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

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36 Abstract:

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

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

51

52 Introduction

53 Efficient communication to coordinate the actions of up to a million specialised nestmates is  
54 fundamental to the success of social insects, especially ants. Various modes of signalling have  
55 been identified, including the release of semio-chemicals, visual behavioural displays involving  
56 movement or posture, tactile interactions, and the comparatively poorly studied use of acoustic  
57 signals (Hölldobler & Wilson, 1990, 2009). As hotspots of resources in their environment, ants  
58 fiercely defend their colonies using a wide range of weapons (e.g. gland secretions,  
59 mandibles, sting), which are deployed in the manner of co-ordinated attacks by legions of  
60 intercommunicating workers. Nevertheless, ant nests are also magnets for other organisms  
61 that have evolved means to overcome the hostility of the host ants. Thus, an estimated  
62 ~10,000 invertebrate species live as obligate social parasites of ants, able to penetrate and  
63 exploit the resources within host colonies in order to complete their life-cycle (Thomas,  
64 Schönrogge, Elmes, 2005). The large majority of these adaptations evolved in many separate  
65 lines, especially among Coleoptera, Diptera, Lepidoptera and other Hymenoptera, from a ten-  
66 times greater number of commensals or mutualists (Fiedler, 1998; Hölldobler & Wilson, 1990;  
67 Nash & Boomsma, 2008; Pierce et al., 2002; Thomas, Schönrogge et al., 2005). All these  
68 myrmecophiles show morphological, behavioural, chemical or acoustic adaptations to interact  
69 with ants (Cottrell, 1984; Donisthorpe, 1927; Hinton, 1951; Lenoir, D'Ettorre, Errard, & Hefetz,  
70 2001; Malicky, 1969; Wasmann, 1913; Wheeler, 1910; Witek, Barbero, & Marko, 2014).  
71 Armour, stealth and the secretion of attractive food rewards are frequently sufficient for  
72 unspecific or facultative myrmecophiles to access the enemy-free spaces of ants. However,  
73 the subversion of the ants' chemical and/or acoustic signalling is generally required to enable  
74 true social parasites (*sensu* Nash & Boomsma, 2008) to live for long periods as undetected  
75 intruders in close contact with their hosts.

76 A key element of successful co-habitation in ant nests is to circumvent the host's ability to  
77 differentiate between nestmates and intruders. Nestmate recognition is a dynamic process,

78 primarily based on the detection of distinctive species- or colony-specific cocktails of cuticular  
79 hydrocarbons (CHC) covering the surface of all individuals (Hölldobler & Wilson, 1990;  
80 Howard, 1993; vander Meer & Morel, 1998; Winston, 1992). Social interactions such as  
81 allogrooming ensure an exchange between the CHC mixtures among nestmates and give rise  
82 to a shared CHC *gestalt* odour (vander Meer & Morel, 1998). The role that chemical  
83 communication and nestmate recognition have in maintaining the cohesion of ant societies  
84 and those of other social insects has been subject to extensive study, with excellent recent  
85 reviews, for example by Martin & Drijfhout (2009) and van Wilgenburg, Symonds, & Elgar  
86 (2011): The deployment of chemical communication by obligate social parasites to subvert  
87 host recognition systems is equally well reviewed (e.g. Lenoir et al., 2001; von Thienen,  
88 Metzler, Choe, & Witte, 2014).

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

96 We first examine ant sound producing organs and convergent adaptations that allow non-ant  
97 organisms to mimic and subvert ant–ant communications, focussing on advances in  
98 knowledge since the reviews by Hölldobler & Wilson (1990), Fiedler (1998), Pierce and  
99 colleagues (2002), Thomas and colleagues (2005) and Nash & Boomsma (2008), or covered  
100 cursorily by Witek and colleagues (2014). We then review recent insights concerning the ant  
101 acoustic signals themselves and their corruption by social parasites. This includes both the  
102 morphological adaptations to produce acoustic signals, the behavioural responses to them,  
103 and thus the impact on ant – social parasite/guest interactions. Much of this builds on the  
104 pioneering work of Markl (1965, 1967), DeVries (1991a, 1991b), Hölldobler, Braun,

105 Gronenberg, Kirchner, & Peeters (1994) and Kirchner (1997). Finally we present new data  
106 relating the intimacy of interactions of lycaenid butterfly larvae to phylogeny and the similarity  
107 of acoustic signalling.

### 108 *Acoustic signalling in ants*

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

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

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

147 Nestmate recruitment is the most frequently reported function for ant–ant acoustic signalling.  
148 For example, outside the nest, *Atta cephalotes* uses vibratory signals to attract foraging  
149 workers towards newly found food sources (Roces & Hölldobler 1995). The same authors also  
150 observed that in the presence of parasitic phorid flies, foragers used acoustics to recruit minor  
151 workers for defence, thus also employing vibrations as alarm signals (Roces & Hölldobler,  
152 1995, 1996). Finally, although created by a scraper and file organ located on the first gastric  
153 tergite and the post-petiole, Tautz and colleagues (1995) observed that vibrations travelled  
154 the length of the body to the mandibles, aiding the cutting of soft young leaf tissue by

155 stiffening it. Behavioural experiments, however, suggest that this is a secondary effect and  
156 that communication is the main function for these vibrations (Roces & Hölldobler, 1996).

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

166 Acoustic patterns also signal caste and hierarchical status in at least two genera of Myrmicinae  
167 ants: *Myrmica* (Barbero, Thomas et al., 2009) and *Pheidole* (Di Giulio et al., 2015). In both  
168 taxa, the queens produce distinctive stridulations which, when played back to kin workers,  
169 elicit additional 'royal' protective behaviours compared with responses to worker signals  
170 (Barbero, Bonelli, Thomas, Balletto, & Schönrogge, 2009; Barbero & Casacci, 2015; Barbero,  
171 Thomas et al., 2009; Casacci et al., 2013; Ferreira, Poteaux, Delabie, Fresneau, & Rybak,  
172 2010). In addition, in *Pheidole pallidula* the soldier and minor worker castes also make  
173 distinctive vibroacoustic signals (Di Giulio et al., 2015). Unlike *Pachycondyla* species, little  
174 inter-specific variation was detected in either the queen- or worker-sounds made by closely-  
175 related sympatric species of *Myrmica* (Barbero et al., 2012; Barbero, Thomas et al., 2009;  
176 Thomas, Schönrogge, Bonelli, Barbero, & Balletto, 2010), which are instead clearly  
177 demarcated by unique hydrocarbon profiles (Elmes, Akino, Thomas, Clarke, & Knapp, 2002).  
178 Although the young stages of tested ants are mute (e.g. DeVries, Cockerill, & Thomas, 1993),  
179 Casacci and colleagues (2013) found that acoustic signalling appears to act as a substitute  
180 for other forms of communication in developing *Myrmica* pupae. The various stages of ant



181 brood, from egg to pupa, are afforded ascending levels of priority based on tactile and  
182 chemical cues (Brian, 1975). Most are mute, but the older “brown”, sclerotised pupae of  
183 *Myrmica* species produce calls, emitted as single pulses, similar to those of workers (Casacci  
184 et al. 2013). This coincides with a presumed reduced ability to secrete brood recognition  
185 pheromones during this period, and brown pupae that were experimentally silenced fell  
186 significantly behind their mute white siblings in social standing.

### 187 *Acoustic signals of myrmecophiles*

188 Derived acoustic signals that enhance interactions with ants are increasingly being confirmed  
189 in both juvenile and adult stages of myrmecophiles. To date, most studies involve riodinid and,  
190 especially, lycaenid butterfly larvae and pupae (e.g. Barbero, Thomas et al., 2009; DeVries,  
191 1990, 1991a; Pierce et al., 2002). However, similar phenomena were recently described from  
192 adults of a socially parasitic beetle, *Paussus favieri* (Di Giulio et al., 2015), where males and  
193 females emit mimetic stridulations using a row of scrapers on the proximal abdominal segment  
194 rasping across a file located on the hind femora (see Fig. 1p-t).

### 195 *Stridulation organs*

196 With a few exceptions, an ability to produce calls occurs after the third larval moult in riodinid  
197 and lycaenid larvae, coinciding with the development of chemical ‘ant organs’, which perhaps  
198 suggests they act synergistically (DeVries, 1991a). In most riodinids, acoustic signals are  
199 generated by grooved vibratory papillae. These are typically found in pairs on the prothorax,  
200 and grate against specialised epicranial granulations when the larva rotates its head (see Fig  
201 1a-e), especially when walking or under attack, generating low amplitude substrate-borne calls  
202 (DeVries, 1991a). The tribe Eurybiini lacks vibratory papillae; instead, caterpillars generate  
203 calls by scraping teeth on a prothoracic cervical membrane against the epicranial granulations  
204 in at least some mutualists or entomophagous predators of ant-tended Homoptera (DeVries  
205 & Penz, 2002; Travassos, DeVries, & Pierce, 2008). The detection of dedicated organs in

206 lycaenid larvae that produce calls has been elusive, apart from a file-and-scraper described  
207 between the 5<sup>th</sup> and 6<sup>th</sup> abdominal segments of *Arhopala madytus* (C. J. Hill, 1993) and a  
208 putative organ in *Maculinea rebeli* larvae (see Fig.1fg). In other species strong substrate-borne  
209 vibrations (and apparently weak air-borne sounds) may be generated by muscular  
210 contractions of the abdomen, which compress air through the tracheae to produce distinctive  
211 rhythms and intensities in the manner of a wind instrument, as described by Schurian and  
212 Fiedler (1991) for *Polyommatus dezinus*. These vibroacoustic signals range from low  
213 background calls punctuated by pulses in mutualists (DeVries, 1991a) to the grunts, drumming  
214 and hisses of the host-specific *Jalmenus evagoras* (Travassos & Pierce, 2000), to the mimetic  
215 calls of *Maculinea* larvae (Barbero, Bonelli et al., 2009; DeVries et al., 1993; Sala, Casacci,  
216 Balletto, Bonelli, & Barbero, 2014).

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

### 223 *Acoustic signalling in ant–myrmecophile interactions*

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

227 First, DeVries (1991c) showed that fewer ants attended larvae of the mutualistic riodinid  
228 *Thisbe irenea* that had been artificially silenced compared with controls that were able to call,  
229 establishing that at least one function of riodinid calls is to attract ants. Similarly, Travassos  
230 and Pierce (2000) demonstrated that pupae of the lycaenid *Jalmenus evagoras* stridulated  
231 more frequently in the presence of *Iridomyrmex anceps* ants, and attracted and maintained a

232 larger number of guards than muted ones. The calls convey the pupa's value as a provider of  
233 nutritious secretions to the ants, which does however, represent a significant cost to the  
234 pupae. Tended pupae have been shown to lose 25% of weight and take longer to eclose than  
235 untended ones (Pierce, Kitching, Buckley, Taylor, & Benbow, 1987). In further behavioural  
236 experiments Travassos and Pierce (2000) showed that pupae used acoustic signalling to  
237 adjust the number of attendant ants. They provided a path from an *I. anceps* nest to signalling  
238 pupae and scored the rate of worker movement in relation to signal strength once the pupa  
239 was discovered. This appears to be an important fitness component evolved to attract no more  
240 than an adequate number of ant guards against enemy attacks. The larvae of *J. evagoras*  
241 produce more varied acoustic signals than pupae - grunts, hisses and drumming – and are  
242 also heavily attended and guarded by their mutualist ant (Pierce et al., 2002). Hisses are  
243 emitted briefly after encountering a worker, whereas grunts are produced throughout ant  
244 attendance. The ability of *J. evagoras* juveniles to produce distinct vibrations, some probably  
245 with different functions, suggests the evolution of a finely-tuned acoustic system of  
246 communication with their hosts, which might be elucidated using play-back experiments.

247 In parasitic interactions with ant colonies, the clearest evidence to date that some acoustic  
248 signals are mimetic involves the highly specialized species of the *Myrmica* ant - *Maculinea*  
249 butterfly and *Pheidole* ant - *Paussus* beetle systems. Initially, DeVries and colleagues (1993)  
250 showed that the calls made by larvae of four *Maculinea* species differed from those of  
251 phytophagous lycaenids in showing distinctive pulses that resembled the stridulations of  
252 *Myrmica* worker ants. This was the first suggestion of mimicry of an adult host attribute by the  
253 caterpillars, which appeared to be genus- rather than species-specific. The insects in early  
254 experiments were unavoidably alarmed, being held with forceps during the recording, but a  
255 similar genus-specific result was later obtained using modern equipment and unstressed ants  
256 and butterflies. Both the pupae and larvae of *Maculinea* species closely mimicked three  
257 attributes of their hosts' acoustic signals: dominant frequency, pulse length, pulse repetition  
258 frequency (Barbero, Bonelli et al., 2009, Barbero, Thomas et al., 2009). However, the calls of

259 both stages were significantly more similar to queen ant calls than they were to worker calls,  
260 despite each being generated in a different way (see Fig.1f-j). Behavioural bioassays, where  
261 the calls of butterflies and ants were played back to unstressed *Myrmica* workers, revealed  
262 that the calls of juvenile *Maculinea*, especially those of pupae, caused workers to respond as  
263 they do to queen ant calls. Both types of acoustic stimuli caused worker ants to aggregate,  
264 antennate the source of sound, and show significantly higher levels of guarding behaviour  
265 than was elicited in response to worker ant calls (Barbero, Thomas et al., 2009).

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

276 [insert Figure 1]

#### 277 *Larval acoustic signals and phylogeny in the Lycaenidae*

278 Various authors (e.g. DeVries, 1991a, 1991b; Fiedler, 1998; Pech, Fric, Konvicka, & Zrzavy,  
279 2004; Pellissier, Litsios, Guisan, & Alvarez, 2012; Pierce et al., 2002) have analysed the  
280 evolution of myrmecophily in lycaenids and riodinids, including social parasitism in the  
281 Lycaenidae, and most concluded that it also provided a template for diversification and  
282 radiation in these species-rich families. Pierce and colleagues (2002) argued convincingly that  
283 social parasitism (including entomophagy of the domestic Hemiptera of ants) has evolved  
284 independently in at least 20 lineages.

285 The analysis of acoustics as a parameter in evolutionary studies of these taxa was pioneered  
286 by DeVries (1991a, 1991b). In seminal early papers, DeVries (1991a, 1991b) found that only  
287 lycaenids and riodinids that interacted with ants produced calls, while several non  
288 myrmecophilous members of the tribe Eumaeini were silent. Subsequent studies and reviews  
289 confirmed this pattern (e.g. Fiedler, Seufert, Maschwitz, & Idris, 1995) and provided evidence  
290 of the use of lycaenid calls in enhancing the interaction with ants (Pierce et al., 2002; Barbero,  
291 Thomas et al., 2009, Sala et al. 2014). However, some lycaenid and riodinid larvae and pupae  
292 also emit sounds when disturbed by putative predators or parasitoids, even if ants are absent.  
293 In addition, other species classed as having no interaction with ants do emit sound (e.g.  
294 Alvarez et al., 2014; Downey & Allyn, 1973; 1978; Fiedler, 1992, 1994; Schurian & Fiedler,  
295 1991). The most recent study, by Riva and colleagues (in press), found that lycaenid sounds  
296 are highly specific and are emitted by both non- and myrmecophilous species. Calls by species  
297 that are least associated with ants consist of shorter and more distant pulses relative to those  
298 of species that are highly dependent on them.

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

309 Fourth instar caterpillars were recorded using customized equipment, as described by Riva  
310 and colleagues (in press). We analyzed recordings of three individuals per species, randomly

311 selecting two trains of five pulses in each trace. Fourteen sound parameters were measured  
312 using Praat v. 5.3.53 (Boersma & Weenink, 2013). These included the lower and higher  
313 quartiles of the energy spectrum (Hz), power (dB<sup>2</sup>), intensity (dB), the root-mean-square  
314 intensity level (dB) and the relation of the frequency peak energy to the call total energy (%).  
315 Two temporal variables were measured from the oscillogram: the duration of the pulse (s) and  
316 the Pulse Rate (calculated as  $1/t_{\text{start}}(x) - t_{\text{start}}(x+1); s-1$ ). Six additional variables were estimated  
317 on each pulse by inspection of power spectra: the frequency of the first, second and third peak  
318 amplitudes (Hz), the intensity of the first two peaks (dB) and the center of gravity (Hz).

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

332 Two trees for species' phylogenetic distance and for the similarity of acoustic profiles are  
333 presented in Figure 2, together with the score for myrmecophily of each species. Similarities  
334 in sound profiles neatly match the spectrum of observed strengths and specificities in  
335 myrmecophily across the study species, much more closely than does phylogeny. Overall,  
336 PC1 of the acoustic parameters explained 56% and PC2 a further 27% of variation, and both  
337 were significantly correlated with the differences in myrmecophilous relationships (PC1:  $F_{1,13}$

338 = 11.146,  $P = 0.005$ ; PC2:  $F_{1,13} = 6.959$ ,  $P = 0.020$ ) after accounting for phylogeny using  
339 phylogenetic regression.

340 It is apparent that the sound profiles of *Ma. rebeli* and *Ma. alcon* (average Euclidean distance  
341 ( $\pm 1$ SD) between *Ma. rebeli* and *Ma. alcon* =  $1.65 \pm 0.14$ ) are far removed from all other  
342 species, including from their congeners *Ma. arion* and *Ma. teleius* (Barbero, Bonelli et al.,  
343 2009; Sala et al., 2014). Indeed, the mean Euclidean distances in the acoustic signals of *Ma.*  
344 *alcon* or *Ma. rebeli* from other lycaenid species are among the highest measured to date  
345 (mean Euclidean acoustic distance of *Ma. alcon* vs. lycaenids other than *M. rebeli*:  $7.41 \pm$   
346  $1.00$ ; *Ma. rebeli* vs lycaenids other than *Ma. alcon*:  $7.66 \pm 1.01$ ; see also Riva et al. in press).  
347 This is consistent with the intimate level of social integration these species achieve within host  
348 ant nests, an association that is so close that in times of shortage the ants kill their own brood  
349 to feed to these ‘cuckoos’ in the nest (Thomas, Elmes, Schönrogge, Simcox, & Settele, 2005).  
350 It is also notable that the acoustics of *Plebejus argus*, the only host-specific myrmecophile  
351 among the mutualistic species, is less similar to its nearest relative *Plebejus argyrognomon*,  
352 and appears to converge with the two ‘predatory’ *Maculinea* social parasites even though its  
353 ‘host’ ant, *Lasius niger*, has no known stridulation organs and belongs to a different subfamily  
354 to *Myrmica* (mean Euclidean acoustic distance of *P. argus* vs. *P. argyrognomon*:  $4.33 \pm 0.30$ ;  
355 *P. argus* vs *M. arion*:  $2.51 \pm 0.55$ ; paired  $t$  test:  $t_{16} = -8.723$ ,  $P < 0.001$ ; distance of *P. argus* vs.  
356 *Ma. teleius*:  $3.79 \pm 0.28$ ; paired  $t$  test:  $t_{16} = -3.963$ ,  $P = 0.001$ ). *Scolitantides orion* perhaps  
357 represents selection in the opposite direction to *P. argus*, being less host specific than its  
358 ancestry or relatives might suggest, as, less convincingly, may *Polyommatus icarus*. Yet  
359 despite *L. coridon* and *L. bellargus* being close congeners, sounds emitted by *L. bellargus* are  
360 much more similar to those produced by *P. argyrognomon* (belonging to the same  
361 myrmecophilous category - 3) rather than to *L. coridon* (mean Euclidean acoustic distance of  
362 *L. coridon* vs *L. bellargus*:  $3.87 \pm 0.15$ ; *P. argyrognomon* vs *L. bellargus*:  $1.54 \pm 0.20$ ; paired  $t$   
363 test:  $t_{16} = 27.775$ ,  $P < 0.001$ ). A possible, but untested, explanation is that this reflects a similar  
364 disruptive selection via acoustics to that described in sympatric lineages of the ant

365 *Pachycondyla*, since the juveniles of these congeneric butterflies overlap largely in  
366 distribution, sharing the same single species of foodplant and often the same individual plant.

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

376 [insert Figure 2]

### 377 *Conclusions & Future Research*

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

384 Sound may be used synergistically with other modes of signalling. Hölldobler and colleagues  
385 (1994) studied the role of audible vibrational signals made by the Ponerine ant *Megaponera*  
386 *foetens*, a raider of termite colonies, in the context of trail following and column building. They  
387 found that stridulations were emitted only during disturbances and for predator avoidance. It  
388 is also known that *M. foetens* has a distinctive pheromone to signal alarm (Janssen,  
389 Bestmann, Hölldobler, & Kern, 1995). These observations suggest that vibrations may be used



390 to qualify a general alarm signal that is chemical, but again this requires formal testing. This  
391 is in contrast to the observations by Casacci and colleagues (2013) described above where  
392 acoustic signalling appears to replace chemical and tactile signal apparently with the same  
393 function of signalling rank, but this is not truly a case of multimodal communication.

394 To date, no direct evidence exists for the behavioural consequences of full synergistic  
395 multimodal communication involving acoustics. Yet the interactions of *Maculinea* butterfly  
396 larvae and their *Myrmica* host ant societies illustrate the importance of both chemical and  
397 acoustic mimicry. Here, the acceptance (or rejection) of larvae as members of their host colony  
398 appears to be based entirely on a mimetic mixture of chemical secretions, but on this cue  
399 alone intruders are treated simply like the low-ranking kin brood (Akino, Knapp, Thomas, &  
400 Elmes, 1999; Thomas et al., 2013; Thomas, Schönrogge et al., 2005). It is the ability  
401 simultaneously to emit acoustic calls that mimic adult hosts, and furthermore mimic queen  
402 sounds, that is believed to explain the observed priority 'royal' behaviour that workers regularly  
403 afford to social parasites, giving them a status that exceeds that of large ant larvae. Not only  
404 do these brood parasites gain priority in the distribution of food by nursery workers to the  
405 extent that workers feed younger kin ant brood to the *Maculinea* larvae when food is short, but  
406 they are also carried ahead of kin ant brood when moving nest or during rescues (Elmes,  
407 1989; Gerrish, 1994; Thomas, Schönrogge, et al., 2005). Anecdotal observations of the  
408 manipulation of *Paussus favieri* by the beetle *Pheidole pallidula* suggests a similar chemical-  
409 acoustic mechanism (Di Giulio et al., 2015), but as with ant-ant communication itself, the  
410 putative use of acoustics in multimodal communication requires rigorous testing. About 10,000  
411 species of invertebrates from 11 orders are estimated have evolved adaptations to infiltrate  
412 ant societies and live as parasites inside nests (Hölldobler & Wilson, 1990). Current studies  
413 have largely focussed on the family Lycaenidae among the Lepidoptera and a few selected  
414 species of Coleoptera. While the study systems used today provide some variety in the type  
415 of interactions with their host ants, there is clearly a vast variety still to be discovered to

416 understand respective roles of signalling modes and the social interactions in ants and other  
417 social insects.

418 The important role that acoustic signalling has in ant- and other social insect societies is well  
419 established and it is perhaps unsurprising that other, interacting species show adaptations  
420 that relate to the hosts acoustic traits. In only a few cases, however, has the role of vibro-  
421 acoustics in mediating myrmecophile - host interactions been investigated experimentally. The  
422 modalities of signal production, transmission and reception remain largely unknown for most  
423 species of myrmecophiles or indeed their hosts, but the greatest future challenge is to  
424 understand how different modes of signalling interact. Social insects are well known to  
425 interpret stimuli in a context-dependent manner, where the same stimulus can trigger a  
426 different behaviour when encountered under different circumstances (Hölldobler & Wilson  
427 1990). Other aspects of insect social behaviour have been subject to sophisticated and  
428 successful experimentation, and it should be possible to unravel this essential aspect of  
429 communication. Hunt and Richards (2013) suggested that understanding the suites of  
430 modalities in signalling enables a clearer view of the adaptive role of multimodal  
431 communication, and while that has been true for rare examples such as the honey bee waggle  
432 dance, research into understanding the role of ant acoustics is in its infancy. With the  
433 development of recording equipment that is portable, affordable, which can focus on  
434 individuals and record sound and behaviour at the same time, our understanding of social  
435 interactions should become more specific. Such instruments, laser-vibrometers and hand-held  
436 “noses” for acoustic and chemical analyses, are being developed for engineering applications  
437 and could be deployed to record acoustic and chemical signals in behavioural science in the  
438 near future. Technological developments in both recording equipment and behavioural  
439 experimentation will allow designing studies following the same principles to investigate  
440 synergistic effects of multiple chemical signals.

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450 Ethical Note

451 The authors confirm that their work adheres to the ASAB/ABS and ARRIVE Guidelines. The  
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455 The authors declare that there is no conflict of interest.

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689 Figures

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

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

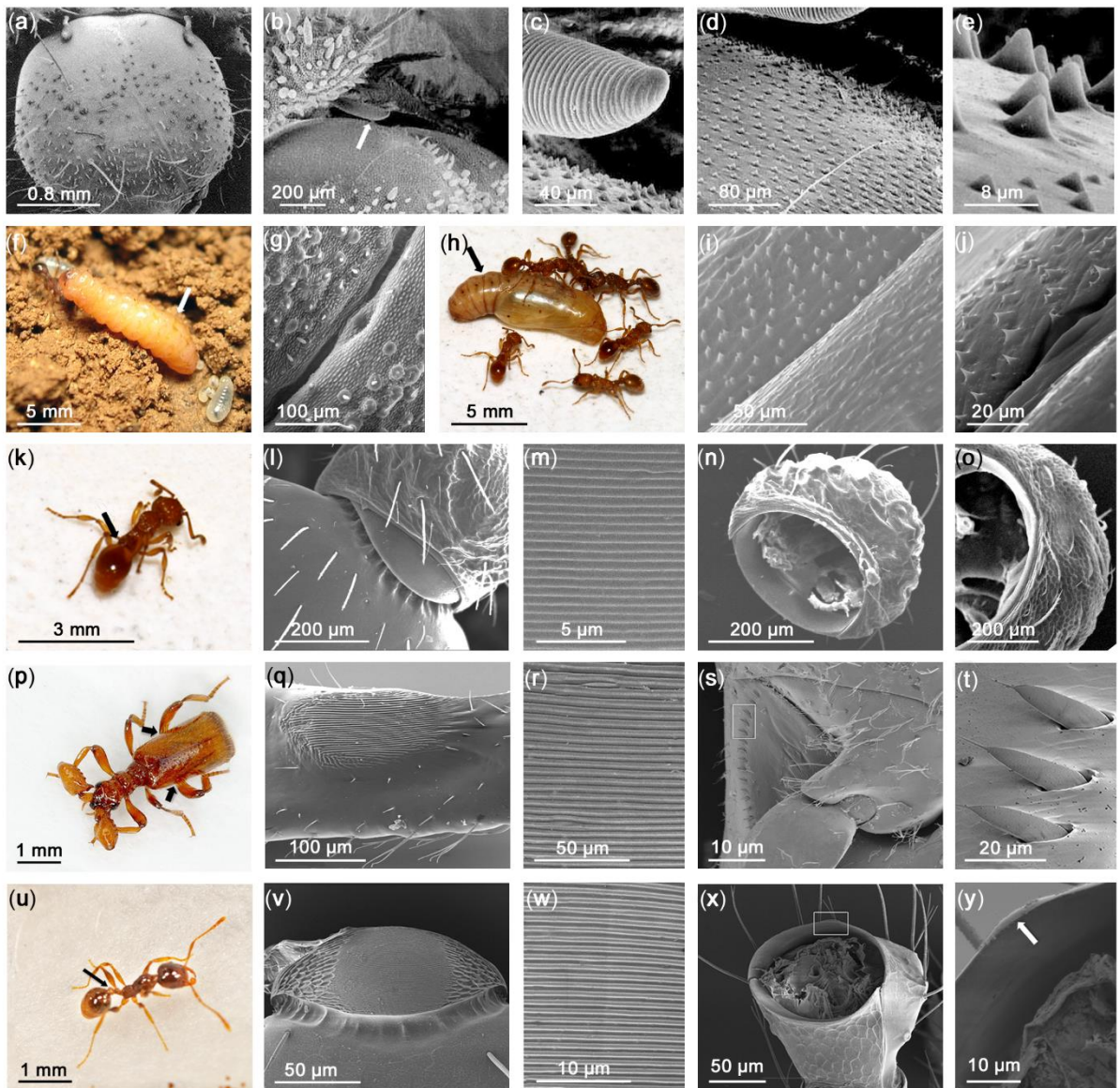
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717 Figure 1:



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