

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/330451323>

Brain evolution in social insects: advocating for the comparative approach

Article in *Journal of Comparative Physiology* · January 2019

DOI: 10.1007/s00359-019-01315-7

CITATIONS

4

READS

305

2 authors:



R. Keating Godfrey
The University of Arizona

4 PUBLICATIONS 4 CITATIONS

[SEE PROFILE](#)



Wulfila Gronenberg
The University of Arizona

84 PUBLICATIONS 3,426 CITATIONS

[SEE PROFILE](#)



Brain evolution in social insects: advocating for the comparative approach

R. Keating Godfrey^{1,2} · Wulfila Gronenberg¹

Received: 31 May 2018 / Revised: 9 January 2019 / Accepted: 11 January 2019
© Springer-Verlag GmbH Germany, part of Springer Nature 2019

Abstract

Sociality is classified as one of the major transitions in the evolution of complexity and much effort has been dedicated to understanding what traits predispose lineages to sociality. Conversely, studies addressing the role of sociality in brain evolution (e.g., the social brain hypothesis) have not focused on particular traits and instead relied largely on measurements of relative brain composition. Hymenoptera range from solitary to advanced social species, providing enticing comparisons for studying sociality and neural trait evolution. Here we argue that measuring the role of sociality in brain evolution will benefit from attending to recent advances in neuroethology and adopting existing phylogenetic comparative methods employed in analysis of non-neural traits. Such analyses should rely on traits we expect to vary at the taxonomic level used in comparative analyses and include phylogenetic structure. We outline the limits of brain size and volumetric interpretation and advocate closer attention to trait stability and plasticity at different levels of organization. We propose neural traits measured at the cellular, circuit, and molecular levels will serve as more robust variables for evolutionary analyses. We include examples of particular traits and specific clades that are well-suited to answer questions about the role of sociality in nervous system evolution.

Keywords Social brain hypothesis · Brain size · Division of labor · Evolutionary framework

Abbreviations

EQ	Encephalization quotient
IN	Local interneurons
KC	Kenyon cells
MB	Mushroom body
MBON	Mushroom body output neurons
PCM	Phylogenetic comparative methods
PN	Projection neurons
SN	Sensory neurons entering antennal lobe

Introduction

In an attempt to find a unifying theory for differences in brain size across primates, the middle of the twentieth century saw the formalization of a number of hypotheses addressing primary drivers in primate brain expansion. Hypotheses ranged from those proposing that expansion was driven by release from energetic constraints (Hofman 1983a, b) to those giving primacy to selective forces such as ecological factors (Parker and Gibson 1977; Harvey et al. 1980) or the maintenance of complex social relationships in group-living (Dunbar 1992). The latter, the social brain hypothesis, has garnered much attention for highlighting a potentially unique contribution of sociality to brain expansion. It posits that predation pressure, as the strongest selective force in primate evolution, resulted in the formation of social groups to mitigate predation risk, leading to novel cognitive functions required to manage multiple, non-familial relationships (social complexity, Humphrey 1976). These behaviors are characterized by Dunbar and colleagues as having uniquely complex cognitive demands and requiring expansion in higher cortical regions (Dunbar 2009). While group size

R. Keating Godfrey, Wulfila Gronenberg contributed equally to this review.

✉ R. Keating Godfrey
rkeatinggodfrey@email.arizona.edu

¹ Department of Neuroscience, University of Arizona, Tucson, AZ 85721, USA

² Neuroscience Interdisciplinary Graduate Program, University of Arizona, Tucson, AZ 85721, USA

or grooming clique size (Kudo and Dunbar 2001) appears to explain variation in certain measures of relative brain size (Fig. 1a), more recent studies show that inclusion of ecological components better predicts differences in relative brain measures (Walker et al. 2006). Mixed findings on the effect of group size on relative brain measures across vertebrates led the original author to advocate for a more inclusive definition of social complexity, one that acknowledges pair bonding as a socially complex behavior

(Dunbar 2009). Furthermore, recent large-scale comparisons of primate behaviors and brain sizes add weight to the hypothesis that ecological drivers (e.g., foraging behavior, diet composition), not group size *per se*, best correlate with brain expansion in primates (Reader et al. 2011; Parker 2015; DeCasien et al. 2017).

In a manner similar to the conversation about brain evolution in primates, there has been speculation about the factors driving brain expansion across lineages of insects. Higher-order associative centers, the mushroom bodies, are larger, layered structures in many Hymenoptera (Dujardin 1850; Molina and O'Donnell 2008; Strausfeld et al. 2009) and Blattodea (Farris and Strausfeld 2003), orders represented by numerous social or gregarious species. This observation led to the suggestion that large, expanded mushroom bodies may be related to the cognitive demands of sociality (Jaffe and Perez 1989; O'Donnell et al. 2007, 2011). However, in Hymenoptera, this morphological feature preceded the evolution of sociality (Farris and Schulmeister 2011), and relatively large, duplicated mushroom body calyces are seen in non-social Coleoptera (Farris 2013) and Lepidoptera (Montgomery and Ott 2015; Montgomery et al. 2016).

Currently, two descriptive social brain hypotheses suggest sociality may have a predictable effect on brain characteristics in insects (Gronenberg and Riveros 2009; O'Donnell et al. 2015). Both propose that changes in colony size produce consistent changes in individual worker behavior and that these behavioral changes require less advanced cognitive capacities compared to those of individuals of solitary species. Combined with the observation that neural tissue is costly to develop and maintain (Ames 1992; Hasenstaub et al. 2010), both hypotheses predict reduced brain investment in species with large colony sizes, or consistent reductions in relative investment in brain structures responsible for higher cognitive functions across species that vary greatly in colony size.

Gronenberg and Riveros (2009) hypothesized that the transition from solitary or facultatively gregarious to a colony-based social structure occurred concurrent with expansion of brain regions related to communication (Riveros et al. 2012), but that behavioral specialization of individuals in species with large colony sizes may have led to reduced investment in brain regions required for large behavioral repertoires or behavioral flexibility (Fig. 1b). This hypothesis has two implicit assumptions: that species in large colonies have more individual behavioral specialization and that large behavioral repertoires require larger brains. Findings supporting reduced brain sizes in large-colony have been described by v. Alten (1910) for social bees and by Jaffe and Perez (1989) for ants. Riveros et al. (2012) reported the kind of parabolic relationship predicted from this hypothesis in the antennal lobe of leaf-cutting ants, a finding in line with an expansion of communication systems in the transition to

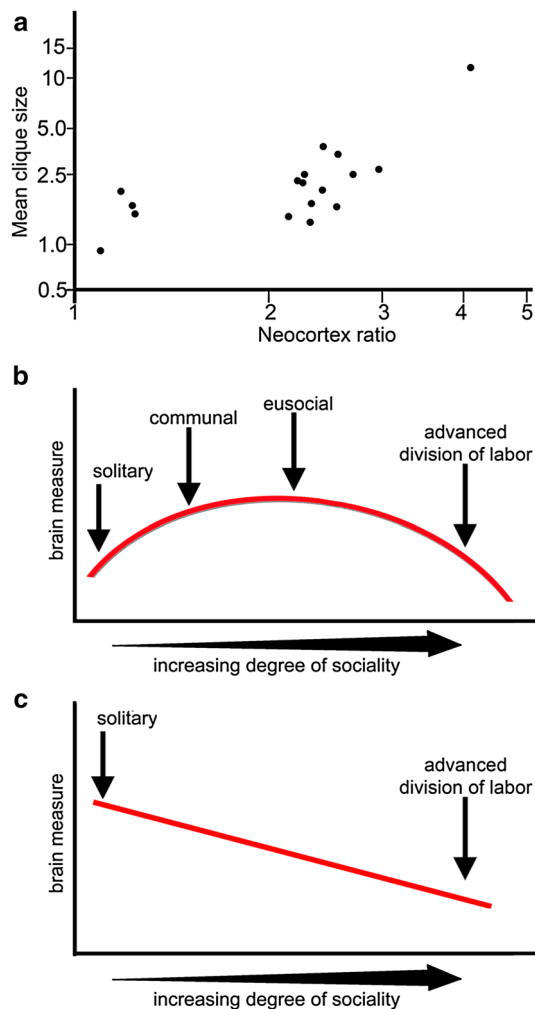


Fig. 1 Correlations between the degree of sociality and brain or brain component size in primates (**a**) and insects (**b**, **c**). **a** Grooming clique size plotted against relative cortex volume of primates (strepsirrhines, anthropoids and hominoids). Based on Kudo and Dunbar (2001). **b** Hypothetical relationship between the degree of sociality and brain measures (e.g., brain size) expected for insects. Note that this hypothesis suggests an increase in brain size associated with the evolution of sociality, but a subsequent decrease for eusocial insects with an advanced system of division of labor. Modified after Gronenberg and Riveros (2009). **c** Hypothetical linear relationship between the degree of sociality and brain measures as suggested by O'Donnell et al. (2015). Note that these hypotheses (**b**, **c**) are not supported by empirical data

moderate-sized colonies, followed by a reduction in large-colony species.

O'Donnell et al. (2015) proposed the Distributed Cognition Hypothesis, postulating that within-group communication may supplant some individual information processing needs and result in decreased cognition in individuals of social species as compared with their solitary relatives. This hypothesis explicitly addresses transitions from solitary to social life histories but can be extended to predict a negative linear relationship between social structures more generally and brain regions associated with higher-order associative processing (Fig. 1c). It assumes that larger behavioral repertoires require larger brain regions for associative processing (namely the mushroom bodies). Across-species comparisons of Vespidae wasps provided support for this hypothesis (O'Donnell et al. 2015).

Attempts to link social structure and brain evolution in insects have responded largely to the causal links made in the social brain hypothesis, with Gronenberg and Riveros (2009) and Donnell et al. (2015) citing Dunbar's work and suggesting that the presumed increase in cognitive demands for individuals in social primate lineages may not hold true for individuals of social insect lineages. In a terse and informative review, Lihoreau et al. (2012) pointed out that social brain hypotheses built for insects neglect core questions about the nature of social neuroethology: (1) whether unique cognitive processes are required for social interactions among insects; (2) from which existing behaviors of less social species these qualities might be derived; (3) if such behaviors require novel neural machinery, and (4) what kind of neuronal changes would be required. Authors of that review suggested that behaviors required for sociality may drive more specialized brains or circuitry and they called for a better understanding of the cognitive processes that underlie social interactions.

There is great value in the neuroethological approach suggested by Lihoreau et al. (2012), but it is important to note that social brain hypotheses are often interested in indirect effects, in particular how the evolution of social behaviors affect other behaviors and cognitive functions in individuals of social species, not merely how social behaviors themselves are coordinated. And hypotheses about social insect brains, being derived within the framework of the social brain hypothesis developed for primates instead of work on trait evolution in insects, suffer three more primary flaws. First, the methods of testing these hypotheses have developed independent of extensive work on trait evolution in other systems, e.g., wings in stick insects (Whiting et al. 2003), wing shape in damselflies (Outomuro et al. 2013), trap-jaws in spiders (Wood et al. 2016), olfactory receptors in insects (Missbach et al. 2014), opsins in beetles (Sharkey et al. 2017), and rely largely on volumetric traits that are not robust enough for evolutionary analyses at the species level,

where most comparisons are made. Second, social structure is often classified as simple or advanced, or by colony size, with both classification systems too coarse to detect consistent changes in the nervous system associated with sociality. Third, the social brain hypothesis in primates attempts to connect a selective pressure (predation) with changes in behavior (group-living) that drove neural trait change, while insect social brain hypotheses do not state nor explicitly test fitness aspects of trait evolution. This also contributes to the neglect of alternate hypotheses for observed changes in neural structure. Given existing criticisms, we suggest studying the role of sociality in brain evolution through the evolutionary comparative approach commonly used for non-neural traits and doing so in tandem with studies conducting more detailed neuroethological studies of particular behaviors and circuits. Here we limit our review to Hymenoptera, but such analyses could be employed at a larger scale or within other insect orders with social lineages.

Adopting the comparative approach

Phylogenetic comparative methods (PCM) have been crucial in understanding trait evolution (Schluter et al. 1997; Pagel 1999; Cornwell and Nakagawa 2017). These methods employ statistical models and an underlying phylogenetic relationship to propose how traits have changed through time and are correlated with environmental or other characteristics of taxa along the phylogenetic tree. PCM are common to studies of non-neural traits seeking to understand how traits themselves arise and rates of change over time (Tallamy and Schaefer 1997; Wood et al. 2016; Gottardo et al. 2016), including of course social structure itself (Cardinal and Danforth 2011; Gibbs et al. 2012). However, social brain studies usually involve the classification of species into a few discrete social categories and do not always take phylogenetic relatedness into account. Here we suggest including phylogenetic structure to best test hypotheses about the effects of sociality on brain evolution. Not only can PCM account for evolutionary history and relatedness (Martins and Hansen 1997), they can be used to map neural characteristics onto phylogenetic trees, infer evolutionary history of particular traits, and study correlated evolution of traits across lineages (Pagel 1999; Dobson and Sherwood 2011; Caetano and Harmon 2017). This could be done across multiple instances of social structure shifts in Hymenoptera (see “[Measuring social complexity](#)” section) to test if trends in elaboration or simplification correlate with shifts to sociality or changes in social structure. This method allows us to test if homologous or analogous genes, cellular phenotypes, or neural circuits are associated with social structures or particular social behaviors across lineages. Importantly, analysis

methods are now attempting to account for intraspecific trait variation (Kostikova et al. 2016), something that may be particularly important for neural traits. And, while here we discuss hypotheses of how changes in sociality affect neural traits, these analyses can also be used to test if particular neural characteristics predispose lineages to social behaviors or structures (Ng and Smith 2014).

Measuring trait evolution

To put PCM to use, we need well-developed phylogenies for comparisons (even if only to control for shared evolutionary history), sufficient understanding of social behavior in lineages used for comparison, and more detailed quantification of neural characteristics than just relative volumes of brain components. Admittedly, comparing neural trait differences across a few genera that vary in social structure is far easier than mapping these traits onto broader phylogenies. But the level of detail required for neural trait reconstructions may not be as precise as that needed to ascribe function to changes in neural traits. For the purpose of studying sociality and brain evolution, it may be sufficient to describe increases and decreases in number and diversity of components of these systems.

To connect social structure with measured changes in the brain, we must ask what characteristics of brains might show the appropriate variation at the evolutionary scale (i.e., at the level of species or genus) and in what behavioral differences may be ascribed to or linked with these changes. Fitness consequences must also eventually be included in such analyses, but initial correlative studies of these traits do not require this. We suggest measuring brain change in size or architecture at three levels of organization: synapses, cells, and circuits. In terms of interspecific comparison of the kind measured for evolutionary study, differences in brains likely reflect changes that can be grouped into four broad categories: the addition of cells (of an existing function/phenotype or of novel function), reallocation of existing cells to a new function (through changes in connections or gene expression), changes in connectivity among neurons, or changes in the density of cell connections. In the following sections, we discuss how social complexity might be classified and what neural traits might be useful in such comparative analyses. In addition to their value in PCM, we suggest traits that can be quantified as functional units because of the interest in social and behavioral complexity across social brain hypotheses. It is our hope that this framework can be used to ground future studies of brain evolution in Hymenoptera (and other social insects). But first, we need to briefly touch on alternate hypotheses for brain evolution

in insects as they are an important consideration in comparative studies.

Alternate hypotheses

The literature supporting a strong link between sociality and primate brain evolution is often focused on disproving alternatives (Table 1 in Dunbar 1998). The social brain hypothesis, both its iteration in vertebrates and insects, is a functional explanation for changes in brain size or brain component size across lineages. Mechanistic explanations addressing genetic, developmental, or metabolic changes that allow for brain expansion remain largely neglected in insect brain evolution research. These explanations address constraints on brain size evolution and can be used to test underlying assumptions about constraints. They may also help distinguish functional explanations in instances where different mechanisms would best explain observed changes.

The strong focus on directional effects of sociality in Hymenoptera brain evolution has resulted in a body of the literature deficient in tests of competing or complementary functional explanations. Hypotheses addressing diet shifts and foraging behaviors as drivers in brain evolution have been put forth by vertebrate biologists for decades (Gittleman 1986; King and Cowlshaw 2007; Dunbar and Shultz 2017; Rosati 2017), in large part because they offer an explicit link between release from constraints and functional explanations for specific changes in the nervous system. For example, diet shifts that provide novel caloric resources may allow for increased growth in cell size or number and require more acute sensory systems or better associative learning capability, traits which often show natural variation within a species upon which selection could act. Diet shifts that result in higher efficiency exploitation of a single resource may affect sensory system specificity (Goldman-Huertas et al. 2015), and theoretically, relax pressure on associative learning networks.

Studies of insects should include additional alternate hypotheses from vertebrate literature such as the role of maternal care, home range size, and spatial navigation in predictable changes in brain size or structure. These are not completely absent in insect brain evolution literature; Farris and Schulmeister (2011) proposed that cognitive demands of locating hosts, including spatial learning, associated with a parasitoid habit may have driven mushroom body expansion in these lineages long before sociality emerged. In this case, it is unclear if regional expansion preceded and facilitated host shifts or if host shifts provide caloric and cognitive drive for regional expansion. The cognitive demands of central place foraging have been offered as a driver in multi-modal circuit evolution in the mushroom bodies (Avauguès-Weber and Giurfa 2013), whereas the role of maternal care in brain evolution has received relatively little attention.

Given that both social and solitary Hymenoptera exhibit variation in these behaviors, these kinds of questions may be addressed through comparative study. We suggest more explicit inclusion of these hypotheses in studies addressing sociality and brain evolution.

Classification of social structure

Colony size is not an accurate predictor of behavioral complexity

Generally, insect social brain hypotheses predict that changes in colony size correlate with individual-level traits, with both hypotheses reviewed above supposing simplification or specialization of individual behaviors at larger colony sizes due to information sharing or division of tasks. The differences in expected individual cognitive loads in individual-based societies (e.g., in primates) versus those of social insects drive opposite predictions about behavior and brains. We choose the term ‘reduced behavioral complexity’ here to encompass the general idea that individuals of social insect species have been hypothesized to show behavioral specialization (Gronenberg and Riveros 2009), or reduced individual cognitive abilities (O'Donnell et al. 2015), recognizing that these are not equivalent (or sometimes even comparable) descriptions of behavior. We review evidence for these predictions below, but first it is important to acknowledge that there may be behavioral or neural changes related to colony size that are unrelated to adaptive change in individual behaviors. Indeed, colony size might indicate important aspects of colony organization such as division of labor (Pacala et al. 1996; Thomas and Elgar 2003; Ferguson-Gow et al. 2014), morphological variation within the worker caste (Wilson 1953; Wetterer 1994, 1999), or coordination of cooperative behaviors (Pacala et al. 1996; Bourke 1999). Species that form large colonies are often ecologically dominant and colony size likely has tangible consequences for survival and competitive advantage. For example, species that vary in mature colony size may experience different selective pressures due to shifts in life-history traits such as effective population size (Romiguier et al. 2014) or colony size may affect individual resource provisioning (Ferguson-Gow et al. 2014; Wills et al. 2018), a variable controlling body and brain size in many insect species.

Are colony size and individual reduction in behavioral complexity positively correlated as our current theoretical structure assumes? First, while mature colony size is often treated as a stable, species-level trait or variable, colony age and environmental conditions such as resource distribution (Williams et al. 2012) and competition likely affect colony size (though this is an understudied topic); within a species there may exist much variation in colony size. Even in

species where mature colony size is stable, as is true in many ant species, size can covary with latitude across species, suggesting it may function as a buffer against environmental variation and therefore be responsive in part to environmental conditions (Kaspari and Vargo 1995).

Perhaps more important for predictions about individual behavior and underlying neural traits is to consider that, regardless of mature colony size, most ant and wasp and many bee colonies start small; division of labor and task specialization often vary with colony maturity within a species. Temporal or age polyethism, wherein task allocation is determined by worker age, is common in ants (Seid and Traniello 2006; Oettler and Johnson 2009) and bees (Johnson 2010) and exists but more weakly in wasps (Hurd et al. 2007; Torres et al. 2012; Brahma et al. 2018). This allocation structure itself does not support the prediction of increased individual specialization, since each individual progresses through most if not all tasks, or expands their repertoire with age (Seid and Traniello 2006). Behavioral complexity, which can be approximated by repertoire size (Traniello 1978; Cole 1980, 1985; Retana and Cerdá 1991; Sempo and Detrain 2004; Seid and Traniello 2006) would be expected to be comparable across individuals when their entire lives are taken into account.

Further complicating this predicted link between colony size and reduced behavioral complexity, the mechanism of task allocation itself may change as colonies grow to maturity. In the Ponerine green-head ant *Rhytidoponera metallica*, temporal polyethism arises only when colonies reach large sizes and may be in some way triggered by greater frequency of worker contact (Thomas and Elgar 2003). Furthermore, workers from large colonies spend more time foraging than age-matched controls from small colonies (Thomas and Framenau 2005), indicating worker age alone does not determine task performance or behavior in the larger *R. metallica* colonies. These levels of behavioral plasticity—in workers across the life of the colony and within workers over their lives—may itself be a very useful characteristic for comparison across social insect taxa when trying to understand social structure or measure complexity. These observations do require a note of caution: since colony size varies over time and sometimes within a species, it is difficult to account for observed differences in behavior or brain characteristics using ultimate explanations unless we understand underlying patterns of change in behavior or brains, or individuals from all potential colony sizes and colony ages are sampled.

Despite the fact that colony size itself is not always a good predictor of worker specialization (Fjerdingstad and Crozier 2006), there are instances where members of a colony are measurably specialized and these examples come primarily from species that form large colonies, particularly when those species reach their largest colony size. However, evidence for worker specialization exists largely in cases where

there are distinct morphological castes or high worker size polymorphism, rather than large colony size alone. In ants, in particular in the fire ant *Solenopsis invicta* (Tschinkel 1988) and leaf-cutter *Atta cephalotes* (Wetterer 1994), colonies are initially comprised of monomorphic workers and size polymorphism emerges at intermediate colony sizes, likely due to changes in larval provisioning (Wills et al. 2018). Worker size is correlated with behavioral specialization in some species, though there is more overlap in behavioral repertoires between morphological castes than originally expected (Sempo and Detrain 2004). In the leaf-cutter ant *Atta wollenweideri* the smallest workers, which perform a limited task set, have reduced investment in particular regions of the antennal lobe (Kelber et al. 2010). Given overwhelming evidence to the contrary, we cannot *a priori* assume that species forming larger colonies contain workers exhibiting smaller behavioral repertoires or greater specialization. Studies quantifying behavioral repertoires in solitary and social insects, alongside the adoption of a more explicit classification of social structure, will allow us to test assumptions about correlations between social structure and behavioral specialization or individual cognitive capacities.

Comparative studies quantifying behavioral and neural differences across closely related species with and without task specialists will bolster our understanding of specialization and brain evolution, but will not necessarily provide insight into unifying effects of sociality on brain evolution. Behavioral specialization is theorized to provide release from the neural investment costs (Bernays 2001), but it is possible that selection in social lineages has provided a mean of flexibly regulating metabolic costs of brain tissue by capitalizing on environmental control of developmental pathways for neural investment (O'Donnell et al. 2018). Sociality may result in an increased capacity for regulating gene expression (suggested for bees in Kapheim et al. 2015), potentially a response to genetic constraints associated with kin selection (Hughes et al. 2008) and effective population size. Greater flexibility in gene expression may indeed be a hallmark of sociality, but resulting changes in neural investment would be derived from a different predictive structure than group size driving reduced investment due to increased task specialization.

While the generalist–specialist framework may still be useful in instances where specialization exists, we have shown that it is difficult to link specialization to sociality through group size (e.g., honey bees form large, socially advanced colonies but show no morphological specialization while bumble bees form small, socially less advanced colonies but feature a very large range of body (and brain) size within colonies). Another means of addressing whether sociality and differences in social structure predictably affect neural traits is to begin by asking whether selective pressures on solitary, semi-social, and eusocial species have

predictably affected individual traits in divergent lineages with shared social structure and if so, which traits. Here we can fold in the thinking on specialization by mapping traits onto phylogenies to ask which traits are derived or lost in social lineages. With this information, we can build a better predictive structure to address the way behavior and underlying neural traits change in social lineages and if they do so in consistent or novel ways.

Measuring social complexity

Social complexity describes the number and diversity of parts in a social system and connectivity among parts, with complexity scaling with increases in any of these components (Bergman and Beehner 2015). Maybe more salient in a social insect context, in the social brain hypothesis social complexity is approximated by the size of a particular network where an individual manages social relationships, often group size (Dunbar 1992) or grooming clique size (Kudo and Dunbar 2001). The use of group size as a measure of social complexity in vertebrates contributed to the use of colony size as a metric of social complexity against which to regress brain characteristics in insects (Riveros et al. 2012; O'Donnell et al. 2015; Amador-Vargas et al. 2015; Kamhi et al. 2016). While some parallels can be drawn, vertebrate social groups and insect social groups are not analogous. Social groups in vertebrates (individual-based societies) are often formed by generations of individuals that vary at least moderately in relatedness, each of which retains the capacity to reproduce; a colony of social insects (colonial society) is formed by highly related individuals, the majority of which do not or cannot mate and reproduce (Wells et al. 2003). In individual-based societies each organism is expected to navigate the physical and social environment to maximize direct fitness, whereas in colonial societies individuals work to maximize indirect (colony-level) fitness, displaying behaviors or traits that contradict direct fitness predictions (Wilson and Hölldobler 2005). Insect social brain hypotheses leverage this erosion of individuality in making predictions. It also means that increasing social complexity in social lineages is defined by more individual units in a network, but given what we know about individual interactions in colonies (Pacala et al. 1996; Mersch et al. 2013) and the general lack of an individual recognition systems, not necessarily by the management of a greater number of unique social connections.

Attempts to link sociality and neural trait evolution need to adopt more explicit measures of social complexity. Recent reviews summarizing classifications of sociality and social structure (Kocher and Paxton 2014; Rehan and Toth 2015; Boomsma and Gawne 2018) provide more satisfying frameworks for comparing species based on differences in social complexity. The transitions in social

structures put forth by Boomsma and Gawne (2018), with an important distinction between eusociality and superorganismality being the presence of a queen–worker polymorphism, will be useful in choosing future comparisons (Fig. 2a). In particular, the resuscitation of the concept of the superorganism as a distinct, irreversible transition in life history strategy provides backing for the division of comparisons between solitary and subsocial or eusocial

taxa and those involving taxa that are superorganismal (e.g., ants).

Although social structures can be ranked according to social complexity, it can be difficult to compare classifications based broadly on social structure given nuances in social behavior across independent origins of sociality (Fig. 2). Instead, we suggest studies of brain evolution and sociality choose comparisons based on sets of specific social

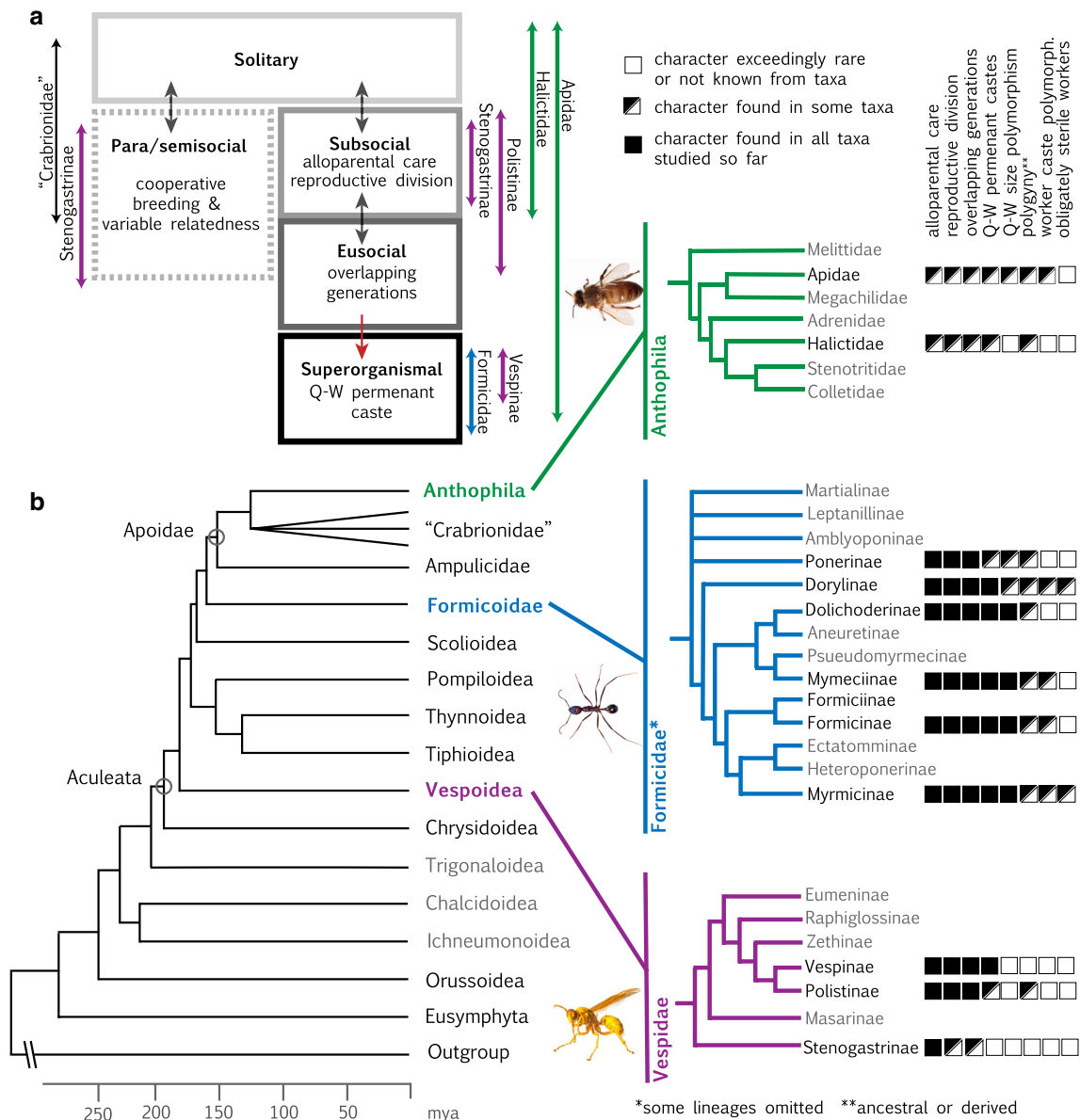


Fig. 2 **a** Transitions in social structure modeled after Boomsma and Gawne (2018) with ranges of social characteristics and structures exhibited by taxa displayed by arrows. **b** Major lineages of Hymenoptera with advanced social characteristics, including those classified as eusocial or superorganismal. **c** Example lineages (in black) containing variation in social structure useful in employing the comparative approach. Social characteristics listed across the top and lineages

coded based on whether taxa possess characteristics and if there is variation. Hymenoptera phylogeny adapted from Peters et al. (2017), family-level Anthophila phylogeny adapted from Danforth et al. (2013), subfamily-level Formicidae phylogeny adapted from Ward (2014), and subfamily-level Vespiidae phylogeny adapted from Bank et al. (2017)

characteristics associated with changes in social complexity rather than broader classifications of social structure (Fig. 2; classifications of social structure included as general reference). Contrasts of related taxa that vary in particular social characteristics help control for shared evolutionary history and allow us to ask questions about lineage-specific neural adaptations and changes associated with social structure. Multiple comparisons of this kind across independent origins of sociality allow us to ask if consistent neural character shifts are observed over comparable changes in social structure. In particular, the use of taxa for which we have well-supported phylogenies and fairly comprehensive behavior and life history traits will allow us to also parse the relative contribution of social and non-social factors in brain evolution. Lineages currently used to study the evolution of sociality and social complexity may be the best place to start.

The vast majority of bee and wasp taxa are solitary, but a number of lineages show astounding diversity in social structure and some multiple instances of the emergence of social characteristics. The bee families Apidae and Halictidae are particularly diverse, and both provide opportunity to study the transition between solitary and social forms. Halictids in particular may be useful in studying sensory and neural characteristics after reversions from social forms to solitary ones (Wittwer et al. 2017), as this occurred a dozen times in this lineage (Danforth 2013). The Halictidae subfamily Halictinae contains some of the greatest diversity in what are often called ‘primitively eusocial’ bees—often workers are totipotent (still able to mate and reproduce and some species actually do) or colonies lack numerous overlapping generations. However, species in this subfamily do range from solitary reproducers to colonies with distinct reproductive division of labor, making them ideal for comparative studies looking at trait diversity and plasticity across this range of the social spectrum (Gibbs et al. 2012). Furthermore, this subfamily is notorious for species displaying facultatively subsocial behaviors, changing strategies based on habitat characteristics or larval provisioning (Smith et al. 2007; Brand and Chapuisat 2012). This provides opportunity for within-species comparisons of the effects of sociality on neural traits (Smith et al. 2010; Kocher and Paxton 2014). Though different in many behavioral aspects, hover wasps (Stenogastrinae) span a range of communal nesting and subsocial behaviors which overlap with those exhibited by Halictids (Turillazzi 1989; Francescato et al. 2002). These may serve as good comparisons for tests of neural character shifts with changes in social structure across independent origins of social behavior in highly divergent lineages.

Apidae, the largest family of bees, contains taxa that range from solitary to superorganismal. The sheer size and span of divergence time across taxa in this lineage requires comparisons be chosen carefully, but because taxa span the transition from eusocial to superorganismal, with superorganismality

having evolved independently in the honeybee (Apini) and the stingless bee (Meliponini) lineages, this family allows us to ask questions about neural character shifts at that transition. Comparisons of Polistinae and Vespinae wasps, sister subfamilies within Vespidae, also allow the study of this transition as the former display a range of subsocial and eusocial behaviors while the latter are all superorganismal. A fairly comprehensive neuromorphological comparison of Vespidae did not find differences associated with sociality after accounting for phylogenetic relatedness (O’Donnell et al. 2015), but this may be due to the broad classification of species as social or solitary. We suggest ascribing particular social characteristics to taxa for comparison may provide necessary resolution to detect differences.

Comparing neural traits across social taxa and at the transition to advanced eusociality allows us to test if shifts in reproductive opportunity or reductions in totipotency consistently affect these traits. Comparing differences in social structure within superorganismal taxa are useful in understanding how very advanced forms of eusociality, wherein workers of a colony form morphologically specialized castes, are sterile, or even show low levels of relatedness, may affect nervous system investment or structure. For example, beyond the loss of mating capacity, the evolution of worker sterility (loss of egg production or complete loss of ovaries) in some lineages represents a final step in the separation of reproductive and worker tasks (Aoki and Moody 1981). Less commonly, a number of advanced eusocial taxa have evolved a morphologically specialized worker sub-caste (e.g., *Cephalotes* ants) or lost the morphologically specialized queen caste (e.g., *Dinoponera* ants). The tolerance of polygyny exists as a derived trait in a number of ant genera (Boomsma et al. 2014; Fig. 2). The ants (Formicidae) show the greatest diversity of forms and represent multiple independent origins of many socially advanced traits. There may also be greater diversity of traits in other superorganismal taxa than approximated by current research. For example, it has generally been thought that morphologically distinct worker subcastes exist only in ants, not superorganismal bee or wasp genera. However, measurements of specialized guard bees in the stingless bee *Tetragonisca angustula* found morphological worker subcastes outside of Formicidae (Gruter et al. 2012).

Rethinking neural characteristics

Behavioral complexity and brain size

“... no one supposes that the intellect of any two animals or of any two men can be accurately gauged by the cubic contents of their skulls. ... Under this point of view, the brain of an ant is one of the most marvelous atoms of matter in the

world, perhaps more so than the brain of a man.” Darwin, 1871, *The descent of man*.

Despite Darwin’s assertion that such comparisons are conceptually problematic, an underlying assumption of most comparative studies is that larger behavioral repertoires or greater behavioral flexibility (the number of different behaviors an individual can perform and the ability to switch among behaviors opportunistically, respectively) require larger brains or greater investment in particular regions. While many morphometric brain studies in insects do not specifically refer to the insects’ degree of sociality, the majority of them compare the brain size or the relative size of brain components to the insects’ behavioral complexity. There are real statistical relationships between volumetric differences in regional brain investment and behavior with the general trend that greater volumes correlate with more acute or additional behavioral abilities (Reader and Laland 2002; Changizi 2003; Roth and Dicke 2012). However, changes in nervous system complexity may not result in measurable volumetric investment differences. Therefore, just as with social complexity, measuring number and diversity of components of the brain or brain regions will be useful (suggestions for the olfactory system in Fig. 3). If the functional subunits of a system are known and can be quantified along discrete 3D boundaries, volumetric comparisons may be suitable. The antennal lobe of many insects is one such example where volume and system complexity are related. The peripheral number of sensory receptor

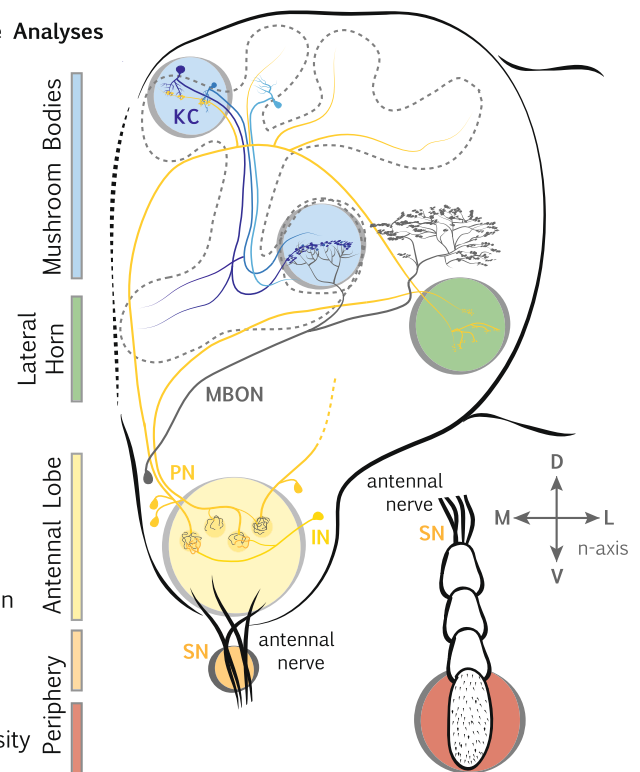
neurons for a particular odorant underlies the sensitivity for that odorant and also determines the size of the particular antennal lobe subunit (glomerulus) where all the neurons tuned to this specific odorant terminate. The best-known example is the tremendous number of receptor neurons in male moths that allow males to detect the presence of female pheromones at extremely low concentrations. These pheromone-sensitive neurons terminate in particularly large glomeruli (macroglomeruli). The number of diverse sensory receptor types on the antenna determines the number of glomeruli within the antennal lobe, which, in turn, underlies the number of odorants that the insect can discriminate. These relationships are reviewed in Hansson and Stensmyr (2011). In this case, we understand the basic circuitry of the antennal lobe and because glomeruli are functional units that can be measured volumetrically, their numbers and volumes are a fitting way to describe the general diversity of parts.

On the other hand, volumetric measurement in the visual system may not be a good analog for system complexity. Like most other social insects, *Polistes* paper wasps recognize their nest mates olfactorily, by their nest odor, a complex mixture of odorants (in particular cuticular hydrocarbons) that differs among colonies (Lorenzi and Caprio 2000). In contrast, two *Polistes* species (*P. fuscatus*, *P. dominulus*) can individually recognize their nestmates visually by their black and yellow facial patterns, which differ among individuals in a colony (Tibbetts 2004). One might expect this novel visual behavior to require advanced visual capabilities and

Fig. 3 Functional units of the nervous system. A simplified version of the Hymenoptera olfactory system as example of characteristics of the nervous system that may be used in comparative analysis. Regions and cells color coded: red antennal club, orange antennal nerve; yellow antennal lobe; blue mushroom body input and output regions; green lateral horn. Cells shown include sensory neurons (SN) in tracts entering antennal lobe. Projection neurons (PN) and local interneurons (IN) of the antennal lobe in shades of yellow, three types of Kenyon cells (KC) of the mushroom bodies in shades of blue, a mushroom body output neuron (MBON) in gray

Characteristics for Comparative Analyses

- KC phenotypes & number
- PN - KC connectivity
- KC - MBON connectivity
- Number of output layers
- Synaptic morphology
- Plasticity gene expression
- Input cell types & regions
- Connectivity patterns
- Glomeruli number & sizes
- PN innervation patterns
- IN neurotransmitters
- SN - IN - PN connectivity
- Neuromodulatory innervation
- Sensory neuron tracts
- Tract distribution in AL
- Sensilla phenotypes & density



to correlate with an expansion of visual and/or memory processing centers in the wasps' brains. However, a volumetric comparison of these two wasp species and two related species that do not show these advanced visual capabilities did not reveal any significant differences in either the optic lobes or the presumable visual memory centers [the mushroom body (MB) collar] (Gronenberg et al. 2008). This suggested that pronounced behavioral differences may not manifest themselves in volumetric brain changes but in subtle differences in the connectivity of certain neurons or the capacity for experience-dependent plasticity in these networks. Functional units of optic lobe structures may be better described by their connectivity or neural phenotypes (Li et al. 2013). Similarly, recent detailed neuroanatomical description of the silkworm lateral accessory lobe, a functional region of the insect brain without clear anatomical delineation among parts under standard volumetric staining techniques, provides a nice example of the characterization of a brain region into functional units suitable for comparison across insects (Namiki and Kanzaki 2016).

While comparing brain or brain component size and behavior does have its merits in closely related species, in a way it seems analogous to comparing the computational powers of electronic computers by measuring the physical size of their processors and forgetting that a 1975 Cray supercomputer weighed almost a ton while a much smaller modern smart phone has almost a thousand times the computational powers of a 40-year-old supercomputer. In this section, we review the use of volumetric measurement, highlighting for which questions and comparisons it may be most useful, and discuss synaptic, cellular, and circuit-level characteristics more suitable for a PCM approach asking how sociality affects brain evolution in insects.

Measuring neural traits

Analogous to the social brain hypothesis in primates, insect social brain hypotheses propose what may be deemed an emergent property of sociality to be reflected the brain—they propose sociality itself acts as a unique selective force and directional changes in social structure or complexity should be reflected in the anatomy of higher-order brain regions. Many studies on social vertebrate brains focused on the relative size of the cerebral cortex or certain cortical regions (e.g., Reader and Laland 2002; Dunbar and Shultz 2007). Similarly, morphometric research on social insect brains in the context of advanced behavioral capabilities focuses on the relative size of mushroom bodies (MB; see below) in particular (e.g., Howse 1975; Jaffe and Perez 1989; Farris and Schulmeister 2011; Farris 2016), an associative brain region where neuropils comprised of multiple populations of input and output neurons. Sensory structures and first-order sensory neuropil receive far less attention (a recent

exception being Wittwer et al. 2017), even though they may serve as more direct targets of selection and could similarly show directional changes with increasing social complexity. In addition to advocating for the use of functional units of neural traits that can be compared across species, we suggest peripheral structures should receive more attention in comparative studies (Fig. 3).

Volumetrics

Historically, research focused on comparing brain size and later also brain component size, across animals of different perceived behavioral or cognitive capabilities. These measures respond to the idea that neural tissue is costly to maintain and therefore costs should be reduced whenever possible (Ames 1992; Aiello and Wheeler 1995; Isler and Van Schaik 2006) and were influenced by the fact that humans, arguably the most cognitively advanced of animals, have particularly large brains for their body size. The fact that brain size correlates most strongly with body size across all animals (Jerison 1973), needed to be taken into account when trying to interpret brain size in any evolutionary, ecological, behavioral or cognitive context. This leads to the formulation of the encephalization quotient (EQ; Jerison 1977), an approach to express the allometric brain/body size correspondence as an exponential relationship. More recent studies suggested other statistical ways of correcting for body size (e.g., Krebs et al. 1989). Research has focused in particular on the comparison of primate brains, from prosimians to monkeys, apes and man, and using different brain-related measures (EQ, relative size of the cerebral cortex, etc.). Those measures correlate relatively well with the respective primate species' behavioral capacities and presumed intelligence (reviewed in Roth and Dicke 2012) and also allowed inferences into the intellectual capacities of our ancestors by estimating the brain volumes of hominids from the cranial capacity of fossil skulls (reviewed by Schoenemann 2013). Recent meta-analyses suggest that absolute brain size might be a better predictor for cognitive abilities of primates than relative brain measures or the size of brain components (Deaner et al. 2007), a measure unfortunately often not reported in comparative insect studies. In addition, as indicated above, there are the more general reservations about the validity of correlating brain measures and sociality (Barrett et al. 2007; DeCasien et al. 2017).

It is important to acknowledge that relative brain investment and relative investment in particular structures within the brain answer slightly different questions. The first seeks to quantify the investment in central nervous system tissue relative to total tissue investment and is best suited for asking questions about fitness trade-offs and limits to brain investment. The second measures the relative allocation to particular brain regions (described by

detectably different architectures and/or ascribed functions) and is better suited to asking questions about intranervous system trade-offs and developmental, behavioral, or evolutionary constraints to reallocation. Brain investment (brain–body size relationship) values are usually not reported for insects (exceptions include O'Donnell et al. 2018; Mares et al. 2005; Seid et al. 2011; Van Der Woude et al. 2013; Muscedere et al. 2014), but may be an important avenue for understanding how changes in allocation occur in social lineages and what the fitness trade-off driving differences might be. In the solitary cabbage white butterfly, there is a trade-off between neural investment and egg production seemingly due to the cognitive costs of making well-informed oviposition decisions (Snell-Rood et al. 2011). While the metabolic cost of neural tissue is often cited as a reason reductions will occur under selection, to our knowledge, the relationship between sociality, brain investment, and fitness has not been tested. We recognize that testable hypotheses for these questions may be methodologically challenging, if not intractable, and that a number of groups are trying to more carefully quantify costs by measuring metabolic rates from brains, individuals, and colonies.

A reason for the popularity of volumetric brain measurements and quantification of relative investment in structures is that they are relatively easy to obtain (although the traditional approach of estimating brain component volumes from serial sections may be tedious), and data are quite reliable if they are based on the appropriate number of brain sections and proper use of statistical methods to estimate volumes. Originally, volumes were crudely extrapolated from just a very few brain sections (Alten 1910; Pietschker 1911; Pandazis 1930), but more recently volumes have been calculated from extensive series of brain sections, and as in vertebrates, unbiased stereological estimates resulted in reliable datasets (Withers et al. 1993). Because of the typically small size of insect brains, in addition to the traditional section-by-section analysis, recent studies use whole-brain confocal microscopy or micro-CT methods in combination with computer-aided reconstruction, which allow much faster measurements (el Jundi et al. 2009b; Smith et al. 2016). Because of this work a number of standard insect brain atlases are now available including the desert locust (*Schistocerca gregaria*, Kurylas et al. 2008), the tobacco hawk moth (*Manduca sexta*, el Jundi et al. 2009b), the European honeybee (*Apis mellifera*, Rybak et al. 2010), the red flour beetle (*Tribolium castaneum*, Dreyer 2010) and a myrmecine ant (*Cardiocondyla obscurior*, Bressan et al. 2015). These provide 3D maps upon which other variables (e.g., circuitry, cellular phenotypes, gene or protein expression) can be arranged and compared across individuals or species (el Jundi et al. 2009a; Rybak et al. 2010; Arganda-Carreras et al. 2018).

As noted above, problems with the use of relative volumetric measurement in interspecies comparisons also include the variety of cellular mechanisms that can contribute to volumetric differences, and conversely, the various non-volumetric changes that result in appreciable changes in behavior. This makes it difficult to attribute directional differences in regional investment to underlying life history traits (brain–body) or behavioral differences (brain regions). In cases where we can reasonably assume that the development and gross morphology of the circuitry are similar across individuals, as in many intraspecific comparisons, brain volume has provided insight into phenotypic plasticity in the nervous system (Brown et al. 2002; Krubitzer 2007; Rehan et al. 2015; Amador-Vargas et al. 2015), including experience-dependent changes in volume (Hourcade et al. 2009; Arenas et al. 2012). This helps illustrate another problem with interspecific volumetric comparisons—relative volume is a trait that may show a great deal of intraspecific variation, making it a difficult candidate for use in evolutionary analyses.

Synapses

If the volume of brains or their components do not reflect cognitive capacities in a reliable way, a better way to establish correlations between specific behavioral or cognitive capacities would be to analyze the neuronal architecture of particular brain components, as suggested for the cortical architecture of primates (Kaas 2012). One approach that is used by an increasing number of researchers, and which has as similar goal, although at a much more restricted level: Comparing the number or density of synaptic complexes in the mushroom bodies (MB). Much experimental evidence, in particular based on fruit flies and honey bees, shows that the MB is involved in learning and memory [reviewed by Davis (2011) and by Menzel (2014)]. The MB also plays an important role in decision making (Zhang et al. 2007), action selection and spatial learning (reviewed in Strausfeld 2012), behavioral features that are particularly relevant for social insects. A part of the MB referred to as the calyx is present in many (but not all) insect taxa (Strausfeld et al. 2009). It represents an important sensory input region and is particularly pronounced in advanced Hymenoptera (Farris and Schulmeister 2011). The calyx of most insects is composed of microglomeruli (Trujillo-Cenóz and Melamed 1962; Schürmann 1974, 2016), large synaptic complexes in which higher-order sensory projection neuron boutons terminate on more than 100 post-synaptic spines (Groh et al. 2012). These microglomeruli are the site of synaptic plasticity and pruning and their numbers correlate with experience in honey bees and ants (Stieb et al. 2010; Falibene et al. 2015) and potentially with their learning

ability (Hourcade et al. 2010; Rössler and Groh 2012), although the interpretation of the number and density of microglomeruli in the context of behavior or experience can be problematic (Fahrbach and Van Nest 2016).

Calycal microglomeruli are present in most insects (Groh et al. 2012) and can be resolved using standard bright-field microscopy, but they are particularly easy to quantify using immunostaining and confocal microscopy. This may be a major reason for the recent increase in the number of published studies focusing on social insect microglomeruli in the context of different behavioral aspects. However, differences in the estimated numbers or densities of microglomeruli are notoriously difficult to interpret in any behavioral context. Neuronal plasticity underlying behavioral changes varies in temporal characteristics and includes the enhancement of synapses or the formation of new ones as well as the pruning of existing synapses, and a lack of understanding of the microcircuits in the MB makes it difficult to predict a summed, directional change. For example, the most accepted models of mushroom body function in learning and memory (e.g., Heisenberg 2003) assume that repeated co-activation of Kenyon cell (KC) synapses by associated stimuli (e.g., an odor and a reward or punishment) strengthen involved synapses through coincident firing (Finelli et al. 2008). As proposed by Hebb (1949), the repeated coincident activation of an olfactory projection neuron and its post-synaptic KC, called spike-timing-dependent plasticity, results in a strengthening of this synapse which, over time, would lead to a morphological consolidation and potential expansion of the synaptic complex (microglomerulus). By the same token, synapses that are not used (e.g., coding for behaviorally irrelevant odors) would over time be reduced and eventually eliminated (pruned), meaning learning could in some cases or at particular intervals reduce the number of synaptic complexes in a region. In the case of microglomeruli both processes could take place simultaneously across the mushroom body and this may result in a zero net change in the number and/or density, similar to the case of analyzing total brain (or MB) volumes at the more macroscopic level. This difficulty explains why different studies may even show opposite results (compare e.g., Li et al. 2017 and; Van Nest et al. 2017). Just as it has not yet been possible to unequivocally establish the changes in microglomerular composition presumably underlying learning and memory, the use of microglomerular features does not seem particularly promising for comparing social attributes across different insect species. However, if we are able to quantify the degree of microglomerular plasticity at the species level, this might be a useful trait in comparative analyses, although it would be time-consuming at the current technological state as it would involve analyzing relatively large numbers of brains for each species. We expect it will be more useful to estimate the number of synapses formed by particular neurons,

something that could be achieved with a combination of tracers and synaptic markers.

Cells

Plasticity at the cellular level is inherent to the function of the nervous system; changes in kinase activity, receptor expression, and membrane production are hallmarks of neural plasticity. This inherent plasticity often causes difficulty separating proximate and ultimate causes in studies of brain evolution—volumetric differences across individuals may be due to developmental stage, environment, experience, or even time of day (proximate causes), or they may be attributed to allelic or epigenetic differences at the population or species level (ultimate causes). We may expect highly related individuals to show low trait variation, but a recent study of isogenic zebrafish indicates epigenetic mechanisms may produce discernable behavioral variation (Román et al. 2018). Probing gene expression differences across cellular phenotypes (e.g., KCs of the MB by Kaneko et al. 2013) will allow us to assess whether responsiveness to the environment—plasticity in the brain's plasticity response, if you will—is greater in particular insect lineages, as has been suggested for the capacity for gene regulation in social lineages (Kapheim et al. 2015). In all species, but particularly those with life history characteristics that rely on high relatedness, selection may act to leverage environmental responsiveness in the nervous system through intragenerational epigenetic control (Jaenisch and Bird 2003; Moczek et al. 2011), as is presumably the case in other tissues where morphological polymorphisms arise in closely related individuals (e.g., caste size polymorphism). Additionally, trait evolution models are beginning to include intraspecific variation, allowing us to more explicitly test if this variation is linked to particular life history traits (Kostikova et al. 2016).

Though cells themselves may show plasticity in size, connectivity, or synapse number, most neural precursor cells divide a discrete number of times and cell lineages can be traced within a species (Liu et al. 2015; Lacin and Truman 2016) and compared across species (Urbach and Technau 2003). Therefore, cell number may be a fairly stable trait within a species and a good one to use in interspecific comparisons. The addition of new cells of existing types might provide greater acuity (as with the addition of peripheral sensory neurons), or, in higher-order networks, increased associative capacity. The addition of cells that form a novel population may result in functional change, either because these cells allow for the extraction of a different kind of information from the neuronal network that supplies them, or modulate network functions by introducing different neurotransmitters or neuromodulators to networks, or provide novel connections among neurons. The latter may be the case for insect mushroom bodies where number of KC

subtypes appears to correlate with phylogenetic position (Oya et al. 2017) and is suggested to correlate with the emergence of nursing and foraging in social insects (Kaneko et al. 2016). Reallocation, on the other hand, may indicate positive selection on particular brain components under conditions of energetic constraint, therefore distinguishing reallocation may be particularly interesting in the context of sociality and brain evolution.

Counting cell numbers

Assessing connectivity as described is admittedly not a fast or easy task and another first and valuable step towards the goal of assessing an insect's potential neuronal processing capabilities would be the ability to count or estimate the number of neurons in an insect's brain. While this is technically feasible, it takes a tremendous amount of work to do so. Traditionally, brains have to be sectioned and neuronal cell bodies have to be counted or estimated section by section. Stereological approximation procedures and confocal image stacks simplify those estimates, but they work best when the particle sizes and distributions are homogeneous. As insect neurons and glia are distributed in many different clusters, some of which comprise a large range of cell sizes, it is still difficult to estimate numbers for entire brains even with the help of modern computational and imaging tools. This is the reason why there are very few trustworthy estimates of insect neuron numbers. In fact, we are only aware of a few species for which neuron numbers have been estimated, typically only of a single brain and generally not up to current standards for reliable cell number estimates: the tiny parasitoid *Megaphragma mymaripenne* (7,400 neurons; Polilov 2012), the house fly *Musca domestica* (340,000 neurons; Strausfeld 1976), the honey bee *Apis mellifera* (worker: 850,000 neurons; drone: 1,200,000 neurons; Witthöft 1967), and the fruit fly *Drosophila melanogaster* ["in the region of 100,000 neurons"; Armstrong and van Hemert (2009) citing Power (1943); however, Power does not seem to give any number. Other authors mention 135,000 neurons (Alivisatos et al. 2012), but we were not able to find the original source for those numbers either]. Incidentally, the same was true for vertebrates until quite recently, and original sources for neuron numbers mentioned for vertebrate brains were hard to verify (Herculano-Houzel 2009).

Estimating vertebrate neuron numbers became much easier with the introduction of the isotropic fractionator by Herculano-Houzel and Lent (2005). This method entails homogenizing a particular amount of brain or brain component in a known volume of buffer, destroying the cells but keeping the nuclei intact. After being stained with a fluorescent DNA marker, many small samples of the nuclei homogenate are then counted using a hemocytometer or a cell counter. Using this technique, Herculano-Houzel and

coworkers have transformed our knowledge not just of neuron numbers across mammals and birds, but also of morphometric trends in vertebrate brain evolution (Herculano-Houzel 2009; Gabi et al. 2010). Among many other interesting findings, Herculano-Houzel's work revealed that primate brains follow different scaling rules than rodent brains and that the human brain is not special—it is almost exactly the size and comprises the number of neurons estimated for a primate of the human body size. For a given body size, primates not only have larger brains than other mammals, but more importantly they have more neurons. It is not so much the size of their brains, but the number of neurons that supports the advanced behavioral capabilities of primates; based on Herculano-Houzel (2011) calculations, it also appears that brain energy consumption depends on the number of neurons rather than on total brain size. This still needs to be tested in insects, but may have important implications for fitness trade-offs under different life history strategies (see "Volumetrics" Section above).

We point out these findings because we think that this technique (the isotropic fractionator) would be a welcome tool to analyze insect brains and to establish more meaningful relationships between brain composition and behavioral and cognitive parameters. If neuron number, rather than brain size, determines insect brain metabolism, it would add a new twist to the idea that social insect brains may potentially be less metabolically expensive than solitary insect ones (O'Donnell et al. 2011; Kamhi et al. 2016). We think that reliable estimates of insect neuron numbers will help figure out if there is anything special about social insect brains. Who knows, it may turn out that they have fewer neurons than related solitary insects of similar brain and body size. Social insects and their solitary relatives in the order Hymenoptera are holometabolous and therefore for the purpose of studying potential allocation trade-offs, it will also be important to study cell numbers in larvae and adults. For instance, since larvae of socially advanced Hymenoptera are cared for by their nestmates and do not need to search for food, avoid predators or perform other demanding behaviors, one might assume that their nervous systems and neuron numbers would be reduced compared to the larvae of solitary insects. An altricial larval stage may provide the opportunity to allocate particular populations of neurons to the adult stage through delayed onset of division or differentiation of neuroblasts; the former appears to be the case in the gestated tsetse fly (*Glossina pallidipes*) (Truman 1990) and latter has been suggested for honeybee KCs (Jef-feris and Luo 2005, pp. 451).

Counting cells will be particularly valuable for studying brain scaling in social insects, but where differences are detected, we will undoubtedly want to know which populations contribute to these differences. Cell-lineage tracing can help understand where new cells or new cell types are added

to regions of the brain and if additions or losses coincide with behavioral or life history traits. Where markers exist, *in situ* hybridization may be used in combination with cell counts to classify cellular-level phenotypes to track gains and losses of particular populations of cells across lineages (Oya et al. 2017). Cell lineage tracing may also help define functional units useful in evolutionary comparisons. Neuroblast maps created using positions and gene expression profiles through development may be particularly useful in tracing cell lineages and gene profile (i.e., phenotypic) shifts across species (Biffar and Stollewerk 2014). In vertebrates, cell-lineage and gene expression studies have contributed to the hypothesis that whole-circuit duplication led to the development of song learning in particular avian lineages (Chakraborty and Jarvis 2015).

Cell regions

While number of cells correlates with advanced behavioral capabilities in vertebrates, in insect brains functional units may be smaller than a cell as a few individual neurons may perform tasks similar to what large groups of neurons do in vertebrates. Rather than having cell bodies embedded within neuropil, insect brains develop as a kind of cell bodies that project dendritic and axonal processes into a center mass to form specific regions. Pre- and post-synaptic regions of a single cell may be in close proximity to one another, giving rise to local circuits and potentially allowing individual insect neurons to contribute to different computational processes simultaneously. Polyadic synapses, where presynaptic neurons contact multiple post-synaptic cells in very close proximity, such as MB microglomeruli, may allow insects to have greater connectivity in smaller volumes of space (Cardona et al. 2010). Furthermore, the same neuron may synapse on cells in different regions of the brain with different functions, or receive different neuromodulatory input across subregions (Aso et al. 2014, Fig. 3). These structural observations suggest subunits of cells might act as functional units in the insect brain; cell regions or collections of synapses may act in a manner similar to cell types in vertebrates. This is something to keep in mind when trying to measure functional units we predict to change with changes in social or behavioral complexity.

Circuits

Given the tangled connectivity described above, it has been difficult to conclusively describe cellular-level circuitry in most insects. As noted above, herculean efforts are under way to create connectomes (Takemura et al. 2017) but much of our current understanding of cell connectivity comes from studies in a single insect, *Drosophila melanogaster*. As noted in context to microglomeruli studies, a survey of all synapses

in a given species' brain may provide the best measure for estimating an insect's cognitive capacities; however, it would seem intractable to trace behaviorally relevant modifications at the synaptic level. This would require analyzing the connections of individual identifiable neurons across castes or species, a challenging task with current technologies. This connectomics approach is currently possible only for very small brains (e.g., the worm *Caenorhabditis elegans*; Varshney et al. 2011) and partially for another model system, the brain of *Drosophila* (Eichler et al. 2017; Thum and Gerber 2019). In a great, concerted effort involving advanced technology and multi-million dollar funding, more than 20 researchers from six high-tech research institutions are reconstructing the connections of all neurons in the fruit fly brain from serial electron microscopical images. Currently, this has been realized only for certain brain components, e.g., the larval MB (Eichler et al. 2017) or the MB alpha lobe (Takemura et al. 2017). While this is an interesting and daring project, a similar approach would be orders of magnitude more difficult in a mouse brain, not to mention the human brain. By the same token, it appears unfeasible within the next 10 years or so to perform a similar study comparing a number of social insect species at the level of individual neuronal connections.

A map of every synapse in the brain may be more precise than we need and less accurate than we would want given the inherent plasticity of neurons in general and synapses specifically. Given the structure of insect brains (Fig. 3), connectivity maps indicating synapses in close proximity would provide sufficient information on connectivity to improve neuroethological and evolutionary studies of social insect brains. Due to the meticulous work of a century of neuroanatomists and physiologists, a great deal of information on cellular anatomy, phenotypes, and at times physiology, already exists for a small number of insects—fruit flies, cockroaches, locusts, crickets, tobacco hawk moths, red flour beetles and honey bees (reviewed in Strausfeld 2012)—but not for any other social insects. A combination of classical neuroanatomical descriptions, 3D brain atlases, neuronal tracers, and immunohistochemistry for pre- and post-synaptic proteins would provide enough insight into connectivity to compare whether sociality modifies particular circuits in number or diversity.

In conclusion, we suggest rethinking the current approach of comparing the brains of social insects. Instead of repeating the history and pitfalls of comparative brain research in primates and other social vertebrates, we propose to first establish an evolutionary framework using the comparative approach to assess neural trait variation across closely related insects with discernably different social characteristics. This approach includes using life history traits to characterize the degree of sociality beyond colony size or broad definitions of social structure.

In terms of quantifying neural traits for evolutionary analyses, the current focus on brain-volumetric approaches may not be able to tell us much more about brain evolution in social insects because the size of brains may be only weakly correlated with their processing powers and the volume of recognized brain components may be larger than functional units useful in analyzing shifts in complexity. Cellular and subcellular properties will have more predictive value, even though they are more difficult to quantify. In particular, we point out the isotropic fractionator method could be used to quantify neuron numbers in insects. Additionally, we suggest using existing histological techniques for tracing circuits and identifying cellular phenotypes to quantify functional units of the brain for evolutionary comparison. Based on evolutionary insights and the rapidly advancing molecular, genomic and neurobiological technologies, in the near future we should be able to make considerable progress understanding the contribution sociality to nervous systems evolution in insects.

Finally, where the comparative method highlights correlated changes in neural structures and the evolution of social behaviors, we can adopt a more neuroethological approach to study whether social interactions play a direct or indirect role in these changes. First employing the comparative approach will make possible meaningful predictions regarding the required neuronal machinery necessary to support social behaviors or potential trade-offs with other behaviors. Because social insects rely heavily on their chemosensory abilities for kin recognition and advanced pheromone communication, olfactory and gustatory pathways may be a promising starting point for this analysis.

Acknowledgements We thank Skye Long for helpful comments on the manuscript. This work was supported by a grant from the University of Arizona Graduate Student and Professional Council (GPSC) to RKG and NSF grant ISO-1354191 to WG.

References

- Aiello LC, Wheeler P (1995) The expensive-tissue hypothesis: the brain and the digestive system in human and primate evolution. *Curr Anthropol* 36:199–221. <https://doi.org/10.1086/204350>
- Alivisatos AP, Chun M, Church GM, Greenspan RJ, Roukes ML, Yuste R (2012) The brain activity map project and the challenge of functional connectomics. *Neuron* 74:970–974. <https://doi.org/10.1016/j.neuron.2012.06.006>
- Alten V (1910) Zur Phylogenie des Hymenopterengehirns. *Jena Z Med Naturwiss* 46:511–590
- Amador-Vargas S, Gronenberg W, Wcislo WT, Mueller U (2015) Specialization and group size: brain and behavioural correlates of colony size in ants lacking morphological castes. *Proc Biol Sci* 282:20142502. <https://doi.org/10.1098/rspb.2014.2502>
- Ames a (1992) Energy requirements of CNS cells as related to their function and to their vulnerability to ischemia: a commentary based on studies on retina. *Can J Physiol Pharmacol* 70 Suppl:S158–164. <https://doi.org/10.1139/y92-257>
- Aoki K, Moody M (1981) One- and two-locus models of the origin of worker behavior in hymenoptera. *J Theor Biol*. [https://doi.org/10.1016/0022-5193\(81\)90362-3](https://doi.org/10.1016/0022-5193(81)90362-3)
- Arenas A, Giurfa M, Sandoz JC, Hourcade B, Devaud JM, Farina WM (2012) Early olfactory experience induces structural changes in the primary olfactory center of an insect brain. *Eur J Neurosci* 35:682–690. <https://doi.org/10.1111/j.1460-9568.2012.07999.x>
- Arganda-Carreras I, Manoliu T, Mazuras N, Schulze F, Iglesias JE, Bühler K, Jenett A, Rouyer F, Andrey P (2018) A statistically representative atlas for mapping neuronal circuits in the *Drosophila* adult brain. *Front Neuroinform*. <https://doi.org/10.3389/fninf.2018.00013>
- Armstrong JD, van Hemert JJ (2009) Towards a virtual fly brain. *Philos Trans A Math Phys Eng Sci* 367:2387–2397. <https://doi.org/10.1098/rsta.2008.0308>
- Aso Y, Sitaraman D, Ichinose T, Kaun KR, Vogt K, Belliard-Guérin G, Plaçais PY, Robie AA, Yamagata N, Schnaitmann C, Rowell WJ, Johnston RM, Ngo TTB, Chen N, Korff W et al (2014) Mushroom body output neurons encode valence and guide memory-based action selection in *Drosophila*. *Elife* 3:e04580. <https://doi.org/10.7554/eLife.04580>
- Avarguès-Weber A, Giurfa M (2013) Conceptual learning by miniature brains. *Proc R Soc B Biol Sci* 280:19–21. <https://doi.org/10.1098/rspb.2013.1907>
- Bank S, Sann M, Mayer C, Meusemann K, Donath A, Podsiadlowski L, Kozlov A, Petersen M, Krogmann L, Meier R, Rosa P, Schmitt T, Wurdack M, Liu S, Zhou X et al (2017) Transcriptome and target DNA enrichment sequence data provide new insights into the phylogeny of vespid wasps (Hymenoptera: Aculeata: Vespidae). *Mol Phylogenet Evol* 116:213–226. <https://doi.org/10.1016/j.ympev.2017.08.020>
- Barrett L, Henzi P, Rendall D (2007) Social brains, simple minds: does social complexity really require cognitive complexity? *Philos Trans R Soc B* 362:561–575. <https://doi.org/10.1098/rstb.2006.1995>
- Bergman TJ, Beehner JC (2015) Measuring social complexity. *Anim Behav* 103:203–209. <https://doi.org/10.1016/j.anbehav.2015.02.018>
- Bernays EA (2001) Neural limitations in phytophagous insects: implications for diet breadth and evolution of host affiliation. *Annu Rev Entomol* 46:703–727. <https://doi.org/10.1146/annurev.ento.46.1.703>
- Biffar L, Stollewerk A (2014) Conservation and evolutionary modifications of neuroblast expression patterns in insects. *Dev Biol* 388:103–116. <https://doi.org/10.1016/j.ydbio.2014.01.028>
- Boomsma JJ, Gawne R (2018) Superorganismality and caste differentiation as points of no return: how the major evolutionary transitions were lost in translation. *Biol Rev* 93:28–54. <https://doi.org/10.1111/brev.12330>
- Boomsma JJ, Huszár DB, Pedersen JS (2014) The evolution of multiqueen breeding in eusocial lineages with permanent physically differentiated castes. *Anim Behav* 92:241–252. <https://doi.org/10.1016/j.anbehav.2014.03.005>
- Bourke FG (1999) Colony size, social complexity and reproductive conflict in social insects. *J Evol Biol* 12:245–257. <https://doi.org/10.1046/j.1420-9101.1999.00028.x>
- Brahma A, Mandal S, Gadagkar R (2018) Emergence of cooperation and division of labor in the primitively eusocial wasp *Ropalidia marginata*. *PNAS* 115:756–761. <https://doi.org/10.1073/pnas.1714006115>
- Brand N, Chapuisat M (2012) Born to be bee, fed to be worker? The caste system of a primitively eusocial insect. *Front Zool* 9:1–9. <https://doi.org/10.1186/1742-9994-9-35>
- Bressan JMA, Benz M, Oettler J, Heinze J, Hartenstein V, Sprecher SG (2015) A map of brain neuropils and fiber systems in the ant

- Cardiocondyla obscurior*. Front Neuroanat 8:1–13. <https://doi.org/10.3389/fnana.2014.00166>
- Brown SM, Napper RM, Thompson CM, Mercer AR (2002) Stereological analysis reveals striking differences in the structural plasticity of two readily identifiable glomeruli in the antennal lobes of the adult worker Honeybee. J Neurosci 22:8514–8522. <https://doi.org/10.1523/jneurosci.22-19-08514.2002>
- Caetano DS, Harmon LJ (2017) ratematrix: an R package for studying evolutionary integration among several traits on phylogenetic trees. Methods Ecol Evol 8:1920–1927. <https://doi.org/10.1111/2041-210X.12826>
- Cardinal S, Danforth BN (2011) The antiquity and evolutionary history of social behavior in bees. PLoS One 6:e21086. <https://doi.org/10.1371/journal.pone.0021086>
- Cardona A, Saalfeld S, Preibisch S, Schmid B, Cheng A, Pulokas J, Tomancak P, Hartenstein V (2010) An integrated micro- and macroarchitectural analysis of the *Drosophila* brain by computer-assisted serial section electron microscopy. PLoS Biol 8:e1000502. <https://doi.org/10.1371/journal.pbio.1000502>
- Chakraborty M, Jarvis ED (2015) Brain evolution by brain pathway duplication. Philos Trans R Soc B Biol Sci 370:20150056–20150056. <https://doi.org/10.1098/rstb.2015.0056>
- Changizi MA (2003) Relationship between number of muscles, behavioral repertoire size, and encephalization in mammals. J Theor Biol doi. <https://doi.org/10.1006/jtbi.2003.3125>
- Cole BJ (1980) Repertoire convergence in two Mangrove ants, *Zacryptocerus varians* and *Camponotus (Colobopsis)* sp. Insectes Soc 27:265–275. <https://doi.org/10.1007/BF02223668>
- Cole BJ (1985) Size and behavior in ants: constraints on complexity. Proc Natl Acad Sci 82:8548–8551. <https://doi.org/10.1073/pnas.82.24.8548>
- Cornwell W, Nakagawa S (2017) Phylogenetic comparative methods. Curr Biol 27:R333–R336. <https://doi.org/10.1016/j.cub.2017.03.049>
- Danforth BN (2013) Social insects: are ants just wingless bees? Curr Biol 23:R1011–R1012. <https://doi.org/10.1016/j.cub.2013.10.026>
- Danforth BN, Cardinal S, Praz C, Almeida EAB, Michez D (2013) The impact of molecular data on our understanding of bee phylogeny and evolution. Annu Rev Entomol 58:57–78. <https://doi.org/10.1146/annurev-ento-120811-153633>
- Davis RL (2011) Traces of *Drosophila* memory. Neuron 70:8–19. <https://doi.org/10.1016/j.neuron.2011.03.012>
- Deaner RO, Isler K, Burkart J, van Schaik C (2007) Overall brain size, and not encephalization quotient, best predicts cognitive ability across non-human primates. Brain Behav Evol 70:115–124. <https://doi.org/10.1159/000102973>
- DeCasien AR, Williams SA, Higham JP (2017) Primate brain size is predicted by diet but not sociality. Nat Ecol Evol 1:0112. <https://doi.org/10.1038/s41559-017-0112>
- Dobson SD, Sherwood CC (2011) Correlated evolution of brain regions involved in producing and processing facial expressions in anthropoid primates. Biol Lett 7:86–88. <https://doi.org/10.1098/rsbl.2010.0427>
- Dreyer (2010) 3D standard brain of the red flour beetle *Tribolium castaneum*: a tool to study metamorphic development and adult plasticity. Front Syst Neurosci 4:1–13. <https://doi.org/10.3389/neuro.06.003.2010>
- Dujardin F (1850) Mémoire sur le système nerveux des insectes. Ann Sci Nat é 14:195–205
- Dunbar RIM (1992) Neocortex size as a constraint on group size in primates. J Hum Evol 22:469–493. [https://doi.org/10.1016/0047-2484\(92\)90081-J](https://doi.org/10.1016/0047-2484(92)90081-J)
- Dunbar RIM (2009) The social brain hypothesis and its implications for social evolution. Ann Hum Biol 36:562–572. <https://doi.org/10.1080/03014460902960289>
- Dunbar RI, Shultz S (2007) Understanding primate brain evolution. Philos Trans R Soc B Biol Sci 362:649–658. <https://doi.org/10.1098/rstb.2006.2001>
- Dunbar RIM, Shultz S (2017) Why are there so many explanations for primate brain evolution? Philos Trans R Soc B Biol Sci 372:20160244. <https://doi.org/10.1098/rstb.2016.0244>
- Eichler K, Li F, Litwin-Kumar A, Park Y, Andrade I, Schneider-Mizell CM, Saumweber T, Huser A, Eschbach C, Gerber B, Fetter RD, Truman JW, Priebe CE, Abbott LF, Thum AS et al (2017) The complete connectome of a learning and memory centre in an insect brain. Nature 548:175–182. <https://doi.org/10.1038/nature23455>
- el Jundi B, Heinze S, Lenschow C, Kurylas AE, Rohlfing T, Homburg U (2009a) The locust standard brain: a 3D standard of the central complex as a platform for neural network analysis. Front Syst Neurosci 3:1–15. <https://doi.org/10.3389/neuro.06.021.2009>
- el Jundi B, Huetteroth W, Kurylas AE, Schachtner J (2009b) Anisometric brain dimorphism revisited: implementation of a volumetric 3D standard brain in *Manduca sexta*. J Comp Neurol 517:210–225. <https://doi.org/10.1002/cne.22150>
- Fahrbach SE, Van Nest BN (2016) Synapsin-based approaches to brain plasticity in adult social insects. Curr Opin Insect Sci 18:27–34. <https://doi.org/10.1016/j.cois.2016.08.009>
- Falibene A, Roces F, Rössler W (2015) Long-term avoidance memory formation is associated with a transient increase in mushroom body synaptic complexes in leaf-cutting ants. Front Behav Neurosci 9:1–13. <https://doi.org/10.3389/fnbeh.2015.00084>
- Farris SM (2013) Evolution of complex higher brain centers and behaviors: behavioral correlates of mushroom body elaboration in insects. Brain Behav Evol 82:9–18. <https://doi.org/10.1159/000352057>
- Farris SM (2016) Insect societies and the social brain. Curr Opin Insect Sci 15:1–8. <https://doi.org/10.1016/j.cois.2016.01.010>
- Farris SM, Schulmeister S (2011) Parasitoidism, not sociality, is associated with the evolution of elaborate mushroom bodies in the brains of hymenopteran insects. Proc R Soc B Biol Sci 278:940–951. <https://doi.org/10.1098/rspb.2010.2161>
- Farris SM, Strausfeld NJ (2003) A unique mushroom body substructure common to basal cockroaches and to termites. J Comp Neurol 456:305–320. <https://doi.org/10.1002/cne.10517>
- Ferguson-Gow H, Sumner S, Bourke AFG, Jones KE (2014) Colony size predicts division of labour in attine ants. Proc R Soc B Biol Sci. <https://doi.org/10.1098/rspb.2014.1411>
- Finelli LA, Haney S, Bazhenov M, Stopfer M, Sejnowski TJ (2008) Synaptic learning rules and sparse coding in a model sensory system. PLoS Comput Biol. <https://doi.org/10.1371/journal.pcbi.1000062>
- Fjerdingstad EJ, Crozier RH (2006) The evolution of worker caste diversity in social insects. Am Nat 167:390–400. <https://doi.org/10.1086/499545>
- Francescato E, Massolo A, Landi M, Gerace L, Hashim R, Turillazzi S (2002) Colony membership, division of labor, and genetic relatedness among females of colonies of *Eustenogaster fraterna* (Hymenoptera, Vespidae, Stenogastrinae). J Insect Behav 15:153–170. <https://doi.org/10.1023/A:1015489532040>
- Gabi M, Collins CE, Wong P, Torres LB, Kaas JH, Herculano-Houzel S (2010) Cellular scaling rules for the brains of an extended number of primate species. Brain Behav Evol 76:32–44. <https://doi.org/10.1159/000319872>
- Gibbs J, Brady SG, Kanda K, Danforth BN (2012) Phylogeny of halictine bees supports a shared origin of eusociality for *Halictus* and *Lasioglossum* (Apoidea: Anthophila: Halictidae). Mol Phylogenet Evol 65:926–939. <https://doi.org/10.1016/j.ympev.2012.08.013>

- Gittleman JL (1986) Carnivore brain size, behavioral ecology, and phylogeny. *J Mammal* 67:23–36. <https://doi.org/10.1126/science.95.2469.427-b>
- Goldman-Huertas B, Mitchell RF, Lapoint RT, Faucher CP, Hildebrand JG, Whiteman NK (2015) Evolution of herbivory in Drosophilidae linked to loss of behaviors, antennal responses, odorant receptors, and ancestral diet. *Proc Natl Acad Sci* 112:3026–3031. <https://doi.org/10.1073/pnas.1424656112>
- Gottardo M, Dallai R, Mercati D, Hörschemeyer T, Beutel RG (2016) The evolution of insect sperm—an unusual character system in a megadiverse group. *J Zool Syst Evol Res* 54:237–256. <https://doi.org/10.1111/jzs.12136>
- Groh C, Lu Z, Meinertzhagen IA, Rössler W (2012) Age-related plasticity in the synaptic ultrastructure of neurons in the mushroom body calyx of the adult honeybee *Apis mellifera*. *J Comp Neurol* 520:3509–3527. <https://doi.org/10.1002/cne.23102>
- Gronenberg W, Riveros AJ (2009) Social brains and behavior: past and present. In: *Organization of insect societies*. pp 377–401
- Gronenberg W, Ash LE, Tibbetts EA (2008) Correlation between facial pattern recognition and brain composition in paper wasps. *Brain Behav Evol* 71:1–14. <https://doi.org/10.1159/000108607>
- Gruter C, Menezes C, Imperatriz-Fonseca VL, Ratnieks FLW (2012) A morphologically specialized soldier caste improves colony defense in a neotropical eusocial bee. *Proc Natl Acad Sci* 109:1182–1186. <https://doi.org/10.1073/pnas.1113398109>
- Hansson BS, Stensmyr MC (2011) Evolution of insect olfaction. *Neuron* 72:698–711. <https://doi.org/10.1016/j.neuron.2011.11.003>
- Harvey PH, Clutton-Brock TH, Mace GM (1980) Brain size and ecology in small mammals and primates. *Proc Natl Acad Sci U S A* 77:4387–4389. <https://doi.org/10.1073/pnas.77.7.4387>
- Hasenstaub A, Otte S, Callaway E, Sejnowski TJ (2010) Metabolic cost as a unifying principle governing neuronal biophysics. *Proc Natl Acad Sci U S A* 107:12329–12334. <https://doi.org/10.1073/pnas.0914886107>
- Hebb DO (1949) *The organization of behavior; a neuropsychological theory*. Wiley, Oxford, England
- Heisenberg M (2003) Mushroom body memoir: from maps to models. *Nat Rev Neurosci* 4:266–275. <https://doi.org/10.1038/nrn1074>
- Herculano-Houzel S (2009) The human brain in numbers: a linearly scaled-up primate brain. *Front Hum Neurosci* 3:1–11. <https://doi.org/10.3389/neuro.09.031.2009>
- Herculano-Houzel S (2011) Scaling of brain metabolism with a fixed energy budget per neuron: Implications for neuronal activity, plasticity and evolution. *PLoS One* 6:e17514. <https://doi.org/10.1371/journal.pone.0017514>
- Herculano-Houzel S, Lent R (2005) Isotropic fractionator: a simple, rapid method for the quantification of total cell and neuron numbers in the brain. *J Neurosci* 25(10):2518–2521
- Hofman MA (1983a) Evolution of brain size in neonatal and adult placental mammals: a theoretical approach. *J Theor Biol* 105:317–332. [https://doi.org/10.1016/S0022-5193\(83\)80011-3](https://doi.org/10.1016/S0022-5193(83)80011-3)
- Hofman MA (1983b) Energy metabolism, brain size and longevity in mammals. *Q Rev Biol* 58:495–512
- Hourcade B, Perisse E, Devaud J-M, Sandoz J-C (2009) Long-term memory shapes the primary olfactory center of an insect brain. *Learn Mem* 16:607–615
- Hourcade B, Muenz TS, Sandoz J-C, Rössler W, Devaud J-M (2010) Long-term memory leads to synaptic reorganization in the mushroom bodies: a memory trace in the insect brain? *J Neurosci* 30:6461–6465. <https://doi.org/10.1523/JNEUROSCI.0841-10.2010>
- Howse PE (1975) Brain structure and behavior in insects. *Annu Rev Entomol* 20:359–379
- Hughes WOH, Oldroyd BP, Beekman M, Ratnieks FLW (2008) Ancestral monogamy shows kin selection is key to the evolution of eusociality. *Science* 320:1213–1216. <https://doi.org/10.1126/science.1200609>
- Humphrey N (1976) The social function of intellect. In: Bateson PPG, Hinde RA (eds) *Growing points in ethology*. Cambridge University Press, Cambridge, pp 303–317
- Hurd CR, Jeanne RL, Nordheim EV (2007) Temporal polyethism and worker specialization in the wasp, *Vespula germanica*. *J Insect Sci* 7:1–13. <https://doi.org/10.1673/031.007.4301>
- Isler K, Van Schaik CP (2006) Metabolic costs of brain size evolution. *Biol Lett* 2:557–560. <https://doi.org/10.1098/rsbl.2006.0538>
- Jaenisch R, Bird A (2003) Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. *Nat Genet* 33:245. <https://doi.org/10.1038/ng1089>
- Jaffe K, Perez E (1989) Comparative study of brain morphology in ants. *Brain Behav Evol* 33:25–33
- Jefferis GSX, Luo L (2005) Development of the olfactory system. In: Gilbert LI (ed) *Comprehensive molecular insect science volume 1*. Elsevier B.V., Amsterdam, pp 421–463
- Jerison HJ (1973) *Evolution of the brain and intelligence*. Academic Press, Inc., New York
- Jerison HJ (1977) The theory of encephalization. *Ann N Y Acad Sci* 299:146–160. <https://doi.org/10.1111/j.1749-6632.1977.tb41903.x>
- Johnson BR (2010) Division of labor in honeybees: form, function, and proximate mechanisms. *Behav Ecol Sociobiol* 64:305–316. <https://doi.org/10.1007/s00265-009-0874-7>
- Kaas JH (2012) The evolution of neocortex in primates. *Prog Brain Res* 195:91–102. <https://doi.org/10.1016/B978-0-444-53860-4.00005-2>
- Kamhi JF, Gronenberg W, Robson SKA, Traniello JFA (2016) Social complexity influences brain investment and neural operation costs in ants. *Proc R Soc B Biol Sci* 283:20161949. <https://doi.org/10.1098/rspb.2016.1949>
- Kaneko K, Ikeda T, Nagai M, Hori S, Umatani C, Tadano H, Uga-jin A, Nakaoka T, Paul RK, Fujiyuki T, Shirai K, Kunieda T, Takeuchi H, Kubo T (2013) Novel middle-type Kenyon cells in the honeybee brain revealed by area-preferential gene expression analysis. *PLoS One* 8:e71732. <https://doi.org/10.1371/journal.pone.0071732>
- Kaneko K, Suenami S, Kubo T (2016) Gene expression profiles and neural activities of Kenyon cell subtypes in the honeybee brain: identification of novel “middle-type” Kenyon cells. *Zool Lett* 2:14. <https://doi.org/10.1186/s40851-016-0051-6>
- Kapheim KM, Pan H, Li C, Salzberg SL, Puiu D, Magoc T, Robertson HM, Hudson ME, Venkat A, Fischman BJ, Hernandez A, Yandell M, Ence D, Holt C, Yocum GD et al (2015) Genomic signatures of evolutionary transitions from solitary to group living. *Science* 348:1139–1143. <https://doi.org/10.1126/science.aaa4788>
- Kaspari M, Vargo EL (1995) Colony size as a buffer against seasonality: Bergmann’s rule in social insects. *Am Nat* 145:610–632. <https://doi.org/10.1086/285758>
- Kelber C, Rössler W, Kleineidam CJ (2010) Phenotypic plasticity in number of glomeruli and sensory innervation of the antennal lobe in leaf-cutting ant workers (*A. vollenweideri*). *Dev Neurobiol* 70:222–234. <https://doi.org/10.1002/dneu.20782>
- King AJ, Cowlishaw G (2007) When to use social information: the advantage of large group size in individual decision making. *Biol Lett* 3:137–139. <https://doi.org/10.1098/rsbl.2007.0017>
- Kocher SD, Paxton RJ (2014) Comparative methods offer powerful insights into social evolution in bees. *Apidologie* 45:289–305. <https://doi.org/10.1007/s13592-014-0268-3>
- Kostikova A, Silvestro D, Pearman PB, Salamin N (2016) Bridging inter- and intraspecific trait evolution with a hierarchical Bayesian approach. *Syst Biol* 65:417–431. <https://doi.org/10.1093/sysbio/syw010>

- Krebs JR, Sherry DF, Healy SD, Perry VH, Vaccarino DL (1989) Hippocampal specialization of food-storing birds. *Proc Natl Acad Sci USA* 86:1388–1392
- Krubitzer L (2007) The magnificent compromise: cortical field evolution in mammals. *Neuron* 56:201–208. <https://doi.org/10.1016/j.neuron.2007.10.002>
- Kudo H, Dunbar RIM (2001) Neocortex size and social network size in primates. *Anim Behav* 62:711–722. <https://doi.org/10.1006/anbe.2001.1808>
- Kurylas AE, Rohlfing T, Kroficzek S, Jenett A, Homberg U (2008) Standardized atlas of the brain of the desert locust, *Schistocerca gregaria*. *Cell Tissue Res* 333:125–145. <https://doi.org/10.1007/s00441-008-0620-x>
- Lacin H, Truman JW (2016) Lineage mapping identifies molecular and architectural similarities between the larval and adult *Drosophila* central nervous system. *Elife* 5:e13399
- Li X, Erclik T, Bertet C, Chen Z, Voutev R, Venkatesh S, Morante J, Celik A, Desplan C (2013) Temporal patterning of *Drosophila* medulla neuroblasts controls neural fates. *Nature* 498:456–462. <https://doi.org/10.1038/nature12319>
- Li L, MaBouDi H, Egertová M, Elphick MR, Chittka L, Perry CJ (2017) A possible structural correlate of learning performance on a colour discrimination task in the brain of the bumblebee. *Proc Biol Sci* 284:20171323. <https://doi.org/10.1098/rspb.2017.1323>
- Lihoreau M, Latty T, Chittka L (2012) An exploration of the social brain hypothesis in insects. *Front Physiol* 3:442. <https://doi.org/10.3389/fphys.2012.00439>
- Liu Z, Yang CP, Sugino K, Fu CC, Liu LY, Yao X, Lee LP, Lee T (2015) Opposing intrinsic temporal gradients guide neural stem cell production of varied neuronal fates. *Science* 350:317–320. <https://doi.org/10.1126/science.aad1886>
- Lorenzi MC, Caprio P (2000) Nest recognition in neighboring colonies: a comparison of two European species of *Polistes* wasps (*P. dominulus* and *P. nimphus*, Hymenoptera: Vespidae). *J Ethol* 18:65–68. <https://doi.org/10.1007/s101640070001>
- Mares S, Ash L, Gronenberg W (2005) Brain allometry in bumblebee and honey bee workers. *Brain Behav Evol* 66:50–61. <https://doi.org/10.1159/000085047>
- Martins EP, Hansen TF (1997) Phylogenies and the comparative method: a general approach to incorporating phylogenetic information into the analysis of interspecific data. *Am Nat* 149:646–667. <https://doi.org/10.1086/286013>
- Menzel R (2014) The insect mushroom body, an experience-dependent recoding device. *J Physiol Paris* 108:84–95. <https://doi.org/10.1016/j.jphysparis.2014.07.004>
- Mersch DP, Crespi A, Keller L (2013) Tracking individuals shows spatial fidelity is a key regulator of ant social organization. *Science* 340:1090–1093. <https://doi.org/10.1126/science.1234316>
- Missbach C, Dweck HKM, Vogel H, Vilcinskas A, Stensmyr MC, Hansson BS, Grosse-Wilde E (2014) Evolution of insect olfactory receptors. *Elife* 3:e02115. <https://doi.org/10.7554/eLife.02115>
- Moczek AP, Sultan S, Foster S, Ledón-Rettig C, Dworkin I, Nijhout HF, Abouheif E, Pfennig DW (2011) The role of developmental plasticity in evolutionary innovation. *Proc R Soc B Biol Sci* 278:2705–2713
- Molina Y, O'Donnell S (2008) Age, sex, and dominance-related mushroom body plasticity in the paperwasp *Mischocyttarus mastigophorus*. *Dev Neurobiol* 68:950–959. <https://doi.org/10.1002/dneu.20633>
- Montgomery SH, Ott SR (2015) Brain composition in *Godyris zavaleta*, a diurnal butterfly, reflects an increased reliance on olfactory information. *J Comp Neurol* 523:869–891. <https://doi.org/10.1002/cne.23711>
- Montgomery SH, Merrill RM, Ott SR (2016) Brain composition in *Heliconius* butterflies, posteclosion growth and experience-dependent neuropil plasticity. *J Comp Neurol* 524:1747–1769. <https://doi.org/10.1002/cne.23993>
- Muscudere ML, Gronenberg W, Moreau CS, Traniello JF a (2014) Investment in higher order central processing regions is not constrained by brain size in social insects. *Proc Biol Sci* 281:20140217. <https://doi.org/10.1098/rspb.2014.0217>
- Namiki S, Kanzaki R (2016) Comparative neuroanatomy of the lateral accessory lobe in the insect brain. *Front Physiol* 7:1–14. <https://doi.org/10.3389/fphys.2016.00244>
- Ng J, Smith SD (2014) How traits shape trees: new approaches for detecting character state-dependent lineage diversification. *J Evol Biol* 27:2035–2045. <https://doi.org/10.1111/jeb.12460>
- O'Donnell S, Donlan N, Jones T (2007) Developmental and dominance-associated differences in mushroom body structure in the paper wasp *Mischocyttarus mastigophorus*. *Dev Neurobiol* 67:39–46. <https://doi.org/10.1002/dneu.20324>
- O'Donnell S, Clifford M, Molina Y (2011) Comparative analysis of constraints and caste differences in brain investment among social paper wasps. *Proc Natl Acad Sci* 108:7107–7112. <https://doi.org/10.1073/pnas.1017566108>
- O'Donnell S, Bulova SJ, Deleon S, Khodak P, Miller S, Sulger E, Donnell SO, O'Donnell S, Bulova SJ, Deleon S, Khodak P, Miller S, Sulger E (2015) Distributed cognition and social brains: reductions in mushroom body investment accompanied the origins of sociality in wasps (Hymenoptera: Vespidae). *Proc R Soc B Biol Sci* 282:20150791-. <https://doi.org/10.1098/rspb.2015.0791>
- O'Donnell S, Bulova S, Barrett M, von Beeren C (2018) Brain investment under colony-level selection: soldier specialization in *Eciton* army ants (Formicidae: Dorylinae). *BMC Zool* 3:3. <https://doi.org/10.1186/s40850-018-0028-3>
- Oettler J, Johnson R (2009) The old ladies of the seed harvester ant *Pogonomyrmex rugosus*: foraging performed by two groups of workers. *J Insect Behav* 22:217–226. <https://doi.org/10.1007/s10905-008-9167-7>
- Outomuro D, Adams DC, Johansson F (2013) The evolution of wing shape in ornamented-winged damselflies (Calopterygidae, Odonata). *Evol Biol* 40:300–309. <https://doi.org/10.1007/s11692-012-9214-3>
- Oya S, Kohno H, Kainoh Y, Ono M, Kubo T (2017) Increased complexity of mushroom body Kenyon cell subtypes in the brain is associated with behavioral evolution in hymenopteran insects. *Sci Rep* 7:1–11. <https://doi.org/10.1038/s41598-017-14174-6>
- Pacala S, Gordon D, Godfray H (1996) Effects of social group size on information transfer and task allocation. *Evol Ecol* 10:127–165. <https://doi.org/10.1007/BF01241782>
- Pagel M (1999) Inferring the historical patterns of biological evolution. *Nature* 401:877–884. <https://doi.org/10.1038/44766>
- Pandazis G (1930) Über die relative Ausbildung der Gehirnzentren bei biologisch verschiedenen Ameisenarten. *Zeitschrift für Morphol und Ökol der Tiere* 18:114–169. <https://doi.org/10.1007/BF00419207>
- Parker ST (2015) Re-evaluating the extractive foraging hypothesis. *New Ideas Psychol* 37:1–12. <https://doi.org/10.1016/j.newideapsych.2014.11.001>
- Parker ST, Gibson KR (1977) Object manipulation, tool use and sensorimotor intelligence as feeding adaptations in cebus monkeys and great apes. *J Hum Evol* 6:623–641. [https://doi.org/10.1016/S0047-2484\(77\)80135-8](https://doi.org/10.1016/S0047-2484(77)80135-8)
- Peters RS, Krogmann L, Mayer C, Donath A, Gunkel S, Meusemann K, Kozlov A, Podsiadlowski L, Petersen M, Lanfear R, Diez PA, Heraty J, Kjer KM, Klopstein S, Meier R et al (2017) Evolutionary history of the hymenoptera. *Curr Biol* 27:1013–1018. <https://doi.org/10.1016/j.cub.2017.01.027>
- Pietschker H (1911) Das Gehirn der Ameise. *Jenaische Zeitschrift für Naturwiss* 47:43–114

- Polilov AA (2012) The smallest insects evolve anucleate neurons. *Arthropod Struct Dev* 41:29–34. <https://doi.org/10.1016/j.asd.2011.09.001>
- Power ME (1943) The brain of *Drosophila melanogaster*. *J Morphol* 72:517–559. <https://doi.org/10.1002/jmor.1050720306>
- Reader SM, Laland KN (2002) Social intelligence, innovation, and enhanced brain size in primates. *Proc Natl Acad Sci* 99:4436–4441. <https://doi.org/10.1073/pnas.062041299>
- Reader SM, Hager Y, Laland KN (2011) The evolution of primate general and cultural intelligence. *Philos Trans R Soc B Biol Sci* 366:1017–1027. <https://doi.org/10.1098/rstb.2010.0342>
- Rehan SM, Toth AL (2015) Climbing the social ladder: the molecular evolution of sociality. *Trends Ecol Evol* 30:426–433. <https://doi.org/10.1016/j.tree.2015.05.004>
- Rehan SM, Bulova SJ, O'Donnell S (2015) Cumulative effects of foraging behavior and social dominance on brain development in a facultatively social bee (*Ceratina australensis*). *Brain Behav Evol* 85:117–124. <https://doi.org/10.1159/000381414>
- Retana J, Cerdá X (1991) Behavioral repertoire of the ant *Cataglyphis cursor* (Hymenoptera: Formicidae): is it possible to elaborate a standard specific one? *J Insect Behav* 4:139–155. <https://doi.org/10.1007/BF01054608>
- Riveros AJ, Seid MA, Wcislo WT (2012) Evolution of brain size in class-based societies of fungus-growing ants (Attini). *Anim Behav* 83:1043–1049. <https://doi.org/10.1016/j.anbehav.2012.01.032>
- Román A-C, Vicente-Page J, Pérez-Escudero A, Carvajal-González JM, Fernández-Salguero PM, de Polavieja GG (2018) Histone H4 acetylation regulates behavioral inter-individual variability in zebrafish. *Genome Biol* 19:55. <https://doi.org/10.1186/s13059-018-1428-y>
- Romiguer J, Lourenco J, Gayral P, Faivre N, Weinert LA, Ravel S, Ballenghien M, Cahais V, Bernard A, Loire E, Keller L, Galtier N (2014) Population genomics of eusocial insects: the costs of a vertebrate-like effective population size. *J Evol Biol* 27:593–603. <https://doi.org/10.1111/jeb.12331>
- Rosati AG (2017) Foraging cognition: reviving the ecological intelligence hypothesis. *Trends Cogn Sci* 21:691–702. <https://doi.org/10.1016/j.tics.2017.05.011>
- Rössler W, Groh C (2012) Plasticity of synaptic microcircuits in the mushroom-body calyx of the honey bee. In: Galizia CG, Eisenhardt D, Giurfa M (eds) *Honeybee neurobiology and behavior*. Springer, Dordrecht, pp 141–153
- Roth G, Dicke U (2012) *Evolution of the brain and intelligence in primates*, 1st edn. Elsevier B.V., Amsterdam
- Rybak J, Kuß A, Lamecker H, Zachow S, Hege H-C, Lienhard M, Singer J, Neubert K, Menzel R (2010) The digital bee brain: integrating and managing neurons in a common 3D reference system. *Front Syst Neurosci* 4:1–15. <https://doi.org/10.3389/fnsys.2010.00030>
- Schluter D, Price T, Mooers A, Ludwig D (1997) Likelihood of ancestor states in adaptive radiation. *Evolution* 51:1699–1711. <https://doi.org/10.1111/j.1558-5646.1997.tb05095.x>
- Schoenemann PT (2013) Hominid brain evolution. In: *A companion to paleoanthropology*. Blackwell Publishing Ltd, Oxford, pp 136–164
- Schürmann FW (1974) Bemerkungen zur Funktion der Corpora Pedunculata im Gehirn der Insekten aus morphologischer Sicht. *Exp Brain Res* 19:406–432. <https://doi.org/10.1007/BF00234464>
- Schürmann FW (2016) Fine structure of synaptic sites and circuits in mushroom bodies of insect brains. *Arthropod Struct Dev* 45:399–421. <https://doi.org/10.1016/j.asd.2016.08.005>
- Seid MA, Traniello JAJA (2006) Age-related repertoire expansion and division of labor in *Pheidole dentata* (Hymenoptera: Formicidae): a new perspective on temporal polyethism and behavioral plasticity in ants. *Behav Ecol Sociobiol* 60:631–644. <https://doi.org/10.1007/s00265-006-0207-z>
- Seid MA, Castillo A, Wcislo WT (2011) The allometry of brain miniaturization in ants. *Brain Behav Evol*. <https://doi.org/10.1159/000322530>
- Sempo G, Detrain C (2004) Between-species differences of behavioural repertoire of castes in the ant genus *Pheidole*: a methodological artefact? *Insectes Soc* 51:48–54. <https://doi.org/10.1007/s00040-003-0704-2>
- Sharkey CR, Fujimoto MS, Lord NP, Shin S, McKenna DD, Suvorov A, Martin GJ, Bybee SM (2017) Overcoming the loss of blue sensitivity through opsin duplication in the largest animal group, beetles. *Sci Rep* 7:1–10. <https://doi.org/10.1038/s41598-017-00061-7>
- Smith AR, Wcislo WT, O'Donnell S (2007) Survival and productivity benefits to social nesting in the sweat bee *Megalopta genalis* (Hymenoptera: Halictidae). *Behav Ecol Sociobiol* 61:1111–1120. <https://doi.org/10.1007/s00265-006-0344-4>
- Smith AR, Seid MA, Jiménez LC, Wcislo WT (2010) Socially induced brain development in a facultatively eusocial sweat bee *Megalopta genalis* (Halictidae). *Proc Biol Sci* 277:2157–2163. <https://doi.org/10.1098/rspb.2010.0269>
- Smith DB, Bernhardt G, Raine NE, Abel RL, Sykes D, Ahmed F, Pedrosa I, Gill RJ (2016) Exploring miniature insect brains using micro-CT scanning techniques. *Sci Rep* 6:21768. <https://doi.org/10.1038/srep21768>
- Snell-Rood EC, Davidowitz G, Papaj DR (2011) Reproductive tradeoffs of learning in a butterfly. *Behav Ecol* 22:291–302. <https://doi.org/10.1093/beheco/arq169>
- Stieb SM, Muenz TS, Wehner R, Rössler W (2010) Visual experience and age affect synaptic organization in the mushroom bodies of the desert ant *Cataglyphis fortis*. *Dev Neurobiol* 70:408–423. <https://doi.org/10.1002/dneu.20785>
- Strausfeld NJ (1976) *Atlas of an insect brain*. Springer, Berlin
- Strausfeld NJ (2012) *Arthropod brains: evolution, functional elegance, and historical significance*. Belknap Press of Harvard University Press, Cambridge
- Strausfeld NJ, Sinkevitch I, Brown SM, Farris SM (2009) Ground plan of the insect mushroom body: functional and evolutionary implications. *J Comp Neurol* 513:265–291. <https://doi.org/10.1002/cne.21948>
- Takemura S, Aso Y, Hige T, Wong A, Lu Z, Xu CS, Rivlin PK, Hess H, Zhao T, Parag T, Berg S, Huang G, Katz W, Olbris DJ, Plaza S et al (2017) A connectome of a learning and memory center in the adult *Drosophila* brain. *Elife*. <https://doi.org/10.7554/eLife.26975>
- Tallamy DW, Schaefer C (1997) Maternal care in the Hemiptera: ancestry, alternatives, and current adaptive value. In: Choe JC, Crespi BJ (eds) *The evolution of social behavior in insects and arachnids*. Cambridge University Press Cambridge, pp 94–115
- Thomas ML, Elgar M (2003) Colony size affects division of labour in the ponerine ant *Rhytidoponera metallica*. *Naturwissenschaften* 90:88–92. <https://doi.org/10.1007/s00114-002-0396-x>
- Thomas ML, Framenau VW (2005) Foraging decisions of individual workers vary with colony size in the greenhead ant *Rhytidoponera metallica* (Formicidae, Ectatomminae). *Insectes Soc* 52(1):26–30
- Thum AS, Gerber B (2019) Connectomics and function of a memory network: the mushroom body of larval *Drosophila*. *Curr Opin Neurobiol* 54:146–154. <https://doi.org/10.1016/j.conb.2018.10.007>
- Tibbetts EA (2004) Complex social behaviour can select for variability in visual features: a case study in *Polistes* wasps. *Proc R Soc B Biol Sci* 271:1955–1960. <https://doi.org/10.1098/rspb.2004.2784>
- Torres VO, Montagna TS, Raizer J, Antonialli-Junior WF (2012) Division of labor in colonies of the eusocial wasp,

- Mischocyttarus consimilis*. J Insect Sci 12:21–47. <https://doi.org/10.1673/031.012.2101>
- Traniello JFA (1978) Caste in a primitive ant: absence of age polyethism in *Amblyopone*. Science 202:770–772. <https://doi.org/10.1126/science.202.4369.770>
- Trujillo-Cenóz O, Melamed J (1962) Electron microscope observations on the calyces of the insect brain. J Ultrastruct Res 7:389–398. [https://doi.org/10.1016/S0022-5320\(62\)90035-7](https://doi.org/10.1016/S0022-5320(62)90035-7)
- Truman JW (1990) Metamorphosis of the central nervous system of *Drosophila*. J Neurobiol 21:1072–1084. <https://doi.org/10.1002/neu.480210711>
- Tschinkel WR (1988) Colony growth and the ontogeny of worker polymorphism in the fire ant, *Solenopsis invicta*. Behav Ecol Sociobiol 22:103–115. <https://doi.org/10.1007/BF00303545>
- Turillazzi S (1989) The origin and evolution of social life in the Stenogastrinae (Hymenoptera, Vespidae). J Insect Behav 2:649–661. <https://doi.org/10.1073/pnas.0610140104>
- Urbach R, Technau GM (2003) Early steps in building the insect brain: neuroblast formation and segmental patterning in the developing brain of different insect species. Arthropod Struct Dev 32:103–123. [https://doi.org/10.1016/S1467-8039\(03\)00042-2](https://doi.org/10.1016/S1467-8039(03)00042-2)
- Van Nest BN, Wagner AE, Marrs GS, Fahrbach SE (2017) Volume and density of microglomeruli in the honey bee mushroom bodies do not predict performance on a foraging task. Dev Neurobiol 77:1057–1071. <https://doi.org/10.1002/dneu.22492>
- Van Der Woude E, Smid HM, Chittka L, Huigens ME (2013) Breaking Haller's rule: brain-body size isometry in a minute parasitic wasp. Brain Behav Evol. <https://doi.org/10.1159/000345945>
- Varshney LR, Chen BL, Paniagua E, Hall DH, Chklovskii DB (2011) Structural properties of the *Caenorhabditis elegans* neuronal network. PLoS Comput Biol 7:e1001066. <https://doi.org/10.1371/journal.pcbi.1001066>
- Walker R, Burger O, Wagner J, Von Rueden CR (2006) Evolution of brain size and juvenile periods in primates. J Hum Evol 51:480–489. <https://doi.org/10.1016/j.jhevol.2006.06.002>
- Ward PS (2014) The phylogeny and evolution of ants. Annu Rev Ecol Syst 45:23–43. <https://doi.org/10.1146/annurev-ecolsys-120213-091824>
- Wells RS, De Waal FBM, Tyack P (2003) Animal social complexity: intelligence, culture, and individualized societies. Harvard University Press, Cambridge
- Wetterer JK (1994) Ontogenetic changes in forager polymorphism and foraging ecology in the leaf-cutting ant *Atta cephalotes*. Oecologia 98:235–238. <https://doi.org/10.1007/BF00341478>
- Wetterer JK (1999) The ecology and evolution of worker size distribution in leaf cutting ants (Hymenoptera: Formicidae). Sociobiology 34:119–144
- Whiting MF, Bradler S, Maxwell T (2003) Ups and downs of evolution: Insects that lost: then re-evolved—the ability to fly. Nature 421:264–267. <https://doi.org/10.1038/nature01274.1>
- Williams NM, Regetz J, Kremen C (2012) Landscape-scale resources promote colony growth but not reproductive performance of bumble bees. Ecology 93:1049–1058
- Wills BD, Powell S, Rivera MD, Suarez AV (2018) Correlates and consequences of worker polymorphism in ants. Annu Rev Entomol. <https://doi.org/10.1146/annurev-ento-020117-043357>
- Wilson EO (1953) The origin and evolution of polymorphism in ants. Q Rev Biol 28:136–156. <https://doi.org/10.1086/399512>
- Wilson EO, Hölldobler B (2005) Eusociality: origin and consequences. Proc Natl Acad Sci U S A 102:13367–13371. <https://doi.org/10.1073/pnas.0505858102>
- Withers GS, Fahrbach SE, Robinson GE (1993) Selective neuroanatomical plasticity and division of labour in the honeybee. Nature 364:238
- Witthöft W (1967) Absolute Anzahl und Verteilung der Zellen im Hirn der Honigbiene. Z Morph Tiere 61:160–184
- Wittwer B, Hefetz A, Simon T, Murphy LEK, Elgar MA, Pierce NE, Kocher SD (2017) Solitary bees reduce investment in communication compared with their social relatives. Proc Natl Acad Sci 114:6569–6574. <https://doi.org/10.1073/pnas.1620780114>
- Wood HM, Parkinson DY, Griswold CE, Gillespie RG, Elias DO (2016) Repeated evolution of power-amplified predatory strikes in trap-jaw spiders. Curr Biol 26:1057–1061. <https://doi.org/10.1016/j.cub.2016.02.029>
- Zhang K, Guo JZ, Peng Y, Xi W, Guo A (2007) Dopamine-mushroom body circuit regulates saliency-based decision-making in *Drosophila*. Science 316:1901–1904. <https://doi.org/10.1126/science.1137357>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.