

Opinion

Deconstructing
Superorganisms and
Societies to Address Big
Questions in Biology

Patrick Kennedy,¹ Gemma Baron,² Bitao Qiu,³ Dalial Freitak,⁴
Heikki Helanterä,⁴ Edmund R. Hunt,¹ Fabio Manfredini,²
Thomas O'Shea-Wheller,¹ Solenn Patalano,⁵
Christopher D. Pull,^{2,6} Takao Sasaki,⁷ Daisy Taylor,¹
Christopher D.R. Wyatt,^{8,9} and Seirian Sumner^{1,10,*,@}

Social insect societies are long-standing models for understanding social behaviour and evolution. Unlike other advanced biological societies (such as the multicellular body), the component parts of social insect societies can be easily deconstructed and manipulated. Recent methodological and theoretical innovations have exploited this trait to address an expanded range of biological questions. We illustrate the broadening range of biological insight coming from social insect biology with four examples. These new frontiers promote open-minded, interdisciplinary exploration of one of the richest and most complex of biological phenomena: sociality.

Expanding Horizons for Social Insect Research

A pivotal point in biological research was the realization that life, across all levels of biological organization, can be explained via a series of **major evolutionary transitions** (see [Glossary](#)) [1]. One such transition is the shift from solitary to highly social living, in which previously independent units come together to form a new level of individuality – the society [1,2]. The transition to social living is apparent across levels of biological organization and governed by similar principles. Major evolutionary transitions include the origin of the genome, the evolution of multicellularity from free-living cells and the evolution of insect sociality from individual insects.

However, insect societies (the ants, termites and certain bees and aculeate wasps) are unique amongst the products of the major transitions in that the component parts (individual insects) can be easily deconstructed and manipulated ([Figure 1](#)), allowing the mechanistic and evolutionary basis of the complexities of life to be scrutinized. We are now witnessing a revolution in the scope of study offered by social insects. Conceptual and methodological advances allow researchers to capitalize on the ease of deconstructing the society; as a result, social insects are now being used to unlock questions as diverse as the evolution of innate immunity [3], how specialist phenotypes can emerge from a common genome [4], the evolution of heritability [5] and the roles played by personality and heterogeneity in biological systems [6]. Here we argue that social insect research is helping solve general problems in biology. We invited the opinions of 120 biologists (delegates at the 2015 meeting of the North West European Section of the

Trends

Many social insect colonies are closely analogous to multicellular organisms, possessing key 'organismal' traits. Most prominently amongst these is the obligate differentiation of individuals into germ and soma, accompanied by extreme levels of functional integration and a colony-level immune system. This striking echo in evolution means that social insects can provide highly accessible models for questions that have traditionally been the domain of whole-organism and cell biology.

The expanded scope of model organisms amongst the social insects, novel techniques and recent conceptual advances suggest that social insect biology can make profound contributions to diverse fields – from immunology to the study of heredity itself – to which it has previously had little connection.

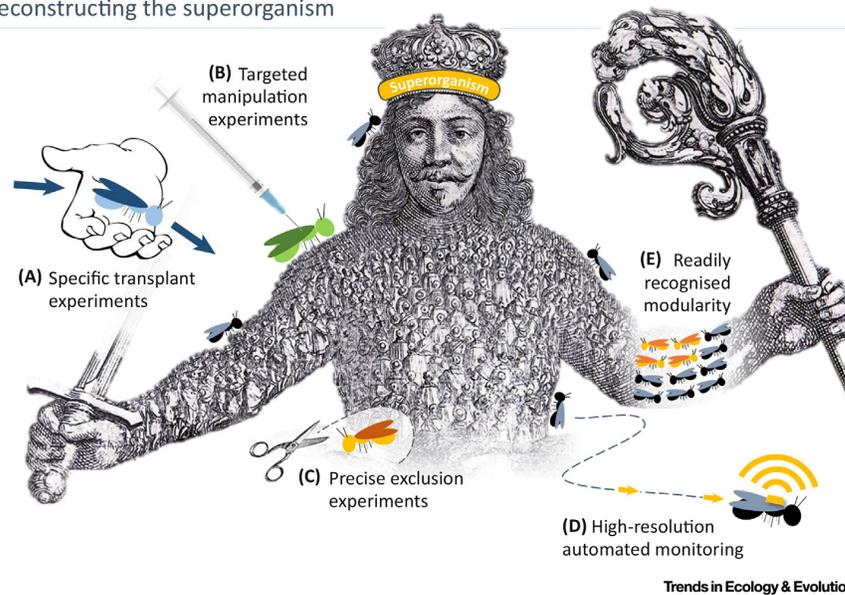
¹Life Sciences Building, University of Bristol, 24 Tyndall Avenue, Bristol BS8 1TQ, United Kingdom

²School of Biological Sciences, Royal Holloway University of London, Egham TW20 0EX, United Kingdom

³Centre for Social Evolution, Department of Biology, University of Copenhagen, Universitetsparken 15, DK-2100 Copenhagen, Denmark

⁴Centre of Excellence in Biological

Deconstructing the superorganism



Trends in Ecology & Evolution

Figure 1. Functionally, Eusocial Insect Colonies Often Resemble Multicellular Organisms, and Are Said to Be 'Superorganismal'. Social insect species possess organismal traits to greater or lesser degrees. By echoing the internal functions of multicellular organisms, social insects can provide insights into research questions that have traditionally been the domain of cellular biology and medicine. Unlike multicellular organisms, social insect colonies are amenable to manipulation, including (A) transplantation of 'graft tissue' (in the form of individual insects) between or within colonies, (B) targeted manipulation of specific individuals within the colony and (C) targeted exclusion of specific parts of the colony to identify function. Observation and experiment can now utilize (D) automatic monitoring of movement and interactions at an individual level. (E) Distinct modules within the colony can be readily identified (morphologically and behaviourally). We represent the colony with the most famous 'superorganism' of all: Thomas Hobbes' Leviathan.

International Union for the Study of Social Insects) on general topics in biology that social insect research is revolutionizing. Nominated topics were discussed in workshops to define the conceptual advances offered by social insect research, and discuss emerging challenges and solutions. We showcase four of these emerging trends. This list is nonexhaustive and biased by the delegates' research interests, but they serve well in illustrating the point that social insects are no longer limited as models for classical behavioural ecology and evolution, and that they are now being offered as solutions to key challenges in 21st century biology (see Outstanding Questions).

Trend 1: Superorganisms and a Unified Theory of Social Evolution

The Problem

The evolution of societies with distinct **castes** of sterile workers and reproductive **queens** is the latest major evolutionary transition (Figure 1). **Inclusive fitness theory** explains the complex interplay of kinship, conflict and cooperation among individuals in the evolution of insect societies. This transition means that selection increasingly scrutinizes group-level traits; however, we understand surprisingly little about how the evolution of group-level traits compares to the evolution of individual-level traits, the latter of which have been the traditional focus of evolutionary biology.

Conceptual Advances

The most elaborate form of social living in the social insects is **eusociality**, typically defined by three characteristics: cooperative care of young, **reproductive division of labour** and overlapping generations. Describing eusocial insect societies as '**superorganisms**' – social

Interactions, Department of Biosciences, University of Helsinki, Viikinkaari 1, P.O. Box 65, 00014 University of Helsinki, Finland
²The Babraham Institute, Cambridge CB22 3AT, United Kingdom
³IST Austria (Institute of Science and Technology Austria), Am Campus 1, A-3400 Klosterneuburg, Austria
⁴Department of Zoology, University of Oxford, The Tinbergen Building, Parks Road, Oxford OX1 3PS, United Kingdom
⁵Centre for Genomic Regulation (CRG), The Barcelona Institute for Science and Technology, Dr. Aiguader 88, 08003 Barcelona, Spain
⁶Universitat Pompeu Fabra (UPF), Barcelona, Spain
¹⁰Current address: Centre for Biodiversity & Environment Research, Department of Genetics, Evolution & Environment, University College London, Gower Street, London WC1E 6BT, United Kingdom
 *Twitter: @WaspWoman

*Correspondence: s.sumner@ucl.ac.uk (S. Sumner).

colonies that possess the fundamental qualities of organisms to such an extent that we can consider them organismal in their own right – is compelling, and dates to the early 1900s [7]. Currently, the superorganism concept is moving from an appealing metaphor to a more precise formal analogy [8]. The superorganism view has often been limited to physical and functional analogies between multicellular organisms and eusocial societies, but recent work shows how the concept can provide an explicit comparison of new kinds of organisms; this promises a more unified understanding of how evolution works at different hierarchical levels [2].

Challenges and Solutions

To determine how traits of superorganisms evolve, and how similarities in evolution at different biological levels arise, we need to understand the building blocks of natural selection at the superorganism level, that is, the fitness effects and heritability of variation in colony-level traits. Colonies vary consistently in traits such as reproductive allocation [9] and foraging [5]. The fitness effect of variation in colony-level traits (including caste ratios, genetic diversity, colony size and foraging thresholds) has been demonstrated in the laboratory and in the wild [10] using fitness proxies such as foraging success, productivity or disease resistance. To set colony-level trait variation into an evolutionary context, we need to understand how colony traits respond to selection.

The heritability, and response to selection, of group traits is poorly understood [11]. We outline three areas where significant progress can be made. First, long-term field studies allow mapping of fitness effects at the levels of lifetime reproductive output [12] and heritability of colony-level traits as they are transmitted from parent colonies to daughter colonies [5]. Colony longevity, long dispersal distances and pervasive plasticity in colony traits, however, make gathering such field data challenging. This is why, second, experimental approaches are needed. Experimental work in honeybees [13] and ants [14] has revealed the complex genetic architecture of traits such as foraging preferences and allocation decisions. Third, theory needs to reveal how traits of superorganisms with internal genetic diversity, conflicts of interest between individual and group levels, complex **indirect genetic effects** [15], caste antagonistic selection pressures [16] and extensive phenotypic plasticity respond to selection. To achieve this, it will be necessary to move seamlessly between the quantitative genetics view point offered by multilevel selection and the optimality logic of inclusive fitness theory [17]. Finally, understanding how individual-level variation translates (during ontogeny, and in the genotype–phenotype map of the superorganism) to colony-level variation is nontrivial, and continues to be modelled extensively [18].

The concept of a superorganism combines disparate approaches into a general framework for studying how traits of colonies evolve. There is potential in comparative tests of adaptive hypotheses over key colony-level life history traits [2] and analyses of ongoing selection of superorganismal traits, both in the field and in the laboratory. The variation in insect societies (Box 1), and a mechanistic understanding of the behaviour of the parts that make the whole, provides an excellent setting for taking the study of evolutionary transitions to the next level, to understand how a transition in heritable, fitness-relevant variation happens and to compare evolutionary processes across hierarchical levels.

Trend 2: The Functional Roles of Biological Heterogeneity

The Problem

Superficially similar individual units in cooperative groups – such as cells in the pancreas or workers in ant colonies – often show dramatic variation in how they behave [19]. Such **heterogeneity** is emerging as an adaptive trait of the group itself, not noise around an optimum [20]. Social insect colonies provide good models for identifying adaptation amid the noise, due to their specialized task-allocation strategies (e.g., among reproductive and non-

Glossary

Behavioural syndrome: a suite of behaviours that differ consistently between entities (also termed ‘personality’). Behavioural syndromes are typically analysed at the level of the individual, but colony-level behavioural syndromes are also possible.

Biological heterogeneity: differences between members of a group, which can be adaptive, maladaptive or neutral.

Caste: a specialized subset of individuals in a colony, which are morphologically or behaviourally distinct from other individuals. The most profound caste distinction is between a reproductive caste and a non-reproductive (worker) caste, but the term is also used for distinct subsets of the non-reproductive workforce that specialize in different tasks (and can be dramatically different in morphology); for example, soldier and forager castes in some leaf-cutting ant species.

Eusociality: a form of social organization typically defined by three traits: (i) cooperative care of young, (ii) differentiation of individuals into reproductive and non-reproductive castes and (iii) overlapping generations.

Epigenetics: modifications to chromosomes that influence gene expression without altering the DNA sequence itself.

Genomic plasticity: aspects of the genome (e.g., gene transcription, translation, regulation) that facilitate rapid changes in phenotype (e.g., changes in gene expression or methylation in response to an environmental cue).

Immunological memory: the ability of the adaptive immune system to mount a targeted response upon re-encountering a specific pathogen.

Inclusive fitness theory: a central paradigm in social evolution, which sees individual organisms as maximizing the sum of direct fitness (the actor’s own fitness) and indirect fitness (the actor’s effects on the fitness of others, weighted by relatedness).

Indirect genetic effects: effects on an individual’s phenotype caused by the genes of other conspecifics.

Innate immunity: the branch of the immune system that presents general, nonspecific responses to pathogens.

Box 1. Diversifying Model Organisms: Going beyond the Honeybee

The biological diversity exhibited by social insect species is astounding, from variation in social organization and mating strategies, to physiology, behaviour and life history. Despite this immense variability, social insect research has focused historically on a relatively small coterie of accessible model organisms, in particular the honeybee (*Apis mellifera*). The range of model organisms is now expanding, opening new research possibilities (Figure 2).

Models of Simple Sociality

In the quest to understand the mechanisms and processes that occurred at the origin of sociality, it is necessary to study model organisms that have very simple societies, and thus are likely to represent an early stage of social evolution. These so-called primitively eusocial insects – including the *Polistes* paper wasps (Figure 2B) and various halictid ‘sweat bees’ – offer snapshots in evolutionary time into the conditions favouring the origin of group living. Workers in these species retain reproductive potency; the distinction between queen and worker can be temporary, and discernible only through behaviour and basic reproductive physiology. Such simple societies offer unique opportunities to explore fundamental questions in biology, including the role of plasticity in adaptation [45], the emergence of distinct behavioural roles from a common genome [34], the relative importance of direct and indirect fitness in the formation of social groups and the first stages in a major evolutionary transition [1,59].

Models of Complex Sociality

Species with complex societies offer insights into the extraordinary level of coordination required to maintain functioning colonies of thousands of individuals. Here, division of reproductive labour is complex and derived: queen and worker roles are fixed during development in response to an environmental cue, or (more rarely) a genetic basis [37]. Adults are therefore committed to their roles, and thus are excellent models for understanding the loss of phenotypic plasticity, and a major transition in evolution [8]. Fire ants (*Solenopsis invicta*; Figure 2C) provide such a model. As an invasive species with dramatic ecological effects, they have become a focus in a high-stakes struggle to understand the role of sociality in invasions. Remarkably, genetic research in *S. invicta* has uncovered the existence of a ‘social chromosome’ that explains variation in social structure [60], providing a rare example of the elusive ‘greenbeard’ traits predicted by evolutionary theory. Similar advances are being made in the attine leafcutter ants, the agriculturalists of the hymenopteran world, that live in a symbiotic association with a fungus that they farm to produce food for their developing brood: comparative genomics are now revealing in fine detail the evolution of these complex societies and mutualisms [61].

Comparative Social Biology

Phylogenetic analyses of social traits provide key insights into the patterns and processes of evolution. With large clades encompassing different levels of sociality and a wide range of quantifiable traits, social insects provide valuable models for this emerging field [62].

The bumblebee genus (*Bombus* spp.) provides a fascinating case study, in which comparative social biology can recreate in detail the genetics of adaptation. The genus includes species representing a range of social complexity, and certain species (in the subgenus *Psithyrus*) are workerless social parasites of the social *Bombus* colonies. These parasites invade foreign colonies, overthrow the queen and exploit the host workforce. Given that genes in each of these lineages will experience radically different social contexts, such systems provide an opportunity to reveal the effects of sociality on the dynamics of natural selection [63]. With such opportunities now emerging, we expect that the social insects will make some of the strongest contributions to future comparative social biology.

reproductive castes) and their propensity for self-organization. At a given moment, workers will be distributed across a range of tasks – specializing (permanently or temporarily) as a forager, a cemetery cleaner or a nurse, for instance. This allocation of workers to distinct tasks promotes colony efficiency and productivity [21]. However, recent studies have revealed extraordinary heterogeneity within physical worker castes.

Conceptual Advances

The heterogeneous nature of social phenotypes within the colony appears to be adaptive at the colony level. For example, honeybee colonies with high genetic diversity (generated from multiply mated queens) experience reduced fluctuation in temperature compared with less genetically diverse colonies; this is because the response thresholds of individuals are genetically determined, and so varied patriline deliver varied response thresholds [21]. Variations in

Major evolutionary transition: an event in the history of life in which lower-level units (such as solitary insects) evolve to form highly integrated higher-level units (such as social insect colonies). Other major transitions include the origin of the genome and the multicellular organism.

Phenotypic plasticity: the characteristic of expressing different phenotypes from the same genotype in different environments.

Queen: a female in social insect colonies accepted by the workers as a sustained egg layer. Colonies can have multiple queens.

Reproductive division of labour: the condition in which only a subset of individuals within groups reproduce, aided in brood rearing by non-reproductive individuals.

Social immunity: the additional level of disease protection arising in a eusocial society due to the social disease defences of its members.

These traits are selected for to maximise the inclusive fitness of the society’s members.

Social parasite: social organisms that exploit the socially acquired resources of other (social) colonies, such as slave-making ants that raid colonies and ‘enslave’ workers, and parasitic reproductives that infiltrate foreign colonies to lay their own eggs.

Superorganism: a group of individual organisms that possesses the fundamental characteristics of an organism itself. Classically, this requires obligate differentiation of individuals into morphologically distinct reproductive and non-reproductive castes, echoing the distinction between gonads and somatic tissue in multicellular organisms, accompanied by tight functional integration.

Transcriptomics: the study of messenger RNA molecules, which communicate the ‘commands’ of the genome to the cell for the creation of proteins.

Transgenerational immune priming: the transmission of disease resistance, developed through experience, from parents to offspring.

Box 2. The Rise of Automated Monitoring

In recent years, developments in tracking technology have led to more detailed study of individual-level insect behaviour. The long-standing method that involves marking individuals with unique paint marks [64] is now being complemented with automated methods, opening new vistas of research.

Methods are varied and adaptable to address a wide spectrum of questions. Time-lapse video can be used to track aggregate activity levels over time [65], while individual trajectories of two-dimensional movements can also be recovered from video using various tracking software [66]. Radio-frequency identification chips are attached to ants and wasps to automatically monitor visits to particular locations [67,68]. Tracking small ants over long distances is a challenge for a fixed-camera system, and so a moveable gantry-based camera can also be effective for detailed studies of individual movement [69]. Recent research has successfully employed paper barcode tags to study the colony behaviour of a large species of ant [70]. For flying insects such as bumblebees, small transponders can be attached and individually tracked using harmonic radar; this technique has been used successfully in long-term behavioural studies [71]. If individual animals are sufficiently different in appearance, computer algorithms can identify a 'fingerprint' for individual-level recognition [72]. Noninvasive tracking is preferable as marking animals might modify behaviour.

With ever-improving video recording quality, even colonies of numerous, small and apparently identical insects can be revealed as a collection of behaviourally heterogeneous individuals. Automated monitoring of social insects therefore offers unprecedented opportunities to study the mechanisms and evolution of the superorganism. At the same time, social insect research is inspiring parallel developments in the tracking of cells [73] and human crowds [74].

response thresholds are also important in decision making. For example, among house-hunting ants, some individuals have high thresholds for accepting a new nest [22], making it more likely that the colony finds the best available option [23]. Social insects are suited to answering general questions about the adaptive value of biological heterogeneity at a fine scale, due to their large colony sizes and observability, alongside recent breakthroughs in individual-level automated monitoring (Box 2).

Challenges and Solutions

We identify five areas in which social insects can offer insights into biological heterogeneity. First, the contributions of some forms of heterogeneity are not obvious. A striking example is 'lazy workers' in social insect colonies, whose low activity levels leave other group members to perform a large proportion of the work [6]. Some forms of heterogeneity in activity can be traced to underlying heterogeneity in physiology; for example, heterogeneous distributions of fat stores regulate foraging activity in ants [24].

Second, how is heterogeneity linked across organizational scales? Higher-level variation can emerge from lower-level heterogeneity. **Behavioural syndromes** (consistent expression of behavioural traits across different environments or conditions) are observable at the colony level [25] but it is not clear how these relate to individual-level heterogeneity. It has been suggested that heterogeneous traits at the colony level might be most important for species-level ecology, allowing for the coexistence of complementary types [10].

Third, continuous heterogeneous traits are rarely normally distributed; the adaptive reasons for skewed distributions of traits remain elusive. Social insects, given their remarkable accessibility, offer opportunities to both measure and – crucially – experimentally manipulate the distributions of heterogeneous traits *in situ*. With their readily observed behavioural heterogeneity [6], ant workers are especially suited to this form of manipulation. To exploit this opportunity, we require a close understanding of how flexible individual thresholds are in response to, for example, the removal of workers with specific thresholds/roles and over what timescale responses should be expected.

Fourth, if heterogeneity is functional, then it must be a target of selection, but the mechanisms by which this occurs are unknown. Social insects offer insights into the role of life history and social information in generating and maintaining adaptive heterogeneity. Life-history traits

include physical maturity, nutritional and environmental factors [26,27]; social factors include learning and experience [28]. Exploitation of individual tracking technology allows behaviours to be followed over long periods under different mechanistic scenarios, to investigate how thresholds can be altered (Box 2). Social information provides a mechanism for shaping individual-level variation in behaviour [29,30], although it is not always reliable, due to conflicts between the group and individual [31] or because accuracy must be traded-off against speed when making colony-level decisions [32].

Trend 3: Phenotypic Plasticity Is Key to Coping with Changing Environments

The Problem

Phenotypic plasticity is an adaptive mechanism whereby a single genotype can respond to changing environments by expressing different phenotypes under different environmental conditions [33]. Such plasticity is especially important in rapidly changing environments where there is not time for populations to evolve or disperse. The molecular mechanisms underlying plasticity are poorly explored; this information is essential for understanding how organisms respond to their environment, and predicting how they will cope with environmental change. Social insects display plasticity in behavioural and physiological traits that can be easily measured and manipulated; moreover, genomic tools for examining plasticity at the molecular level are now accessible for any species (Box 3).

Conceptual Advances

Social insect species exhibiting simple societies are excellent models for studying phenotypic plasticity, as individuals retain caste flexibility throughout life. Accordingly, adults in simple societies can often respond to changes in their environment by switching caste (Box 1) [4,34]. Next-generation methods in molecular biology (Box 3) mean these relatively understudied species can now be used to reveal the molecular mechanisms of phenotypic plasticity [35–37]. For example, high levels of plasticity have been associated with subtle differences in transcriptional networks, molecular pathways, DNA methylation and tissue-specific gene expression [4,38]. By contrast, low levels of plasticity appear to be governed by large-scale shifts in molecular processes: functional enrichment and canalization of gene expression might limit plasticity [39].

Exploring the mechanisms of phenotypic plasticity in social insects is an opportunity to move the focus beyond a single individual, as plasticity is a feature that applies also to whole colonies [40]. The network of interactions among colony members can reach different levels of plasticity, depending on fluctuating colony needs, similar to what we observe in molecular networks of coexpressed genes. However, this aspect of social behaviour has not been fully characterized. From an applied perspective, understanding phenotypic plasticity in social insects is of value due to their roles as both invasive species and providers of ecosystem services [41]; moreover, the remarkable difference in longevity between queens and workers means that social insects are now models for understanding ageing [42]. The rich diversity of phenotypic plasticity in social insects (Box 1), together with new molecular tools (Box 2), places this taxonomic group centre stage in advancing our understanding of plasticity at multiple levels of analysis, and the interplay of these different levels with the environment.

Challenges and Solutions

We identify three fundamental issues on the nature of phenotypic plasticity on which social insects can shed light. First, we understand little about the molecular processes underlying plasticity. The tractability of experimental work in the field and laboratory (Box 1) and their relatively small genomes (Box 3) make social insects good models here. The range of plasticity exhibited by social insects (Box 1) is helping reveal molecular signatures of plasticity, at the **transcriptomic** and **epigenetic** level [4]. Such insights can provide new solutions to urgent

Box 3. 'Omic' Technologies Are Revolutionary but Require Careful Standardization

Recent advances in whole-genome high-throughput sequencing now facilitate testing of evolutionary and mechanistic hypotheses using a wide range of taxa, generating unprecedented data sets. Social insects have taken centre stage in this due to their availability and small genome sizes. Genomic, transcriptomic, epigenetic and proteomic approaches showcase the potential of these analyses in social insects to answer wider biological questions. For example, comparative genomic analyses have revealed the expansion of olfactory receptors in ants [75]; the identification of caste-associated gene expression has probed the molecular differentiation between simple and complex social species [4]; epigenetic studies explain behavioural plasticity across social evolution [76] and proteomic, lipidomic and metabolomic approaches are on the horizon [77]. However, methodologies need to be standardized, and well reported, to ensure future-proofing of data and facilitate comparisons across studies and species. We summarize some of the major challenges.

Sampling

Field samples reflect the natural conditions, whilst laboratory environments can result in artefacts [78]. Choice of tissue is also important. Whole-body transcriptomic, proteomic or methylomic analyses are of limited use due to the huge variability across tissues. Analyses of highly specialized tissues, such as brain mushroom bodies, will accurately reflect their specificity but will preclude identification of upstream signalling pathways linked with individual phenotype. Most sequencing studies use pooled samples, which average the variability among individuals as well as increasing the amount of starting material, making replication affordable; but, pooling loses information on individual-level heterogeneity. The sampling methods chosen should be appropriate to the biological question: for example, effects of diet on the developing phenotype might be better studied using whole-body analyses under the controlled environment of the laboratory, whilst behavioural studies require analyses of brain tissue in an environmentally relevant context.

Sequencing

RNA extraction and library preparation influence the quality and concentration of cDNA. For example, different concentrations of cDNA can result in differences in sequences [79]. Choice of extraction kit (RNA only, combined RNA–DNA) and library preparation method (number of PCR cycles, concentration of adapters) are key for library optimization; RNA spike-in controls help to standardize large-scale comparisons across data sets [80]. A second consideration is batch effects (confounding variation in the experience of different samples during sequencing). Barcoding and sequencing barcoded mixed samples in multiple lanes can reduce batch effects [81]. Finally, different sequencing platforms might not generate comparable data, for example, between 454 and Illumina platforms [82]. With new platforms appearing almost annually, reproducibility across platforms is one of the major challenges facing genomic studies.

Data Analysis

Evolutionary analyses and cross-species gene-expression analyses are annotation dependent. Thus, the quality of genome annotations influences detection of gene losses and gains, and can bias analyses of gene expression across species. The use of single-copy orthologues (through KEGG orthology) to assess genome assembly and annotation quality might improve the analysis [83]. However, ultimately, the power (and relevance) of annotation is limited by the range of genes and species that have been subjected to functional genomic analyses. In particular, advancing social insect genomics will require using social insect models rather than traditional models such as *Drosophila*. Finally, to compare gene expression across species, it is important to normalise for GC (guanine–cytosine) biasing of RNA-sequencing, exon–intron structure and gene length that can differ between species [84].

In conclusion, the tandem power of 'omics' technologies (this box) and diversity of social insect biology (Box 1) provides an unprecedented tool for answering a range of biological questions. However, close dialogue between social insect researchers, molecular biologists and bioinformaticians is essential to fully exploit this potential, and future-proof genomic data sets within meaningful ecological contexts.

biodiversity issues; for example, molecular indicators of plasticity could help prioritize conservation of species that lack the capacity of a plastic response.

A second challenge is understanding the causes of variation in plasticity. Some species are remarkably resilient to environmental change (e.g., invasive ant species [43]), whilst others are not (e.g., to habitat loss, novel toxins or pathogens [44]). Differences in the mechanistic basis of **genomic plasticity** might explain variation in species-level resilience to environmental change,

whilst the nature of the plastic response will depend on the nature of the challenge (e.g., chemicals, temperature fluctuations, pathogens). Using a comparative approach (Box 1) and exploiting molecular tools (Box 3), traits that explain variation in plastic responses can be identified, and used to advise biodiversity management. Of particular importance is understanding what genomic traits might limit plasticity. Social insects exhibit an evolutionary loss of plasticity in the form of caste differentiation, with more complex societies exhibiting low levels of plasticity relative to simpler societies [4]. An extreme example of caste reduction is found in the **social parasites**, which have evolved multiple times within the social insects and rely on social species to survive: they exhibit varying levels of losses in phenotypic plasticity depending on the species. These insects might reveal how genomes change with extreme losses of phenotypic plasticity [45].

A third challenge is to understand when (and why) phenotypes become more plastic. This is urgent as invasive species with high levels of plasticity are threatening ecosystems globally. Is plasticity a preadaptation to invasion or does plasticity emerge due to the invasion process? Can molecular indicators of plasticity (e.g., epigenetic marks) predict vulnerability to environmental challenges and thus improve predictions of (and mitigate) population declines [46]? Social insects are an important and accessible group to address these questions, as ecosystem service providers (e.g., pollinators, predators and seed dispersers [47]) and successful invasive species [43].

Trend 4: Towards a Unified Framework for Understanding Disease Defence

The Problem

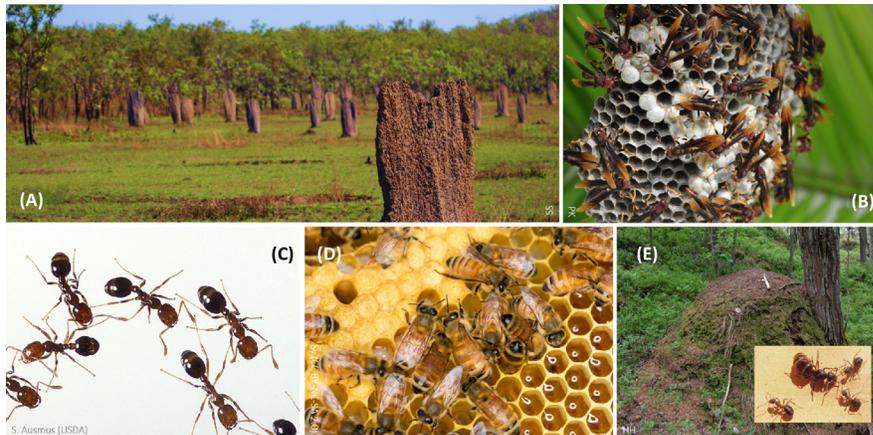
Research on the vertebrate adaptive immune system has led to major advances in our understanding of host disease defence [48]. However, despite the potency of **immunological memory**, it is often **innate immunity** that makes the difference between survival and death [49]. The discovery that invertebrates, including social insects, can prime their immune responses and transfer immune protection across generations is paradigm shifting [50]. This development comes at a time of rising interest in the links between sociality and disease dynamics [51]. In general, we lack a solid understanding of the interplay between physiological immunity, behavioural responses and social interactions, yet this is essential to the study of disease defence, host–pathogen coevolution and epidemiology across all taxa [48].

Conceptual Advances

The natural variation and plasticity present in social insect colonies (Trends 2 and 3) makes them ideal candidates for ecoimmunological work and powerful models to study the evolution of immune defences from the gene to the society [52]. The diversity of social complexity in social insects (Box 1 and Figure 2) facilitates the study of disease dynamics and epidemiology in groups of different sizes and social structures. Studying disease dynamics in social insects has already led to new concepts: **social immunity** is the additional layer of defence arising from collectively performed disease defences that reduce the disease susceptibility of the colony. It is thereby analogous to the physiological immune system of multicellular organisms, and can plausibly provide insights into the evolution of immune defences across these domains [53].

Challenges and Solutions

We identify three critical issues that are of general importance to the study of disease defence in animals. The first concerns the molecular mechanisms underpinning **transgenerational immune priming**, where insect parents can enhance the resistance of their offspring to disease without antibodies. A mechanism for maternal immune priming in insects was recently discovered in the honeybee: the egg yolk protein vitellogenin transfers bacterial fragments from food to developing eggs [3]. Do mothers pass on other molecules (e.g., mRNA or specific proteins) that shape the phenotype of the developing embryo? It has been argued that the



Trends in Ecology & Evolution

Figure 2. Social Insect Colonies Are Richly Diverse, Illustrating the Surprising Innovations Borne of Social Insect Cooperation. Examples range from (A) the architectural grandeur of termite mounds to (B) small colonies of simple eusocial wasps, (C) the strict organisation of fire ants and (D) honeybees to (E) the vast societies of wood ants collaborating to form sizeable nest mounds.

evolution of immune priming in insects is dependent on the longevity of the species and mechanism of dispersal [50], but strong evidence is lacking. As they exhibit large variation in longevity and dispersal strategies, social insects offer ideal candidates to test these hypotheses.

Second, social insects can provide a window into the costs of immune defence. It is plausible that resistance is traded-off against other fitness-related traits and depends on life history [48]. Indeed, the costs (and need) for immune defence can differ between colony members. The range of model systems (Box 1) and the accessibility of genomic tools (Box 3) mean that social insects have the potential to reveal the costs of immunity on fitness-related traits and determine the interaction between physiological and behavioural immunity.

To study the impacts of behavioural defences on survival and fitness, longer-term studies are required; the short generation times and amenability to experimental manipulation of behaviour make social insect promising models [54]. For example, nest entrances can be closed to prevent the removal of corpses, a key social immunity behaviour [55]. Moreover, with the increasing number of sequenced genomes and RNAi-mediated knockdown techniques, genes that regulate behavioural phenotypes can be targeted directly to obtain the desired behavioural modification [56].

Finally, studies of complete social groups are needed to understand the role of social networks in disease transmission and susceptibility [57]. Social insects are ideal candidates for studies of epidemiological networks as whole colonies can be observed and manipulated, and their responses can be intimately linked to the functions of subsets of individuals within the colony [58]. More generally, the fact that natural enemies, including microbial parasites, pathogens and multicellular antagonists (such as social parasites), can target two distinct levels of organization (individuals or colonies) presents a unique arena in which to examine the role of hierarchical organization in immunity and defence. With the development of tracking techniques and advances in network analysis (Box 2), disease outbreaks can be studied across space and

in real time within the 'physiology' of the superorganism, revealing the impact of infection on social interactions [57].

Concluding Remarks

Social insect research is one of the historic triumphs of modern biology. A comprehensive body of natural history has been documented for a broad range of species. Theory explaining the mechanisms and evolutionary processes underpinning sociality is well developed. Social insects have provided important models for studying behaviour, development and cooperation for over a century. Given this springboard of knowledge, social insects now offer solutions to some of the big questions in biology (see Outstanding Questions). Precise investigation of the fact that convergent forms of social organization occur at multiple levels of biological organization, a renewed fascination with the extraordinary diversity of the social insects (Box 1) and a powerful new ability to exploit this diversity through technological (Box 2) and molecular (Box 3) advances represent an exciting triad of recent trends, responsible for the rise of the social insects as small solutions to big problems in biology.

Acknowledgements

We thank participants at the International Union for the Study of Social Insects (North West Europe Section) for their invaluable contributions to discussions on these topics at the Winter Meeting of 2015, at the University of Bristol. We also thank A.F.G. Bourke, M.J.F. Brown and three anonymous reviewers for helpful comments.

Supplemental Information

Supplemental information associated with this article can be found online at <http://dx.doi.org/10.1016/j.tree.2017.08.004>.

References

- Szathmáry, E. (2015) Toward major evolutionary transitions theory 2.0. *Proc. Natl. Acad. Sci. U. S. A.* 112, 10104–10111
- Helanterä, H. (2016) An organismal perspective on the evolution of insect societies. *Front. Ecol. Evol.* 4 (6), Published online February 11. <http://dx.doi.org/10.3389/fevo.2016.00006>
- Salmela, H. *et al.* (2015) Transfer of immunity from mother to offspring is mediated via egg-yolk protein vitellogenin. *PLoS Pathog.* 11, 1–12
- Patalano, S. *et al.* (2015) Molecular signatures of plastic phenotypes in two eusocial insect species with simple societies. *Proc. Natl. Acad. Sci. U. S. A.* 112, 13970–13975
- Gordon, D.M. (2013) The rewards of restraint in the collective regulation of foraging by harvester ant colonies. *Nature* 498, 91–93
- Charbonneau, D. and Dornhaus, A. (2015) Workers "specialized" on inactivity: behavioral consistency of inactive workers and their role in task allocation. *Behav. Ecol. Sociobiol.* 69, 1459–1472
- Wheeler, W.M. (1911) The ant-colony as an organism. *J. Morphol.* 22, 307–325
- Boomsma, J.J. and Gawne, R. (2017) Superorganismality and caste differentiation as points of no return: how the major evolutionary transitions were lost in translation. *Biol. Rev.* Published online May 15, 2017. <http://dx.doi.org/10.1111/brv.12330>
- Meunier, J. *et al.* (2008) Split sex ratios in the social Hymenoptera: a meta-analysis. *Behav. Ecol.* 19, 382–390
- Jandt, J.M. *et al.* (2014) Behavioural syndromes and social insects: personality at multiple levels. *Biol. Rev.* 89, 48–67
- Okasha, S. (2006) *Evolution and the Levels of Selection*, Oxford University Press
- Ingram, K.K. *et al.* (2013) Colony life history and lifetime reproductive success of red harvester ant colonies. *J. Anim. Ecol.* 82, 540–550
- Linksvayer, T.A. *et al.* (2009) Honeybee social regulatory networks are shaped by colony-level selection. *Am. Nat.* 173, E99–E107
- Linksvayer, T.A. (2006) Direct, maternal, and sibsocial genetic effects on individual and colony traits in an ant. *Evolution* 60, 2552–2561
- Wolf, J.B. *et al.* (1999) Interacting phenotypes and the evolutionary process. II. Selection resulting from social interactions. *Am. Nat.* 153, 254–266
- Holman, L. (2014) Caste load and the evolution of reproductive skew. *Am. Nat.* 183, 84–95
- Goodnight, C. (2013) On multilevel selection and kin selection: contextual analysis meets direct fitness. *Evolution* 67, 1539–1548
- Planas-Sitja, I. *et al.* (2015) Group personality during collective decision-making: a multi-level approach. *Proc. Biol. Sci.* 282, 20142515
- Blodgett, D.M. *et al.* (2017) Surprising heterogeneity of pancreatic islet cell subsets. *Cell Syst.* 3, 330–332
- Clobert, J. *et al.* (2009) Informed dispersal, heterogeneity in animal dispersal syndromes and the dynamics of spatially structured populations. *Ecol. Lett.* 12, 197–209
- Jones, J.C. *et al.* (2004) Honey bee nest thermoregulation: diversity promotes stability. *Science* 305, 402–404
- Robinson, E.J.H. *et al.* (2009) Do ants make direct comparisons? *Proc. Biol. Sci.* 276, 2635–2641
- Masuda, N. *et al.* (2015) Computational model of collective nest selection by ants with heterogeneous acceptance thresholds. *R. Soc. Open Sci.* 2, 140533
- Robinson, E.J.H. *et al.* (2012) Experience, corpulence and decision making in ant foraging. *J. Exp. Biol.* 215, 2653–2659
- Bengston, S.E. *et al.* (2014) Be meek or be bold? A colony-level behavioural syndrome in ants. *Proc. Biol. Sci.* 281, 20140518
- Seeley, T.D. (1982) Adaptive significance of the age polyethism schedule in honeybee colonies. *Behav. Ecol. Sociobiol.* 11, 287–293
- Kühn-Bühmann, S. and Wehner, R. (2006) Age-dependent and task-related volume changes in the mushroom bodies of visually guided desert ants, *Cataglyphis bicolor*. *J. Neurobiol.* 66, 511–521

Outstanding Questions

Trend 1: Superorganisms and a Unified Theory of Social Evolution

- Can the concept of the superorganism answer the need for a unified theory of social evolution, combining the logic of inclusive fitness theory with measures of response to selection at multiple levels?
- Do superorganisms show variation in superorganismal phenotypes, and how does this influence life history?
- How do superorganism traits affect colony life-time fitness?
- How heritable are superorganismal traits? How can colony-level traits be heritable with genetic heterogeneity, phenotypic plasticity and complex life cycles?

Trend 2: The Functional Roles of Biological Heterogeneity

- Some forms of heterogeneity are clearly important for colony function, but are all forms important? If not, why not? If so, are they inter-related?
- How is heterogeneity linked across different spatial scales?
- What shape does heterogeneity take? Normality is assumed, but is unlikely to be realistic.
- What are the mechanisms generating heterogeneity? What are the relative roles of genetics, life history, social information use and learning?
- How important are 'innovators' in the evolution of individual heterogeneity?

Trend 3: Phenotypic Plasticity Is Key to Coping with Changing Environments

- How is plasticity regulated at the molecular level?
- What traits (genomic, life history, environmental) cause variation in plastic responses and when does plasticity arise?
- What is the relationship between phenotypic plasticity and conservation (e.g.,

28. Jeanson, R. and Weidenmüller, A. (2014) Interindividual variability in social insects – proximate causes and ultimate consequences. *Biol. Rev.* 89, 671–687
29. Seeley, T.D. *et al.* (2012) Stop signals provide cross inhibition in collective decision-making by honeybee swarms. *Science* 335, 108–111
30. Sasaki, T. *et al.* (2015) Flexibility of collective decision making during house hunting in *Temnothorax* ants. *Behav. Ecol. Sociobiol.* 69, 707–714
31. Grüter, C. and Leadbeater, E. (2017) Insights from insects about adaptive social information use. *Trends Ecol. Evol.* 29, 177–184
32. Franks, N.R. *et al.* (2003) Speed versus accuracy in collective decision making. *Proc. Biol. Sci.* 270, 2457–2463
33. Kelly, S.A. *et al.* (2012) Phenotypic plasticity: molecular mechanisms and adaptive significance. *Compr. Physiol.* 2, 1417–1439
34. Ferreira, P.G. *et al.* (2013) Transcriptome analyses of primitively eusocial wasps reveal novel insights into the evolution of sociality and the origin of alternative phenotypes. *Genome Biol.* 14, R20
35. Yan, H. *et al.* (2014) Eusocial insects as emerging models for behavioural epigenetics. *Nat. Rev. Genet.* 15, 677–688
36. Sumner, S. *et al.* (2006) Differential gene expression and phenotypic plasticity in behavioural castes of the primitively eusocial wasp, *Polistes canadensis*. *Proc. Biol. Sci.* 273, 19–26
37. Smith, C.R. *et al.* (2008) Genetic and genomic analyses of the division of labour in insect societies. *Nat. Rev. Genet.* 9, 735–748
38. Corona, M. *et al.* (2016) Molecular mechanisms of phenotypic plasticity in social insects. *Curr. Opin. Insect Sci.* 13, 55–60
39. Hatle, J.D. *et al.* (2003) Plasticity and canalization in the control of reproduction in the lubber grasshopper. *Integr. Comp. Biol.* 43, 635–645
40. Norman, V.C. *et al.* (2016) The effects of disturbance threat on leaf-cutting ant colonies: a laboratory study. *Insectes Soc.* 64, 1–11
41. New, T. (2012) *Hymenoptera and Conservation*, John Wiley & Sons
42. Parker, J.D. (2010) What are social insects telling us about aging? *Myrmecol. News* 13, 103–110
43. Holway, D.A. *et al.* (2002) The causes and consequences of ant invasions. *Annu. Rev. Ecol. Syst.* 33, 181–233
44. Goulson, D. *et al.* (2015) Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. *Science* 347, 1255–1257
45. Cini, A. *et al.* (2015) Social parasitism and the molecular basis of phenotypic evolution. *Front. Genet.* 6, 32
46. Davidson, A.M. *et al.* (2011) Do invasive species show higher phenotypic plasticity than native species and, if so, is it adaptive? A meta-analysis. *Ecol. Lett.* 14, 419–431
47. Klein, A.-M. *et al.* (2007) Importance of pollinators in changing landscapes for world crops. *Proc. Biol. Sci.* 274, 95–96
48. Schmid-Hempel, P. (2011) *Evolutionary Parasitology: The Integrated Study of Infections, Immunology, Ecology, and Genetics*, Oxford University Press
49. Medzhitov, R. (2001) Toll-like receptors and innate immunity. *Nat. Rev. Immunol.* 1, 135–145
50. Pigeault, R. *et al.* (2016) Evolution of transgenerational immunity in invertebrates. *Proc. Biol. Sci.* 283, 20161136
51. Kappeler, P.M. *et al.* (2015) Sociality and health: impacts of sociality on disease susceptibility and transmission in animal and human societies. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 370, 20140116
52. Wilson-Rich, N. *et al.* (2009) Genetic, individual, and group facilitation of disease resistance in insect societies. *Annu. Rev. Entomol.* 54, 405–423
53. Cremer, S. and Sixt, M. (2009) Analogies in the evolution of individual and social immunity. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 364, 129–142
54. Ugelvig, L.V. *et al.* (2010) Rapid anti-pathogen response in ant societies relies on high genetic diversity. *Proc. Biol. Sci.* 277, 2821–2828
55. Diez, L. *et al.* (2014) Keep the nest clean: survival advantages of corpse removal in ants. *Biol. Lett.* 10, 20140306
56. Zhou, X. *et al.* (2006) Social exploitation of hexamerin: RNAi reveals a major caste-regulatory factor in termites. *Proc. Natl. Acad. Sci. U. S. A.* 103, 4499–4504
57. Stroeymeyt, N. *et al.* (2014) Organisational immunity in social insects. *Curr. Opin. Insect Sci.* 5, 1–15
58. Otterstatter, M.C. and Thomson, J.D. (2007) Contact networks and transmission of an intestinal pathogen in bumble bee (*Bombus impatiens*) colonies. *Oecologia* 154, 411–421
59. Leadbeater, E. *et al.* (2011) Nest inheritance is the missing source of direct fitness in a primitively eusocial insect. *Science* 333, 874–876
60. Buechel, S.D. *et al.* (2014) Social chromosome variants differentially affect queen determination and the survival of workers in the fire ant *Solenopsis invicta*. *Mol. Ecol.* 23, 5117–5127
61. Nygaard, S. *et al.* (2016) Reciprocal genomic evolution in the ant-fungus agricultural symbiosis. *Nat. Commun.* 7, 1–9
62. Ferguson-Gow, H. *et al.* (2014) Colony size predicts division of labour in attine ants. *Proc. Biol. Sci.* 281, 20141411
63. Fouks, B. and Lattorff, H.M.G. (2016) Contrasting evolutionary rates between social and parasitic bumblebees for three social effect genes. *Front. Ecol. Evol.* 4 (64), 2016, Published online April 19. <http://dx.doi.org/10.3389/fevo.2016.00064>
64. O'Shea-Wheller, T.A. *et al.* (2016) A social mechanism facilitates ant colony emigrations over different distances. *J. Exp. Biol.* 219, 3439–3446
65. Boi, S. *et al.* (1999) Coupled oscillators and activity waves in ant colonies. *Proc. Biol. Sci.* 266, 371–378
66. Pinter-Wollman, N. *et al.* (2013) Harvester ants use interactions to regulate forager activation and availability. *Anim. Behav.* 86, 197–207
67. Sumner, S. *et al.* (2007) Radio-tagging technology reveals extreme nest-drifting behavior in a eusocial insect. *Curr. Biol.* 17, 140–145
68. Robinson, E.J.H. *et al.* (2009) Radio tagging reveals the roles of competence, experience and social information in ant decision making. *Behav. Ecol. Sociobiol.* 63, 627–636
69. Hunt, E.R. *et al.* (2016) Ants determine their next move at rest: motor planning and causality in complex systems. *R. Soc. Open Sci.* 3, 150534
70. Mersch, D.P. *et al.* (2013) Tracking individuals shows spatial fidelity is a key regulator of ant social organization. *Science* 340, 1090–1093
71. Woodgate, J.L. *et al.* (2016) Life-long radar tracking of bumblebees. *PLoS One* 11, 1–22
72. Pérez-Escudero, A. *et al.* (2014) idTracker: tracking individuals in a group by automatic identification of unmarked animals. *Nat. Methods* 11, 743–748
73. Xu, B. *et al.* (2014) An accurate multi-cell parameter estimate algorithm with heuristically restrictive ant system. *Signal. Process.* 101, 104–120
74. Kok, V.J. *et al.* (2016) Crowd behavior analysis: a review where physics meets biology. *Neurocomputing* 177, 342–362
75. McKenzie, S.K. *et al.* (2014) Comparative genomics and transcriptomics in ants provide new insights into the evolution and function of odorant binding and chemosensory proteins. *BMC Genomics* 15, 718
76. Simola, D.F. *et al.* (2013) Social insect genomes exhibit dramatic evolution in gene composition and regulation while preserving regulatory features linked to sociality. *Genome Res.* 23, 1235–1247
77. Valcu, C.M. and Kempenaers, B. (2015) Proteomics in behavioral ecology. *Behav. Ecol.* 26, 1–15
78. Jandt, J.M. *et al.* (2015) Lab rearing environment perturbs social traits: a case study with *Polistes* wasps. *Behav. Ecol.* 26, 1274–1284
79. Marioni, J.C. *et al.* (2008) RNA-seq: an assessment of technical reproducibility and comparison with gene expression arrays. *Genome Res.* 18, 1509–1517
80. Risso, D. *et al.* (2014) Normalization of RNA-seq data using factor analysis of control genes or samples. *Nat. Biotechnol.* 32, 896–902

declining populations, resilience to environmental change and propensity to become an invasive species)? To what extent can measures of plasticity be used to predict, and mitigate, changes in species ranges and abundance?

Trend 4: Towards a Unified Framework for Understanding Disease Defence

- What are the mechanisms behind trans-generational immune priming?

- Which kind of costs are associated with disease defence?

- What is the impact of behavioural disease defence on survival and fitness?

- How do social network interactions influence disease transmission and susceptibility?

These four trends are far from exhaustive, but serve to showcase the range of broad biological questions that can be addressed using social insects. Other equally important trends include:

- How are conflicts of interest over fitness resolved among group members?

- What is the relative importance of direct and indirect fitness in the formation of social groups?

- What roles are played by 'social chromosomes' in the evolution of sociality?

- What insights can be provided by a comparative approach to social biology?

- How can insights in automated tracking of social insects inform developments in other fields, including monitoring of cells and human crowds?

- What can epigenetics teach us about behavioural plasticity? Is there an applied role for epigenetics in identifying species for conservation priorities?

- Will new 'omics' approaches (proteomic, lipidomic and metabolomic) alter our understanding of the concept of castes?

81. Gilad, Y. and Mizrahi-Man, O. (2015) A reanalysis of mouse ENCODE comparative gene expression data. *F1000Research* 121, 1–32
82. Li, S. *et al.* (2014) Multi-platform assessment of transcriptome profiling using RNA-seq in the ABRF next-generation sequencing study. *Nat. Biotechnol.* 32, 915–925
83. Simão, F.A. *et al.* (2015) BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. *Bioinformatics* 31, 3210–3212
84. Loverso, P.R. and Cui, F. (2015) A computational pipeline for cross-species analysis of RNA-seq data using R and bioconductor. *Bioinform. Biol. Insights* 9, 165–174