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Antoine Couto, Simon Marty, Erika H Dawson, Patrizia d’Ettorre, Jean-christophe Sandoz, et al..
Evolution of the neuronal substrate for kin recognition in social Hymenoptera. *Biological Reviews*,
2023, 98 (6), pp.2226 - 2242. 10.1111/brv.13003 . hal-04295088

HAL Id: hal-04295088

<https://hal.science/hal-04295088>

Submitted on 20 Nov 2023

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Evolution of the neuronal substrate for kin recognition in social Hymenoptera

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ABSTRACT

In evolutionary terms, life is about reproduction. Yet, in some species, individuals forgo their own reproduction to support the reproductive efforts of others. Social insect colonies for example, can contain up to a million workers that actively cooperate in tasks such as foraging, brood care and nest defence, but do not produce offspring. In such societies the division of labour is pronounced, and reproduction is restricted to just one or a few individuals, most notably the queen(s). This extreme eusocial organisation exists in only a few mammals, crustaceans and insects, but strikingly, it evolved independently up to nine times in the order Hymenoptera (including ants, bees and wasps). Transitions from a solitary lifestyle to an organised society can occur through natural selection when helpers obtain a fitness benefit from cooperating with kin, owing to the indirect transmission of genes through siblings. However, this process, called kin selection, is vulnerable to parasitism and opportunistic behaviours from unrelated individuals. An ability to distinguish kin from non-kin, and to respond accordingly, could therefore critically facilitate the evolution of eusociality and the maintenance of non-reproductive workers. The question of how the hymenopteran brain has adapted to support this function is therefore a fundamental issue in evolutionary neuroethology. Early neuroanatomical investigations proposed that social Hymenoptera have expanded integrative brain areas due to selection for increased cognitive capabilities in the context of processing social information. Later studies challenged this assumption and instead pointed to an intimate link between higher social organisation and the existence of developed sensory structures involved in recognition and communication. In particular, chemical signalling of social identity, known to be mediated through cuticular hydrocarbons (CHCs), may have evolved hand in hand with a specialised chemosensory system in Hymenoptera. Here, we compile the current knowledge on this recognition system, from emitted identity signals, to the molecular and neuronal basis of chemical detection, with particular emphasis on its evolutionary history. Finally, we ask whether the evolution of social behaviour in Hymenoptera could have driven the expansion of their complex olfactory system, or whether the early origin and conservation of an olfactory subsystem dedicated to social recognition could explain the abundance of eusocial species in this insect order. Answering this question will require further comparative studies to provide a comprehensive view on lineage-specific adaptations in the olfactory pathway of Hymenoptera.

Key words: eusociality, kin selection, Hymenoptera, brain adaptation, social recognition, olfactory system, cuticular hydrocarbons, basiconic sensilla, olfactory receptors.

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I. INTRODUCTION

Interactions between individuals of the same species can take many forms, from strictly competitive relationships to mutually beneficial behaviours. While the evolutionary origin of competition is easily explained by natural selection, the emergence of cooperation is less readily understood (Axelrod & Hamilton, 1981; Sachs *et al.*, 2004; Nowak, 2006). In practice, animals can form affiliative groups with different functional arrangements of communal tasks (Espinass, 1877; Rubenstein & Abbot, 2017). This diversity is well illustrated in insects, including familiar species like ants, bees and wasps that are known for their eusocial lifestyle which features a division of labour and hierarchical reproduction. In fact, the broad spectrum of social organisations observed across insect societies involves many forms of cooperation, offering abundant material to study the evolutionary path from solitary to social life (Wheeler, 1928).

The diversity of social organisations is traditionally classified and ranked according to behavioural traits that reflect an increased commitment of individuals to the reproductive success of their colony (Fig. 1). In the highest levels observed among social animals, cooperative behaviour goes beyond mutually beneficial interactions as some individuals demonstrate extreme forms of altruism (West, Griffin & Gardner, 2007b). Most females of eusocial colonies lack the ability to reproduce, and instead provide support to the colony (nourishment, brood care, nest defence and thermoregulation), while one or a few reproductive individuals (the queens) generally outlive several generations, being protected and tended to in the nest (Wilson, 1971). The sequence leading to this reproductive skew with altruistic individuals (workers) forgoing their own reproduction to support the reproductive efforts of others is considered to be one of the major transitions in the evolutionary history of life (Maynard Smith & Szathmari, 1997). Notably, the emergence of sterile, altruistic individuals represented a critical difficulty for Darwin's theory of natural selection (Darwin, 1876; Herbers, 2009; Ratnieks, Foster & Wenseleers, 2011). A solution to this problem was offered by the concept of inclusive fitness, quantifying an organism's overall genetic success (direct and indirect), and the kin selection theory (Hamilton, 1964a; Foster,

Wenseleers & Ratnieks, 2006; Keller & Chapuisat, 2017). Thus, in spite of their infertility, workers that help closely related individuals indirectly promote the transmission of their own genes, increasing their inclusive fitness. Although a eusocial lifestyle exists in a few mammals (mole-rats), crustaceans (snapping shrimps), and other insects (including termites), it is Hymenoptera (ants, bees and wasps) that provide most known examples of this lifestyle, and a well-documented illustration of kin selection theory (Hamilton, 1964b).

Since kinship appears to be a major driving force in the evolution of cooperation, eusocial evolution is vulnerable to exploitation due to the loss of indirect fitness benefits when altruistic behaviours support non-kin. To counteract this, mechanisms have evolved that allow eusocial species to recognise cooperation partners and ensure that the benefits of altruism are received by the appropriate recipients, thereby preventing costly opportunism from unrelated individuals (Gamboa, Reeve & Pfennig, 1986; Hepper, 1986; Waldman, 1987, 1988; Penn & Frommen, 2010; Elgar & Riehl, 2021). Such a function is undoubtedly performed by sensory organs and the nervous system which detect, process, and trigger adequate behavioural responses. On this basis, the comparative investigation of brain circuits, sensory systems, and the expression of associated genes across solitary and social lineages can provide major insights into the interplay between neural adaptations and the evolution of social behaviours (Kocher & Paxton, 2014; Godfrey & Gronenberg, 2019). As illustrated in this review, the Hymenoptera provide unique features for this endeavour. They represent a highly diverse insect order (Forbes *et al.*, 2018; Blaimer *et al.*, 2023), encompassing a wide range of evolutionary radiations, including phytophagous sawflies, parasitoid wasps, and carnivorous as well as palynivore (bee) species (Fig. 2A). One of their most noteworthy characteristics, though, is the repeated evolution of eusociality, with up to nine independent origins, and the presence of all levels of social organisation in extant species (Fig. 2B,C), including social parasites (slave-making species) and species with extensive supercolonies. This unique feature offers exceptional comparative power for studying how the brain and sensory systems have adapted for the evolution and maintenance of a eusocial lifestyle.

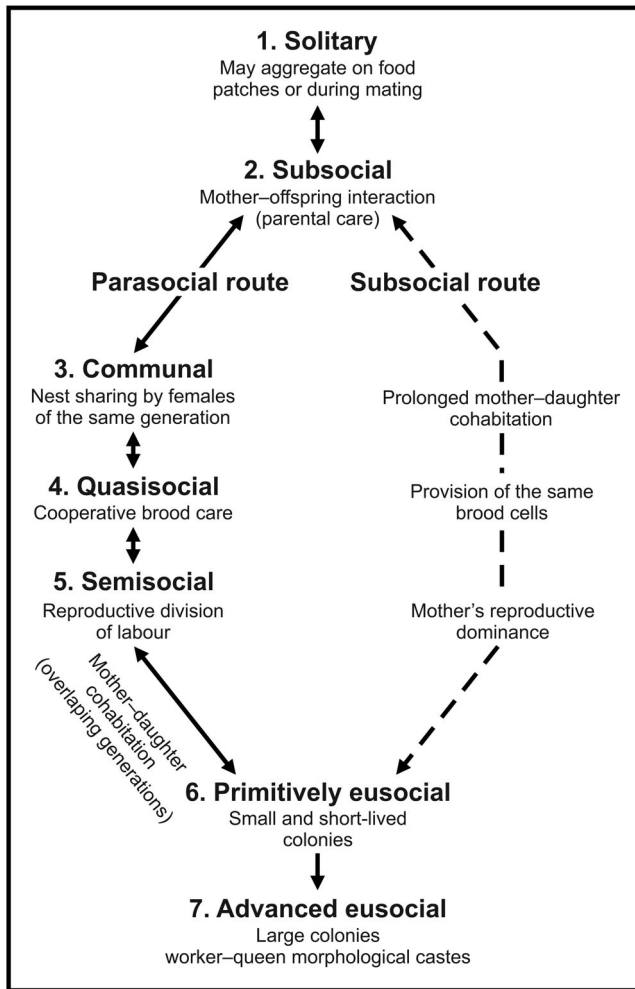


Fig. 1. Evolutionary routes to sociality. Conceptual diagram showing the possible routes to eusociality with the type of social organisation (in bold) and their associated behavioural traits (adapted from da Silva, 2021). Arrows show the reversibility of most evolutionary transitions but advanced eusociality is thought to be irreversible. The subsocial route involves a prolonged mother–daughter cohabitation at the adult stage (i.e. females stay together) whereas the parasocial route involves affiliative groups made of individuals from the same generation (e.g. sisters/cousins). In Hymenoptera, semisociality often precedes eusociality, with the exception of the Vespidae as there is no evidence of parasociality in this clade.

This review explores both historical and contemporary hypotheses regarding the cognitive demands and neural adaptations associated with the eusocial lifestyle in Hymenoptera. Specifically, we emphasise the significance of olfactory recognition in ensuring that cooperative behaviours benefit the intended, related recipient(s), at all levels of social evolution. In insects, social identity is generally signalled by blends of cuticular hydrocarbons (CHCs) which may have evolved and diversified to support changes in social ecology and communication across species. We then report advances in neuroanatomical descriptions of the olfactory system, and the identification of a peculiar sensory subsystem,

likely involved in CHC detection. The molecular phylogeny of olfactory receptor (ORs) genes putatively linked to this subsystem also reveals a highly dynamic evolutionary history in Hymenoptera. Although converging lines of evidence point to a possible role of this sensory specialisation in social evolution, its exact contribution remains to be elucidated. We propose two competing evolutionary hypotheses which assume the ‘convergence’ or the ‘exaptation’ of this sensory subsystem, and highlight essential lines of future investigation, which will help decipher how enhanced chemical communication has interplayed with the multiple emergences of complex social organisations in Hymenoptera.

II. THE SOCIAL BRAIN HYPOTHESIS AND BEYOND

(1) Early hypotheses linking social cognition and mushroom body expansion

Species-specific behavioural traits are generally determined by the structure and function of their nervous system. Proponents of the social brain hypothesis (Jolly, 1966; Dunbar & Shultz, 2007), as applied to social Hymenoptera, argue that superior levels of social organisation pose higher demands on cognitive abilities to support the integration of group features and the synchronisation of colonial activities. Accordingly, the evolution of eusociality could be associated with enlarged brain areas granting increased power for the processing and storage of social information (Lihoreau, Latty & Chittka, 2012). This hypothesis arose from early comparative investigations attempting to relate insect behavioural traits to the composition of their brains (Dujardin, 1850). Dujardin reported that a conspicuous brain structure which he had recently discovered, the mushroom bodies, increases in size and anatomical complexity when comparing a cockchafer or locust to a range of Hymenoptera, including a parasitic wasp (*Ichneumon*), a carpenter bee (*Xylocopa*), a solitary bee, a honey bee (*Apis mellifera*) and an ant. With the belief that ‘intelligence’ dominates over instinct in such industrious insects as social Hymenoptera (Cuvier, 1831), Dujardin suggested that the relative size of this brain structure mirrors an insects’ ‘intelligence’ and their degree of sociality. This link between the mushroom bodies, cognition and social ecology has persisted in the literature, and has become intertwined with theories of vertebrate brain evolution which elevate social cognition to the primary driver of brain expansion (Jolly, 1966; Dunbar & Shultz, 2007).

Since Dujardin’s initial descriptions, knowledge on the structure and function of the mushroom bodies has considerably increased (Strausfeld *et al.*, 1998; Fahrbach, 2006; Farris, 2016). The mushroom bodies are a bilateral neuropil made up of thousands of intrinsic neurons called Kenyon cells (KCs) (Kenyon, 1896a,b). This structure includes a sensory projection area known as the calyx, a peduncle formed by axon bundles of the KCs, and lobes where these neurons

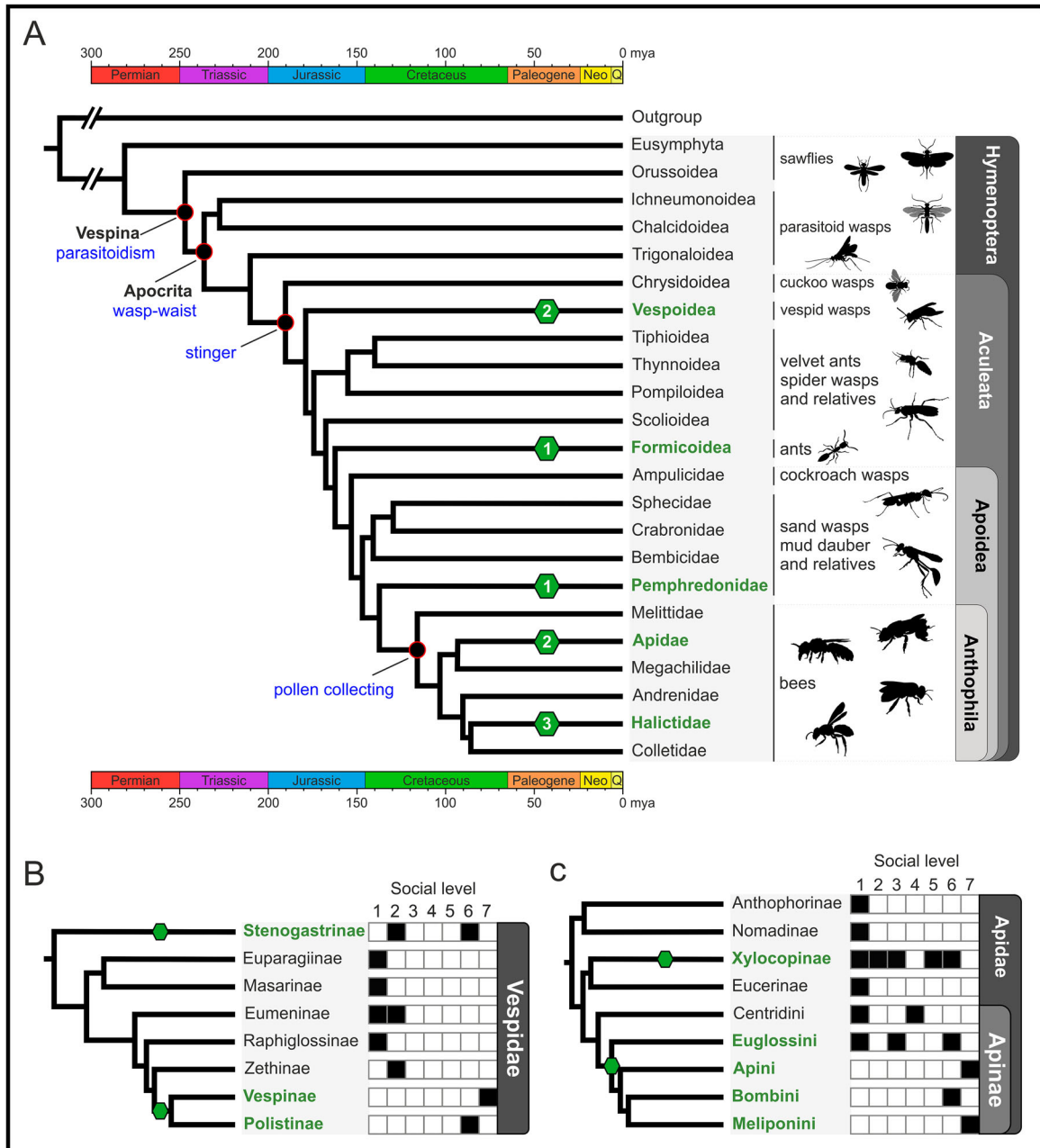


Fig. 2. Phylogeny of the Hymenoptera. (A) Simplified phylogeny of the main Hymenoptera lineages, adapted from Peters *et al.* (2017), Branstetter *et al.* (2017) and Sann *et al.* (2018). The major evolutionary innovations are shown in blue, while green hexagons show the number of times eusociality evolved in a lineage. (B) Phylogeny of the Vespidae, adapted from Piekarski *et al.* (2018) and Bank *et al.* (2017). (C) Phylogeny of the Apidae, adapted from Bossert *et al.* (2019). The black squares indicate the presence of species with a particular level of social organisation (see Fig. 1) within the associated lineage. Social levels: 1, solitary; 2, subsocial; 3, communal; 4, quasisocial; 5, semisocial; 6, primitively eusocial; 7, advanced eusocial.

synapse with output neurons (Heisenberg, 2003). Although this general layout is prevalent across insects, there is extensive morphological variation, particularly in the composition of the calyces (Farris, 2005; Strausfeld *et al.*, 2009). These differences seem to coincide with variation in the relative importance of sensory inputs, with small mushroom bodies predominantly receiving projections from one sensory

modality, typically the olfactory system (Farris, 2005, 2013), while large mushroom bodies display a higher number of KCs and additional functional subdivisions in the calyces, receiving input from several sensory modalities [notably olfactory and visual in Hymenoptera (Gronenberg, 2001; Ehmer & Gronenberg, 2002)]. Shifts in mushroom body size, structure, and sensory profile have repeatedly evolved

in numerous insect lineages, in both social and non-social species, suggesting that they evolve dynamically in response to, presumably diverse, ecological selection pressures (Farris & Roberts, 2005; Nishino *et al.*, 2012; Kinoshita *et al.*, 2015; Montgomery, Merrill & Ott, 2016; Couto *et al.*, 2023).

(2) Contradictory clues from more recent data across Hymenoptera

In Hymenoptera, relatively small mushroom bodies that typically receive only olfactory inputs are found in the basal phytophagous families of sawflies (Rössler & Zube, 2011). However, novel visual projections colonised the calyces early in hymenopteran evolution, and were likely present in the last common ancestor of Vespina [Orussoidea and Apocrita, ~250 million years ago (Mya); Fig. 2A]. This precedes the transition from wandering herbivorous insects (sawflies) to parasitoidism, ~90 Mya before the emergence of eusocial behaviours in Aculeata (Farris & Schulmeister, 2011). Consequently, large mushroom bodies with visually elaborate calyces are found across all Aculeata, regardless of their level of social organisation. In contrast to the predictions of the social brain hypothesis, expanded mushroom bodies in Hymenoptera have therefore been linked with advanced abilities for visually oriented navigation between learned locations of hosts, food sources or nest sites, rather than with enhanced social cognition (Farris & Schulmeister, 2011). These enhanced abilities could however support higher behavioural flexibility required at early stages of sociality, for instance for central place foraging and mass provisioning of offspring (Farris, 2016).

Even if the early expansion of the mushroom bodies is not linked to social evolution, the possibility that they underwent additional adaptations in eusocial lineages, following the acquisition of social traits, cannot be excluded. For example, morphologically diverse mushroom bodies are found across independent emergences of eusociality in Vespidae (Ehmer & Hoy, 2000). Most notably, relative mushroom body size appears to decrease, rather than increase, with social complexity in the Polistinae subfamily (O'Donnell *et al.*, 2015, 2019). An alternative phenomenon has therefore been suggested, called distributed cognition, whereby eusocial colony members rely on information sharing and task specialisation, leading to decreased reliance on individual cognition and potentially relaxed selection for expanded mushroom bodies (O'Donnell *et al.*, 2015). In addition, recent allometric comparisons across anthophilic bees have shown that feeding diet (i.e. specialist over generalist) and life-history traits, rather than sociality, correlate with increased brain investment (Sayol *et al.*, 2020). Nevertheless, a comparison between two species of sweat bees (Halictidae, *Augochlorini*) demonstrated a reduction of mushroom body size associated with a loss of sociality (Pahlke *et al.*, 2020). There are therefore contrasting data on how social ecology drives variation in mushroom body size and structure. This may reflect the species-specific nature of social interactions, but it is also possible that simple volumetric comparisons might not be sufficient to capture the effects of selection for social cognition, if these

operate at finer neurological scales. Future comparative work would therefore benefit from a narrower focus on the structure and metabolic costs of particular neural circuits functionally linked to social evolution (Kamhi *et al.*, 2016; Coto & Traniello, 2021). Regardless, for the time being, the popular assumption that complex social ecologies have prompted major expansions and innovation in insects' learning and memory centres, the mushroom bodies, does not appear well supported, and so consideration of other brain structures and neural processes may be informative.

(3) Olfactory processing and the central role of social recognition in social behaviour

Following the description of Hamilton's rule, which specified the kinship conditions under which reproductive altruism evolves, it was hypothesised that social animals should possess the ability to assess relatedness between themselves and their potential partners (Hepper, 1986; Holmes, 2004). Indeed, most species can recognise their conspecifics and adapt their behavioural responses in varying contexts, such as during mating or social interactions (Waldman, 1988). For essential and costly social traits such as parental care, the identification of their own young by adults ensures that resources and energy are invested in the propagation of their own genes rather than in those of unrelated individuals, maximising their direct fitness. Extending recognition to kinship identity may enhance individuals' inclusive (both direct and indirect) fitness when investing in cooperative breeding, by enabling the acceptance of less closely related individuals while still promoting cooperative relationships with close kin over non-kin (Penn & Frommen, 2010). In eusocial species, however, high relatedness among colony members may render the need for kinship recognition unnecessary, with nestmates recognising a common nest phenotype (Crozier, 1987). This high relatedness, combined with a shared environment, leads to phenotypic homogenisation of recognition cues, making within-colony discrimination challenging or even unsolvable (Van Zweden *et al.*, 2010; Nehring *et al.*, 2016). Nestmate discrimination abilities are displayed by many eusocial Hymenoptera, suggesting that maintaining a robust kinship recognition system in social Hymenoptera is crucial, as it allows discrimination between strangers and colony members, providing protection against competitors and parasites, and ensuring colony survival. This ability may also help to resolve conflicts within the colony (Greene & Gordon, 2003; Ratnieks, Foster & Wenseleers, 2006), and may explain how workers can rely solely on indirect fitness (Dani *et al.*, 2004). The sensory systems that underlie these animals' perception of kinship identity may therefore play a role in the formation and maintenance of social organisation.

In Hymenoptera, both visual and olfactory cues have been demonstrated to play a role in mediating recognition. Remarkably, visual recognition of facial coloration patterns is used by several species of *Polistes* wasps to distinguish nestmates from non-nestmates (Tibbetts, 2002; Baracchi *et al.*, 2015; Cervo, Cini & Turillazz, 2015). This reliance on

visual cues may reduce the need for olfactory processing, as evidenced by the smaller size of primary olfactory neuropil in these species (Gronenberg, Ash & Tibbetts, 2008). Interestingly, however, the wasp *Polistes dominula* shows a degree of plasticity in the use of sensory cues in social contexts, increasing its reliance on olfactory over visual cues when the number of individuals increases during colony growth (Cini *et al.*, 2019). Thus, reliance on the olfactory channel seems more prevalent and suitable in larger groups, as well as in closed nests where visual recognition becomes impractical as interactions occur in the dark. In fact, empirical evidence supports the notion that olfaction is the primary channel of recognition across Hymenoptera (Vandenabeele & Schmitt, 2023). First, recent experiments in ants clearly illustrate the key role of chemical detection in social interactions. For example, genetic disruption of the olfactory co-receptor *orco* hampers the development of the olfactory system and results in highly defective social behaviour (Yan *et al.*, 2017; Tribble *et al.*, 2017). Secondly, it has been demonstrated that functional ORs are necessary for non-nestmates discrimination in *Camponotus floridanus* (Ferguson *et al.*, 2020). Accordingly, the transition to higher social complexity may be associated with expansions of brain regions supporting olfactory communication, as has been observed between ant species with different colony sizes (Riveros, Seid & Weislo, 2012; Godfrey *et al.*, 2021). This idea is also supported by observations made in Halictidae (sweat bees), in which a higher investment in chemosensory structures on the antennae has been observed in social bees compared to solitary ones, at both species and population levels (Wittwer *et al.*, 2017; Wenseleers & van Zweden, 2017; Boulton & Field, 2022). It should be noted that beyond kinship recognition, many aspects of eusocial organisation, such as coordinated division of labour, concerted actions and reproductive dominance, also require highly efficient communication systems (Leonhardt *et al.*, 2016). Most of these messages are relayed through the use of pheromones, which are also detected and processed by the olfactory system. Thus, observations of an increased olfactory investment in social clades may relate to an increased ability to recognise kinship and/or more elaborate pheromonal communication. In any case, available data strongly suggest that the olfactory system may play a pivotal role in advanced social organisation as it allows for the detection of chemical signals used by social insects in a wide variety of contexts.

III. EVOLUTION OF THE CHEMICAL COMMUNICATION OF IDENTITY

Chemical messages are the most ancient and widespread mode of communication, from unicellular organisms to animals living in large and complex societies. Many chemical messages are species-specific signals, called pheromones, which coordinate interactions in essential contexts, including alarm, presence of food, breeding receptivity, reproductive status, or territorial marking. Other chemical messages are personalised and

communicate identity at different levels, from the individual, through to family, group, or species. These chemicals are usually less volatile than pheromones and are typically part of the body odour, both in vertebrates and invertebrates. These chemical profiles have been defined as ‘signature mixtures’ (Wyatt, 2014). In contrast to pheromones, which elicit stereotypical responses in the receiver, responses to signature mixtures are more context dependent, because they are unique to certain individuals, families, or colonies in social insects.

Typically, chemical signals are derived from ancestral traits that initially served another role. The body surface of insects is covered with a layer of CHCs, with the original function to prevent desiccation and protect against pathogen infection. Although the CHCs that are most suited for waterproofing are the long-chained linear *n*-alkanes (as they have the highest melting temperature), most insects carry a mixture which also includes methylated (branched) alkanes and unsaturated (non-linear) alkenes (Gibbs & Rajpurohit, 2010). These compounds might have been co-opted for communication purposes because the position of a methyl group or of the double bond may act as discriminative feature, enabling the transmission of more complex information despite their lower waterproofing efficiency (Châline *et al.*, 2005; Dani *et al.*, 2005; Guerrieri *et al.*, 2009). This creates a potential trade-off between waterproofing and communication functions that shapes the evolution of CHC profiles across species (Menzel, Blaimer & Schmitt, 2017; Chung & Carroll, 2015), but such trade-offs are poorly investigated.

The ancestral use of CHCs for communication was likely in species recognition. In insects, each species has its own CHC blend which enables conspecific and heterospecific recognition, conducive to the avoidance of interspecific breeding (Chung & Carroll, 2015). In *Drosophila*, for instance, each species shows a unique CHC blend (Jallon & David, 1987) which facilitates reproductive boundaries between closely related species (Shahandeh, Pischedda & Turner, 2018). CHCs are also sexually dimorphic and serve as sex pheromones in many solitary insects, including *Drosophila* (Bontonou & Wicker-Thomas, 2014) and long-horned beetles (Cerambycidae), where they play an important role in maintaining reproductive isolation (Ginzel, 2010).

In addition to the identification of conspecifics, CHCs can play a central role in family-level recognition. For example, German cockroaches (*Blattella germanica*) are able to distinguish between kin and non-kin using CHCs and prefer to join shelters occupied by siblings rather than by unrelated individuals (Lihoreau & Rivault, 2008). Kin recognition has also been shown to play a role in inbreeding avoidance, with male cockroaches preferring to mate with unrelated females (Lihoreau, Zimmer & Rivault, 2007). Limited evidence also suggest that some social insects discriminate between kin and non-kin to avoid mating with related individuals (Foster, 1992; Oppelt *et al.*, 2008).

Kin recognition is particularly important in insects that live in groups of related individuals because recognition of identity helps to direct cooperative behaviours specifically to kin, avoiding social parasitism (Hamilton, 1987). Typically, closely related individuals share a group-specific

signature CHC blend, which is used as a template to identify intruders (van Zweden & d'Etterre, 2010). The main route to eusociality is *via* family groups, headed by a single female mated with only one male (lifetime monogamy), therefore social insect colonies are thought to have evolved from groups of highly related individuals (Boomsma, 2009). This structure persists in many species, although some social insect species have become secondarily polygynous (more than one queen) or polyandrous (queen mated with multiple males), decreasing relatedness among colony members due to the presence of multiple matrilineal and/or patrilineal. In this case, true kin recognition would allow disruptive within-colony nepotism (Boomsma & d'Etterre, 2013). Yet, nestmate recognition is still functional, and aggression towards outsiders can be observed in these species with higher intra-colony genetic diversity (e.g. Rosset, Schwander & Chapuisat, 2007), albeit to a lesser extent in some species (e.g. Sundström, 1997). Although nestmate recognition has a heritable component (Van Zweden *et al.*, 2010), it is likely that in these cases individuals are identified as coming from the same colony, rather than from a particular genetic background.

Perhaps consistent with a role in fine-grained inter- and intra-colony recognition, social Hymenoptera are characterised by complex chemical profiles including all three main groups of CHCs: saturated *n*-alkanes, alkenes (with double bonds) and methylalkanes (with one or more methyl groups). In some species, the diversity of hydrocarbon compounds present on the cuticle can be very high, with more than 100 different compounds detected (Martin & Drijfhout, 2009). It is likely that not all the CHCs, but only specific components of the CHC profile, are used for recognition, and that different compounds are used in different contexts or at different levels of recognition (species, nestmate, caste, mate recognition). In ants, wasps and bees, studies point to differential and/or complementary roles of alkenes, methyl-branched alkanes and linear alkanes in nestmate recognition across species (Dani *et al.*, 2001, 2005; Ruther, Sieben & Schircker, 2002; Greene & Gordon, 2007; Martin *et al.*, 2008; Guerrieri *et al.*, 2009). Differential olfactory conditioning experiments have been performed to study the learning and discrimination abilities of ants and bees with the different types and concentrations of CHCs (Châline *et al.*, 2005; Bos *et al.*, 2012; van Wilgenburg *et al.*, 2012; di Mauro *et al.*, 2015). Generally, these studies show that social insects learn most (but not all) CHCs well, and can discriminate between individual CHCs based on chemical features like chain length, double-bond position or methyl branch position. However, remarkable differences in the response to different CHC types were also observed. For instance, honey bees learn and discriminate alkenes better than alkanes (Châline *et al.*, 2005), while Argentine ants (*Linepithema humile*) learn tri-methyl branched alkanes better than mono-methyl or linear alkanes (van Wilgenburg *et al.*, 2012). Discrimination success also depends on CHC structure. Argentine ants can discriminate between CHCs with different branch positions and the same chain length, but not always between CHCs with the same branching patterns but different chain lengths. Carpenter ants (*Campenotus aethiops*) have been found to discriminate

accurately between CHCs that differ in the presence or absence of a methyl group, whereas their discrimination abilities are lower between pairs of methyl-alkanes (Bos *et al.*, 2012). In addition, they exhibit asymmetric discrimination towards similar CHCs of different chain lengths, being able to distinguish CHCs with longer chains from those learned with shorter chains, but not *vice versa* (Bos *et al.*, 2012). Thus, social Hymenoptera are generally able to learn these cues and their relative proportions to build neural representations of nestmate phenotypes (Bos & d'Etterre, 2012). These internal templates are acquired through association with familiar individuals, or by self-evaluation of their own CHC profile as a self-referent phenotype. Since environmental factors, such as food, affect the composition of CHC profiles, these templates need to be updated throughout the insect's life, and multiple templates may be acquired (Neupert *et al.*, 2018). Recognition is therefore based on the degree of similarity between the label of encountered individuals and the learned template of nestmate odours (phenotype matching), with non-matching phenotypes rejected as non-nestmate.

It is now clear that in Hymenoptera some CHCs also act as a queen pheromone informing nestmates about her fertility status (Holman, Lanfear & d'Etterre, 2013; Van Oystaeyen *et al.*, 2014; Oi *et al.*, 2015; Smith & Liebig, 2017). This places these compounds as key signals in the evolution of reproductive altruism. However, the identity of the CHCs acting as recognition compounds remains elusive. A pertinent question is then to what extent has sociality shaped the evolution of CHCs in Hymenoptera? While both social and solitary species use CHCs to communicate, the occurrence of multidimensional levels of communication in social insects led to the hypothesis that their CHC profiles should be more diverse and more complex than those of solitary Hymenoptera (Leonhardt *et al.*, 2016). A meta-analysis of 241 species, including solitary and social species from Symphyta (sawflies), Parasitica (parasitoid wasps) to Aculeata (wasps, bees, and ants) could not verify this hypothesis and suggested that sociality does not drive CHC complexity. Indeed, all the main classes of CHCs are already present in the basal Symphyta and Parasitica, and some parasitoid wasps show very complex CHC profiles (Kather & Martin, 2015). Therefore, given the presumed high complexity of the chemical signal early in Hymenoptera evolution, it is likely that these insects' detection and perception abilities have had to be adapted to use these cues in different social contexts. This implies a heightened importance of olfactory perception and processing in the evolution of social insects.

IV. SPECIALISED SENSORY SYSTEM FOR CHCS?

(1) The olfactory system of insects

In insects, olfactory stimuli are detected within cuticular structures called sensilla, generally located on the antennae and housing olfactory sensory neurons (OSNs) (Keil, 1999). These OSNs express OR proteins, localised at the neuron's

dendrites within the sensillum. Insect ORs form heteromeric complexes with a unique and obligate co-receptor named Orco (Larsson *et al.*, 2004; Benton *et al.*, 2006), producing a ligand-gated non-selective cation channel that opens upon activation by appropriate ligands (Sato *et al.*, 2008; Wicher *et al.*, 2008; Butterwick *et al.*, 2018; del Marmol, Yedlin & Ruta, 2021). In general, each OSN expresses a single OR gene together with Orco, and the functional properties of the OR are thus responsible for the response spectrum of the OSN, although some exceptions, with multiple ORs expressed in a single OSN, have been observed (Ebrahim *et al.*, 2015; Herre *et al.*, 2022). Some OSNs also express another family of chemosensory receptors, the ionotropic receptors (IRs) (Rytz, Croset & Benton, 2013; Wicher & Miazzi, 2021). Bundles of OSN axons form the antennal nerve, which projects to a primary olfactory centre in the brain, the antennal lobe (AL), within a number of roughly spherical neuropil units called glomeruli. All OSNs expressing a particular OR gene converge in the same glomerulus (Vosshall, Wong & Axel, 2000; Couto, Alenius & Dickson, 2005). There is high variability in the number of OR genes expressed by different insect species, and accordingly in the number of glomeruli in the AL, from roughly 50 (54 glomeruli in *Drosophila melanogaster*; Grabe *et al.*, 2015) to several hundred [166 glomeruli in the honey bee (Arnold, Masson & Budharugsa, 1985), 440 and 630 respectively in the leaf cutter ants *Atta vollenweideri* (Kelber, Rössler & Kleineidam, 2010), and *Apterostigma cf. mayri* (Kelber *et al.*, 2009)]. In species with a high number of glomeruli, the AL is generally divided into several distinct clusters, innervated by different OSN tracts that materialise from the antennal nerve at the entrance of the AL [*Apis mellifera* (Suzuki, 1975), carpenter ant *Camponotus floridanus* (Zube *et al.*, 2008), hornet *Vespa velutina* (Couto *et al.*, 2016)]. Within the AL, olfactory information is refined by local interneurons connecting the glomeruli, before being conveyed by projection neurons (PNs) to higher-order brain centres, the lateral horn and the mushroom bodies. While the former is thought to be involved in innate behaviours (Jefferis *et al.*, 2007; Roussel *et al.*, 2014), the latter is a higher-order integration centre involved in simple and complex learning tasks (Heisenberg, 1998; Devaud *et al.*, 2015).

(2) Structure of the basiconic subsystem

Antennal sensilla are categorised in several different classes based on their shape, distribution, and structure, from hair-like trichoid sensilla to flat placoid sensilla, *via* a range of other forms (Zacharuk, 1980). On the strength of electron microscopic data showing a sex-biased distribution, it has been postulated that a certain type of antennal sensilla, the basiconic sensilla (BaS), could be responsible for host detection in some parasitic wasps (Borden, Miller & Richerson, 1973; Norton & Vinson, 1974). This sensillum always consists of a peg-like structure perforated by multiple minute pores and standing in the centre of a socket. However, it displays remarkable structural diversity, from a

large and short cylindrical shape with a porous apical surface (e.g. in vespid wasps; Fig. 3D), to a longer and thinner peg apparatus with pores distributed more laterally (e.g. in ants; Fig. 3B) (Walther, 1983; but see Gellert *et al.*, 2022). Experimental evidence of its behavioural function was provided by Anton & Gnatzy (1998) who showed that females of the digger wasp, *Liris niger*, use the distal and dorsal part of their antenna flagellum to probe the cuticle of prospective cricket prey before attacking or rejecting them. The authors showed that covering this region of the antenna, where the BaS are distributed, resulted in the wasp's inability to recognise prey. Because the wasp's antennae had to contact the prey during hunting behaviour, the authors suspected a role of low-volatility compounds, like CHCs, in this process (Anton & Gnatzy, 1998).

In Hymenoptera, BaS are generally located on the most distal segments of the antennae, where social or predatory contact is made with other individuals (Nakanishi *et al.*, 2009; Couto *et al.*, 2017). In ants, OSNs from BaS were shown to project exclusively into a single glomerular cluster of the AL, initially termed T₆ in ants on account of the relative position of its afferent sensory tract (Zube & Rössler, 2008; Nakanishi *et al.*, 2010; Kelber *et al.*, 2010; McKenzie *et al.*, 2016). The same projection pattern of BaS OSNs was found in hornets, in which the cluster was named T_B in an attempt at inter-species standardisation (Couto *et al.*, 2016, 2017). For the sake of consistency, we therefore refer to this group of glomeruli as T_B in the remainder of this review, irrespective of the species being discussed. In both lineages, this cluster is located dorsally (according to neuroaxis; Strausfeld, 2002), close to the antennal nerve entrance (caudal), and is composed of a relatively high number of small and similar-size glomeruli (Nakanishi *et al.*, 2010; Kelber *et al.*, 2010; Zube *et al.*, 2008; Couto *et al.*, 2017, 2021). This cluster also contrasts with other areas of the AL, as immunohistochemical studies revealed an identical lack of serotonergic innervation in the T_B cluster of carpenter ants and hornets (Fig. 3C,E), whereas serotonin-immunopositive neurons are present in all other regions of the AL (Dacks, Christensen & Hildebrand, 2006; Tsuji *et al.*, 2007; Zube & Rössler, 2008; Nakanishi *et al.*, 2010; Couto *et al.*, 2017). Additionally, an early study on an ant, *Camponotus vagus*, and a vespid wasp, *Polistes gallicus*, also described a difference in both succinate dehydrogenase and acetylcholinesterase enzymatic activities in two subregions of the AL (termed 'lobules'; Masson & Strambi, 1977). The small size and position of the glomeruli of sensory lobule 2 correspond to those of the T_B cluster, possibly defining another shared feature of these glomeruli in ants and vespid wasps. Lastly, data from a paper wasp, *Polistes fuscatus* (Vespidae), suggests that the T_B glomeruli may receive sparser innervation from a population of histaminergic local interneurons compared to other glomeruli (Dacks *et al.*, 2010).

In ants, neuronal tracing experiments further showed that the T_B cluster is poorly connected to the rest of the AL as local interneurons innervate either the T_B glomeruli or the non-T_B glomeruli, with none found to innervate both groups

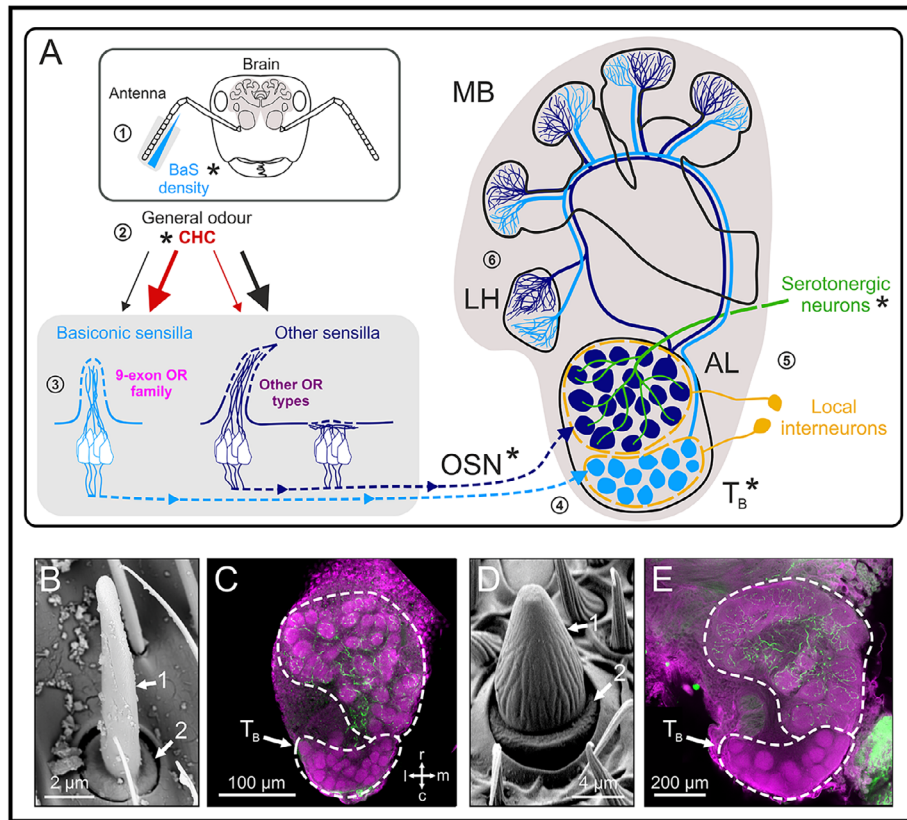


Fig. 3. The basiconic subsystem. (A) Illustrative example of the basiconic subsystem within the olfactory system of a *Camponotus* ant. ① The basiconic sensilla (BaS) are more abundant in distal segments of the antenna; ② they preferentially respond to cuticular hydrocarbons (CHCs) compared to general odours. ③ The BaS sensory neurons are thought to express the 9-exon olfactory receptor (OR) family; ④ they project exclusively into a dorso-caudal cluster of glomeruli (T_B). ⑤ The T_B cluster and other glomeruli have distinct patterns of innervation. ⑥ Olfactory projection neurons from BaS glomeruli terminate in separated regions of the mushroom bodies (MB) and the lateral horn (LH). Stars indicate features present in both ants and hornets. OSN, olfactory sensory neuron. (B, D) Basiconic sensilla of *Camponotus aethiops* (Formicidae; B) and *Vespa velutina* (Vespidae; D). This sensillum consists of a peg like structure (1) standing in the centre of a socket (2). (C, E) Optical sections of the antennal lobe (AL) in *C. aethiops* (C) and *V. velutina* (E) after immunohistochemical staining of serotonin fibres (green). The ALs are shown with the same orientation, based on the neuro-axis (l, lateral; m, median; c, caudal; r, rostral). In both species, the dorso-caudal cluster (T_B) does not receive innervation from serotonergic neurons.

(Nishikawa, Watanabe & Yokohari, 2012). Nishikawa *et al.* (2012) also reported a segregation of T_B projection neurons, which terminate in a restricted part of higher-order olfactory centres, the mushroom bodies and the lateral horn, and do not overlap with non- T_B projections. Together, these observations suggest the existence of a distinctive olfactory pathway (Fig. 3A) present in ants and hornets which is partially independent from the general olfactory system, from sensilla to higher-order brain centres. Henceforth, we name this pathway, the BaS subsystem.

Despite the many similarities observed between the BaS subsystems of ants and hornets, a stark difference is also present. In ants, the males lack BaS and accordingly the T_B cluster is absent (Nishikawa *et al.*, 2008; Nakanishi *et al.*, 2010) or drastically reduced (Zube & Rössler, 2008). Such sexual dimorphism is absent in hornets, and both males and females possess BaS and a T_B cluster (Couto *et al.*, 2017). In honey bees, like in ants, BaS are only present in females, and OSNs from BaS preferentially project to a sexually dimorphic

cluster of glomeruli, called T3 (Kropf *et al.*, 2014). This subpart of the honey bee T3 cluster is reminiscent of the T_B , but contains less glomeruli than in ants or hornets and, in contrast to these other species, does not lack serotonin innervation (Rehder, Bicker & Hammer, 1987). There are therefore remarkable differences between the BaS subsystems of ants, bees and hornets despite evidence of numerous shared features across large phylogenetic distances. The reasons for these differences are yet unknown.

(3) Function of the basiconic subsystem

To date, functional studies on the BaS subsystem are scarce and have not yet determined its precise role in CHC blend detection and in nestmate/kin discrimination. Early electrophysiological recordings of the two AL subregions in *Camponotus vagus* suggest that the delimitation may be modality dependent with a mechanical/tactile area (lobule 2, possibly the T_B) and an olfactory area (the rest

of the AL; Masson & Strambi, 1977). Later, when BaS were first identified as CHC-receptive structures in *Camponotus japonicus*, it was proposed that ants would be anosmic with regard to their nestmate odour due to sensory adaptation (Ozaki *et al.*, 2005). According to the ensuing prefilter hypothesis, prolonged exposure to self-odour (presumably identical to nestmate odour) causes the desensitisation of OSNs that would consequently only respond when stimulated by non-nestmate odours. BaS would thereby act as a filter, removing nestmate odour information at the periphery of the olfactory system (Ozaki & Hefetz, 2014). However, this idea was challenged by optical imaging recordings of projection neuron activity in the AL of *Camponotus floridanus*, which showed odour-evoked responses to both nestmate and non-nestmate extracts (Brandstaetter & Kleineidam, 2011; Brandstaetter, Rössler & Kleineidam, 2011). One possible explanation for these differences would be that different types of BaS exist, with CHC blend-specific ones that are able to adapt and other differently tuned sensilla that do not (Bos & d'Etterre, 2012). Brandstaetter & Kleineidam (2011) also found that neural responses elicited by the gland extracts were not restricted to a specific subregion of the AL. In opposition to the desensitisation hypothesis, these authors rather proposed that using the power of the whole AL would be consistent with the high discriminatory demand required to process subtle variations in CHC blends, such as those existing between nestmates' odours (different castes) and non-nestmate odours. More recently, a study using immuno-histochemistry found that a CHC, (Z)-9-tricosene triggered activity specifically from the T_B cluster (Uebi *et al.*, 2022). This controversy is not yet settled, but the available data suggest that the BaS subsystem may be involved in parallel processing of CHC blend information with a putative predominant role in discriminative function (d'Etterre, Deisig & Sandoz, 2017).

In *Camponotus* ants, electrophysiological recording of BaS in response to a wide panel of odorants showed the existence of different functional subtypes of BaS as well as clear responses to both nestmate and non-nestmate odours (Sharma *et al.*, 2015). Similar recordings in hornets highlighted that BaS preferentially respond to long-chain alkanes (present in CHC blends) compared to more volatile (shorter carbon chain length) aliphatic compounds (Couto *et al.*, 2017). Investigations of the ultrastructure of BaS revealed that a remarkably high number of OSNs are sheltered within each sensillum (>100 in *Camponotus japonicus*), compared to other sensillum types (<10) (Nakanishi *et al.*, 2009). Additionally there is existence of gap junctions between these OSNs, potentially providing lateral inhibition through electrical and ephaptic coupling (Takeichi *et al.*, 2018). It was thereby suggested that the BaS might act as an intensity filter at the periphery of the olfactory system ensuring a low response probability for the evaluation of concurrent and countervailing olfactory cues (Ng, Wu & Su, 2020; Watanabe *et al.*, 2023).

Despite the controversies, clear evidence suggests that CHC information reaches the T_B cluster in the AL, where processing by local neurons presumably assures gain control and increases odorant separation, as shown in the general

olfactory systems of flies and bees (Sachse & Galizia, 2002; Silbering & Galizia, 2007; Olsen & Wilson, 2008; Deisig, Giurfa & Sandoz, 2010; Carcaud, Giurfa & Sandoz, 2018). Local processing rules may however differ between the T_B and other parts of the AL, as in ants T_B local neurons have been shown to be restricted to this subsystem (Nishikawa *et al.*, 2012). The refined combinatorial CHC signal of the T_B is then relayed to higher-order brain centres, the mushroom bodies, then the lateral horn, *via* the median tract of uniglomerular projection neurons, the m-ALT (Zube & Rössler, 2008; Couto *et al.*, 2016). Mushroom body intrinsic neurons, the KCs, are numerous, highly specific neurons that are only activated by combinatorial input from many incoming PNs (Szyszka *et al.*, 2005; Perez-Orive *et al.*, 2002). They are therefore the ideal candidates for a central representation of individual CHC profiles in the brain. In *Drosophila*, the associative learning of odorants was shown to be based on the specific depression of synapses between learned odour-specific KCs and particular mushroom-body output neurons (MBONs) that trigger behavioural output circuits (Aso *et al.*, 2014; Aso & Rubin, 2016). It has therefore been proposed that the learned templates of CHC profiles associated with nestmate odours might be stored as weakened connections between a set of KCs and specific aggression-related MBONs in the mushroom bodies of ants (Neupert *et al.*, 2018). When a newly detected CHC profile is encountered, it would be compared to the stored template(s) of nestmate odours that have been previously learned or experienced, and a mismatch would trigger an antagonistic response. This sensory information would be integrated in the mushroom body lobes along with other environmental stimuli, such as those from the non-BaS olfactory system as well as from other sensory modalities (visual, tactile, etc.), instructing output neurons with a kind of pre-decision, *i.e.* whether to attack or ignore an encountered individual (Neupert *et al.*, 2018; Strube-Bloss & Rössler, 2018). At this stage, the role of the lateral horn in nestmate discrimination is unclear, but some MBONs are known to connect the mushroom bodies with the lateral protocerebrum, including the lateral horn (Rybak & Menzel, 1993; Dolan *et al.*, 2019; Das Chakraborty & Sachse, 2021). Both structures may thus interplay in this context.

Overall, the exact role of each processing step along the olfactory pathway is still ambiguous, and more work is needed to uncover how and where social identities are processed within the brain. However, multiple lines of convergent evidence point to an important role of the BaS subsystem for supporting recognition and social behaviour.

V. CORRELATED EVOLUTION OF OLFACTORY RECEPTOR DIVERSITY AND THE BaS?

If the evolution of complex social ecologies is accompanied by an enhanced ability to discriminate social cues, a reasonable expectation is that this would be manifest in expansions of gene families responsible for detecting identity signals,

such as CHCs. Evidence of an olfactory subsystem putatively linked to CHC detection and social recognition suggests the presence of OR proteins with specialised tuning to CHCs. ORs present at OSN dendrites are usually tuned to a range of chemical compounds (Pelz *et al.*, 2006; Andersson, Löfstedt & Newcomb, 2015). Because olfactory information processing is based on combinatorial activation of AL glomeruli, having more OR genes, and hence more glomeruli, increases the number of possible combinations and is thought to enhance olfactory discrimination ability (Su, Menz & Carlson, 2009). Indeed, comparative analyses of OR repertoires across sequenced hymenopteran genomes suggest ORs have a dynamic evolutionary history, with multiple OR sub-clades showing independent expansions and gene losses across the phylogeny (Zhou *et al.*, 2012, 2015; Sadd *et al.*, 2015; Brand & Ramírez, 2017; Obiero *et al.*, 2021; Legan *et al.*, 2021). In addition several lineages show evidence of adaptive sequence evolution in some ORs (Engsontia *et al.*, 2015; Sadd *et al.*, 2015).

One subfamily of ORs that have received particular attention are the 9-exon ORs. This subfamily was initially highlighted by being the most expanded subfamily in obligatorily eusocial ants, representing around 30% of all ORs (Zhou *et al.*, 2012, 2015; Engsontia *et al.*, 2015). Their putative role in CHC detection was further bolstered by exhibiting female-biased gene expression in the antennae (Zhou *et al.*, 2012; McKenzie *et al.*, 2016), as predicted by most eusocial traits also being female specific, and aligned with the female-biased morphology of the BaS subsystem. In support of this hypothesis, the number of 9-exon OR genes is suggested to correlate with glomerular number within the BaS AL cluster (the T_B) of the ants *Camponotus floridanus* and *Ooceraea biroi* (McKenzie *et al.*, 2016; Engsontia *et al.*, 2015; Zhou *et al.*, 2015; Zube *et al.*, 2008). In addition, the expression of 9-exon ORs appears enriched on the antennal side where BaS are located (McKenzie *et al.*, 2016), and electrophysiological responses to CHC stimulations were recently recorded using heterologous expression of 9-exon OR genes in the *Drosophila* empty neuron system (Pask *et al.*, 2017).

CHC detection forms the basis of nestmate recognition as a proxy of kinship identity, therefore the combined evidence in ants suggests a potentially intimate link between 9-exon ORs, the BaS subsystem and the evolution of eusociality. However, whether or not this link can be generalised across Hymenoptera is currently unclear. Initial studies of 9-exon OR evolution were taxonomically biased towards ants, all of which are obligatorily eusocial species. It should be noted that in this clade, convergent losses of 9-exon ORs have been observed across independent origins of slave-making parasites (Jongepier *et al.*, 2021). Expanding surveys of OR evolution across Hymenoptera reveal a complex, and at times contradictory relationship with social complexity. While 9-exons are found in large numbers in eusocial ants and in phylogenetically distant primitively eusocial *Polistes* wasps (Legan *et al.*, 2021), large OR repertoires have also been reported for some solitary and parasitic/predatory wasps such as *Ampulex compressa* (Apoidea) (Obiero *et al.*, 2021),

and the phylogenetic structure of gene trees suggests these reflect partially independent expansions. It is also notable that all Apidae genomes to date contain many fewer 9-exon ORs (~12–50) than some non-social Apoidea (Sadd *et al.*, 2015; Zhou *et al.*, 2015; Brand & Ramírez, 2017; Obiero *et al.*, 2021), despite their highly derived social ecologies, while 9-exon OR repertoire expansions are even absent in obligatorily eusocial Meliponini bees (Brand & Ramírez, 2017). Some patterns of variation are therefore inconsistent with a simple, systematic link between 9-exon ORs and eusocial ecology. Whether or not this also reflects a more complex relationship between 9-exon ORs and the BaS subsystem, or between the BaS subsystem and eusociality, is currently unclear.

Much of this apparent inconsistency may reflect the limitations of incomplete sampling of the hymenopteran phylogeny. To date, the OR repertoires of only a handful of families have been characterised and current taxonomic sampling does not represent the diversity of hymenopteran social organisations. A lack of data for key, non-social lineages at the base of Aculeata, and close outgroups to each of the social wasps, ants and bees, limits accurate reconstructions of OR birth and death rates during key transitions in their social ecology. This is further compounded by the high turnover rate of ORs in insects, which results in a high degree of lineage specificity (Zhou *et al.*, 2012, 2015; Engsontia *et al.*, 2015; Brand & Ramírez, 2017; Karpe *et al.*, 2017; Obiero *et al.*, 2021; Legan *et al.*, 2021). In addition, McKenzie *et al.* (2016) particularly highlighted one clade within 9-exon ORs, the α -clade, as being key candidates for CHC receptors. This clade appears to have evolved from a single gene in the last common ancestor of Formicidae and Apoidea, with independent gains in both lineages. Detailed catalogues of this clade have not been widely explored in other analyses of hymenopteran OR evolution. As a result, described patterns of 9-exon OR expansions currently provide an incomplete picture of ancestral and derived OR repertoires and their functions across Hymenoptera.

Two pieces of evidence suggest that the convergent evolution of nestmate recognition in phylogenetically separated lineages of social Hymenoptera could be underpinned by independent molecular, and potentially also neural, mechanisms. First, phylogenomic comparisons to date show major expansions in OR clades outside the 9-exon ORs. For example, Brand & Ramírez (2017) highlight the OR subfamily G02A, which is expanded in all obligate eusocial bees and in ants, but not in *Lasioglossum albipes*, a facultatively social halictid bee. Second, electrophysiological responses to CHC stimulations are not, as previously hypothesised, specific to 9-exon ORs (Slone *et al.*, 2017). Instead, in Hymenoptera there is currently no evidence for a strict partitioning of receptive roles, including CHC detection, between OR subfamilies, suggesting that all, or many, ORs are CHC sensitive, not just the 9-exon ORs (Slone *et al.*, 2017). This may not be unexpected due to the coarse tuning of ORs and the importance of combinatorial coding in odour detection, but given the correlative link between the 9-exon ORs and the BaS subsystem in ants, which has a distinct projection

pathway to higher-order brain centres (Nishikawa *et al.*, 2012; McKenzie *et al.*, 2016), the different pathways of the olfactory system may process certain aspects of CHC information (d’Ettorre *et al.*, 2017). Together, these observations also raise the possibility that independent acquisitions of nestmate/kin recognition systems and eusociality may co-opt different OR subfamilies and olfactory pathways.

VI. COMPETING EVOLUTIONARY HYPOTHESES FOR THE ORIGIN OF A SPECIALISED RECOGNITION SYSTEM

The order Hymenoptera is remarkable for its frequent evolution of eusociality, making it a key group in which to study the factors that may have facilitated the evolution of this behaviour (Quiñones & Pen, 2017). Traditionally this research has focussed on the genetic component, due to the perceived connection between the hymenopteran sex-determination system and the high degree of relatedness among cohabiting sisters (Trivers & Hare, 1976). However, while many ecological and behavioural factors, such as limited dispersal, as well as genetic characteristics, such as haplodiploidy, contribute to relatedness within a population, less attention has been given to the factors that impact the cost/benefit balance of Hamilton’s rule (West, Griffin & Gardner, 2007a; West *et al.*, 2021). The accurate recognition of cooperation partners may logically be one of these factors, as it allows selective assistance to individuals sharing similar genes, hence ensuring the benefits of cooperation (Sheehan, Miller & Reeve, 2017).

In social Hymenoptera, previous research has intensively documented the chemical signals involved in recognition and how they might reflect attributes of the signaller, but the features ensuring adequate reception of the signals and how it is enabled by receptor organs of the receiver have received much less consideration. So far, the BaS and the 9-exon ORs have emerged as key elements of CHC detection, and they are considered as components of the nestmate recognition system in the current literature (Nishikawa *et al.*, 2012; Zhou *et al.*, 2015; McKenzie *et al.*, 2016; Godfrey *et al.*, 2021; Gellert *et al.*, 2022). The apparent dichotomy of the BaS subsystem with the general olfactory system conveniently allows the study of recognition at the neuronal level with little interference from the general odour-detection system. Beyond the use of CHCs as nestmate recognition cues, and thereby as a proxy of kinship identity by eusocial Hymenoptera, CHCs are also reported to be involved in prey, host and/or mate selection in solitary species (Anton & Gnatzy, 1998; Mair *et al.*, 2017). Therefore, how the neuronal system evolved to support the potentially competing demands of recognising odour cues in social and non-social contexts remains to be elucidated. Several evolutionary scenarios linking recognition with social complexity emerge from the above discussion.

First the ‘exaptation or precursor hypothesis’ proposes that ancestral solitary species would already have had an

olfactory subsystem resembling the BaS. This subsystem may have originated during hymenopteran diversification in response to selection for recognition of host, prey, optimal mate cues, or in response to the arms race with clepto- and brood parasites (Castillo *et al.*, 2022). This scenario would be supported by the presence of CHC-sensitive BaS which project exclusively into a distinct, isolated area of the AL (or sensory lobule) in most non-social Hymenoptera. Furthermore, the number of T_B glomeruli and 9-exon ORs, which determines the neural system’s discriminatory capabilities, is expected to vary across species. Such variations could be influenced by the level of similarity among recognition cues and the level of discrimination effort required between interacting agents (such as host, prey, mate, parasite and/or nestmate and non-nestmates), rather than social complexity. In this scenario such a subsystem, once established, may have facilitated the evolution of kin-directed assistance, through a simple shift of its function to the recognition of cooperative partners, using similar cues (i.e. CHCs) but in a specific social context. Under this circumstance, eusociality may be more likely to evolve in lineages already equipped with an efficient recognition system.

By contrast, under the ‘convergence hypothesis’, the BaS subsystem would have evolved convergently in ants and eusocial wasps (Vespidae) as suggested by the dynamic and convergent expansions of 9-exon OR genes (McKenzie *et al.*, 2016; Legan *et al.*, 2021). This would be supported by BaS with lower CHC sensitivity and/or non-exclusive projections of their OSNs to a particular region of the AL in solitary Hymenoptera. In this scenario, the numbers of glomeruli and 9-exon OR genes would have increased with social complexity, and only eusocial clades would display a clear BaS subsystem, revealing a specialisation of the olfactory system selected for social recognition.

A final, third hypothesis, the ‘social conflict’ hypothesis proposes that recognition requirements peak at intermediate levels of sociality in primitively eusocial species when reproductive conflicts among non-sterile females are most likely to occur (Nehring & Steiger, 2018). Consequently, nest-mate recognition would become simpler in eusocial species, as individuals mostly interact with surrounding relatives. In our opinion, this hypothesis can be rejected in the case of the BaS subsystem, because the system is well developed in obligate eusocial species such as ants and hornets (Nakanishi *et al.*, 2010; McKenzie *et al.*, 2016; Couto *et al.*, 2021).

The most plausible evolutionary scenarios therefore have quite clear, contrasting predictions about the evolution and distribution of olfactory specialisations, providing ample opportunity for direct hypothesis testing using comparative data. Of course, these scenarios assume that the evolution of eusociality is tightly linked to recognition requirements. The repeated evolution of eusociality in Hymenoptera is however likely the result of an intricate interplay between the evolution of recognition cues, the neural substrate of recognition, relatedness, ecological constraints and the life-history traits of the species. We therefore believe that only large-scale analyses of the BaS subsystem across multiple solitary and eusocial hymenopteran lineages, including diverse

ecologies and colony structure (e.g. number of gynes and mated males), will allow us to disentangle these multiple contributions to eusocial evolution and decide between the competing scenarios presented above.

VII. CONCLUSIONS

(1) An ability to recognise the social identity of others must be a pivotal component of eusociality, allowing individuals to help kin discerningly instead of intruders, which optimises the inclusive fitness of colony members and prevents social parasitism. In insects, social recognition appears to be mediated by chemical signals which are detected and processed by the olfactory system. Understanding the unparalleled density and independent origins of eusocial lineages in Hymenoptera will benefit from an increased understanding of the mechanisms underlying sensory discrimination of colony mates and foreign individuals.

(2) The description of a neuronal subsystem involved in CHC sensing, namely the BaS subsystem, has brought increased attention to the role of chemical communication in kin recognition and the evolution of complex social behaviour. However, this knowledge is currently fragmented, and it remains unclear whether the evolution of social behaviour could have driven adaptations in the olfactory system, or conversely be facilitated by a preadapted chemosensory apparatus. To fill these gaps, several functional and evolutionary aspects of the BaS subsystem remain to be investigated.

(3) Evidence of a specialised BaS subsystem has been observed with poor phylogenetic resolution in only two independent eusocial lineages (Vespidae and Formicidae). Broader sampling is therefore required to reveal how the anatomical organisation of the subsystem varies across Hymenoptera, and to integrate these anatomical data with information on social organisation. By testing whether this variation is associated with differences in social structure, it will be possible to reconstruct the evolutionary history of key traits across the phylogeny and to place behavioural and neuronal changes in chronological order. This will help us understand the roles of the BaS subsystem in the evolution of hymenopteran social diversity, and the level at which behavioural convergence is linked to convergent neural adaptation.

(4) A clear demonstration that the 9-exon OR gene family is expressed by the OSN of BaS would enable a more comprehensive integration of molecular and neuroanatomical data, as this link is so far only supported by indirect evidence. *In situ* hybridisation experiments could allow the precise localisation of 9-exon OR expression and strengthen the connection with particular antennal equipment, while broader genomic assessments of OR repertoires across Hymenoptera would permit formal tests of co-evolution with social ecology.

(5) The complexity of each species' CHC profile has not been included as a possible explanation in the expansion of OR repertoires. Bringing together chemical and genomic information across solitary and eusocial lineages may reveal

that changes in selection regimes during evolutionary transitions between social systems are mainly dependent on species' specific chemical complexity.

(6) To understand the biological significance of such a specialised olfactory subsystem and how it evolved in response to selection for higher-order social organisation, further functional investigations are required. Central recordings (e.g. *in vivo* calcium imaging) of glomerular responses in the AL to a range of odorant compounds, including CHCs, may uncover the functional tuning of each AL compartment and allow exploration of BaS subsystem involvement in parallel processing of identity signals. Moreover, comparing the discriminative capabilities of the BaS subsystem towards CHC blends might provide evidence of an ancestral function in solitary species (such as prey or mate recognition), as well as potential adaptations that support nestmates recognition in eusocial ones.

(7) To date, whether the BaS subsystem is a conserved, ancestral feature of Hymenoptera, or has evolved convergently in eusocial lineages is not known. Evidence that the same neural specialisation evolved in independent eusocial lineages would strongly suggest sociality as a driver of the evolutionary innovations and divergences observed in the hymenopteran olfactory system. By contrast, if we find that this subsystem is conserved across solitary and social Hymenoptera, this could suggest that the hymenopteran brain was pre-adapted to facilitate kin recognition, and explain why these insects have evolved eusociality so often compared to all other orders.

(8) The acquisition of a comprehensive view on the evolution of social recognition in Hymenoptera and its implication in the repeated emergence of eusociality is crucial for explaining the diversity of social ecology across this order. This will require integrative studies that combine neuroanatomy, molecular phylogeny, physiology, and behavioural and chemical ecology within a clear comparative, evolutionary framework that spans eusocial and solitary lineages.

VIII. ACKNOWLEDGEMENTS

A. C. and S. H. M. were supported by the research project grant of the Leverhulme trust (RPG-2019-287) and NERC IRF (NE/N014936/1); S. M., E. H. D., P. d. E. and J.-C. S. were funded by the ANR (ANR-20-CE02-0012).

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(Received 11 August 2022; revised 18 July 2023; accepted 20 July 2023)