

Acoustical mimicry in a predatory social parasite of ants

F. Barbero^{1,2}, S. Bonelli¹, J. A. Thomas³, E. Balletto¹ and K. Schönrogge²

¹Department of Animal and Human Biology, University of Turin, 10123 Turin, Italy, ²Centre for Ecology and Hydrology, Maclean Building, Wallingford, Oxfordshire, OX10 8BB, UK and ³Department of Zoology, University of Oxford, Tinbergen Building, Oxford, OX1 3PS, UK

Author for correspondence (simona.bonelli@unito.it)

Accepted 3 June 2009

SUMMARY

Rapid, effective communication between colony members is a key attribute that enables ants to live in dominant, fiercely protected societies. Their signals, however, may be mimicked by other insects that coexist as commensals with ants or interact with them as mutualists or social parasites. We consider the role of acoustics in ant communication and its exploitation by social parasites. Social parasitism has been studied mainly in the butterfly genus *Maculinea*, the final instar larvae of which are host-specific parasites of *Myrmica* ants, preying either on ant grubs (predatory *Maculinea*) or being fed by trophallaxis (cuckoo *Maculinea*). We found similar significant differences between the stridulations of model queen and worker ant castes in both *Myrmica sabuleti* and *Myrmica scabrinodis* to that previously reported for *Myrmica schencki*. However, the sounds made by queens of all three *Myrmica* species were indistinguishable, and among workers, stridulations did not differ significantly in two of three species-pairs tested. Sounds recorded from the predatory caterpillars and pupae of *Maculinea arion* had similar or closer patterns to the acoustics of their host *Myrmica sabuleti* than those previously reported for the cuckoo *Maculinea rebeli* and its host *Myrmica schencki*, even though *Maculinea rebeli* caterpillars live more intimately with their host. We conclude that chemical mimicry enables *Maculinea* larvae to be accepted as colony members by worker ants, but that caterpillars and pupae of both predatory and cuckoo butterflies employ acoustical mimicry of queen ant calls to elevate their status towards the highest attainable position within their host's social hierarchy.

Key words: Lycaenidae butterfly, *Myrmica* ant, *Maculinea*, *Phengaris*, acoustic mimicry, stridulation, cuckoo, predatory parasite.

INTRODUCTION

Effective communication is fundamental to the ability of social insects to live in complex hierarchical societies, in which different castes or individuals perform different activities, yet each type of behaviour is so well integrated that the colony functions as a 'superorganism' (Hölldobler and Wilson, 2009). In ants, the primary method of communication involves chemical and, to a lesser extent, tactile cues (Hölldobler and Wilson, 1990; Hölldobler and Wilson, 2009). Acoustics also plays a role among the adults of four ant subfamilies (Ponerinae, Nothomyrmecinae, Pseudomyrmecinae, Myrmicinae) that can stridulate (Markl, 1973; Taylor, 1978), and others that drum the substrate, by inducing specific behaviours in receiving individuals (Markl, 1965; Barbero et al., 2009) or by amplifying or modulating the effects of pheromones (Markl and Hölldobler, 1978; Baroni-Urbani et al., 1988; Hölldobler, 1999).

Although acoustics has generally been regarded as 'weakly developed' in ants (Hölldobler and Wilson, 1990; Hölldobler and Wilson, 2009; Keller and Gordon, 2009), it is also the least studied of their communication systems (Barbero et al., 2009). Indeed, knowledge of the structures involved in the production and reception of signals is incomplete. Most sounds or vibrations are produced by a minutely ridged stridulating organ (pars stridens) situated on the mid-dorsal edge of the fourth 'abdominal' segment, and by an embossed spike (plectrum) projecting from the rear edge of the post-petiole (Giovannotti, 1996; Grandi, 1966; Pavan et al., 1997; Grasso et al., 1998; Ruitz et al., 2006). When an ant raises and lowers her gaster, the two structures rub together and emit a series of 'chirps' (Hölldobler and Wilson, 1990; Roces and Hölldobler, 1996; Ruitz et al., 2006). Stridulations are

defined by the transmitting medium, and may be sounds transmitted by air or vibrations transmitted by the substrate. Many myrmecologists maintain that ants cannot hear the aerial component of stridulations and perceive only substrate-transmitted vibrations (Fielde and Parker, 1904; Roces and Tautz, 2001; Hölldobler and Wilson, 2009), an idea supported by the discovery of a subgenual organ in *Camponotus* ants (Menzel and Tautz, 1994). However, evidence for the perception of air-transmitted sounds is accumulating, at least over distances of a few centimetres (Hickling and Brown, 2000; Hickling and Brown, 2001; Roces and Tautz, 2001). Possible receptors of air-borne sounds are the Johnston's organ (Masson and Gabouriaux, 1972) or the trichoid organs on the tips of the antennae (Dumpert, 1972), although the latter, in ants, differ in structure from the filiform hair trichoid sensilla used by other insects and spiders for auditory reception (Tautz, 1977; Kumagai et al., 1998; Barth, 2000).

However sounds are detected, ant acoustics frequently signal antagonistic or distress behaviours between workers in a colony, including alarm (Markl, 1965; Markl, 1985; Roces and Hölldobler, 1995), intimidation (Stuart and Bell, 1980; Ware, 1994), aposematic 'threatening' (Santos et al., 2005), or a call for rescue after a cave-in of the nest (Markl, 1985). In addition, when combined with pheromones or in isolation, several species stridulate or drum during foraging to enhance worker recruitment to food sources (Markl and Hölldobler, 1978; Baroni-Urbani et al., 1988; Roces et al., 1993; Hölldobler and Roces, 2000): in *Atta* stridulations are most frequent when leaves of the highest quality for fungal cultures are found (Hölldobler and Roces, 2000). *Myrmica* workers frequently stridulate during trophallaxis, particularly the receiving worker when

food decreases (Stuart and Bell, 1980; Zhantiev and Sulkanov, 1977).

Inter-caste acoustical communication has been recorded in only a few instances. Mating queens of *Pogonomyrmex badius* stridulate to signal to males when their spermathecae are full (Markl et al., 1977), whereas in *Atta* those workers that cut leaves stridulate when they are ready to return to the nest. This recruits individuals of the smallest minor caste to climb onto the leaf fragment from where they protect their larger sisters from attack by phorid flies during the journey home (Roces and Hölldobler, 1995). Until recently, there was no direct evidence that different members of an ant society produced distinctive caste-specific sounds to induce appropriate patterns of behaviour in fellow or other castes, although this was implied when Markl (Markl, 1968) found that the major workers of *Atta cephalotes* produce more intense sounds, that carry further, than their smaller nestmates, and by Grasso and colleagues' (Grasso et al., 1998) demonstration that the space between the ridges of the pars stridens in queens of *Messor* species was greater than in the workers. Working with *Myrmica schencki*, we reported that the difference in width between the ridges of workers and queens was larger than in *Messor*, and that despite a considerable overlap, the queens made distinctive sounds from the workers (Barbero et al., 2009). When recordings of unstressed adult *M. schencki* were played back to laboratory cultures of workers, the sounds of both castes induced benign responses including aggregation and antennation at the speaker. Moreover, when workers were played their queen's sounds, they stood 'on guard' on the speaker to a much greater extent than when worker sounds were played, each holding the characteristic posture adopted by a *Myrmica* worker when protecting an object of high value to the colony (Barbero et al., 2009).

The chemical component of ant communication systems is highly specific, enabling colony members to recognise kin ants as well as conspecifics. Nevertheless, approximately 10,000 other invertebrate species live as social parasites within ant colonies, where they exploit the rich resources concentrated inside nests (Thomas et al., 2005a). The mechanism whereby they penetrate and often integrate with their host society has been studied in very few social parasites, but generally involves corrupting the honest signals of the ants (Hölldobler and Wilson, 1990; Akino et al., 1999; Lenoir et al., 2001; Thomas et al., 2005a; Hojo et al., 2009). European species of *Maculinea* butterflies are among the better understood examples. The adult butterfly is free-living and oviposits on a specific food plant(s), on which the first three larval instars feed. On entering the fourth and final instar, weighing just 1–2% of its ultimate biomass, the small larva (hereafter called a caterpillar) falls from its plant and secretes semio-chemicals that mimic the surface hydrocarbons of *Myrmica* ants, causing any forager that encounters it to carry the caterpillar to the brood chambers of the underground ant nest (Thomas, 1984; Thomas, 2002; Elmes et al., 1991a). Two strategies have evolved in *Maculinea* to exploit *Myrmica* resources. Caterpillars of the cuckoo species, *Maculinea rebeli* and *M. alcon*, remain in the brood chambers for 11–23 months, where they are tended and fed directly by the nurse ants on regurgitations and other food (Elmes et al., 1991a; Elmes et al., 1991b). Caterpillars of *M. arion* and *M. teleius* are predators of ant larvae, and inhabit peripheral cells from which they periodically foray to binge-feed in the brood chambers (Thomas and Wardlaw, 1992).

With a few apparent exceptions (Barbero, 2007; Tartally et al., 2008), all *Maculinea* species or populations are specific to one primary host species or local genotype of *Myrmica* at a regional scale (Thomas et al., 1989; Thomas et al., 2005a; Thomas et al., 2005b; Nash et al., 2008). In cuckoo species, host specificity is

explained by chemical mimicry of the host's communication system. Pre- and newly adopted caterpillars of cuckoo *Maculinea* are weak chemical mimics of their regional host (Akino et al., 1999; Nash et al., 2008), but within a few days are given preferential care. Thus, in western Europe where its host is *Myrmica schencki*, *Maculinea rebeli* caterpillars are rescued ahead of the ant brood when a colony is disturbed, and are fed in preference to *M. schencki* larvae when food is scarce (Thomas et al., 1998). This deeper infiltration coincides with the secretion of additional hydrocarbons that more closely mimic the distinctive odour of *M. schencki*, but inevitably make this genotype of *M. rebeli* a poor (and seldom tolerated) mimic of other *Myrmica* species (Elmes et al., 2004; Schönrogge et al., 2004).

Neither chemical mimicry nor their begging behaviour explains why *M. rebeli* caterpillars are treated in preference to host ant brood. Instead, we have suggested that acoustical cues are employed (Barbero et al., 2009). It is well known that certain pupae and caterpillars of Lycaenidae produce sounds; the former from tooth-and-comb stridulatory organs between the fifth and sixth segments (Downey, 1966; Downey and Allyn, 1973; Downey and Allyn, 1978; Pierce et al., 2002), whereas caterpillar sounds probably emanate from muscular contraction and air compression through the trachea (Schurian and Fiedler, 1991). The acoustics of mutualistic lycaenid species do not obviously mimic ant stridulations, and an attraction to ants has been demonstrated only in the pupae of one extreme mutualist (Travassos and Pierce, 2000; Pierce et al., 2002). The calls of socially parasitic *Maculinea* caterpillars, however, more closely resemble *Myrmica* worker stridulations, although the putative mimicry appeared to be modelled on the genus rather than a host species of *Myrmica* (DeVries et al., 1993). The same study showed that *Myrmica* larvae are mute, suggesting that in this trait *Maculinea* caterpillars are mimicking an adult ant cue.

The recordings by DeVries et al. (DeVries et al., 1993) were restricted to distressed worker ants and caterpillars, and were not played back to the ants. Using modern equipment, we recently found that unstressed *Maculinea rebeli* caterpillars and pupae were close acoustical mimics of *Myrmica schencki*, and that the sounds produced by both butterfly stages were significantly closer to those of the queen ants than the workers (Barbero et al., 2009). Playing *M. rebeli* sounds back to laboratory cultures of *M. schencki* workers resulted in similar enhanced 'on guard' (and other) benign behaviours as when the ants were played the stridulations of their own queens, especially when pupal calls were played. We concluded that although chemical mimicry is used to gain and maintain acceptance in a *M. schencki* society, the social parasite simultaneously employs acoustical mimicry inside the nest to advance its status to that of the highest member in its host's hierarchy.

DeVries et al. (DeVries et al., 1993) showed that caterpillars of predatory *Maculinea* species also produce sounds that appear to mimic *Myrmica* (worker) stridulations, although in nature they are less closely integrated with their host's society (Thomas et al., 2005a). We speculated that they might be less perfect acoustical mimics of their hosts. Here we test this idea by comparing the acoustics of unstressed *Maculinea arion* caterpillars and pupae with those of the queens and workers of its host ant, *Myrmica sabuleti*, and with our published data for *Maculinea rebeli* and *Myrmica schencki*. We also compare the worker and queen sounds of *M. sabuleti*, and those of another ant *Myrmica scabrinodis*, to determine whether the distinctive acoustical communication made by different castes of *M. schencki* exists in its congeners.

MATERIALS AND METHODS

Materials

Myrmica colonies were excavated in the field and set up as laboratory ant colonies of >100 workers in 28 cm×15 cm×10 cm Perspex containers and maintained on a diet of sugar and *Drosophila* larvae. We collected *Myrmica schencki* Emery 1894 colonies ($N=12$) in May 2006 at Colle di Tenda, Piedmont, Italy where this species is host to *Maculinea rebeli* (Barbero et al., 2009). Two colonies of *M. sabuleti* Meinert 1861 were collected from Italy and four from Somerset, UK from sites where they are host to *Maculinea arion*. Eleven colonies of *M. scabrinodis* Nylander 1846 were taken from Dorset, UK from sites with no *Maculinea* parasites, although this ant is host to *Maculinea alcon* and *M. teleius* elsewhere in Europe (Thomas et al., 2005a). The post-adoption larvae and pupae of *Maculinea arion* Linnaeus 1758 were collected in Somerset, UK and kept with *M. sabuleti* colonies until they were recorded.

Stridulation organ morphology

M. sabuleti and *M. scabrinodis* ants (three queens and three workers of each species) were kept in 70% ethanol and dissected between the post-petiole and the abdomen to expose the pars stridens and the plectrum. The two ant parts were mounted on the same steel stub, coated with gold, and the distances between adjacent ridges of the pars stridens were measured automatically (10 measurements per individual) using a Cambridge Stereoscan S360 scanning electron microscope (SEM). The SEM operated at 20–25 kV.

Sound recording

Recordings were made of individual workers and queens of the three *Myrmica* species (Table 1) using the procedures described by Barbero et al. (Barbero et al., 2009). We also recorded four pre-adoption caterpillars of *Maculinea arion*, i.e. before they came into contact with the ants, three post-adoption caterpillars and three pupae. The sounds for *M. rebeli* and *Myrmica schencki* used in the analyses are the same as those used in Barbero et al. (Barbero et al., 2009) (Table 1).

The recording equipment consisted of a 12.5 cm×8 cm×2 cm recording chamber with a moving-coil miniature microphone attached through the centre. A second microphone of the same type was used to record ambient noise but in anti-phase. An amplifier was attached to each microphone and calibrated to maximise the noise cancellation of ambient noise from the two microphones, leaving the signal from the recording chamber. The resulting signal was processed through a two-stage low-noise amplification before being recorded digitally on a laptop computer using Audacity v. 1.2.4 (<http://audacity.sourceforge.net/>). To further reduce ambient noise and interference, the equipment was powered by a 12 V gel cell battery, and the recording chamber and microphones were placed inside an anechoic chamber. Sounds were recorded for 15 min periods starting 5 min after an insect was introduced and had become calm.

Statistical analysis

The sound parameters used by DeVries et al. (DeVries et al., 1993) and Barbero et al. (Barbero et al., 2009), dominant frequency (DF; Hz), pulse repetition frequency (the reciprocal of the duration of one pulse; PRF; s⁻¹) and pulse length (PL; s), were measured using Audacity 1.2.4. To test whether sound differed between groups we calculated the pairwise normalized Euclidean distance over all three parameters and used a nested ('Individual' within 'Group') ANalysis Of SIMilarity implemented in Primer v. 6.1.12 (Primer-E Ltd.). Groupings were visualised using non-parametric multi-dimensional scaling (nMDS) with sound fragments averaged within individuals. To further test whether the overall sounds produced by butterfly pupae and larvae were more similar to queens or workers of the two host ants *M. sabuleti* and *M. schencki*, we estimated mean Euclidean distances between groups and used Student's *t*-test to estimate the significance of the differences. Student's *t*-test was also used to establish the differences in the morphometric measurements on the stridulation organs of *M. sabuleti* and *M. scabrinodis* queens and workers.

Table 1. Source and number of the sound fragments analysed in this study

Species	Group	No. of individuals	No. of sound fragments
<i>Maculinea arion</i>	Pre-adoption larvae	4	98
	Post-adoption larvae	3	89
	Pupae	3	89
<i>Maculinea rebeli</i>	Post-adoption larvae	10	292
	Pupae	4	115
<i>Myrmica sabuleti</i>	Queens	8	226
	Workers	8	240
<i>Myrmica schencki</i>	Queens	11	285
	Workers	13	345
<i>Myrmica scabrinodis</i>	Queens	6	180
	Workers	5	180

Sounds from *Maculinea rebeli* and *Myrmica schencki* are the identical fragments used in Barbero et al., 2009.

Table 2. Average values of the three sound parameters for the casts and stages of the three *Myrmica* species and *Maculinea arion* and *M. rebeli*

Group	Pulse length (s)	Pulse repetition frequency (s ⁻¹)	Dominant frequency (Hz)
<i>M. arion</i> pre-adoption larvae	0.029±0.002	10.08±0.71	561.6±8.79
<i>M. arion</i> post-adoption larvae	0.038±0.005	4.55±0.34	240.6±29.9
<i>M. arion</i> pupae	0.012±0.003	13.74±0.66	1070.0±47.6
<i>M. rebeli</i> larvae	0.036±0.005	13.32±1.40	404.3±39.23
<i>M. rebeli</i> pupae	0.040±0.005	12.84±1.02	530.9±13.51
<i>M. sabuleti</i> queens	0.019±0.001	36.99±1.96	832.8±33.18
<i>M. sabuleti</i> workers	0.017±0.001	40.39±1.99	1285.3±80.6
<i>M. scabrinodis</i> queens	0.023±0.003	31.62±4.04	810.7±31.1
<i>M. scabrinodis</i> workers	0.021±0.002	29.93±2.61	1480.3±161.4
<i>M. schencki</i> queens	0.021±0.001	34.50±2.53	812.2±40.1
<i>M. schencki</i> workers	0.021±0.002	39.03±3.37	1132.3±105.2

Values are means ± s.e.m.

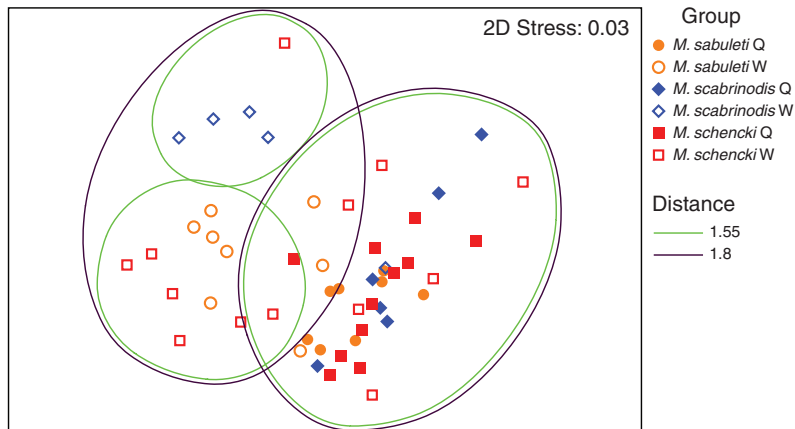


Fig. 1. MDS plot of the normalised Euclidean distances of the queens and workers of the three model ants, *Myrmica sabuleti*, *M. scabrinodis* and *M. schencki*. Solid symbols indicate data from queens (Q), open symbols are data from workers (W). The contours indicate the normalised Euclidean distance separating the groups.

RESULTS

Worker and queen acoustics in three species of *Myrmica* ant

The workers of all *Myrmica* species recorded to date have been shown to stridulate. We recorded both workers and queens of *Myrmica sabuleti*, *M. scabrinodis* and *M. schencki* to compare species and castes. Average measurements for the three sound parameters PL, PRF and DF are listed in Table 2. Using a multivariate approach over the three sound parameters, the normalised Euclidean distance within each group were (mean \pm s.e.m.): *M. sabuleti* queens: 0.68 ± 0.05 , workers: 0.97 ± 0.08 ; *M. scabrinodis* queens: 1.26 ± 0.19 , workers: 1.38 ± 0.22 ; *M. schencki*: queens: 0.97 ± 0.06 , worker: 1.74 ± 0.05 . The sounds of *M. schencki* workers are quite diffuse (Fig. 1), whereas those of the other workers are more clustered. However, the sounds of queens of all three species were very similar and closely overlap, as seen in Fig. 1. This is also reflected in the results of a nested ANOSIM to test for pairwise differences between groups. Among the ant groups, *M. schencki* workers were significantly different only from their own queens (ANOSIM $R=0.12$, $P=0.04$), and did not separate from the workers or queens of the other species ($R < 0.12$, $P > 0.17$). More intriguingly, there was no significant difference in the sounds made by the queens of the three species tested (*M. sabuleti* versus *M. scabrinodis* $R=0.05$, $P=0.22$; *M. sabuleti* vs *M. schencki* $R=0.002$, $P=0.43$; *M. scabrinodis* vs *M. schencki* $R=0.11$, $P=0.15$).

All other pairwise comparisons among groupings show significant separation ($R \geq 0.49$, $P \leq 0.01$). In other words, as previously demonstrated in *M. schencki*, the queens of both *M. sabuleti* and *M. scabrinodis* made distinctive sounds that were significantly different from those of their workers. This difference reflects the distinct stridulatory organ morphology of the two castes (Fig. 2). As shown in *M. schencki*, the distance between the pars stridens ridges in *M. sabuleti* (queens: $1.61 \pm 0.18 \mu\text{m}$, workers: $1.35 \pm 0.11 \mu\text{m}$) and *M. scabrinodis* (queens: $1.86 \pm 0.50 \mu\text{m}$, workers: $1.27 \pm 0.48 \mu\text{m}$) differs between queen and worker ants (two-sample *t*-test $t_{M.sabuleti}=6.53$, d.f.=58, $P < 0.001$; $t_{M.scabrinodis}=4.66$, d.f.=58, $P < 0.001$).

Sound similarities of larvae and pupae of the predatory *Maculinea arion* and workers and queens of its host ant *Myrmica sabuleti*

Using a nested ANOSIM we found that sounds from *M. arion* pupae differ from those of pre-adoption larvae ($R=1$, $P=0.029$), yet they apparently do not differ from the post-adoption larvae ($R=1$, $P=0.1$; Fig. 3). However, we interpret the results in Fig. 3, as an artefact of small sample size. Since data were available for only three pupae and three post-adoption larvae, there were only 10 possible permutations, and in one instance R , after permutation, was greater than the observed R , giving a level for P of 0.1.

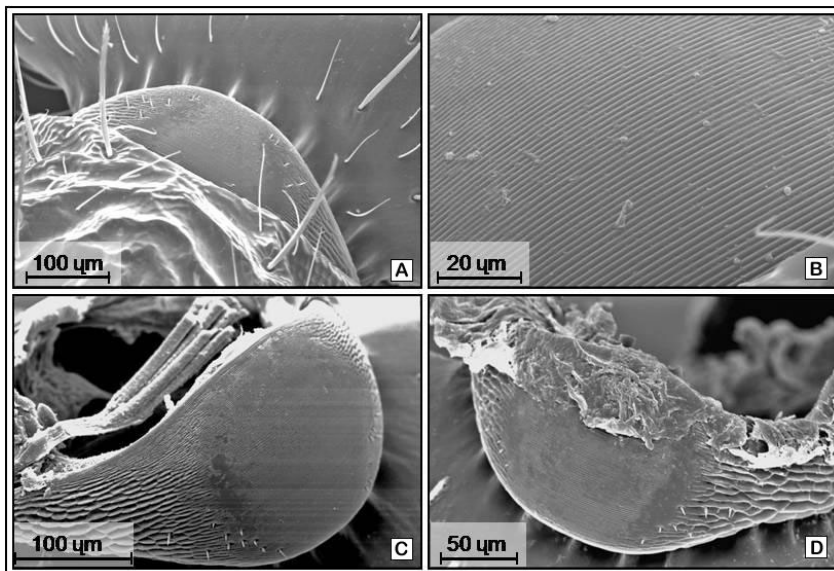


Fig. 2. The *pars stridens* on the stridulatory organs of a *M. sabuleti* queen (A), a worker (B), and of a *M. scabrinodis* queen (C) and worker (D).

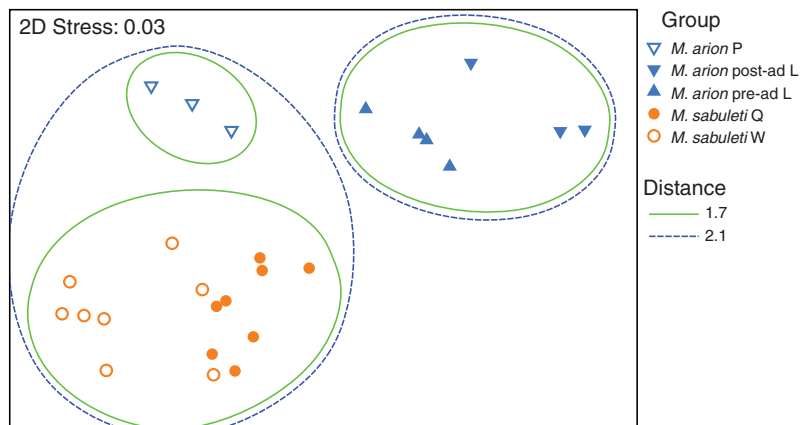


Fig. 3. MDS plot of the normalised Euclidean distances of the developmental stages of the predatory social parasite *Maculinea arion* and the queens and workers of its host ant *Myrmica sabuleti*. Open symbols indicate the pupal stage of *M. arion* and the workers of *M. sabuleti*. The final instar larval stages of *M. arion* are separated into pre-adoption (pre-ad), before the larvae had contact with ants, and post-adoption (post-ad) recorded after they were in contact with ants. The contours indicate the normalised Euclidean distance separating the groups. ANOSIM indicates significant separation between ant queens and workers and the larval and pupal stages of *M. arion* (see text). Pupae also differed from *M. sabuleti* queens ($R=0.99$, $P=0.006$) and workers ($R=0.99$, $P=0.006$), as did pre-adoption larvae (queens: $R=1$, $P=0.002$; workers: $R=1$, $P=0.002$) and post-adoption larvae (queens: $R=1$, $P=0.006$; workers: $R=1$, $P=0.006$). P, pupa; L, larva; Q, queen; W, worker.

The acoustical signals of all three groups of butterfly differed significantly from both *M. sabuleti* queens and workers (Fig. 3). *t*-Tests comparing the between-group normalised Euclidean distances of the butterfly to workers and queens showed that the acoustics made by all three stages of *M. arion* were significantly closer to the stridulations of the host queens rather than workers (Pupae: distance_{queens}=1.98±0.07, distance_{worker}=3.33±0.09, $t=11.3$, d.f.=41, $P<0.001$; pre-adoption larvae: $d_{\text{queens}}=2.32\pm0.08$, $d_{\text{worker}}=3.13\pm0.08$, $t=7.18$, d.f.=61, $P<0.001$; and post-adoption larvae: $d_{\text{queens}}=3.33\pm0.09$, $d_{\text{worker}}=4.22\pm0.11$, $t=6.13$, d.f.=45, $P<0.001$).

Comparison of *Maculinea arion* (predator) and *M. rebeli* (cuckoo) as mimics of their host ants

Owing to their cuckoo feeding life style, *M. rebeli* larvae, although not the pupae, are in more frequent and closer contact with ant workers than those of *M. arion*. We therefore predicted that *M. rebeli* might be a closer acoustical mimic of its host *M. schencki* than *M. arion* of *M. sabuleti*. In the absence of behavioural data, we compared the normalised Euclidean distances of sounds of the larval and pupal stages of both *Maculinea* species to the stridulations of both *Myrmica* species' queens and workers (Table 3).

We found no evidence that *M. rebeli* is a closer mimic of *M. schencki* than *M. arion* is to *M. sabuleti*. In fact, with one exception (*M. arion* pre-adoption larvae and *M. rebeli* pupae are equidistant to *M. schencki* workers), in all comparisons between *M. arion* pupae

or pre-adoption larvae and *M. rebeli* larvae or pupae with queens and workers of either ant species, the acoustics of *M. arion* were significantly closer to *Myrmica* stridulations than were those of *M. rebeli*. Only the acoustics of *M. arion* post-adoption larvae were found to be more distant than either stage of *M. rebeli* to both castes of either ant species (Table 3). Overall, the acoustics made by the immature stages of *M. arion* were 19.7% closer to *Myrmica* stridulations than those from *M. rebeli* (max=39.3% *M. arion* pupa and *M. rebeli* larva vs *M. sabuleti* workers, min=6.5% *M. arion* pre-adoption larva and *M. rebeli* larva vs *M. sabuleti* workers). This comfortably encompasses the range of acoustical variation found between *M. rebeli* larvae and pupae, where differences in the worker responses to butterfly sounds have been demonstrated (Barbero et al., 2009).

DISCUSSION

Our results demonstrate that stridulating queens from two additional *Myrmica* species make distinctive sounds to those of their workers using morphologically distinct organs. Indeed, intra-specific inter-caste differences were more clear-cut in *M. scabrinodis* and *M. sabuleti* than we previously reported for *M. schencki* (Barbero et al., 2009). Less expected was the fact that queen stridulations from the three *Myrmica* species were indistinguishable, as were worker stridulations in two of the three pairs of species tested. This suggests that acoustics plays little or no part in the cues used by *Myrmica*

Table 3. Pairwise *t*-test of normalized Euclidian distances between the predatory social parasite *Maculinea arion*, the cuckoo feeding parasite *Maculinea rebeli* and the queens and workers of their respective host ants *Myrmica sabuleti* and *Myrmica schencki*

	<i>M. sabuleti</i> queens	<i>M. arion</i> pupa (t; d.f.;P)	<i>M. arion</i> pre-adoption larva (t; d.f.;P)	<i>M. arion</i> post-adoption larva (t; d.f.;P)
<i>M. rebeli</i> pupae		5.61; 46; <0.001*	3.19; 50; =0.002*	3.36; 53; =0.001†
<i>M. rebeli</i> larvae		4.85; 101; <0.001*	2.59; 108; =0.011*	3.60; 94; =0.001†
<i>M. sabuleti</i> workers				
<i>M. rebeli</i> pupae		9.99; 46; <0.001*	2.94; 55; =0.005*	4.09; 53; <0.001†
<i>M. rebeli</i> larvae		9.96; 101; <0.001*	3.07; 109; =0.003*	3.65; 86; <0.001†
<i>M. schencki</i> queens				
<i>M. rebeli</i> pupae		4.47; 69; <0.001*	3.15; 79; =0.002*	3.41; 74; =0.001†
<i>M. rebeli</i> larvae		4.22; 137; <0.001*	2.90; 147; =0.004*	3.69; 115; <0.001†
<i>M. schencki</i> workers				
<i>M. rebeli</i> pupae		4.17; 74; <0.001*	1.63; 100; n.s.	2.62; 84; =0.011†
<i>M. rebeli</i> larvae		5.76; 160; <0.001*	2.35; 125; =0.02*	2.49; 77; =0.015†

*The mean distance of the *M. arion* pupae or larvae is closer; †the mean distance to *M. rebeli* larvae or pupae is closer; n.s., not significant.

to distinguish between non-kin or other species of ant and members of their own society, and indeed numerous studies demonstrate the predominant role of chemical cues and the gestalt odour in colony recognition or physiological states within an ant society (Hölldobler and Wilson, 1990). However, our recent results suggest that acoustical communication, in isolation, is capable of signalling at least the caste and the status of a colony member, and of inducing appropriate behaviour towards it by the workers (Barbero et al., 2009). It is possible that caste differences are yet more distinctive than the data presented here (Figs 1, 2), since to date we have measured and compared only the three attributes most commonly studied in ant–butterfly acoustics: dominant frequency, pulse repetition frequency and pulse length. Furthermore, when analysing our recordings of *Myrmica* acoustics, we noted that a wide variety of sound sequences was made by an individual queen or worker (see Barbero et al., 2009) (Fig. 2). During bioassays, we played the full repertoire of a test ant to cultures of workers, but it is possible that different component phrases induce one or other of the three behaviours observed in these tests (aggregation, antennation or guard attendance). It is also probable that individual ants can alter the rhythms, speed and intensity of stridulations to communicate other information under more natural or different conditions to those imposed by our experiments. For example, our equipment and procedures ensured that the ants were unstressed both at the time of recording and when receiving signals; and we observed only benign responses, with none of the antagonistic or alarm behaviours induced in early experiments, when ants were often distressed; and *vice versa* (Hölldobler and Wilson, 1990; Barbero et al., 2009).

Yet our experimental procedures were far from natural, and we suspect that a wider spectrum of information might involve acoustical communication. To draw a parallel from vertebrates, the main benefit of acoustical communication is its flexibility, allowing many short-lived signals to convey different information in a short time, by changing the pitch, harmonies or volume of the sound (Krebs and Davies, 1993; Greenfield, 2002). We have not yet studied whether different castes of *Myrmica* ant respond differently when exposed to the same sounds, although this seems probable, because queen *Myrmica schencki* respond aggressively when introduced to *Maculinea rebeli* pupae (which mimic queen sounds) whereas the workers tend them gently (Barbero et al., 2009) (Supporting Online Material: <http://www.sciencemag.org/cgi/content/full/323/5915/782/DC1>). And it is of course probable that the distinctive acoustical signals made by different members of an ant society modulate chemical and tactile cues in different ways.

Confirmation that the queens in other *Myrmica* societies make distinctive sounds from their workers indicates that this model is available to other mimetic *Maculinea* species or races that parasitise other *Myrmica* species, as we confirm here for *Maculinea arion* and *Myrmica sabuleti*. However, the demonstration that queens make the same sound in all three host ants studied suggests that acoustical mimicry functions strictly to raise the hierarchical status of a social parasite once it has been successfully accepted as a chemical mimic by a host society. In other words, acoustical mimicry is genus rather than species specific, as DeVries et al. (DeVries et al., 1993) concluded, albeit after recording highly stressed ants and *Maculinea* caterpillars. Although this presumably conveys a considerable benefit when resources in a nest are scarce – a frequent occurrence when *Myrmica* colonies are parasitised by supernumerary caterpillars of predatory (Thomas and Wardlaw, 1992) or cuckoo *Maculinea* caterpillars (Thomas et al., 1993) – it does not influence host specificity. For example, despite their similar acoustics, mortality of *Maculinea arion* caterpillars is more than

five times greater in *Myrmica scabrinodis* nests than when adopted by *M. sabuleti*, whereas mortality of (western) *Maculinea rebeli* caterpillars is more than 30 times higher when adopted by *M. sabuleti* than by their primary host, *M. schencki* (Thomas et al., 1989; Thomas et al., 2005a).

In our earlier study of *M. rebeli*–*M. schencki* interactions, we found that the pupal stage of the social parasite was a closer acoustical mimic of host queens than the caterpillar. This was demonstrated by worker ant behaviour, where pupal and queen sounds elicited characteristic ‘on guard’ behaviour at equal frequency, as did larval sounds, but to a slightly lesser degree (Barbero et al., 2009). That and this study also illustrate the amount of variation in acoustic signals found within and between the groups. The only guidance currently available of how much difference in acoustical similarity results in a difference in worker behavioural response comes from the original study, where, for instance, there were 27% more differences between the pupal and the worker calls than between the pupal and the queen calls, resulting in behaviour frequencies that were the same as towards queens, but significantly different from workers (Barbero et al., 2009). In this study, between-group differences in acoustical similarity ranged from ~6% to ~40%, and those of *M. arion* pupae and larvae tended to be closer to the queens than those of *M. rebeli* pupae and larvae, suggesting that the predatory species is at least as good a mimic as the cuckoo-feeding one. Further behavioural work should focus on the response norm of the receiving partner, i.e. the variation in the cue that is still accepted to trigger a behaviour, which in this case would be both workers and queens. This could be achieved by varying particular aspects of the sounds used for behavioural assays by computer manipulation. That *M. arion* caterpillars are apparently closer mimics of queen *Myrmica* would suggest that this interaction evolved as a basal trait in the *Phengaris*–*Maculinea* clade of Lycaenidae, for molecular studies suggest that the predatory life style preceded the evolution of the cuckoo forms (Als et al., 2004). Behavioural assays and further recordings will allow acoustic signalling to be used to address these evolutionary hypotheses.

As in ant communication, we suspect that the role of acoustics has been underestimated in the few studies made to date of the adaptations with which an estimated 10,000 species of invertebrate social parasite succeed in cheating ant societies. Promising taxa for future research are *Lepidochrysops* and 11 other lines of lycaenid butterfly that have independently evolved social parasitism from mutualistic, presumably sound-producing, ancestors (Fiedler, 1998): *Myrmecophila* species of cricket; the stridulations of staphilinid beetle social parasites; and the many inquiline ‘queen’ ants that parasitise other ants, including their close relatives.

We thank Luca Casacci for help during fieldwork and Mark Charles for designing and building the acoustic equipment. This study was supported by the Italian Ministry of the Environment and the Biodiversa project CLIMIT (CLimate change impacts on Insects and their MITigation).

REFERENCES

- Als, T. D., Vila, R., Kandul, N. P., Nash, D. R., Yen, S. H. and Hsu, Y. F. (2004). The evolution of alternative parasitic life histories in large blue butterflies. *Nature* **432**, 386–390.
- Akino, T., Knapp, J. J., Thomas, J. A. and Elmes, G. W. (1999). Chemical mimicry and host specificity in the butterfly *Maculinea rebeli*, a social parasite of *Myrmica* ant colonies. *Proc. R. Soc. Lond. B* **266**, 1419–1426.
- Barbero, F. (2007). *Myrmecophily and Interspecific Communication in Maculinea rebeli* (Lepidoptera: Lycaenidae). PhD thesis, Turin University.
- Barbero, F., Thomas, J. A., Bonelli, S., Balletto, E. and Schönrogge, K. (2009). Queen ants make distinctive sounds that are mimicked by a butterfly social parasite. *Science* **323**, 782–785.
- Baroni-Urbani, C., Buser, M. W. and Schilliger, E. (1988). Substrate vibration during recruitment in ant social organization. *Insect. Soc.* **35**, 241–250.

- Barth, F. G. (2000). How to catch the wind: spider hairs specialized for sensing the movement of air. *Naturwissenschaften* **87**, 51-58.
- DeVries, P. J., Cocroft, R. B. and Thomas, J. A. (1993). Comparison of acoustical signals in *Maculinea* butterfly caterpillars and their obligate host *Myrmica* ants. *Biol. J. Linn. Soc.* **49**, 229-238.
- Downey, J. C. (1966). Sound production in pupae of Lycaenidae. *J. Lepid. Soc.* **20**, 129-155.
- Downey, J. C. and Allyn, A. C. (1973). Butterfly ultrastructure: 1. Sound production and associated abdominal structures in pupae of Lycaenidae and Riodinidae. *Bulletin of the Allyn Museum of Entomology* **14**, 1-39.
- Downey, J. C. and Allyn, A. C. (1978). Sounds produced in pupae of Lycaenidae. *Bulletin of the Allyn Museum of Entomology* **48**, 1-14.
- Dumpert, K. (1972). Bau und Verteilung der Sensillen auf der Antennengeißel von *Lasius fuliginosus* (Latr.) (Hymenoptera, Formicidae). *Zeitschrift für Morphologie und Ökologie der Tiere* **73**, 95-116.
- Elmes, G. W., Thomas, J. A. and Wardlaw, J. C. (1991a). Larvae of *Maculinea rebeli*, a large blue butterfly, and their *Myrmica* host ants: wild adoption and behaviour in ant nests. *J. Zool.* **223**, 447-460.
- Elmes, G. W., Wardlaw, J. C. and Thomas, J. A. (1991b). Larvae of *Maculinea rebeli*, a large blue butterfly and their *Myrmica* ant hosts: patterns of caterpillar growth and survival. *J. Zool.* **224**, 79-92.
- Elmes, G. W., Wardlaw, J. C., Schönrogge, K. and Thomas, J. A. (2004). Food stress causes differential survival of socially parasitic larvae of *Maculinea rebeli* (Lepidoptera, Lycaenidae) integrated in colonies of host and non-host *Myrmica* species (Hymenoptera, Formicidae). *Entomol. Exp. Appl.* **110**, 53-63.
- Fiedler, K. (1998). Lycaenid-ant interactions of the *Maculinea* type: tracing their historical roots in a comparative framework. *J. Insect Conserv.* **2**, 3-14.
- Fieele, A. M. and Parker, G. H. (1904). The reactions of ants to material vibrations. *Proc. Acad. Nat. Sci. Philadelphia* **56**, 642-650.
- Giovannotti, M. (1996). The stridulatory organ of five Ponerinae species. A SEM study (Hymenoptera, Formicidae). *Fragmenta entomologica* **28**, 157-165.
- Grandi, G. (1966). *Istituzioni di Entomologia Generale*. Bologna: Calderini.
- Grasso, D. A., Mori, A., Le Moli, F., Giovannotti, M. and Fanfani, A. (1998). The stridulatory organ of four *Messor* ant species (Hymenoptera, Formicidae). *Ital. J. Zool.* **65**, 167-174.
- Greenfield, M. D. (2002). *Signalers and Receivers: Mechanism and Evolution of Arthropod Communication*. New York: Oxford University Press.
- Hickling, R. and Brown, R. L. (2000). Analysis of acoustic communication by ants. *J. Acoust. Soc. Am.* **108**, 1920-1929.
- Hickling, R. and Brown, R. L. (2001). Response to "Ants are deaf" [J. Acoust. Soc. Am. **109**, 3080]. *J. Acoust. Soc. Am.* **109**, 3083.
- Hojo, M. K., Wada-Katsumata, A., Akino, T., Yamaguchi, S., Ozaki, M. and Yamaoka, R. (2009). Chemical disguise as particular caste of host ants in the ant inquiline parasite *Niphanda fusca* (Lepidoptera: Lycaenidae). *Proc. R. Soc. Lond. B* **276**, 551-558.
- Hölldobler, B. (1999). Multimodal signals in ant communication. *J. Comp. Physiol. A* **184**, 129-141.
- Hölldobler, B. and Roces, F. (2000). The behavioural ecology of stridulatory communication in leafcutting ants. In *Model systems in Behavioural Ecology: Integrating Empirical, Theoretical and Conceptual Approaches* (ed. L. Dugatkin), pp. 92-109. Princeton: Princeton University Press.
- Hölldobler, B. and Wilson, E. O. (1990). *The Ants*. Cambridge, MA: Belknap Press, Harvard University.
- Hölldobler, B. and Wilson, E. O. (2009). *The superorganism*. London: WW Norton.
- Keller, L. and Gordon, E. (2009). *The Lives of Ants*. Oxford: Oxford University Press.
- Krebs, J. R. and Davies, N. B. (1993). *An Introduction to Behavioural Ecology*. Sunderland, Massachusetts: Sinauer Associates.
- Kumagai, T., Shimozawa, T. and Baba, Y. (1998). Mobilities of the cercal wind-receptor hairs of the cricket, *Gryllus bimaculatus*. *J. Comp. Physiol. A* **183**, 7-21.
- Lenoir, A., D'Etterre, P. and Errard, C. (2001). Chemical ecology and social parasitism in ants. *Annu. Rev. Entomol.* **46**, 573-599.
- Markl, H. (1965). Stridulations in leaf-cutting ants. *Science* **149**, 1392-1393.
- Markl, H. (1968). Die Verständigung durch Stridulationssignale bei Blattschneiderameisen. II Erzeugung und Eigenschaften der Signale. *Z. Vergl. Physiol.* **60**, 103-150.
- Markl, H. (1973). The evolution of stridulatory communication in ants. *Proceedings of the International Congress IUSSI*, London **7**, 258-265.
- Markl, H. (1985). Manipulation, modulation, information, cognition: some of the riddles of communication. In *Experimental Behavioural Ecology and Socio-Biology* (ed. B. Hölldobler and M. Lindauer), pp. 163-185. Stuttgart: Fischer.
- Markl, H. and Hölldobler, B. (1978). Recruitment and food-retrieving behaviour in *Novomessor* (Formicidae, Hymenoptera). Vibration signals. *Behav. Ecol. Sociobiol.* **4**, 183-216.
- Markl, H., Hölldobler, B. and Hölldobler, T. (1977). Mating behavior and sound production in harvester ants (*Pogonomyrmex*, Formicidae). *Insect. Soc.* **24**, 191-212.
- Masson, C. and Gabouriau, D. (1972). Ultrastructure de l'organe de Johnston de la Forme *Camponotus vagus* Scop. (Hymenoptera, Formicidae). *Zeitschrift für Zellforschung und Mikroskopische Anatomie* **140**, 39-75.
- Menzel, J. G. and Tautz, J. (1994). Functional morphology of the subgenital organ of the carpenter ant. *Tissue Cell* **26**, 735-746.
- Nash, D. R., Ais, T. D., Maile, R., Jones, G. R. and Boomsma, J. J. (2008). A mosaic of chemical coevolution in a large blue butterfly. *Science* **319**, 88-90.
- Pavan, G., Priano, M., De Carli, P., Fanfani, A. and Giovannotti, M. (1997). Stridulatory organ and ultrasonic emission in certain species of Ponerinae ants (Genus: *Ectatomma* and *Pachycondyla*, Hymenoptera, Formicidae). *The International Journal of Animal and its Recording* **8**, 209-221.
- Pierce, N. E., Braby, M. F., Heath, A., Lohman, D. J., Mathew, J., Rand, D. B. and Travassos, M. A. (2002). The ecology and evolution of ant association in the Lycaenidae (Lepidoptera). *Annu. Rev. Entomol.* **47**, 733-771.
- Roces, F. and Hölldobler, B. (1995). Vibrational communication between hitchhikers and foragers in leaf-cutting ants (*Atta cephalotes*). *Behav. Ecol. Sociobiol.* **37**, 297-302.
- Roces, F. and Hölldobler, B. (1996). Use of stridulation in foraging leaf-cutting ants: mechanical support during cutting or short-range recruitment signal? *Behav. Ecol. Sociobiol.* **39**, 293-299.
- Roces, F. and Tautz, J. (2001). Ants are deaf. *J. Acoust. Soc. Am.* **109**, 3080-3082.
- Roces, F., Tautz, J. and Hölldobler, B. (1993). Stridulation in leaf-cutting ants. *Naturwissenschaften* **80**, 521-524.
- Ruiz, E., Martinez, M. H., Martinez, M. D. and Hernandez, J. M. (2006). Morphological study of the stridulatory organ in two species of *Crematogaster* genus: *Crematogaster scutellaris* (Olivier 1792) and *Crematogaster auberti* (Emery 1869) (Hymenoptera: formicidae). *Ann. Soc. Entomol. Fr.* **42**, 99-105.
- Santos, J. C., Korndörfer, A. P. and Del Claro, K. (2005). Defensive behavior of the weaver ant *Camponotus* (*Myrmobrachys*) *senex* (Formicidae, Formicinae): drumming and mimicry. *Sociobiology* **46**, 1-10.
- Schönrogge, K., Wardlaw, J. C., Peters, A. J., Everett, S., Thomas, J. A. and Elmes, G. W. (2004). Changes in chemical signature and host specificity from larval retrieval to full social integration in the myrmecophilous butterfly *Maculinea rebeli*. *J. Chem. Ecol.* **30**, 91-107.
- Schurian, K. G. and Fiedler, K. (1991). Einfache Methoden zur Schwallwahrnehmung bei Blaulings-Larven (Lepidoptera: Lycaenidae). *Entomologische Zeitschrift* **101**, 393-412.
- Stuart, R. J. and Bell, P. D. (1980). Stridulation by workers of the ant, *Leptothorax muscorum* (Nylander) (Hymenoptera, Formicidae). *Psyche* **87**, 199-210.
- Tartally, A., Nash, D. R., Lengyel, S. and Varga, Z. (2008). Patterns of host ant use by sympatric populations of *M. alcon* and *M. rebeli* in the Carpathian Basin. *Insect Soc.* **55**, 370-381.
- Tautz, J. (1977). Reception of medium vibration by thoracic hairs of caterpillars of *Barathra brassicae* L. (Lepidoptera, Noctuidae). I. Mechanical properties of the receptor hairs. *J. Comp. Physiol. A* **118**, 13-31.
- Taylor, R. W. (1978). *Nothomyrmecia macrops*: A living-fossil ant rediscovered. *Science* **201**, 979-985.
- Thomas, J. A. (1984). The behaviour and habitat requirements of *Maculinea nausithous* (the dusky large blue butterfly) and *M. teleius* (the scarce large blue) in France. *Biol. Conserv.* **28**, 325-347.
- Thomas, J. A. (2002). Larval niche selection and evening exposure enhance adoption of a predacious social parasite, *Maculinea arion* (large blue butterfly), by *Myrmica* ants. *Oecologia* **122**, 531-537.
- Thomas, J. A. and Wardlaw, J. C. (1992). The capacity of a *Myrmica* ant nest to support a predacious species of *Maculinea* butterfly. *Oecologia* **91**, 101-109.
- Thomas, J. A., Elmes, G. W., Wardlaw, J. C. and Woyciechowski, M. (1989). Host specificity among *Maculinea* butterflies in *Myrmica* ant nests. *Oecologia* **79**, 452-457.
- Thomas, J. A., Elmes, G. W. and Wardlaw, J. C. (1993). Contest competition among *Maculinea rebeli* larvae in ant nests. *Ecol. Entomol.* **18**, 73-76.
- Thomas, J. A., Elmes, G. W. and Wardlaw, J. C. (1998). Polymorphic growth in larvae of the butterfly *Maculinea rebeli*, a social parasite of *Myrmica* ant colonies. *Proc. R. Soc. Lond. B* **265**, 1895-1901.
- Thomas, J. A., Schönrogge, K. and Elmes, G. W. (2005a). Specializations and host associations of social parasites of ants. In *Insect Evolutionary Ecology* (ed. M. D. E. Fellowes, G. J. Holloway and J. Rolff), pp. 479-518. Wallingford: CABI.
- Thomas, J. A., Elmes, G. W., Schönrogge, K., Simcox, D. J. and Settele, J. (2005b). Primary hosts, secondary hosts and 'non-hosts': common confusions in the interpretation of host specificity in *Maculinea* butterflies and other social parasites of ants. In *Studies in the Ecology & Conservation of Butterflies in Europe 2. Species ecology along a European gradient: Maculinea butterflies as a model* (ed. J. Settele, E. Kühn and J. A. Thomas), pp. 99-104. Moscow Sofia: Pensoft Press.
- Travassos, M. A. and Pierce, N. E. (2000). Acoustics, context and function of vibrational signalling in a lycaenid butterfly-ant mutualism. *Anim. Behav.* **60**, 13-26.
- Ware, A. B. (1994). Factors eliciting stridulation by the ponerine ant *Streblognathus aethiopicus* Smith (Hymenoptera, Formicidae). *Afr. Entomol.* **2**, 31-36.
- Zhantiev, R. D. and Sulkanov, A. V. (1977). Sounds of ants of the genus *Myrmica*. *Zool. Zh.* **56**, 1255-1258. (In Russian with English summary).