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Acoustic communication within ant societies and its mimicry by mutualistic and socially parasitic myrmecophiles

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Abstract:

This review focusses on the main acoustic adaptations that have evolved to enhance social communication in ants. We also describe how other invertebrates mimic these acoustic signals in order to coexist with ants in the case of mutualistic myrmecophiles, or, in the case of social parasites, corrupt them in order to infiltrate ant societies and exploit their resources. New data suggest that the strength of each ant-myrmecophile interaction leads to distinctive sound profiles and may be a better predictor of the similarity of sound between different myrmecophilous species than their phylogenetic distance. Finally, we discuss the evolutionary significance of vibrations emitted by specialised myrmecophiles in the context of ant multimodal communication involving the use of chemical and acoustic signals in combination and identify future challenges for research including how new technology might allow a yet better understanding of the study systems.

Keywords: Acoustic communication, ants, mutualists, social parasites, social structure

Introduction

Efficient communication to coordinate the actions of up to a million specialised nestmates is fundamental to the success of social insects, especially ants. Various modes of signalling have been identified, including the release of semio-chemicals, visual behavioural displays involving movement or posture, tactile interactions, and the comparatively poorly studied use of acoustic signals (Hölldobler & Wilson, 1990, 2009). As hotspots of resources in their environment, ants fiercely defend their colonies using a wide range of weapons (e.g. gland secretions, mandibles, sting), which are deployed in the manner of co-ordinated attacks by legions of intercommunicating workers. Nevertheless, ant nests are also magnets for other organisms that have evolved means to overcome the hostility of the host ants. Thus, an estimated ~10,000 invertebrate species live as obligate social parasites of ants, able to penetrate and exploit the resources within host colonies in order to complete their life-cycle (Thomas, Schönrogge, Elmes, 2005). The large majority of these adaptations evolved in many separate lines, especially among Coleoptera, Diptera, Lepidoptera and other Hymenoptera, from a ten-times greater number of commensals or mutualists (Fiedler, 1998; Hölldobler & Wilson, 1990; Nash & Boomsma, 2008; Pierce et al., 2002; Thomas, Schönrogge et al., 2005). All these myrmecophiles show morphological, behavioural, chemical or acoustic adaptations to interact with ants (Cottrell, 1984; Donisthorpe, 1927; Hinton, 1951; Lenoir, D'Ettorre, Errard, & Hefetz, 2001; Malicky, 1969; Wasmann, 1913; Wheeler, 1910; Witek, Barbero, & Marko, 2014). Armour, stealth and the secretion of attractive food rewards are frequently sufficient for unspecific or facultative myrmecophiles to access the enemy-free spaces of ants. However, the subversion of the ants' chemical and/or acoustic signalling is generally required to enable true social parasites (*sensu* Nash & Boomsma, 2008) to live for long periods as undetected intruders in close contact with their hosts.

A key element of successful co-habitation in ant nests is to circumvent the host's ability to differentiate between nestmates and intruders. Nestmate recognition is a dynamic process, primarily based on the detection of distinctive species- or colony-specific cocktails of cuticular hydrocarbons (CHC) covering the surface of all individuals (Hölldobler & Wilson, 1990; Howard, 1993; vander Meer & Morel, 1998; Winston, 1992). Social interactions such as allogrooming ensure an exchange between the CHC mixtures among nestmates and give rise to a shared CHC *gestalt* odour (vander Meer & Morel, 1998). The role that chemical communication and nestmate recognition have in maintaining the cohesion of ant societies and those of other social insects has been subject to extensive study, with excellent recent reviews, for example by Martin & Drijfhout (2009) and van Wilgenburg, Symonds, & Elgar (2011): The deployment of chemical communication by obligate social parasites to subvert host recognition systems is equally well reviewed (e.g. Lenoir et al., 2001; von Thienen, Metzler, Choe, & Witte, 2014).

In contrast, the function, the origin and role of acoustic signals in ants and their corruption by social parasites are much less well studied. In this review, we therefore focus on the state of the art concerning acoustic signaling in ants, and then consider the acoustic signaling of obligate and facultative myrmecophiles. In both cases we emphasize the insights that have resulted from recent technological advances that allow unalarmed ants and their guests to be recorded and to receive broadcasts of their acoustic signals under semi-natural conditions (Barbero, Thomas, et al., 2009; Riva, Barbero, Bonelli, Balletto, Casacci, in press).

We first examine ant sound producing organs and convergent adaptations that allow non-ant organisms to mimic and subvert ant–ant communications, focussing on advances in knowledge since the reviews by Hölldobler & Wilson (1990), Fiedler (1998), Pierce and colleagues (2002), Thomas and colleagues (2005) and Nash & Boomsma (2008), or covered cursorily by Witek and colleagues (2014). We then review recent insights concerning the ant acoustic signals themselves and their corruption by social parasites. This includes both the morphological adaptations to produce acoustic signals, the behavioural responses to them, and thus the impact on ant – social parasite/guest interactions. Much of this builds on the pioneering work of Markl (1965, 1967), DeVries (1991a, 1991b), Hölldobler, Braun, Gronenberg, Kirchner, & Peeters (1994) and Kirchner (1997). Finally we present new data relating the intimacy of interactions of lycaenid butterfly larvae to phylogeny and the similarity of acoustic signalling.

Acoustic signalling in ants

The use of acoustics, whether through receiving pressure waves through the air (i.e. sounds *stricto sensu*) or substrate vibrations, is a common means of communication in insects, whose functions include defence, displays of aggression, territorial signalling and mate attraction (Bennet-Clark, 1998; Gerhardt & Huber, 2002). Its advantage as a signal over chemical volatiles lies in instantaneous reception that pinpoints a distant, but exact, location to the receiver, for example in social insects to attract help (Markl, 1965, 1967; Roces, Tautz, & Hölldobler, 1993). The physics, use and effects of substrate-borne vibrations of ants and other insects are comprehensively reviewed by P.S. Hill (2009). A simple form involves "drumming", where the substrate is tapped by part of the exoskeleton to produce vibrations. Drumming is employed by many ant taxa, but at least four of the eleven subfamilies also stridulate by rasping a 'plectrum' across a 'file' (*pars stridens*), both chitinous organs being located on opposite segments of the anterior abdomen (see Fig. 1 k-o, u-y) (Barbero, Thomas, Bonelli, Balletto, & Schönrogge, 2009b; Golden & P.S. Hill, 2016; Ruiz, Martinez, Martinez, & Hernandez, 2006). Although these stridulations produce air-borne (as well as substrate-borne) pressure waves that are audible to the human ear, it remains uncertain whether ants can perceive sound as pressure waves through the air (Hickling & Brown, 2000, 2001; Roces & Tautz, 2001). In contrast, there is no controversy about the ants' ability to perceive substrate vibrations and two types of sensor have been proposed to receive substrate vibrations: campaniform sensilla measuring the tension in the exoskeleton; and the subgenual organ, a spherical arrangement of sensory cells in the tibia, as described from *Camponotus ligniperda* (Gronenberg, 1996; Menzel & Tautz, 1994).

Most studies that measure insect acoustics have used accelerometers, moving coil- or particle velocity microphones, often with phase inversion focussing on the vibrational part of the signal rather than pressure waves through the air. Hereafter in this review we use the term “sound” *sensu latu* in its broadest sense, as we do the terms: calls, vibrations, vibro-acoustics and stridulations.

Early studies suggested that acoustic signals were a minor means of communication among ants, largely confined to activities outside the nest and mainly signalling alarm or calls for rescue, for instance when parts of nests collapse (Markl 1965, 1967). Due to a perceived preponderance of stridulatory organs among soil nesting ant species, Markl (1973) hypothesised that stridulation evolved initially as a burial/rescue signal when volatile chemicals would be ineffective, whereas substrate borne vibrations would at least travel short distances. However, this is not supported by Golden and P.S. Hill (2016), who showed that stridulation organs have evolved independently multiple times in ants. In addition, whereas Markl (1973) suggested that they would probably become vestigial over time in arboreal ant species, due to the rarity of burial by soil, there was instead a strong positive association between the presence of functional stridulation organs and the possession of an arboreal life–style (Golden & P.S. Hill, 2016).

Nestmate recruitment is the most frequently reported function for ant–ant acoustic signalling. For example, outside the nest, *Atta cephalotes* uses vibratory signals to attract foraging workers towards newly found food sources (Roces & Hölldobler 1995). The same authors also observed that in the presence of parasitic phorid flies, foragers used acoustics to recruit minor workers for defence, thus also employing vibrations as alarm signals (Roces & Hölldobler, 1995, 1996). Finally, although created by a scraper and file organ located on the first gastric tergite and the post-petiole, Tautz and colleagues (1995) observed that vibrations travelled the length of the body to the mandibles, aiding the cutting of soft young leaf tissue by stiffening it. Behavioural experiments, however, suggest that this is a secondary effect and that communication is the main function for these vibrations (Roces & Hölldobler, 1996).

It has recently become clear that acoustic signals are also used to transmit more abstract information, including a species' identity or an individual's caste and status (Barbero, Thomas et al., 2009; Casacci et al., 2013; Ferreira, Cros, Fresneau, & Rybak, 2014). For example, modern molecular analyses revealed the neotropical ponerine ant species, *Pachycondyla apicalis*, to be a species complex of five cryptic lineages. The stridulations of three largely sympatric lineages are also distinctive, suggesting that morphological characters on the *pars stridens* differ in length, width and ridge gap in each lineage (Ferreira, Cros, Fresneau, & Rybak, 2014; Wild, 2005). By contrast, two allopatric lineages had very similar acoustics, suggesting disruptive selection on this trait where sympatric overlap is high.

Acoustic patterns also signal caste and hierarchical status in at least two genera of Myrmicinae ants: *Myrmica* (Barbero, Thomas et al., 2009) and *Pheidole* (Di Giulio et al., 2015). In both taxa, the queens produce distinctive stridulations which, when played back to kin workers, elicit additional 'royal' protective behaviours compared with responses to worker signals (Barbero, Bonelli, Thomas, Balletto, & Schönrogge, 2009; Barbero & Casacci, 2015; Barbero, Thomas et al., 2009; Casacci et al., 2013; Ferreira, Poteaux, Delabie, Fresneau, & Rybak, 2010). In addition, in *Pheidole pallidula* the soldier and minor worker castes also make distinctive vibroacoustic signals (Di Giulio et al., 2015). Unlike *Pachycondyla* species, little inter-specific variation was detected in either the queen- or worker-sounds made by closely-related sympatric species of *Myrmica* (Barbero et al., 2012; Barbero, Thomas et al., 2009; Thomas, Schönrogge, Bonelli, Barbero, & Balletto, 2010), which are instead clearly demarcated by unique hydrocarbon profiles (Elmes, Akino, Thomas, Clarke, & Knapp, 2002). Although the young stages of tested ants are mute (e.g. DeVries, Cocroft, & Thomas, 1993), Casacci and colleagues (2013) found that acoustic signalling appears to act as a substitute for other forms of communication in developing *Myrmica* pupae. The various stages of ant brood, from egg to pupa, are afforded ascending levels of priority based on tactile and chemical cues (Brian, 1975). Most are mute, but the older “brown”, sclerotised pupae of *Myrmica* species produce calls, emitted as single pulses, similar to those of workers (Casacci et al. 2013). This coincides with a presumed reduced ability to secrete brood recognition pheromones during this period, and brown pupae that were experimentally silenced fell significantly behind their mute white siblings in social standing.

Acoustic signals of myrmecophiles

Derived acoustic signals that enhance interactions with ants are increasingly being confirmed in both juvenile and adult stages of myrmecophiles. To date, most studies involve riodinid and, especially, lycaenid butterfly larvae and pupae (e.g. Barbero, Thomas et al., 2009; DeVries, 1990, 1991a; Pierce et al., 2002). However, similar phenomena were recently described from adults of a socially parasitic beetle, *Paussus favieri* (Di Giulio et al., 2015), where males and females emit mimetic stridulations using a row of scrapers on the proximal abdominal segment rasping across a file located on the hind femora (see Fig. 1p-t).

Stridulation organs

With a few exceptions, an ability to produce calls occurs after the third larval moult in riodinid and lycaenid larvae, coinciding with the development of chemical ‘ant organs’, which perhaps suggests they act synergistically (DeVries, 1991a). In most riodinids, acoustic signals are generated by grooved vibratory papillae. These are typically found in pairs on the prothorax, and grate against specialised epicranial granulations when the larva rotates its head (see Fig 1a-e), especially when walking or under attack, generating low amplitude substrate-borne calls (DeVries, 1991a). The tribe Eurybiini lacks vibratory papillae; instead, caterpillars generate calls by scraping teeth on a prothoracic cervical membrane against the epicranial granulations in at least some mutualists or entomophagous predators of ant-tended Homoptera (DeVries & Penz, 2002; Travassos, DeVries, & Pierce, 2008). The detection of dedicated organs in lycaenid larvae that produce calls has been elusive, apart from a file-and-scraper described between the 5th and 6th abdominal segments of *Arhopala madytus* (C. J. Hill, 1993) and a putative organ in *Maculinea rebeli* larvae (see Fig.1fg). In other species strong substrate-borne vibrations (and apparently weak air-borne sounds) may be generated by muscular contractions of the abdomen, which compress air through the tracheae to produce distinctive rhythms and intensities in the manner of a wind instrument, as described by Schurian and Fiedler (1991) for *Polyommatus dezinus*. These vibroacoustic signals range from low background calls punctuated by pulses in mutualists (DeVries, 1991a) to the grunts, drumming and hisses of the host-specific *Jalmenus evagoras* (Travassos & Pierce, 2000), to the mimetic calls of *Maculinea* larvae (Barbero, Bonelli et al., 2009; DeVries et al., 1993; Sala, Casacci, Balletto, Bonelli, & Barbero, 2014).

In contrast, the pupae of all lycaenids studied (Pierce et al., 2002) and a minority of riodinids (DeVries, 1991a; Downey & Allyn, 1973; 1978; Ross, 1966) have a well-developed file-and-scraper organ (two pairs in the case of riodinids) situated between opposite segments of the abdomen, that emit substrate- and air-borne calls often audible to humans (see Fig 1h-j). In lycaenids, the plate against which teeth are rubbed may be complex, consisting of tubercles, reticulations or ridges (Alvarez, Munguira, & Martinez-Ibanez, 2014).

Acoustic signalling in ant–myrmecophile interactions

Evidence that the acoustics of myrmecophiles are adaptive to their interactions with ants has progressed from correlative studies to two experimental approaches: muting the myrmecophile or recording and playing back their calls to undisturbed ant colonies.

First, DeVries (1991c) showed that fewer ants attended larvae of the mutualistic riodinid *Thisbe irenea* that had been artificially silenced compared with controls that were able to call, establishing that at least one function of riodinid calls is to attract ants. Similarly, Travassos and Pierce (2000) demonstrated that pupae of the lycaenid *Jalmenus evagoras* stridulated more frequently in the presence of *Iridomyrmex anceps* ants, and attracted and maintained a larger number of guards than muted ones. The calls convey the pupa's value as a provider of nutritious secretions to the ants, which does however, represent a significant cost to the pupae. Tended pupae have been shown to lose 25% of weight and take longer to eclose than untended ones (Pierce, Kitching, Buckley, Taylor, & Benbow, 1987). In further behavioural experiments Travassos and Pierce (2000) showed that pupae used acoustic signalling to adjust the number of attendant ants. They provided a path from an *I. anceps* nest to signalling pupae and scored the rate of worker movement in relation to signal strength once the pupa was discovered. This appears to be an important fitness component evolved to attract no more than an adequate number of ant guards against enemy attacks. The larvae of *J. evagoras* produce more varied acoustic signals than pupae - grunts, hisses and drumming – and are also heavily attended and guarded by their mutualist ant (Pierce et al., 2002). Hisses are emitted briefly after encountering a worker, whereas grunts are produced throughout ant attendance. The ability of *J. evagoras* juveniles to produce distinct vibrations, some probably with different functions, suggests the evolution of a finely-tuned acoustic system of communication with their hosts, which might be elucidated using play-back experiments.

In parasitic interactions with ant colonies, the clearest evidence to date that some acoustic signals are mimetic involves the highly specialized species of the *Myrmica* ant - *Maculinea* butterfly and *Pheidole* ant - *Paussus* beetle systems. Initially, DeVries and colleagues (1993) showed that the calls made by larvae of four *Maculinea* species differed from those of phytophagous lycaenids in showing distinctive pulses that resembled the stridulations of *Myrmica* worker ants. This was the first suggestion of mimicry of an adult host attribute by the caterpillars, which appeared to be genus- rather than species-specific. The insects in early experiments were unavoidably alarmed, being held with forceps during the recording, but a similar genus-specific result was later obtained using modern equipment and unstressed ants and butterflies. Both the pupae and larvae of *Maculinea* species closely mimicked three attributes of their hosts' acoustic signals: dominant frequency, pulse length, pulse repetition frequency (Barbero, Bonelli et al., 2009, Barbero, Thomas et al., 2009). However, the calls of both stages were significantly more similar to queen ant calls than they were to worker calls, despite each being generated in a different way (see Fig.1f-j). Behavioural bioassays, where the calls of butterflies and ants were played back to unstressed *Myrmica* workers, revealed that the calls of juvenile *Maculinea*, especially those of pupae, caused workers to respond as they do to queen ant calls. Both types of acoustic stimuli caused worker ants to aggregate, antennate the source of sound, and show significantly higher levels of guarding behaviour than was elicited in response to worker ant calls (Barbero, Thomas et al., 2009).

Similar, but more sophisticated communication, was recently described between the carabid beetle *Paussus favieri,* an obligate social parasite in all stages of its life-cycle, and their host ant *Pheidole pallidula* (Di Giulio et al., 2015). Here the adult beetle can generate three types of call when it stridulates, which respectively mimic the calls made by the queens, the soldiers and the minor worker caste of its host. These calls elicit a range of responses when played back to worker ants, consistent with the intruder's more diverse activities (compared to juvenile *Maculinea*) in different parts of the host's society and nest. Thus *P. favieri*'s various stridulations can elicit recruitment, including digging (rescue) behaviour, as well as the enhanced level of 'royal' (queen ant) protection observed towards *Maculinea* pupae and larvae.

[insert Figure 1]

Larval acoustic signals and phylogeny in the Lycaenidae

Various authors (e.g. DeVries, 1991a, 1991b; Fiedler, 1998; Pech, Fric, Konvicka, & Zrzavy, 2004; Pellissier, Litsios, Guisan, & Alvarez, 2012; Pierce et al., 2002) have analysed the evolution of myrmecophily in lycaenids and riodinids, including social parasitism in the Lycaenidae, and most concluded that it also provided a template for diversification and radiation in these species-rich families. Pierce and colleagues (2002) argued convincingly that social parasitism (including entomophagy of the domestic Hemiptera of ants) has evolved independently in at least 20 lineages.

The analysis of acoustics as a parameter in evolutionary studies of these taxa was pioneered by DeVries (1991a, 1991b). In seminal early papers, DeVries (1991a, 1991b) found that only lycaenids and riodinids that interacted with ants produced calls, while several non myrmecophilous members of the tribe Eumaeini were silent. Subsequent studies and reviews confirmed this pattern (e.g. Fiedler, Seufert, Maschwitz, & Idris, 1995) and provided evidence of the use of lycaenid calls in enhancing the interaction with ants (Pierce et al., 2002; Barbero, Thomas et al., 2009, Sala et al. 2014). However, some lycaenid and riodinid larvae and pupae also emit sounds when disturbed by putative predators or parasitoids, even if ants are absent. In addition, other species classed as having no interaction with ants do emit sound (e.g. Alvarez et al., 2014; Downey & Allyn, 1973; 1978; Fiedler, 1992, 1994; Schurian & Fiedler, 1991). The most recent study, by Riva and colleagues (in press), found that lycaenid sounds are highly specific and are emitted by both non- and myrmecophilous species. Calls by species that are least associated with ants consist of shorter and more distant pulses relative to those of species that are highly dependent on them.

Here we further explore the hypothesis that the strength of ant-myrmecophile interactions (using Fiedler's 1991 definitions) leads to characteristic sound profiles that may be a better predictor of the similarity of sound between species than their phylogenetic distance. We present a new analysis of the acoustic profiles made by 13 species of European lycaenids, ranging from highly integrated 'cuckoo' social parasites (*Maculinea alcon, Ma. rebeli*) via one host-specific mutualist (*Plebejus argus*) and a spectrum of generalist myrmecophiles, to species for which little or no interaction is known (*Lycaena* spp.). The 13 species (see Fig. 2) are a subset of the commensal or mutualistic species used by Riva and colleagues (in press), with three species of *Maculinea* added to represent the two levels of intimate integration found in this socially parasitic genus (Thomas, Schönrogge et al., 2005).

Fourth instar caterpillars were recorded using customized equipment, as described by Riva and colleagues (in press). We analyzed recordings of three individuals per species, randomly selecting two trains of five pulses in each trace. Fourteen sound parameters were measured using Praat v. 5.3.53 (Boersma & Weenink, 2013). These included the lower and higher quartiles of the energy spectrum (Hz), power (dB^2), intensity (dB), the root-mean-square intensity level (dB) and the relation of the frequency peak energy to the call total energy (%). Two temporal variables were measured from the oscillogram: the duration of the pulse (s) and the Pulse Rate (calculated as $1/t_{start}(x) - t_{start}(x+1)$; s-1). Six additional variables were estimated on each pulse by inspection of power spectra: the frequency of the first, second and third peak amplitudes (Hz), the intensity of the first two peaks (dB) and the center of gravity (Hz).

Hierarchical Cluster analyses was performed on a matrix of normalized Euclidean distances over sound parameters, averaged by individual using unweighted pair-group average (UPGMA) in Primer v. 6.1.12 (Primer-E Ltd.). A two-sample *t* - test was used to compare differences between group distances. To test whether species differences reflect degrees of myrmecophily, we used Phylogenetic Regression as implemented in the library “phyreg” (Grafen, 1989) using R (R Core Team, 2015). Principal components, derived by PCA on log-transformed sound parameters, were correlated with the degree of myrmecophily while controlling for phylogenetic relatedness among species. To assemble a working phylogeny, we used cytochrome oxidase subunit 1 (COI) sequences of the 13 lycaenid species from two recent studies on the Romanian and Iberian butterflies (Dinca et al., 2015; Dinca, Zakharov, Hebert, & Vila, 2011). Geneious Pro 4.7.5 (Biomatters, http://www.geneious.com/) was used to align COI sequences and to produce a neighbor-joining (NJ) tree. We also included in the phylogeny *Hamearis lucina* (Riodinidae) and *Pieris rapae* (Pieridae) as outgroups.

Two trees for species' phylogenetic distance and for the similarity of acoustic profiles are presented in Figure 2, together with the score for myrmecophily of each species. Similarities in sound profiles neatly match the spectrum of observed strengths and specificities in myrmecophily across the study species, much more closely than does phylogeny. Overall, PC1 of the acoustic parameters explained 56% and PC2 a further 27% of variation, and both were significantly correlated with the differences in myrmecophilous relationships (PC1: $F_{1,13} = 11.146$, $P = 0.005$; PC2: $F_{1,13} = 6.959$, $P = 0.020$) after accounting for phylogeny using phylogenetic regression.

It is apparent that the sound profiles of *Ma. rebeli* and *Ma. alcon* (average Euclidean distance (± 1SD) between *Ma. rebeli* and *Ma. alcon* = 1.65 ± 0.14) are far removed from all other species, including from their congeners *Ma. arion* and *Ma. teleius* (Barbero, Bonelli et al., 2009; Sala et al., 2014). Indeed, the mean Euclidean distances in the acoustic signals of *Ma. alcon* or *Ma. rebeli* from other lycaenid species are among the highest measured to date (mean Euclidean acoustic distance of *Ma. alcon vs.* lycaenids other than *M. rebeli*: 7.41 ± 1.00; *Ma. rebeli* vs lycaenids other than *Ma. alcon*: 7.66 ± 1.01; see also Riva et al. in press). This is consistent with the intimate level of social integration these species achieve within host ant nests, an association that is so close that in times of shortage the ants kill their own brood to feed to these 'cuckoos' in the nest (Thomas, Elmes, Schönrogge, Simcox, & Settele, 2005). It is also notable that the acoustics of *Plebejus argus*, the only host-specific myrmecophile among the mutualistic species, is less similar to its nearest relative *Plebejus argyrognomon*, and appears to converge with the two 'predatory' *Maculinea* social parasites even though its 'host' ant, *Lasius niger*, has no known stridulation organs and belongs to a different subfamily to *Myrmica* (mean Euclidean acoustic distance of *P. argus vs. P. argyrognomon*: 4.33 ± 0.30; *P. argus vs M. arion*: 2.51 ± 0.55; paired *t* test: $t_{16} = -8.723$, $P < 0.001$; distance of *P. argus vs. Ma. teleius*: 3.79 ± 0.28; paired *t* test: $t_{16} = -3.963$, $P = 0.001$). *Scolitantides orion* perhaps represents selection in the opposite direction to *P. argus*, being less host specific than its ancestry or relatives might suggest, as, less convincingly, may

Polyommatus icarus. Yet despite *L. coridon* and *L. bellargus* being close congeners, sounds emitted by *L. bellargus* are much more similar to those produced by *P. argyrognomon* (belonging to the same myrmecophilous category - 3) rather than to *L. coridon* (mean Euclidean acoustic distance of *L. coridon* vs *L. bellargus*: 3.87 ± 0.15; *P. argyrognomon* vs *L. bellargus*: 1.54 ± 0.20; paired t test: $t_{16} = 27.775$, $P < 0.001$). A possible, but untested, explanation is that this reflects a similar disruptive selection via acoustics to that described in sympatric lineages of the ant *Pachycondyla*, since the juveniles of these congeneric butterflies overlap largely in distribution, sharing the same single species of foodplant and often the same individual plant.

However, given the small number of species studied, we caution against over-interpreting the apparent patterns depicted in Figure 2, and suggest they be tested by comparative behavioural experimentation. We also recognise that vibrations of less- or non-myrmecophilous lycaenids (and other taxa) may have very different functions, such as repelling natural enemies (Bura, Fleming, & Yack, 2009; Bura, Rohwer, Martin, & Yack, 2011). We tentatively suggest that ancestral species in the Lycaenidae were preadapted to myrmecophily through an ability to make sounds, and that once behavioural relationships with ants evolved, the selection regime changed resulting in adaptive mimetic sound profiles, at least among obligate myrmecophiles.

[insert Figure 2]

Conclusions & Future Research

Ants are known to sometimes use multiple cues to moderate kin behaviour, for example by combining posturing, tactile and chemical interactions to convey complex or sequential information and to elicit particular responses between members of their society (Hölldobler & Wilson, 1990). To date little is known of how acoustic signalling might interact with other means of communication, and less still of whether myrmecophiles manipulate behaviour using multiple cues.

Sound may be used synergistically with other modes of signalling. Hölldobler and colleagues (1994) studied the role of audible vibrational signals made by the Ponerine ant *Megaponera foetens*, a raider of termite colonies, in the context of trail following and column building. They found that stridulations were emitted only during disturbances and for predator avoidance. It is also known that *M. foetens* has a distinctive pheromone to signal alarm (Janssen, Bestmann, Hölldobler, & Kern, 1995). These observations suggest that vibrations may be used to qualify a general alarm signal that is chemical, but again this requires formal testing. This is in contrast to the observations by Casacci and colleagues (2013) described above where acoustic signalling appears to replace chemical and tactile signal apparently with the same function of signalling rank, but this is not truly a case of multimodal communication.

To date, no direct evidence exists for the behavioural consequences of full synergistic multimodal communication involving acoustics. Yet the interactions of *Maculinea* butterfly larvae and their *Myrmica* host ant societies illustrate the importance of both chemical and acoustic mimicry. Here, the acceptance (or rejection) of larvae as members of their host colony appears to be based entirely on a mimetic mixture of chemical secretions, but on this cue alone intruders are treated simply like the low-ranking kin brood (Akino, Knapp, Thomas, & Elmes, 1999; Thomas et al., 2013; Thomas, Schönrogge et al., 2005). It is the ability simultaneously to emit acoustic calls that mimic adult hosts, and furthermore mimic queen sounds, that is believed to explain the observed priority ‘royal’ behaviour that workers regularly afford to social parasites, giving them a status that exceeds that of large ant larvae. Not only do these brood parasites gain priority in the distribution of food by nursery workers to the extent that workers feed younger kin ant brood to the *Maculinea* larvae when food is short, but they are also carried ahead of kin ant brood when moving nest or during rescues

(Elmes, 1989; Gerrish, 1994; Thomas, Schönrogge, et al., 2005). Anecdotal observations of the manipulation of *Paussus favieri* by the beetle *Pheidole pallidula* suggests a similar chemical-acoustic mechanism (Di Giulio et al., 2015), but as with ant-ant communication itself, the putative use of acoustics in multimodal communication requires rigorous testing. About 10,000 species of invertebrates from 11 orders are estimated have evolved adaptations to infiltrate ant societies and live as parasites inside nests (Hölldobler & Wilson, 1990). Current studies have largely focussed on the family Lycaenidae among the Lepidoptera and a few selected species of Coleoptera. While the study systems used today provide some variety in the type of interactions with their host ants, there is clearly a vast variety still to be discovered to understand respective roles of signalling modes and the social interactions in ants and other social insects.

The important role that acoustic signalling has in ant- and other social insect societies is well established and it is perhaps unsurprising that other, interacting species show adaptations that relate to the hosts acoustic traits. In only a few cases, however, has the role of vibro-acoustics in mediating myrmecophile - host interactions been investigated experimentally. The modalities of signal production, transmission and reception remain largely unknown for most species of myrmecophiles or indeed their hosts, but the greatest future challenge is to understand how different modes of signalling interact. Social insects are well known to interpret stimuli in a context-dependent manner, where the same stimulus can trigger a different behaviour when encountered under different circumstances (Hölldobler & Wilson 1990). Other aspects of insect social behaviour have been subject to sophisticated and successful experimentation, and it should be possible to unravel this essential aspect of communication. Hunt and Richards (2013) suggested that understanding the suites of modalities in signalling enables a clearer view of the adaptive role of multimodal communication, and while that has been true for rare examples such as the honey bee waggle dance, research into understanding the role of ant acoustics is in its infancy. With the development of recording equipment that is portable, affordable, which can focus on individuals and record sound and behaviour at the same time, our understanding of social interactions should become more specific. Such instruments, laser-vibrometers and hand-held "noses" for acoustic and chemical analyses, are being developed for engineering applications and could be deployed to record acoustic and chemical signals in behavioural science in the near future. Technological developments in both recording equipment and behavioural experimentation will allow designing studies following the same principles to investigate synergistic effects of multiple chemical signals.

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Ethical Note

The authors confirm that their work adheres to the ASAB/ABS and ARRIVE Guidelines. The guide to ethical information required for papers published in the journal has been consulted as well. *Maculinea* caterpillars were collected under permit from The Italian Ministry for the Environment (protocol numbers: 446/05. DPN/2D/2005/13993 & 0012494/PNM/2015). The authors declare that there is no conflict of interest.

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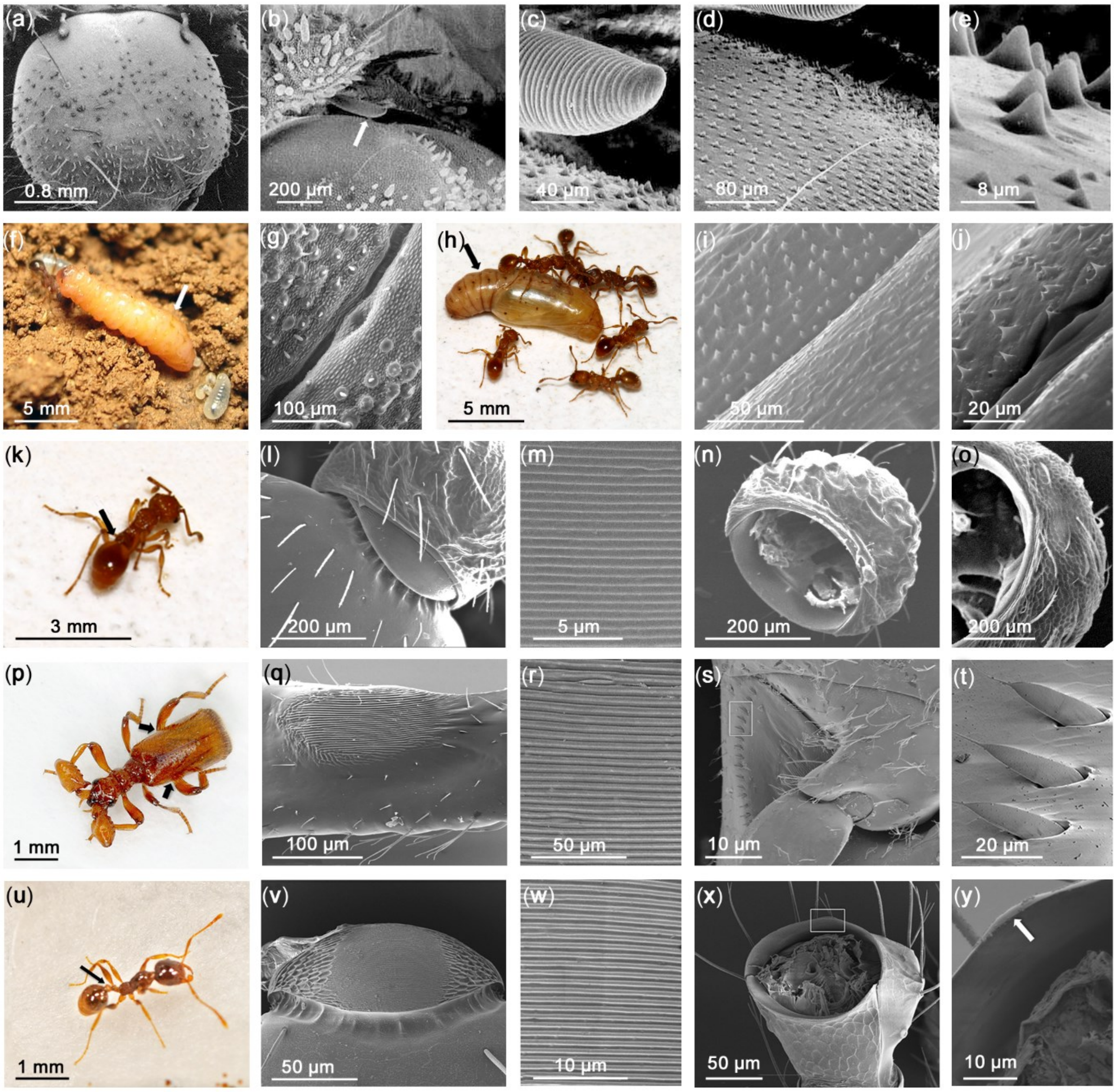
Figures

Figure 1. The comparative morphology of sound production organs in myrmecophiles and host ants. (a-e) the riodinids *Synargis gela* and *Thisbe irenea* (Riodinidae); larva (f, g) and pupa (h-j) of the obligate lycaenid social parasite *Maculinea rebeli* and its adult host ant *Myrmica schencki* (k-o); the adult beetle *Paususs favieri* (p-t) and its host *Pheidole pallidula* (u-y). (a) Frontal view of *Synargis gela* head showing typical position of the riodinid vibratory papillae; (b) general view of *Thisbe irenea* anterior edge of segment T-1 showing a vibratory papilla (arrow) and the surface of the epicranium where the vibratory papilla strikes; (c) detail of the vibratory papilla showing the annulations on its shaft and the epicranial granulations; (d) enlarged view of the epicranial granulation and vibratory papilla; (e) details showing two sizes of epicranial granulations. (f) Position of (g) the presumed sound producing organ of *Maculinea rebeli* caterpillars and of its pupa (h), formed by a stridulatory plate (*pars stridens*) placed on the fifth abdominal segment and a file (*plectrum*) in the sixth abdominal segment. (k,p,u) Respective positions of the stridulatory organs of *Myrmica schencki*, *Paussus favieri* and *Pheidole pallidula*; the organs are composed of suboval pars stridens (l,q,v) with minute ridges (m,r,w) and a plectrum (n, x) consisting of a medial cuticular prominence (t,y) that originates from the posterior edge of the postpetiole in the two ant species or of a curved row of small cuticular spines in *P. favieri* (s,t). (a, modified by De Vries 1991; b-e modified by DeVries 1988; p-y modified by Di Giulio et al. 2015).

Figure 2. A diagram of the phylogeny (left) and the cluster analysis constructed from a matrix of pairwise normalized Euclidean distances of the sound profiles from three caterpillars of 13 species of lycaenid. Symbols and values refer to the intensity of interaction of the lycaenid species with their host ants (0 = none; 4 = social parasite), following Fiedler (1991).

Figure 1:


(a)
0.8 mm
(b)
200 µm
(c)
40 µm
(d)
80 µm
(e)
8 µm
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1 mm
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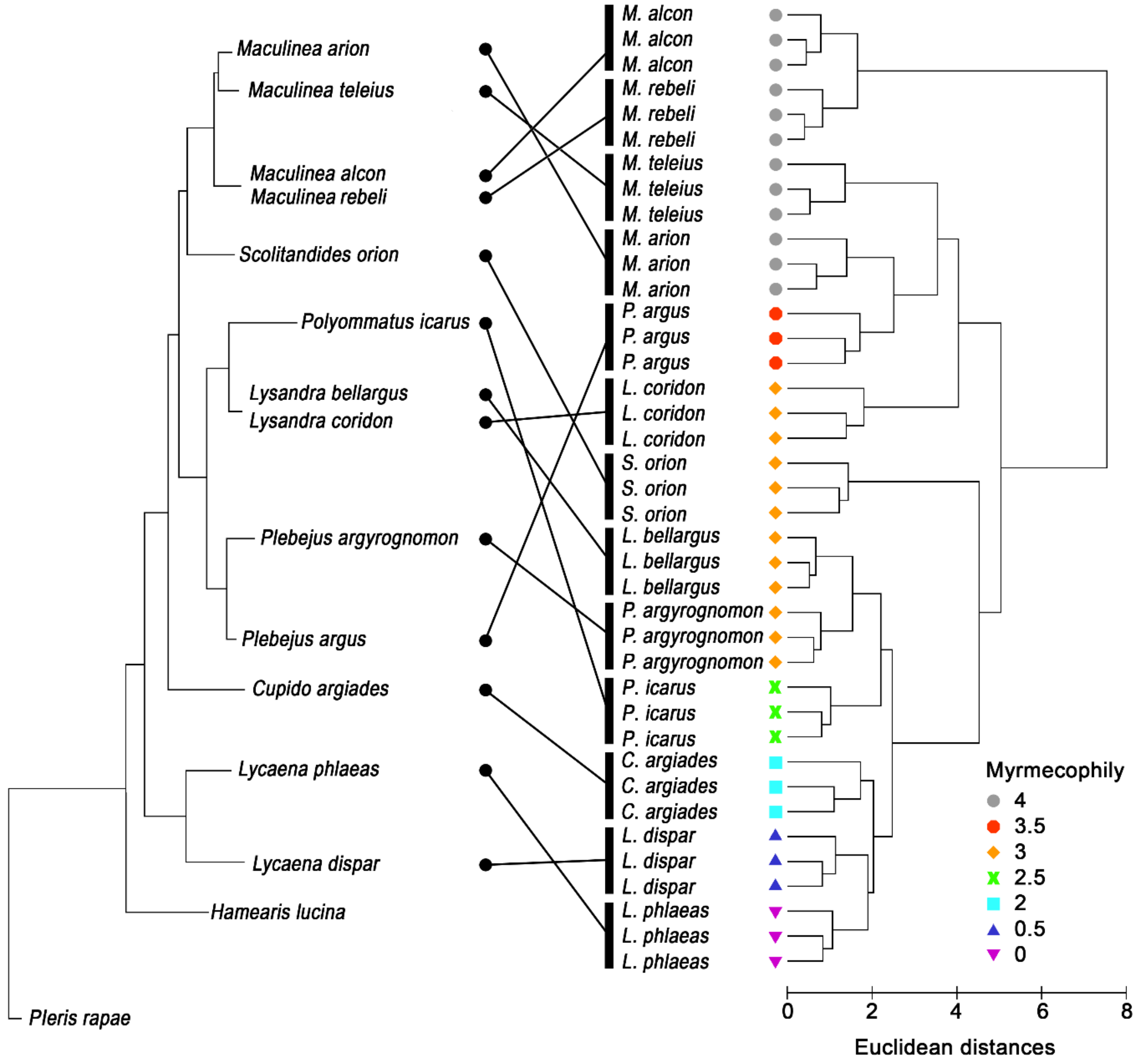
(x)
50 µm
(y)
10 µm

Figure 2:


Maculinea arion
Maculinea teleius
Maculinea alcon
Maculinea rebeli
Scolitandides orion
Polyommatus icarus
Lysandra bellargus
Lysandra coridon
Plebejus argyrognomon
Plebejus argus
Cupido argiades
Lycaena phlaeas
Lycaena dispar
Hamearis lucina
Pleris rapae
M. alcon
M. alcon
M. alcon
M. rebeli
M. rebeli
M. rebeli
M. teleius
M. teleius
M. teleius
M. arion
M. arion
M. arion
P. argus
P. argus
P. argus
L. coridon
L. coridon
L. coridon
S. orion
S. orion
S. orion
L. bellargus
L. bellargus
L. bellargus
P. argyrognomon
P. argyrognomon
P. argyrognomon
P. icarus
P. icarus
P. icarus
C. argiades
C. argiades
C. argiades
L. dispar
L. dispar
L. dispar
L. phlaeas
L. phlaeas
L. phlaeas
Myrmecophily
● 4
● 3.5
◆ 3
✗ 2.5
■ 2
▲ 0.5
▼ 0
0
2
4
6
8
Euclidean distances