, particularly from the genera *Dilobopterus* and *Mesogonia*. Males of the phereurhinine genera *Phereurhinus* and *Clydacha* were also numerous here. The dominant species were similar between puddling sites at Rio Huallaga near Tingo Maria (CD & RR) and at Rio Tambopata (EV & DB), but the diversity at the first site was higher. Within the Tambopata site, which includes mineral-rich clays of several types (Brightsmith & Aramburú Muñoz-Najar 2004), some of the species tended to clump in different areas, possibly reflecting different preferences with regard to the substrate chemistry or attraction to conspecifics. The aggregations were observed at day time and lasted only several hours, subsequently disappearing for days. Long-term observations currently conducted at Rio Tambopata suggest that these happen sporadically, apparently being correlated with weather. The males were intensively drinking the ground water and profusely excreting. Surprisingly, none of the species collected was found on the nearby vegetation (nor elsewhere on plants), suggesting that the males travel distances to these ground sites. Near two other streams in Peru, we collected from the ground 27 females of *Proconosama* sp. 1 (Cicadellinae) at one site and 60 females of *Proconosama* sp. 2 at another. Neither conspecific males, nor other leafhopper species were found at these sites. In both cases, we failed to collect any *Proconosama* on nearby vegetation, again suggesting that the females came from a distance.

Other subfamilies. Besides the two above dominant subfamilies, the larger puddling swarms observed in Peru contained representatives (males) of three subfamilies with poorly known biology, Neobalinae, Arrugadinae, and

Nioniinae, plus much smaller numbers of Coelidiinae, Deltocephalinae, and Evacanthinae (Nirvanini). The nocturnal collections made by Adler (1982) in Pennsylvania included a small number of Deltocephalinae (2.9%), mostly *Osbornellus*. Males of three species of Scarinae, predominantly *Dragonana dracontea* (Gibson), were observed in rock pools near mountain streams and at the edges of a swimming pool in southern Arizona (Knull & Knull 1960). Surprisingly, these males were typically found partly submerged in water and vibrating.

To summarize, puddling was observed in 8 leafhopper subfamilies representing various dietary guilds. Mesophyll-feeding Typhlocybinae and xylem-feeding Cicadellinae appear to be the most common puddlers, while the phloem-feeders are only rarely found at the ground. Characteristically, other Auchenorrhyncha do not participate in puddling. Only a small fraction of species occurring in a particular area comes to puddling sites. Composition of the puddling communities differs between sites both at the species and the higher taxonomic levels. In all the observed species, except two species of *Proconosama*, the puddlers are exclusively or predominantly males. As was correctly pointed out by Adler (1982), unlike Lepidoptera, leafhoppers are not attracted to sweat, urine, excrements, or carrion, which suggests that they may either "look" for different chemicals or use different cues to locate the same. Our preliminary analyses of the chemical composition of the excreta produced by puddling cicadellines compared to the water extracted from the ground at Rio Tambopata (EV & DB) indicate significant retention of sodium, through a hypothesized ionic exchange. To test whether these chemicals play role in enhancing reproductive success of the males, as in Lepidoptera, further experimental work is needed. A recent study demonstrated that at least in one leafhopper species, *Bothrogonia ferruginea* (Cicadellinae), males produce relatively large spermatophores, which may contain male-derived chemicals incorporated into developing eggs (Hayashi & Kamimura 2002). Therefore, the physiological basis for passing nutrients or chemicals otherwise increasing reproductive success of the male from males to females does exist in leafhoppers. One enigmatic aspect of puddling in leafhoppers is its sometimes drastic and seemingly unpredictable dynamics (e.g., at our rainforest sites in Peru), apparently related to weather changes. It is possible that this behavior is finely tuned to the time periods when the moisture at the puddling sites is at the optimal level. Finally, puddling aggregations on mud near water may create unique opportunities for fossilization of leafhopper remains. The biased composition of these aggregations should be kept in mind when fossil assemblages are interpreted.

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Comparison of the Feeding Behaviors of Three Age Groups of *Umbonia crassicornis* (Hemiptera: Membracidae) Using Electrical Penetration Graph Monitoring

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Membracid feeding has received little attention because most detailed studies of feeding have focused on agricultural pest species and few treehoppers are considered pests. The few exceptions of membracids in which feeding behaviors have been studied are the three-cornered alfalfa hopper, *Spissistilus festinus* (Say), on soybean and alfalfa and the buffalo treehopper, *Stictocephala bisonia* Kopp & Yonke, on grape (Johnson and Mueller 1988, Hicks 1984 for *S. festinus*; Vidano 1970 for *S. bisonia*). Nonetheless, rigorous studies of feeding behavior using electrical penetration graph (EPG) technology have not been performed with treehoppers. The goal of our study was to investigate treehopper feeding using EPG combined with histology in the treehopper *Umbonia crassicornis*. In addition, we wanted to better understand how the feeding behavior of nymphs compares to adult feeding in *U. crassicornis*.

Methods and Materials

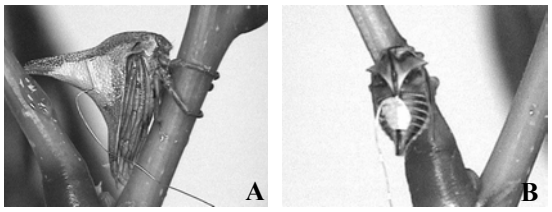

Fig 1. (A). A wired adult female *U. crassicornis* resting on a young *Albizia julibrissin* stem. (B). A wired fourth instar nymph on an *A. julibrissin* petiole.

We used EPG monitoring combined with feeding site histology to compare waveform types and feeding sites among three age groups of insects. Each insect was recorded for a 20 hour long period using an AC-EPG system (Backus and Bennett 1992). Acquired waveforms were characterized, measured and descriptive and analytical statistics were performed to identify feeding differences. The associated feeding sites were sectioned and stained for each insect and the feeding tissues, salivary sheaths and associated feeding damage was compared among each group.

Results

We found that there are distinct sets of waveforms representing the feeding behaviors of each age group. In addition, we found that individual age groups exhibit unique probing durations and frequencies. We also found that *U. crassicornis* fed on both phloem and xylem, although each age group spent different amounts of time ingesting from each tissue type. Furthermore, examination of salivary sheaths showed differences in structure among age groups, with young nymphs producing incomplete sheaths made up of discontinuous salivary blobs scattered along the path leading to feeding tissues and older nymphs producing more complete sheaths directly into feeding tissues.

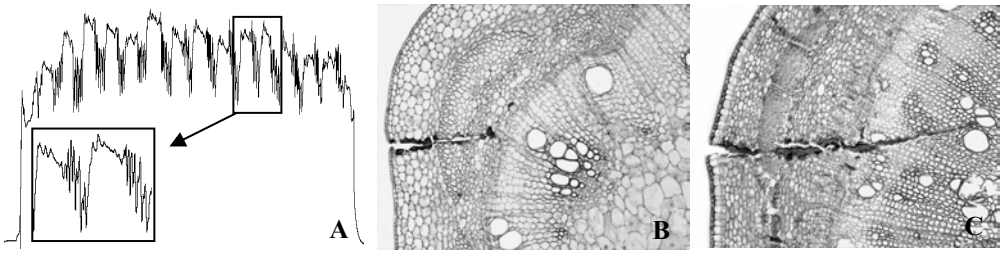

Fig. 2. (A). A waveform excerpt showing an adult probe lasting 48.5 seconds in duration. Boxed inset shows enlargement of waveform A2. (B). A fourth instar salivary sheath. (C). An adult salivary sheath.

Discussion

Our study is unique in that comprehensive studies of nymphal feeding are rarely carried out using EPG. We not only determine the preferred feeding tissues and associated waveforms for adults but also for two age groups of nymphs and found striking differences in the ways that each group fed, both in ingestion durations and in ingestion tissues.

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ECOLOGY POSTERS

Diversity of Cicadellidae (Hemiptera: Auchenorrhyncha: Membracoidea) and potential vectors of *Xylella fastidiosa* in coffee plantations

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Coffee plantations represent one of most common agroforestry systems in Middle America and the Caribbean. These systems play important roles, not only from an agronomic and economic standpoint, but also as a refuge for biodiversity, including birds and insects (Rojas *et al.* 2001). However, one recently discovered component of coffee plantations in Costa Rica could potentially cause serious damage – *Xylella fastidiosa*, a bacterium associated with many diseases, including coffee leaf scorch (Rodriguez *et al.* 2001). This bacterium, which is confined to the xylem in its host plants, is known to haves a large number of vectors in other agroecosystems, principally species of Cicadellinae (Cicadellidae) and Clastopteridae (Cercopoidea). Because the vectors in coffee plantations are unknown, we undertook a survey of the Cicadellinae that are present and are presently in process of determining which of these are potential vectors for the disease in coffee.

Methods and Materials

Three coffee plantations were evaluated between November 2002 and December 2004: Alajuela province, Pavas (in Carrizal), a monoculture coffee plantation; in the same province, Los Angeles (in Grecia), coffee with citrus; and San José province, San Isidro (in Leon Cortés), coffee monoculture. In each plantation we selected 4 plots for sampling, each of which was 120 m² (four rows with ten plants in each); the plots were separated from one another by 50 m. The leafhoppers were collected by yellow sticky traps (25 x 25.5 cm); two traps were placed in each plot, each of which was attached near the top of a pole (the height of the pole was the same as that of the coffee plants) that was fixed in the ground between the second and third rows, one at each end of the plot. Leafhoppers on sticky traps were counted under a dissecting microsope and were identified to species with the aid of a reference collection; in some cases specimens were removed from the traps in order to dissect male genitalia. Live specimens (captured with a vacuum apparatus) were tested with enzyme-linked immunosorbent assay (ELISA) to detect for the possible presence of *X. fastidiosa*.

Results and discussion

Ten species of Cicadellinae were found in the three coffee plantations. *Graphocephala bivittata* was the most abundant species in two of the three plantations, and was the second most abundant species in the other plantation, where it was slightly surpassed in abundance by *G. permagna* (Table 1). Of six species for which more than ten samples (each sample consisting of two specimens) have been tested with ELISA, all showed several positive results: *Dilobopterus instratus* (27 positive samples, 0 negative), *Fusigonalia lativittata* (58 positive, 46 negative), *Graphocephala bivittata* (14 positive, 3 negative), *G. permagna* (16 positive, 21 negative), *Graphocephala* n.sp. (18 positive, 9 negative), and *Kapateira* n.sp. (11 positive, 20 negative). However, transmission tests are now needed to determine whether any of these species are functioning as vectors of *X. fastidiosa* in coffee plantations.

Table 1. Cicadellinae in three coffee plantations in Costa Rica: number of individuals and percent of total number of Cicadellinae.

	Pavas	Pavas	Los Ángeles	Los Ángeles	San Isidro	San Isidro
Species	Number	Percent	Number	Percent	Number	Percent
Dilobopterus hyalinatulus	29	0,117	153	0,953	20	1,744
Dilobopterus instratus	50	0,202	166	1,034	41	3,575
Erythrogonia sonora	5	0,020	17	0,106	23	2,005
Fusigonalia lativittata	9	0,036	2525	15,722	30	2,616
Graphocephala bivittata	21214	85,537	5115	31,849	846	73,758
Graphocephala permagana	1335	5,383	5558	34,608	100	8,718
Graphocephala n. sp.	2110	8,508	1614	10,050	54	4,708
Hortensia similis	28	0,113	45	0,280	1	0,087
Kapateira n. sp.	12	0,048	226	1,407	27	2,354
Macugonalia testudinaria	9	0,036	641	3,991	5	0,436
Total	24801	100,000	16060	100,000	1147	100,000

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Long-distance migrations of brown planthopper *Nilaparvata lugens* (Homoptera: Delphacidae) across the border between the East Asian and Southeast Asian Population

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The brown planthopper *Nilaparvata lugens* is a major migratory pest of rice. The insect's insecticide susceptibility, wing polymorphism and feeding on resistant rice varieties affect reproductive rate, and consequently, damage to rice plants. These properties differ among brown planthopper populations, which are grouped into three populations: the East Asian, the Southeast Asian and South Asian Population, based on biotype shifts (Sogawa, 1992). It is thought that these populations are maintained independently without migrations between them. However, migrations that occurred between the East Asian and Southeast Asian Population were estimated with a three-dimensional backward trajectory analysis. This study discusses the effect of such migrations on the East Asian Population.

Methods and Materials

Capture data observed at Kin (26.4 ˚N, 127.9 ˚E) on Okinawa island in south-western Japan in June 1999 and 2000 and at Shao-Ma (23.2 ˚N, 121.3 ˚E) in eastern Taiwan in August 1978 were analyzed using the backward trajectory analysis method (Otuka et al., 2005a) to find migration source, or migration paths across the border between the East Asian and Southeast Asian population.

In response to the results of the above analysis, forward migration simulations with 10-year weather data from 1995 to 2004 were conducted using a migration simulation model (Otuka et al. 2005b) to find possible migrations from the Philippines to southern China in the early season (i.e. April to mid-June). Southern China, especially Fujian province, is estimated to be a major migration source of planthoppers immigrating into western Japan.

Results

Backward trajectories from Kin as well as Shao-Ma reached over the Luzon in the Philippines. The Philippines is in a region inhabited by the Southeastern Asian Population, and Okinawa and Taiwan are located in the range of the East Asian Population. Therefore, migrations from the Southeast Asian Population to East Asian Population were estimated (bold arrows in Fig. 1).

The 10-year migration analysis found 21 possible migrations from the Philippines to southern China in the early season (the dotted arrow in Fig. 1) Weather conditions under which these migrations might have happened were mainly depressions located in the South China Sea, which caused southeasterly winds blowing from the Philippines to southern China.

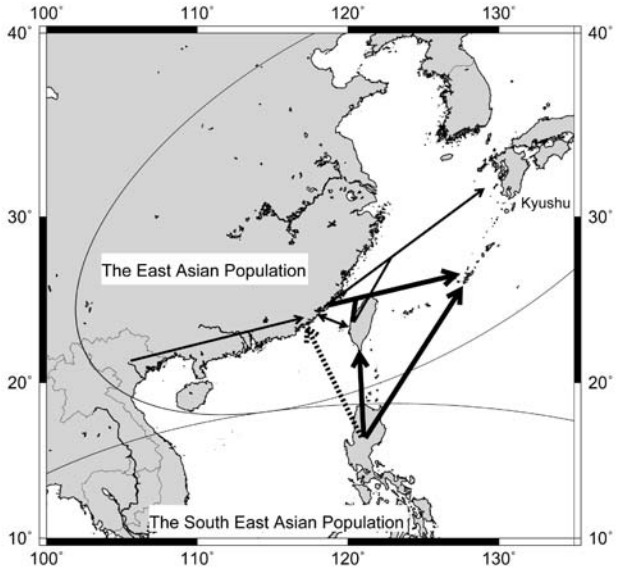

Fig.1. Migration paths across the border of the East Asian and Southeast Asian Population. Bold and dotted arrows indicate those path found in this study, and a feasible path, respectively.

Discussion

The backward trajectory analysis strongly suggested the migration of *N. lugens* from the Philippines to Okinawa and Taiwan. Immigrants from a different population likely invaded southern Japan. In other words, there were probably mixtures of the Southeast Asian and the East Asian population that entered south-western Japan.

The 10-year migration analysis suggested that the planthoppers in the Philippines can invade southern China in the early months before mid-June, given that appropriate weather conditions occur. This is another migration path from the Philippines. One of the most important consequences would be that the planthoppers from the Southeast Asian population might indirectly migrate to Japan after a few generations of reproduction in southern China. If so, special care should be taken for pest management in Japan, because immigrants from the different populations, which show different properties, could invade Japan.

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Abundance of leafhoppers (Hemiptera: Cicadellidae) in organic and conventional coffee farms within the Turrialba-Jiménez Biological Corridor, Costa Rica

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Multistrata, shaded, organic coffee farms are known to support higher biodiversity (including insects) than conventional full-sun coffee plantations. As a consequence, shaded organic coffee farms are being promoted as a sustainable land use within biological corridors in Central America. However, the impact of these practices on coffee pests is not well understood. We investigated the effect of shade structure and management (organic vs. conventional) on the abundance and community composition of leafhoppers. Leafhoppers in the subfamily Cicadellinae are considered potential vectors of *Xylella fastidiosa*, the bacteria that causes "crespera" disease in coffee. We selected four treatments of combined management and structure types: a) certified organic two strata systems with *Erythrina* shade, b) certified organic three strata systems with *Cordia-Musa-Erythrina* shade, c) conventional two strata systems with *Erythrina* shade and d) conventional three strata systems with *Cordia-Musa-Erythrina* shade. We used yellow sticky traps to survey the leafhoppers in farms. Results of samplings will be presented.

Leafhopper diversity in natural and rehabilitated grassland at Wonderwater Coal strip-mine, Sasolburg

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A qualitative study of leafhoppers (Cicadellidae: Auchenorrhyncha) was conducted in three types of rehabilitated grasslands and undisturbed, or so-called natural grassland.

Methods
The study site is in the center of the grassland biome. Eight surveys were conducted, mainly in summer and autumn. Rehabilitation was achieved by translocation from a nursery of grass plants with soil collected before mining, spontaneous natural re-growth of the seed-bank in the top soil and by sowing a commercial seed mix. D-Vac suction sampling was used to collect the specimens in 4x4m plots.

Results
The total of leafhopper species collected was 79, including one new genus and species, planthoppers, 15 and other Aunchenorrhyncha, 6. Wingless leafhoppers such as *Basutoia brachyptera* Linnavuori, *Chiasmus hyalinus* (Evans), *C. undulatus* Theron and *Tzitzikamaia silvicola* Linnavuori were found only in natural and translocated grassland. More species were found in translocated rehabilitation (54 species) than in seeded and spontaneous natural rehabilitation (42, 33 respectively). Natural grassland produced 58 species, with 75 species common to all the sampled habitats. The wetland habitats at the mine, that were usually characterized by the presence of sedges (Cyperaceae) had a unique complement of leafhoppers (*Samuraba elegans* Linnavuori, *Teyasteles divisfrons* Theron and *Vecaulis* sp.n.), as well as other groups of insects such as plant hoppers, spittlebugs and tingids.

Discussion
The hypothesis that required testing was whether wingless species could possibly be used as indicators of successful rehabilitation due to their inability to migrate rapidly and their sole occurrence in undisturbed or climax grassland. Leafhoppers in seeded and spontaneous natural rehabilitation were the more migrant species, usually the stronger fliers, and could be associated with typical inhabitants of the savanna biome. This result greatly improved the knowledge of grassland leafhoppers to that published by Theron (1987, 1982). Mining is to cease in a few years, after which follow-up is envisaged with quantitative sampling.

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Egg parasitoids (Hymenoptera: Mymaridae and Trichogrammatidae) of *Cuerna* sharpshooters (Hemiptera: Cicadellidae) in the USA

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Cuerna Melichar is the only genus of the predominantly tropical New World leafhopper tribe Proconiini of the subfamily Cicadellinae that has substantially radiated in the temperate zone. The range of the genus extends from Costa Rica to Alaska. Among the 31 recognized species the majority are known from the USA. *Cuerna* occur primarily in open habitats, ranging from extreme deserts to moist prairies, where they feed on a variety of plants, including but not limited to grasses, yuccas, pinyon pines, saltbush, and numerous perennial Asteracea and Fabaceae. Eggs are laid in clusters under the lower epidermis of leaves. Some species have dual host plants, with the immatures and adults feeding on evergreens with tough or resinous leaves and eggs laid into a different plant. All species north of Mexico overwinter as adults, and are mono- or, more rarely, bivoltine. The second author is currently conducting a comparative evolutionary study of egg-laying behaviors of *Cuerna* (see Rakitov et al. this volume). It includes sampling natural populations of most species, providing a unique opportunity to survey the egg parasitoids, which is of interest because:

(1) little is known about egg parasitoids of this genus and their host specificity; identification of field-collected parasitized leafhopper eggs is often difficult;

(2) only a few studies of insect egg parasitoids surveyed multiple species of a host genus;

(3) species of *Cuerna* are associated with diverse habitats and host plants;

(4) these species vary in the presence or absence of a specific maternal behavior, powdering egg nests with brochosomes, which may protect eggs against parasitoids (Rakitov 2004);

(5) it is not well understood how the life cycles of egg parasitoids adjust to those of their monovoltine sharpshooter hosts overwintering as adults (i.e., whether parasitoids overwinter in the eggs of alternative hosts);

(6) parasitoids of *Cuerna* are of potential importance for biocontrol, particularly against the glassy-winged sharpshooter, *Homalodisca coagulata* (Say).

Preliminary results

Prior to this study, egg parasitoids had been known for only one species of *Cuerna*, *C. costalis* (F.) (Turner and Pollard 1959; Huber 1988; Triapitsyn 2003; Hoddle and Triapitsyn 2004). All known records to this date, including our recent findings, are summarized in Table 1 (taxonomic identifications of the sharpshooters (other than *C. costalis*) were made by R.A.R. and those of the egg parasitoids by S.V.T.).

Table 1. Known egg parasitoids of *Cuerna* spp. in the USA.

Host species (*Cuerna*)	Locality (state)	Family (parasitoid)	Egg parasitoid species
C. balli[1] Oman and Beamer	Arizona	Mymaridae	*Gonatocerus* sp. near *impar* Huber
C. costalis[2] (F.)	Georgia	Mymaridae	*Gonatocerus ashmeadi* Girault *Gonatocerus incomptus* Huber
		Trichogrammatidae	*Paracentrobia acuminata* (Ashmead) *Ufens niger* (Ashmead)
C. fenestella[2] Hamilton	Minnesota	Mymaridae	*Anagrus epos* Girault
C. sayi[1] Nielson	Montana	Trichogrammatidae	*Zagella* sp. near *spirita* (Girault)
Cuerna sp. [1] [*C. alpina* Oman and Beamer or *C. septentrionalis* (Walker)]	Montana	Mymaridae	*Gonatocerus* sp. near *incomptus* Huber

[1] Species coating egg masses with brochosomes.
[2] Species not coating egg masses with brochosomes.

Although we were able to collect significant numbers of egg masses for only a few species, no conspicuous difference in the rate of parasitism was noticed between those coating and not coating eggs with brochosomes. The percentage of parasitized eggs ranged from 0 to 80% (the maximum was recorded for a roadside population of *C. sayi* in Montana, parasitized by *Zagella*, N=80).

Discussion

Among the parasitoid species in Table 1, *Gonatocerus* sp. near *impar*, *G.* sp. near *incomptus*, and perhaps *Zagella* sp. near *spirita* display minor but consistent morphological differences from the types of *Gonatocerus impar*, *G incomptus*, and *Zagella spirita*, respectively. It is not yet clear if our material represents mere variants or new, possibly host-specific taxa. The remaining species are known as common egg parasitoids of other proconiine sharpshooters, such as *Homalodisca insolita* (Walker) (*P. acuminata, U. niger*) (Triapitsyn 2003), *H. coagulata* and *H. liturata* Ball (*G. ashmeadi, G. incomptus*) (Huber 1988), as well as *Erythroneura* spp. and other small, non-proconiine leafhoppers (Triapitsyn 1998).

Two of the species reared from *Cuerna* eggs were able to parasitize eggs of *H. coagulata*, a fictitious host, in a laboratory colony at the University of California, Riverside quarantine facility. Following successful establishment of a colony of *A. epos,* reared by R.A.R. from egg masses of *C. fenestella* collected on *Solidago* sp. and *Zigadenus* sp. near Glyndon, Minnesota, in early June 2004, on *H. coagulata* (Hoddle and Triapitsyn 2004), attempts were also made by S.V.T. to establish a quarantine colony of the trichogrammatid *Zagella* sp. near *spirita*, reared from egg masses of *C. sayi,* collected by R.A.R. in Twin Bridges, Montana, in May 2004 on *Sonchus* sp. (Fig. 1). During June and in the beginning of July 2004, females of this *Zagella* species readily parasitized freshly laid (by female glassy-winged sharpshooters also present in the rearing cage) eggs of *H. coagulata* on corn leaves. The next generation of parasitoids then developed to the pupal stage inside *H. coagulata* eggs (several per each host egg), but the adults failed to emerge until next year, when on 7 March 2005 one male of *Zagella* sp. near *spirita* emerged after a long (about 7 month) diapause. One more specimen, a female, emerged from another parasitized egg mass on 29 March 2005. This may suggest that, in the natural habitat, the first generation of the parasitoid emerges from *C. sayi* (a monovoltine species that lays eggs early in the spring) in late spring, then parasitizes eggs of a different host(s), in which it overwinters, and emerges early next spring to again parasitize eggs of *C. sayi*.

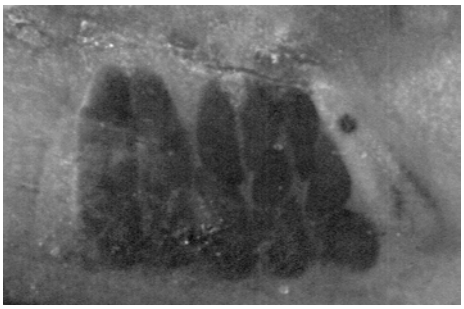

Fig. 1. An egg mass of *C. sayi* with all but one egg containing multiple developing *Zagella* sp. near *spirita*.

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Measuring Habitat Quality of Tallgrass Prairie Remnants Using an Auchenorrhynchous Homoptera Index (Insecta, Hemiptera, Auchenorrhyncha)

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Tallgrass prairies are the most endangered ecosystems in North America, often restricted to small isolated remnants in parks and preserves (Hamilton 2005). Their health is often measured based on information from small subsets of organisms such as plants, vertebrates, and butterflies. Unfortunately, these taxa do not always show a strong correlation in patterns of species richness to those of other under-sampled groups (Anderson and Major 2004), and they may respond differently to different environmental perturbations (Panzer 2001). Thus, assessments of prairie health based on such taxa are incomplete at best. Auchenorrhyncha are potentially excellent bioindicators of prairie health. They represent some of the most speciose and abundant taxa in prairies (Hamilton 1995), are ecologically important (Nickel and Hildebrandt 2003), and are sensitive to environmental perturbations (Harper et al 2000).

The focus of this project is to develop a habitat quality index (i.e., AHI) to measure prairie health along disturbance and moisture gradients. Life histories and species richness variables will be used to develop the index. Each auchenorrynchous species encountered will be assigned a ranked score, from 0 to 4, based on the level of conservatism or tendency for the species to be restricted to a remnant prairie. A score of 0 indicates a low level of conservatism, whereas a score of 4 indicates a high level of conservatism. Life history variables to be scored for each species include voltinism, host plant affinity, microhabitat preference, wing length, and remnant-dependence. For each sampled site, a mean coefficient of conservatism (Cavg)) will be calculated by averaging coefficients over all species encountered. This will be combined with other variables such as species richness and evenness and corrected for site size to produce an Auchenorrhynchous Homoptera Index of habitat quality. The development of an AHI could provide an alternative measure of prairie health to those currently based on less diverse groups of organisms, and preserve management practices

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PHYSIOLOGY POSTERS

Molecular characterization of delta-9 desaturase from two sharpshooters, *Homalodisca coagulata* and *Oncometopia nigricans* (Hemiptera: Cicadellidae)

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Insects depend on lipids for food storage, pheromone production, and many other aspects of growth. We report the isolation and characterization of a delta-9 desaturase from two sharpshooter leafhoppers, the Glassy-winged Sharpshooter, *Homalodisca coagulata*, and the black-winged sharpshooter, *Oncometopia nigricans.* These two leafhoppers are known to transmit the bacterial pathogen of Pierce's Disease of grapes, which also causes disease in many other crop plants such as alfalfa, almonds, oleander, peach, plum, and citrus. Sequencing of the sharpshooters' cDNA showed that it encoded a 367 amino acid protein belonging to 'Family 1' of the ProDomain fatty acid desaturases. Conserved amino acid motifs and *in silico* analyses to known proteins within the desaturases confirmed that these genes code for a delta-9 desaturase-1, especially for a 16-carbon chain structure. Although these leafhopper desaturase proteins appear to have three structural domains similar to other characterized desaturase proteins, a number of significant differences from the previously established desaturase crystalline structures separate them into a Hemiptera-specific clade. Desaturases play important roles in lipid production and utilization, which are important processes for leafhopper survival. Understanding how desaturase proteins function may aid researchers to develop better management strategies.

Natural colonization and feeding of sharpshooters (Hemiptera, Cicadellidae) in relation to citrus phenology and xylem sap composition

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Leafhoppers in the subfamily Cicadellinae, commonly named sharpshooters, are important vectors of the bacterium *Xylella fastidiosa* in Brazilian citrus and coffee, causing citrus variegated chlorosis and coffee stem atrophy, respectively. The sharpshooters feed on young branches and leaves of several host plants, usually by sucking xylem sap, which has relatively low nutrient concentrations (Andersen et al. 1989). In order to fulfill their nutritional needs, sharpshooters exploit a variety of host plants in agricultural and natural habitats over the year. Knowledge about the nutritional and phenological basis for host selection by the sharpshooters might be useful to understanding population dynamics and managing these vectors in the affected crop systems. Thus, a field study was conducted to evaluate natural colonization and feeding of sharpshooters in relation to seasonal variations in citrus tree phenology and xylem sap composition in the Northern region of São Paulo State, Brazil.

Materials and Methods

The study was carried out in a 4-year old sweet orange [*Citrus sinensis* (L.) Osbeck 'Westin'] grove, nearby Monte Azul Paulista, SP. Natural colonization of citrus trees by sharpshooters was monitored fortnightly, by visual inspection of 20 plants (2 min/plant), from August/01 to December/03. Presence and activity of sharpshooters in the grove was also checked by using 9 yellow sticky cards, hung outside the tree canopy at a height of 1.8 m and spaced 40 m apart. Every 2 months, sharpshooter ingestion rates were determined by confining individual *Oncometopia facialis* (Signoret) adults for 48 h on young branches of citrus trees, inside leaf cages similar to those described by Andersen et al. (1992), for measurement of liquid excretion. At the same dates of the excretion observations, samples of citrus xylem sap were extracted from similar branches of the trees in which the insects were confined, by using a pressure chamber (Soilmoisture, model 3005) (Scholander et al. 1965). In the laboratory, the xylem samples were analyzed for amino acids, organic acids and carbohydrates by high performance liquid chromatography (HPLC). The concentration of organic compounds was contrasted among periods with high, low or zero numbers of sharpshooters observed on the citrus plants.

Results

Larger populations of sharpshooters were observed on citrus trees during late spring and summer (rainy season), when the plants were producing young shoots. No sharpshooters were observed on citrus plants during winter and early spring months (dry season), although the yellow sticky traps indicated some activity of these insects during that period. Sharpshooter ingestion rates, which were indirectly measured by excretion, were higher in the rainy season than during late fall and winter seasons, which were characterized by water deficit in the soil. The relative concentration of organic compounds in citrus xylem sap varied seasonally. During the period of higher sharpshooter populations (summer), there was a more balanced nutrient profile, with higher proportions of essential amino acids in relation to total amino acids. Moreover, 18 amino acids were detected in the summer compared with only 8 in the season without sharpshooters (winter). Large proportions of organic acids were detected during fall and winter, when relatively low numbers of sharpshooters were observed. There was an increase in total concentration of amino acids during the winter, which was due to the higher contents of two non-essential aminoacids, asparagine and glutamine.

Discussion

Overall the results indicate that sharpshooter incidence and feeding rates on citrus trees are associated with seasonal variations in plant phenology and xylem sap composition, which are apparently determined by weather and soil conditions. The observations of higher sharpshooter populations and ingestion rates during periods of rainfall and vegetative growth of citrus are consistent with a previous study that shows strong preference of *O. facialis* for young and succulent shoots (Marucci et al., 2004). The low populations and feeding rates observed on citrus trees during the drier and colder months of the year (winter), when young shoots are scarcer and the xylem sap was nutritionally unbalanced, suggest that soil water deficit and/or lower temperature may affect plant suitability for the sharpshooters. In a recent growth chamber (laboratory) study with citrus seedlings, Pereira et al. (submitted) showed that feeding and survival of *O. facialis* under typical summer or winter temperatures were significantly reduced when the plants were submitted to water stress.

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Interaction Between the Plant Pathogenic Bacterium *Spiroplasma kunkelii* and a Parasitic Wasp (*Gonatopus bartletti*) within the leafhopper *Dalbulus maidis.*

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Mollicutes (phytoplasmas and some species of spiroplasmas) are plant pathogenic, cell wall-free bacteria that cause diseases in several hundred plant species. Mollicutes must multiply in the hemolymph of leafhopper (Cicadellidae) and psyllid (Psyllidae) vectors before being transmitted to plants. These vectors not only host the mollicutes in the hemolymph but also host parasitoid larvae of Diptera, Hymenoptera, and Strepsiptera. The larval stages of these parasitoids feed in the hemolymph of the insect vectors (Moya-Raygoza *et al*., 2004)

No studies have described the relationship between plant pathogenic bacteria and parasitoid larvae within hemipteran vectors. To initiate such a study, I used bacterium *Spiroplasma kunkelii* (Mycoplasmatales: Spiroplasmataceae), parasitoid *Gonatopus bartletti* (Hymenoptera: Dryinidae), and vector *Dalbulus maidis* (Hemiptera:

Cicadellidae). The **objective** of this study was to investigate the relationship between *S. kunkelii* and *G. bartletti* when both are present in *D. maidis* adults. Results of this study have implications in the control of plant pathogenic bacteria with persistent-propagative transmission, such as hundreds of phytoplasmas that damage plants, and *S. kunkelii*, considered an important disease pathogen of maize, *Zea mays*, in Latin America.

Methods and Materials

Development of larva and adult parasitoid with presence of bacterium. Three experiments with multiple treatments were conducted. Experiment 1 investigated parasitism in leafhoppers before and after they acquired *S. kunkelii*. Experiment 2 investigated parasitism during the incubation period of the bacterium in the leafhopper. Experiment 3 was designed to determine whether the presence of *S. kunkelii* prevents the parasitoid from reaching the adult stage.

Development of bacterium with presence of larval parasitoid. Two experiments, experiments 4 and 5, were designed to determine whether *S. kunkelii* is killed within the leafhopper if the leafhopper is parasitized.

Results and Discussion

S. kunkelii did not affect the development of the larval parasitoid *G. bartletti* when both coexisted in *D. maidis* adults. Parasitoids completed development in leafhoppers that acquired *S. kunkelii* either before or after parasitism and when *S. kunkelii* had median (10 d) and long (20 d) incubation periods in the leafhopper before the leafhopper was parasitized. A long incubation period of *S. kunkelii* usually results in high titers of the spiroplasma in *D. maidis* (Alivizatos and Markham, 1986). The presence of *S. kunkelii* did not affect parasitoid development to the adult stage. These findings could explain why *G. bartletti* parasitizes a high percentage of *D. maidis* adults throughout the same altitudinal distribution as *S. kunkelii* in central Mexico. However, another study reported that Secondary symbionts (bacteria) of the pea aphid, *Acyrthosiphon pisum* produced negative effects on the parasitoid. Oliver *et al*. (2003) found that secondary symbionts increased resistance to parasitoid wasp *Aphidius ervi* by causing high mortality of developing parasitoid larva. In contrast, polymerase chain reaction showed that parasitoid larva negatively affected the presence (survival) of *S. kunkelii* in *D. maidis*. Few leafhoppers had the spiroplasma before and after parasitism, compared with leafhoppers that acquired the Spiroplasma but were unparasitized. This negative effect suggests that parasitoids decrease wall-free bacteria such as *S. kunkelii* and perhaps other mollicutes that damage plant species of economic importance.

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SYSTEMATICS POSTERS

Older males have increased mating success in the thornbug, *Umbonia crassicornis* (Hemiptera: Membracidae)

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Age-based mating preferences have received increased attention in recent years from researchers interested in evaluating the benefits of mate choice. Both positive and negative relationships between male age and female choice have been proposed in many theoretical studies, with some empirical support for both existing (Brooks and Kemp, 2001). Some researchers argue that older males provide better parental care or are genetically superior because their age indicates their proven viability (Kokko and Lidström, 1998; Alatalo et al., 1986). Another benefit of choice for older males that has also been proposed is inbreeding avoidance. This is thought to occur in semelparous species where siblings mature in family groups and encounter one another frequently during mate searching (Wood and Dowell, 1985).

In the thornbug, *Umbonia crassicornis*, siblings develop together and following adult eclosion individuals remain aggregated with their family for up to one month before dispersing from their natal site (Wood, 1974). Adults reach sexual maturity prior to dispersing, and following dispersal siblings often encounter one another as they search for mates (Wood and Dowell, 1985). Masters (1997) showed that the offspring produced from matings between siblings have reduced fitness compared to those sired by non-kin. Taken together, these factors strongly suggest that female thornbugs might use some form of inbreeding avoidance mechanism to prevent mating with brothers (Pusey and Wolf, 1996). Wood and Dowell (1985) proposed that inbreeding avoidance could occur if females preferred older males, thus enabling the avoidance of same-age brothers as mates. In our to evaluate their hypothesis we conducted this study with 2 goals: (1) to examine female mating patterns in the context of inbreeding avoidance and age-based choice, and (2) to assess the influence of age on male mating success.

Methods and Materials

We performed this study within a greenhouse at the University of Missouri in 2004 using adult insects derived from a natural population of *U. crassicornis* occurring in Miami, Florida. We wanted to simulate the social environment of adult thornbugs in order to obtain realistic measures of mating behavior. To accomplish this we used 2 identical cages (183 x 122 x 76 cm) that we stocked with adults of varying age and relatedness, in a manner approximating the social conditions normally experienced by thornbugs during mate searching (Wood and Dowell, 1985). Each cage contained 5 *Albizia julibrissin* (Leguminosae) host plants that each measured approximately 70 cm in height.

Within each cage we placed 3 groups of adults, each on their own plant. Two of the groups were each composed of a family of 10 male and 10 female siblings that were approximately 7 days old post-eclosion. The third group was comprised of 10 sibling males that were about 17 days old. The 3 plants containing insects were set equidistant from one another. Individuals had unrestricted movement in the cages and were allowed to behave as they would in nature. All 20 females in each cage thus had "free choice" of three kinds of potential mates: 10 siblings, 10 non-siblings of the same age, and 10 older non-siblings. Individuals were uniquely marked with a small numbered tag glued to their pronotum for identification.

Once cages were stocked with insects we conducted hourly observations of their behavior from 08:00-18:00 hours every day until all females mated (a period that took between 2-3 weeks). For each pair we found courting or mating we recorded their identities and the plant location. This study was replicated 3 times in 2004 (July, September and November), and for each new study period we used individuals drawn from new families. In total 15 families were used: 10 provided the same-age males and females, and 5 supplied the older males.

Results

Patterns of movement

Older males moved off their host plant and started courting both groups of females on the first day of observations. Younger males, however, did not begin courting females until approximately 5 days later. Younger males from both families courted their sisters on their host plant, but they also moved off and courted non-sisters on other plants in the enclosure. Most of the females were courted by males from all 3 groups (siblings, non-siblings of equal age, and older non-siblings) for several days before the first matings were observed. Thus, the mating patterns we observed represent decisions made by females following exposure to all 3 types of potential mates.

Courtship behavior

We observed a total of 328 courtship behaviors summed across all 3 study periods. Our analysis revealed that the study period and cage did not significantly affect the proportions of females courted by the different male groups. The family a female was from did not have a significant effect on the type of male that courted her. The relatedness of a male to a female also did not have a strong effect on the proportions of females courted by the different male groups. However, there was as a significant effect of male age on the proportions of females courted by the 3 groups of males. Older males performed 44% of the total courtships, while siblings and non-siblings of the same age performed 24% and 32%, respectively.

Mating patterns

We observed 106 copulations across the 3 study periods. Each female mated only once. Our analysis indicated that the study period and cage had no significant effect on the proportions of females mating to the different male groups. The family a female was from did not have a significant effect on the type of male that she mated with. The relatedness of a male to a female also did not have a strong effect on the proportions of females that mated to the different male groups. However, there was a significant effect of age on the proportions of females mated by the 3 male groups. Older males obtained 44% of the matings, while siblings and non-siblings of the same age each obtained 28% of the total copulations.

We also found the effect of male weight on mating success to be age-dependent. For older but not younger males mass was significantly positively correlated with the number of copulations obtained.

Discussion

Our results demonstrate the importance of male age in the mating behavior of *U. crassicornis*. Females were courted significantly more often and mated to a greater proportion of older males compared to younger males. Conversely, females did not discriminate between siblings and non-siblings of the same age as mates. These findings offer empirical support to the hypothesis that mating with older males facilitates outbreeding in thornbugs.

Sibling mating in *U. crassicornis* results in inbreeding depression (Masters 1997). Species vulnerable to inbreeding depression are thought to use some type of inbreeding avoidance mechanism to reduce the frequency of these costly mating errors. A widespread method of inbreeding avoidance, kin discrimination, has been shown to operate in many species where siblings mature together in family groups (Hepper, 1991). However, the fact that female thornbugs did not distinguish brothers from non-brothers of the same age suggests that they may be unable to identify kin based solely on relatedness. Rather, females may be exercising age-based discrimination to mate with older, unrelated males and thus avoid costly inbreeding mistakes.

An unexpected finding from this study was the positive effect of male mass on mating success for older, but not younger males. In many species of animals larger size provides an advantage in male-male competition irrespective of age (Andersson, 1994). Some studies have also shown, however, that size and age are important factors influencing the outcome of male competition (Kemp, 2002; Otronen, 1995; Petersson, 1989). We sometimes observed more than one male courting a female at the same time, thus age and size may be key determinants of success for thornbug males during courtship when other males are also present.

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Polyphyletic Origin of the New World Erythroneurini (Cicadellidae: Typhlocybinae)

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Comprising 176 genera and nearly 1700 described species, Erythroneurini is the largest tribe of the leafhopper subfamily Typhlocybinae. Species of this tribe are distributed worldwide, but appear to be particularly diverse and abundant in tropical Asia and in temperate North America. The fauna of the Old World tropics comprises more than 1000 species grouped into more than 150 genera. In contrast the Nearctic fauna comprises approximately 712 described species, but only four described genera: *Arboridia (Erythridula), Erythroneura, Hymetta,* and *Zygina*.

Based on current taxonomy, the fauna of the Old World appears to be much more phyletically diverse than that of the New World. Part of this disparity in genus-level diversity may be attributable to different generic concepts of the principal workers on the group (i.e., Dworakowska for the Old World fauna; Young and Hepner for the New World fauna). However, the paleotropical fauna exhibits considerably greater variation in body proportions, the structure and chaetotaxy of the male pygofer, and the shape of the connective and styles compared to those of New World species. This relative lack of structural diversity, and the extremely depauperate erythroneurine fauna of the New World tropics, suggest that most of the diversification of the tribe occurred in the Old World with relatively few lineages reaching the New World and diversifying mostly in the Nearctic region. Indeed, in his pre-cladistic estimate of the phylogeny of Erythroneurini (as *Erythroneura*, sensu lato) based on an intuitive assessment of morphological evolution Ross (1965) placed paleotropical taxa at the base of the tree and implied that the New World fauna arose through at least three separate dispersal/colonization events.

Since the publication of Ross' (1965) paper, over 150 new genera and nearly 1000 new species of Erythroneurini have been described. Unfortunately, the taxonomy of the New World fauna is more rudimentary than that of the Old World fauna, largely due to the different philosophies and practices of the principal recent workers in these regions. Irena Dworakowska, the most productive recent worker, has focused almost exclusively on the Old World fauna, describing over 100 erythroneurine genera based mostly on distinctive features of the head, wings, and male genitalia. Her papers (Dworakowska 1970 and subsequent) include detailed drawings of the head, fore- and hindwing venation, and chaetotaxy of the male genital capsule, as well as the genitalia. In contrast, Leon Hepner, the most productive recent North American worker, described 234 species of *Erythroneura* but never described a new genus (Hepner 1966 and subsequent). His work focused on establishing accurate host associations for *Erythroneura* species through field rearing and, through hybridization experiments, determining the degree to which variation in the male genitalia reflects reproductive isolation among populations and species. Hepner's species treatments included many reliable host records, but only the minimum amount of morphological character information needed to distinguish the species from its congeners, usually a line drawing of the aedeagus, style apex, and/or pygofer process. Thus, Dworakowska, by emphasizing the recognition of distinctive groups of species, discovered many putatively monophyletic units within Erythroneurini defined by numerous new presumably apomorphic characters, particularly in the chaetotaxy of the genital capsule. Hepner's work demonstrated that *Erythroneura* populations differing slightly in their male genitalia are good biological species and that the *Erythroneura* fauna of temperate North American deciduous forests is hyperdiverse.

Methods and Materials

We conducted a preliminary phylogenetic analysis using morphological characters to examine the status and relationships of the New World erythroneurine genera and provide a framework for a species-level revision of the group. We compiled a dataset comprising 108 adult morphological characters scored for 73 exemplar taxa, including several representatives of all the presently recognized New World erythroneurine genera, various Old World genera, and outgroups representing typhlocybine tribes Alebrini, Dikraneurini, Empoascini, Jorumini, and Typhlocybini. It was not practical to include all of the recognized genera of Erythroneurini in the analysis, so exemplars were selected to represent a broad spectrum of the morphological diversity of the tribe.

Results and Discussion

Analysis of these data using parsimony methods (PAUP*, 300 random addition sequence replicates, TBR branch swapping) largely confirms Ross' (1965) finding that the New World fauna of Erythroneurini is polyphyletic. On the resulting cladograms, several alternative optimizations of the binary character "geographic distribution", with states (0) Old World and (1) New World, are possible. Nevertheless, under the optimization criterion of maximum parsimony, the results require at least two, and as many as four, independent origins of the various New World erythroneurine lineages. At the same time, the results imply two to four subsequent dispersals of New World lineages back into the Palearctic and/or Oriental regions.

The analysis supported the monophyly of the Nearctic genus *Hymetta* and the subgenera *Erythridula*, *Erythroneura* (s.s.) and *Erasmoneura*, but indicated that *Eratoneura* gave rise to *Erythroneura* and suggested that *Arboridia* (sensu lato), *Erythroneura* (sensu lato), and *Zygina*, as currently defined, are polyphyletic. The New World species currently placed in *Zygina* did not group with Old World representatives of the genus, but instead formed two groups corresponding to the informal "*ceonothana*" and "*ritana*" species groups recognized by Young (1952). Some newly discovered undescribed erythroneurine species from Mexico and South America were placed in separate lineages and should therefore be placed in new genera. These results indicate that substantial revisions to the classification of New World Erythroneurini are needed.

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The phylogenetic relationships among the higher taxa of the Dictyopharidae (Hemiptera: Fulgoroidea)

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The planthopper family Dictyopharidae consists approximately 489 species in 119 genera worldwide (Metcalf 1946, O'Brien and Wilson 1985). Traditionally, the family consists of 2 subfamilies and four tribes: the Orgeriinae with two tribes, the Orgeriini (31 gen., 108 sp., Mediterranean, Palearctic and North American) and the Lyncidini (5 gen., 8 sp., African), and the Dictyopharinae with two tribes, Cladodipterini (3 gen., 14 sp., Central and South American) and Dictyopharini (74 gen., 350 sp., Cosmopolitan). These designations are primarily based on overall morphological similarities with few inferences of primitive or advanced characters. Emeljanov (1983) further divided the Dictyopharini into 9 tribes based on fossil evidence, though many modern genera were not treated in his tribal designations. Currently, analyses of the phylogenetic relationships among the higher taxa are lacking and the higher taxonomy of the group remains unclear.

Here we present a portion of an ongoing research project on the phylogeny of the Dictyopharidae. Included are the preliminary results of morphological maximum parsimony analyses (using PAUP*, version 4.0b1) of relationships among the higher taxa of the Dictyopharidae. Our analyses includes representatives of all 4 traditional tribes, as well as representatives of 7 of 8 of Emeljanov's tribes, using the sister taxon Fulgoridae as an outgroup. The results will be used as a guideline in determining phylogenetic and taxonomic relationships between the genera within the traditional tribe Dictyopharini, with emphasis on New World Groups.

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Auchenorrhyncha on Postage Stamps of the World from 2000-2005

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The Auchenorrhyncha on postage stamps of the world up to the 21st Century was covered in a paper at the Wales meeting in 1999, and was published the next year (Freytag, 2000). At that time the stamps that were known to have been issued up to and through 1999 were covered.

Results

There have been 24 stamps issued with Auchenorrhyncha on them in the last five years. Included are 15 with cicadas on them (at least one each year), 1 with a spittlebug, 7 with planthoppers and 1 with a leafhopper. Many of these will be illustrated in the format, or formats, in which they were issued. Also, a new up-to-date list of the Auchenorrhyncha on postage stamps will be available for those who are interested.

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New Records of Membracidae (Hemiptera: Membracoidea) in Costa Rica

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Treehoppers use several groups of herbaceous and woody plants as hosts, including both gymnosperms and angiosperms (none are known from ferns). The degree of host specialization varies considerably, including some species that are specialists (monophagous; i.e. utilizing plants in just one plant genus) and others that are generalists (polyphagous). The latter range from those that utilize plants in related genera to those that use plants belonging to different families (Ballou 1936; Dietrich and Dietz 1991; Wood, 1984; 1993). A better understanding of patterns of host plant utilization by Membracidae requires more field observations, especially in tropical regions, as well as a careful distinction between plants harboring only adults versus those harboring all developmental stages. In this study new host plant records are presented for 28 genera of Membracidae occurring in Costa Rica.

Methods and Materials

This study was carried out in various regions of Costa Rica and involved searching for plants harboring both adults and eggs and/or nymphs. Specimens of the host plants were collected for later identification by botanists at the National Biodiversity Institute of Costa Rica (INBio) or at the University of Costa Rica. Membracids were identified to species, either named species or morphospecies, and in both cases voucher specimens are deposited in the membracid collection at INBio.

Results and Discusion

Adults with eggs and/or nymphs of 28 membracid genera were collected from 44 species of plants, belonging to 22 families. The plant family from which most treehoppers were collected was Asteraceae with 14 records, followed by Fabaceae and Melastomataceae with 8 records each. Treehopper genera with the most host records include *Membracis*, collected from 8 plant families and *Bolbonota* from 5 families. The results of this study, together with a summary of host records from the literature, will be included in an illustrated guide to neotropical treehoppers (Godoy *et al*., 2005)

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A Review of *Toya* (Hemiptera: Delphacidae) from the New World

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The genus *Toya* is currently represented in the New World by five species: *Toya argentinensis* (Muir 1929) from Argentina, *T. boxi* (Muir 1929) from Brazil; *T. venilia* (Fennah 1959) from the West Indies (Leeward Islands); *T. iaxartes* (Fennah 1959) from St. Lucia; and *T. propinqua* (Fieber 1866) from throughout the region. Specimens were collected during the Great Smoky Mountains All Taxon Biotic Inventory (ATBI) (Sharkey 2001) that were either undescribed North American species of *Toya*, or species belonging to *Toya* currently assigned to the wrong genus. This led to this review of New World *Toya*. In this review, all available New World *Toya* specimens were examined along with specimens of the type species, *Toya attenuata* Distant, 1906, from Sri Lanka. The definition of the genus is examined with reference to the New World species, and the generic placement of each New World species is reconsidered. In addition, some species from the polyphyletic genus *Delphacodes* appear to belong in, or are related to, *Toya* and were compared to *Toya* for possible placement, including *D. wetmorei* (Muir & Giffard 1924), *D. fallax* Muir 1926, *D. dolosa* Muir 1926, *D. idonea* Beamer 1947, *D. axonopi* (Crawford 1914), and *D. nigra* (Crawford 1914).

The genus *Toya* is best defined through a series of genitalic features, particularly a pygofer greatly expanded on the dorsocaudal angles and a produced and apically bifurcate or lobed genital diaphragm. Using this definition, it appears that *Toya venilia, T. argentinensis*, and *T. iaxartes* (the latter species based on illustrations, pending the examination of the holotype requested from the Bernice P. Bishop Museum, Hawaii) do not belong within the genus. While they exhibit the expanded pygofer similar to *Toya*, they do not possess a bifurcated armature of the diaphragm. Rather, the former two species have an elongated armature which narrows at the apex, thereby more closely fitting the definition of the genus *Syndelphax* Fennah 1963. *Toya iaxartes* will not be placed until the holotype is examined. Descriptions of *T. boxi* suggest that it is not properly placed as well. *Toya boxi* fits neither the definition for *Toya* nor *Syndelphax.* Instead, this species more closely resembles certain species currently in *Delphacodes*, in particular *Delphacodes idonea*.

Several species of *Delphacodes* were examined with structural similarities to *Toya.* One species, *D. wetmorei*, belongs to *Toya* and is hereby transferred to the genus. Other species studied, *Delphacodes axonopi*, *D. nigra*, and *D. idonea,* while externally similar to *Toya*, exhibited genitalic differences, particularly with the genital diaphragm. The diaphragm in these species is a concave shape, sometimes with a pair of small projections. It appears that *Delphacodes axonopi*, *D. nigra*, and *D. idonea* represent a distinct grouping and should be segregated from *Delphacodes,* with *Toya boxi,* into their own genus. *Delphacodes fallax* and *D. dolosa* also appear very similar to *Toya*. While the armature of the diaphragm is very well-developed and produced caudally, it lacks a bifurcated apex, and forms a support structure extending along the basal third of the aedeagus in both species suggesting their possible placement in *Syndelphax*.

During the progress of this study, the specimens from the ATBI that motivated this study were found to be *T. propinqua* and *D. idonea*. The specimen of *T. propinqua* was within the range of variation found among specimens of this species. *Delphacodes idonea* is a species which was not expected to range into the Park. Although neither specimen was found to be a new species, the identification of *D. idonea* provided a new record for the GSMNP ATBI project.

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High diversification of *Pagaronia* leafhoppers (Cicadellidae, Cicadellinae, Pagaroniini) in East Asia

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Leafhoppers of the genus *Pagaronia* Ball are much diversified in East Asia, especially in Japan and the Korean Peninsula. At present, 70 species have been described; 48 species from Japan, 23 from Korea, 2 from the Russian Far East, and 1 from NE China (Anufriev 1970; Okada 1976, 1978; Kwon & Lee 1978; Hori 1994; Hayashi & Arai 1990; Hayashi & Yoshida 1995; etc.). On the basis of detailed morphological investigations for Japanese material, we could further recognize at least 70 undescribed species to the Japanese fauna. To clarify and understand the diversification of *Pagaronia* leafhoppers in East Asia systematically, we attempted to make the species-group classification by morphological data.

For the species grouping, we used both described and undescribed species (about 140 species in total), noting several important characters, such as shape of head, black markings on head, shape of female 7th abdominal sternum, and structure of male genitalia (pygofer lobe, genital plates, aedeagus, etc.).

Results and Discussion

In the shape of head, two morphological states are recognized: 1) triangulary tumid anteriad, 2) rounded exapanded anteriad. In the black markings on head, four states are recognizable; bearing 1) three black spots on dorsal angle of clypeus, 2) same except an addition of a small central spot near anterior tip of head (dorsal spots sometimes developed and fused, forming a transverse black band), 3) two lateral spots without a central one, 4) no spots. The female 7th abdominal sternum is divided into three states; 1) quadrate with caudal margin emarginate at middle, 2) trapezoidal with caudal slightly incised at middle, 3) long and elliptical with caudal margin strongly convex caudad. Furthemore, in the male genitalia, 15 states are recognizable in the combination of the shape of pygofer lobe, shape and relative length of genital plates, and aedeagus.

As a result of our intensive comparison and discussion of above-mentioned characters, the shape of aedeagus must be particularly well-defined to species grouping of the leafhoppers. And consequently, E Asian *Pagaronia* species can be classified into 15 species-groups. In Table 1, the number of species in each species-group is enumerated (except for a Korean species *incertae sedis*); the number in bracket indicating undescribed species.

Judging from our field surveys in Japan, every species classified into same species-group is allopatric in distribution; the range is generally narrow, clearly separated by locality and/or altitude. That is to say that no other species in the same species-group occur in a same locality, and that in case more than two species inhabit same area they belong to different species-groups. We conclude that this fact may be much related to high diversification of the *Pagaronia* leafhoppers.

In certain species-groups, their morphological characters show similarity to several genera of Pagaroniini and Evacanthini (partly); *Epiacanthus* Matsumura, *Kurotsuyanus* Ishihara, *Babacephala* Ishihara, and *Tengirhinus* Ishihara. Additionally, we discuss the relationships among them.

Table 1 Species-groups of *Pagaronia*.

	species-group	no. of species	
1	*aurantia*	5	[+6]
2	*grossa*	3	
3	*minor*	4	[+7]
4	*guttigera*	18	[+26]
5	*hakusanensis*	3	[+7]
6	*evansi*	15	
7	*yakuensis*	1	
8	*impunctata*	1	
9	*montana*	1	[+4]
10	*togashii*	3	[+4]
11	*harpagonis*	1	[+4]
12	*caudata*	1	[+2]
13	*okadai*	5	[+2]
14	*jenjouristi*	4	[+2]
15	*protecta*	4	[+8]

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Taxonomic Study of Cicadelline Leafhoppers (Hemiptera:Cicadellidae: Cicadellinae) of the Indian Subcontinent

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Cicadellinae is one of the economically important subfamilies of the family Cicadellidae. The present study was confined to the genera of the subfamily from the Indian subcontinent. The first comprehensive study on the Indian Cicadellinae was done by Distant (1908, 1918) wherein he dealt with 20 genera and 86 species. Young (1986) revised the subfamily for the Old World and included 32 genera and 278 species. Of these, 12 genera and 55 species were recorded from India. The present study did not include all the Indian genera and species due to non- availability of specimens.

Out of 710 specimens examined, the majority came from the rich collection of leafhoppers housed at the Department of Entomology, University of Agricultural Sciences Bangalore. Seven genera were included in this study : *Anagonalia* Young, *Anatkina* Young, *Atkinsoniella* Distant, *Bothrogonia* Melichar, *Cofana* Melichar, *Kolla* Distant, and *Tettigoniella* Jacobi. In these, we were able to examine 30 species in 7 genera. Additionally, one genus was newly described based on a single species from the sub-Himalayan region. Among the examined taxa, *Anagonalia* is represented by three species and all the three occur on the subcontinent. However, only female specimens of *A. melichari* were available for the study; the species was redescribed based on external markings and female genitalia characters. Of the 9 species of *Anatkina* recorded from the subcontinent, *A. infecta* Young, *A. kharavela* (Distant) and *A. kotagiriensis* (Distant) were studied. Additionally two species of *Anatkina* were discovered. In *Atkinsoniella*, (12 spp. recorded from India) seven species were examined: *A. anabella* Young, *A. atronotata* (Distant), *A. opponens* (Walker), *A. gregalis* (Distant), *A. tigrina* (Distant), *A. thalia* (Distant), and *A. cyclops* (Melichar). *A. tigrina* was treated as a species distinct from *A. opponens* based on coloration and male genitalia characters. *A. cyclops* (Melichar) is recorded for the first time from the subcontinent. *Bothrogonia* is closely related to *Bharagonalia* Young (not included in this study). The genus is represented in the subcontinent by 11 species, six of which were redescribed during this study. *B. addita* is a species complex which could not be resolved completely based on the shape of the seventh abdominal sternum, used for this pupose by Young (1986). We separated *B. addita* from the other species based on the shape of apex of the male pygophore process and castaneous coloration. *Cofana* and *Plesiommata* Provancher (not included) are closely related genera. In *Cofana*, in addition to the previously known five species; *C. spectra* (Distant), *C. subvirescens* (Stål), *C. lineata* (Distant), *C. nigrilinea* (Stål), and *C. unimaculata* (Signoret), two new species were discovered. These new species are externally very close to *C. lineata* and *C. nigrilinea* but differ in the structure of aedeagus. In *Kolla* (8 spp. in the subcontinent), three species were examined: *K. diaphana* Distant, *K. insignis* (Distant) and *K. paulula* (Walker). *K. paulula* (Walker) displays significant variation in external markings, especially between the specimens collected from northern and southern part of the subcontinent. The study discovered a new monotypic genus, closely related to *Bhooria* Distant and *Anagonalia* Young, but distinct in male genitalia characters. Several males and females of an undescribed species, possibly related to *Tettigoniella iocasta* Distant, were also found. Young (1986) illustrated the female holotype of *Tettigoniella iocasta* and listed it as a species of uncertain position that " probably does not belong in the Cicadellinae." The undescribed species resembles *T. iocasta* in the color pattern of the head and the venation of the hind wing, but differs in the shape of the abdominal sternite VII. The male aedeagus and connective resemble those of *Kolla*, but have a distinctly articulated sclerite between these two structures which is not present in previously described *Kolla* species.

Based on the material available for this study, the divesity of cicadelline genera is the highest in northeastern India (10 genera) followed by southern India (6 genera). However, many areas of potentially high species diversity have not yet been sampled, while other areas (e.g., Karnataka where this study was conducted) are overrepresented in collections, so these patterns may represent sampling artifacts.

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Molecular taxonomy of the Membracidae from Korea (Homoptera: Auchenorrhyncha)

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Korean membracids number 14 known species in 7 genera belonging to 2 tribes, based on their morphological characters (Kwon and Huh, 2001). Nevertheless, the phylogenetic relationships have not yet been analyzed at the molecular level. This study is the first attempt to gather molecular data to determine phylogenetic relationships within the Korean Membracidae, and to clarify the usefulness of the mitochondrial cytochrom oxidase gene. Genomic sequences of CO unit I gene were determined among 4 species and compared with each other and with those from American membracids.

Methods and Materials

An MJ-research thermal cycler (PTC-150) was used for CO gene amplification. The cycling profile began with one cycle of DNA denaturation at 94°C/2 min. and followed by 37 cycles of sequence amplification (94°, 52°, 72°C /1 min).

Results

A region of mitochondrial COI gene with 1224 nucleotides was sequenced and aligned in 14 membracids. Among these nucleotides, 506 characters were constant, 356 characters were variable and 362 characters were informative for parsimony analysis. The sequences revealed high proportions in A+T (69% on average). The nucleotide differences among genera were ranged from 13% to 23%. The largest genetic distance was found between *Machaerotypus sibiricus* and *Gargara genistae.* The inferred phylogenetic tree indicated that *M. sibiricus* and *Thunozemia paradoxa* are clustered together and related as a sister-group with that of *Gargara parvula* and *G. genistae* that are clustered at the bootstrap support. There were intraspecific variabilities of nucleotide sequences among different habitats and also among different host plants within a species. The differences in pairwise comparison within a species ranged from 1 to 10%.

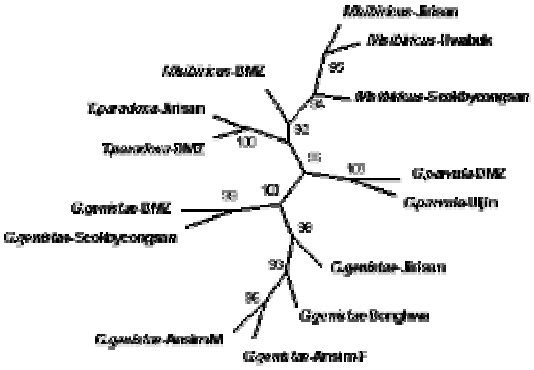

Fig. 1. Phylogenetic relationships of Korean treehoppers inferred from neighbor-joining unrooted tree using

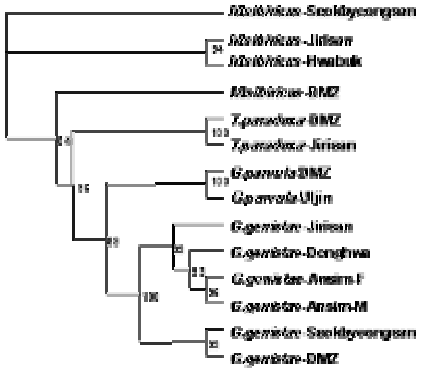

Fig. 2. Phylogenetic relationships of Korean treehoppers inferred from neighbor-joining rectangular cladoglam

Discussion

The above partial information may not provide a complete resolution of intraspecific variabilities in allopatric population. Therefore, the extent of intraspecific variabilities needs to be expanded beyond the limited data presented here to reflect the more geographic ranges. To survey any relevant interspecific or intraspecific diversity throughout the geographic range of East Asian membracids requires more variable mitochondrial genes and extensive sampling.

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Stuart McKamey - see end of Systematics posters for abstract

Evolutionary and distributional scenario of Bathysmatophorini (Hemiptera: Cicadellidae)

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Representatives of the family Cicadellidae are known since the Berriasian of Lower Cretaceous (Shcherbakov & Popov 2002). Palaeogene record of Cicadellidae seems to be quite rich (Szwedo 2002), but most described taxa need revision. Among the Eocene Baltic amber inclusions, representatives of various subfamilies are mentioned, but most old descriptions are insufficient and poor. Quite recently, representatives of Cicadellinae, Ledrinae, Mileewinae (Szwedo 2002) and new subfamily Nastlopiinae (Szwedo & Gębicki 2002) were described. From Oligocene/Miocene Dominican amber representatives of Agalliinae, Aphrodinae: Xestocephalini, Cicadellinae: Cicadellini, Evacanthinae: Nirvanini, Iassinae: Krisnini, Neocoelidiinae and Typhlocybinae are recorded (Dietrich & Vega 1995, Dietrich 2003). Representatives of some other groups were mentioned from Oligocene and Miocene strata (Metcalf & Wade 1966, Oman et al. 1990), but in most cases need to be re-examined and their systematic position proved.

As to the distributional patterns and possible origin of Cicadellidae lineages discussed by Nielson & Knight (2000), some additional remarks can be made in respect to new proposals for the classification of some Cicadellidae lineages and new data on fossils. The tribe Bathysmatophorini comprises extant genera: *Ankosus* Oman et Mus., *Bathysmatophorus* J. Shlb., *Carsonus* Oman, *Diodontophorus* Huh et Kwon, *Errhomus* Oman, *Erronus* Hmlt. et Zack, *Hylaius* Oman et Mus. and *Thatuna* Oman. All of them with exception of *Bathysmatophorus* and *Diodontophorus* are restricted in distribution to western regions of North America. Phylogeny, geographical patterns and evolutionary scenario of the lower rank taxa of *Errhomus* and *Erronus* were discussed by Hamilton & Zack (1999). These genera probably originated during or before the Miocene, and some subspecies can be tracked at least 6 million years. The other Nearctic genera also are believed to be relicts, survived only in limited refugia (Oman & Musgrave 1975). The genus *Bathysmatophorus* is distributed in Northern Palaearctic, to western Altai Mts., Mongolia, Manchuria, Japan and Korea, *Diodontophorus* is known from Japan and Korea.

Extinct genera are known from Upper Cretaceous of North America and from Middle Eocene Baltic amber, therefore the question of origin and dispersal of the group arises. If Bathysmatophorini originated from North America, it seems very probable that the ancestors of Old World genera migrated along northern Pacific arc, via Bering Strait, probably well before glaciation periods, as suggested also for some other cicadellines, e.g. Pagaronini (Knight & Nielson 2000). The second scenario is possible in respect to finding Bathysmatophorini in the Eocene Baltic amber inclusions (Szwedo, in press). Cretaceous ancestors of Bathysmatophorini, originating in North America had the opportunity to reach the Scandinavian Peninsula through the Thulean route, the most favourable period for dispersal being the earliest part of the Early Eocene (Sanmartín et al. 2001). The global climate during the Cretaceous and the Early Cenozoic is thought to have been warmer than the present climatic conditions, and for at least first 10 Ma of the Eocene a large part of Earth, including continental interiors, had climates with winter temperatures much higher than today (Greenwood & Wing 1995). Extant Bathysmatophorini are adapted to more cold and dry conditions, to areas of temperate, cold-temperate and montane conditions, and seems to be weak dispersers. It seems probable that extant representatives of the group are descendants of leafhoppers, which are adapted to more severe conditions. A similar case, where a group originated in warm, tropical conditions is now restricted to the temperate zone is observed in Raphidoptera (Aspöck 1998). Alaska does not harbour Raphidiidae and Inocelidae, and Bathysmatophornini as well, and all groups are present in northern Europe. Aspöck believes that Raphidioptera have apparently never crossed the Pacific in the north along the Bering straits in either direction. It was suggested, that the Atlantic route, via the Thulean Bridge, has been more important than the Beringian route in the Early Palaeogene. According to palaeogeographic reconstructions of this time period, Beringia was situated closer to the North Pole than it is today. This means that it would have been located in considerable higher palaeolatitude than the North Atlantic Bridge (McKenna 1983). Dispersal across Beringia should have been severely constrained by climate and light (Tiffney 1985). The first Beringian Bridge (Early-Mid Palaeogene), which partly coincided with the Thulean Bridge, was covered with the mixed-mesophytic forest and was presumably dominated by dispersal of associated plants. The second bridge (Neogene, 20–3 Mya) was covered with taiga forest and inhabited by associated faunal elements, while the third (Late Neogene-Quaternary, 3–0 Mya) was dominated by tundra habitats. Two other, more northern trans-Atlantic bridges continued to exist after the disappearance of the Thulean Bridge: De Geer route and the Greenland–Faeroes bridge. The first was far on the north and was presumably restricted to cold-adapted groups, the second was no more than a chain of islands (Sanmartín et al. 2001). Results of analysis provided by Sanmartín et al. (2001) suggest that the first Beringian Bridge was relatively unimportant for intercontinental faunal exchange, Early Palaeogene intercontinental exchange appears to have been dominated by the trans-Atlantic Thulean Bridge. This statement seems to be correct also for Bathysmatophorini. Numerous Eastern Palaearctic groups evolved and dispersed in the Palaeogene, as tropical and temperate Asia was a major centre of diversification (Tangelander 1988, Nordlander et al. 1996). This region was topologically diverse and encompassed a rich mosaic of habitats and climates, which presumably accelerated speciation rates compared to other infraregions (Qian & Ricklefs 2000). Ancestors of extant Eastern Palaearctic Bathysmatophorini probably reached this region after terminal Eocene event (Prothero 1994).

This migration (evolution and perhaps host-plant shifting) is to be related to cooling and drying of the climate in Late Palaeogene and Neogene. The glaciations have been associated with mass extinction, but Sanmartín et al. (2001) suggested that differences between eastern and western Palaearctic faunal richness cannot be explained simply by Pleistocene extinctions. This asymmetry in species richness is observed in Bathysmatophorini: one species of *Bathysmatophorus* is present in western Palaearctic, while East Palaearctic fauna encompasses 10 species of two genera. The tribe Malmaemichungiini (3 genera, 5 species), close to Bathysmatophorini is also restricted in distribution to Eastern Palaearctic. Some proposals for explanation of this faunal asymmetry (Sanmartín et al. 2001) could be applied for Bathysmatophorini, e.g. lower and decreasing rate of diversification in the western Palaearctic, and extinction due to glaciation events. It seems that presence of single and wide distributed species in Western Palaearctic could be related rather to interglacial and postglacial expansions (from refugial areas?), and its highly successful adaptation to the particular habitat condition of the cold temperate zone. Western Nearctic Bathysmatophorini appears to have developed in a different fashion. Diversification and increment of taxonomic diversity was accelerated due to increased lithospheric and climatic complexity in the region since the Miocene, as presented by Hamilton & Zack (1999).

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Coevolution of sharpshooters (Hemiptera: Cicadellidae) and their two bacterial endosymbionts

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The leafhopper subfamily Cicadellinae is the most diverse lineage of xylem-feeding herbivores and some of its members (= sharpshooters) are major vectors of bacterial diseases of economically important plants. Many insect groups with specialized nutritionally unbalanced diets, such as plant sap, rely on mutualistic associations with bacteria to supplement amino acid or vitamin deficiencies of their diets (Moran 1998). These bacterial symbionts typically live within host cells called bacteriocytes that form specialized organs called bacteriomes. Auchenorrhyncha are characterized by some of the most complex assemblages of obligate symbiotic microbes known in any animal group. Most of these insects contain two or more bacterial endosymbionts that are transmitted between generations through eggs (Buchner 1965, Nault *et al*. 1985). For example, treehoppers (Membracidae) may contain up to six distinct symbiont types.

Bacteriomes of sharpshooters are paired structures positioned laterally within the first three abdominal segments, and usually colored yellow, orange or red (Buchner 1965, Kaiser 1980, Moran *et al*. 2003). Bacteriocyte-associated symbionts have been previously described based on light and electron microscopy from sharpshooter species such as *Cicadella viridis* (Buchner 1965), *Graphocephala coccinea* (Kaiser 1980), and *Helochara communis* (Chang & Musgrave 1972). To date, very few symbionts of leafhoppers or related families have been characterized using DNA sequences. Moran *et al.* (2003) recently published the first molecular characterization of bacteriome-associates in leafhoppers, based on bacterial 16S rDNA sequences recovered from five species of sharpshooters. Candidatus *Baummania cicadellinicola*, found in both yellow and red portions of the bacteriomes and believed to be the primary symbiont of sharpshooters, formed a strongly supported clade within the gamma-3 Proteobacteria. Results supported congruence of insect and *Baumannia* phylogenies, but the evidence for co-diversification was tentative because so few species were included. Additionally, another endosymbiont, belonging to the Bacteroidetes phylum, was found restricted to the yellow portions of the bacteriome of one of the host species. Numerous studies have documented long-term codiversification of insects and symbionts, following infection of a shared insect ancestor by a single symbiotic lineage. Additional symbionts, when present, have been sporadically distributed and may thus represent more recent infections. Usually these secondary symbionts are phylogenetically diverse, consisting of several lineages of bacteria in different members of a host lineage, as shown for aphids (Sandström *et al*. 2001), psyllids (Thao *et al*. 2000b), and mealybugs (Thao *et al*. 2002).

In the present study, 24 additional leafhopper species, spanning six tribes, with emphasis on sharpshooters, were screened for both symbionts and for additional bacterial types. We performed separate phylogenetic analyses using symbiont rDNA sequences and four nuclear and mitochondrial insect gene sequences to elucidate the evolution of each of these taxa, and to assess the evidence for their host-symbiont coevolution.

Materials and Methods

Included in these analyses were 29 species (including five studied by Moran *et al.* 2003) belonging to four leafhopper subfamilies. Insect specimens were collected in the field directly into 95-100% ethanol and stored at –20°C until processed. Genomic DNA was extracted from a single hind leg and associated muscles of sharpshooters. Additionally, DNA was extracted from isolated bacteriomes, to facilitate amplification of bacterial genes.

Partial sequences of insect genes cytochrome oxidase I and II (COI and COII), 16S rDNA, and histone H3 (Hex) were amplified and combined into a molecular dataset of 2,183 bp. Most of the 16S rDNA was amplified for *Baumannia* (1,495 bp) and the Bacteroidetes symbiont (1,489 bp) using different eubacterial primers. Models of molecular evolution were estimated by the hierarchical likelihood ratio test of Modeltest 3.06. Models chosen were TVM+G+I for the host dataset, GTR+G+I for *Baumannia*, and TrN+G+I for the Bacteroidetes symbiont. Maximum parsimony and likelihood analyses were run in PAUP*. Branch support was assessed by non-parametric character bootstraping (Felsenstein 1985) and Bremer decay indices (Bremer 1994), the latter being partitioned for the sharpshooter dataset.

To assess the evidence for codiversification of the two symbionts with sharpshooters, three statistical tests were conducted. A parsimony-based incongruence length difference (ILD) test was used to test the null hypothesis that the host dataset and each symbiont rDNA dataset are congruent, suggesting a history of cospeciation. Congruence of host and symbiont topologies was also assessed with a Shimodaira-Hasegawa likelihood-based test (Shimodaira & Hasegawa 1999, Goldman *et al*. 2000). Considering the null hypothesis that the log-likelihood score of a given host tree calculated using the host dataset and model of evolution is the same as the score calculated using the symbiont dataset and model of

evolution (and vice-versa), a failure to reject H_0 is suggestive of a perfect cospeciation scenario. Finally, an event-based tree-fitting method was used to hypothesize parallel evolutionary events based on host and symbiont trees and, by comparing host trees with 1,000 randomly generated symbiont trees, test if cospeciation events hypothesized are more numerous than expected by chance (Ronquist 2002). The advantage of the ILD test over the other two methods is that it is not biased by topological uncertainty.

Results and Discussion

Of the 29 species tested, *Baumannia* was present in 25, including *Clydacha catapulta* (Phereurhininae) and all species of Proconiini and Cicadellini, except *Cicadella viridis*. *Baumannia* was not detected in *Hylaius oregonensis* (Bathysmatophorini) and in *Jikradia olitoria* (Coelidiinae: Teruliini). All newly tested species possessed the additional symbiont from the Bacteroidetes phylum. For every 16S rDNA sequence recovered, the first BLASTn hit against the non-redundant GenBank nucleotide database was the single sequence of this symbiont type from the glassy-winged sharpshooter previously deposited in GenBank (Moran *et al*. 2003). Although the symbiont appears to be related to some other known insect symbionts, including *Blattabacterium* and symbionts of coccinellid beetles, the sequence divergence from the closest relatives (>14% in 16S rDNA) and the restricted localization within bacteriomes warrant description of this symbiont as a new bacterial taxon in the future.

Both bacterial symbionts show a congruent evolutionary history with their sharpshooter hosts, based on all (*Baumannia*) or most (Bacteroidetes symbiont) statistical tests. Although the Shimodaira-Hasegawa test did not corroborate a scenario of coespeciation between the Bacteroidetes symbiont and their sharpshooter hosts, we view this incongruent result as an artifact of the low amount of information present in the symbiont 16S rDNA, which yielded poorly supported, polytomous topologies. Therefore, both symbionts appear to have been associated with their sharpshooter hosts for a long time. Our results are in agreement with Buchner´s (1965) views that different leafhopper subfamilies and tribes may show long-term coevolution with two or more bacterial associates. Furthermore, in his evolutionary scenario, based in part on the work of H. J. Müller, one symbiotic association was ancestral to the Auchenorrhyncha ("*a*" symbiont), with different host lineages acquiring and losing symbionts during the radiation of this large clade, resulting in a mosaic of different symbiont combinations across modern subfamilies and tribes. Our data corroborate this view. Preliminary observations suggested that, unlike *Baumannia*, the Bacteroidetes symbiont may be broadly distributed within Auchenorrhyncha (Moran, unpublished data), and thus may represent the ancestral symbiotic lineage. Under this view, the bilateral bacteriomes are homologous organs retained by most groups of Auchenorrhyncha during their evolution from an ancestor containing an original symbiont type.

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Phylogeny of the sharpshooter tribe Proconiini based on morphology and DNA sequences (Membracoidea: Cicadellidae: Cicadellinae)

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The sharpshooter tribe Proconiini is endemic to the Western Hemisphere and currently comprises approximately 350 species in 56 genera. Proconiines are, like most other sharpshooters, generally polyphagous xylem-feeders (Novotny & Wilson 1997, Basset & Charles 2000) and are the main vectors of *Xyllela fastidiosa*, a bacterium pathogenic to several agricultural crops, causing phony peach disease, Pierce´s disease of grape, coffee leaf scorch, and variegated chlorosis of *Citrus* among other diseases (Redak *et al.* 2004). Some proconiines display a unique ovipositional behavior, in which females cover egg-nests with Malpighian tubule products (brochosomes), possibly providing extra protection to eggs (Rakitov 2004). This egg-powdering behavior may have facilitated a shift from endophytic (in most leafhoppers) to exophytic oviposition, the latter displayed only by species of the genus *Acrogonia* (e.g., Marucci *et al*. 2002).

Mejdalani (2000), using morphological characters of the external morphology and male genitalia, investigated the phylogenetic relationships among 21 Proconiini genera belonging to a group having the posterior meron exposed (Young 1968), recovering two main lineages (the *Cleusiana* and the *Abana* generic groups), but there has been no previous attempt to reconstruct the phylogenetic relationships of the whole tribe. Ceotto & Mejdalani (2005) studied the relationships among the genera of the *Abana* group, increasing taxon sampling within genera and adding female genitalia characters.

The main objectives of this study were to provide the first phylogenetic hypotheses for the entire tribe Proconiini based on both morphological and DNA sequence data, test the monophyly of the tribe as currently defined, and propose a revised classification.

Materials and methods

Previous phylogenetic analyses of higher groups of leafhoppers have recovered the close relatedness of the tribes Cicadellini and Proconiini, and have also suggested that Phereurhininae is closely related to proconiines (Dietrich 1999, Dietrich *et al.* 2001). Thus, the present dataset included outgroup taxa from the tribe Cicadellini, subfamily Phereurhininae, and unplaced cicadelline genera, as well as at least one species of all available Proconiini genera. Multiple congeneric species were included from the *Oncometopia* group, which contains species-rich genera that are difficult to characterize and delimit morphologically. Parsimony analyses were run heuristically in PAUP* and branch support was estimated by non-parametric character bootstrapping, Bremer support for the morphological dataset and partitioned Bremer support for the molecular dataset.

Based on a hierarchical log-likelihood ratio test, Modeltest chose GTR+I+G as the appropriate model of molecular evolution for the molecular dataset. This model was then used to calculate likelihood scores of the trees obtained from parsimony analysis of the molecular dataset and test these topologies using the Shimodaira-Hasegawa likelihood-based test (Shimodaira & Hasegawa 1999, Goldman *et al*. 2000). Topologies tested included those from the unconstrained molecular analysis, and those reflecting previous classifications of Proconiini including and excluding the genera *Ochrostacta* (currently in Proconiini), and *Pamplona*+*Pamplonoidea* (currently in Cicadellini).

Results and Discussion

The morphological dataset consisted of 96 characters of the external morphology and internal male genitalia of 115 taxa, while the molecular consisted of alignments of 697bp of COI, 558bp of COII, 404 of 12S, 481 of 16S, and 322bp of H3 (total of 2,462 bp) for 94 taxa (not all taxa had every gene sequenced). Respectively in the morphological and molecular datasets, these taxa represented the same twenty Cicadellini genera (1 undescribed), three and two Phereurhininae genera, two unplaced Cicadellinae genera scored for morphology only, and 89 and 72 species representing 58 and 45 genera of Proconiini (out of 58) including two still undescribed (Godoy 2005, Rakitov & Godoy 2005).

Proconiini, as currently defined, was not recovered as monophyletic. The morphological dataset supports Proconiini excluding the genus *Ochrostacta* and including *Pamplona*+*Pamplonoidea*, currently placed in Cicadellini. The molecular dataset supports a more complicated scenario with several Cicadellini lineages more closely related to one Proconiini lineage. Statistical likelihood tests found no significant difference between this scenario and one in which Proconiini was constrained to be monophyletic. Among the various topologies tested, only the one representing the current classification (which includes *Ochrostacta*, but not *Pamplona*+*Pamplonoidea*) was significantly less likely.

Young (1968), based on a subjective morphological analysis, divided the tribe Proconiini into two main groups: those genera with the posterior meron concealed and those with the posterior meron exposed (due to a straightening of the costal margin of forewings). The present preliminary analyses also recovered the division of Proconiini in two main

lineages, but they do not agree with Young´s groups – the group with the posterior meron exposed was polyphyletic in both molecular and morphological analyses. Three genera with the posterior meron concealed (*Anacuerna*, *Cuerna,* and *Dechacona*) appear related to the *Oncometopia* group, which contains most of the genera with the posterior meron exposed. On the other hand, the remaining six genera of the exposed meron group (the *Abana* group as redefined by Ceotto & Mejdalani (2005) are well nested within the group with the posterior meron concealed.

Rakitov (1999) noted that proconiines belonging to the *Oncometopia* group (= *Cleusiana* group, *sensu* Mejdalani 2000) share a unique suite of morphological, physiological, and behavioral traits related to the egg-powdering behavior. He later (Rakitov 2004) pointed out that two related genera of the Cicadellini, *Pamplona* and *Pamplonoidea*, and the phereurhinine genus *Dayoungia* also share some or all of these traits with the *Oncometopia* group. *Pamplona* and *Pamplonoidea* in both morphological and molecular analyses are recovered as belonging to Proconiini. They are apparently closely related to the genus *Acrogonia* and uncertainly placed at the base of the *Oncometopia* group. On the other hand, the evidence for the relationship of phereurhinines (currently composed of three genera) with proconiines is contradictory. Morphologically, phereurhinines appear to be related to the *Oncometopia* group and should include the unplaced genus *Homalogoniella*. On the other hand, the two phereurhinine genera included in the molecular analysis tended to group with outgroups from Cicadellini.

The strongly modified external morphology of the southern South American genus *Ochrostacta* obscures its relationship to other Proconiini genera. Species of this genus have a strongly produced and atypical head and are flattened dorsoventrally, like other sharpshooter specialists on *Eryngium* (Takiya & Mejdalani 2004). *Ochrostacta* does not group with any Proconiini lineage and appears to be related to Cicadellini genera, particularly in the molecular analysis, where it groups with all included members of the *Erythrogonia* generic group (Young 1977) with high bootstrap and Bremer support. Furthermore, *Ochrostacta* shares with other Cicadellini the presence of supranumerary setae on the AD row of the hindleg and the uncommon stalked paraphyses with two pairs of rami; the latter also present in the Cicadellini genus *Exogonia*.

Some genera of the better sampled *Oncometopia* group were not recovered as monophyletic units. Most of the confusion lies in the position of species currently assigned to *Homalodisca*. *Homalodisca elongata* tends to group with *Phera obtusifrons* + *P. luciola* and at the same time the position of *Phera lanei* is uncertain. The type-species of *Homalodisca*, *H. triquetra* was included in the morphological analyses, grouping with high branch support with *Propetes schmidti,* and this clade appears to be unrelated to any other *Homalodisca* lineage. Two *Homalodisca* clades appear well supported – the North American *H. liturata*, *H. coagulata*, and *H. ichthyocephala*, and the South American *H. ignorata* and *Homalodisca* n. *apicalis* – but their relationship to each other is unclear. Finally, the position of *Homalodisca insolita* cannot be established with certainty due to contradictory morphological and molecular data.

The completion of sequencing the studied partial genes for the taxa sampled at present will probably enhance the support and change some relationships presented herein. Given the availability of fresh specimens for molecular studies, taxon sampling will be increased to include representatives of genera not sampled and more species of the *Oncometopia* group to clarify some of the generic limits. More taxa of the *Oncometopia* group and characters of the male and female genitalia will be included in the morphological dataset.

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Leafhopper biodiversity in Arizona —a dynamic reassessment

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Given the large number of leafhopper (Homoptera:Cicadellidae) species known from Arizona, the state is usually considered to be "well collected." This assumption now seems to be unfounded. In three relatively short field trips in the spring and monsoonal seasons, we have found about 30 new species, and we have just begun to explore understudied habitat subregions [insularized subdivisions of larger vegetational regions of sufficient size to generate endemics]. Much more collecting will be needed throughout the year and in undercollected subregions before we can consider that we know even a portion of the Arizona leafhopper fauna. Our search was aided by a preliminary understanding of some of the elements that generate cicadellid biodiversity. Host plants are only one of the niche dimensions of cicadellid habitats. Spatiotemporal features of niches such as geographical contiguity (or lack of it) and phenological factors generated by climate are also of great importance and in some cases may be more important than the identity of the host species. Thus, in our studies of Canadian and American grasslands, we often find that cicadellid species have different hosts in different geographic regions.

A final niche dimension is the cicadellid species itself, which, but for a happenstance evolutionary event, would not be available to form part of the niche structure. In the Southwestern United States, there is a plethora of niches for leafhopper species, and a large number of cicadellid species have evolved to partially fill the available niche space. The most influential factor driving niche proliferation in this region appears to be habitat fragmentation. The Southwest—particularly Arizona—appears on vegetation maps at all but the coarsest grains to be an intricate mosaic of vegetational fragments. California is subdivided similarly, accounting for a richness in endemic leafhopper species even greater than that in Arizona. Vegetational regions are not in themselves niche dimensions; the ranges of many plant species cross the borders of vegetational zones. Nevertheless, a large number of highly fragmented regions are predictors of a high degree of niche fragmentation. Some specialists accompany their plant hosts over part of the plant host range, across the boundaries of two or even more vegetational zones. However, at some level of disparity, climate proves to be limiting for almost all specialists of hosts that inhabit widely dissimilar climatic regions. For example, the transition from Mediterranean climate (Californian) to Sonoran and other Arizonan climates is especially limiting. Because climate limits cicadellid species more severely than it limits their plant hosts, the geographic ranges of leafhopper species that specialize on widely distributed host species are almost always much smaller than the range of their host. A high position of the host plant in its dominance hierarchy seems to be necessary for a host-cicadellid relationship to escape extinction. The ranges of plant species that dominate today's grasslands and that probably have been prominent throughout the Pleistocene, provide stages for host-cicadellid relationships that have survived one or more speciation events. In contrast to the few handfuls of grass species that dominate the Southwest, the vast majority of the grass species there occur so patchily that no niche involving them exists for a would-be specialist to exploit. Many of these grass species are accessible to cicadellid "tramps", proving that they are suitable hosts for leafhoppers that can locate and colonize them. But, for these patchily distributed plant hosts, the spatiotemporal constraints that govern extinction/colonization equilibria are a powerful force that prevents them from forming part of an enduring host-specialist relationship. Hyperspecialists are able to create niches that would otherwise not exist by "cueing in" on host semiochemicals; this minimizes—to some extent—the negative effects of host patchiness. But there are very few hyperspecialists among the assemblage of grassland leafhoppers.

As a result of the circumstances we describe, the diversity of leafhoppers in New Mexican grasslands is accounted for by a relatively large number of climatic regions, not a large number of suitable host plant species (Whitcomb and Hicks, 1992). Although New Mexico has a large number of vegetational zones as compared with the United States east of the Rocky Mountains, Arizona habitats are even more fragmented than those of New Mexico. Like New Mexico, Arizona has a very diverse grass flora (Hendricks, 1985), accounting for a portion of the cicadellid species richness there. However, to a much larger degree than New Mexico, Arizona's desert steppes are characterized by communities of broad-leaved desert-adapted shrubs. The dynamics of cicadellid speciation in shrublands are governed by principles similar to those that operate in grasslands. As with grasses, only dominant shrubs appear to have specific leafhopper colonists, and the overall cicadellid diversity in steppe habitats is explained by habitat fragmentation resulting from climate and topography. The number of habitat subregions in Arizona is much higher than in New Mexico, because Arizona is the more topologically complex of the two states, and because more than half of Arizona's land area receives less than 10 inches of annual precipitation (Hendricks, 1985). These semidesert regions in Arizona provide barriers to cicadellid movement that most New Mexico subregions do not impose.

In addition to the insularity of Southwestern subregions, the course of Pleistocene history profoundly influenced biodiversity in the Southwest. The primary characteristic of extant cicadellid species is that they have escaped Pleistocene extinction. Avoidance of extinction is, of course greatly facilitated by the absence of glaciers and/or alpine or boreal or even cold temperate ecosystems that the leafhopper species of the northern half of the United States had to endure—if they did endure—during the Ice Ages. The existing cicadellid fauna of the Southwest is for this reason an

assemblage of many species of many different ages—even on a geologic time scale. Some portions of the western states are fragmented as much as Arizona and California but were strongly impacted by glaciation and, as a result, have smaller cicadellid biota. The importance for biodiversity of long periods (on a geologic time scale) without catastrophic disturbance is underscored by the large number of endemic plant species on the southeastern Coastal Plain, which lacks — totally — topographic diversity.

The degree to which vegetational maps are useful in the search for endemic species varies among faunal taxa. For example, coarse-grained maps might be sufficient for predicting avian distributions. However, much finer-grained vegetational maps are required to explain cicadellid distributions. Maps that identify habitat islands characterized by elevations that are higher than those of the surrounding region (in many cases ranges of hills or mountains) are especially useful. Our search for new leafhopper species in Arizona was greatly abetted by the existence of vegetation maps that identify insularized biogeographic regions. However, the choice of maps is critical. Kuchler's classical vegetation map (1964), which is very suitable for study of cicadellid host relationships east of the Rockies, is much too coarse-grained to be useful in the Southwest. Other maps of the Southwest oriented toward native seed search, river systems, and biotic conservation (with a necessary emphasis on plants and vertebrates) are also too coarse-grained. A map of vegetational regions of New Mexico proved useful but is unnecessarily fine-grained. A map of the biotic communities of the Southwest prepared by D. E. Brown in 1982 is subdivided at an appropriate grain to be useful as a guide to cicadellid searches in Arizona's understudied vegetational regions. This map shows that fully half of the Southwestern grassland/steppe habitats are south of the U. S. border. Even Brown's map, however, fails to identify regions in which insularization is mediated by unusual soil substrates (e.g., sand or gypsum), or unusual contrasts in local availability of water (wetlands or perennial desert waterholes). We recognize that there is taxic variation in response to habitat patchiness even among cicadellid species. *Athysanella* species, which are in general brachypterous and dependent on macropters for dispersal are more restricted to narrow climatic regions or subregions than are species of *Flexamia* which, although they have reduced wing length, never produce brachypterous forms. Past collection efforts in Arizona tended to be repetitive, with some areas (e.g., Mogollon Rim and Chiricahua and Huachuca Mountains), receiving a disproportionate amount of attention. But also, previous workers did not focus, systematically, on all dominant host plant species in each habitat subregion. In particular, plant species that are dominant in only one or a few subregions have been undersampled. Our understanding of the underlying factors generating cicadellid diversity in the Southwest (though extremely incomplete), has helped us greatly in directing our collection efforts. And we now realize that, given the immense topological and climatic diversity in Arizona, a large amount of its cicadellid diversity remains undiscovered.

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What are true Issidae?! (Hemiptera Fulgoroidea)

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The family Issidae, as used in its widest sense, is one of the largest families of the fulgoroid planthoppers with around 2000 species and is distributed worldwide. Traditionally, the features of external morphology, in particular those of the wings, have been used for erecting the taxa of the family groups. Over the past 100 years various authors such as Melichar, Muir, Dlabola, and Fennah have distinguished tribes and subfamilies and transferred genera to other families such as Nogodinidae, while at the same time genera in other families have been recognised as Issidae s.l.

Phylogenetic analysis by Emeljanov (1990), indicates that the family Issidae s. l., including the subfamilies Issinae, Caliscelinae, Acanaloniinae,Tonginae, Trienopinae, and Bladininae, has no autapomorphies. Bourgoin with coauthors (1997) also indicated probable paraphyly of the family. Molecular and morphological data also indicate the heterogeneity of Issidae s. l. (Yeh et al., 1998; Emeljanov, 1999). Emeljanov (1999) after examination of external morphology including male and female genitalia proposed to recognise the separate families **Issidae**, **Caliscelidae**, and **Acanaloniidae** (with subfamilies Acanaloniinae, Tonginae, and Trienopinae).

Recently Gnezdilov (2002, 2003a, 2003b) suggested the family Issidae sensu stricto comprises one subfamily with 5 tribes: Issini, Parahiraciini, Hemisphaeriini, Thioniini, Colpopterini. He showed that according to the structure of ovipositor the family Issidae s. str. distinctly differs from the family Caliscelidae as well as from the families Nogodinidae, Acanaloniidae and Ricaniidae, but all are closely related according to the ovipositor structure.
The identification of true taxonomic position of species, genera or tribe is possible only on basis of examination of all available morphological features. The following diagnosis of the true Issidae is provided here:

Issidae *s. str.* *Diagnosis* (after Gnezdilov, 2003b). Adults with body more or less oval or hemisphaerical. Coryphe often short and broad. Metope often with median and sublateral keels. Frontoclypeal suture arched or almost straight. Postclypeus without lateral keels. Pronotum with large disc and narrow lateral lobes. Mesonotum relatively short or long and broad. Fore wing dull, rigid and convex (elytriform), often with hypocostal lobe and knee prominence. Hind wing normally developed or rudimentary. Legs usually short and strong (but may be more or less long), with lateral teeth. The axis of coxa – trochanter articulation of hind leg, as a rule, more or less horizontal. Metatarsomere I ventrally with a row of apical setae on high socles.
Male. Penis with a sclerotized phallobase; the latter, as a rule, with dorso–lateral and ventral lobes, the first ones often bearing teeth or processes. Aedeagus with ventral hooks or without it. Style with distinct capitulum bearing apical and lateral teeth.
Female. Gonoplacs convex, sometimes with transverse keels outside, fused basally (in this case, their medial margins distinctly pigmented in form of a fork) or fused entirely along median line. Gonapophyses IX fused in proximal part and joined with scoop-shaped gonospiculum bridge. Posterior connective laminae of gonapophyses IX with convex proximal part and arcuated (or curved at angle) or straight distal parts. Median field weakly convex and often bilobed distally, or in form of a large process curved to the base of gonapophyses. Lateral fields flat or in shape of projections. Endogonocoxal process either simple or 2-3-lobed. Anterior connective lamina of gonapophyse VIII, as a rule, in shape of relatively broad denticated plate with apical and lateral groups of teeth (these groups include 1-3 and 1-5 teeth, respectively) or narrow without teeth (*Colpoptera* sp.). Hind margin of gonocoxa VIII often protruded as a lobe over triangular sclerotized plate.

Recently two projects concerning a revision of the family Issidae were supported by the Royal Society (London, UK): "Planthoppers of the family Issidae of West Palaearctic Region" (2003) and "Systematic and biogeographic studies on world Issidae" (2005). Within these projects the issid collections of National Museum of Wales (Cardiff, UK), the Natural History Museum (London, UK), Universiteit van Amsterdam, Zoölogisch Museum (Amsterdam, The Netherlands), Museum of Natural History Naturalis (Leiden, The Netherlands), Institut Royal des Sciences Naturelles de Belgique (Bruxelles, Belgium), and Museum National d`Histoire Naturelle (Paris, France) were examined. Descriptions of new taxa, new combinations, and new data on distribution of Issidae sensu lato were provided (Gnezdilov et al., 2004; Gnezdilov & Wilson, 2005).

At present the revision of the family Issidae sensu lato is in progress on the basis of external morphological features including male and female genitalia. This includes studies of the species in the large genus *Hysteropterum*, considered as a world-wide distributed taxon, but is distributed only in the Mediterranean and Middle Europe and comprises 7 species (Gnezdilov, 2003a, 2004; Gnezdilov & O'Brien, in press),. All other species described in the genus from other parts of the World belong to other genera.

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Taxonomic Catalogue of the Leafhoppers (Membracoidea). Part 1. Cicadellinae

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This work, derived from the Leafhoppers of the World Database, catalogues the changes to the classification of Cicadellinae (Hemiptera: Cicadellidae)—the sharpshooters— world-wide from 1956 through 2005 and provides a current list of all valid taxa and synonyms, as well as their distribution and citations of their original descriptions. All subsequent references discovered by May, 2005, were also incorporated. World Cicadellinae now consists of 2 tribes, 328 genera (62 Proconiini), and 2,259 species (421 Proconiini). Literature citations through 1955 correspond to references given in a previous bibliography of the Cicadellidae; a bibliography is provided for subsequent taxonomic publications.

VECTOR POSTERS

Biology of the recently introduced aster yellows phytoplasma and aster leafhopper (Hemiptera, Cicadellidae) in Hawaii

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Aster leafhopper (AL) (*Macrosteles* sp.) is the vector of Watercress Yellows Phytoplasma (WYP), a phloem-limited bacterium that causes disease in watercress in Hawaii. AL and WYP were both first found in Hawaii in 2001. Diseased watercress (*Nasturtium officinale*) has symptoms of reduced leaf size, leaf yellowing and crinkling, and occasionally witches' brooms. The threat of WYP causing disease in other susceptible crops is of concern to Hawaii's diversified agriculture industry. PCR-based tests using primers specific for phytoplasmas were positive for phytoplasma presence for all symptomatic plants, but not from healthy, non-symptomatic plants. Sequence analysis of cloned PCR products indicate that the watercress is infected with a phytoplasma nearly identical to the severe strain of western North American aster yellows (SAY) and to onion yellows (OY). Aster leafhopper adults collected from the field were also found to be positive for this bacterium. We studied this leafhopper's host range and its ability to vector WYP, using twenty-five different plant species, including crops of economic importance and native Hawaiian plants, as test plants for oviposition and adult survival experiments. We have found that this leafhopper breeds on watercress in commercial fields. We have also shown by confining field-collected insects on susceptible host plants that AL transmits WYP to watercress. Transmission experiments using *Plantago major* and *Lactuca sativa* as host plants also showed the capacity of this insect to vector WYP. The information on host plant range and WYP transmission will help to improve control strategies for this serious disease of watercress in Hawaii.

Use of immunofluorescence confocal laser scanning microscopy to study the distribution of plant viruses and pathogenic bacteria in vector insects and in host plants

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The distribution of maize mosaic virus (MMV, *Rhabdoviridae*) and of corn stunt spiroplasma (CSS, *Spiroplasma kunkelii,* Prokaryotes, Mollicutes) in their insect vectors was studied by immunofluorescence confocal laser scanning microscopy (iCLSM). MMV is transmitted in a propagative manner by the planthopper *Peregrinus maidis* (Hemiptera, Delphacidae), whereas CSS is similarly transmitted mainly by the leafhopper *Dalbulus maidis* (Hemiptera, Cicadellidae). Three preparation methods were used in conjunction with iCLSM on these vectors: dissected whole-mount organs, thick (6-8 µm) paraffin sections, and hemocyte smears. These preparations were incubated with primary antibodies (antisera to MMV or CSS), followed by the secondary fluorescent antibody Alexa Fluor 488 (green fluorescence), then treated with the nuclear stain propidium iodide (Ammar and Hogenhout, 2005; Ammar et al. 2005). The latter stained the nuclei red, which helped in the identification of the tissues observed and in the subcellular localization of the above pathogens.

Using iCLSM to localize MMV in its planthopper vector *P. maidis*, 2-4 wk post-exposure to MMV-diseased plants for 1 wk, MMV antigens/accumulations were found associated with the nuclei in almost all the vector tissues examined including the midgut, anterior diverticulum, Malpighian tubules, hemocytes, epidermis, muscles and fat tissues. Additionally, extensive perinuclear accumulations of MMV were found in tracheal cells and in cells of the brain and other nerve ganglia of this vector. In the principle salivary glands MMV accumulations were associated with the cell periphery in addition to the nuclei. Earlier studies by transmission electron microscopy (TEM) indicated that in maize leaf cells and in most of the vector tissues examined, virions of MMV mainly assemble by budding through nuclear membranes then accumulate in perinuclear space (Ammar and Nault, 1985; McDaniel et al., 1985). However, in secretory cells of the salivary glands, MMV virions were found to bud through the plasma membrane accumulating in intercellular and extracellular spaces, apparently facilitating release of virions into the salivary ductules and ducts, which is necessary for virus transmission by the vector (Ammar and Nault, 1985).

The three preparation methods mentioned above for iCLSM were also successful in studying the distribution of the bacterial mollicute CSS in its leafhopper vector *D. maidis*. CSS antigens/accumulations were detected in the midgut, filter chamber, Malpighian tubules, hindgut, fat and muscle tissues, hemocytes, tracheae and in several lobes of the salivary glands. However, CSS was not detected in nerve cells of the brain or other nerve ganglia of *D. maidis*. The percentage of leafhoppers with detected CSS in various organs/tissues 2-3 wk post-exposure to CSS-infected plants for 1 wk, was up to 95%, and 75% of these leafhoppers inoculated CSS into maize test seedlings prior to iCLSM processing.

To localize MMV or CSS in the plant host by iCLSM, paraffin sections as well as free-hand sections of fixed un-embedded leaves from infected maize were used. CSS accumulations were detected mainly in phloem tissue, whereas MMV accumulations were detected in epidermal, mesophyll, and phloem tissues of infected maize leaves, which is consistent with previous TEM studies of these two pathogens in maize plants (McDaniel at al., 1985; Nault and Bradfute, 1979).

Although TEM provides higher resolution for the localization of viruses, mollicutes or other pathogens in host plants and in insect vectors/hosts at the cellular and subcellular levels, iCLSM has the following advantages compared to TEM: a. both processing and examination of specimens are considerably faster; b. much larger and more numerous samples can be processed and examined; and c. the distribution of pathogens can be studied at the tissue and organ levels, or even in the entire insect through thick sectioning. Additionally, compared to epifluorescence microscopy, iCLSM provides three-dimensional images of the studied organs indicating their spatial relationships, which can be valuable for studying the routes of pathogens in their vectors.

Because of these advantages, iCLSM is being used at present to study the temporal movement of MMV and of CSS in tissues and organs of their insect vectors at various times post acquisition, which is necessary to determine the routes and transmission barriers for these pathogens in their vectors (Ammar, 1994; Hogenhout et al., 1993). Additionally, iCLSM can be used to study the localization of various pathogen associated/induced proteins in insect vectors or host plants, which may provide important clues to elucidate various aspects of pathogen-vector/host relationships.

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Can insecticides prevent transmission of phytoplasmas?

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The control of phytoplasma diseases often relies on vector control but hoppers and psyllids can transmit phytoplasmas with short feeding periods, so that insecticides should act very rapidly and persist in their activity to provide satisfactory protection. Chrysanthemum yellows, CY, is a disease of several herbaceous crops (Conti *et al*., 1998) associated with phytoplasmas belonging to the genetic group 16Sr-IB (Candidatus "*Phytoplasma asteris"*) and transmitted by at least three species of *Cicadellidae Deltocephalinae* leafhoppers (Palermo *et al*., 2001). The aim of this study was to investigate the effect of some organophosphates (OPs) and a neonicotinoid on the transmission and acquisition of CY phytoplasmas by the vector. The OPs tested in the trials are used in Italian vineyards against *Scaphoideus titanus* Ball vector of Flavescence dorée of grape (Mazzoni et al., 2003). Imidacloprid was also included in the study since neonicotinoids showed a good activity in preventing persistent transmission of arthropod-borne plant pathogens (Ahmed et al., 2001). CY-*Chrysanthemum carinatum-Macrosteles quadripunctulatus* association was used for the study because it is a convenient experimental model.

Methods and Materials

Nymphs of *M. quadripunctulatus* Kirschbaum were allowed to acquire CY from untreated infected daisy plants (*Chrysanthemum carinatum* Schousboe) for one week, maintained on oat plants for two weeks and then transferred in groups of 5 to test daisy seedlings for an inoculation period (IAP) of 48 hr. Insectricides were sprayed on the foliage of *C. carinatum* test plants with Fenitrothion at 750 ppm active ingredients (a.i.), chlorpyrifos ethil at 525 ppm a.i., malathion at 600 ppm a.i., drench treated with imidacloprid with 7 mg a.i. per plant. Negative controls were treated with water only. About 20 plants for each treatment were exposed to infective leafhoppers 1, 4, 7, 10, 15 and 20 days after treatment. The survival of leafhoppers exposed to organophosphates were recorded every 30 min [*do you mean every 30 minutes on each of the 3 days observed?] at 1, 7 and 15 days after treatments. The number of infected [*do you mean symptomatic plants? Or did you test for CYP some other way (in which case you should describe the method used.)?] plants were recorded one month after inoculation.

In a second experiment CY-infected plants were treated as above and 40 days later 100 nymphs of *M. quadripunctulatus* were caged for 48 h to acquire CY. The surviving leafhoppers were maintained for two weeks on oat plants and then checked for the presence of CY by PCR and for the infectivity by transmission assays.

Results

Survival of leafhoppers following exposure to OPs at different times after treatments are summarised in Table 1.

Percentages of test plant protected by transmission of CY by *M. quadripunctulatus* at different times after treatments are summarised in Figure 1.

No leafhoppers fed on CY-infected plants treated with imidacloprid survived the 2-day AAP, while some leafhoppers fed on CY-infected OPs survived. At the end of the latency, these latter were able to transmit CY to test plants, nevertheless fewer insects successfully acquired CY from treated compared to untreated source plants.

Discussion

The insecticidal activity measured in terms of protection from infection (instead of insect mortality) provides interesting results. In spite of the high mortality induced by OPs, these insecticides have shown a very limited activity in preventing phytoplasma transmission; Imidacloprid was the most effective. [*you do not include any mortality data for imidacloprid. Do you want to add this to Table 1?] Due to the rapid death of leafhoppers on treated plants, the transmission of CY has been effective also within very short periods, and 1 h of IAP proved to be enough for efficient transmission. When the main purpose is to protect crops from incoming infected insects, the use of OPs is not advisable. On the other hand, they could reduce the vector population, limiting secondary spread of the disease. The activity of the neonicotinoid insecticide, imidacloprid, can be explained by its rapid, irreversible effects to stop feeding activity, with effects comparable to those of knock-down insecticides. [*A caption is needed for Figure 1. I suggest adding an explantion in the figure caption of how the data in the figure were calculated. If possible, please add some indication of statistical significance of differences among insecticides for each time after treatment.]

Table 1.

Insecticide	Days after treatment	Lethal time$_{50}$ (hours)	Lethal time$_{100}$ (hours)
Fenitrothion	1	1.5	4
	7	1.5	4
	14	2	5
Chlorpyrifos ethyl	1	1	2.5
	7	1> <1.5	4
	14	1.5	5
Malathion	1	0.5> <1	1.5
	7	0.5> <1	1.5

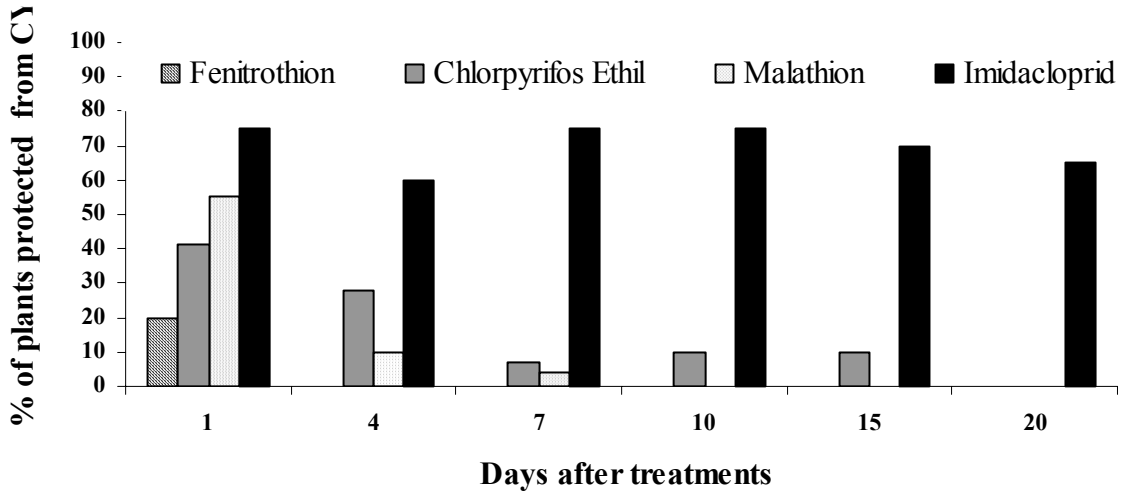

Figure 1

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Microinjection and insect feeding medium bioassays to test the vector specificity of Flavescence dorée phytoplasma by some Hemiptera species

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Phytoplasmas are a group mollicute plant pathogens transmitted by insect vectors in the Hemiptera. Although vector species have been identified for many of plant diseases associated with phytoplasmas, little is known about the specificity of vector transmission. Some phytoplasma strains seem to be specifically transmitted, for example phytoplasmas in the Elm yellow group seem to be transmitted by only a few leafhopper species. Other strains have low vector specificity, such as those clustered in the Aster yellows group, which are transmitted by several leafhopper species.

Flavescence dorée of the grapevine is associated with a phytoplasma (FDP) that seems to be specifically transmitted in the field by the leafhopper vector *Scaphoideus titanus* Ball (Hemiptera, Cicadomorpha, Cicadellidae). However earlier works revealed that, in experimental conditions, other leafhopper species were alternative vectors of FDP to herbaceous hosts, i.e. *Euscelidius variegatus* Kirschbaum (Hemiptera, Cicadomorpha, Cicadellidae) (Caudwell et al. 1972).

In the present work we studied the vector specificity of FDP by 15 Hemiptera Cicadomorpha and Fulgoromorpha species collected in European vineyards. To test vector transmission we adopted needle injection to deliver phytoplasma suspensions into the abdomen of candidate vector species (Purcell 1996), and after an incubation period we fed the injected insects on artificial diets through a Parafilm membrane (Zhang et al. 1998, Tanne et al. 2001) and tested the feeding medium for the presence of FDP DNA by a PCR procedure.

Materials and Methods

Healthy colonies of *E. variegatus* were maintained on maize inside cubical Plexiglas cages. They were periodically collected as nymphs or young adults for injection assays and for feeding acquisition and transmission to maintain phytoplasma strains (Caudwell and Larrue 1977).

Suspensions of FDP were obtained from FDP-infective *E. variegatus* following a procedure described by Whitcomb and Coan (1982). Optimization of the concentration of viable phytoplasma extracts and latency in vectors were monitored by injecting healthy-reared *E. variegatus* leafhoppers. On the basis of these preliminary results, insects were injected using phytoplasma extracts that ensured the highest rate of FDP acquisition and transmission by *E. variegatus*. Transmission was attempted to an artificial diet (feeding medium) through a Parafilm membrane about three weeks after insect injection.

Leafhopper and planthopper specimens were collected by using a D-vac or a sweep-net in viticulture areas located in the Mosel Region (Germany), Burgundy Region (France) and Veneto Region (Italy), other specimens were kindly provided by some specialized laboratories. Insects were caged on suitable host plants. Univoltine species were directly used for injection assays, while plurivoltine species were reared for at least one generation and the progeny was used in injection assays. All the insect species were maintained or reared in a climatic chamber (23 ± 1 ºC, L16:D8).

To confirm transmissibility of FDP by some tested insects, we attempted transmission assays by insect feeding on plants. To accomplish this, insects were confined on FD-infected broadbeans or on healthy broadbean seedlings (as control) for an acquisition period of 15 days and then confined to healthy broadbean seedlings after an incubation period of about 35 days.

Detection of FDP in insects that acquired FDP by injection or by feeding, in plants and in feeding medium was done using polymerase chain reaction (PCR) amplification of phytoplasma DNA using FDP-specific primers (Clair et al. 2003).

Results

Among the batches of FDP-injected insects belonging to 15 Hemiptera species, that were confined in cages and fed through a Parafilm membrane in the medium for a 4-5 days inoculation access period (IAP), FDP DNA was detected by PCR in the feeding medium inoculated by the leafhoppers (Cicadellidae) *Anoplotettix fuscovenosus* (Ferrari), *Aphrodes makarovi* Zachvatkin, *Euscelidius variegatus* Kirschbaum and *Euscelis incisus* Kirschbaum.

FDP was not detected in the feeding medium where injected insects of the other 11 species were confined: the leafhoppers *Agallia consobrina* Curtis *Circulifer haematoceps* (Mulsant & Rey), *Fieberiella florii* (Stål), *Psammotettix* sp.; the spittlebug (Cercopidae) *Philaenus spumarius* (Linneus); the treehopper (Membracidae) *Stictocephala bisonia* (Kopp &Yonke); the Fulgoromorpha: *Agalmatium flavescens* (Olivier), *Hyalesthes obsoletus* Signoret, *Laodelphax striatellus* (Fallén), *Metcalfa pruinosa* (Say), and *Pentastiridius* sp.. Detection of FDP was positive in injected insects of

all the Hemiptera species although band intensities in the agarose gels were positively associated with the transmissibility of FDP to artificial diets.

The ability/inability to transmit FDP was confirmed for *E. variegatus*, *E. incisus*, *C. haematoceps* and *F. florii* by feeding on FDP-infected broadbeans and transmission to healthy broadbean seedlings. FDP in inoculated broadbeans was confirmed by symptom expression and by PCR detection. *E. variegatus* and *E. incisus* that transmitted to feeding medium also inoculated broadbean; *C. haematoceps and F. florii* transmitted neither to feeding medium nor to broadbean.

Discussion

Injection technique is a potential useful tool for searching for insect vectors (Whitcomb and Coan 1982). Injecting pathogens into the insect haemocoel increases the transmission efficiency by vectors by increasing the rate of phytoplasma acquisition and reducing the latent period. Additionally it is possible to test insects with different feeding habits or insects that have different host plant preferences and that could not feed well on plants used as source for acquisition. As a result injection of pathogen in the abdomen of the vectors suppresses the effects of host plant-vector interactions in the acquisition process.

The use of feeding medium assays to test the inoculative potential of insects allows a significant reduction of time if compared with transmission to host plants, considering the time necessary for symptom expression in inoculated plants (incubation period). Also, it eliminates the effects of host plant-vector interactions in the inoculation process. In addition, the technique allows in increasing the efficiency of vector transmission when compared to feeding transmission to host plants (Ge and Maixner 2003).

The four insect species that transmitted FDP after injection belong to the family Cicadellidae, as well as the economic vector of FDP, *S. titanus*. The other Hemiptera species tested could not transmit FDP after abdominal injection of the phytoplasma suspension. Therefore we may assume that the latter species are not potential vectors of FDP or are extremely inefficient in transmitting the mollicute. Passage of plant pathogens from the haemocoel to the salivary glands and subsequent transmission is not enough by it self to recognize if one insect is a vector. Actually, in natural conditions, phytoplasma cells should overcome at least the two physical barriers that are the gut and the salivary glands (Lefol et al. 1994, Fletcher et al. 1998). Therefore other assays based on feeding acquisition from FDP-infected host plants are in progress to confirm the ability to transmit FDP by *A. fuscovenosus* and *A. makarovi*.

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Phytoreovirus and the glassy-winged sharpshooter (Hemiptera: Cicadellidae)

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The Glassy-winged sharpshooter (GWSS), *Homalodisca coagulata,* has become established in California and has drawn attention as an important vector for Pierce's Disease of grapes, however recent findings suggest that other crops are also at risk from plant diseases which are being transmitted by this fast spreading insect pest. A Rice Dwarf-like virus was isolated from the salivary glands of the GWSS collected in southern California. California is the third largest rice-growing state in the USA, producing over 2.50 million tons of rice in 2004. The Reoviridae are large, architecturally-complex viruses containing segmented double-stranded RNA genomes that infect plant hosts and that are transmitted by leafhopper vectors. Rice Dwarf Virus (RDV) is a leafhopper-transmitted member of the Phytoreoviridae that infects graminaceous hosts. The presence of Rice Dwarf-like virus indicates that the GWSS may be causing more economic damage than just as a vector of Pierce's Disease, and expanded monitoring in other crops that are susceptible to viruses within the Phytoreoviridae would be prudent.

Characteristics of transmission by *Pentastiridius leporinus* (Hemiptera, Cixiidae) of a phytopathogenic bacterium-like organism closely related to bacterial endosymbionts of hemiptera

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The disease of sugar beet known as the syndrome “basses richesses” (SBR) has repeatedly affected sugar beet crops in Burgundy and Jura regions of Eastern France since 1991. It causes a loss of root sugar content which can have dramatic economic consequences for growers and local sugar beet industry: in 1992 the loss of income was about 50 % over 1000 ha. On the basis of microscope observations and PCR diagnosis, the SBR disease of sugar beet has been associated with two uncultivable phloem-restricted organisms: a stolbur phytoplasma and a bacterium-like organism (BLO) (Gatineau et al. 2001; 2002). The BLO is the major etiological agent of SBR. It is a γ-3 proteobacteria phylogenetically related to endosymbionts of hemiptera (aphids, psyllids, whiteflies) (3).

SBR is transmitted by the cixiid planthopper *Pentastiridius leporinus* (Fig 1) with a high efficiency, i.e. 100% of transmission after an inoculation access period (IAP) of 1 hour. The univoltine insect species is rare in natural environment. It appears that the cropping system of sugar beet and the associated cultural rotation are ideal conditions for the species to complete its biological cycle and reach high populations.

Fig 1. Male *Pentastiridius leporinus* on a sugar beet leaf.

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