Genome **Biology**

REVIEW

Ant genomics sheds light on the molecular regulation of social organization

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Abstract

Ants are powerful model systems for the study of cooperation and sociality. In this review, we discuss how recent advances in ant genomics have contributed to our understanding of the evolution and organization of insect societies at the molecular level.

Keywords: Ant; Social insect, Caste determination, Communication, Division of labor, Epigenetics, Genome, Genomics, Immunity, Model, Mutualism

Introduction

Ants are among the most ecologically diverse and successful animals on our planet. They have colonized most terrestrial environments, where they occupy keystone positions and have strong ecological impacts owing to their crucial roles as scavengers, predators, granivores, herbivores and mutualists [1]. Understanding the ecological dominance of ants requires investigation into the complex organization of their societies.

The past three years have seen the publication of seven ant genomes [2-8] (Table 1; Figure 1), an achievement that has given the community studying social insects an unprecedented opportunity to investigate ant societies at the molecular level. The ant genomes provide insights into social insect biology that are complementary to those provided by the honeybee genome published in 2006 [9] because ants have a wider variety of social structures, morphotypes, behaviors, colony sizes and diets.

In this review, we focus on six core aspects of ant biology: the production of alternative morphological castes, division of labor, chemical communication, alternative social organization, social immunity and mutualisms (Figure 2). While these issues have been extensively studied from a behavioral and physiological perspective,

only now are we beginning to understand them at the molecular level [10-13]. For each issue, we discuss the advances provided by the ant genomes and their use in subsequent studies (Figure 3). Finally, we propose some avenues of research for future molecular studies.

Caste determination and the division of labor

One of the most striking features of ants is the presence of queens and workers that can differ greatly in morphology, physiology and behavior. In some species, workers (which are all females) can also differ in size and morphology (such as minors, majors and soldiers). In some species, caste determination stems mostly from a developmental switch controlled by environmental factors, whereas, in others, strong genetic effects can also influence the process of caste determination. These genetic influences range from plastic genotypes that are biased toward queen or worker development to a strictly genetic determination [14]. Thus, ants provide an ideal system to investigate how differences in the social environment trigger developmental switches, changes in gene expression and the production of alternative phenotypes.

Recent studies implicate epigenetic processes in caste determination during larval development. Contrary to *Drosophila* fruit flies and *Tribolium* flour beetles, all sequenced ant and bee species have a complete set of DNA methylation enzymes [15]. Genome methylation in mammals and plants is widespread and primarily occurs in repetitive, transposable and regulatory elements, whereas it is found mostly in gene bodies in ants and bees, suggesting a fundamentally different function of DNA methylation [16-18]. In the carpenter ant *Camponotus floridanus* and the jumping ant *Harpegnathos saltator*, DNA methylation is correlated with caste-specific gene expression and alternative splicing, and patterns of DNA methylation change during development [16]. The genomes of the harvester ant *Pogonomyrmex barbatus* and the Argentine ant *Linepithema humile* revealed that annotated genes in the networks that underlie reproductive development, apoptosis and wing polyphenism have fewer CpG sites, the main target sites of DNA methylation, than the genome average [4,5]. This was

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Table 1. Summary of key parameters of seven sequenced ant genomes

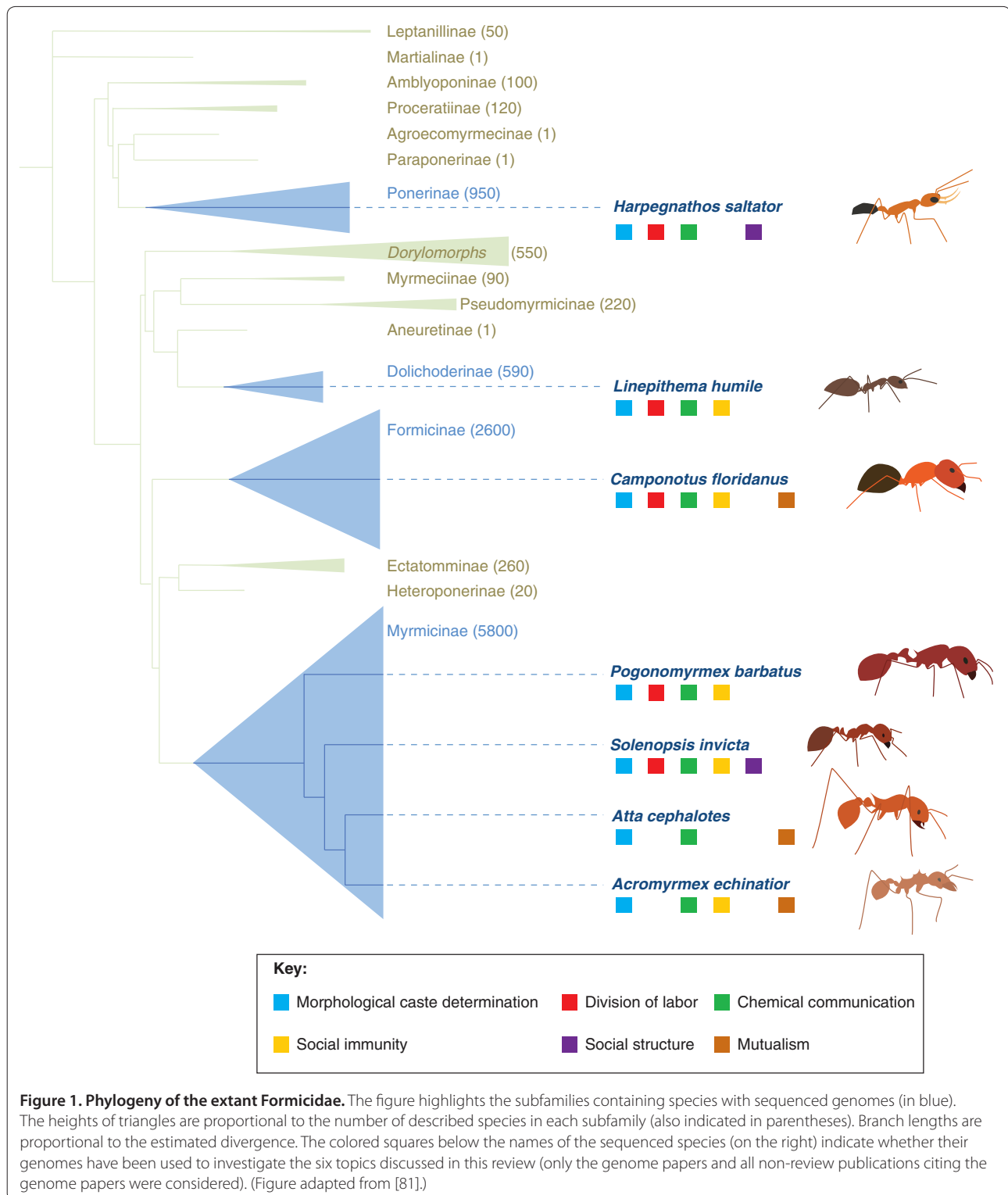|	*Harpegnathos saltator*	*Linepithema humile*	*Camponotus floridanus*	*Pogonomyrmex barbatus*	*Solenopsis invicta*	*Atta cephalotes*	*Acromyrmex echinatior*	Average	Range
Depth of coverage	104x	23x	102x	12x	70x	19x	123x	64.7x	(12x-123x)
Assembly size (Mb)	297	215.6	240	235	353	317	300	279.7	(215.6-353)
Total genome size (Mb)	330	250.8	313	267	608.15	303	335	343.9	(250.8-608.15)
Contig N50 (bp)	38,027	35,858	24,134	11,606	14,674	14,240	62,705	28,749.1	(11,606-62,705)
Scaffold N50 (bp)	59,8192	138,6360	602,923	793,749	720,578	5,154,504	1,094,267	1,478,653.3	(598192-5154504)
G+C composition (%)	45	38	34	37	36	33	34	36.7	(33-45)
Protein-coding genes	18,564	16,123	17,064	17,177	16,569	18,093	17,278	17,266.9	(16,123-18,564)
Orthologs + co-orthologs	11,695	12,860	11,433	12,857	12,590	12,617	12,121	-	-
Species-specific genes	6,869	3,263	5,631	4,320	3,979	5,476	5,157	4,956.4	(3,263-6,869)
Manually curated genes	400	1,000	400	1,200	0	522	200	531.7	(0-1,200)
Genes with EST support (%)	84	51	81	43	56	40	84	62.7	(40-84)
microRNA	159	71	96	100	NA	68	93	97.8	(68-159)
Total repeat content (%)	26.9	23.5	15.1	11.5	NA	25.1	28	21.7	(11.5-28)

EST, expressed sequence tag; NA, not available.

interpreted as evidence for elevated rates of methylation in these genes because DNA methylation leads to increased mutation rates and thereby a depletion of CpG sites [19,20]. By contrast, the opposite pattern was observed in the leaf-cutting ant *Atta cephalotes* [6], suggesting that there might be important interspecific variation in how DNA methylation affects the production of alternative phenotypes. This is consistent with the recent finding that ant genomes exhibit distinct genome-wide depletion of observed relative to expected CpG sites [20]. Changes in gene transcription are also associated with histone modifications and changes in chromatin structure between castes and developmental stages in *H. saltator* and *C. floridanus* [2,21]. Both genome methylation and histone modification might therefore influence caste determination through transcriptional control and alternative splicing.

A recent comparative analysis suggested that the abundance and diversity of transcription factor binding sites (TFBSs) might also play a role in the evolution of caste-specific patterns of gene expression [20]. TFBSs were found to be more divergent within ants than between social and solitary insects, and genes exhibiting important changes in the abundance of TFBSs between social and solitary insects showed higher levels of gene expression plasticity between castes in *C. floridanus* (high polymorphism and reproductive division of labor) compared with *H. saltator* (low polymorphism and reproductive division of labor). Furthermore, the ant genomes revealed that the networks commonly known to exhibit phenotypic plasticity between castes (such as the neuroendocrine system) were preferentially targeted for regulatory changes during the evolution of sociality [20].

The genome sequences also facilitate the identification and study of candidate genes in the process of caste differentiation [5-7,20]. For example, analysis of insulin signaling, juvenile hormone and vitellogenin expression during artificial hibernation and hormone manipulation revealed how the environmental cues experienced by *Pogonomyrmex rugosus* queens are translated into the production of new queens and workers [22]. Similarly, the fire ant (*Solenopsis invicta*) was found to harbor two insulin receptors, which might play a role in the process of caste determination [23]. Finally, a study on molecular evolution in *S. invicta* revealed a positive association between caste-biased gene expression and the rate of gene evolution, the latter being driven largely by variation in the strength of purifying selection [24]. This study also showed that high rates of gene evolution actually preceded gene expression bias associated with the evolution of castes, suggesting that fast-evolving genes are more likely to be recruited to the processes underlying phenotypic plasticity [24].

Genetic effects on morphological caste determination have been found in *L. humile* [25] and the leaf-cutting ant *Acromyrmex echinatior* [26,27]. An extreme case of genetic caste determination occurs in the genus *Pogonomyrmex*, where some populations contain differentiated genetic lineages, most or all of which derive from historical hybridization between the harvester ants *P. rugosus* and *P. barbatus* [28-32]. These lineages always occur in pairs [33], and queens in each lineage-pair mate multiple times with males of their own as well as with males of the alternative lineage. Inter-lineage offspring develop into workers, whereas intra-lineage offspring develop into queens. Thus, the only way that a queen can

Figure 1. Phylogeny of the extant Formicidae. The figure highlights the subfamilies containing species with sequenced genomes (in blue). The heights of triangles are proportional to the number of described species in each subfamily (also indicated in parentheses). Branch lengths are proportional to the estimated divergence. The colored squares below the names of the sequenced species (on the right) indicate whether their genomes have been used to investigate the six topics discussed in this review (only the genome papers and all non-review publications citing the genome papers were considered). (Figure adapted from [81].)

produce a colony with both workers and queens is by mating with males of both lineages. Crossing experiments revealed that intra-lineage individuals are developmentally constrained to become queens [32]. Inter-lineage individuals have partly retained plasticity and can develop into queens under some conditions, but the association of genotype and caste is very strong, with almost no adult females presenting a mismatch between
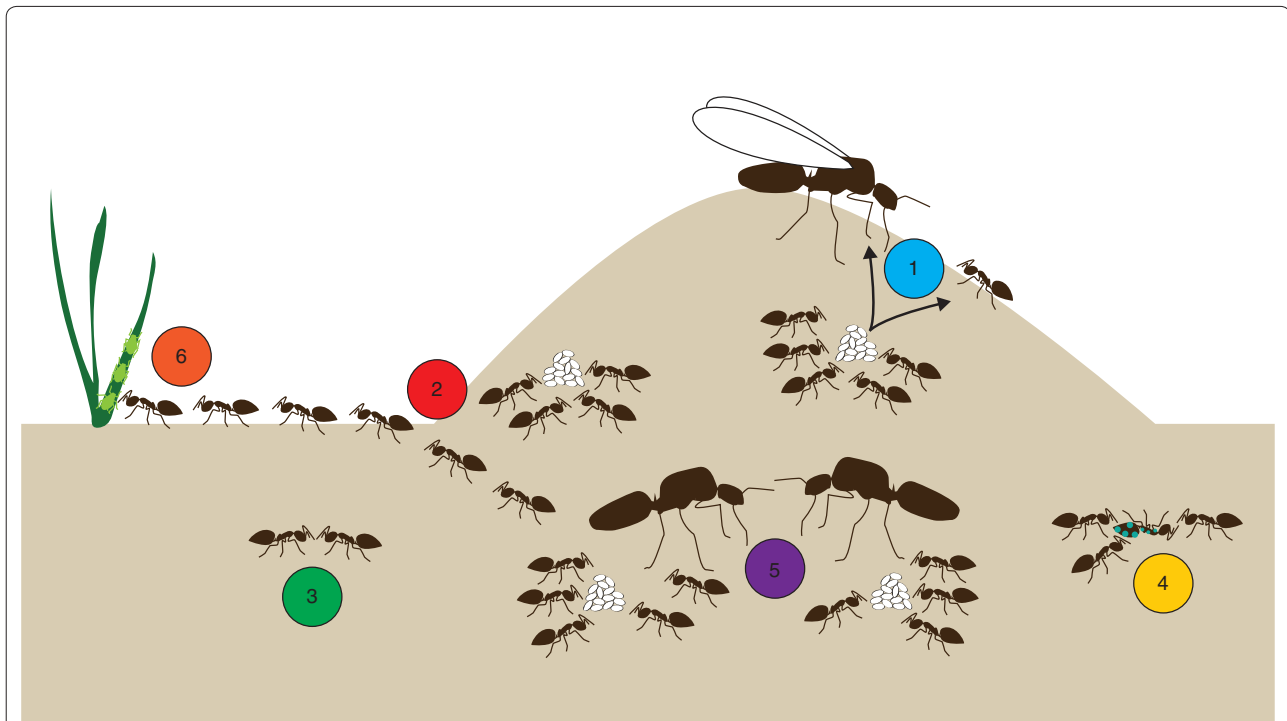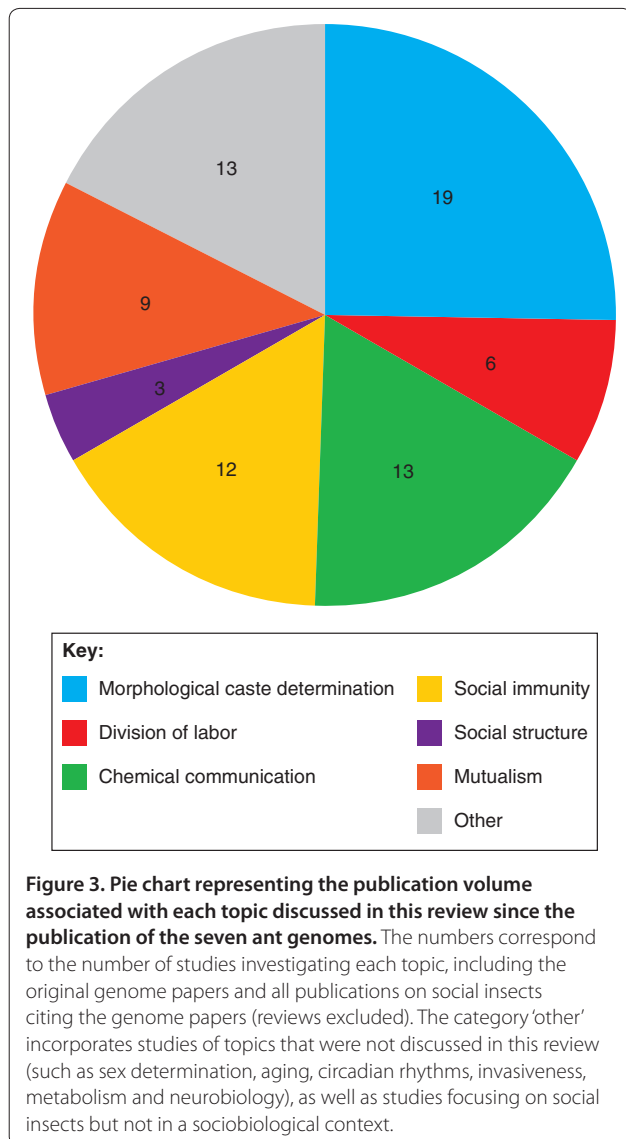
Figure 2. Diagram of a typical ant colony representing the six topics discussed in this review. (1) Morphological caste determination. In most ant species, a switch during larval development triggers alternative trajectories toward different female castes. Only the queen and one-worker caste are represented here, but some species also have different morphological worker castes. **(2)** Division of labor. Different groups of workers perform different tasks. Here, some workers nurse the brood inside the nest, whereas others forage for food outside. **(3**) Chemical communication. Ants rely on chemical communication for many aspects of their social organization. **(4)** Social immunity. Ants use both behavioral and physiological defenses to limit the transmission of pathogens and diseases in their societies. **(5**) Social structure. Ant species differ in the number of queens found in one nest, as well as the number of males that the queens mate with (not shown here). In some cases, these numbers vary between colonies of the same species. **(6)** Mutualism. Many ant species engage in mutualistic interactions with other organisms. Here, some ants tend and protect aphids in exchange for the sugary honeydew they produce.

the genotype and expected phenotype [34]. This provides an interesting system to compare the epigenetic and gene expression changes across developmental stages between individuals for which caste fate is already known [17].

In addition to the reproductive division of labor between queens and workers, there is also usually a strong division of labor between workers, which specialize in different tasks. Worker behavior is influenced by several factors, including size and morphology [35-37], age [38,39], individual experience [40,41] and genetic background [42,43]. Worker behavior and task specialization in ants are often regulated by genes that also affect behavior in solitary insects (the *foraging* gene and circadian clock genes, for example) [44-48].

Reproduction and behavior are often interconnected in animals. In solitary wasps, for instance, females with developed ovaries lay eggs, whereas females with un-developed ovaries forage for food. Accordingly, studies in the honeybee *Apis mellifera* suggest that physiological pathways regulating reproduction and behavior in

solitary insects have been co-opted for the regulation of worker behavior in social insect species [49,50]. Studies in the ant *Pogonomyrmex californicus* revealed that nurses and foragers differ in ovary activity [51] and juvenile hormone levels [52], which are known to affect the production of vitellogenin (typically involved in reproduction) [22]. Interestingly, phylogenetic analyses revealed the existence of multiple genes encoding vitello-genin in most of the ant genomes sequenced [53], suggesting that an initial duplication of the ancestral vitellogenin gene occurred after ants diverged from bees and wasps. The ant vitellogenin genes cluster in two paralogous gene families that show caste- and behavior-specific expression in *S. invicta* [7] and *P. rugosus* [53], suggesting that vitellogenin in ants not only regulates reproduction but also the behavior of sterile workers [53]. The finding that vitellogenin plays similar roles in ants and bees, which evolved sociality independently, supports the hypothesis that the co-option of repro-ductive pathways plays a major role in social evolution.

Figure 3. Pie chart representing the publication volume associated with each topic discussed in this review since the publication of the seven ant genomes. The numbers correspond to the number of studies investigating each topic, including the original genome papers and all publications on social insects citing the genome papers (reviews excluded). The category 'other' incorporates studies of topics that were not discussed in this review (such as sex determination, aging, circadian rhythms, invasiveness, metabolism and neurobiology), as well as studies focusing on social insects but not in a sociobiological context.

Chemical communication

Effective communication is an important ingredient for any society to function efficiently. Ants rely heavily on chemical communication for social organization and to discriminate nestmates from non-nestmates [1]. The importance of chemical communication in ants is reflected by large expansions of chemosensory gene families and metabolic pathways for cuticular hydrocarbons in all ant genomes that have been studied in this respect compared with other sequenced hymenopterans [2,4,5,7,54]. A comparative analysis of antennal transcriptomes between *C. floridanus* and *H. saltator* identified many chemo-receptors differentially expressed between males and females, as well as between species, suggesting that sex- and species-specific biology is likely to have shaped the expression patterns of genes involved in communication [54].

A recent study of chemosensory protein genes revealed contrasting modes of evolution between genes occurring only in ants and those also found in the honeybee [55]. Clades with ant-specific expansions showed evidence of faster evolution and elevated levels of positive selection compared with clades of one-to-one orthologs across all ants and the honeybee. A possible explanation is that the more conserved chemosensory protein genes that occur in both ants and bees are associated with more general features of social insect biology, whereas genes in ant-specific expansions might be related to more idiosyncratic environmental and social conditions [55]. At the same time, ant-specific desaturase gene families, which are involved in the production of chemical signals, show an elevated number of genes and high variability in both sequence and expression, possibly reflecting an increased demand for diversity in the chemical signals used in ant communication [20].

Social structure, social immunity and mutualisms

There is tremendous variation across ant species in social organization and the number of queens per nest. Such variation in the breeding system can also occur within species, as in *S. invicta*, where colonies can have one queen (monogyne) or many queens (polygyne). As is the case in other ants, the two social forms not only differ in the number of queens but also in many other traits (such as the reproductive potential of queens, odor and size of both queens and workers, aggressiveness of workers and number of sperms produced by males) [7,56-58]. The social phenotype of *S. invicta* is completely associated with two allelic variants at a single locus (*Gp-9*) encoding an odorant binding protein [56,59,60]. *Gp-9* was recently found to be located on a pair of heteromorphic social chromosomes (SB and Sb) comprising a large (12.7 Mb) non-recombining genomic region [57]. It is likely that several of the 600-plus genes in the non-recombining region are involved in the many phenotypic differences characterizing individuals harboring the alternative social chromosomes. Comparative studies revealed that these social chromosomes have many properties typical of sex chromosomes. First, the lack of recombination is also associated with several inversions. Second, one of the two variants (the Sb chromosome) occurs only in one type of social organization (the polygyne form), just as the Y chromosome occurs only in males. Third, the Sb chromosome cannot recombine with itself because individuals having two copies of this chromosome die within weeks after reaching the adult stage. Finally, the inability of the Sb chromosome to recombine with itself or with the SB chromosome has been associated with the accumulation of many repetitive elements, in a manner similar to that of the Y chromosome [57]. While this is the first description of a social chromosome, it is likely

that such supergenes also exist in other social insects. Polymorphism in social organization has evolved independently numerous times in ants, where many species have both monogyne and polygyne colonies. The occurrence of the polygyne social form is associated almost invariably with a *polygyny syndrome* whereby, as in *S. invicta*, polygyne queens are smaller, accumulate reduced amounts of fat during sexual maturation, have lower fecundity and initiate new colonies with the help of workers, rather than independently [61]. Interestingly, it appears that variation in queen number is also associated with a single non-recombining region in the ant *Formica selysi* (J Purcell, A Brelsford and M Chapuisat, unpublished data).

Another important aspect of social life is that it facilitates the transmission of pathogens and diseases owing to the associated high population densities and frequent social contacts [62]. Thus, one could expect social insects to have more efficient immune systems and more genes involved in immunity compared with solitary species. Surprisingly, early comparative analyses reported that both the honeybee and the ants have fewer immune genes than do *Drosophila melanogaster* and *Tribolium castaneum* [4-6,9,63-66]. Social insects have multiple collective behavioral defenses against pathogens, such as grooming other colony members [67] or the intake of tree resin with anti-pathogenic properties [68]. It has thus been proposed that such prophylactic behaviors might reduce the selective pressure for increasing the number of immune genes in the genome [4-6]. However, a recent comparative-genomic analysis shows that only 3 of 16 immune-gene families differed significantly between social and solitary insect species [20]. This finding, combined with the fact that the non-social parasitoid wasp *Nasonia vitripennis* also contains fewer immune genes than do flies and beetles [69], suggests that the depletion of immune genes in social insects is not as dramatic as initially proposed and might not be directly associated with sociality.

Specific features of the ant immune system could also account for the lower number of immune genes in their genomes. The genome of *C. floridanus* revealed the antimicrobial peptide hymenoptaecin to be a large precursor protein with multiple bioactive domains [65,66], suggesting a diverse array of possible immune functions. Hymenoptaecin is present in all other ants, the honeybee, as well as *Nasonia vitripennis* [5,65,69]. Finally, behavioral analyses combined with RNA-seq of genes implicated in physiological immune defenses in *A. echinatior* confirmed the existence of efficient prophylactic behaviors and showed that, in most conditions, pathogenic challenges triggered an increase in immune gene expression. However, ants challenged with a fungus-garden pathogen showed a decrease in immune gene expression while displaying more prophylactic behaviors, suggesting that trade-offs might occur between physiological and behavioral immune responses [70].

Another key type of interspecific interaction in ants lies in the evolution of diverse and ecologically important forms of mutualisms with plants (*Acacia* trees, for example) [71] and other insects (such as aphids) [72]. Approximately 50 million years ago, one ant clade also evolved a complex system of fungus farming [73]. The current repertoire of sequenced ant genomes includes two species of fungus farming leaf-cutting ants (*A. cephalotes* and *A. echinatior*), which cultivate their food fungus on leaf fragments harvested from the vegetation surrounding their nest [1]. The analysis of their genomes revealed that the genes necessary for the biosynthesis of the amino acid arginine are lacking (in contrast to *C. floridanus*, *S. invicta* and *H. saltator*), but that they are present in their symbiotic fungus [3,6]. This suggests that leaf-cutting ants have become completely dependent on their food fungus over evolutionary time. These findings highlight the benefit of genomic resources for identifying the potential physiological consequences of evolution in complex societies and pave the way for further studies to better understand the genomic impact of obligate mutualisms in ants and other organisms.

Concluding remarks

Analysis of the seven sequenced ant genomes has already led to significant advances in our understanding of important aspects of ant biology. Below, we highlight three avenues of research that we believe will prove fruitful as this endeavor continues.

First, large comparative analyses of the genomes of ants, social bees and social wasps (which evolved sociality independently) with the genomes of solitary bees, wasps and other insects will be needed to investigate the key evolutionary changes associated with sociality in the Hymenoptera. The only study that has conducted such a comparative analysis so far has provided valuable information on the evolution of several aspects of social organization (caste determination, chemical communication and social immunity), as discussed above [20].

A second important step will be to perform functional studies to validate experimentally the numerous hypotheses generated by comparative analyses. So far, only a handful of studies have manipulated gene expression in ants using RNA interference [74,75], hormonal treatments [22,76] or pharmacological manipulations [47], and none of them investigated the consequences at the genome scale. Forward genetics, using, for example, random mutagenesis, has been prohibitive in ants and other social insects because these approaches require substantial subsequent crossing. This is not feasible in ants mainly owing to their long generation times and the

difficulty to breed most species in the laboratory. However, reverse-genetic approaches for social insects using new and highly effective tools for targeted genome editing have now come within reach. These approaches use engineered nucleases, such as zinc-finger nucleases (ZFNs) [77], transcription activator-like effector nucleases (TALENs) [78] or CRISPR RNA-guided Cas9 nucleases [79,80]. Although these techniques will not be able to overcome the fundamental experimental limitations posed by many ant species, they are now opening up the possibility of creating transgenics and genetic knockouts for a subset of carefully chosen model species that can be propagated in the laboratory. Studies using combinations of genetic, pharmacological, social, hormonal and phero-monal manipulations will be necessary to provide a better understanding of the actual roles of different genes and physiological pathways in regulating ant social life.

Finally, understanding the molecular organization of ant societies will require precise behavioral data at the individual level to investigate the links between com-munication, gene expression, physiology and behavior. Collecting such data has long been a difficult task, but a new automated tracking system now allows the automatic quantification of all social interactions between all individuals in an ant colony over the course of several weeks. Using this system has already demonstrated the importance of spatial distribution in the regulation of age-related division of labor in insect societies [39]. The use of such sophisticated behavioral tracking and quantification, combined with next-generation genomic data, could well provide the next answers to the big questions in the biology of social insects.

In conclusion, social insects have played a central role in our understanding of the organization and behaviors of complex animal societies, principally from ethological and ecological perspectives. The advent of next-genera-tion sequencing techniques now provides opportunities to use social insects to study how genetic and environmental contributions interact to control societal organization.

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