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Parallel evolutionary paths of rove beetle myrmecophiles: replaying a deep-time tape of life[☆]

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The rise of ants over the past ~100 million years reshaped the biosphere, presenting ecological challenges for many organisms, but also opportunities. No insect group has been so adept at exploiting niches inside ant colonies as the rove beetles (Staphylinidae) — a global clade of > 64,000 predominantly free-living predators from which numerous socially parasitic 'myrmecophile' lineages have emerged. Myrmecophilous staphylinids are specialized for colony life through changes in behavior, chemistry, anatomy, and life history that are often strikingly convergent, and hence potentially adaptive for this symbiotic way of life. Here, we examine how the interplay between ecological pressures and molecular, cellular, and neurobiological mechanisms shape the evolutionary trajectories of symbiotic lineages in this ancient, convergent system.

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Introduction

Convergent systems, where organismal clades have recurrently evolved similar phenotypes, are the focus of intense scrutiny in evolutionary biology [1,2]. The repeated evolution of analogous features under similar selection pressures is often taken as evidence of adaptation, and raises questions about the extent of determinism in the emergence of novel traits. Among the most striking and widely studied convergent systems are clades exhibiting a high frequency of repetitive evolution. These include host plant ecotypes and color morphs of *Timema* stick insects [3,4], recurrent losses of defensive traits in freshwater sticklebacks [5,6], and cichlid ecomorphs in African Great Lakes [7]. A common property of these systems is their comparatively young age, the convergent lineages often sharing a common ancestor as recently as ~10^4–10^6 years ago. This relative recency implies similar ancestral genomic starting conditions across lineages, a feature likely to elevate their probability of converging [8,9]. In multiple other convergent systems, the likelihood of convergence is increased instead by the focal traits sharing a relatively simple genetic architecture. Examples include toxin resistance by protective mutations in orthologous Na^+ pumps and channels [10–12], or the evolution of venoms via repeated recruitment of paralogs from the same gene families [13].

In contrast to these examples, cases of convergent evolution of highly complex traits evolving at high frequency across evolutionary ancient clades are scarce. One compelling example, however, is the rove beetles (Staphylinidae). This clade of 64 000 species, comprising Metazoa's most speciose family, is composed predominantly of free-living predators that have radiated extensively in leaf litter and soil habitats across the planet [14]. From this ancestral condition, numerous lineages — perhaps hundreds — have evolved into symbionts that exploit the colonies of social insects, ants in particular [15–18]. Termed 'myrmecophiles', ant-symbiotic rove beetles provide one of the most dramatic cases of deep-time convergent evolution. Repeatedly, myrmecophilous rove beetles have evolved that exhibit relatively stereotyped behavioral, chemical, anatomical, and life history modifications, adapting the beetles to socially parasitic niches inside colonies. Some of these convergent lineages share a common ancestor > 10^8 years ago [19–21]. Studies in other convergent systems have investigated whether parallel mechanisms explain phenotypic convergence across lineages, focusing on the genomic, molecular, and cellular phenomena underlying trait development or function [22]. Here, we extend this question to rove beetles, enquiring how such

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mechanisms may combine with ecological forces to shape the phenotypes and fates of symbiont lineages.

Emergence of a deep-time convergent system

The rise of ants in terrestrial ecosystems over the past ~100 million years placed tremendous selection pressure on other taxa, but also created exploitable, resource-rich niches for species that could accommodate or attenuate ant aggression [23]. Myrmecophilous organisms specialized for colony infiltration have evolved in many arthropod groups [17,24], but the greatest number of origins of this lifestyle occurs in the Staphylinidae. Myrmecophily has arisen in 9 of 32 staphylinid subfamilies, the majority of lineages clustered into the 2 largest: Aleocharinae (~17 000 species) and Pselaphinae (~10 000 species) [18]. Why these groups? The ancestral lifestyle in both clades is free living. Phenotypically, however, members of both subfamilies sport a suite of traits posited to enable their coexistence in ant-dominated habitats, and which secondarily outfit the beetles for facultative colony exploitation. These traits are a predatory diet, small body size, and, perhaps most critically, robust defenses to counter ant aggression [18]. In Pselaphinae, ant defense is physical, stemming from a thick, internally reinforced integument and the ability to conglobate (fold into a protective ball) [19]. In Aleocharinae, defense is chemical, the beetles possessing a benzoquinone-secreting tergal gland that is targetable, and can be directly smeared on ants during hostile interactions [25–28].

These plesiomorphic traits can be regarded as 'potentiations' that facilitated opportunistic entry of free-living lineages into a new adaptive zone — the ant colony [18]. Crucially, however, the phenotypic ground plans of both clades also seem to have provided versatile material for subsequent specialization inside the nest. The outcome has been a set of relatively repeatable evolutionary trajectories, manifesting as ecomorphs adapted to comparable niches inside colonies of different ant species [16,18] (Figure 1). In an extension of this scenario, termite colonies have also been exploited, giving rise to convergent lineages of termitophiles (Figure 1). Below, we describe parallel evolutionary processes hypothesized to have shaped the adaptation of rove beetles to social insect colonies. We focus primarily on Aleocharinae, where more is known.

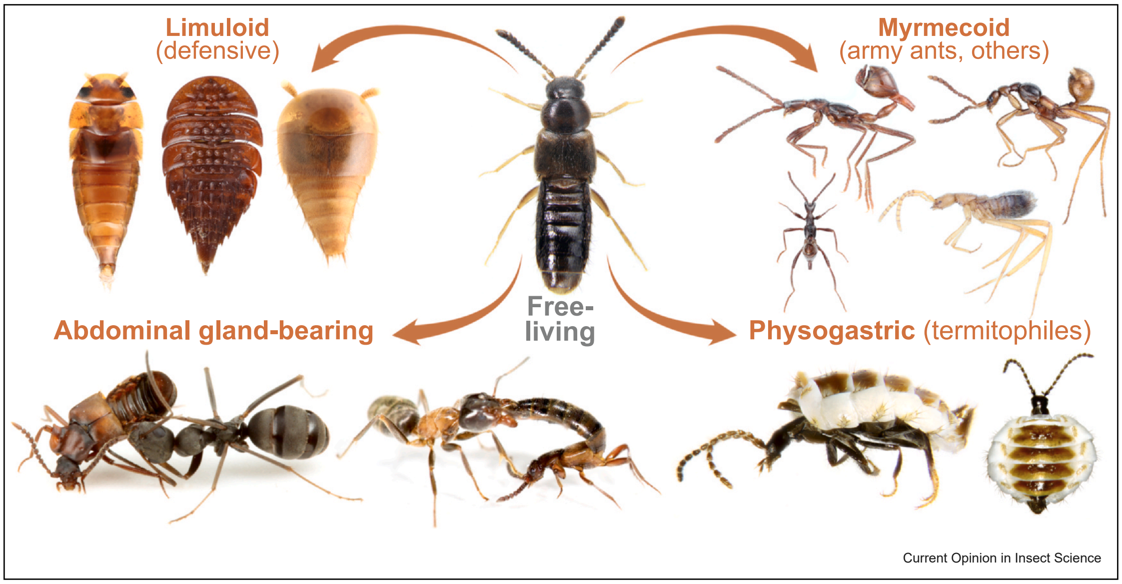
Evolution of chemical integration

The evolutionary transition from a free-living predator to a social symbiont is a multifaceted transformation of the phenotype (Figure 2 and 3). Common to perhaps all but the least specialized myrmecophiles are changes in chemistry and behavior that adapt rove beetles to colony-associated niches. Chemosensation is perhaps the principal modality by which ants respond to other organisms; hence, changes in chemistry enabling staphylinids to circumvent ant aggression are likely among the earliest steps toward specialization. The primary cues used by ants for nestmate recognition are cuticular hydrocarbons (CHCs) — blends of very long chain alkanes and alkenes (C25–C40) that coat the body (Figure 2a). Present in all insects as a barrier against desiccation, ants and other eusocial insects exapted CHCs as contact cues, resulting in complex gestalt chemical profiles used for nestmate discrimination [29]. Through the interaction of genetic and environmental factors, the blend of hydrocarbons varies not only between ant species but also between conspecific nests [29,30], and it is generally accepted that at least a subset of CHCs mediates nestmate recognition.

Unsurprisingly, the literature contains multiple examples of myrmecophiles (and termitophiles) that seemingly mimic their hosts' CHC profile [31], including several rove beetles [32–37]. CHC mimicry is likely a convergent feature of the majority of aleocharines that are at least partly socially integrated inside host colonies, but the mechanistic bases of CHC mimicry nevertheless remain murky. Two mechanistic explanations are commonly put forward: innate mimicry, where the beetle synthesizes a mimetic CHC profile *de novo*, and acquired chemical mimicry, where the beetle reduces or silences endogenous CHC production, creating a chemical blank slate onto which host-derived CHCs may be horizontally transferred (Figure 2b) [38]. Although widely invoked, these opposing mechanisms remain to be demonstrated unequivocally. For example, some highly integrated aleocharines of both Neotropical and South-East Asian army ants exhibit near-identical CHC profiles to their hosts [34,35] — a purported consequence of host CHC acquisition via frequent grooming interactions with workers. Grooming behavior (Figure 2b) is observed widely in aleocharines associated with army ants and some other host taxa [16,39–42], but its relation to chemical mimicry nevertheless remains ambiguous. To date, all studied cases of myrmecophile chemical mimicry have relied on indirect methods to ascertain the source of the myrmecophile's CHCs. Inference is typically based on whether novel CHCs are acquired when the myrmecophile is introduced to colonies, or retained or lost following isolation from ants [e.g. 43–45]. Yet, such observations do not preclude alternative mechanisms: endogenous CHC production may be plastic, with synthesis of a mimetic profile occurring only in the presence of hosts (giving the impression of horizontal acquisition); conversely, host-derived CHCs might be stably maintained on the body for a significant time (giving the illusion of endogenous biosynthesis).

Experiments with a myrmecophilous *Malayatelura* silverfish, in which isotopically labeled alkanes were placed

Figure 1

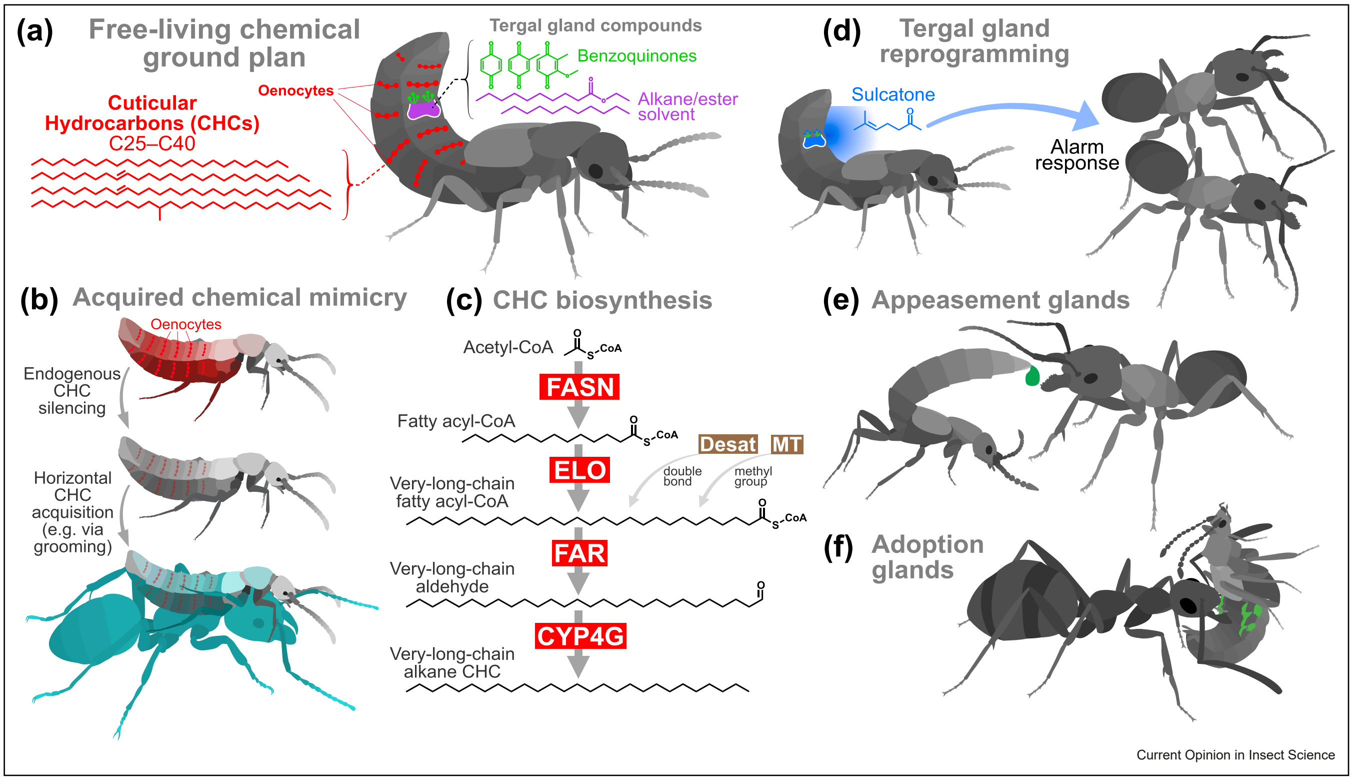


Convergent evolution of social insect symbiont ecomorphs in aleocharine rove beetles. From a chemical and behavioral ground plan that is highly conserved across free-living aleocharines (beetle in center), multiple independent lineages have evolved into symbiotic ecomorphs that are adapted to niches inside social insect colonies. Clockwise from top right: the 'myrmecoid' ecomorph anatomically (and where known, chemically) mimics host ants. This phenotype has evolved repeatedly in rove beetles associated with doryline army ants and some other ant genera. The massively expanded body of the 'physogastric' ecomorph has evolved convergently in beetles that are integrated into host termite colonies. The 'gland-bearing' ecomorph embodies all aleocharine species that have evolved abdominal gland-based mechanisms for chemically manipulating host behavior. Finally, the 'limuloid' ecomorph is a physically protective body plan that has evolved in many myrmecophile and termitophile taxa.
Photo credits: T. Shimada, T. Kanao, D. Miller, M. Maruyama, J. Parker.

onto host workers, and were seen to transfer onto the silverfish's body, indicate that compounds can indeed be acquired from hosts, but the origin of the mimetic CHCs themselves has proven elusive [44]. Verifying the source of CHCs — whether myrmecophile or host — can be more directly established on the premise that different dietary sources of carbon and hydrogen, as well as the different pathways of carbon and hydrogen routing during CHC biosynthesis, should produce distinct ^{13}C and ^{2}H incorporation patterns, thus exposing their origins. Strong support for the innate versus acquired mimicry models may therefore come from using compound-specific isotope analysis [46] to determine the $\delta^{13}C$ or $\delta^{2}H$ values of host and myrmecophile CHCs.

At the cellular level, how CHC mimicry is achieved is still more obscure. Recent studies have delineated a conserved CHC pathway in insects that is likely the target for adaptive changes in CHC production (Figure 2c). The CHC pathway uses fatty acid synthase enzymes (FASNs) to convert units of acetyl- and malonyl-CoA into medium-chain fatty acids, which are then increased in chain length by elongase enzymes (ELOs). Subsequent reduction by fatty acyl-CoA reductases (FARs) and decarbonylation by a 4G-class cytochrome P450 (CYP4G) yield very long chain alkanes; desaturase and methyltransferase enzymes can further modify CHCs into alkenes or methyl-branched alkanes [47]. The CHC pathway is expressed in oenocytes — specialized secretory cells, typically distributed in the insect abdomen [48]. To date, no studies have examined the CHC pathway or oenocytes of myrmecophiles. Based on findings in ants, CHC profiles are highly evolvable [49], and in insects more broadly they can exhibit significant phenotypic plasticity [50]. Mimetic CHC profiles may thus be relatively facile to achieve during evolution, raising the possibility of parallel changes underlying CHC profiles in myrmecophiles. Adaptive molecular evolution in CHC pathway components in myrmecophilous lineages relative to free-living outgroups has thus far not been demonstrated. We speculate that cases of innate mimicry may be correlated with duplication or accelerated birth and death within CHC pathway enzyme families, or signatures of positive selection in sequences of specific enzymes. In contrast, the reduction or loss of endogenous CHC production required for acquired chemical mimicry may typically arise from diminished expression or pseudogenization/loss of CHC pathway components, or perhaps reduction or loss of oenocytes.

Figure 2



Chemical ecology of free-living and myrmecophile rove beetles. **(a)** The chemical ground plan of free-living rove beetles: cuticular hydrocarbons (CHCs) are produced by oenocytes (red) and secreted onto the body surface. A defensive secretion containing benzoquinones in a medium-chain alkane/ester solvent is produced by the abdominal tergal gland. **(b)** Acquired chemical mimicry is accomplished by reducing or eliminating CHC biosynthesis, combined with horizontal transfer of CHCs from hosts, a possible function of grooming behavior. **(c)** The CHC pathway in insects. Acetyl- and malonyl-CoA are converted into very long chain alkanes, alkenes, and methyl branched alkanes that are secreted onto the body surface. Myrmecophiles evolve innate chemical mimicry by modifying CHC chain lengths, double bond positions, and methyl-branching patterns to match those of their host ants. These chemical changes may stem from molecular evolution in the FASN, ELO, desaturase (Desat), and methyltransferase (MT) enzyme families. **(d)** An example of tergal gland reprogramming. Here, the beetle secretes its host ant's alarm pheromone, sulcatone, dispersing the ants to avert aggression. **(e)** Use of an appeasement gland, located at the tip of the abdomen, to reduce ant aggression. **(f)** Adoption glands of some beetles induce ants to carry them into the nest or transport them on emigrations.

CHC mimicry and life history tradeoffs

The mode of CHC mimicry likely has major ramifications for myrmecophile life history, influencing both the degree of integration inside colonies and the ability to survive away from them. The complexity and plasticity of ant CHC profiles imply that innate mechanisms of chemical mimicry are unlikely to yield a perfectly matched CHC profile. Consequently, myrmecophiles that employ innate CHC mimicry as the principal means of countering host aggression may be relatively weakly socially integrated. There is a tradeoff, however, because the dual role of CHCs as anti-desiccants [30] means such species may not be unremittingly dependent on host colonies. Dispersal may be unchallenging for these species, their one-template-fits-all CHC profile permitting ready (albeit peripheral) association with newly encountered colonies of the same host, or perhaps even a range of chemically similar host species. Such a pattern of host promiscuity is seen in weakly integrated species of *Drusilla*, *Zyras*, *Tetradonia* [35], and *Pella* [51], some members of the latter genus being culturable for long periods away from host colonies.

In contrast, acquired chemical mimicry can achieve near-perfect chemical resemblance, minimizing hostility toward the myrmecophile. Perfect template matching presumably facilitates deeper penetration of the colony, access to novel trophic resources such as brood, as well as the potential for social interactions. The tradeoff is that such species can become obligately tied to host colonies, strikingly evident in the high mortality of certain species within hours to days of being removed from nests [39,42]. Continual acquisition of host CHCs, in their additional role as anti-desiccants, may underlie this tight dependence, but other, currently unknown host-derived cues may also contribute. The energetic investment in behaviors thought to underlie host CHC acquisition, such as grooming, seem to be huge: under experimental observation, some army ant-associated aleocharines groom workers almost constantly [39,52], while grooming accounts for 50–60% of the time budget of the *Liometopum*-associated aleocharine *Sceptobius* [42]. Dispersal of such species can be massively constrained, with loss of flight being a common (though not universal) trait. During their evolutionary specialization, perhaps the majority of myrmecophile and termitophile lineages navigate this life history tradeoff stemming from innate versus acquired CHC mimicry.

Biosynthetic innovation

Beyond modifications to CHC biosynthesis, a convergent feature of most rove beetle myrmecophiles is the secretion of chemicals that actively manipulate ant behavior. The beetles' short elytra expose a flexible abdomen that has been repurposed as a gland-bearing appendage [18,53], fostering impressive biochemical novelty [54]. Recent work in the laboratory model rove beetle *Dalotia coriaria* has uncovered how the defensive tergal gland of free-living aleocharines was functionally assembled during evolution [28]. The gland (Figure 2a) is composed of two secretory cell types that are unique to aleocharines: the 'BQ cells,' which synthesize the toxic but solid benzoquinones, and the 'solvent cells,' which form a chemical reservoir inside the abdomen, secreting the alkane and esters into which the benzoquinones dissolve. The solvent cells are an evolutionary hybrid: a cuticular cell type that has gained a major gene expression program from two ancient biosynthetic cell types: adipocyte-like 'fat body' cells and the CHC-producing oenocytes. These parental cell types also contributed distinct branches of a bifurcating fatty acid pathway, one leading to the esters, the other producing the alkane (undecane). In contrast, the BQ cells synthesize benzoquinones via a chimeric pathway built from enzyme components with ancestral functions in mitochondrial ubiquinone (CoQ10) synthesis and tyrosine-dependent cuticle maturation. The tergal gland was thus evolutionarily pieced together by recycling ancient transcriptomic modules and pathway motifs that enable the BQ and solvent cells to cooperate in the synthesis of a potent, defensive cocktail.

The biosynthetic functions of the tergal gland cell types have been targets of selection in myrmecophilous lineages. Detailed studies come from aleocharines associated with *Lasius fuliginosus* ants, which employ undecane as an aggregation pheromone. To counter the undecane solvent in its own tergal gland secretion (Figure 2a), one *Pella* species has added an additional terpenoid ketone, sulcatone, into the secretion (Figure 2d), mimicking the *L. fuliginosus* alarm pheromone, thereby dispersing instead of attracting the ants [55]. A second *Pella* species has replaced undecane with tridecane, which like undecane also functions as an effective benzoquinone solvent, but one that is neutral with regard to *L. fuliginosus* behavior [56]. Two further *Pella* species, in this case associated with *Lasius spathepus*, are thought to synthesize the terpene citronellal — the host's alarm pheromone [57]. Terpene synthesis has also been reported in two *L. fuliginosus*-associated *Zyras* species that produce α-pinene, β-pinene, myrcene, and limonene, possibly mimicking cues of ant-mutualist aphids [58]. In our own work on the tergal gland of the *Liometopum* myrmecophile *Liometoxenus*, we have observed production of aromatic esters that seem to intoxicate ants, impairing locomotion. These examples show that chemical strategies employed by beetles will often match the peculiarities of host ant biology. Presently, nothing is known about the genetic mechanisms that reprogram the tergal gland to produce novel compounds. How the transcriptomes and gene regulatory architectures of the BQ and solvent cells differ between *Dalotia* and myrmecophilous taxa may reveal genomic substrates that enable tergal gland pathways to undergo further modifications, yielding new chemistries.

In addition to modifying the tergal gland, novel abdominal exocrine organs have emerged in many myrmecophiles and termitophiles. Often — but not always — their appearance is coupled to the degeneration of the tergal gland, implying that the latter structure and its chemistry are no longer of utility, but that other compound types have instead been recruited for the symbiosis. Most unique glands have only been characterized anatomically, typically only in gross detail, but their repeated evolution in symbiont lineages implies functional relevance to this lifestyle [16,18]. In a few cases, functions have been inferred. Some secrete so-called 'appeasement compounds' on which ants feed, and which appear to attenuate ant aggression (Figure 2**e**). Appeasement secretions have been documented from a novel gland at the abdominal tip of some Lomechusini, including certain *Pella* [56, 59], and *Lomechusa* and allied genera [60,61]. An analogous appeasement gland and associated behavior have been reported in the distantly related *Dinarda* (Oxypodini) [62]. Chemical appeasement may be transiently deployed by otherwise weakly integrated species to counteract detection by hosts [56,59,62]. Appeasement may also facilitate initial colony entry in more tightly integrated species [60,61], potentially to pacify aggression before acquisition of the host CHC profile.

Lomechusa and allies also bear segmentally repeated 'adoption' glands, secretions from which induce workers to carry beetles into brood galleries where they oviposit (Figure 2f) [60]. This pattern of interaction is evocative of brood recognition, or perhaps of a food item to be provided to brood (an explanation for the treatment of the pselaphine *Claviger testaceus* inside colonies [63]). Hijacking chemical cues mediating brood care has been proposed for the *Aenictus*-associated *Trichotobia gracilis* and *Rosciszewskia gracilis*, each of which bear unique abdominal glands that may produce ant larva-specific compounds [34]. The identities and mechanisms of action on the ant nervous system are, however, unknown for any of the substances emanating from novel aleocharine glands, which represent a frontier for biosynthetic and neurobiological exploration.

Evidence for multisensory mimicry

All indications point to chemistry playing a preeminent role in successful colony exploitation. Yet, nestmate recognition may be a multi-layered phenomenon involving additional sensory modalities. Tactile or mechanical cues, in particular, may be relevant in some ants, and have been invoked to explain the dramatic myrmecoid (ant-mimicking) body plans of army ant myrmecophiles (Figure 1). The myrmecoid ecomorph has arisen at least a dozen times independently in Aleocharinae, in clades targeting almost all known doryline army ant genera, as well as scattered non-doryline hosts [21]. The physogastric form of termitophiles has also evolved on multiple occasions (Figure 1) [16,64]. Both ecomorphs are among the most tightly integrated symbionts known, and their mimetic body shapes have been argued to confer tactile mimicry [16,18,37,41,65]. Further support for tactile nestmate recognition and its mimicry has recently emerged in socially parasitic ants. Malagasy *Pheidole* ants comprise an endemic radiation in which one clade has evolved to parasitize multiple other distantly related *Pheidole* hosts. Through detailed morphometric study, it was revealed that the sizes and shapes of certain parasitic *Pheidole* body parts had evolved to match those of their hosts [66]. All evidence for anatomical/tactile mimicry nevertheless remains circumstantial, and efforts are needed to experimentally demonstrate that ants truly surveil features of nestmate anatomy, providing a selective agent for changes in symbiont form. The evolutionary correlation between anatomical resemblance of hosts and intimate social acceptance is, however, compelling, and implies that many of the most specialized lineages have experienced parallel selection to achieve host-like morphology. The correlation is not absolute, however, since anatomically non-mimetic symbionts can also be tightly integrated. Potentially, the latter taxa may rely more heavily on chemical strategies, such as appeasement, than attaining what might approximate 'nestmate status' through combined CHC and anatomical resemblance [67].

Neural basis of host recognition and social interaction

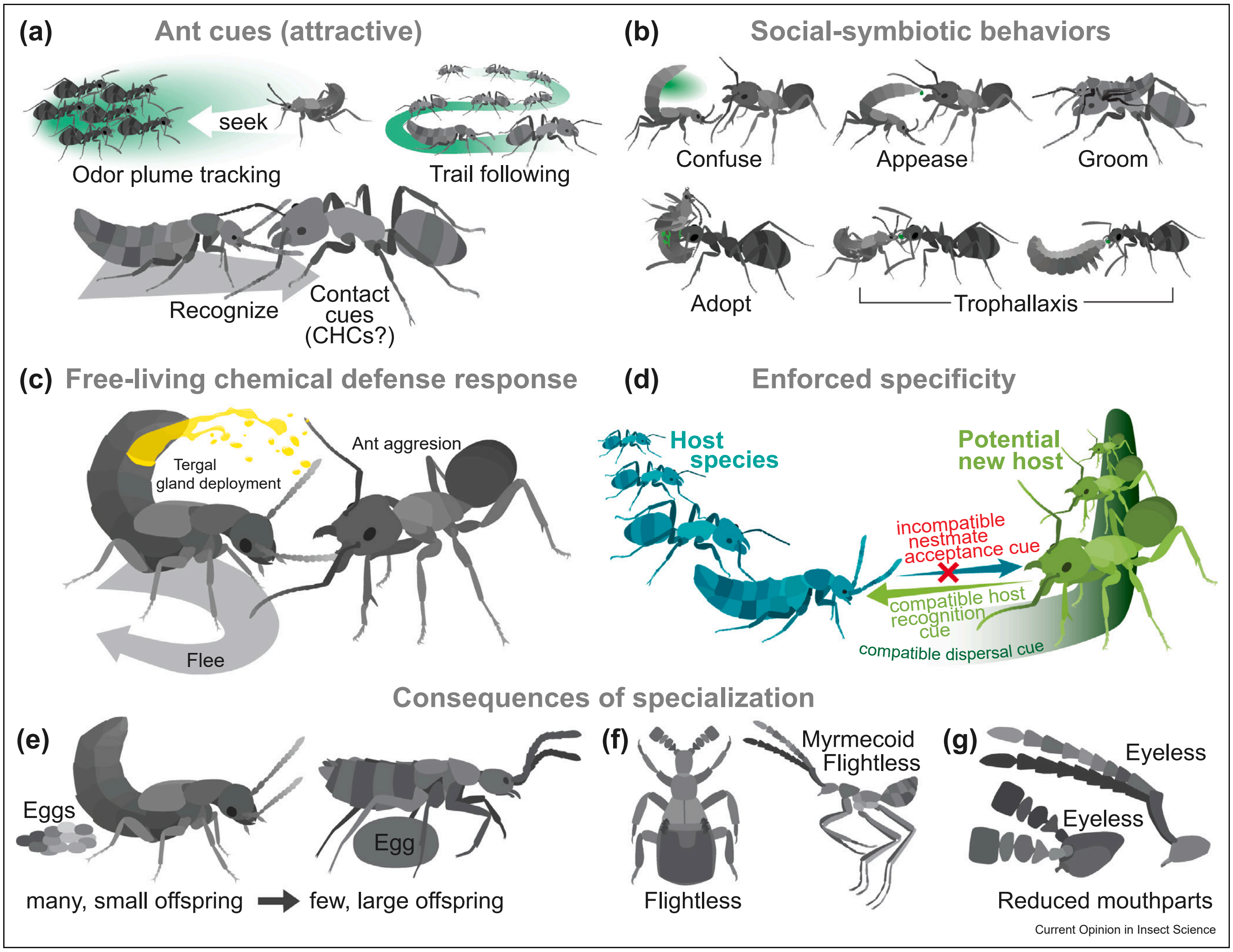
The adjustment to life inside colonies involves integrated changes in chemistry and behavior, modifications in each enabling subsequent changes in the other. Association with hosts requires recognition of and attraction to host-derived cues, but the nature of these stimuli, and the sensorimotor pathways that detect them and execute behavioral responses remain unstudied. Aleocharine myrmecophiles have been shown to eavesdrop on long-range colony cues that guide dispersal and host finding (Figure 3a). Multiple genera have convergently evolved to follow chemical foraging trails of ants [68,69], while others reportedly track volatile plumes of colony odors [70]. In addition, short-range or contact-based detection of individual ants occurs; based on studies in other insect symbionts, ant CHCs are candidate recognition cues [71–73].

A remarkable and convergent feature of myrmecophilous aleocharines is how such recognition cues are interpreted as attractive stimuli, promoting social interactions. Direct engagement with ants via stereotyped behavioral sequences such as grooming, trophallaxis, appeasement and other physical or phoretic actions contrasts dramatically with the typically aversive response that most insects display toward ants (Figure 3b). In free-living aleocharines, ant encounters elicit deployment of the tergal gland [25,26,28], combined with a rapid escape (Figure 3c). Myrmecophilous lineages have thus seemingly undergone a behavioral reversal toward these cues. Indeed, some species capable of chemically appeasing hosts do so despite retaining a functional tergal gland with primitive benzoquinone defensive chemistry [25,56,59,61,62,74]. This ability to select between glands for appeasement or defense implies a capacity for decision-making based on the perception of host cues. Evolution of valence toward chemosensory cues has been described between *Drosophila* species, and traced to changes in central brain circuitry [75,76]. We posit that across convergent myrmecophile lineages, parallel valence changes have occurred in sensorimotor pathways responsible for the perception of ant cues. The altered valence has rendered such cues attractive, or at least non-aversive and responsible for promoting interspecies social behaviors. Possible neurobiological mechanisms underlying valence evolution in interspecies contexts are discussed by Kanwal and Parker in this issue of COIS [77].

Mechanisms of host specificity

Aleocharine myrmecophiles commonly target a narrow host range, with highly integrated species often associating with single ant species. For example, each of at least a dozen myrmecoid clades parasitize single army ant genera, with beetle species within these clades usually associating with one ant species [21,78]. In the tribe Sceptobiini, the two constituent genera are thought to have speciated via cocladogenesis with *Liometopum* host ants [42,79]. Even members of some relatively weakly integrated taxa, such as *Pella* and *Tetradonia*, can have extremely narrow host ranges [51,80]. The narrowing of host range is likely a self-reinforcing phenomenon: phenotypic changes that enable more effective exploitation of specific hosts may be

Figure 3



Parallel behavioral and life history evolution in myrmecophile rove beetles. **(a)** Myrmecophiles associate ant cues with positive valence, and are attracted to long-range cues such as nest odors and trail pheromones, which facilitate host finding. Myrmecophiles likely use short range/contact cues from ant bodies — potentially CHCs — for host recognition and to initiate social-symbiotic behaviors. **(b)** Examples of behavioral interactions between myrmecophiles and hosts: confusing otherwise aggressive ants (for example via alarm pheromone release), chemical appeasement, grooming, soliciting adoption and oral trophallaxis. **(c)** Ancestrally, aleocharine rove beetles respond to perceived threats like ants by deploying their tergal gland and fleeing. **(d)** Enforced specificity. Many host ant dispersal and recognition cues that may be detected by myrmecophiles are not ant species-specific. Other ant species may therefore be compatible with the sensory recognition system employed by the myrmecophile. The potential novel host may nevertheless recognize the myrmecophiles as an outsider and reject it, thereby enforcing host specificity. **(e, f)** Long-term obligate specialization on ants is coupled to phenotypic changes that may hinder reversion to the free-living condition. Myrmecophile lineages show reduced fecundity but increased investment in single offspring. **(e)** Reductive evolution occurs, evident in recurrent loss of flight, vision, and mouthpart minimization. **(f)** Some of these changes may be adaptive, others arising from genetic drift in small populations under relaxed selection.

adaptive, but can also confine the subsequent selective environment, triggering further iterations of this process. Such 'runaway specialization' [81] has been posited to select for more efficient neural mechanisms of host finding [82]. In phytophagous insects and parasitoid wasps, specificity has been proposed to stem at least in part from sensory tuning to host cues [83–85]. The extent to which sensory tuning explains host specificity in myrmecophiles remains unexamined, however, and we argue that additional features of myrmecophile biology might constrain host interactions and should also be considered.

First, the close physical association of myrmecophiles with territorial host colonies may limit interactions with other ant species — a restriction possibly extending to the poorly documented dispersal mechanisms of many myrmecophiles. For flightless species, these may include vertical transmission during colony fission (e.g. some symbionts of army ants), phoresy on foundresses, or dispersal along chemical foraging trails. Even flighted genera, such as *Homoeusa*, have been found to diligently follow host trails 20 m from colonies [69]. Compounding these constraints is the inviability of some species when isolated from ants for even short time periods, discussed above [39,42]. For these reasons, opportunities for interactions with alternative hosts may be vanishingly scarce for certain species. Second, should such interactions occur, incompatibility of CHCs or other cues may lead to rejection. In this way, host specificity may be externally enforced through negative fitness interactions with alternative hosts (Figure 3d). Evidence consistent with enforced specificity is seen in *Maculinea* butterflies, where adopted larvae experience drastic differences in survival inside nests of different *Myrmica* species [86], their CHCs likely determining the outcome [87]. Similarly, in aleocharines, *Lomechusa pubicollis* is attracted to odors of different *Myrmica* species, which show different levels of aggression toward the beetle [60]. These examples argue against pronounced sensory specialization on optimal hosts, and instead imply that some myrmecophiles can exhibit latent promiscuity: a potential to associate with alternative hosts, even if these interactions incur negative fitness. Enforced specificity may even be the norm if myrmecophiles eavesdrop on compounds such as alarm and trail pheromones. These chemicals are often shared by related ant species or even members of different subfamilies; hence, their reliability for optimal host-finding or following may be far from absolute.

The fates of symbiotic lineages

Once a lineage undergoes the transition to myrmecophily, its long-term evolutionary fate is likely contingent on several factors. These include persistence of its host, its ability to host switch, and life history attributes that impact population genetic processes. Deep-time host associations, dating to the Cretaceous or even Jurassic, have been proposed for aleocharine termitophiles of the tribes Trichopseniini and Corotocini based on the present-day zoogeography of hosts and beetles [16]. Discovery of fossilized trichopseniines in mid-Cretaceous amber [88] is consistent with the age of this tribe, but inconclusive with respect to the fidelity of these beetles' host associations over time. The largest clades of obligate myrmecophiles — the clavigerite pselaphine rove beetles [89], haeteriine histerids [90], and paussine carabids [91] — show a pervasive pattern of host-switching across phylogeny, with transitions occurring between novel host ant taxa up to the subfamily level. Host switching facilitates speciation, prevents symbionts undergoing coextinction with hosts, and has presumably contributed to the antiquity of these ancient myrmecophile clades and their radiation into hundreds of species [90,92]. Host switching presents a paradox, in that species that are overtly specialized on certain hosts nevertheless seem adept at moving to new ones. For speciose myrmecophile clades, an explanation may be that the strategy these beetles use to integrate into colonies, as well as their physiology and life history, are sufficiently plastic to be compatible with alternative ant hosts ('ecological fitting' [93]). In Aleocharinae, switching to phylogenetically distant hosts is, in general, nowhere near as pronounced, being largely confined to weakly integrated genera such as *Drusilla* and *Zyras* [94] (some of which may in reality be facultatively myrmecophilous), and to a lesser extent *Pella*, which targets four host genera from two ant subfamilies [51]. Restricted host associations correlate with the relatively low species richness of individual clades of aleocharine myrmecophiles and termitophiles, and hints at barriers to moving to novel hosts, potentially related to the enforcement of specificity hypothesized above.

At present, we have little knowledge of how narrow host ranges impact symbiont lineage persistence. The most specialized lineages seem to have experienced selection for modified life histories that channel reproductive investment into few, high-quality offspring. The outcome is manifested in the production of giant eggs, each almost filling the female abdomen — a convergent feature of myrmecoid species [95] — as well as remarkable viviparity seen in physogastric termitophiles [96]) (Figure 3e). Numerical reduction of progeny may promote both offspring and host viability, but also contribute to the seeming natural rarity of many highly specialized symbionts. The pull of specialization is coupled to reductive losses of features critical in free-living species, including the tergal gland, flight capacity, robust predatory mouthparts, and in some cases vision (Figure 3f) [18]. In socially parasitic ants, marked losses of chemoreceptors have been demonstrated [97,98]; eye loss in some myrmecophiles implies comparable degenerate evolution of staphylinid sensory systems. The shedding of obsolete free-living features may initially be adaptive, but likely imposes constraints on gene flow leading to small effective population sizes. If genetic drift rather than natural selection dominates, genome erosion may ensue. The most extreme cases may be species hypothesized to undergo vertical transmission with host colonies — analogous to heritable endosymbiotic bacteria that are terminally confined to host insect lineages [99]. The genomic consequences of long-term obligate host dependence have yet to be surveyed in rove beetle myrmecophiles.

Conclusion

Rove beetles comprise an ancient system of complex evolutionary convergence in which numerous lineages have transitioned from free-living organisms into obligate symbionts of social insect colonies. From a relatively conserved chemical and behavioral ground plan, adaptive changes in pheromonal and glandular chemistry have evolved in parallel across symbiont lineages, facilitating mimicry and host manipulation; these have arisen in coordination with parallel changes in behavior that enable the beetles to interact socially with hosts. Future comparative studies, involving both symbiotic taxa and free-living outgroups, are needed to illuminate the molecular, cellular, and neurobiological substrates that have been remodeled to enable the repeated emergence of these symbiotic lifestyles. Examination of the population genetic forces that shape myrmecophile life histories may shed light on the fates of symbiotic lineages.

Conflict of interest statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest

•• of outstanding interest.

1. Morris SC: Life's Solution. Cambridge University Press; 2003.
2. Losos JB: Improbable Destinies: Fate, Chance, and the Future of Evolution. Penguin; 2018.
3. Soria-Carrasco V, Gompert Z, Comeault AA, Farkas TE, Parchman TL, Johnston JS, Buerkle CA, Feder JL, Bast J, Schwander T, *et al*.: **Stick insect genomes reveal natural selection's role in parallel speciation**. *Science* 2014, **344**:738-742.
4. Nosil P, Villoutreix R, Carvalho CF, de, Farkas TE, Soria-Carrasco V, Feder JL, Crespi BJ, Gompert Z: **Natural selection and the predictability of evolution in *Timema* stick insects**. *Science* 2018, **359**:765-770.
5. Colosimo PF, Hosemann KE, Balabhadra S, Villarreal G, Dickson M, Grimwood J, Schmutz J, Myers RM, Schluter D, Kingsley DM: **Widespread parallel evolution in sticklebacks by repeated fixation of Ectodysplasin alleles**. *Science* 2005, **307**:1928-1933.
6. Chan YF, Marks ME, Jones FC, Villarreal G, Shapiro MD, Brady SD, Southwick AM, Absher DM, Grimwood J, Schmutz J, *et al*.: **Adaptive evolution of pelvic reduction in sticklebacks by recurrent deletion of a *Pitx1* enhancer**. *Science* 2012, **327**:1-5.
7. Kratochwil CF, Liang Y, Gerwin J, Woltering JM, Urban S, Henning F, Machado-Schiaffino G, Hulsey CD, Meyer A: **Agouti-related peptide 2 facilitates convergent evolution of stripe patterns across cichlid fish radiations**. *Science* 2018, **362**:457-460.
8. Conte GL, Arnegard ME, Peichel CL, Schluter D: **The probability of genetic parallelism and convergence in natural populations**. *Proc R Soc B Biol Sci* 2012, **279**:5039-5047.
9. Ord TJ, Summers TC: **Repeated evolution and the impact of evolutionary history on adaptation**. *BMC Evolut Biol* 2015, **15**:137.
10. Feldman CR, Brodie ED, Pfrender ME: **Constraint shapes convergence in tetrodotoxin-resistant sodium channels of snakes**. *Proc Natl Acad Sci USA* 2012, **109**:4556-4561.
11. Dobler S, Dalla S, Wagschal V, Agrawal AA: **Community-wide convergent evolution in insect adaptation to toxic cardenolides by substitutions in the Na, K-ATPase**. *Proc Natl Acad Sci USA* 2012, **109**:13040-13045.
12. Zhen Y, Aardema ML, Medina EM, Schumer M, Andolfatto P: **Parallel molecular evolution in an herbivore community**. *Science* 2012, **337**:1634-1637.
13. Fry BG, Roelants K, Champagne DE, Scheib H, Tyndall JDA, King GF, Nevalainen TJ, Norman JA, Lewis RJ, Norton RS, *et al*.: **The Toxicogenomic multiverse: convergent recruitment of proteins into animal venoms**. *Ann Rev Genom Hum Genet* 2009, **10**:483-511.
14. Thayer MK: **Staphylinidae latreille, 1802**. In *Handbuch der Zoologie/Handbook of Zoology, Vol. IV (Arthropoda: Insecta), Part 38 Coleoptera, Beetles. Volume 1: Morphology and systematics (Archostemata, Adephaga, Myxophaga, Polyphaga partim)*. Edited by Beutel RG, Leschen RAB. Walter de Gruyter; 2005:296-344.
15. Seevers CH: **The systematics, evolution and zoogeography of staphylinid beetles associated with army ants (Coleoptera, Staphylinidae)**. *Fieldiana Zoology* 1965, **47**:139-351.
16. Kistner DH: **Social and evolutionary significance of social insect symbionts**. In *Social Insects*. Edited by Hermann HR. Academic Press; 1979:339-413.
17. Kistner DH: **The social insects' bestiary**. In *Social Insects*. Edited by Hermann HR. Academic Press; 1982:1-244.
18. Parker J: **Myrmecophily in beetles (Coleoptera): evolutionary patterns and biological mechanisms**. *Myrmecol News* 2016, **22**:65-108.
19. Parker J: **Emergence of a superradiation: pselaphine rove beetles in mid-Cretaceous amber from Myanmar and their evolutionary implications**. *Syst Entomol* 2016, **41**:541-566.
20. Yamamoto S, Maruyama M, Parker J: **Evidence for social parasitism of early insect societies by Cretaceous rove beetles**. *Nat Commun* 2016, **7**:13658.
21. Maruyama M, Parker J: **Deep-time convergence in rove beetle symbionts of army ants**. *Curr Biol* 2017, **27**:920-926.
22. Stern DL: **The genetic causes of convergent evolution**. *Nat Rev Genet* 2013, **14**:751-764.
23. Parker J, Kronauer DJC: **How ants shape biodiversity**. *Curr Biol* 2021, **31**:R1208-R1214.
24. Hölldobler B, Wilson EO: The Ants. Harvard University Press; 1990.
25. Jordan K: **Zur Morphologie und Biologie der myrmecophilen Gattungen *Lomechusa* und *Atemeles* und einiger verwandter Formen**. *Z Wiss Zool* 1913, **107**:346-386.
26. Brand JM, Blum MS, Fales HM, Pasteels JM: **The chemistry of the defensive secretion of the beetle, *Drusilla canaliculata***. *J Insect Physiol* 1973, **19**:369-382.
27. Steidle JLM, Dettner K: **Chemistry and morphology of the tergal gland of freeliving adult Aleocharinae (Coleoptera: Staphylinidae) and its phylogenetic significance**. *Syst Entomol* 1993, **18**:149-168.
28. •• Brückner A, Badroos JM, Learsch RW, Yousefelahiyeh M, Kitchen SA, Parker J: **Evolutionary assembly of cooperating cell types in an animal chemical defense system**. *Cell* 2021, **184**:6138-6156.

Brückner et al. demonstrate how the tergal gland of aleocharine rove beetles was constructed during evolution via the assembly of two unique cell types — the BQ and solvent cells — which cooperate in the synthesis of the beetle's defensive secretion. The functions of each cell type evolved via repurposing of gene expression programs and pathway components from other, more ancient metabolic or biosynthetic cell types present in insects.

29. Sturgis SJ, Gordon DM: **Nestmate recognition in ants (Hymenoptera: Formicidae): a review**. *Myrmecol News* 2012, **16**:101-110.

30. Sprenger PP, Menzel F: **Cuticular hydrocarbons in ants (Hymenoptera: Formicidae) and other insects: how and why they differ among individuals, colonies, and species**. *Myrmecol News* 2020, **30**:1-26.

31. Bagnères A-G, Lorenzi MC: **Chemical deception/mimicry using cuticular hydrocarbons**. In *Insect Hydrocarbons*. Edited by Blomquist GJ, Bagnères A-G. Cambridge University Press; 2010:282-324.

32. Howard RW, McDaniel CA, Blomquist GJ: **Chemical mimicry as an integrating mechanism: cuticular hydrocarbons of a termitophile and its host**. *Science* 1980, **210**:431-433.

33. Akino T: **Chemical camouflage by myrmecophilous beetles *Zyras comes* (Coleoptera: Staphylinidae) and *Diartiger fossulatus* (Coleoptera: Pselaphidae) to be integrated into the nest of *Lasius fuliginosus* (Hymenoptera: Formicidae)**. *Chemoecology* 2002, **12**:83-89.

34. Maruyama M, Akino T, Hashim R, Komatsu T: **Behavior and cuticular hydrocarbons of myrmecophilous insects (Coleoptera: Staphylinidae; Diptera: Phoridae; Thysanura) associated with Asian *Aenictus* army ants (Hymenoptera; Formicidae)**. *Sociobiology* 2009, **54**:19-35.

35. •• Beeren C, von, Brueckner A, Maruyama M, Burke G, Wieschollek J, Kronauer DJC: **Chemical and behavioral integration of army ant-associated rove beetles - a comparison between specialists and generalists**. *Front Zool* 2018, **15**:8.
von Beeren et al. investigated a number of beetles associated with *Eciton* army ants. The most socially integrated species associate with a single host ant, exhibit a myrmecoid morphology, and accurately mimic the cuticular hydrocarbons of the host ant. Conversely, the more loosely associated beetle species exhibit more divergent CHC profiles from the ants, are found with multiple species of *Eciton*, and possess a more typical rove beetle morphology.

36. Rosa CS, Cristaldo PF, Florencio DF, Marins A, Lima ER, DeSouza O: **On the chemical disguise of a physogastric termitophilous rove beetle**. *Sociobiology* 2018, **65**:38-47.

37. • von Beeren C, Brückner A, Hoenle PO, Ospina-Jara B, Kronauer DJC, Blüthgen N: **Multiple phenotypic traits as triggers of host attacks towards ant symbionts: body size, morphological gestalt, and chemical mimicry accuracy**. *Front Zool* 2021, **18**:46.
von Beeren et al. compare multiple phenotypic traits of army ant myrmecophiles and argue that certain morphologies result in lower ant aggression. Ant nestmate recognition mechanisms may therefore integrate multisensory information.

38. Beeren C, von, Pohl S, Witte V: **On the use of adaptive resemblance terms in chemical ecology**. *Psyche J Entomol* 2012, **2012**:1-7.

39. Akre RD, Rettenmeyer CW: **Behavior of staphylinidae associated with army ants (Formicidae: Ecitonini)**. *J Kansas Entomol Soc* 1966, **39**:745-782.

40. Kistner DH, Jacobson HR: **Cladistic analysis and taxonomic revision of the ecitophilous tribe Ecitocharini with studies of their behaviour and evolution (Coleoptera, Staphylinidae, Aleocharinae)**. *Sociobiology* 1990, **17**:333-480.

41. Kistner DH: **Cladistic analysis, taxonomic restructuring and revision of the Old World genera formerly classified as Dorylomimini with comments on their evolution and behavior (Coleoptera: Staphylinidae)**. *Sociobiology* 1993, **22**:147-383.

42. Danoff-Burg JA: **An ethogram of the ant-guest beetle tribe Sceptobiini (Coleoptera: Staphylinidae; Formicidae)**. *Sociobiology* 1996, **27**:287-328.

43. Meer RKV, Wojcik DP: **Chemical mimicry in the myrmecophilous beetle *Myrmecaphodius excavaticollis***. *Science* 1982, **218**:806-808.

44. Beeren C, von, Schulz S, Hashim R, Witte V: **Acquisition of chemical recognition cues facilitates integration into ant societies**. *BMC Ecol* 2011, **11**:1-12.

45. Lenoir A, Chalon Q, Carvajal A, Ruel C, Barroso Á, Lackner T, Boulay R: **Chemical integration of myrmecophilous guests in *Aphaenogaster* ant nests**. *Psyche J Entomol* 2012, **2012**:1-12.

46. Whiteman JP, Smith EAE, Besser AC, Newsome SD: **A guide to using compound-specific stable isotope analysis to study the rates of molecules in organisms and ecosystems**. *Diversity* 2019, **11**:8.

47. Blomquist GJ, Ginzel MD: **Chemical ecology, biochemistry, and molecular biology of insect hydrocarbons**. *Ann Rev Entomol* 2021, **66**:45-60.

48. Makki R, Cinnamon E, Gould AP: **The development and functions of oenocytes**. *Ann Rev Entomol* 2014, **59**:405-425.

49. Menzel F, Schmitt T, Blaimer BB: **The evolution of a complex trait: cuticular hydrocarbons in ants evolve independent from phylogenetic constraints**. *J Evolut Biol* 2017, **30**:1372-1385.

50. Otte T, Hilker M, Geiselhardt S: **Phenotypic plasticity of cuticular hydrocarbon profiles in insects**. *J Chem Ecol* 2018, **44**:235-247.

51. Maruyama M: **Revision of the Palearctic Species of the Myrmecophilous Genus *Pella* (Coleoptera, Staphylinidae, Aleocharinae)**. *Natl Sci Mus Monogr* 2006, **32**:1-207.

52. Akre RD, Torgerson RL: **The behavior of *Diploeciton nevermanni*, a staphylinid beetle associated with army ants**. *Psyche J Entomol* 1968, **75**:211-215.

53. Seevers CH: **A generic and tribal revision of the North American Aleocharinae (Coleoptera: Staphylinidae)**. *Fieldiana Zool* 1978,71 i–289.

54. Kishi Y, Parker J: **Cell type innovation at the tips of the animal tree**. *Curr Opin Genet Dev* 2021, **69**:112-121.

55. Stoeffler M, Maier TS, Tolasch T, Steidle JLM: **Foreign-language skills in rove-beetles? Evidence for chemical mimicry of ant alarm pheromones in myrmecophilous *Pella* beetles (Coleoptera: Staphylinidae)**. *J Chem Ecol* 2007, **33**:1382-1392.

56. Stoeffler M, Tolasch T, Steidle JLM: **Three beetles—three concepts. Different defensive strategies of congeneric myrmecophilous beetles**. *Behav Ecol Sociobiol* 2011, **65**:1605-1613.

57. Kistner DH, Blum MS: **Alarm Pheromone of *Lasius* (*Dendrolasius*) *spathepus* (Hymenoptera: Formicidae) and its possible mimicry by two species of *Pella* (Coleoptera: Staphylinidae)**. *Ann Entomol Soc Am* 1971, **64**:589-594.

58. Stoeffler M, Boettinger L, Tolasch T, Steidle JLM: **The tergal gland secretion of the two rare Myrmecophilous Species *Zyras collaris* and *Z. haworthi* (Coleoptera: Staphylinidae) and the effect on *Lasius fuliginosus***. *Psyche J Entomol* 2013, **2013**:1-5.

59. Hölldobler B, Möglich M, Maschwitz U: **Myrmecophilic relationship of *Pella* (Coleoptera: Staphylinidae) to *Lasius fuliginosus* (Hymenoptera: Formicidae)**. *Psyche J Entomol* 1981, **88**:347-374.

60. Hölldobler B: **Zur Physiologie der Gast-Wirt-Beziehungen (Myrmecophilie) bei Ameisen. II. Das Gastverhältnis des imaginalen *Atemeles pubicollis* Bris. (Col. Staphylinidae) zu *Myrmica* und Formica (Hym. Formicidae)**. *Z. Vgl Physiol* 1970, **66**:215-250.

61. Hölldobler B, Kwapich CL, Haight KL: **Behavior and exocrine glands in the myrmecophilous beetle *Lomechusoides strumosus* (Fabricius, 1775) (formerly called *Lomechusa strumosa*) (Coleoptera: Staphylinidae: Aleocharinae)**. *PLOS One* 2018, **13**:e0200309-e0200338.

62. Hölldobler B, Kwapich CL: **Behavior and exocrine glands in the myrmecophilous beetle *Dinarda dentata* (Gravenhorst, 1806) (Coleoptera: Staphylinidae: Aleocharinae)**. *PLOS One* 2019, **14** e0210524-22.

63. Cammaerts R: **Transport location patterns of the guest beetle *Claviger testaceus* (Pselaphidae) and other objects moved by workers of the ant, *Lasius flavus***. *Sociobiology* 1999, **34**:433-475.

64. Seevers CH: **A monograph on the termitophilous Staphylinidae (Coleoptera)**. *Fieldiana Zool* 1957, **40**:1-334.

65. Sands WA, Lamb RW: **The systematic position of *Kaudernitermes* gen.n. (Isoptera: Termitidae, Nasutitermitinae) and its relevance to host relationships of termitophilous staphylinid beetles**. *J Entomol Ser B Taxon* 1975, **44**:189-200.

66. •• Fischer G, Friedman NR, Huang J-P, Narula N, Knowles LL, Fisher BL, Mikheyev AS, Economo EP: **Socially parasitic ants evolve a mosaic of host-matching and parasitic morphological traits**. *Curr Biol* 2020, **30**:3639-3646.

This study provides evidence that a clade of socially parasitic ants have evolved to morphologically match their hosts. The morphology-matching trend cannot be explained by the parasite phylogeny, suggesting that it is an adaptive strategy to fool nestmate recognition systems, presumably those based on tactile cues.

67. •• Parker J, Rabeling C: **Evolution: shape-shifting social parasites**. *Curr Biol* 2020, **30**:R1049-R1051.

This brief review of reference 66 discuss the weight of evidence for tactile/morphological sensing in nestmate recognition and its potential mimicry by social parasites.

68. Akre RD, Rettenmeyer CW: **Trail-following by guests of army ants (Hymenoptera: Formicidae: Ecitonini)**. *J Kansas Entomol Soc* 1968, **41**:165-174.

69. Quinet Y, Pasteels JM: **Trail following and stowaway behaviour of the myrmecophilous staphylinid beetle, *Homoeusa acuminata*, during foraging trips of its host *Lasius fuliginosus* (Hymenoptera: Formicidae)**. *Insectes Soc* 1995, **42**:31-44.

70. Hölldobler B: **Host finding by odor in the myrmecophilic beetle *Atemeles pubicollis* Bris. (Staphylinidae)**. *Science* 1969, **166**:757-758.

71. Colazza S, Aquila G, Pasquale CD, Peri E, Millar JG: **The egg parasitoid *Trissolcus basalis* uses n-nonadecane, a cuticular hydrocarbon from its stink bug host *Nezara viridula*, to discriminate between female and male hosts**. *J Chem Ecol* 2007, **33**:1405-1420.

72. Sakata I, Hayashi M, Nakamuta K: ***Tetramorium tsushimae* ants use methyl branched hydrocarbons of aphids for partner recognition**. *J Chem Ecol* 2017, **43**:966-970.

73. Awater-Salendo S, Schulz H, Hilker M, Fürstenau B: **The importance of methyl-branched cuticular hydrocarbons for successful host recognition by the larval ectoparasitoid *Holepyris sylvanidis***. *J Chem Ecol* 2020, **46**:1032-1046.

74. Ikeshita Y, Taniguchi K, Kitagawa Y, Maruyama M, Ito F: **The rove beetle *Drusilla sparsa* (Coleoptera: Staphylinidae) is a myrmecophilous species associated with a myrmicine ant, *Crematogaster osakensis* (Hymenoptera: Formicidae)**. *Entomol Sci* 2017, **20**:437-442.

75. •• Seeholzer LF, Seppo M, Stern DL, Ruta V: **Evolution of a central neural circuit underlies *Drosophila* mate preferences**. *Nature* 2018, **559**:64-569.

This study demonstrates how changes in neural circuit wiring in related fruit fly species shift the valence of a behavioral response to a pheromone cue from attractive to aversive. Both species share homologous peripheral mechanisms to detect the pheromone, but a difference in central brain circuitry yields opposite responses to the same CHC signal.

76. • Auer TO, Khallaf MA, Silbering AF, Zappia G, Ellis K, Álvarez-Ocaña R, Arguello JR, Hansson BS, Jefferis GSXE, Caron SJC, *et al.*: **Olfactory receptor and circuit evolution promote host specialisation**. *Nature* 2020, **579**:402-408.

The authors investigate neural differences in two species of fruit fly (*D. melanogaster* and *D. sechellia*) to understand the specialization of *D. sechellia* on noni fruit. They uncover higher sensitivity in tuning of an odorant receptor in *D. sechellia* to the volatile cues generated by noni fruit. They also observe differences in central circuit connectivity in *D. sechellia*. The changes in odorant receptor tuning and circuit wiring together likely underlie attraction to noni fruit in *D. sechellia.*

77. Kanwal J, Parker J: **The neural basis of interspecies interactions in insects**. *Curr Opin Insect Sci* 2022, **51**:100891.

78. Beeren C, von, Blüthgen N, Hoenle PO, Pohl S, Brückner A, Tishechkin AK, Maruyama M, Brown BV, Hash JM, Hall WE, *et al.*: **A remarkable legion of guests: diversity and host specificity of army ant symbionts**. *Mol Ecol* 2021, **30**:5229-5246.

79. Danoff-Burg JA: **Evolving under myrmecophily: a cladistic revision of the symphilic beetle tribe Sceptobiini (Coleoptera: Staphylinidae: Aleocharinae)**. *Syst Entomol* 1994, **19**:25-45.

80. Beeren C, von, Maruyama M, Kronauer DJC: **Community sampling and integrative taxonomy reveal new species and host specificity in the army ant-associated beetle genus *Tetradonia* (Coleoptera, Staphylinidae, Aleocharinae)**. *PLOS One* 2016, **11**:e0165056.

81. West-Eberhard MJ: **Sexual selection, social competition, and evolution**. *Proc Am Philos Soc* 1979, **123**:222-234.

82. Bernays EA, Wcislo WT: **Sensory capabilities, information processing, and resource specialization**. *Q Rev Biol* 1994, **69**:187-204.

83. Jaenike J: **Host Specialization in phytophagous insects**. *Ann Rev Ecol Syst* 1990, **21**:243-273.

84. Bruce TJA, Pickett JA: **Perception of plant volatile blends by herbivorous insects – finding the right mix**. *Phytochemistry* 2011, **72**:1605-1611.

85. Godfray HCJ: Parasitoids: Behavioral and Evolutionary Ecology. Princeton University Press; 1994.

86. Thomas JA, Schönrogge K, Elmes GW: **Specializations and host associations of social parasites of ants**. In *Insect Evolutionary Ecology: Proceedings of the Royal Entomological Society's 22nd Symposium*. Edited by Fellowes M, Holloway G, Rolff J. CABI; 2005:479–518.

87. •• Casacci LP, Schönrogge K, Thomas JA, Balletto E, Bonelli S, Barbero F: **Host specificity pattern and chemical deception in a social parasite of ants**. *Sci Rep* 2019, **9**:1619.

The authors demonstrate local variation in survival rates of an obligate ant social parasite with different potential hosts and how variable CHCs in the symbiont correlate with these survival patterns. These data suggest that chemical integration with a particular host also enforces specificity, as other potentially compatible symbiotic partners exclude symbionts based on their chemical phenotype.

88. Cai C, Huang D, Newton AF, Eldredge KT, Engel MS: **Early evolution of specialized termitophily in cretaceous rove beetles**. *Curr Biol* 2017, **27**:1229-1235.

89. Parker J, Grimaldi DA: **Specialized myrmecophily at the ecological dawn of modern ants**. *Curr Biol* 2014, **24**:2428-2434.

90. •• Zhou Y-L, Ślipiński A, Ren D, Parker J, Mesozoic A: **A Mesozoic clown beetle myrmecophile (Coleoptera: Histeridae)**. *eLife* 2019, **8**:e44985.

Zhou et al. report the discovery of a mid-Cretaceous myrmecophile: a haeteriine histerid beetle recovered from Burmese amber, a deposit that houses the earliest known definitive ants. The antiquity of the fossil, combined with the absence in Burmese amber of ant subfamilies that host haeteriine beetles today, implies that host-switching has been key to the long-term persistence and species richness of certain myrmecophile clades.

91. Moore W, Robertson JA: **Explosive adaptive radiation and extreme phenotypic diversity within ant-nest beetles**. *Curr Biol* 2014, **24**:2435-2439.

92. • Hlaváč P, Parker J, Maruyama M, Fikáček M: **Diversification of myrmecophilous Clavigeritae beetles (Coleoptera: Staphylinidae: Pselaphinae) and their radiation in New Caledonia**. *Syst Entomol* 2021, **46**:422-452.

This paper discusses evidence for widespread host switching in clavigerite pselaphines — a species-rich myrmecophile clade, and argues that ecological fitting may underlie the ability of these beetles to associated with novel hosts.

93. Agosta SJ, Klemens JA: **Ecological fitting by phenotypically flexible genotypes: implications for species associations, community assembly and evolution**. *Ecol Lett* 2008, **11**:1123-1134.

94. Hlaváč P, Newton AF, Maruyama M: **World catalogue of the species of the tribe Lomechusini (Staphylinidae: Aleocharinae)**. *Zootaxa* 2011, **3075**:1-151.

95. Wasmann W: **Zur Kenntniss der Gäste der Treiberameisen und ihrer Wirthe am obern Congo, nach den Sammlungen und

Beobachtungen von P. Herm. Kohl, C.S.S.C. bearbeitet. *Zoologische Jahrbücher Supplementheft* 1904, **7**:611-682.

96. Zilberman B, Pires-Silva CM, Moreira IE, Pisno RM, Bezerra-Gusmão MA: **State of knowledge of viviparity in Staphylinidae and the evolutionary significance of this phenomenon in *Corotoca* Schiødte, 1853**. *Pap Avulsos Zool* 2019, **59**:e20195919.

97. Jongepier E, Séguret A, Labutin A, Feldmeyer B, Gstöttl C, Foitzik S, Heinze J, Bornberg-Bauer E: **Convergent loss of chemoreceptors across independent origins of slave-making in ants**. *Mol Biol Evol* 2021, **39**:msab305.

98. •• Schrader L, Pan H, Bollazzi M, Schiøtt M, Larabee FJ, Bi X, Deng Y, Zhang G, Boomsma JJ, Rabeling C: **Relaxed selection underlies genome erosion in socially parasitic ant species**. *Nat Commun* 2021, **12**:2918.

References 97 and 98 document large-scale losses of chemoreceptors in socially parasitic ants, implying that these species have experienced reductive evolutionary changes in the chemosensory systems, a probable consequence of their symbiotic lifestyle.

99. McCutcheon JP, Moran NA: **Extreme genome reduction in symbiotic bacteria**. *Nat Rev Microbiol* 2012, **10**:13-26.