COMMENTARY

Singing the blues: from experimental biology to conservation application

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Summary

Chemical communication plays a major role in the organisation of ant societies, and is mimicked to near perfection by certain large blue (*Maculinea*) butterflies that parasitise *Myrmica* ant colonies. The recent discovery of differentiated acoustical communication between different castes of ants, and the fact that this too is mimicked by the butterflies, adds a new component of coevolutionary complexity to a fascinating multitrophic system of endangered species, and it could inspire new ways to engage the public in their conservation.

Key words: acoustical communication, ant–butterfly interaction, chemical communication, coevolution, endangered species, multi-trophic system.

Introduction: ants and their specialisations

Ants form some of the most sophisticated societies known to biology (Hölldobler and Wilson, 1990). In the most complex examples, such as in a colony of more than a million of leafcutter ants, individuals are far from being equal. Just one queen may be responsible for all reproduction over an extended period, after initial mating with perhaps three to eight short-lived males. The other hundreds of thousands of females are sterile workers, specialised to work all their lives, often in very different ways, for the benefit of a colony whose members are so completely integrated that it is sometimes referred to as a single 'super-organism' (Hölldobler and Wilson, 2009). Specialisation between different workers may be morphological or physiological, or may simply involve changing behaviour over an individual's lifespan. Sometimes all three are combined, resulting in distinct castes that range from soldiers, foragers for food, tenders of domesticated aphids or fungi, and nursemaids to – in the most extreme cases – 'kamikaze warriors', whose abdomens have evolved to explode as a sticky bomb that incapacitates enemies attacking its colony (Maschwitz and Maschwitz, 1974) or repletes, whose grossly distorted bodies have no function other than to store honeydew for the future needs of the colony (Hölldobler and Wilson, 1990).

Acoustic communication in ants

Recent work on the organisation and flexibility of ant societies shows that even the most complex societies obey simple behavioural rules that can be modelled, responding bottom-up in binary or other simple ways to chemical, physical or acoustic signals (e.g. Bonabeau et al., 1997; Krieger et al., 2000). Until now, it was thought that acoustics – which has been found in four subfamilies of ants, including some of the most dominant and species-rich – was a very minor part of ant communication; indeed, strictly speaking not communication at all, rather a simple signal conveying alarm or used as a beacon by other ants for orientation.

This partly reflected the technical difficulties of studying the acoustics of ant interactions under natural, benign situations. But now that this constraint has been resolved, it is evident that, for example, individual queen ants can make different sounds to workers and that the workers respond differently and appropriately to them (Barbero et al., 2009a; Barbero et al., 2009b; Thomas et al., 2010).

Penetration of ant colonies by mutualistic insects

Because the individual communications that govern cohesion, recognition, status, defence and other activities in an ant society are relatively predictable (involving standard reactions to combinations of physical shape, behaviour, chemistry and now acoustics), this has provided a route for pre-adapted predators, detritus feeders and, in some taxa, mutualistic (symbiotic) insects to penetrate ant colonies and eventually evolve into social parasites, which live safely inside the nest and exploit the rich resources concentrated there. Many bizarre examples of morphological and behavioural mimicry were described in these myrmecophiles (literally, 'ant-lovers') approximately a century ago by Wasmann (Wasmann, 1899), Wheeler (Wheeler, 1910) and Donisthorpe (Donisthorpe, 1927), culminating in Hölldobler's (Hölldobler, 1969; Hölldobler, 1970) classic demonstration of the existence of chemical manipulation and behavioural mimicry by *Lomechusa* (=*Atemeles*), Kistner's (e.g. Kistner, 1982) reviews of diversity in myrmecophiles and termitophiles, and Pierce et al. and Fielder's (Pierce et al., 2002; Fielder, 2006) accounts of lycaenid–ant interactions. Among this diversity of adaptations, Akino et al. (Akino et al., 1999) showed not only that *Maculinea rebeli* larvae were chemical mimics of their *Myrmica* host species, but also identified the specific cocktails of cuticular hydrocarbons that were involved. Now we can add acoustics, first postulated by DeVries et al. (DeVries et al., 1993), as a mimetic mechanism.

Mimics preferred over kin offspring!

A fascinating attribute of social parasites is that, by using a combination of cues, they can achieve extraordinary levels of host-colony penetration or acceptance (Thomas and Settele, 2004). Examples include the extreme host specificity to a single super-colony of its host species found in individual populations of the syrphid fly *Microdon mutabilis* (Schönrogge et al., 2006; Schönrogge et al., 2008), and the behaviour of host worker ants to integrated caterpillars of the butterfly *Maculinea rebeli* (Barbero et al., 2009a). The latter, although being chemical and physical mimics of their host ant's larvae, are actually treated as superior members of the colony. Consequently, in times of stress, workers rescue the *M. rebeli* caterpillars first (Thomas et al., 1998), and in times of starvation, they slaughter their own young (eggs, larvae) to feed them to the hungry caterpillars, just as if the parasites were adult queens (Thomas et al., 2005). Although based on current knowledge chemical mimicry cannot explain this superior status, we now know that acoustic mimicry of the adult queen ant's sounds provides a likely mechanism.

Do acoustic and chemical communication complement each other's evolution?

If, as seems increasingly probable, certain ant species can modulate acoustical signals to convey different information suited to particular circumstances, we might expect this system to complement that of chemical communication. While scientists are developing a clearer picture of the chemical world of these creatures, we should also consider the implications of placing an acoustic world of considerable complexity on top of it. It is here where subtle differences might lead to quite different relationships, especially because in any communicative interaction within or between species it is not just the properties of the signal that are important but also the reciprocal evolution in the other partner to have the sensory equipment to receive the signal. Thus, an answer to some of the little understood observed complexities might lie in the phylogenetic history of the interacting partners and how well-developed the use of chemical or acoustic signals was among the ancestral species.

On the basis of current knowledge, it seems clear that major to minor variations in ant hydrocarbon profiles enable ants and their mimics to distinguish between ant species (and within species between regional, non-kin and kin colonies), providing a mosaic of models that are duly mimicked at specific and sub-specific levels by the cocktails of chemicals secreted by social parasites (Akino et al., 1999; Schönrogge et al., 2004; Nash et al., 2008). In contrast, little interspecific variation in acoustics has been observed to date in the ant genus *Myrmica* or in their *Maculinea* mimic(s). Here the observed variation is between castes. The queens of three species studied (*Myrmica sabuleti*, *Myrmica scabrinodis* and *Myrmica schencki*) emit a very similar signal that is distinctive from those made by their workers and that elicits increased protection from them (Barbero et al., 2009a; Barbero et al., 2009b). It is these generic queen calls that at least three species of *Maculinea* social parasite mimic in their pupal and (less successfully) their larval stages, generating the same royal protection from the workers whose colony has been infiltrated (Barbero et al., 2009a; Barbero et al., 2009b). In other words, these social parasites appear to use chemical mimicry to gain acceptance by a putative host society but, once accepted, use acoustics to elevate their social standing to the highest attainable status in a colony (Thomas et al., 2010).

It is interesting that each mode of communication seems to work well in isolation. Both Akino et al. (Akino et al., 1999), studying

semio-chemicals, and Barbero et al. (Barbero et al., 2009a; Barbero et al., 2009b), studying acoustics, removed the other source of signal by exposing host workers to mute dummies or sounds played through speakers, respectively, and obtained clear behavioural responses. It is likely, however, that both modes of communication have synergistic or modulating effects in combination, a possibility that has yet to be explored in *Myrmica–Maculinea* or indeed any ant–myrmecophile communications. But in ants, some sounds or vibrations are used as accessory signals during activities that are stimulated or initiated by chemical, tactile or behavioural cues, or as a part of a multi-signalled process. For example, when alarmed, certain ants release both sounds and alarm pheromones (Hölldobler and Wilson, 1990); the end of mating, which is initiated by chemicals, is signalled by sound in at least one species (Markl et al., 1977); the minor castes of leafcutter ants use sound to indicate they are ready to hitch a ride back on the major workers carrying leaves (Roces and Hölldobler, 1995); and both *Myrmica* and *Leptothorax* adults produce sounds during mouth-to-mouth exchanges of regurgitated food and recognition pheromones (trophallaxis) (Zhantiev and Sulkanov, 1977; Stuart and Bell, 1980). So far, intraspecific variation in sound has been measured only in *Myrmica* ants, and this variable has yet to be studied in combination with chemical or tactile cues. Moreover, a wide range of sounds has been recorded from each individual *Myrmica* host or *Maculinea* parasite studied, and only the whole repertoire has been played back during behavioural bioassays. Within this range there may well be individual sequences (or 'tunes') that evoke different responses from the receiver.

Possible cost and other uses of acoustic mimicry

Just as the chemical profiles – especially of polygynous (multi-queened) ant colonies – and acoustical communication systems of certain ants make them vulnerable to infiltration through mimicry (Gardner et al., 2007), so too do the *Maculinea* social parasites of *Myrmica* support high loads of equally specialised genus- and perhaps species-specific wasps (parasitoids), which inject their eggs into the tissues of a caterpillar. Each parasitic wasp larva then grows to adulthood feeding on the host caterpillar's fat bodies, before killing it in the pupal stage, when, instead of a butterfly, an equally beautiful wasp emerges from the host pupa. The *Ichneumon* parasitoids of the well-integrated cuckoo species of *Maculinea* sting their butterfly hosts as caterpillars in the ant nests. They first detect which species of ant is the primary host and investigate only the nests of that species; they then somehow detect from above ground which primary host nests also support a few *Maculinea* among the abundant ant larvae in the brood chambers, situated 10–20 cm below ground in the case of *M. schencki* (Thomas and Elmes, 1993). Because the *Maculinea* caterpillars produce allomones that are such close mimics of ant pheromones that they fool their ant hosts when in physical contact, and because the chemicals identified so far are non-volatile, chemistry seems an unlikely cue in the detection of underground caterpillars by the parasitic wasps. Acoustics seem a more likely mechanism, although this has not been studied and has yet to be confirmed.

Although the acoustical signals of *Maculinea* larvae contain certain components that closely mimic the stridulations of ants, they also contain non-mimetic elements that, when amplified, are clearly distinguishable from ant songs, even to human ears. The initial behaviour of the parasitoid is consistent with the use of acoustic cues. Having identified a host *Myrmica* species' nest, it puts its head in the entrance, provoking a violent reaction from the guarding ants. At this stage it almost certainly releases some of the four to

five chemicals identified by Thomas et al. (Thomas et al., 2002) that induce the following sequence of ant behaviour: (1) workers are attracted to the parasitoid; (2) on contact, additional chemicals provoke violent hostility, resulting in them attacking any object in the vicinity; and finally (3) a touch-allomone causes repulsion from parasitoid and results in the ants fighting each other, leaving their colony poorly guarded. We hypothesise that it is inevitable that the initial response by the guard ants would send a wave of chemically and acoustically induced alarm through the colony, and it is logical that this would cause the *Maculinea* caterpillars to sing in distress too, thereby identifying them to the parasitoid. If so, there may be a trade-off for the caterpillars (in regions where these rare parasitoids exist) between identifying themselves to ants at times of general distress and making themselves more vulnerable when the stressor is a parasite.

We also envisage that it might be rewarding to apply the latest (more natural) recording techniques to reexamine the putative role played by acoustics in linking ants with mutualistic myrmecophiles, which tend to be two orders of magnitude more abundant and species-rich than their socially parasitic relatives. For example, the larvae and pupae of many of the lycaenid and riodinid butterflies studied generate substrate-borne vibrations which, although they do not mimic the acoustics of associated ants in obvious ways, nevertheless attract workers to some of the more strongly mutualistic species (DeVries, 1991; Fiedler et al., 1996; Travassos and Pierce, 2000).

Outlook: how to engage the public in the evolutionary ecology and conservation of myrmecophiles

When amplified, the calls of the caterpillars and pupae of *Maculinea* butterflies are haunting, yet beautiful, to human ears. This eeriness, and the endangered status of each species, make a happy play on words of their vernacular name of 'large blues', and remind us that today we should be singing the blues to bring attention to their decline and galvanise efforts for their conservation.

Large blue butterflies have already become a flagship taxon for conservation (Thomas and Settele, 2004; Thomas et al., 2009). Despite being comparatively small, these organisms have attracted wide public attention owing to their endangered status, their beauty as adults and their extraordinary life history based on deception, social infiltration, exploitation and predation. This contrasts starkly with people's perceptions of butterflies as elegant creatures, yet the lifestyle also opens people's mind towards the need to include trophic interactions in conservation planning; not only are these relationships between species at different levels in the food chain fascinating in their own right, they make people aware of the complexity of life in their immediate surroundings.

It is a fascination that is already entering human culture. For example, from the world of art, at least three European nations have featured large blue butterflies on their postage stamps, a major UK chain of stores is currently selling hessian 'bags for life' depicting a stylised large blue and, under the recent EU project MacMan, art students across Europe competed to design a poster using large blues to highlight the loss of biodiversity. Large blues also feature frequently in the European media, including in 2009 when, in a broadcast heard by millions, Sir David Attenborough included this butterfly as one of the twenty 'life stories' from the world of geology, biology and anthropology that had inspired him most. Meanwhile, in the performing arts, in November 2008 a theatrical presentation in a southern German town was given on the life history of large blue butterflies, and in Cornwall, UK, a folk dance was recently choreographed and performed, again inspired by the large blue. These performances were produced before the new insights into large blue acoustics. How much more evocative might future shows be if played to the rhythms and timbres of the butterflies' ant-mimetic tunes?

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