# PROCEEDINGS B

## Editorial

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# The interactions of ants with their biotic environment

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This *special feature* results from the symposium 'Ants 2016: ant interactions with their biotic environments' held in Munich in May 2016 and deals with the interactions between ants and other insects, plants, microbes and fungi, studied at micro- and macroevolutionary levels with a wide range of approaches, from field ecology to next-generation sequencing, chemical ecology and molecular genetics. In this paper, we review key aspects of these biotic interactions to provide background information for the papers of this *special feature*. After listing the major types of biotic interactions that ants engage in, we present a brief overview of ant/ant communication, ant/plant interactions, ant/fungus symbioses, and recent insights about ants and their endosymbionts. Using a large molecular clock-dated Formicidae phylogeny, we map the evolutionary origins of different ant clades' interactions with plants, fungi and hemiptera. Ants' biotic interactions provide ideal systems to address fundamental ecological and evolutionary questions about mutualism, coevolution, adaptation and animal communication.

## 1. Introduction

With over 13 000 named species [1], ants (Formicidae) are the largest group of eusocial insects. Like other eusocial organisms, ant societies have cooperative brood care, overlapping generations living in the same nest and a division of labour with reproductive and non-reproductive individuals (workers). With an estimated 10 000 trillions of individuals, they equal the global human biomass, and constitute most of the animal biomass of rainforests [2]. Key to their success are the myriad of interactions ants engage in with members of their own colony, other insects, fungi, microbes and plants (figure 1 and table 1). These interactions have long been used as systems in which to address evolutionary questions about mutualism, coevolution, adaptation and animal communication. In this review, we highlight some of the most important interactions between ants and their biotic environment, centred on four points relevant to this special feature: (i) ant/ant communication, (ii) interactions between ants and plants, (iii) ant/fungus symbioses, and (iv) ants and their endosymbionts. We also provide a timeline for the evolution of some of the major ant biotic interactions.

## 2. Ant communication with other ants

Ants interact with a wide range of the organisms that are part of their biotic environment. These interactions are mediated by semiochemicals, including cuticular hydrocarbons (CHCs), chemical footprints, trail pheromones and alarm pheromones, and these chemicals are central to cooperation and conflict at distinct scales. This section and two papers in this special feature [4,34] focus on chemical communication.

While solitary insects mostly communicate for mating, eusocial insects have self-organized societies, where individuals communicate local information to mediate tasks, such as the division of labour, collective resource utilization and collective defensive actions [35]. CHCs are present on the cuticle of virtually all insects, and play central roles as waterproofing agents, but also

**Figure 1.** The diversity of ant biotic interactions. (*a,b*) Ant/ant interactions. (*a*) Slavemaking ant *Protomognathus americanus* (black) and its host *Temnothorax longispinosus*. (*b*) Parabiosis between *Crematogaster modiglianii* (small, left) and *Camponotus rufifemur* (large, right). (*c,d*) Ant/other arthropod interactions. (*c*) ant/lycaenid interaction. (*d*) The rove beetle *Diploeciton nevermanni* is a social parasite of the army ant *Neivamyrmex pilosus*. (*e*–*k*) Ant/plant interactions. (*e*) Ant foraging on *Senna scabriuscula* extrafloral nectary. (*f*) *Pheidole pallidula* ant dispersing a *Borderea chouardii* seed. (*g*) *Philidris nagasau* ant farm of *Squamellaria* plants in Fiji. (*h*) *Pseudomyrmex concolor* living in *Tachigali* domatium and cultivating Chaetothyriales fungi inside the domatium (black patches). (*i*–*k*) Ant/pitcher plant interactions (*Nepenthes*). (*i*) Ant foraging on peristome nectaries. When it rains, the peristome undulates with raindrops, acting as a mechanism to catch ant prey. (*j,k*) Mutualistic interaction between *Camponotus schmitzi* and *Nepenthes bicalcarata*. (*j*) *Camponotus schmitzi* ants live inside the hollow petiole of *N. bicalcarata* (arrowhead) and are able to walk inside the pitcher and swim to steal *Nepenthes* prey (*k*). (*l,m*) Ant/fungus interaction (see also (*l*) *Trachymyrmex* ants farm fungus cultivar). (*m*) *Allomerus* ants cultivate fungi to make carton scaffold to catch insect prey, here a horsefly. (*n*) Ant/microorganism interaction. *Blochmannia* endosymbionts in bacteriocytes (green) in the midgut tisue of a pupa (shortly after pupation) of *Camponotus floridanus* ants. Red cells are midgut cells that do not (yet) contain any bacteria. Photo credit: (*a*) Susanne Foitzik. (*b*) Florian Menzel. (*c*) School of Ecology and Conservation, UAS Bangalore, India. (*d*) Christoph von Beeren. (*e*) Brigitte Marazzi. (*f*) María García, Xavier Espadaler, Jens Olesen. (*g*) Guillaume Chomicki. (*h*) Rumsais Blatrix. (*i,k*) Ulrike Bauer. (*l*) Scott Solomon. (*m*) Claude Delhaye. (*n*) Sascha Stoll.

function as semiochemicals. Because CHCs primarily serve a waterproofing role, one can wonder about the extent to which climate, especially air humidity and rainfall, shapes CHC evolution. Menzel *et al*. [4] address this question and find that the amount of rainfall in an ant's environment indeed influences CHC profiles.

Particularly important to ant colonies is *queen signalling*, which indicates the queen's presence and fertility, and signals workers to abandon their own reproduction and to help with brood care [3,34,36]. If the queen's fertility diminishes, non-sterile workers or subordinate reproductives can take over her function [36]. Queen signalling is mediated either by CHC compounds [37–39] or volatile pheromones, as in the fire ant *Solenopsis invicta* [40]. Caste influences the CHC profiles [34,41,42], thereby facilitating the self-organization

of the division of labour [43]. Another crucial type of signal for the survival of the colony is nest-mate recognition, highlighted in this special feature [4].

Ant communication among nest-mates also concerns food sources: ant workers alert and motivate their nest-mates, and direct them to the food source using tandem running. Ant trail pheromones originate from several glands (ventral venom gland, poison gland, Dufour's gland, sternal gland or hindgut), and the origin of trail pheromones is often subfamily-specific [44–47]. Quality of the food source, and its quantity and distance from the nest, can be reflected either in the strength of the trail [48] or in the abundance of chemicals produced by distinct glands [44]. Finally, alarm pheromones occur in all ant species studied [2], with the responses depending on colony size and ant species. In the

**Table 1.** Main types of ant biotic interactions.

| partners involved | type of interaction | references |
|---|---|---|
| ant/ant interactions | | |
| within colony interactions | cooperative | [3] |
| between colony interactions | antagonistic | [3] |
| between species interactions | mutualistic (parabiosis); parasitic (social parasitism) | [4,5]; figure 1a,b |
| ant/other arthropod interactions | | |
| ant/hemipteran interactions | mutualistic to parasitic | [6,7] |
| ant/butterfly interactions | mutualistic to parasitic | [8]; figure 1c |
| ant/mollusc interactions | parasitic | [9] |
| ant/diptera interactions | parasitic | [10] |
| ant/spider interactions | parasitic | [11] |
| ant/beetle interactions | parasitic | [12–14]; figure 1d |
| ant/wasp interactions | parasitic | [15] |
| ant/silverfishes interactions | parasitic | [16] |
| ant/plant interactions | | |
| extrafloral nectary/ant | mutualistic | [17]; figure 1e |
| elaiosome-based dispersal by ants | mutualistic | [18]; figure 1f |
| seed predation (harvester ants) | parasitic | [19] |
| domatium-based symbioses | mutualistic (rarely parasitic) | [20,21]; figure 1h |
| plant farming | mutualistic | [22,23]; figure 1g |
| ant/pitcher plant interactions | mutualistic or parasitic | [24,25]; figure 1i–k |
| ant/fungus interactions | | |
| attine-fungus farming | mutualistic (parasitic [Escovopsis]) | [26,27]; figure 1l |
| ant-Chaetothyriales-domatium interactions | mutualistic | [28,29]; figure 1h |
| ant–Chaetothyriales–carton interactions | mutualistic | [30]; figure 1m |
| ant/microorganism interactions | | |
| ant/bacterial endosymbionts | mutualistic or parasitic | [31–33]; figure 1n |
| ant/bacterial gut symbionts | mutualistic (but also likely commensal and parasitic) | [6] |
| ant/fungal gut symbionts | mutualistic | [6] |

ant genus *Cardiocondyla*, when sexual conflict occurs, winged males mimic the CHC profile of females to protect themselves against wingless males attacks [49,50].

The interactions of ants with other ant species are of three main types, mutualistic, parasitic or competitive. In this special feature, two studies focus on the evolution of chemicals that mediate mutualistic (parabiotic) [4] and parasitic ant/ant interactions [34]. Parabiosis ([51]; figure 1b) is a mutualistic symbiosis between different ant species that involves nest sharing, joint foraging and aphid tending, whereas brood are kept separate [5,52]. The relation is asymmetric in that only one of the species locates the pheromone trails of the other [53,54]. This asymmetry is reminiscent of parasitic ant/ant interactions (see below) and thus suggests that parabiosis might have evolved from parasitic associations [55–57]. Two factors appear to maintain parabiosis as mutualistic and prevent aggression: distinct CHC profiles with long carbon chains (more than C35; [4], this special feature) allow an ant colony to differentiate its parabiont from other ant species, and appeasement pheromones on the cuticle suppress aggressiveness, as shown for a *Camponotus/Crematogaster* parabiosis [58]. Non-parabiotic ants, which have different

CHC profiles and lack appeasement pheromones, are attacked as intruders [59,60].

About 220 ant species (out of more than 13 000) are parasites of other ant species, often living in the same nest as the 'host' ([61], figure 1a). Such parasites either exploit other species by using their pheromone trails to find the discovered food source [62] or use 'eavesdropping'—using auditory cues as information on food sources [63]. Trail following is always unilateral [62]. Distinct classes of social parasites exist, depending on whether or not they keep their brood separate from the host, and whether or not they kill the queen (see [61] for a review). When parasites locate the host nest, they need to prevent attacks from the host. This can be achieved by parasites: (i) mimicking the CHC signature of their hosts [61]; (ii) acquiring it via wiping themselves against the host's surface (allogrooming) [64]; (iii) secreting a substance to appease the host [65]; (iv) chemically inducing fights among host workers to ease nest takeover [66,67]; and/or (v) reducing the CHC recognition cue so they become 'invisible' [34]. A particular type of social parasitism is slavemaking in which ants capture broods in raids to increase the worker force of their colony. In this special feature,

Kleeberg *et al.* [34] show that 'chemical camouflage' evolved several times in slavemaking ants (figure 1*a*) and that the shifts in the CHC profiles that mediated the evolution of parasitism affected mostly the worker caste, but hardly the reproductive caste.

## 3. Interactions between ants and plants

Ants engage in a range of interactions with plants, including non-symbiotic defence mutualisms mediated by extrafloral nectaries (EFNs) ([17]; figure 1*e*), spore, seed or fruit dispersal (myrmecochory) ([18]; figure 1*f*), plant farming ([22,23,68]; figure 1*g*) and ant/plant symbioses ([20,21]; figure 1*h*). Ants are prey for carnivorous plants, such as *Nepenthes*, but at least one carnivorous species has domatia and houses ants (figure 1*i–k* and table 1). Defence mutualism and dispersal by ants are widespread both in the tropics and temperate regions, whereas ant/plant symbioses and plant farming are restricted to the tropics. Dispersal by ants involves lipid- and protein-rich appendages on seeds that serve as rewards for the ants that disperse the seeds. Ant/plant symbioses involve plants that provide nesting spaces (domatia) to ants in return for extra nutrients brought in by the ants and the killing of insect herbivores and vines that shade, and directly or indirectly damage, the ant-hosting plant [21]. Plant farming occurs in so-called ant-gardens, where ants plant the seeds of selected epiphytes inside their carton nest in return for nest stabilization and food, and an extreme case of plant farming occurs in Fiji, where the ant *Philidris nagasau* obligately farms six species of the Rubiaceae genus *Squamellaria* [23]. These ant/plant interactions must involve chemical communication in: (i) the selection of the particular seed species farmed in the gardens; (ii) the detection of host plants by founding queens; (iii) the discrimination of plants to prune from or near the host (but obviously not the host itself), (iv) the selective patrolling on young developing host shoots (but not other shoots); and (v) host damage-induced more active protection [69].

Ant-garden ants are able to identify their host plant's seeds by chemical cues present on the seed coat [70], and seeds from distantly related species that may be part of the same ant gardens have evolved convergent seed chemical signatures [70,71]. One of the key compounds (6-MMS) is also found on the ant head [71], suggesting that ant-gardens might involve chemical mimicry, as initially hypothesized by Ule [72]. Such seed-collecting behaviour occurs in southeast Asian ant gardens [73]. In Fiji the dolichoderine ant *Philidris nagasau* obligately farms not only by planting, but also fertilizing, six *Squamellaria* species (Rubiaceae) by defecating in the plant-provided nesting side (domatium) of these plants from the seedling stage on, in return for nesting space and food rewards ([23]; figure 1*g*). The chemical basis of the latter interaction is as yet unknown. In this issue, Chomicki *et al.* [68] reveal new aspects of the macroevolution of southeast Asian and Australasia ant gardens, including the independent origin of 13 ant-garden fern and flowering plant lineages, and the acquisition during evolution of further host lineages by the ants' broadening of their gardens' diversity.

An implicit consequence of specialized farming mutualisms, such as those between *Squamellaria* and *Philidris nagasau* in Fiji, is a structured population (with high relatedness on both the ant and the plant side) [23]. In this special feature, a theoretical study [74] reveals that population structure reduces the benefits from partner choice (a mechanism that stabilizes mutualisms by allowing active partner discrimination), as long as the benefits to symbionts are *undirected*. The *Squamellaria*/*P. nagasau* mutualism, however, shows population structure and partner choice with rewards *directed* to a specific partner, namely *P. nagasau* [23,75], suggesting that population structure and the specificity of benefits indeed need to be modelled together.

The efficiency of herbivore protection by plant-nesting ants (in the context of local herbivore pressure) plays a central role in the stability of ant/plant and ant/plant/fungus symbioses. In this special feature, Orivel *et al.* [76] reveal trade-offs in ant–plant–fungus mutualisms as a result of the same traits being simultaneously involved in two mutualisms. Ants nest in domatia, but cultivate fungi in a carton scaffold that workers use to hide and trap insects (figure 1*m*). More investment in fungi leads to less plant defence, and species that provide too little defence should be displaced in the long term [76]. Reduction in plant defence can also occur as a result of abiotic stress, such as that experienced at high altitudes [77]. An example of this is provided by the results of Plowman *et al.* [78], who report on ant/plant mutualistic networks along an elevation gradient in Papua New Guinea and find that the benefits for the plant are reduced at high altitudes.

Convergent evolution is frequent in species interactions [79], probably because of similar biotic, abiotic and phylogenetic constraints. A particular form of convergence is parallel evolution, wherein similar traits evolved in related species but from different lineages. Phylogenetic analysis revealed such parallel evolution in the iconic ant/plant symbiosis involving *Pseudomyrmex* ants that protect *Vachellia* trees [80], and in this special feature, Ward & Branstetter [81] now use a phylogenomic approach to show that two closely related *Pseudomyrmex* lineages independently evolved traits related to obligate domatium-living. Detecting such cases of independent evolution, impossible without abundant genomic data, is important for our understanding of the flexibility of specialization and generalization.

## 4. Ant interactions with fungi

A particularly fascinating mutualism is that between attine ants and fungi (figure 1*l*). Attine ants obligately depend on mutualistic fungi for food. Five types of fungal farming by attine ants are known, from the generalist agriculture practised by the basal-most attine lineages to the specialized agriculture of a single fungal clone practised by the higher attines (including leafcutter ants) (reviewed in [26]). Attine farming mutualisms involve two further protagonists, the parasitic fungi in the genus *Escovopsis* [27], which also coevolved as part of tripartite associations [82], and the mutualistic *Pseudonocardia* bacteria that densely cover the lower part of these ants' thorax and that produce an antibiotic effective against *Escovopsis* [83,84].

Ants also interact with Chaetothyriales fungi, either as part of the carton nest building process where arboreal ants use fungal hyphae to strengthen the nest or trap insects ([30,85], figure 1*m*) or inside plant domatia as part of an ant/plant symbiosis ([28,29], figure 1*h*). Some domatium-living
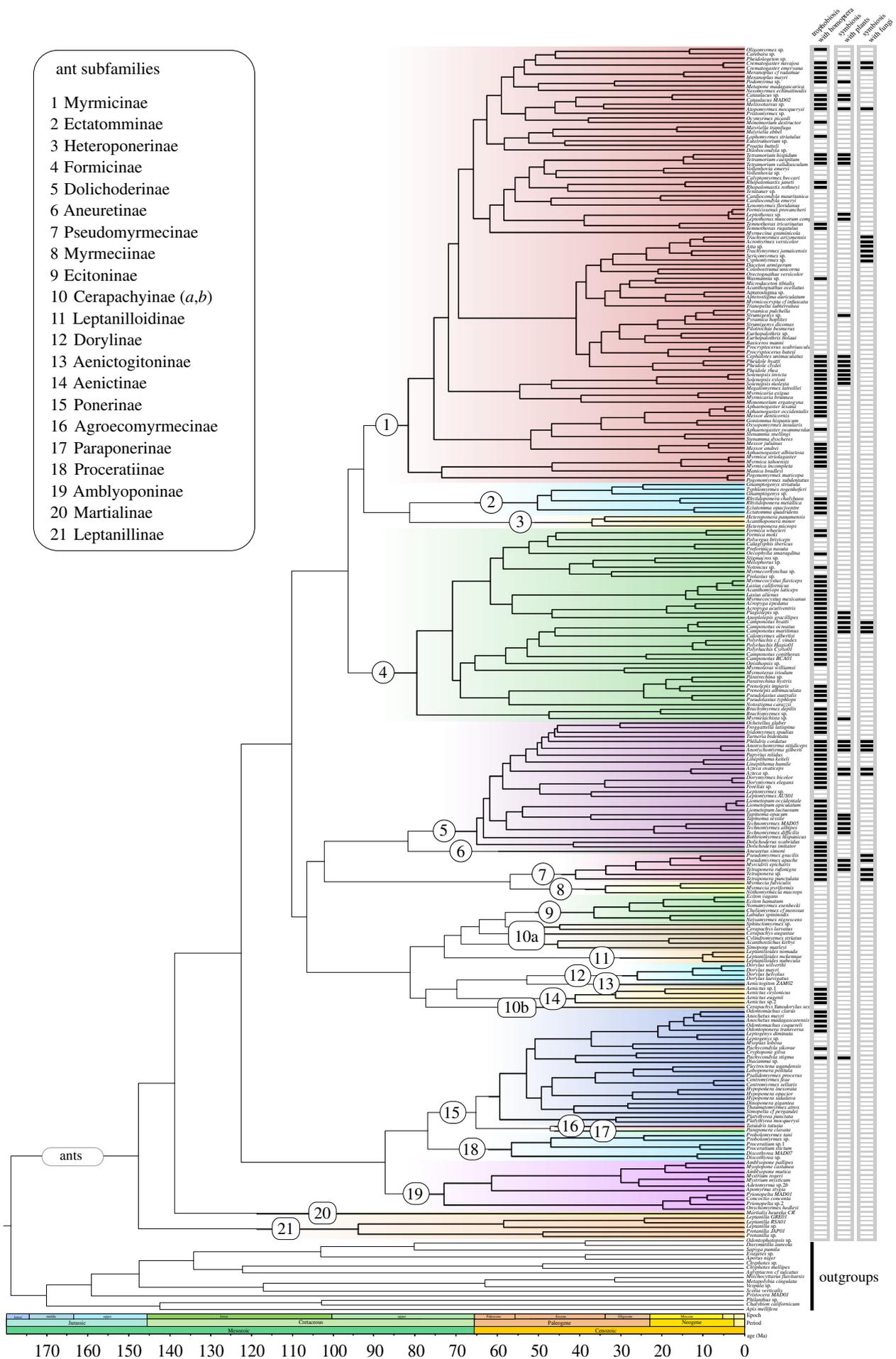
**Figure 2.** Phylogenetic distribution of ant symbioses with hemipterans, plants, and fungi. The tree from Moreau & Bell [99] was kindly provided by Corrie Moreau.

Chaetothyriales seem to be cultivated by ants and are involved in a complex trophic mutualism where the ants (especially larvae) feed on the fungi, which they actively fertilize by defecation [86], and the fungi play a role in nutrient recycling as well as facilitating nutrient uptake by breaking down materials [87]. In this special feature, Vasse *et al*. [88] show that ant/Chaetothyriales interactions evolved multiple times and that carton nest fungi and domatium fungi form different lineages. Another breakthrough published in this special feature is the finding by Baker *et al*. [89], who report community-level differences in domatium fungi and evidence that ant queens transport fungal communities when colonizing new trees.

## 5. Ants and their endosymbionts

Ant endosymbionts are microorganisms living either in specialized intracellular structures or in the ant gut. Endosymbiotic microbiomes may be central to the success of ants in tropical canopies and in other areas depleted in proteins ([6,90], this special issue). The so far best-known (obligate) bacterial endosymbiosis with ants involves *Camponotus* (the largest ant genus with more than 1000 species) and *Blochmannia* bacteria living in structures called bacteriocytes ([91], figure 1*n*), and these endosymbionts are also found in related genera [31]. Bacteriocytes in ovules are transmitted maternally [32,33], which has led to cospeciation between *Blochmannia* and *Camponotus* [92]. The bacteria provide the ants with essential amino acids and potentially recycle nitrogen by using ant urea that they can break down with their functional urease [31,93]. Besides these intracellular endosymbionts, ants have extracellular bacterial endosymbionts in the gut. This has been documented in *Tetraponera* (Pseudomyrmecinae) ants, which have a dense aggregation of gut bacteria that fix nitrogen and supplement the ants' phloem-based diet [94]. In this special feature, Pringle & Moreau [6] reveal new parts of the endosymbiont puzzle: ants share gut endosymbionts with their hemipteran trophobionts (via the honeydew they feed on). Moreover, ants have many facultative symbionts, and for the first time, a fungal endosymbiosis is discovered in *Azteca* [6].

## 6. The timeline of ant interactions with fungi, hemiptera and plants

The first unambiguous ant fossils date from the Lower Cretaceous [95,96], and molecular clock analyses have dated the ant crown group to 120–160 million years ago (Ma) [97–99]. Attine fungiculture is 50–60 million years old [100,101] and has a single evolutionary origin, but leafcutter ants appear to have evolved only 8–12 Ma [100]. However, several other ant lineages also use and farm fungi (figure 2). Interactions between ants and hemiptera (a suborder of Rhynchota) vary from generalist and facultative to specialized and obligate, and involve a wide range of aphids that provide honeydew or 'meat' [7]. They have evolved multiple times during ant evolutionary history (figure 2), and dating associations with hemipterans thus

will require densely sampled phylogenies for both groups. Fossils in amber have revealed ant/hemipteran associations in the Eocene (34–55 Ma) [102] and Early Miocene (15–20 Ma) [103,104], but comparative phylogenetic analyses are still lacking.

Regarding interactions with plants, phylogenetic dating and trait analyses suggest that elaiosomes (fatty seed appendages) that mediate plant dispersal by ants evolved as early as 75 Ma [105]. Non-symbiotic defence mutualisms based on EFNs are documented in the fossil record since the Oligocene (23–34 Ma; [106]), and evolved over 450 times in vascular plants [17], with the earliest EFNs originating at least 35–40 Ma [107–109]. Ant/plant symbioses mediated by plant-formed nesting sites (domatia) have at least 158 independent origins, starting some 15 Ma in the Neotropics and Australasia, and as recently as 5 Ma in Africa [21].

## 7. Conclusion

In organizing the symposium 'Ants 2016: ant interactions with their biotic environments' and this special feature issue, our aim was to bring together workers in this field to foster exchange and encourage integrative approaches. This Introduction has highlighted some of the ways through which ants interact with their biotic environment, and we also offer a new timeline (figure 2) for the evolution of interactions with hemipterans, plants and fungi. Studying interactions between ants and other species provides an opportunity to address fundamental and timely ecological and evolutionary questions.

A central theme that remains poorly studied are the genetic bases of many ant biotic interactions, which will probably emerge in the next decade, with several ant genomes published (e.g. the fire ant: *Solenopsis invicta* [110]; the Argentine ant: *Linepithema humile* [111]; the red harvester ant: *Pogonomyrmex barbatus* [112]; the leafcutter ant *Atta cephalotes* [113] and *Pseudomyrmex* plant ants [114]) and ever faster and low-cost, next-generation sequencing approaches and gene expression studies for targeted tissues and genes. Genomic approaches will allow a new understanding of ant biotic interactions by revealing discrete or genome-wide selection, gene duplication or loss or gene expression changes linked to new interactions [8,115]. Another emerging theme is multi-partite interactions, how they evolve and how the benefits are negotiated between species. Unravelling these and other themes relevant to the interactions between ants and their biotic environment will require the integration of multiple approaches, as exemplified in this special feature.

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