The acoustic repertoire of lycaenid butterfly larvae

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

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

Regarding insect sounds, those produced by Lepidoptera were among the first to be studied. Lepidoptera are known to emit sounds at least since Darwin (1871) described a series of “clicks” emanated by the nymphalid Hamadryas feronia. Since then, acoustic communication in Lepidoptera has been extensively investigated, with over 200 studies published in recent decades (Minet & Surlykke 2003), which have primarily focused on hearing and sound production in adults.
Lepidoptera are able to produce substrate-borne vibrations (Yack et al. 2001; Scott et al. 2010) and air-borne signals (Bura et al. 2011) by means of a stridulatory apparatus, tymbal organs, percussion behaviours or expulsion of tracheal air, from audible frequencies to ultrasounds (Capinera 2008). Organs that perceive acoustic stimuli have evolved independently several times within Lepidoptera: in butterflies, tympanal organs can be present at the base of the forewings, while in moths these sensory organs have been observed in mouthparts, thorax or anterior abdominal segments (Capinera 2008).

Sounds are produced by adult moths mostly in the context of defence against bats, to detect and interfere with their predatory communication (Spangler 1986; Conner 1999; Minet & Surrykke 2003). However, larval acoustic emissions have scarcely been investigated and the behavioural context in which signals are produced has rarely been examined. Acoustic signals emitted by caterpillars can function as an enemy deterrent in Antispila nysaeafoliella (Heliozelidae: Low 2008) and Amorpha juglandis (Sphingidae: Bura et al. 2011), or could represent aposematic displays in the case of Antheraea polyphemus larvae (Saturniidae: Brown et al. 2007). The use of vibrations (substrate-borne sounds) for territory defence has been documented for the tortricid moth Sparganothis pilleriana (Russ 1969), in drepanids Drepana arcuata (Yack et al. 2001), Falcaria bilineata (Bowen et al. 2008) and Oreta rosea (Scott et al. 2010) and in the Gracillariidae Caloptilia serotinella (Fletcher et al. 2006).

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

Lycaenid associations with ants may be mutualistic or parasitic and vary from facultative to strictly obligate. Myrmecophilous interactions can be non-specific, especially if facultative, as the ants involved could belong to several species, or even subfamilies, or may be strictly species-specific. Immature butterfly stages could depend on ants for short periods or, conversely, for the majority of their life cycle (for a detailed review about myrmecophily in lycaenids, see Pierce et al. 2002).

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

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

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

Materials and methods

Species pool and sampling

Larvae belonging to 12 species of lycaenids were field-collected from May 2012 to September 2014 at various sites across Northern Italy, with the exception of Lycaena helle (Poland: Table 1). When available, fully developed caterpillars were collected, otherwise we gathered the food plants on which oviposition had occurred in order to rear the larvae in the laboratory.

Specimens and their respective food plants were maintained in a rearing room within transparent boxes (30 × 20 × 20 cm³) covered with fine nets at a temperature of 18 °C (night):25 °C (day) and at cycles of 14 h light/10 h dark. Following the experiments, caterpillars were released at their original location. Species were selected according to their degree of interaction with ants, with five degrees of association according to Fiedler (1991a, 1991b): namely no ant association (0 – myrmecoxenous), very few ant-associations reported, stable ant-associations only formed exceptionally (1 – weakly myrmecophilous), a varying proportion of larvae attended by ants (2 – moderately myrmecophilous), most if not all mature larvae associated with ants (3 – steadily myrmecophilous), larvae fully dependent on ants as commensals or parasites (4 – obligate myrmecophilous) (Table 1). All myrmecophilous species belonged to the Polyommatinae subfamily, while Lycaena helle, L. dispar, and L. phlaeas were members of the subfamily Lycaeninae. The latter three species are not considered truly myrmecophilous, although they do possess morphological characteristics often associated with myrmecophily, such as specialized dendritic setae (Fiedler 1991a).

Sound recording

We used a custom-made (Figure 1(a)) device that allows the recording of undisturbed (unstressed) late-instar caterpillars (three samples per species). The recording equipment consisted of a 12.5 × 8 × 2 cm³ recording chamber with a moving-coil miniature microphone (sensitivity: 2.5 mV/Pa/1.0 kHz) attached through the centre (sampling rate set to 44.10 kHz). A second identical moving-coil microphone was used to record the ambient
Table 1. Species investigated \((N = 36)\) and related collecting locations. Country, Locality, UTM coordinates and altitude, as well as level of association with ants, according to Fiedler’s classification \((1991a, 1991b)\), are reported. Degrees of myrmecophily: 0 = myrmecoxenous; 1 = weakly myrmecophilous; 2 = moderately myrmecophilous; 3 = steadily myrmecophilous; 4 = obligate myrmecophilous \((2/3 \text{ and } 3/4 \text{ are intermediate categories}).

| Species                  | Country | Locality      | UTM coordinates     | Altitude (m) | Level of myrmecophily (Fiedler 1991a, 1991b) |
|--------------------------|---------|---------------|---------------------|--------------|---------------------------------------------|
| Cacyreus marshalli       | Italy   | Torino        | N 32 T 396631       | 234          | 0                                           |
| Lycaena phlaeas          | Italy   | Venaria       | N 32 T 392350       | 258          | 0                                           |
| Lycaena helle            | Poland  | Ogdroniczki   | N 34 U 651141       | 132          | 0                                           |
| Lycaena dispar           | Italy   | Trino         | N 32 T 444750       | 131          | 0\(^{\text{a}}\)                             |
| Cupido argiades          | Italy   | Venaria       | N 32 T 392350       | 258          | 2                                           |
| Polymommatus icarus      | Italy   | Castelnuovo Scrivia | N 32 T 490624     | 92           | 2/3                                         |
| Lycaenidae argyrognomon  | Italy   | Venaria       | N 32 T 392350       | 258          | 3                                           |
| Polymommatus bellargus   | Italy   | Venaria       | N 32 T 392350       | 258          | 3                                           |
| Polymommatus coridon     | Italy   | Terme di Valdieri | N 32 T 362013    | 1373         | 3                                           |
| Scolitantides orion      | Italy   | Padova        | N 32 T 725123       | 25           | 3                                           |
| Plebejus argus           | Italy   | Terme di Valdieri | N 32 T 362013    | 1373         | 3/4                                         |
| Maculinea alcon          | Italy   | Caselette     | N 32 T 380608       | 391          | 4                                           |

\(^{\text{a}}\)In the extensive literature on this locally endangered species and in recent textbooks, no mention of myrmecophilous interaction is given. Only two old records of ant-associations exist. Like all other European Lycaena species for which appropriate information is available, L. dispar is probably not truly myrmecophilous (Fiedler 1991a, 1991b).

Figure 1. Recording and analysis of acoustic parameters of lycaenid larval calls. (a) Customized recording device. (b) Detailed view of the recording chamber. (c) Oscillogram and spectrogram (window size, 1024). Lycaenid calls all share the same basic acoustic structure: pulses are generally repeated in trains and usually consist of at least three frequency components with a harmonic structure, the first of which is the fundamental frequency. (d) Power spectrum. See text for a description of measured parameters.
noise in anti-phase. A mixer and output amplifier (dynamic range: 5 Hz to 40 kHz; gain: 53 dB) combined the signals from the two microphone preamplifiers. Overall, the frequency ranged from 20 Hz to 20 kHz and the gain was approximately 83 dB. The equipment was powered by a 12 V gel cell battery. The recording chamber (Figure 1(b)) and the microphone were located inside an anechoic chamber to further reduce ambient noise and interference. Late-instar caterpillars were individually placed on the microphone surface within the recording chamber and recorded in the morning at room temperature (23–25°C). Samples were recorded for 20-min periods, starting at 5 min after the introduction of the caterpillar in the recording chamber.

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

**Call comparisons and statistical analysis**

We analysed recordings of three individuals per species. Firstly, we assessed variations in call complexity investigating whether the pulse-interval patterns (classified by Sandberg (2011) as monophasic, varied beat-interval, diphasic and grouped, from the simplest to more complex patterns) and the pulse spectrographic characteristics varied within and across species (from pure tones to more complex frequency patterns). We then randomly selected two trains of five pulses for each individual (Figures 1(c) and 2) and measured, for each pulse a total of 21 temporal and spectral parameters (Supplementary Information Table S1). We then computed a pairwise correlation analysis on the 21 acoustic parameters (Spearman-Rank-Correlation; SPSS v22). From a pair of parameters with $r_s > 0.85$, only one was selected for analysis. This method yielded 12 acoustic variables (Figure 3 and Tables S2, S3). Six variables were automatically extracted using a script: the first and third quartiles of the energy spectrum ($Q_{25}$ and $Q_{75}$, Hz; 25 and 75% of the call energy); the relation of the frequency peak energy to the call total energy expressed as a percentage ($\%E_{\text{Peak}}$); the mean intensity of the entire call represented by the root-mean-square signal level (RMS, dB). Two temporal variables were also measured from the oscillogram: the pulse duration ($\Delta t$, s) and the pulse rate (PR, s$^{-1}$; calculated as $1/t_{\text{start}(x)} - t_{\text{start}(x+1)}$).

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

To account for individual differences in sound production, we assessed the variation in each call parameter using generalized linear models (GLM) in which lycaenid species and myrmecophily categories were used as fixed factor and the “individual” as random factor. Post hoc tests with Bonferroni correction reported pairwise differences between categories (Tables S4 and S5). Analyses were performed using SPSS v.22.

To test whether sounds differed between groups, we calculated the pairwise normalized Euclidean distances over sound parameters and used the ANalysis Of SIMilarity (ANOSIM) implemented in Primer v. 6.1.12 (Primer-E Ltd.) to assess differences between species, subfamilies, and myrmecophilous levels. Groupings were visualized through non-parametric multidimensional scaling (nMDS) using single sound pulses (Figure 4). Similarity
percentages (SIMPER) were used to calculate the individual contribution of each sound parameter to the differences between myrmecophilous categories and their contribution to the similarity between species belonging to the same degree of lycaenid–ant association.

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

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

Overall, we did not find any differences in emission complexity (call and pulse structure) between species. We observed that pulses were characterized by the same spectrographic template (i.e. at least three frequency components with a harmonic structure in all our figure 3. Boxplots show median, quartile, maximum, and minimum values; outliers are open circles. Differences between species were tested for each parameter: first frequency component (F₁ – F₁₅₀₁₁ = 47.089; p < 0.001); second frequency component (F₂ – F₂₅₀₁₁ = 18.734; p < 0.001); first frequency quartile (Q₁₅₀₁₁ = 19.749; p < 0.001); third frequency quartile (Q₃₅₀₁₁ = 12.818; p < 0.001); centre of gravity (CG – F₁₅₀₁₁ = 8.798; p < 0.001); percentage energy at the peak frequency (%Epeak – F₆₃₅₀₁₁ = 10.266; p < 0.001); pulse rate (PR – F₁₅₀₁₁ = 30.435; p < 0.001); pulse duration (Δt – F₆₃₅₀₁₁ = 62.345; p < 0.001); intensity of the first peak amplitude (I₁ – F₁₅₀₁₁ = 3.953; p = 0.003); intensity of the second peak amplitude (I₂ – F₁₅₀₁₁ = 14.386; p < 0.001); intensity of the third peak amplitude (I₃ – F₁₅₀₁₁ = 11.381; p < 0.001); Root-Mean-Square signal level (RMS – F₁₅₀₁₁ = 14.134; p < 0.001). The same letter indicates no significant difference between species in pairwise Bonferroni tests (p > 0.05).
samples). Inter-peak values could occur between principal frequencies, and periodicity was obvious in all cases. Pulses exhibit a broadband frequency extending up to around 7 kHz, but most of the energy in the emissions was distributed below 3 kHz (Figure 2). Average measurements for the 12 sound parameters of each species are reported in Figure 3 (mean values are listed in Table S2).

**Call comparisons: univariate analysis**

Sound parameters varied significantly between species (Figure 3), while no significant differences were observed between calls produced by specimens belonging to the same species (for each model $p > 0.05$). Post hoc comparisons between species showed significant differences for most of the investigated parameters (Figure 3, Table S4). Differences were less pronounced between species belonging to the same myrmecophilous category (Table S5). No significant differences were detected in frequency components ($F_1$, $F_2$), intensities ($I_2$, $I_3$), and centre of gravity (CG) between *Lycaena helle* and *L. dispar*, or in pulse duration and centre of gravity between *Polyommatus bellargus* and *P. icarus*. The investigated parameters did not show unequivocal trends on the basis of species taxonomy. Nevertheless, when excluding *Cacyreus marshalli*, given the different biogeography of this species, data showed patterns of variation related to different degree of interaction with ants (Figure 3). Species that were strictly associated with ants produced longer pulses ($\Delta t$), and the interval between the pulses tended to be shorter (PR). The parameters investigated on the pulse spectrum showed that frequency ($F_1$, $F_2$, Q25, Q75, CG) was generally higher in species that were not associated with ants. Conversely, the percentage energy at the peak frequency (EFPeak) appeared to increase in myrmecophilous species, such as measures of signal intensity ($I_1$, $I_2$, $I_3$, and RMS). *Maculinea alcon*, an obligate parasite of *Myrmica* ants, generally showed the
extreme values for all analysed parameters and it significantly differs from other species for the first frequency component, the first and third frequency quartiles, the centre of gravity, and the intensity of the second peak amplitude.

**Call comparisons: multivariate analysis**

nMDS (Figure 4), carried out on all sound parameters recorded from the 12 lycaenid species, showed group separations, and the ANOSIM statistically discriminated the pulses on the factor “species” (ANOSIM: Global $R = 0.768$; $p = 0.001$). Moreover, all pairwise comparisons resulted as being significant (ANOSIM: $0.249 < R < 1$; $p < 0.001$).

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

**Discussion**

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

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

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

Regarding the five species formerly investigated by DeVries (1991), we obtained similar mean values of temporal parameters, but we recorded broader frequency ranges in the case of Polyommatus coridon, P. bellargus, and P. icarus. These variations between the two studies may be due to both technical limits of the recording equipment used 25 years ago and to the method performed for inducing call emission. Lycaenid sounds have previously been collected by stressing larvae with tweezers (e.g. DeVries 1991; Travassos & Pierce 2000), while our recording device allowed us to collect calls without harming the caterpillars, which could therefore behave more naturally (Barbero et al. 2009b).

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

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

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

Some of the sound parameters clearly define different myrmecophilous and myrmecoxenous groups and for some of them, an evident trend reflecting the degree of association with ants exists. This is particularly true for temporal characteristics, such as pulse duration and pulse rate, with species that are less associated with ants producing shorter and more distant pulses compared to those that are highly dependent on ants. At the same time, SIMPER analysis showed that four frequency parameters contributed to more than 50% of the dissimilarity in acoustic emission between myrmecophilous and myrmecoxenous species, suggesting that call effectiveness probably does not rely on a higher sound complexity, but more likely in possessing an appropriate frequency spectrum to better stimulate attending ants.

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

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

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

Even though information on central auditory systems of insects is scarce, it has been showed that neurones have a remarkable selectivity to acoustic signal properties such as frequencies (Gerhardt & Huber 2002). According to the “matched-filter hypothesis”, receptors are hypothesized to match “the physical properties of the sender’s signal” (Wehner 1987) and the receiver would benefit from being selectively tuned to particular signal features. This has been observed in acoustic behaviour of orthopterans, where different neurones are tuned into unique frequencies involved in sexual communication and directional cues for localization (Kostarakos et al. 2008), but could also occur in ants, given the increasing evidence of the important role played by acoustic signals in ant colony communication (Settele et al. 2011).

**Conclusion**

The results reported here, given the widespread occurrence of call production in lycaenids, and considering the biological context in which they are produced, suggest that calls have evolved from repellent or territorial signals towards lure calls for attracting specific ant taxa.

Nevertheless, modalities of signal production, transmission, and reception remain largely unknown for most lycaenid species (including those studied here). Direct comparisons between caterpillar calls and host ant stridulations, coupled with playback bioassays, are required in order to reach any further conclusions. Behavioural experiments for testing ant reactions to lycaenid acoustic stimuli will provide clear-cut insights into the biological meaning of acoustic communication in this complex inter-dependent system.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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