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13 **The acoustic repertoire of lycaenid butterfly larvae**

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33 **The acoustic repertoire of lycaenid butterfly larvae**

34 Sounds and vibrations play vital roles in intra- and inter-specific communication of many insect
35 taxa, for sexual display, defence and social recruitment. In Lepidoptera, sound production occurs
36 in larvae, pupae and adults and has evolved in response to selection of sexual or defensive traits.
37 About 75% of the 6000 estimated lycaenid butterflies are associated with ants (termed
38 “myrmecophilous species”) and many species produce acoustic emissions during pre-imaginal
39 development. It was initially believed that these acoustic emissions were only produced by
40 myrmecophilous species, but later studies showed that the ability to produce sounds may be
41 universal among this butterfly family. The acoustic repertoire of the late-instar larvae of 12
42 lycaenid species (Polyommatainae and Lycaeninae), showing different degrees of interaction with
43 ants, was analysed by investigating 12 acoustic parameters measured on the call fundamental unit
44 (pulse). All samples produced species-specific calls whose spectra were characterized by
45 harmonic frequency components. The inter-specific call diversity better reflects the level of
46 association with ants than the phylogenetic relationships between species. Our results support the
47 hypothesis that the ability to emit acoustic signals is widespread in lycaenids, and that these
48 emissions play a role in myrmecophilous interactions.

49 Keywords: acoustic signals, vibrations, sounds, butterfly, myrmecophily

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52 **Introduction**

53 Acoustic signals are an extraordinary example of diversity in the animal kingdom. In insects,
54 vibrational communication conveys both intra- and inter-specific information (Kowalski et al.
55 2014; Schmidt and Balakrishnan 2015), covering a wide spectrum of purposes (Huber and
56 Thorson 1985; Hughes et al. 2009; Polidori et al. 2013; Cocroft et al. 2014) and varying in the
57 ways in which the sound emissions are produced (Drosopoulos and Claridge 2005).

58 Regarding insect sounds, those produced by Lepidoptera were among the first to be studied.
59 Lepidoptera are known to emit sounds at least since Darwin (1871) described a series of
60 “clicks” emanated by the nymphalid *Hamadryas feronia*. Since then, acoustic communication
61 in Lepidoptera has been extensively investigated, with over 200 studies published in recent
62 decades (Minet and Surlykke 2003), which have primarily focused on hearing and sound
63 production in adults.

64 Lepidoptera are able to produce substrate-borne vibrations (Yack et al. 2001; Scott et al.
65 2010) and air-borne signals (Bura et al. 2011) by means of a stridulatory apparatus, tymbal
66 organs, percussion behaviours or expulsion of tracheal air, from audible frequencies to
67 ultrasounds (Capinera 2008). Organs that perceive acoustic stimuli have evolved
68 independently several times within Lepidoptera: in butterflies, tympanal organs can be present
69 at the base of the forewings, while in moths these sensory organs have been observed in
70 mouthparts, thorax or anterior abdominal segments (Capinera 2008).

71 Sounds are produced by adult moths mostly in the context of defense against bats, to detect
72 and interfere with their predatory communication (Spangler 1986; Conner 1999; Minet and
73 Surlykke 2003). However, larval acoustic emissions have scarcely been investigated and the
74 behavioural context in which signals are produced has rarely been examined. Acoustic signals
75 emitted by caterpillars can function as an enemy deterrent in *Antispila nysaefoliella*
76 (Heliozelidae: Low 2008) and *Amorpha juglandis* (Sphingidae: Bura et al. 2011), or could

77 represent aposematic displays in the case of *Antherea polyphemus* larvae (Saturniidae: Brown
78 et al. 2007). The use of vibrations (substrate-borne sounds) for territory defence has been
79 documented for the tortricid moth *Sparganothis pilleriana* (Russ 1969), in drepanids *Drepana*
80 *arcuata* (Yack et al. 2001), *Falcaria bilineata* (Bowen et al. 2008) and *Oreta rosea* (Scott et
81 al. 2010) and in the gracillariidae *Caloptilia serotinella* (Fletcher et al. 2006).

82 When considering only “butterfly” acoustics, lycaenids *s.l.* (Lycaenidae and Riodinidae)
83 represent one of the most studied groups. In lycaenids, larval emissions were documented
84 almost one century ago (Farquharson et al. 1922), but their presence, role and mechanisms of
85 production have not been clarified in many species. Most lycaenid species lack obvious larval
86 stridulatory organs (Schurian and Fiedler 1991), although *Arhopala maditus* is an exception,
87 as it possesses a stridulatory apparatus similar to those of pupae (Hill 1993), made of a series
88 of spines rubbing against a continuous surface, which acts like a plectrum (Alvarez et al.
89 2014). Nevertheless, in a survey of 130 butterfly species, DeVries (1990, 1991) revealed that
90 19 species of Riodinidae and 30 species of Lycaenidae were able to produce air-borne sounds,
91 typically as single pulses (i.e. call fundamental unit) or as pulse trains. These species were not
92 necessarily closely related, neither did their geographical distributions overlap. However, all
93 the species able to emit calls showed a certain degree of interaction with ants. Therefore,
94 DeVries (1990, 1991) hypothesized that lycaenid and riodinid emissions may fulfil an
95 important role for interacting with ants (“myrmecophily”), a relationship present in
96 approximately 75% of the 6000 estimated species (DeVries 1990, 1991).

97 Lycaenid associations with ants may be mutualistic or parasitic and vary from facultative to
98 strictly obligate. Myrmecophilous interactions can be nonspecific, especially if facultative, as
99 the ants involved could belong to several species, or even subfamilies, or may be strictly
100 species-specific. Immature butterfly stages could depend on ants for short periods or,

101 conversely, for the majority of their life cycle (for a detailed review about myrmecophily in
102 lycaenids see Pierce et al. 2002).

103 Evidence of the use of lycaenid larval sounds in enhancing the interaction with ants is
104 reported by Travassos and Pierce (2000), who found that the calls produced by pupae and
105 caterpillars of *Jalmenus evagoras* are important in attracting *Iridomyrmex* host ants and in
106 eliciting attendance behaviours. Other studies have highlighted the resemblance between ant
107 stridulations and lycaenid calls (DeVries et al. 1993; Barbero and Casacci 2015), and
108 documented how acoustic mimicry is employed by obligate parasitic *Maculinea* larvae to fool
109 their host ant and live undisturbed within the host nests (Barbero et al. 2009 a, 2009b; Sala et
110 al. 2014).

111 Sound production also occurs in non-myrmecophilous species, but these calls were
112 documented as being emitted less frequently and appeared to be simpler than those of
113 myrmecophilous species (Fiedler et al. 1995), reinforcing the hypothesis that acoustics of
114 lycaenids may have a crucial role in their interactions with ants, while only playing a general
115 defensive function in species that are not associated with ants.

116 The aim of this study was to describe and investigate the acoustic repertoire of 12 European
117 (one of which, *C. marshalli*, was introduced) lycaenid larvae belonging to different
118 subfamilies and representing several degrees of associations with ants (varying from no
119 relationship to obligate parasitism). Specifically, we tested three hypotheses: (i) sounds are
120 species-specific; (ii) myrmecophilous species are able to produce more complex acoustic
121 emissions than species having no relationship with ants (iii) resemblance in acoustic patterns
122 are explained by similarities in associations with ants rather than in phylogenetic distances
123 between species.

124 **Materials and methods**

125 ***Species pool and sampling***

126 Larvae belonging to 12 species of lycaenids were field-collected from May 2012 to
127 September 2014 at various sites across Northern Italy, with the exception of *Lycaena helle*
128 (Poland: Table 1). When available, fully-developed caterpillars were collected, otherwise we
129 gathered the food plants on which oviposition had occurred in order to rear the larvae in the
130 laboratory.
131 Specimens and their respective food plants were maintained in a rearing room within
132 transparent boxes (30 × 20 × 20 cm³) covered with fine nets at a temperature of 18°C (night) :
133 25°C (day) and at cycles of 14 h light/10 h dark. Following the experiments, caterpillars were
134 released at their original location. Species were selected according to their degree of
135 interaction with ants, with five degrees of association according to Fiedler (1991a, 1991b):
136 namely no ant association (0 - myrmecoxenous), very few ant-associations reported, stable
137 ant-associations only formed exceptionally (1 - weakly myrmecophilous), a varying
138 proportion of larvae attended by ants (2 - moderately myrmecophilous), most if not all mature
139 larvae associated with ants (3 - steadily myrmecophilous), larvae fully dependent on ants as
140 commensals or parasites (4 - obligate myrmecophilous) (Table 1). All myrmecophilous
141 species belonged to the Polyommatainae subfamily, while *Lycaena helle*, *L. dispar* and *L.*
142 *phleas* were members of the subfamily Lycaeninae. The latter three species are not considered
143 truly myrmecophilous, although they do possess morphological characteristics often
144 associated with myrmecophily, such as specialised dendritic setae (Fiedler 1991a).

145 ***Sound recording***

146 We used a custom-made (Figure 1(a)) device that allows the recording of undisturbed
147 (unstressed) late-instar caterpillars (three samples per species). The recording equipment
148 consisted of a 12.5 × 8 × 2 cm³ recording chamber with a moving-coil miniature microphone

149 (sensitivity: 2.5 mV/Pa/1.0 kHz) attached through the centre (sampling rate set to 44.10
150 kHz). A second identical moving-coil microphone was used to record the ambient noise in
151 anti-phase. A mixer and output amplifier (dynamic range: 5Hz to 40kHz; gain: 53dB)
152 combined the signals from the two microphone preamplifiers. Overall, the frequency ranged
153 from 20 Hz to 20 kHz and the gain was approximately 83 dB. The equipment was powered by
154 a 12V gel cell battery. The recording chamber (Figure 1 (b)) and the microphone were located
155 inside an anechoic chamber to further reduce ambient noise and interference. Late instar
156 caterpillars were individually placed on the microphone surface within the recording chamber
157 and recorded in the morning at room temperature (23-25°C). Samples were recorded for 20-
158 minute periods, starting at 5 minutes after the introduction of the caterpillar in the recording
159 chamber.

160 Segments containing acoustic recordings were carefully inspected, checked for clipping and
161 digitally saved in WAV format (16-bit amplitude resolution) using Audacity v. 1.2.4
162 (<http://audacity.sourceforge.net/>). The temporal and spectral features of the signals were
163 measured using Praat v. 5.3.53 (<http://www.fon.hum.uva.nl/praat/>).

164 *Call comparisons and statistical analysis*

165 We analysed recordings of three individuals per species. Firstly, we assessed variations in call
166 complexity investigating whether the pulse-interval patterns (classified by Sandberg (2011) as
167 monophasic, varied beat-interval, diphasic and grouped, from the simplest to more complex
168 patterns) and the pulse spectrographic characteristics varied within and across species (from
169 pure tones to more complex frequency patterns). We then randomly selected two trains of five
170 pulses for each individual (Figure 1 (c), Figure 2) and measured, for each pulse a total of 21
171 temporal and spectral parameters (Supplementary Information Table S1). We then computed
172 a pairwise correlation analysis on the 21 acoustic parameters (Spearman-Rank-Correlation;

173 SPSS v22). From a pair of parameters with $r_s > 0.85$, only one was selected for analysis. This
174 method yielded 12 acoustic variables (Figure 3, Table S2, S3). Six variables were
175 automatically extracted using a script: the first and third quartiles of the energy spectrum (Q_{25}
176 and Q_{75} , Hz; 25% and 75% of the call energy); the relation of the frequency peak energy to
177 the call total energy expressed as a percentage ($\%E_{F_{peak}}$); the mean intensity of the entire call
178 represented by the root-mean-square signal level (RMS, dB). Two temporal variables were
179 also measured from the oscillogram: the pulse duration (Δ_t , s) and the pulse rate (PR, s^{-1} ;
180 calculated as $1/t_{start(x)} - t_{start(x+1)}$).

181 The other six variables were manually estimated, for each pulse, by inspection of power
182 spectra: the frequency of the first and second peak amplitudes (F_1 , F_2 , Hz) and the intensity of
183 the first three peak amplitudes (I_1 , I_2 , I_3 , dB – see Figure 1 (d)) In addition we manually
184 estimated the centre of gravity (CG, Hz; an average measure for how high the frequencies in a
185 spectrum are).

186 To account for individual differences in sound production, we assessed the variation in each
187 call parameter by using Generalised Linear Models (GLM) in which lycaenid species and
188 myrmecophily categories were used as fixed factor and the “individual” as random factor.
189 Post hoc tests with Bonferroni correction reported pairwise differences between categories
190 (Table S4, S5). Analyses were performed using SPSS v.22.

191 To test whether sounds differed between groups, we calculated the pairwise normalized
192 Euclidean distances over sound parameters and used the ANalysis Of SIMilarity (ANOSIM)
193 implemented in Primer v. 6.1.12 (Primer-E Ltd.) to assess differences between species,
194 subfamilies, and myrmecophilous levels. Groupings were visualised through nonparametric
195 multi-dimensional scaling (nMDS) using single sound pulses (Figure 4). Similarity
196 percentages (SIMPER) were used to calculate the individual contribution of each sound

197 parameter to the differences between myrmecophilous categories and their contribution to the
198 similarity between species belonging to the same degree of lycaenid-ant association.

199 **Results**

200 All 12 species investigated in our study produced calls, i.e. signals composed of a variable
201 number of pulses (Figure 2). We provide the first evidence of call production for *Cacyreus*
202 *marshalli*, *Lycaena helle*, *L. dispar*, *Cupido argiades*, *Lycaeides argyrognomon*, *Scolitantides*
203 *orion*, and *Plebejus argus*, while we confirm this ability in *Maculinea alcon*, *Lycaena phleas*,
204 *Polyommatus bellargus*, *Polyommatus coridon* and *Polyommatus icarus* (DeVries 1991). Call
205 complexity (i.e. different patterns in pulse/interval succession and pulse spectrum structure)
206 was investigated across the various levels of association, from non-myrmecophilous to
207 myrmecophilous species, but no differences were detected. All larvae emit monophasic
208 signals (constant interval between regular pulses, *sensu* Sandberg 2011) repeating pulses that
209 are stable in tone, duration, and pulse rate. Pulses consist of a short, repeated tone composed
210 of a fundamental frequency followed by a variable number of harmonics (Figure 2) and are
211 generally repeated in trains, but may be rarely delivered as isolated signals.

212 Overall, we did not find any differences in emission complexity (call and pulse structure)
213 between species. We observed that pulses were characterized by the same spectrographic
214 template (i.e. at least three frequency components with a harmonic structure in all our
215 samples). Inter-peak values could occur between principal frequencies, and periodicity was
216 obvious in all cases. Pulses exhibit a broadband frequency extending up to around 7 kHz, but
217 most of the energy in the emissions was distributed below 3 kHz (Figure 2). Average
218 measurements for the 12 sound parameters of each species are reported in Figure 3 (mean
219 values are listed in Table S2).

220 ***Call comparisons: univariate analysis***

221 Sound parameters varied significantly between species (Figure 3), while no significant
222 differences were observed between calls produced by specimens belonging to the same
223 species (for each model $P > 0.05$). Post hoc comparisons between species showed significant
224 differences for most of the investigated parameters (Figure 3, Table S4). Differences were less
225 pronounced between species belonging to the same myrmecophilous category (Table S5). No
226 significant differences were detected in frequency components (F_1 , F_2), intensities (I_2 , I_3), and
227 centre of gravity (CG) between *Licaena helle* and *L. dispar*, or in pulse duration and centre of
228 gravity between *Polyommatus bellargus* and *P. icarus*. The investigated parameters did not
229 show unequivocal trends on the basis of species taxonomy. Nevertheless, when excluding
230 *Cacyreus marshalli*, given the different biogeography of this species, data showed patterns of
231 variation related to different degree of interaction with ants (Figure 3). Species that were
232 strictly associated with ants produced longer pulses (Δ_t), and the interval between the pulses
233 tended to be shorter (PR). The parameters investigated on the pulse spectrum showed that
234 frequency (F_1 , F_2 , Q_{25} , Q_{75} , CG) were generally higher in species that were not associated
235 with ants. Conversely, the percentage energy at the peak frequency (EF_{Peak}) appeared to
236 increase in myrmecophilous species, such as measures of signal intensity (I_1 , I_2 , I_3 and RMS).
237 *Maculinea alcon*, an obligate parasite of *Myrmica* ants, generally showed the extreme values
238 for all analysed parameters and it significantly differs from other species for the first
239 frequency component, the first and third frequency quartiles, the centre of gravity and the
240 intensity of the second peak amplitude.

241

242 ***Call comparisons: multivariate analysis***

243 Nonparametric multi-dimensional scaling (nMDS, Figure 4), carried out on all sound
244 parameters recorded from the 12 lycaenid species, showed group separations, and the
245 ANalysis Of SIMilarity (ANOSIM) statistically discriminated the pulses on the factor

246 “species” (ANOSIM: Global $R=0.768$; $P=0.001$). Moreover, all pairwise comparisons
247 resulted as being significant (ANOSIM: $0.249 < R < 1$; $P=0.001$).

248 In the nMDS graph (Figure 4) individual call pulses produced by species belonging to the
249 subfamily Lycaeninae (*L. dispar*, *L. helle*, *L. phleas*) and Polyommatainae are grouped
250 separately (ANOSIM: $R=0.185$; $P=0.001$). The SIMPER analysis shows that the similarity of
251 acoustic emissions of *Lycaena* species is mainly explained by the length of the pulses, and
252 frequencies (F_1 and F_2) and intensity (I_2 and I_3) of the first three harmonics. Pulses were also
253 distinguishable on the basis of the level of myrmecophily (following the categorization
254 according to Fiedler 1991a,b) (ANOSIM: Global $R=0.356$; $P=0.001$) but, when considering
255 the pairwise similarity tests, sounds produced by non-myrmecophilous species (level=0) did
256 not differ from *C. argiades* calls (level=2; ANOSIM: $R=0.019$; $P=0.341$) or *P. icarus*
257 (level=2/3, ANOSIM: $R=-0.123$; $P=0.998$). We also found no significant differences between
258 the acoustic signals emitted by the latter species and those produced by species classified as
259 “steadily myrmecophilous” (level=3, ANOSIM: $R=-0.108$; $P=0.982$). If we remove the pulses
260 recorded from *C. marshalli* from the analysis, all the previous pairwise comparisons differ
261 significantly, apart from calls emitted by *P. icarus* (level 2/3), which still cluster together with
262 those produced by species having myrmecophilous level 3 (level 2/3 vs. 3; i.e. *P. icarus* vs. *L.*
263 *argyrognomon*, *P. coridon*, *P. bellargus*, *S. orion* - ANOSIM: $R=-0.108$; $P=0.988$). The non-
264 myrmecophilous species (*Lycaena* spp.) and *Maculineaalcon*, which have the highest degree
265 of specialisation on ants, were found to be the most distant groups (SIMPER_{average square distance} =
266 61.51). Four frequency components account for more than 50% of call dissimilarity (i.e. the
267 first frequency component (15.9%), the third (12.3%) and first quartile (12.1%) of frequency
268 and the centre of gravity (11.4%)).

269

270 **Discussion**

271 This study describes and measures similarities between the calls produced by caterpillars of
272 12 lycaenid species, which were selected to represent different subfamilies and degrees of
273 association with ants. All the species investigated produced acoustic signals, providing the
274 first evidence of this ability for seven species, and supporting the hypothesis that acoustic
275 communication is widespread among lycaenids, independently from phylogeny, biogeography
276 and level of association with ants (Fiedler 1991a; Pierce et al. 2002).

277 Our study showed that lycaenid calls are distinct, species-specific signals. Pulses produced by
278 different species were significantly distinguishable, while conspecific individuals shared
279 similar call features.

280 Patterns of sound species-specificity have been previously observed in other insect taxa, and
281 several studies have reported that it is possible to discriminate even “cryptic” species on the
282 basis of their acoustic emissions (e.g. Hymenoptera: Ferreira et al. 2010; Carlos et al. 2014;
283 Heteroptera: Lampson et al. 2013; Orthoptera: Chesmore 2004). However, studies measuring
284 call variation and similarities in lycaenid species are lacking. Recent studies have focused on
285 single species, sometime comparing them to specific host ants (e.g. Travassos and Pierce
286 2000, Barbero et al. 2009 a, b, Barbero et al. 2012), while DeVries (1991), in his seminal
287 study, only described the individual calls made by 30 lycaenid species.

288 Regarding the five species formerly investigated by DeVries (1991), we obtained similar
289 mean values of temporal parameters, but we recorded broader frequency ranges in the case of
290 *Polyommatus coridon*, *P. bellargus* and *P. icarus*. These variations between the two studies
291 may be due to both technical limits of the recording equipment used 25 years ago and to the
292 method performed for inducing call emission. Lycaenid sounds have previously been
293 collected by stressing larvae with tweezers (e.g. DeVries 1991, Travassos and Pierce 2000),

294 while our recording device allowed us to collect calls without harming the caterpillars, which
295 could therefore behave more naturally (Barbero et al. 2009b).

296 All caterpillar specimens emitted calls, despite being unstressed and irrespectively of whether
297 they were myrmecophilous or not.

298 Given that sound production has associated energetic costs (Prestwich 1994), these emissions
299 should provide advantages and increase fitness in both myrmecophilous and myrmecoxenous
300 caterpillars. Thus for those species not interacting with ants (e.g. *Lycaena* spp.), acoustic
301 emissions could be useful to signal and repel natural enemies, as observed in other
302 Lepidoptera (Bura et al. 2009, 2011), but could also be employed in intraspecific contexts.
303 For instance, in the case of a high density of *Cacyreus marshalli* caterpillars sharing the same
304 larval host plant, sounds may be employed as territorial signals to communicate the presence
305 of conspecifics on the food/shelter resource, which has been observed for some moth larvae
306 (see e.g. Yack et al. 2001).

307 Myrmecophilous associations better explain the pattern of acoustic similarity revealed by the
308 present study than taxonomic relationships. For instance, within the Polyommatae
309 subfamily, congeneric species emit rather dissimilar signals (e.g. *Polyommatus icarus* calls
310 are more similar to those of *Lycaeides argyrognomon* than to those produced by *Polyommatus*
311 *coridon*, Figure 4). If we consider only European species (thus excluding *C. marshalli*, an
312 invasive South African species in Europe) calls can be significantly grouped according to
313 their degree of myrmecophily, as displayed by the multi-dimensional scaling plot (Figure 4).
314 The most diverse calls are produced by *Lycaena* spp. and *M. alcon*, representing, respectively,
315 the lowest (not myrmecophilous) and the highest (obligate social parasite) degrees of
316 myrmecophily according to Fielder's classification.

317 Some of the sound parameters clearly define different myrmecophilous and myrmecoxenous
318 groups and for some of them, an evident trend reflecting the degree of association with ants

319 exists. This is particularly true for temporal characteristics, such as pulse duration and pulse
320 rate, with species that are less associated with ants producing shorter and closer pulses
321 compared to those that are highly dependent on ants. At the same time, SIMPER analysis
322 showed that four frequency parameters contributed to more than 50% of the dissimilarity in
323 acoustic emission between myrmecophilous and myrmecoxenous species, suggesting that call
324 effectiveness probably does not rely on a higher sound complexity, but more likely in
325 possessing an appropriate frequency spectrum to better stimulate attending ants.

326 Indeed, in contrast to previous statements (Fiedler et al. 1995, Travassos and Pierce 2000), we
327 did not observe differences in signal complexity between ant-associated and non-
328 myrmecophilous larvae for the studied lycaenids. Calls appeared to be monotonic, and all
329 species displayed a distinctive spectral pattern constituted by a fundamental frequency and a
330 distinctive number of harmonic frequencies (Figure 2). This pattern has also been reported for
331 many other insects (Hung and Prestwich 2004; Gogala and Trilar 2007; Cator et al. 2009), but
332 is described here for lycaenid calls for the first time.

333 The frequencies observed during this study in lycaenid calls were similar to those emitted by
334 ants potentially associated with them (e.g. *gen. Myrmica*, Casacci et al. 2013) and previous
335 bioassays have demonstrated the ability of the call produced by *Maculinea* spp., obligate
336 parasites, to elicit a behavioural response in host ant workers (Barbero et al. 2009 a, Sala et al.
337 2014).

338 Calls of species strictly associated with ants (e.g. *P. coridon*, *P. argus*, *M.alcon*) are
339 characterized by lower fundamental frequency, which means a higher number of harmonics
340 within the frequency range of the ants, potentially increasing the chance to stimulate ant
341 receptors.

342 Even though information on central auditory systems of insects is scarce, it has been showed
343 that neurons have a remarkable selectivity to acoustic signal properties such as frequencies

344 (Gerhardt and Huber 2002). According to the “matched-filter hypothesis”, receptors are
345 hypothesized to match “the physical properties of the sender's signal” (Wehner 1987) and the
346 receiver would benefit from being selectively tuned to particular signal features. This has
347 been observed in acoustic behaviour of orthopterans, where different neurons are tuned into
348 unique frequencies involved in sexual communication and directional cues for localization
349 (Kostarakos et al. 2008), but could also occur in ants, given the increasing evidence of the
350 important role played by acoustic signals in ant colony communication (Settele et al. 2011) .

351

352 *Conclusion*

353 The results reported here, given the widespread occurrence of call production in lycaenids,
354 and considering the biological context in which they are produced, suggest that calls have
355 evolved from repellent or territorial signals towards lure calls for attracting specific ant taxa.
356 Nevertheless, modalities of signal production, transmission and reception remain largely
357 unknown for most lycaenid species (including those studied here). Direct comparisons
358 between caterpillar calls and host ant stridulations, coupled with playback bioassays, are
359 required in order to reach any further conclusions. Behavioural experiments for testing ant
360 reactions to lycaenid acoustic stimuli will provide clear-cut insights into the biological
361 meaning of acoustic communication in this complex inter-dependent system.

362

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486

487 **Tables**

488 Table 1: Species investigated (N=36) and related collecting locations. Country, Locality,
 489 UTM coordinates and altitude, as along with level of association with ants, according to
 490 Fiedler's classification (1991), are reported. Degrees of myrmecophily: 0= myrmecoxenous;
 491 0/1= weakly myrmecophilous; 2= moderately myrmecophilous; 3= steadily myrmecophilous;
 492 4= obligate myrmecophilous (2/3 and 3/4 are intermediate categories). ^aIn the extensive
 493 literature on this locally endangered species and in recent textbooks, no mention of
 494 myrmecophilous interaction is given. Only two old records of ant-associations exist. Like all
 495 other European *Lycaena* species for which appropriate information is available, *L. dispar* is
 496 probably not truly myrmecophilous (Fiedler 1991).

Species	Country	Locality	UTM coordinates	Altitude (m)	Level of myrmecophily (Fiedler 1991)
<i>Cacyreus marshalli</i>	Italy	Torino	N 32 T 396631 4991600	234	0
<i>Lycaena phleas</i>	Italy	Venaria	N 32 T 392350 4998321	258	0
<i>Lycaena helle</i>	Poland	Ogrodniczki	N 34 U 651141 5895299	132	0
<i>Lycaena dispar</i>	Italy	Trino Vercellese	N 32 T 444750 5004777	131	0 ^a
<i>Cupido argiades</i>	Italy	Venaria	N 32 T 392350 4998321	258	2
<i>Polyommatus icarus</i>	Italy	Castelnuovo Scrvia	N 32 T 490624 4980821	92	2/3
<i>Lycaeides argyrognomon</i>	Italy	Venaria	N 32 T 392350 4998321	258	3
<i>Polyommatus bellargus</i>	Italy	Venaria	N 32 T 392350 4998321	258	3
<i>Polyommatus coridon</i>	Italy	Terme di Valdieri	N 32 T 362013 4896424	1373	3
<i>Scolitantides orion</i>	Italy	Padova	N 32 T 725123 5032127	25	3
<i>Plebejus argus</i>	Italy	Terme di Valdieri	N 32 T 362013 4896424	1373	3/4
<i>Maculineaalcon</i>	Italy	Caselette	N 32 T 380608 4996043	391	4

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498

499 **Figure captions**

500 Figure 1: Recording and analysis of acoustic parameters of lycaenid larval calls. (a)
501 Customised recording device. (b) Detailed view of the recording chamber. (c) Oscillogram
502 and spectrogram (window size, 1024). Lycaenid calls all share the same basic acoustic
503 structure: pulses are generally repeated in trains and usually consist of at least three frequency
504 components with a harmonic structure, the first of which is the fundamental frequency. (d)
505 Power spectrum. See text for a description of measured parameters.

506
507 Figure 2: Example waveforms (upper traces) and spectrograms (lower traces) of sounds
508 emitted by the larvae of the 12 lycaenid species. Spectrograms were generated in Praat using a
509 Gaussian window shape, window length from 0.02 to 0.008 s, number of time steps = 1000,
510 number of frequency steps = 500, dynamic range = 50 dB.

511
512 Figure 3: Boxplots show median, quartile, maximum and minimum values; outliers are open
513 circles. Differences between species were tested for each parameter: 1st frequency component
514 ($F_1 - F_{360,11}=47.089$; $P<0.001$); 2nd frequency component ($F_2 - F_{360,11}= 18.734$; $P<0.001$); 1st
515 frequency quartile ($Q_{25} - F_{360,11}=19.749$; $P<0.001$); 3rd frequency quartile ($Q_{75} -$
516 $F_{360,11}=12.818$; $P<0.001$); Centre of gravity ($CG - F_{360,11}=8.798$; $P<0.001$); percentage energy
517 at the peak frequency ($\%E_{F_{peak}} - F_{360,11}=10.266$; $P<0.001$); pulse rate ($PR - F_{360,11}=30.435$;
518 $P<0.001$); pulse duration ($\Delta_t - F_{360,11}=62.345$; $P<0.001$); intensity of the 1st peak amplitude (I_1
519 $- F_{360,11}=3.953$; $P= 0.003$); intensity of the 2nd peak amplitude ($I_2 - F_{360,11}=14.386$; $P<0.001$);
520 intensity of the 3rd peak amplitude ($I_3 - F_{360,11}=11.381$; $P<0.001$); Root-Mean-Square signal
521 level ($RMS - F_{360,11}=14.134$; $P<0.001$).

522 The letters above each column indicate significance in post hoc tests (Table S4). The same
523 letter indicates no significant difference between species in pairwise Bonferroni tests
524 ($P>0.05$).

525

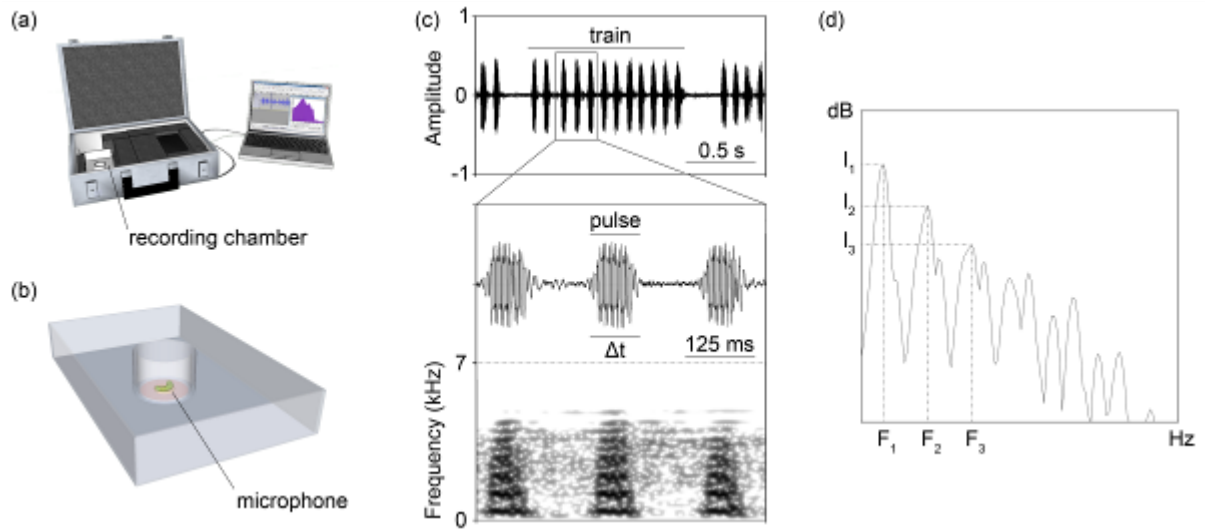
526 Figure 4: 2-dimensional nMDS ordination of the normalised Euclidean distances over all
527 single parameters analysed on individual pulses of the 12 lycaenid species. Each species
528 centroid reports the degree of myrmecophilous interaction, as categorized by Fiedler (1991).

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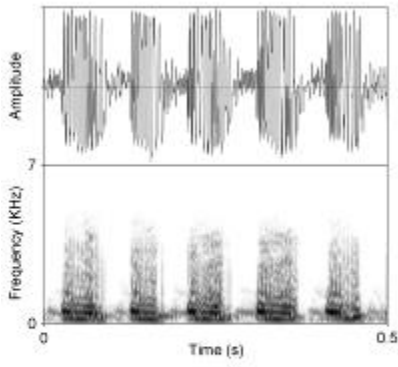
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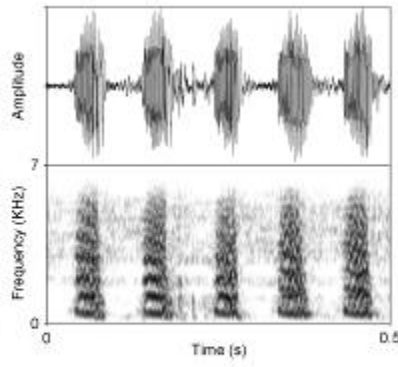
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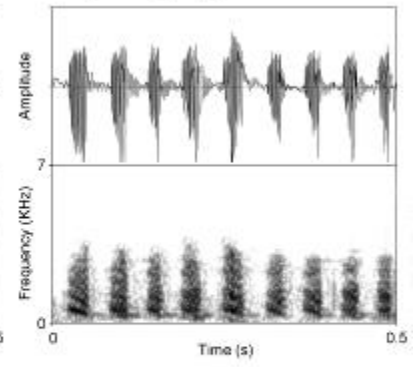
Maculinea alcon



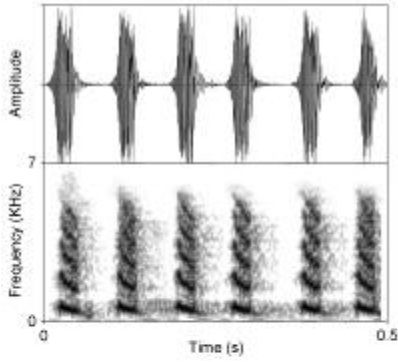
Plebejus argus



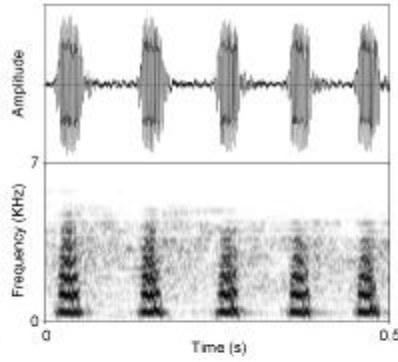
Plebejus argyrognomon



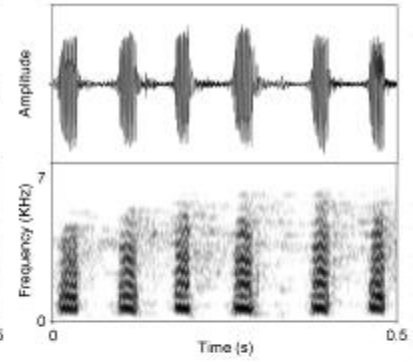
Scolitantides orion



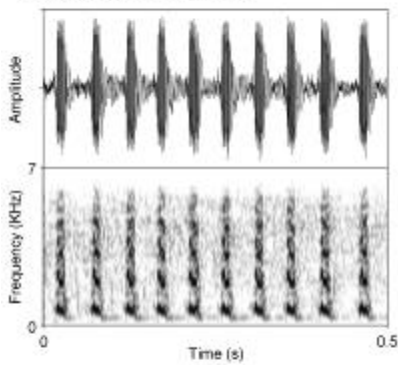
Polyommatus coridon



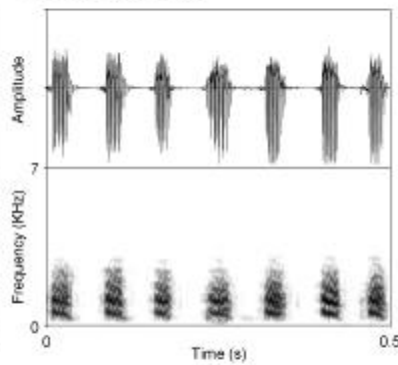
Polyommatus bellargus



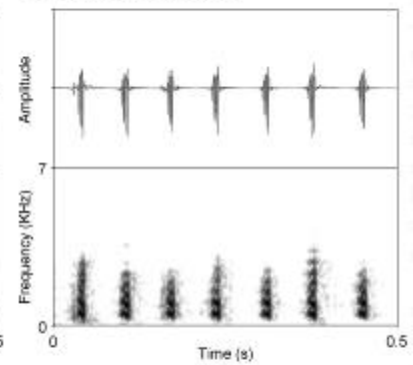
Polyommatus icarus



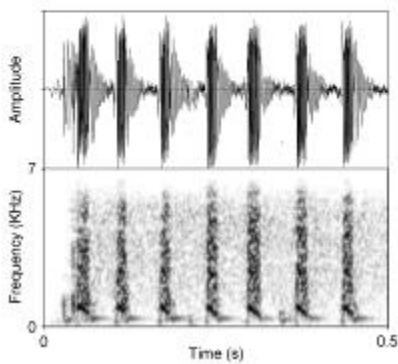
Cupido argiades



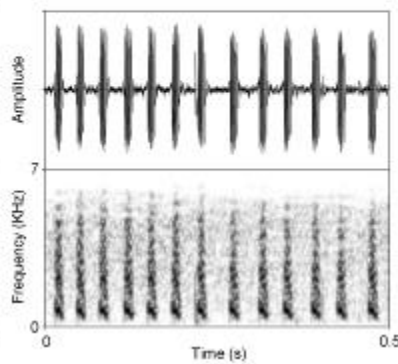
Cacyreus marshalli



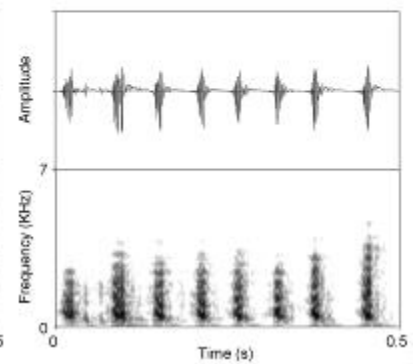
Lycaena helle



Lycaena dispar

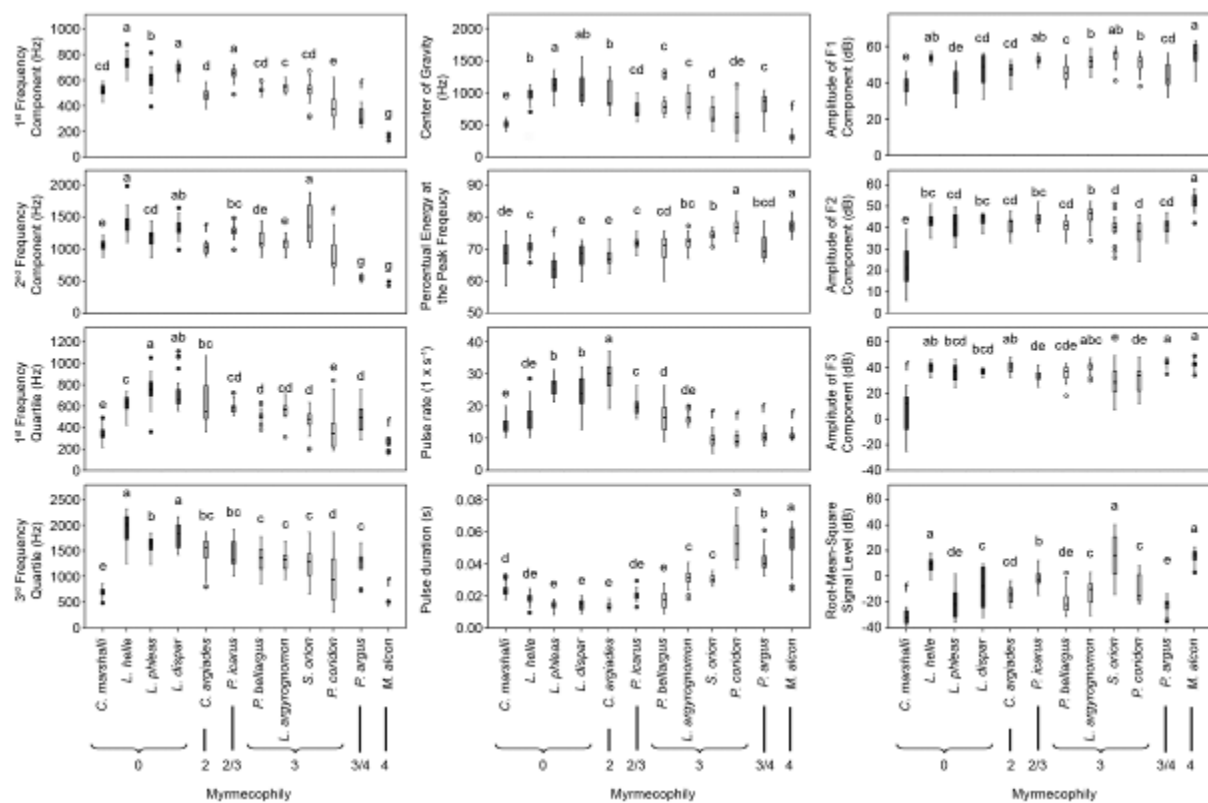


Lycaena phleas



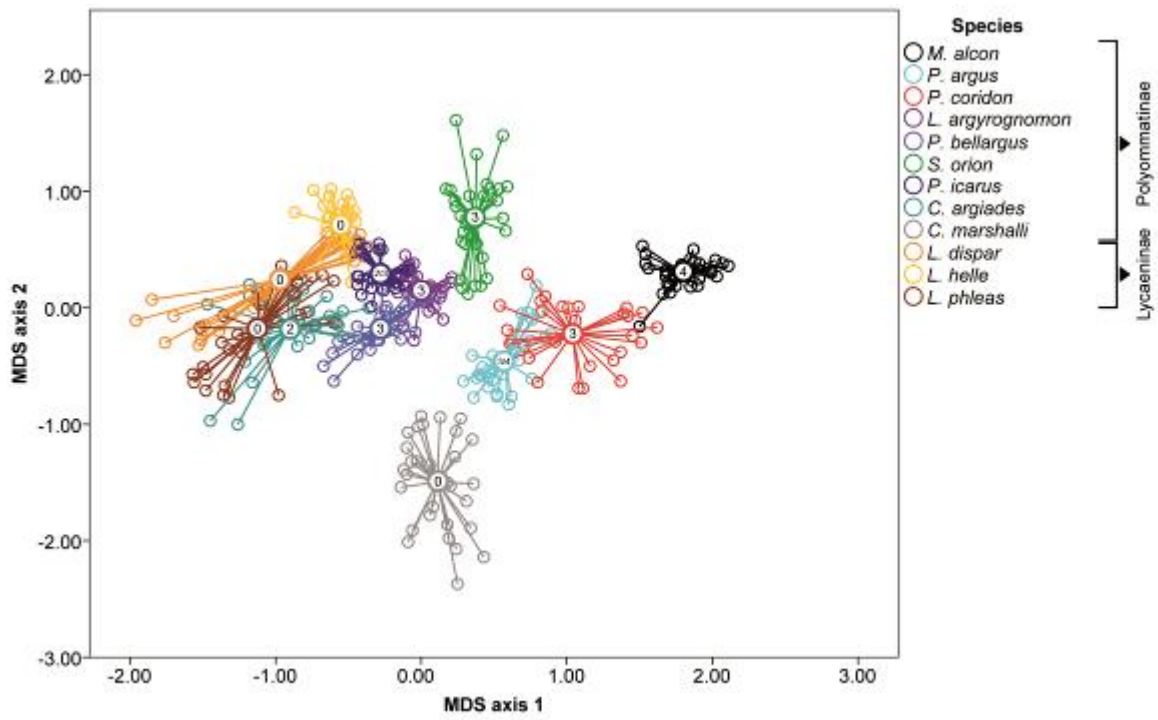
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541 Table S1. List of the 21 acoustic parameters measured on the pulses (i.e. call fundament unit)
 542 of each species

Acoustic parameters		Unit	Measurement
Q ₂₅	Lower quartile of the energy spectrum	Hz	Automatic
Q ₅₀	Central quartile of the energy spectrum	Hz	Automatic
Q ₇₅	Higher quartile of the energy spectrum	Hz	Automatic
SD _{Q50}	Frequency standard deviation	Hz	Automatic
F _{peak}	Frequency peak	Hz	Automatic
E _{Fpeak}	Energy of the frequency peak	Pa ² s ⁻¹	Automatic
%E _{Fpeak}	Relation of the frequency peak energy to the call total energy	%	Automatic
A _a	Mean amplitude	dB	Automatic
P	Power	dB ²	Automatic
E	Energy	Pa ² s ⁻¹	Automatic
I	Intensity value	dB	Automatic
RMS	Root- mean-square signal level	dB	Automatic
Δ _t	Duration of the pulse	s	Automatic
PR	Pulse Rate, calculated as 1/(t _{start(x)} -t _{start(x+1)})	s ⁻¹	Automatic
F ₁	Frequency of the first peak amplitude of the power spectrum	Hz	Manual
F ₂	Frequency of the second peak amplitude of the power spectrum	Hz	Manual
F ₃	Frequency of the third peak amplitude of the power spectrum	Hz	Manual
I ₁	Intensity of the first peak amplitude of the power spectrum	Hz	Manual
I ₂	Intensity of the second peak amplitude of the power spectrum	Hz	Manual
I ₃	Intensity of the third peak amplitude of the power spectrum	Hz	Manual
CG	Centre of gravity	Hz	Manual

543

544 Table S2. Mean values (\pm SD) of the 12 temporal and spectral parameters, collected for the 12
 545 butterfly species

	Δ_t (s)	PR (s^{-1})	F ₁ (Hz)	I ₁ (dB)	F ₂ (Hz)	I ₂ (dB)	I ₃ (dB)	CG (Hz)	RMS (dB)	Q _{2s} (Hz)	Q _{7s} (Hz)	%E _{Fpeak} (%)
<i>C. marshalli</i>	0.023 \pm 0.003	14.260 \pm 2.693	519.846 \pm 45.536	38.115 \pm 4.982	1057.615 \pm 92.366	23.461 \pm 8.276	4.515 \pm 5.428	517.615 \pm 51.482	0.078 \pm 0.046	333.657 \pm 67.024	686.377 \pm 98.565	68.046 \pm 4.041
<i>L. dispar</i>	0.014 \pm 0.003	24.297 \pm 4.753	689.033 \pm 47.347	46.553 \pm 8.760	1330.433 \pm 136.462	43.700 \pm 2.854	35.933 \pm 4.471	1061.6 \pm 240.276	0.309 \pm 0.164	716.689 \pm 147.005	1787.795 \pm 233.564	67.66 \pm 3.993
<i>L. helle</i>	0.018 \pm 0.003	16.407 \pm 4.326	729.566 \pm 62.186	53.433 \pm 1.977	1402.266 \pm 161.318	42.866 \pm 3.501	40.666 \pm 3.950	955.5 \pm 159.160	0.489 \pm 0.061	619.896 \pm 84.788	1917.821 \pm 297.232	70.656 \pm 1.993
<i>L. phleas</i>	0.013 \pm 0.002	25.930 \pm 2.516	608.769 \pm 70.848	41.096 \pm 7.129	1199.153 \pm 123.648	40.95 \pm 6.482	35.950 \pm 7.561	1092.846 \pm 139.122	0.202 \pm 0.114	748.048 \pm 137.203	1586.547 \pm 168.301	64.212 \pm 3.108
<i>C. argiades</i>	0.013 \pm 0.001	29.573 \pm 4.011	476.576 \pm 51.304	44.261 \pm 9.773	1004.192 \pm 79.138	40.357 \pm 3.819	39.673 \pm 3.702	921.5 \pm 242.37	0.240 \pm 0.060	640.87 \pm 22.323	1491.225 \pm 277.280	66.918 \pm 2.206
<i>P. icarus</i>	0.020 \pm 0.002	19.999 \pm 2.498	653.076 \pm 41.904	52.346 \pm 2.152	1290.730 \pm 84.133	44.500 \pm 3.140	34.115 \pm 3.902	723.307 \pm 123.234	0.377 \pm 0.063	585.498 \pm 52.645	1400.419 \pm 261.195	71.967 \pm 1.663
<i>S. orion</i>	0.030 \pm 0.002	9.850 \pm 1.860	507.73 \pm 91.397	53.500 \pm 5.770	1366.115 \pm 281.314	37.576 \pm 7.895	30 \pm 13.514	657.884 \pm 151.996	0.550 \pm 0.169	467.16 \pm 108.901	1322.038 \pm 304.937	74.299 \pm 1.474
<i>P. bellargus</i>	0.018 \pm 0.005	16.508 \pm 4.681	527.961 \pm 25.246	46.307 \pm 5.416	1164.384 \pm 158.214	40.538 \pm 3.088	35.384 \pm 8.913	824.038 \pm 221.363	0.195 \pm 0.093	509.727 \pm 52.009	1379.906 \pm 240.306	70.368 \pm 3.546
<i>P. argyrognomon</i>	0.031 \pm 0.004	15.753 \pm 1.370	531.846 \pm 31.703	50.526 \pm 3.951	1064.500 \pm 88.886	45.719 \pm 4.410	39.619 \pm 5.112	838.73 \pm 176.436	0.260 \pm 0.082	563.715 \pm 80.509	1276.871 \pm 194.715	72.339 \pm 2.422
<i>P. coridon</i>	0.053 \pm 0.011	9.254 \pm 1.601	374.192 \pm 96.326	50.307 \pm 4.654	784.153 \pm 228.309	38.269 \pm 4.065	31.500 \pm 8.936	589.461 \pm 251.978	0.299 \pm 0.111	390.621 \pm 155.782	1073.975 \pm 412.700	76.478 \pm 2.453
<i>P. argus</i>	0.041 \pm 0.006	10.524 \pm 1.836	317.961 \pm 60.027	44.692 \pm 6.442	552.500 \pm 48.228	40.461 \pm 3.679	43.692 \pm 3.495	798.846 \pm 201.968	0.172 \pm 0.072	485.001 \pm 119.232	1211.163 \pm 267.930	70.823 \pm 3.722
<i>M. alcon</i>	0.054 \pm 0.009	10.729 \pm 1.044	160.961 \pm 12.327	56.576 \pm 5.981	491.307 \pm 31.820	51.653 \pm 3.497	41.961 \pm 3.304	303.692 \pm 41.498	0.550 \pm 0.059	189.626 \pm 49.540	501.304 \pm 31.893	77.293 \pm 2.247

546

547 Table S3. Mean values (\pm SD) of temporal and spectral parameters of each myrmecophilous
 548 category (0= myrmecoxenous; 2= moderately myrmecophilous; 3= steadily myrmecophilous; 4=
 549 obliged myrmecophilous)

Myr- mecophi- lous category	PR (s ⁻¹)	F ₁ (Hz)	I ₁ (dB)	F ₂ (Hz)	I ₂ (dB)	I ₃ (dB)	CG (Hz)	Δ_t (s)	RMS (dB)	Q ₂₅ (Hz)	Q ₇₅ (Hz)	%E _{Fpeak} (%)
0	20.233 \pm 6.183	641.982 \pm 9.8069	45.17 \pm 8.522	1255.866 \pm 185.16	38.14 \pm 9.845	29.911 \pm 1.627	914.151 \pm 2.78.04	0.017 \pm 0.005	0.279 \pm 0.185	609.124 \pm 1.95.522	1520.219 \pm 5.21.411	67.752 \pm 4.036
2	29.573 \pm 4.011	476.576 \pm 5.1.304	44.261 \pm 9.773	1004.192 \pm 79.138	40.357 \pm 3.819	39.673 \pm 3.702	921.5 \pm 242.370	0.013 \pm 0.001	0.24 \pm 0.060	640.87 \pm 22.2.323	1491.225 \pm 2.77.28	66.918 \pm 2.206
2/3	19.999 \pm 2.498	653.076 \pm 4.1.904	52.346 \pm 2.152	1290.73 \pm 8.4.133	44.500 \pm 3.140	34.115 \pm 3.902	723.307 \pm 1.23.234	0.02 \pm 0.02	0.377 \pm 0.063	585.498 \pm 5.2.645	1400.419 \pm 2.61.195	71.967 \pm 1.663
3	12.841 \pm 4.275	485.432 \pm 9.4.487	50.16 \pm 5.554	1094.788 \pm 290.255	40.525 \pm 6.038	34.125 \pm 1.0.168	727.528 \pm 2.27.984	0.033 \pm 0.014	0.326 \pm 0.178	482.806 \pm 1.22.641	1263.197 \pm 3.17.058	73.371 \pm 3.415
3/4	10.524 \pm 1.836	317.961 \pm 6.0.027	44.692 \pm 6.442	552.500 \pm 4.8.228	40.461 \pm 3.679	43.692 \pm 3.495	798.846 \pm 2.01.968	0.041 \pm 0.006	0.172 \pm 0.072	485.001 \pm 1.19.232	1211.163 \pm 2.67.93	70.823 \pm 3.722
4	10.729 \pm 1.044	160.961 \pm 1.2.327	56.576 \pm 5.981	491.307 \pm 3.1.820	51.653 \pm 3.497	41.961 \pm 3.304	303.692 \pm 4.1.498	0.054 \pm 0.009	0.55 \pm 0.059	189.626 \pm 4.9.54	501.304 \pm 31.893	77.293 \pm 2.247

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552 Table S4. Bonferroni post hoc tests accounting for species vs. species differences in each
 553 sound parameter.

Species i	Species j	Difference (i-j)											
		PR	F ₁	I ₁	F ₂	I ₂	I ₃	CG	Δ ₁	RMS	Q ₂₅	Q ₇₅	%E _{Peak}
<i>M. alcon</i>	<i>C. argiades</i>	-18.42*	-318.23*	11.33*	-525.13*	10.96*	1.82	-643.47*	0.04*	0.3*	-442.04*	-975.24*	9.96*
	<i>P. argus</i>	0.34	-146.87*	12.3*	-63.07	11.37*	-1.1	-487.5*	0.01*	0.38*	-307.97*	-725.83*	6.88*
	<i>L. argyrognomon</i>	-5.39*	-374.53*	4.45*	-589.97*	6.18*	2.57	-509.9*	0.02*	0.27*	-372.37*	-789.95*	4.98*
	<i>P. bellargus</i>	-5.98*	-362.27*	10.03*	-646.67*	11.37*	7.8*	-508.63*	0.04*	0.35*	-319.44*	-840.3*	7.12*
	<i>P. coridon</i>	1.37	-219.37*	5.23*	-350.93*	14.83*	11.97*	-251.47*	0	0.26*	-177.83*	-494.75*	0.43
	<i>L. dispar</i>	-13.55*	-526.6*	9.21*	-841.37*	8.37*	6.47*	-746.67*	0.04*	0.24*	-527.77*	-1286.96*	9.51*
	<i>L. helle</i>	-5.66*	-567.13*	2.33	-913.2*	9.2*	1.73	-640.57*	0.03*	0.06	-430.97*	-1416.98*	6.51*
	<i>P. icarus</i>	-9.19*	-486.2*	3.5	-788.83*	8.23*	9.2*	-423.53*	0.03*	0.17*	-398.05*	-917.82*	5.33*
	<i>C. marshalli</i>	-3.41*	-355.97*	17.57*	-565.4*	30.4*	36.62*	-195.77*	0.03*	0.47*	-151.85*	-183.97*	8.86*
	<i>S. orion</i>	1.33	-353.97*	1.8	-919.03*	13.8*	12.87*	-334.63*	0.02*	-0.01	-276.32*	-756.67*	2.93*
<i>L. phleas</i>	-15.55*	-437.53*	15.12*	-695.5*	11.15*	6.33*	-790.33*	0.04*	0.37*	-562.88*	-1099.08*	13.45*	
<i>C. argiades</i>	<i>P. argus</i>	18.76*	171.37*	0.97	462.07*	0.4	-2.92	155.97*	-0.03*	0.08*	134.08*	249.41*	-3.07*
	<i>L. argyrognomon</i>	13.03*	-56.3*	-6.89*	-64.83	-4.78*	0.75	133.57*	-0.02*	-0.03	69.67	185.29*	-4.98*
	<i>P. bellargus</i>	12.44*	-44.03	-1.3	-121.53*	0.4	5.98*	134.83*	0	0.05	122.61*	134.95	-2.83*
	<i>P. coridon</i>	19.79*	98.87*	-6.1*	174.2*	3.87	10.15*	392*	-0.04*	-0.04	264.21*	480.5*	-9.52*
	<i>L. dispar</i>	4.87*	-208.37*	-2.12	-316.23*	-2.6	4.65	-103.2	0	-0.06	-85.72	-311.71*	-0.45
	<i>L. helle</i>	12.76*	-248.9*	-9*	-388.07*	-1.76	-0.09	2.9	-0.01*	-0.24*	11.07	-441.74*	-3.45*
	<i>P. icarus</i>	9.23*	-167.97*	-7.83*	-263.7*	-2.73	7.38*	219.93*	-0.01*	-0.13*	44	57.42	-4.63*
	<i>C. marshalli</i>	15.01*	-37.73	6.23*	-40.27	19.44*	34.8*	447.7*	-0.01*	0.17*	290.19*	791.27*	-1.1
	<i>S. orion</i>	19.75*	-35.73	-9.53*	-393.9*	2.84	11.05*	308.83*	-0.02*	-0.31*	165.72*	218.57*	-7.03*
	<i>L. phleas</i>	2.87*	-119.3*	3.78	-170.37*	0.19	4.51	-146.87*	0	0.07	-120.84*	-123.84	3.49*
<i>P. argus</i>	<i>L. argyrognomon</i>	-5.73*	-227.67*	-7.85*	-526.9*	-5.18*	3.67	-22.4	0.01*	-0.1*	-64.41	-64.12	-1.91
	<i>P. bellargus</i>	-6.32*	-215.4*	-2.27	-583.6*	0	8.9*	-21.13	0.02*	-0.02	-11.47	-114.46	0.24
	<i>P. coridon</i>	1.03	-72.5*	-7.07*	-287.87*	3.47	13.07*	236.03*	-0.01*	-0.12*	130.14*	231.09*	-6.45*
	<i>L. dispar</i>	-13.89*	-379.73*	-3.09	-778.3*	-3	7.57*	-259.17*	0.03*	-0.13*	-219.8*	-561.12*	2.62*
	<i>L. helle</i>	-6*	-420.27*	-9.97*	-850.13*	-2.17	2.83	-153.07*	0.02*	-0.31*	-123.01*	-691.15*	-0.37
	<i>P. icarus</i>	-9.53*	-339.33*	-8.8*	-725.77*	-3.13	10.3*	63.97	0.02*	-0.21*	-90.08	-191.98*	-1.55
	<i>C. marshalli</i>	-3.75*	-209.1*	5.27*	-502.33*	19.03*	37.72*	291.73*	0.02*	0.09*	156.11*	541.86*	1.97
	<i>S. orion</i>	0.99	-207.1*	-10.5*	-855.97*	2.43	13.97*	152.87*	0.01*	-0.38*	31.64	-30.84	-3.96*
	<i>L. phleas</i>	-15.89*	-290.67*	2.82	-632.43*	-0.21	7.43*	-302.83*	0.03*	-0.01	-254.91*	-373.25*	6.56*
	<i>L. argyrognomon</i>	<i>P. bellargus</i>	-0.59	12.27	5.59*	-56.7	5.18*	5.23	1.27	0.01*	0.08*	52.94	-50.35
<i>P. coridon</i>		6.76*	155.17*	0.79	239.03*	8.65*	9.4*	258.43*	-0.02*	-0.02	194.55*	295.2*	-4.54*
<i>L. dispar</i>		-8.16*	-152.07*	4.77*	-251.4*	2.18	3.9	-236.77*	0.02*	-0.03	-155.39*	-497.01*	4.53*
<i>L. helle</i>		-0.27	-192.6*	-2.11	-323.23*	3.02	-0.84	-130.67*	0.01*	-0.21*	-58.6	-627.03*	1.53
<i>P. icarus</i>		-3.8*	-111.67*	-0.95	-198.87*	2.05	6.63*	86.37	0.01*	-0.11*	-25.67	-127.87	0.35
<i>C. marshalli</i>		1.97	18.57	13.12*	24.57	24.22*	34.05*	314.13*	0.01*	0.19*	220.52*	605.98*	3.88*
<i>S. orion</i>		6.72*	20.57	-2.65	-329.07*	7.62*	10.3*	175.27*	0	-0.28*	96.05*	33.28	-2.05
<i>L. phleas</i>		-10.16*	-63*	10.67*	-105.53*	4.97*	3.76	-280.43*	0.02*	0.09*	-190.5*	-309.13*	8.47*
<i>P. bellargus</i>		<i>P. coridon</i>	7.35*	142.9*	-4.8*	295.73*	3.47	4.17	257.17*	-0.04*	-0.1*	141.61*	345.55*
	<i>L. dispar</i>	-7.57*	-164.33*	-0.82	-194.7*	-3	-1.33	-238.03*	0	-0.11*	-208.33*	-446.66*	2.38*
	<i>L. helle</i>	0.32	-204.87*	-7.7*	-266.53*	-2.17	-6.07*	-131.93*	0	-0.29*	-111.54*	-576.69*	-0.61
	<i>P. icarus</i>	-3.21*	-123.93*	-6.53*	-142.17*	-3.13	1.4	85.1	0	-0.19*	-78.61	-77.52	-1.79
	<i>C. marshalli</i>	2.57*	6.3	7.53*	81.27	19.03*	28.82*	312.87*	-0.01*	0.11*	167.58*	656.32*	1.73
	<i>S. orion</i>	7.31*	8.3	-8.23*	-272.37*	2.43	5.07	174*	-0.01*	-0.36*	43.11	83.62	-4.19*
	<i>L. phleas</i>	-9.57*	-75.27*	5.08*	-48.83	-0.21	-1.47	-281.7*	0	0.01	-243.44*	-258.79*	6.33*
<i>P. coridon</i>	<i>L. dispar</i>	-14.92*	-307.23*	3.98*	-490.43*	-6.47*	-5.5	-495.2*	0.04*	-0.01	-349.94*	-792.21*	9.07*
	<i>L. helle</i>	-7.03*	-347.77*	-2.9	-562.27*	-5.63*	-10.23*	-389.1*	0.03*	-0.19*	-253.14*	-922.23*	6.08*
	<i>P. icarus</i>	-10.56*	-266.83*	-1.73	-437.9*	-6.6*	-2.77	-172.07*	0.03*	-0.09*	-220.22*	-423.07*	4.9*
	<i>C. marshalli</i>	-4.78*	-136.6*	12.33*	-214.47*	15.57*	24.65*	55.7	0.03*	0.21*	25.98	310.77*	8.42*
	<i>S. orion</i>	-0.04	-134.6*	-3.43	-568.1*	-1.03	0.9	-83.17	0.02*	-0.26*	-98.49*	-261.93*	2.5*
	<i>L. phleas</i>	-16.92*	-218.17*	9.88*	-344.57*	-3.68	-5.64	-538.87*	0.04*	0.11*	-385.05*	-604.33*	13.01*
<i>L. dispar</i>	<i>L. helle</i>	7.89*	-40.53	-6.88*	-71.83	0.83	-4.73	106.1	0	-0.18*	96.79*	-130.03	-3*
	<i>P. icarus</i>	4.36*	40.4	-5.71*	52.53	-0.13	2.73	323.13*	-0.01*	-0.07*	129.72*	369.14*	-4.18*
	<i>C. marshalli</i>	10.14*	170.63*	8.35*	275.97*	22.03*	30.15*	550.9*	-0.01*	0.23*	375.91*	1102.98*	-0.65
	<i>S. orion</i>	14.88*	172.63*	-7.41*	-77.67	5.43*	6.4*	412.03*	-0.02*	-0.25*	251.45*	530.28*	-6.58*
	<i>L. phleas</i>	-2	89.07*	5.9*	145.87*	2.79	-0.14	-43.67	0	0.12*	-35.11	187.87*	3.94*
<i>L. helle</i>	<i>P. icarus</i>	-3.53*	80.93*	1.17	124.37*	-0.97	7.47*	217.03*	0	0.1*	32.93	499.16*	-1.18
	<i>C. marshalli</i>	2.25	211.17*	15.23*	347.8*	21.2*	34.89*	444.8*	0	0.4*	279.12*	1233.01*	2.35*
	<i>S. orion</i>	6.99*	213.17*	-0.53	-5.83	4.6*	11.13*	305.93*	-0.01*	-0.07	154.65*	660.31*	-3.58*
	<i>L. phleas</i>	-9.89*	129.6*	12.78*	217.7*	1.95	4.59	-149.77*	0	0.3*	-131.91*	317.9*	6.94*

<i>P. icarus</i>	<i>C. marshalli</i>	5.77*	130.23*	14.07*	223.43*	22.17*	27.42*	227.77*	0	0.3*	246.19*	733.84*	3.53*
	<i>S. orion</i>	10.52*	132.23*	-1.7	-130.2*	5.57*	3.67	88.9	-0.01*	-0.17*	121.72*	161.15	-2.4*
	<i>L. phleas</i>	-6.36*	48.67*	11.62*	93.33	2.92	-2.87	-366.8*	0.01*	0.2*	-164.83*	-181.26	8.12*
<i>C. marshalli</i>	<i>S. orion</i>	4.74*	2	-15.77*	-353.63*	-16.6*	-23.75*	-138.87*	-0.01*	-0.47*	-124.47*	-572.7*	-5.93*
	<i>L. phleas</i>	-12.14*	-81.57*	-2.45	-130.1*	-19.25*	-30.29*	-594.57*	0.01*	-0.1*	-411.03*	-915.11*	4.59*
<i>S. orion</i>	<i>L. phleas</i>	-16.88*	-83.57*	13.32*	223.53*	-2.65	-6.54*	-455.7*	0.02*	0.37*	-286.56*	-342.41*	10.52*

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556 Table S4. Bonferroni post hoc tests accounting for differences in each sound parameter
 557 between myrmecophilous categories (0= myrmecoxenous; 2= moderately myrmecophilous; 3=
 558 steadily myrmecophilous; 4= obliged myrmecophilous)

		Difference (i-j)											
Cat i	Cat j	PR	F ₁	I ₁	F ₂	I ₂	I ₃	CG	Δ _t	RMS	Q ₂₅	Q ₇₅	%E _{Fpeak}
0	2	-8.88*	153.58*	0.28	228.73*	-3.82	-10.97*	-50.13	0	0.02	-23.67	21.51	0.38
	2/3	0.36	-14.39	-7.56*	-34.97	-6.55*	-3.59	169.8*	0	-0.12*	20.32	78.93	-4.25*
	3	7.38*	144.28*	-5.68*	127.22*	-3.23*	-3.99	192.18*	-0.02*	-0.07*	131.88*	276.33*	-5.71*
	3/4	9.88*	324.94*	1.24	690.8*	-3.41	-13.89*	105.83	-0.02*	0.09	110.4*	270.91*	-2.7*
	4	9.54*	471.81*	-11.06*	753.87*	-14.78*	-12.79*	593.33*	-0.04*	-0.28*	418.37*	996.75*	-9.58*
2	2/3	9.23*	-167.97*	-7.83*	-263.7*	-2.73	7.38	219.93*	-0.01	-0.13*	44	57.42	-4.63*
	3	16.25*	-9.3	-5.96*	-101.52	0.58	6.98*	242.31*	-0.02*	-0.08	155.55*	254.83*	-6.09*
	3/4	18.76*	171.37*	0.97	462.07*	0.4	-2.92	155.97	-0.03*	0.08	134.08*	249.41	-3.07*
	4	18.42*	318.23*	-11.33*	525.13*	-10.96*	-1.82	643.47*	-0.04*	-0.3*	442.04*	975.24*	-9.96*
2/3	3	7.02*	158.67*	1.88	162.18*	3.31	-0.4	22.38	-0.01*	0.05	111.56*	197.4	-1.46
	3/4	9.53*	339.33*	8.8*	725.77*	3.13	-10.3*	-63.97	-0.02*	0.21*	90.08	191.98	1.55
	4	9.19*	486.2*	-3.5	788.83*	-8.23*	-9.2*	423.53*	-0.03*	-0.17*	398.05*	917.82*	-5.33*
3	3/4	2.51	180.67*	6.92*	563.58*	-0.18	-9.9*	-86.34	-0.01*	0.16*	-21.48	-5.42	3.02*
	4	2.17	327.53*	-5.38*	626.65*	-11.55*	-8.8*	401.16*	-0.02*	-0.22*	286.49*	720.42*	-3.87*
3/4	4	-0.34	146.87*	-12.3*	63.07	-11.37*	1.1	487.5*	-0.01*	-0.38*	307.97*	725.83*	-6.88*

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