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Psyllid communication: acoustic diversity, mate recognition and phylogenetic signal

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Abstract. Acoustic signals play an important role in mate selection and speciation in diverse groups of insects. We report reciprocal acoustic mate signalling, often as highly synchronised duetting, for several species of psyllid (Hemiptera : Psylloidea). We reveal that considerable acoustic diversity is present in Australian psyllids belonging to the family Triozidae. The acoustic signals are species and gender specific. Our acoustic analysis and observations suggest that acoustic signals are important in both species recognition and mate selection in psyllids. We found a significant level of phylogenetic signal in the acoustic data when we compared divergence in genetic data (obtained from mitochondrial DNA sequences of the small subunit rRNA) with divergence in acoustic signals in two groups of Australian psyllids. Phylogenetic reconstruction based on DNA sequence data supports the monophyly of the *Eucalyptus*-feeding genus *Schedotrioza* Tuthill & Taylor, 1955, whereas a diverse but little known group on Casuarinaceae hosts appears to be paraphyletic. These two psyllid groups also differ in amounts of geographical and ecological sympatry. We found a significant positive correlation between acoustic distance and genetic distance using pairwise comparisons for all taxa, but the trends within the two groups differ due to a negative association between acoustic and genetic divergence among the sympatric taxa. Phylogenetic information in acoustic data may be greatest in recently speciating and allopatric groups because of increased acoustic divergence in sympatric taxa and greater acoustic convergence in more distantly related species.

Additional keywords: acoustic communication, *Allocasuarina*, Casuarinaceae, *Eucalyptus*, Psylloidea, speciation, substrate vibration, Triozidae.

Introduction

The use of acoustic and visual signals are an important part of sexual advertisement and mate location in animals (van Tets 1965; Bradbury and Vehrencamp 1998; Espmark *et al*. 2000). These mechanisms may also play a central role in speciation processes, particularly through the initiation or reinforcement of divergence by sexual selection (Panhuis *et al*. 2001; Stenseth and Saetre 2003). Several studies have suggested that acoustic behaviour plays an important role in insect speciation (e.g. crickets, flies, cicadas and hoppers: Otte 1989; Butlin 1996; Wells and Henry 1998; Ritchie *et al*. 1999; Marshall and Cooley 2000; Oliveira *et al*. 2001). These and other studies have shown that acoustic behaviour in insects is involved in species recognition, mate selection and the maintenance of reproductive barriers (Butlin 1989; Ewing 1989; Bailey 1991; Claridge and de Vrijer 1994). Acoustic mate recognition systems in insects are typically species and gender specific (Gillham and de Vrijer 1995; Stumpner and Meyer 2001; Bailey 2003) and selection on these signals may differ at different levels of inter- and intraspecific interaction due to variability in songs and preferences (Shaw and Herlihy 2000). In addition, behavioural characters are often considered fast evolving and therefore likely to play a role in the early divergence of taxa (Henry 1985; Irwin 2000; Shaw and Parsons 2002; Martins *et al*. 2004), and a growing number of morphologically cryptic animal taxa identified by their acoustic signals supports a role for acoustics in incipient and early speciation processes (Jones and van Parijs 1993; Henry 1994; Otte 1992; Kingston and Rossiter 2004). Furthermore, studies of crickets have found that sexual selection of acoustic signals plays an important role in the divergence of sympatric taxa (Shaw

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2000; Mendelson and Shaw 2002). Differences in the acoustic signals of sympatric v. allopatric taxa may arise because sympatric taxa compete for acoustic niches, promoting acoustic character displacement in order to avoid signal interference (Hobel and Gerhardt 2003). This displacement may increase if there is strong selection to reinforce reproductive barriers where the sympatric taxa are closely related and likely to interbreed (Marshall and Cooley 2000). We compare acoustic signals in two psyllid groups that differ in their degrees of geographical and ecological sympatry in order to assess whether the evolution of acoustic characters may proceed differently when multiple species are sympatric v. allopatric.

Our study of acoustics in Australian triozids compares one group of taxa that are mainly allopatric geographically (when species occur in the same region there may also be ecological micro-allopatry on different host plants) and a second group where all species occur on the same host plant and are frequently sympatric on the same individual plant. In the same way that freshwater lakes and oceanic islands provide discrete ecological and geographical boundaries for evolutionary studies, the host plants of highly specialised phytophagous insects provide natural isolates (Janzen 1968) that are advantageous in studies of selection and speciation (Percy 2003). The complex spatial structure of host plants within habitats and geographical areas can provide different gradations of sympatry and allopatry useful for comparative studies: insects may occur on the same individual plant; they may occur on the same host plant but in non-overlapping, or only partially overlapping geographical areas; they may be geographically sympatric but on different host plants; or occur on the same individual plants but exploit different plant organs (the latter two may be considered ecologically micro-allopatric if separation at these small spatial scales results in genetic isolation). Each of these gradations of geographic and ecological isolation implies a different level of interspecific interaction that may be reflected and observable in behavioural characters (e.g. studies of cicada communities; Sueur 2002).

To date, there have been relatively few phylogenetic studies that include behavioural data (e.g. Prum 1990; Paterson *et al*. 1995; Kennedy *et al*. 1996; Slikas 1998; Henry *et al*. 1999; McLennan and Mattern 2001). This appears to be due in part to the relative difficulty in obtaining behavioural characters compared with morphological or molecular data, but may also be due to a perception that behavioural characters are highly homoplasious as a result of strong selection, rapid evolution and limited repertoire (Atz 1970; de Queiroz and Wimberger 1993; Proctor 1996; McCracken and Sheldon 1997; Martins *et al*. 2004). Whether there is strong phylogenetic signal in acoustic behavioural characters has been investigated in studies of birds and insects. Two recent examples comparing molecular and acoustic data in a phylogenetic context provide evidence for consistently high phylogenetic signal in songbirds (Price and Lanyon 2002; Päckert *et al*. 2003) and consistently low phylogenetic signal in lacewings (Henry *et al*. 1999). The phylogenetic utility of behavioural characters is therefore likely to remain controversial until more studies are undertaken across different taxon groups. Even then, we may find that phylogenetic utility differs dramatically on a case by case basis due to differing strengths of selection on signal characters.

Acoustic signals in psyllids

Substrate-transmitted vibratory signals used in courtship and mate recognition are characteristic of all Auchenorrhyncha groups in the Hemiptera (cicadas, leafhoppers, planthoppers, treehoppers) (Ossiannilsson 1949; Claridge 1985*a*; Stölting *et al*. 2002; Rodríguez *et al*. 2004; Čokl and Virant-Doberlet 2003). In contrast, in the Sternorrhynchan clade of Hemiptera, which includes four groups: psyllids, aphids, scales and whiteflies, acoustic signals in male whiteflies have been documented (Kanmiya 1996; Kanmiya and Sonobe 2002), but, at present, only psyllids are known to produce reciprocal mate signalling (i.e. duetting) (Tishechkin 2005). Both psyllids and whiteflies are considered to be ancestral within the Sternorrhyncha (von Dohlen and Moran 1995). Thus aphids and scales may have lost the ability to produce stridulatory sound with the development of specialised, often soft bodied anatomies, or lost the requirement for acoustic communication in mating with the evolution of partial asexual reproduction. Alternatively, sound production may be a novel and independent development in both psyllids and whiteflies.

Among the hemipteroid insects, pioneering work on the acoustic signals of leafhoppers and planthoppers was undertaken in an impressive study of the Aunchenorrhyncha by Ossiannilsson (1949). Ossiannilsson (1950) was also the first to discover that psyllids (both males and females) produce sounds accompanied by wing vibrations. Ossiannilsson's initial observations were followed by more extensive investigations by Heslop-Harrison (1960) using at least three taxa from two psyllid families. These early studies of psyllid acoustics used enclosed tubes or bags placed close to the ear to detect the signals (Ossiannilsson 1950; Heslop-Harrison 1960). The first psyllid acoustic signals to be recorded were captured by Campbell (1964) on magnetic tape, followed by Yang *et al*. (1986) using a bamboo membrane and microphone and Tishechkin (1989) using a microphone and gramophone pickup. In some cases sound was produced only by male psyllids and in other cases male and female duetting was recorded. Tishechkin (1989) compared airborne and substrate signals in eight taxa from two psyllid families (Psyllidae and Triozidae) and concluded that psyllids were primarily communicating by vibration of the substrate. Our observations and use of playback support the conclusion that substrate vibration is a primary method of communication in psyllids.

Psyllids lack the complex tymbal mechanisms found in most Auchenorrhyncha (Ossiannilsson 1946; Heslop-Harrison 1960; Claridge 1985*b*). The wing vibrations accompanying sound production in psyllids indicate the use of stridulation and several researchers have examined what appears to be a simple stridulatory mechanism (Heslop-Harrison 1960; Taylor 1962, 1985; Taylor 1990). These studies identified a single row of ridges on the anal vein of the forewing that corresponds with similar structures on protruding ridges on the meso- and metathorax, and which together provide a mechanism for stridulation using vibration of the forewings. However, not all species examined were found to possess these structures and it is therefore not clear whether sound production is ubiquitous in psyllids, or whether different taxa may possess different stridulatory mechanisms. In addition, some environments may be more conducive to the evolution and utilisation of acoustic signals (e.g. Australian dry forests, where acoustic signalling has been found in 26 species from a survey of 35 species in 13 genera (D. Percy unpublished data)).

Australian triozids: taxonomy and biology

The Australian psyllid fauna consists of 354 described species in 57 genera (Hollis 2004). Most Australian psyllids are found on the plant families Myrtaceae (particularly *Eucalyptus* L'Her) and Fabaceae (particularly *Acacia* Mill.) (Yen 2002). The family Triozidae represents a relatively small component of the psyllid fauna in Australia, with 22 native Australian species described and around 25 undescribed species. Relatively few Australian Triozidae feed on Myrtaceae hosts: 16 species, with 13 of these on *Eucalyptus*. The majority of *Eucalyptus*-feeding psyllids in Australia (around 250 species) belong to the family Psyllidae subfamily Spondyliaspidinae, which represents a significant radiation within Australia. The remaining Triozidae feed on Casuarinaceae (*c*. 15 species), Myoporaceae (*c*. three species), Euphorbiaceae (two species), Guttiferae (two species), Asteraceae (one species), Cunoniaceae (one species), Elaeocarpaceae (one species), Moraceae (one species) and Proteaceae (one species).

A revision of the *Eucalyptus*-feeding genus *Schedotrioza* Tuthill & Taylor, 1955 distinguished two species-groups based on the morphology of adults, egg type and gall type (Taylor 1990). The two groups differ in oviposition behaviour and structure of the ovipositor: one group oviposits on the leaf surface and the other group oviposits under the leaf epidermis (Taylor 1990). In contrast, the Casuarinaceae-feeding group is in need of considerable taxonomic and systematic revision. Only two of the Casuarinaceae-feeding psyllids have been described and the taxonomic affiliations of this group are uncertain.

The main objectives of this study were: (*1*) to compare acoustic and genetic divergence in order to assess the phylogenetic utility of acoustic signals in psyllid systematics; (*2*) to establish whether reciprocal acoustic mate signalling (i.e. duetting) is a common behaviour during mating in psyllids, and to analyse the structure of male and female calls in psyllid duets; and (*3*) to assess whether acoustic divergence differs in sympatric versus allopatric taxa.

Materials and methods

The two study groups

We use a comparison of two triozid psyllid groups. The first group consists of 13 closely related and morphologically relatively homogeneous species (genus *Schedotrioza*) that frequently occur in the same geographical area, but are typically associated with different host plants in the genus *Eucalyptus* (we sampled six species from this group, see Appendix 1). The second group is morphologically heterogeneous and species are found on various host plants in the family Casuarinaceae, but all members sampled for our acoustic analysis occur on the same host plant (*Allocasuarina verticellata* (Lam.) L. A. S. Johnson), with several species (up to eight) found sympatrically on the same individual plants (we sampled 11 species from this group). As well as the differing levels of ecological and geographic sympatry in the two groups, there are also some basic biological differences. All the Casuarinaceae-feeding species have free-living nymphal stages, although some species have modified nymphal morphologies (e.g. the genus *Aacanthocnema* Tuthill & Taylor, 1955 has nymphs that develop under a hardened case that is elongate, apparently an adaptation for attachment to the slender stems of the Casuarinaceae host). In the *Schedotrioza*-group, all members produce galls in the nymphal stage on the leaves of their *Eucalyptus* hosts. *Schedotrioza* galls are often easy to detect because of their size and colouration (at maturity galls can be up to a centimetre long and are often red or brown). Some *Schedotrioza* species occur in high densities, severely disfiguring the host plant foliage during gall formation. Often the same tree will be infested in successive years, suggesting either low psyllid vagility or variation in individual host susceptibility to infestation. In contrast, there is no visible change or obvious damage to the plant phenotype to indicate the presence of psyllids, even when present in high densities, in the Casuarinaceae-feeding group.

Molecular sampling

For the molecular analysis two outgroup taxa (*Mesohomotoma hibisci* (Froggatt, 1901) and *Protyora sterculiae* (Froggatt, 1901)) were selected from the family Carsidaridae, which is a putative sister group to the Triozidae based on adult and nymphal morphology (White and Hodkinson 1985). We also included an aphid sequence obtained from GenBank (see Appendix 1). Our ingroup taxon sampling includes two-thirds of the estimated 37 Australian triozid species. Of the three triozid genera sampled, two are endemic to Australia, *Aacanthocnema* and *Schedotrioza*, and the third is a large cosmopolitan genus, *Trioza* Förster, 1848, with ambiguous taxonomic delimitations. The ingroup includes all taxa for which we also have acoustic data, which includes all the taxa on the host plant *Allocasuarina verticellata*. A broader sampling of taxa that occur on other Casuarinaceae hosts for which acoustic data are not available, was added to improve the phylogenetic inference of relationships among the Australian triozids. In order to compare intraspecific genetic divergence in the two *Eucalyptus*-feeding taxa (*S. multitudinea* (Maskell, 1898) and *S. distorta* G. S. Taylor, 1987) that were found on different hosts in different geographical regions, an individual from each host species was sampled. Collection details are provided in Appendix 1.

Deoxyribonucleic acid extraction, amplification and sequencing

Specimens were collected into 100% ethanol. Depending on their size, between one and three insects were bisected and placed with Proteinase K in the buffer provided in the QIAGEN DNeasy Tissue Kit (Qiagen

Ltd, Crawley, UK). These individuals or other individuals from the same collection were then retained as voucher specimens. Negative controls were included with each set of DNA extractions. Specimens were incubated over two nights at 55°C, with the remainder of the extraction following the QIAGEN DNeasy Tissue Kit's specifications. The primers used to amplify the third domain of the small subunit rRNA region (*12S*) were SR-N-14588 and SR-J-14233 (primers supplied by Bioline Ltd, London, UK; Simon *et al*. 1994).

The polymerase chain reaction (PCR) conditions follow those used in Percy (2003) to amplify *12S*. Negative controls were included with each PCR reaction. The amplified PCR products were excised from 2% agarose gels and purified using the QIAGEN QIAquick Gel Extraction Kit. The cleaned PCR products were sequenced on an automated sequencer (Perkin-Elmer ABI 377, Applied Biosystems, Warrington, UK) with ABI Prism Dye Terminator Cycle Ready Reaction Kit (Applied Biosystems), using both the PCR primers. Sequences were aligned manually in the program Se-Al (Rambaut 1998). All the sequences have been deposited in GenBank (www.ncbi.nlm.nih.gov/Genbank/, verified July 2006; accession numbers DQ858180–DQ858204, Appendix 1). The aligned matrix is available from GenBank and the matrix and tree (Fig. 3) are available from TreeBASE (www.treebase.org/, verified July 2006).

Phylogenetic analyses and comparative analysis of acoustic and genetic data

Phylogenetic analyses were performed with PAUP* 4.0b10 (Swofford 2002). The following heuristic search parameters were employed for the maximum parsimony (MP) analysis: 2000 random stepwise addition replicates with TBR (tree bisection reconnection) branch swapping, MULTREES and COLLAPSE. Maximum parsimony bootstrap analyses were performed using 2000 simple addition replicates with TBR branch swapping, with all characters included and with ambiguous alignment regions excluded. Distance neighbour-joining (NJ) analyses were used to provide a comparison of topology and bootstrap. For the comparison of genetic and acoustic divergence, genetic distance (uncorrected 'p' value) was obtained from PAUP* and acoustic divergence was estimated by using the R-Package 4.0 (Casgrain and Legendre 2001) SIMIL function to create a similarity matrix from a combination of binary and quantitative characters (Appendix 2) implementing the Gower (symmetrical) coefficient (option S15). The similarity matrix was then converted to a distance matrix using the CONVERT function.

Acoustic sampling, recording and analyses

Specimens were collected in the field with a sweep net, or directly off the host plant with an aspirator. Live specimens were then placed into a polythene bag with several branches of fresh host plant material and transported to a suitable location for sound recording (in some cases gall material was collected and the newly emerged adults used). Sound recording experiments and observations were undertaken in locations where ambient background vibrations were minimised and usually within 24 h of collecting the specimens.

Acoustic signals and behaviour were recorded and observed in eleven species (Table 1), five in the genus *Schedotrioza*, one species in the genus *Aacanthocnema* and five species in the genus *Trioza*. To compare intraspecific acoustic divergence, five species that are widespread, two species of *Schedotrioza* (*S. multitudinea* and *S. distorta*) and three Casuarinaceae-feeding species (*Trioza*, sp. nov. 2; sp. nov. 3 and sp. nov. 4) were recorded from different geographical regions. Acoustic signals were also compared between populations of *S. multitudinea* and *S. distorta* that occur on two different *Eucalyptus* host plants. Acoustic signals were recorded using substrate pickup methods developed by Claridge *et al*. (1985) and optimised for psyllids (by D. Percy). Recordings were made inside a transparent acrylic tube (15 cm × 4 cm diameter) with cork plugs sealing both ends. A crystal gramophone cartridge was attached firmly to the cork plug at one end with adhesive putty. The cartridge was positioned so the stylus made contact with the surface of a plant stem trimmed from the host plant (*c*. 10–13 cm in length) and placed inside the tube with the base of the plant stem inserted in adhesive putty. Signals from the cartridge were amplified ×10 using an ED1241 Differential Amplifier (designed and constructed by C. Hardy, Department of Electronics and Electrical Engineering, University of Glasgow, UK.) and recorded at a sampling rate of 44100 samples per second (44.1 kHz) on digital audio tape (Sony DAT tape recorder, model TCD-D8). To record the acoustic signals, a female was released into the tube and allowed to settle on the plant stem. Additional females or males were then released into the tube as required. Ambient temperature was ~18–30°C. The recorded sound was analysed using Canary software (version 1.2.4, Cornell Laboratory of Ornithology). Spectrograms were computed with a filter bandwidth of 175 Hz, using a Fast-Fourier transformation (FFT), with a 2048-point window size (frequency = 21.5 Hz) and a 90–98% overlap. We examined the structure and symmetry of the signals (i.e. the number, placement and type of distinct phases in a call), dominant frequency, duration of calls, number of pulses and pulse rates (Table 1, Appendix 2). Where possible, measurements were taken from a minimum of three calls from three individuals, but for some less common taxa only one or two individuals were recorded. To test for levels of inter- v. intra-specific variation, a canonical principle components (PCA) analysis was performed with species as explanatory variables using the first four male call variables in Table 1.

Playback experiments

We carried out preliminary playback experiments using the *Schedotrioza*-group to test female receptivity to interspecific and intraspecific male signals. Recorded calls were played back through a small speaker and transmitted to the host plant surface using a steel pin with one end attached to the cone of the speaker and the other end resting on the host plant. These experiments were carried out with the *Schedotrioza* species because they appear to be a recently derived group and the species have similar call structures. One female of *S. multitudinea* that had previously responded acoustically to live conspecific male calls was allowed to settle on the host plant. Male calls of the four heterospecific *Schedotrioza* were then played back. Playback of heterospecific male calls was alternated with playback of the conspecific male call. *Schedotrioza multitudinea* occurs on two *Eucalyptus* species in different geographical areas and playback of male conspecific calls were from both *Eucalyptus* hosts (therefore only some of the males were from the same *Eucalyptus* species as the target female in the experiment).

Results

Phylogenetic analyses

Our phylogenetic analysis of the *12S* data provides an initial reconstruction of relationships in the Australian triozids and makes possible a comparison of genetic and acoustic divergence.

The aligned length of the *12S* matrix is 365 characters long (mean of 343 base pairs excluding gaps). To evaluate the impact of the alignment and tree reconstruction method used on the resultant tree topologies, analyses were performed using maximum parsimony (MP) and neighbour-joining (NJ) with all characters included and with several regions of ambiguous alignment (a total of 80 characters) excluded. Excluding ambiguous alignment regions resulted in 146 variable sites (103 parsimony informative) and with all characters included there were 211 variable sites (153 parsimony infor-

Table 1. Measurements (± standard deviation) of the acoustic signals of 11 Australian triozids

Species	no. ♂ (calls)	♂ mean call duration S	♂ mean no. pulses	♂ mean pulse rate mS	♂ mean dominant frequency kHz	no. ♀ (calls)	♀ mean call duration S	♀ mean dominant frequency kHz
Schedotrioza apicobystra	4 (14)	1.19 (± 0.13)	50 (± 3.33)	13.49 (± 1.33)	0.615 (± 0.084)	2 (60)	0.75 (± 0.08)	0.499 (± 0.118)
Schedotrioza distorta	4 (12)	0.33 (± 0.05)	3.08 (± 0.29)	81.81 (± 9.55)	0.576 (± 0.124)	2 (23)	0.23 (± 0.04)	0.315 (± 0.116)
Schedotrioza marginata	3 (19)	0.46 (± 0.04)	19.18 (± 3.03)	16.33 (± 0.76)	0.683 (± 0.131)	2 (5)	0.34 (± 0.03)	0.614 ± 0.058)
Schedotrioza multitudinea	5 (32)	0.43 (± 0.14)	7 (± 0.60)	43.68 (± 12.73)	0.869 (± 0.17)	2 (16)	0.3 (± 0.04)	0.602 (± 0.058)
Schedotrioza, sp. nov.	3 (14)	1.09 (± 0.09)	22.75 (± 2.30)	29.53 (± 1.35)	0.765 (± 0.109)	2 (9)	0.7 (± 0.07)	0.268 (± 0.196)
Aacanthocnema dobsoni	1 (9)	1.48 (± 0.38)	48.2 (± 13.22)	27.64 (± 2.04)	0.698 (± 0.434)	1 (10)	0.24 (± 0.02)	0.925 (± 0.043)
Trioza, sp. nov. 2	3 (13)	0.28 (± 0.03)	15 (± 1.28)	17.27 (± 3.05)	1.315 (± 0.186)	–	–	–
Trioza, sp. nov. 3	2 (22)	0.56 (± 0.14)	6.62 (± 0.96)	43.80 (± 15.52)	1.060 (± 0.248)	–	–	–
Trioza, sp. nov. 4	4 (12)	1.61 (± 0.18)	50.67 (± 8.94)	20.27 (± 4.28)	1.449 (± 0.176)	–	–	–
Trioza, sp. nov. 5	1 (5)	0.18 (± 0.01)	–	–	0.690 (± 0.034)	1 (4)	1.18 (± 0.19)	1.096 (± 0.009)
Trioza, sp. nov. 6	1 (2)	0.6 (± 0.03)	32.5 (± 3.54)	13 (± 3.17)	1.699 (± 0.028)	–	–	–

mative sites). The MP analysis (with ambiguous regions excluded) produced a single tree (Fig. 1) of length 467, *CI* (consistency index, excluding uninformative characters) 0.42 and *RI* (retention index) 0.55. This topology is similar to those generated by MP and NJ analyses with all characters included, the exceptions being the placement of taxa not included in the acoustic analysis: the two Euphorbiaceae-feeding taxa (*T. malloticola* (Crawford, 1928) and *T. pallida* (Uichanco, 1919)), *T. adventicia* Tuthill, 1952 and *T.*, sp. nov. 1. In the NJ analysis with all characters included, a well supported (77% bootstrap) split in the *Schedotrioza*-group (*S. multitudinea*, sp. nov., *S. marginata* and *S. apicobystra* G. S. Taylor, 1990 as sister to *S. distorta* and *S. cornuta* G. S. Taylor, 1990) is consistent with a distinct change in song structure in *S. distorta* v. the other four *Schedotrioza* species for which sound data are available (Fig. 1).

All phylogenetic analyses of the *12S* data indicate that *Schedotrioza* (gall forming on *Eucalyptus*) is a monophyletic genus, whereas the Casuarinaceae-feeding taxa appear to be paraphyletic or even polyphyletic. These findings are in agreement with the morphology: *Schedotrioza* is a morphologically homogeneous genus (Taylor 1990), whereas the Casuarinaceae-feeding taxa are morphologically heterogeneous and are placed in several genera. In addition to being monophyletic, *Schedotrioza* is probably a more recently derived Australian group than the Casuarinaceae-feeding taxa (interspecific divergence based on uncorrected pairwise distances of the *12S* data: within *Schedotrioza* 3–15%, among the Casuarinaceae-feeding taxa 9–22%).

Data from other gene regions and a broader sampling of triozids outside Australia are required for a more comprehensive and robust phylogenetic analysis and is currently being undertaken. The general phylogenetic structure reported here is supported by analysis of cytochrome oxidase I and II and the large subunit rRNA (*16S*) data (D. Percy unpublished data). In particular, all these gene regions support the monophyly of *Schedotrioza* and the paraphyly of the Casuarinaceae-feeding species.

Comparative analysis of acoustic and genetic divergence

We compared genetic divergence, estimated using uncorrected pairwise distances in PAUP*, with acoustic distance, using characters of structure and symmetry, dominant frequency, call duration, pulse number and pulse rate (Appendix 2). We found that overall, across all pairwise comparisons, there is a significant positive correlation between acoustic distance and genetic distance, suggesting the presence of phylogenetic signal in the acoustic data. We found a significant correlation ($P = 0.005$) between genetic and acoustic divergence in pairwise comparisons of all 11 taxa (Fig. 2). However, when we looked at the correlation between acoustic and genetic divergence within the two groups (*Eucalyptus*-feeding and *Allocasuarina*-feeding), the trend, though non-significant ($P = 0.07$), was reversed in the *Allocasuarina*-feeding group (i.e. there is a negative association between acoustic and genetic distance), which may reflect increased selection for acoustic divergence in closely related, sympatric taxa. The trend in the *Eucalyptus*-feeding

group was consistent with the total data analysis, showing a positive association ($P = 0.07$) between acoustic and genetic distance (Fig. 2).

Acoustic divergence

The acoustic signals we recorded are unique for each species based on both qualitative and quantitative characters (Table 1, Appendix 2) that are incorporated into the measure of acoustic distance in Fig. 2. The PCA analysis based on four quantitative variables of male calls showed that the difference between species accounted for more than 99% of the total variance ($P < 0.0001$). In the comparative acoustic-genetic analysis the overall correlation in interspecific acoustic and genetic distance ($P = 0.005$) reflects a lower acoustic divergence in the more recently derived *Schedotrioza*-group and a higher acoustic divergence in the apparently older Casuarinaceae-feeding taxa (Figs 1 and 2). Acoustic signals are both species specific and are temporally and structurally consistent when populations occur on different host plants. The two *Schedotrioza* species that are both widespread and oligophagous (*S. multitudinea* and *S. distorta*), feeding on several *Eucalyptus* species, were found to have little

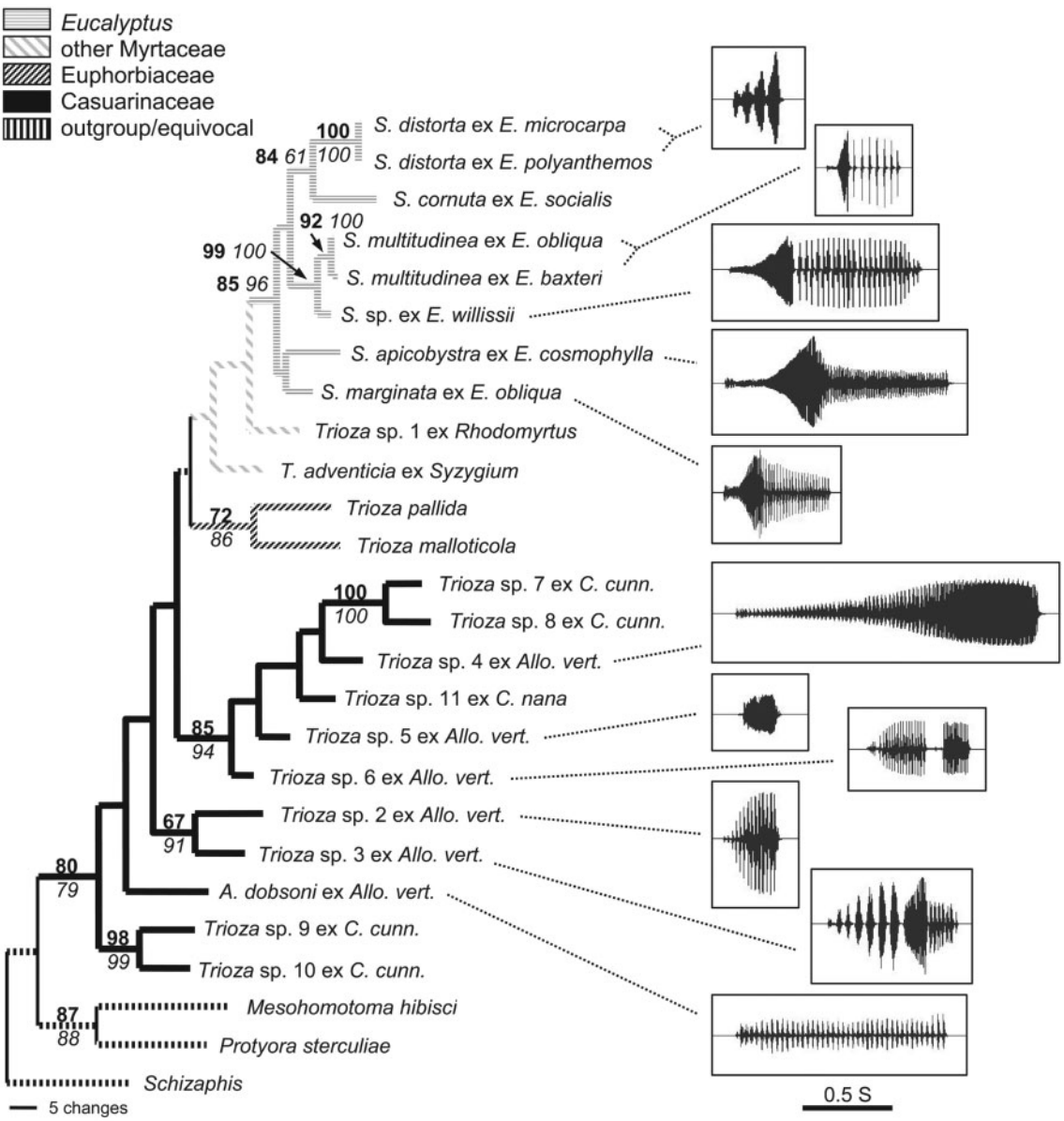

Fig. 1. Single tree obtained from the maximum parsimony analysis (with ambiguous regions excluded) of the *12S* region. Bootstrap values were obtained from maximum parsimony (bold) and neighbour joining (italic) analysis with all characters included. Host plant groups are mapped onto the psyllid tree and host names are given after the psyllid name (host plant names are given in full in Appendix 1). A single male cell is shown for each species included in the acoustic analysis.

intraspecific genetic or acoustic divergence and probably represent recent geographical and host expansions in these taxa. However, some local differences in temporal aspects only (call length and pulse rate) were detected in three taxa (*Trioza*, sp. nov. 2; sp. nov. 3; sp. nov. 4) on the Casuarinaceae host plant, *Allocasuarina verticellata*, from widespread geographic regions (see Appendix 1).

We compared the acoustic divergence of male calls between and within species and found more variability among males within species characterised by long calls with a simple structure v. species with shorter more complex structured calls. Using a comparison of call duration and pulse number within and between individuals (Fig. 3) we found that those males with calls characterised by a simple structure and long duration are more variable in both call duration and pulse number (e.g. calls 3 and 5 in Fig. 3*B*). Species with male calls of short or medium duration showed less variability in call duration and pulse number and tended to have more complex call structures (e.g. Figure 3*A*). We also found some evidence that species with long simple male calls do not engage in tightly synchronised male/female duetting, and these species, though sympatric on the same plant with several other taxa are more distantly related and therefore may have a lower risk of heterospecific mating. We recorded duetting between males and females in seven species (Table 1) and we found that duets are more tightly synchronised in some taxa than others. The species with less temporal variability in male calls also involved tight synchrony during male/female duetting (Fig. 4). In four of the *Schedotrioza* species and *Trioza*, sp. nov. 5, the female response to the male call was rapid with reply latencies of usually less than 30 mS once the duet was underway, and in four species the majority of female replies started before the end of the male call (e.g. Fig. 4*B*, *C*). In contrast, duetting in *Aacanthocnema dobsoni* (Froggatt, 1903), which has a long male call and more variation in call duration and pulse number (Fig. 3*B*), is not tightly synchronised (female reply latency was variable: 97 mS–1.2 S, and the reply sometimes came in the middle of the male call) (Fig. 4). In this species the female response did not seem to have any temporal consistency or to be triggered by a specific cue in the male call. Within both the *Eucalyptus*- and Casuarinaceae-feeding groups the species that are similar in call duration and pulse number can be clearly differentiated using the overall structure and symmetry of the call (e.g. calls 1 and 2 in Fig. 3*A*, *B*).

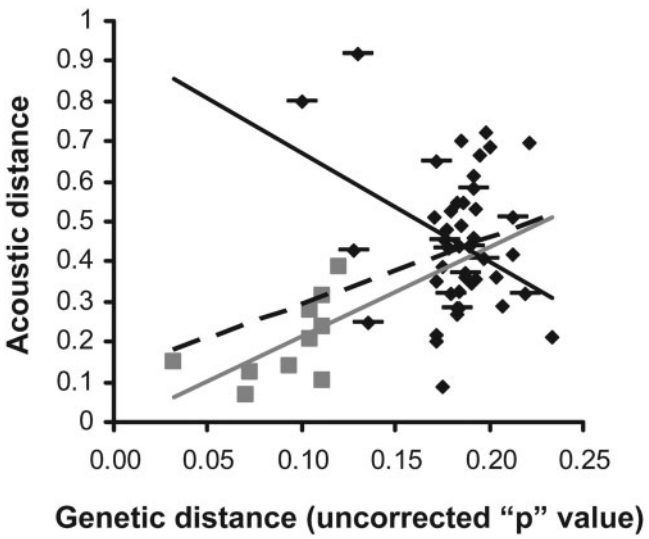

Fig. 2. Comparison of acoustic and genetic distance for 11 triozid species. There is a significant correlation between genetic and acoustic distance using pairwise comparisons of all 11 taxa (dashed line, $P = 0.005$, $R^2 = 0.1393$). An analysis of trends within the two groups showed that within the *Schedotrioza*-group (grey squares) the trend also indicates a positive correlation, though non-significant (grey line, $P = 0.07$, $R^2 = 0.3459$), but within the Casuarinaceae-feeding group (black diamonds with horizontal bar) the trend is reversed, suggesting a negative correlation (black line, $P = 0.07$, $R^2 = 0.2302$).

Males and females have characteristic gender specific calls (Fig. 4). The structure of male and female calls may be similar (i.e. homologous in shape such as in the *Schedotrioza*-group, Fig. 4*C*), differing mainly in call length and dominant frequency, or male and female calls may differ in both temporal and structural aspects (e.g. Casuarinaceae-feeding group, Fig. 4*A*, *B*). In the majority of cases (the five *Schedotrioza* species), the female call is shorter and of lower dominant frequency than the male call (Table 1). The exceptions are *Aacanthocnema dobsoni*, where the male call has a lower dominant frequency and *Trioza*, sp. nov. 5, where the male has a shorter call and lower dominant frequency (Table 1 and Fig. 4). In *Schedotrioza*, the similarity, or homology in shape, between the male and female call structures suggests that divergence of species specific acoustic characters is linked in the sexes. There is no evidence of the same co-divergence in male and female call structure in the two species from the Casuarinaceae-feeding group for which female signals have been recorded, although sampling is currently limited to two species (*Aacanthocnema dobsoni* and *Trioza*, sp. nov. 5).

Observations of mating behaviour

Our observations confirm that the primary transmission and reception of acoustic signals in psyllids is through vibration of the substrate. We also observed the rapid wing vibrations and abdominal flexing which often accompanied the vibratory transmission of sound. On a few occasions the calls could also be detected faintly as airborne sound when insects were contained in a plastic bag, but it is probably unlikely that an airborne sound component is important in the field. In all species for which we recorded male and female signals, the signalling was initiated by males and none of the females emitted spontaneous calls in the absence of male calls. In contrast, males frequently called repeatedly in the absence of females. If no female response is detected calling ceases or becomes more intermittent. On initial contact with the plant

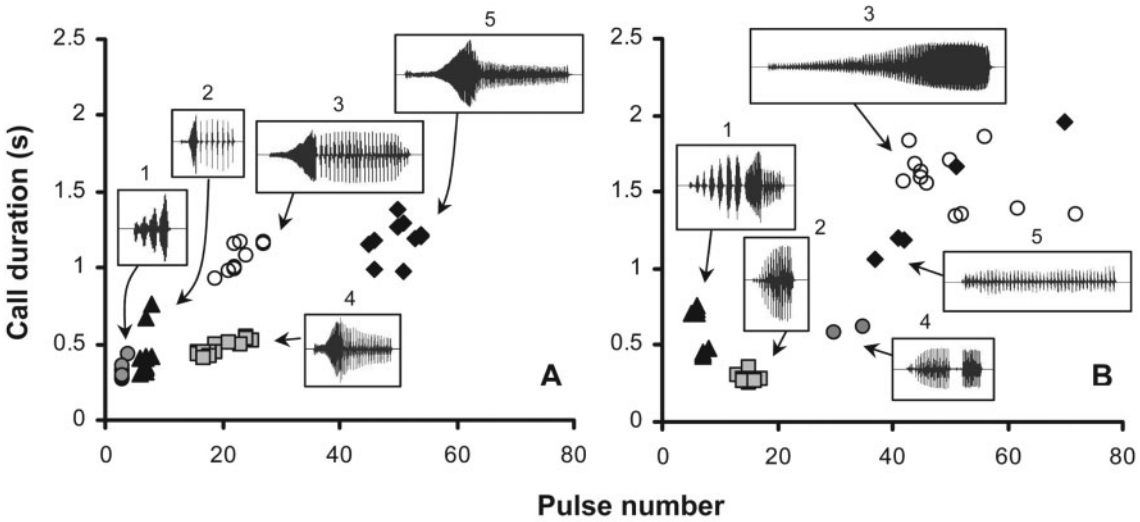

Fig. 3. Plots of two acoustic variables (male call duration and pulse number from Table 1) indicates the extent of variability within and between species. Plot A *Schedotrioza*-group: 1, *S. distorta*; 2, *S. multitudinea*; 3, *S.,* sp. nov.; 4, *S. marginata*; 5, *S. apicobystra*. Plot B *Allocasuarina*-feeding group: 1, *Trioza*, sp. nov. 3; 2, *Trioza*, sp. nov. 2; 3, *Trioza*, sp. nov. 4; 4, *Trioza*, sp. nov. 6; 5, *Aacanthocnema dobsoni*. (*Trioza*, sp. nov. 5 does not have distinct pulses.)

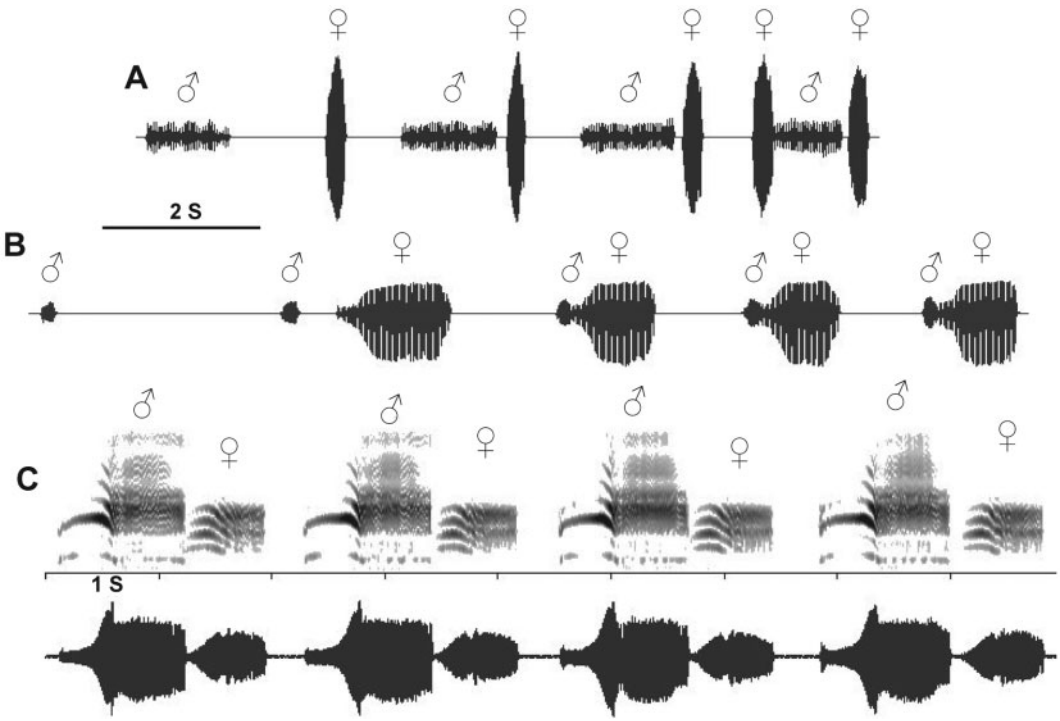

Fig. 4. Examples of male/female duetting. *A*, *Aacanthocnema dobsoni*; *B*, *Trioza*, sp. nov. 5; *C*, *Schedotrioza apicobystra*. In *A*, male and female signals do not become tightly synchronised, whereas in *B* and *C* the timing of the female response to the male call results in tight synchrony once the duet is underway. In the *Allocasuarina*-feeding group (examples A and B) male and female calls do not share the same structure, whereas in the *Schedotrioza*-group (example C) the structure of the call in males and females is similar. Example C also represents the more typical pattern where the female call is shorter and of lower frequency than the male call. The exceptions are (*A*) *Aacanthocnema dobsoni*, where the male call has a lower dominant frequency, and (*B*) *Trioza*, sp. nov. 5, where the male has a shorter call and lower dominant frequency (see Table 1). In all of these examples the females are larger than the males.

the usual male search behaviour involved moving up and down the plant stems stopping periodically to call. If a female response is detected, the male's movements and rate of calling become more rapid, which in turn increases the rate of female response and may aid in faster location of the female and in guarding the male's exchange with the female from interference by satellite males (Bailey 2003). Females are more sedentary than males, usually remaining stationary on the host plant throughout the acoustic exchange. If the acoustically guided location of a female by a male results in physical contact, we observed that mating frequently (but not always) occurred. In some cases, females moved away or turned their abdomens so as to position the genitalia away from the male, and in other cases, females responded only initially to male calls then ceased responding, or responded only intermittently to male calls. When mating did take place, couples remained in copulation for several minutes. Typically, copulating pairs are stationary, but in the *Schedotrioza* taxa the male strikes the female abdomen repeatedly with his forelegs during copulation, a behaviour also observed in some planthopper courtships (Claridge and de Vrijer 1994). After mating, males usually resumed calling, but mated females did not respond to further male calls, supporting previous observations that only virgin females are acoustically responsive to male calls (Taylor 1985).

Playback experiment

For our preliminary playback experiment we used a single target female of *S. multitudinea* that had previously responded to live male conspecific calls, and we noted the presence or absence of her response to playback of males of other *Schedotrioza* species. When we played recorded male conspecific calls, the target *S. multitudinea* female responded with a series of calls and participated in typical acoustic duetting. The response did not differ appreciably with playback of a male from the same host as the female, or playback of a male call from the alternate *Eucalyptus* host species. In contrast, when heterospecific calls of the four other *Schedotrioza* species were played back there was no response, with the exception of a single but only partial initial acoustic response to the male call of *S.*, sp. nov. In the *12S* phylogeny, *S.*, sp. nov. is the sister taxon of *S. multitudinea*, a relationship that is supported by morphology. During this playback session, the target *S. multitudinea* female responded only to the first call of a male *S.*, sp. nov. series of calls, but not to playback of subsequent *S.*, sp. nov. male calls. Throughout the experiment we played conspecific *S. multitudinea* male calls alternately with playback of each of the heterospecific male calls, and the target *S. multitudinea* female continued to respond with a series of strong acoustic signals to conspecifc male calls. There was no response by the *S. multitudinea* female to playback of *S. apicobystra, S. distorta* or *S. marginata* (the latter is found sympatrically on the same individual plants as *S. multitudinea*).

Discussion

Phylogeny and acoustic divergence

Several reasons may account for the few studies that incorporate behavioural data into phylogenetic analyses, including the difficulty in obtaining such data relative to morphological or molecular data, the definition of character homology, and the perception that behavioural data are likely to be more homoplasious than other kinds of data (Atz 1970; Gray and Jordan 2000). Our study suggests that acoustic data from psyllids have significant phylogenetic content, although using this data to reconstruct relationships would require determination of character polarity or an appropriate outgroup. However, our data also suggest that divergence in acoustic signals may occur rapidly under certain conditions (e.g. when taxa are sympatric), potentially confounding phylogenetic interpretations. The Casuarinaceae-feeding taxa appear to be an older group than the *Eucalyptus*-feeding group and, as expected, the overall pattern suggests increased genetic divergence is accompanied by increased acoustic divergence. However, comparative analysis of the *Eucalyptus*- and *Allocasuarina*-feeding groups independently indicates opposing trends, although conclusive support for these trends requires further sampling. More closely related sympatric taxa found on the host plant *Allocasuarina verticellata* are more acoustically divergent than more distantly related taxa on the same host plant. The same pattern is not found in the mostly allopatric taxa in the *Schedotrioza*-group. The inverse trend in the sympatric *Allocasuarina*-feeding taxa may reflect increased selection for acoustic divergence in closely related sympatric taxa (i.e. selection to reinforce reproductive isolation in species more likely to interbreed). Another interpretation, which would also confound phylogenetic inference, is the effect of constraints on acoustic repertoire. If simple stridulatory mechanisms limit acoustic repertoire, acoustic convergence may be more likely in distantly related taxa that do not require reinforcement of reproductive isolation. In contrast, the presence of gradual and consistent acoustic divergence or an 'acoustic clock' similar to the 'glottoclock' or glottochronology of linguistics (Gray and Atkinson 2003) is supported by clearer homologies in acoustic characters in the recently derived *Eucalyptus*-feeding *Schedotrioza*-group. If acoustic characters evolve rapidly through competition or sexual selection in sympatry, and are also prone to convergence due to limited repertoire, then more phylogenetic information may generally be found in acoustic data from recently speciating, allopatric taxa, such as the *Schedotrioza*-group.

Divergence in Australian triozid acoustics and the role of acoustics in speciation

There have been many studies of speciation that invoke either natural or sexual selection as the primary mechanism driving divergence. But the relative contributions of these processes

to speciation within a taxon have rarely been assessed. Studies of fishes from fresh water lake systems (Galis and Metz 1998; McKinnon and Rundle 2002) and island lineages of fruit flies (Kambysellis and Craddock 1997) have provided perhaps the best comparative systems for looking at this question. Psyllids, which are highly host specific and engage in acoustic behaviour during mating, provide a potential model system for comparing natural selection that is linked to host specificity and host selection, with sexual selection related to mate recognition and mate selection.

Acoustic signals are likely to be subject to a combination of stabilising and directional selection. The requirement for signals to convey species specific information may limit call variation within species whereas more variability in calls potentially offers more information to selective mates (Bailey 2003). In addition, there may be a trade off between increased call length, which conveys more information, and the need to avoid attracting predators, such as other invertebrates that may cue in on vibrational signals. Psyllid male and female calls are distinct, with female calls generally shorter and of lower frequency. In crickets, shorter acoustic calls appear to be selected for where there is a need to avoid predators (Simmons *et al*. 2001), and in psyllids the more sedentary females may be at greater risk of predation than the more mobile males. The psyllid species in this study have distinctive species specific acoustic signals and the call type remains consistent in taxa that are widespread or occur on different host plants. Even among the closely related *Eucalyptus*-feeding group, in which the call structure and symmetry share many characteristics, there are distinct differences in call length, pulse rate and pulse number between species. Our preliminary playback experiment, although only using one female, further supports call recognition as species specific and suggests a role for acoustics in maintaining reproductive isolation. Studies of courtship songs in *Drosophila* have shown that male acoustic signals are under sexual selection through female preference (Ritchie *et al*. 1998). Our observations and the experimental evidence from the preliminary playback experiment suggest that females can discriminate between interspecific male calls.

Acoustic signals in psyllids appear to be used during mating to determine both the location and receptiveness of mates. We also found some evidence that acoustic signals are subject to sexual selection because female responses to conspecific male calls are variable, and females do not always mate with males after engaging in acoustic duetting. If females are able to distinguish and differentiate between intraspecific males at both the acoustic and contact stage they may be exercising selection based on multiple cues, a strategy that is likely to have adaptive advantages in mate choice (Candolin 2003). In some species of Orthoptera, male and female calls are extremely brief and additional species specific information is thought to be transmitted via the time delay or latency between caller and respondent (Bailey and Hammond 2004). The psyllid male and female calls we examined have distinct species specific characteristics, but additional information may also be present in the reply latency between the male call and the female response. In six (the *Schedotrioza* species and *Trioza*, sp. nov. 5) of the seven species for which we recorded male/female exchanges, the female response to the male call was rapid. In these species the female often starts her response before the conclusion of the male call. It is notable that in some taxa, the acoustic duets between males and females are more tightly synchronised whereas other taxa have more variable reply latencies. The taxa that have highly synchronised duetting also exhibit less variation in temporal acoustic characters. It may be that the requirement for tightly synchronised duetting is also acting to constrain intraspecific variability in male calls, particularly if species recognition and mate selection rely on temporal constancy as well as other acoustic characters in these taxa. In contrast, duetting in *A. dobsoni*, which has a long male call with considerable intraspecific variation in call duration and pulse number is not tightly synchronised with the female response. The faster call rate observed in the *Schedotrioza* taxa during duetting and the short reply latencies may also serve to 'guard' the interaction from intruding calls (Bailey 2003). Further experiments using manipulated calls in playback experiments could be used to test which aspects of the male signal are responsible for selective responses in females (e.g. Bailey and Hammond 2004). Selection on the rhythmic control of these signals could be further explored with analysis of clock genes such as *period* and *cacophony* (Oliveira *et al*. 2001; Mazzoni *et al*. 2002).

Conclusions

Acoustic signals in psyllids are surprisingly diverse despite the means of sound production being an apparently simple stridulatory mechanism. As with other hemipteroid insects, acoustic signals in psyllids are species specific and can diverge rapidly. The utility of acoustic data in phylogenetic analysis depends not only on the ability to obtain these characters for sufficient taxa, but also on the type and strength of selection acoustic characters are subject to. Acoustic duets between males and females are a primary mechanism for mate location, and in taxa where duets are tightly synchronised between the sexes, synchronised duetting may impose temporal constraints on the variability of male signals. We found positive evidence for the phylogenetic utility of acoustic characters, but the use of these characters to reveal phylogenetic relationships may only be optimal in recently speciating and allopatric taxa owing to selection for character displacement in sympatric taxa and/or convergence in unrelated taxa.

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Appendix 1. Taxa sampled for the molecular and acoustic analyses

An asterisk after the species name indicates taxa for which acoustic data are available. GenBank numbers are given for all haplotypes except those that were only sampled for the acoustic analyses (indicated by a dash). Abbreviations of Australian states: ACT, Australian Capital Territory; NSW, New South Wales; QLD, Queensland; SA, South Australia; VIC, Victoria. Plant family abbreviations: CASU, Casuarinaceae; EUPH, Euphorbiaceae; MALV, Malvaceae; MORA, Moraceae; MYRT, Myrtaceae. Samples were collected by D. Percy unless indicated by initials after the taxon name: CB, Chris Burwell; DH, David Hollis; PD, Pam Dale

Taxon	Origin of sample	Host plant and family	Coll. no.	GenBank no.
Mesohomotoma hibisci	French Polynesia: Moorea	*Hibiscus tiliaceus* MALV	401A	DQ858180
Protyora sterculiae	ACT: Canberra	*Brachychiton populneus* MALV	443	DQ858181
*Aacanthocnema dobsoni**	SA: Fleurieu Peninsula	*Allocasuarina verticellata* CASU	S28A	DQ858192
*Schedotrioza apicobystra**	SA: Adelaide Hills	*Eucalyptus cosmophylla* MYRT	S35	DQ858186
Schedotrioza cornuta	SA: Flinders Ranges	*Eucalyptus socialis* MYRT	NS01	DQ858182
*Schedotrioza distorta**	SA: Adelaide	*Eucalyptus microcarpa* MYRT	S48	DQ858187
*Schedotrioza distorta**	NSW: Albury	*Eucalyptus polyanthemos* MYRT	S55	DQ858188
*Schedotrioza marginata**	SA: Adelaide Hills	*Eucalyptus obliqua* MYRT	S32	DQ858189
*Schedotrioza multitudinea**	SA: Adelaide Hills	*Eucalyptus obliqua* MYRT	S29	DQ858183
*Schedotrioza multitudinea**	VIC: Glenelg Park	*Eucalyptus baxteri* MYRT	S58	DQ858184
Schedotrioza, sp. nov.*	VIC: Glenelg Park	*Eucalyptus willisii* MYRT	S56	DQ858185
Trioza adventicia (PD)	New Zealand: Auckland	*Syzygium* sp. MYRT	NZtri1	DQ858190
Trioza malloticola (CB)	QLD: Rafting Grnd Res	*Mallotus philippensis* EUPH	NS02	DQ858204
Trioza pallida (CB)	QLD: Rafting Grnd Res	*Mallotus philippensis* EUPH	NS03	DQ858203
Trioza, sp. nov. 1 (DH)	NSW: Lansdowne	?*Rhodomyrtus* sp. MYRT	DHT1	DQ858191
Trioza, sp. nov. 2*	SA: Murray Bridge	*Allocasuarina verticellata* CASU	S41A	DQ858193
Trioza, sp. nov. 2*	ACT: Tharwa	*Allocasuarina verticellata* CASU	S73A	–
Trioza, sp. nov. 3*	SA: Murray Bridge	*Allocasuarina verticellata* CASU	S41B	DQ858194
Trioza, sp. nov. 3*	ACT: Tharwa	*Allocasuarina verticellata* CASU	S73B	–
Trioza, sp. nov. 4*	SA: Murray Bridge	*Allocasuarina verticellata* CASU	S51	DQ858200
Trioza, sp. nov. 4*	ACT: Tharwa	*Allocasuarina verticellata* CASU	S73C	–
Trioza, sp. nov. 5*	SA: Murray Bridge	*Allocasuarina verticellata* CASU	S43	DQ858197
Trioza, sp. nov. 6*	SA: Fleurieu Peninsula	*Allocasuarina verticellata* CASU	S28B	DQ858198
Trioza, sp. nov. 7	ACT: Cotter Dam	*Casuarina cunninghamiana* CASU	NS74A	DQ858201
Trioza, sp. nov. 8	ACT: Cotter Dam	*Casuarina cunninghamiana* CASU	NS74B	DQ858202
Trioza, sp. nov. 9	SA: Adelaide	*Casuarina cunninghamiana* CASU	NS38	DQ858195
Trioza, sp. nov. 10	ACT: Cotter Dam	*Casuarina cunninghamiana* CASU	NS74C	DQ858196
Trioza, sp. nov. 11	NSW: Braidwood	*Casuarina nana* CASU	NS05	DQ858199
Schizaphis graminum	[sequence from GenBank]	–	–	AF275249

Appendix 2. Acoustic character list and character matrix used to calculate acoustic distance between psyllid taxa

Acoustic character list

(*1*) *Male mean call duration* S (quantitative).
(*2*) *Male mean number pulses* (quantitative).
(*3*) *Male mean pulse rate* mS (quantitative).
(*4*) *Male structure (symmetry):* 0, asymmetrical; 1, symmetrical
(*5*) *Male structure (pulses):* 0, distinct pulses present; 1, distinct pulses absent
(*6*) *Male location of distinct pulses:* 0, throughout call; 1, at beginning; 2, at end; 3, at beginning and end.
(*7*) *Male mean dominant call frequency* kHz (quantitative).
(*8*) *Female mean dominant call frequency* kHz (quantitative).
(*9*) *Female mean call duration* S (quantitative).
(*10*) *Female call structure (pulses)* 0, distinct pulses present; 1, distinct pulses absent.
(*11*) *Male* v. *female call structure:* 0, male and female call structure similar; 1, different.
(*12*) *Male* v. *female call duration:* 0, male call longer; 1, female call longer.
(*13*) *Male* v. *female call frequency:* 0, male dominant frequency higher; 1, female dominant frequency higher.

Acoustic character matrix

A dash indicates inapplicable character and a question mark indicates missing data

	Character												
Taxon	1	2	3	4	5	6	7	8	9	10	11	12	13
Schedotrioza multitudinea	0.43	7.00	43.68	0	0	2	0.869	0.602	0.30	0	0	0	0
Schedotrioza, sp. nov.	1.09	22.75	29.53	0	0	2	0.765	0.268	0.70	0	0	0	0
Schedotrioza marginata	0.46	19.18	16.33	0	0	2	0.683	0.614	0.34	0	0	0	0
Schedotrioza apicobystra	1.19	50.00	13.49	0	0	2	0.615	0.499	0.75	0	0	0	0
Schedotrioza distorta	0.33	3.09	81.81	1	0	0	0.576	0.315	0.23	0	0	0	0
Schedotrioza dobsoni	1.48	48.20	27.64	1	0	0	0.698	0.925	0.24	1	1	0	1
Trioza, sp. nov. 5	0.18	–	–	1	1	–	0.690	1.096	1.18	0	1	1	1
Trioza, sp. nov. 6	0.60	32.50	13.00	0	0	0	1.699	?	?	?	?	?	?
Trioza, sp. nov. 4	1.61	50.67	20.27	0	0	1	1.449	?	?	?	?	?	?
Trioza, sp. nov. 2	0.28	15.00	17.27	1	0	0	1.315	?	?	?	?	?	?
Trioza, sp. nov. 3	0.56	6.62	43.80	0	0	3	1.060	?	?	?	?	?	?