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Genomic sources of phenotypic novelty in the evolution of eusociality in insects

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Genomic resources are now available for closely related species that vary in social behavior, providing insight on the genomics of social evolution. Changes in the architecture of gene regulatory networks likely influence the evolutionary trajectory of social traits. Evolutionarily novel genes are likely important in the evolution of social diversity among insects, but it is unclear whether new genes played a driving role in the advent or elaboration of eusociality or if they were instead a result of other genomic features of eusociality. The worker phenotype appears to be the center of genetic novelty, but the mechanisms for this remain unresolved. Future studies are needed to understand how genetic novelty arises, becomes incorporated into existing gene regulatory networks, and the effects this has on the evolution of social traits in closely related social and solitary species.

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Introduction

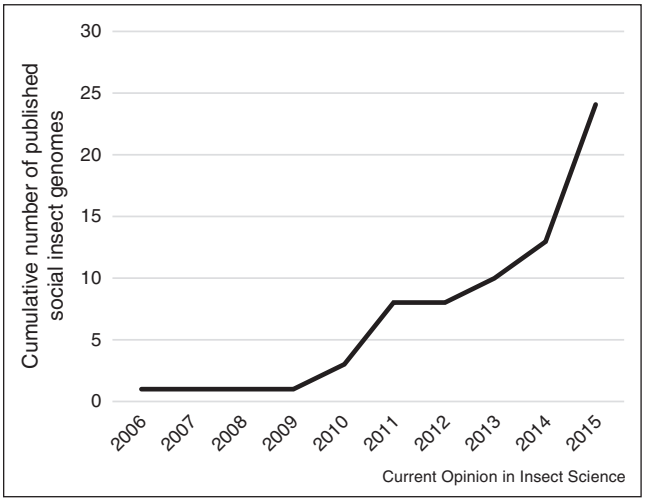
The 2006 publication of the honey bee genome marked a milestone in the field of sociogenomics, the study of social life in molecular terms [1,2]. At the time, this was one of only a few insect species with a published genome, and the first from the group of insects known for their extraordinary social lives — the termites, ants, wasps, and bees. A recent growth of genomic resources available for insects with variable social behavior (Figure 1) provides a refined ability to understand how the genome evolves in association with both the origins of eusociality (i.e., from a solitary lifestyle to the early stages of organized social life) and the further elaborations of complex forms of eusociality from simple societies. This review summarizes recent insights gained from the use of comparative genomics to understand the molecular basis for social evolution and highlights key areas that may be important for future research. The results highlight emerging themes with novel hypotheses related to the conservation of genes and gene networks associated with independent origins of sociality, the origins of evolutionarily novel genes and their role in eusociality, and the evolutionary processes that may lead to genetic novelty associated with the worker phenotype.

Variation in gene regulation as a source of phenotypic novelty

In eusocial insects, individual colony members belong to castes with striking differences in morphology, anatomy, physiology, and behavior, yet share highly similar genomes. Queens specialize on reproductive activity, and workers are responsible for brood care, nest maintenance, nest guarding, and foraging. In bees, ants, and wasps, these roles are divided among adult females, but both sexes in multiple stages of development participate in division of labor in termite colonies. In this way, eusociality is similar to many other complex traits, in that it is multigenic, expressed across multiple tissues, developmental stages, and individuals within a colony, and is highly influenced by variation in both the ecological and social environment. It has long been recognized that evolutionary modifications to the temporal and spatial expression of protein-coding genes play an important role in generating novel phenotypes [3], and early experiments showing that differential expression of certain genes was associated with phenotypic plasticity in social insects [4,5] supported the idea that changes in gene regulation may also be an important component of the evolution of eusociality [6]. Additional support for this has accumulated with myriad studies identifying sets of genes that are differentially expressed between castes or subcastes of a broad range of social insect species [7–16,17••,18–22]. This suggests that changes in gene regulation is a mechanism by which ancestral solitary life histories (i.e., ground plans) could be modified to produce complex societies with division of labor, via changes in the timing [23] or developmental patterns [24–26] of the expression of key genes.

These key genes were proposed to be part of a 'genetic toolkit', defined as a set of conserved genes or molecular pathways that are repeatedly recruited into functions related to eusociality via evolutionary changes in the timing and spatial context of their expression [27]. Support for this hypothesis is mixed. Evidence for a shared set of genes or molecular pathways that are differentially

Figure 1



Line graph of the cumulative total number of social insect genomes (ants, bees, vespid wasps, termites) published each year and available at NCBI (www.ncbi.nlm.nih.gov/genome; accessed 12 August 2015). Two of the genomes currently available on NCBI have not yet been published.

expressed among castes has accumulated across a broad range of taxa [7,14–16,17••,18,19,28,29]. However, the proportion of these differentially expressed genes that are shared between studies is often very small, even if statistically significant, and some studies find little or no overlap between species at all [7,13,29,30•]. This lack of overlap may reflect methodological differences. Eusociality is a dynamic suite of behavioral, physiological, and ecological traits, and there is little reason to expect the same genes to be consistently differentially expressed between castes or subcastes of different species in different tissues, stages of development, and ecological contexts at all times. From studies that have characterized caste-biased gene expression in multiple developmental stages in ants, it has become evident that few genes are expressed exclusively in one caste, and many genes shift the direction of expression bias between castes across developmental stages [18,22]. Yet, a single stage of development and whole organism sampling is often the level at which comparisons are made, and thus represent a snapshot of gene expression at the time of collection, averaged over all the cells in the tissue being sampled. These snapshots probably capture a great deal of variation that render direct comparisons across studies less meaningful. Further sampling of multiple tissues at multiple stages of individual and colony development will provide the necessary data for meaningful tests of the genetic toolkit hypothesis.

There is also a potential evolutionary explanation for the lack of overlap, however. An important component of the genetic toolkit hypothesis is the assumption that the same conserved set of genes regulates physiology and behavior in a broad distribution of solitary ancestors of social insects, such that when they are co-opted for eusociality in different lineages, they are expected to be the same. This assumption has never been tested, and the genetic basis for physiology and behavior in solitary insects representative of the species ancestral to social insects is relatively understudied. There is likely to be a set of conserved genes that are common to reproduction in all insects, but there are also likely to be novel features of these pathways in each species. If it is the lineage-specific elements of these pathways that are recruited into social functions, then this could explain the small proportion of conserved genes differentially expressed between castes among species with independent origins of eusociality. Evidence for lineage-specific evolution of gene families and cis-regulatory relationships involved in social traits was found in a recent comparative study in bees [31••], and thus illustrates how different genes from similar gene networks could be recruited into social functions in each instance of eusociality. This would also explain why more similarity is found at the level of molecular pathways than at the level of individual genes [15]. Tests of the genetic toolkit hypothesis could be substantially improved with detailed characterization and experimental manipulation of key genetic pathways underlying traits related to eusociality (e.g., wing development networks [32]) in closely related solitary and social species. This would help to reveal the origins of lineage-specific differences in gene regulatory networks and the evolutionary processes that determine which components of a network are co-opted for eusociality.

Gene regulatory networks as drivers of evolutionary novelty

As is the case for understanding the evolution of any complex phenotype, an important step in determining how changes in gene regulation function in the evolution of eusociality will be to extend comparisons beyond analyses of differential gene expression to systems-level analyses of gene regulatory networks [33,34]. Gene regulatory networks encode the spatial and temporal patterns of mRNA abundance on the basis of the functional linkages between genes and their regulators, which may include DNA sequences, proteins, and epigenetic tags. Each gene regulatory network is probably comprised of several subnetworks, some of which are more evolutionarily malleable than others [35]. Some recent studies have evaluated gene expression patterns within the context of modules of co-expressed genes [29,36••], but a systems biology approach will require integrating relationships between transcription factors and the genes they regulate [37,38,39•,40•], epigenetic profiles [41–45], chromatin structure and histone modifications [42,46], use of alternative transcription start-sites [39•] and alternative splicing [47], expression of microRNAs [48–50], and patterns of RNA editing [51•]. Additional

research in proteomics [52–54], will also reveal important functional links between transcription and protein products [33]. This may seem like a daunting task, but newly developed technologies, such as single-cell RNA sequencing [55], cap analysis of gene expression (CAGE) [39•], detection of microRNAs [49] and RNA editing events [56] through RNA sequencing should enable the reconstruction of robust regulatory networks in social insects. Additionally, transgenics has recently been developed for honey bees [57••], which will allow direct tests of hypothesized relationships through manipulation of gene expression patterns. This is important, because recent studies have found evidence that regulatory changes are a common component of social evolution [31••,58•,59,60••], but the meaning of these changes is not clear without an understanding of the regulatory architecture in which they function.

Systems-level investigations of gene regulation have also been useful in elucidating the evolutionary processes associated with phenotypic novelty across taxa [61,62], and recent studies with social insects have provided support for two predictions concerning the effects of genetic regulatory architecture on evolution. First, genes with an expression bias among castes are expected to evolve more rapidly than genes expressed similarly in all individuals, because genes with conditional expression are probably under reduced pleiotropic constraint [63] and are less exposed to selection [64]. In turn, expression variation may be more likely to evolve at loci that are already under relaxed selection, and thus removed from selective constraint [65,66]. In support of this relationship, genes with conditional expression patterns also have higher rates of protein divergence within the ant genus *Solenopsis* [65] and in honey bees (*Apis mellifera*) [66]. However, other studies have revealed that this relationship is nuanced, and conditional expression cannot account for all the variation in rates of protein evolution [21,29,60••,67]. A major limitation to resolving these nuances has been that caste-biased genes are typically defined on the basis of a single study of a single tissue (or whole body) at a single developmental stage (but see [18,22]). Reconstructing gene regulatory networks in multiple tissues, castes, and developmental stages will provide more complete expression profiles against which to compare evolutionary rate [22]. It will also provide a comprehensive view of the genomic features that limit or enhance expression of each gene, which could resolve some of the nuances in the relationship between conditional expression and social evolution.

The second prediction regarding genetic architecture and social evolution is that genes at the core of a regulatory network are likely to be more evolutionarily constrained than genes at the periphery. This is because functional changes in genes that are highly connected and central (i.e., 'core') to other genes (i.e., 'peripheral') in a regulatory network are likely to disrupt many biological processes. Support for this comes from analysis of a honey bee transcriptional regulatory network characterizing behavioral traits [59]. Highly connected and centrally located protein coding genes, both transcription factors and their target genes, were under stronger purifying selection than weakly connected or peripherally located genes [59]. It was further found that genes with low connectedness and high tissue specificity are responsible for novel phenotypes via sequence evolution in specialized tissues related to social traits in honey bees, such as the sting gland (used for defense against vertebrates, rather than invertebrates) and the hypopharangeal gland (used by nurse bees to make food for nestmates) [36••].

One exception to this rule seems to be in the brain, the center of behavioral and social plasticity. In honey bees, gene evolution in nervous tissue was not related to network structure, as it was in specialized tissues related to social traits [36••]. Likewise, increases in social complexity across ten bees was accompanied by increasing purifying selection on genes related to brain development, with increasingly rapid evolution of genes involved in splicing, transcription, and translation [31••]. This suggests that brain functions related to eusociality evolve through changes in gene regulation, rather than changes to protein-coding genes that function in brain development [31••], as is known for human brain evolution [68]. As our ability to reconstruct sophisticated gene regulatory networks improves in more species, we will be better poised to distil the causal relationship between a gene's position in a network, its expression profile, and the effect this has on the evolutionary trajectory of social phenotypes.

New genes as drivers of evolutionary novelty

Gene regulatory network reconstruction will not only help to identify the degree to which conserved genes function in social evolution, but can also reveal the role of lineage-specific genes in social evolution. Novel genes, or genes with no known homology, play an important role in evolutionary novelty as they evolve new molecular functions and cellular localizations, contribute to stress resistance, and can influence development [69,70]. These processes are likely to play a role in the evolution of eusociality as well. An important caveat to the study of new genes is that homologs are likely to be found for many novel genes as genomes from more closely related species are sequenced. Nonetheless, a large number of novel genes have been noted in the genomes of many social insects, and in several cases they are among those associated with social traits or are differentially expressed between castes [7,13,36••,60••,71,72,73••], though how prevalent these genes are among those related to social behavior is variable across studies [15,74]. In honey bees, more than 90% of the total expression in simple tissues

with evolved specialized functions for social coordination stemmed from novel genes, but no such enrichment was found in the expression profiles of highly conserved tissues [36••]. A similar pattern of high tissue specificity was also found for novel genes in primates [75], suggesting that there may be foundational links between the evolution of new genes, their expression patterns, and function.

If novel genes are an important source of social novelty, then it will be important to investigate the process by which novel genes arise. As more genomic resources become available for species with variable social behavior, it will be important to distinguish between origins of eusociality from a solitary ancestor and elaborations of complex eusociality, as well as species-specific ecology when discussing the evolutionary role of novel genes. For example, it has been suggested that novel genes that function in sophisticated social coordination would be favored by selection in species that live in highly complex eusocial colonies, and are therefore more likely to be involved in elaborations of eusociality or lineage-specific adaptations than they are to play a significant role in the origins of eusociality from a solitary ancestor [76,77]. There is some evidence to support this secondary role for novel genes. First, the most comprehensive studies of novel genes (i.e., studies that have used rigorous methods to validate that novel genes are robust) have heretofore focused on species with highly derived forms of eusociality, such as ants [58•,73••] and honey bees [36••]. In these species, novel genes function in species-specific aspects of ecology and social biology, rather than in common features of eusociality. Most hymenopteran novel genes originate de novo from intergenic regions, either from precursor open reading frames (ORFs) (i.e., gene birth) or they are remnants of old genes that have been lost by all but one lineage (i.e., gene death) [73••], and species in the order Hymenoptera acquire novel genes at a relatively high rate, as compared to Diptera, when evolutionary distance between species-pairs is accounted for [73••]. This elevated birth/death rate of genes may provide the substrate for lineage-specific adaptations throughout Hymenoptera, independent of social biology. In accordance with this, two species of *Polistes* wasps that share a common origin of eusociality do not share common novel caste-biased genes, suggesting novel genes do not function in the origins of eusociality [15]. (The caste-biased genes were assessed at two different developmental stages in the latter study however, so conclusions based on this result require follow-up.) Additional comparisons of expression profiles of novel genes in lineages of closely related species that vary in social biology will help to determine how often these genes are involved in lineage-specific aspects of socio-ecology or if they are central to the evolution of eusociality.

The causality of this relationship may also be reversed. It is possible that features of eusociality lead to changes in the genome that give rise to new genes. Social insects, and honey bees in particular, have exceptionally high recombination rates throughout the genome [78–80], and especially in regions of high GC content [67,81,82••]. High recombination regions of the genome have recently been identified as an important source for genetic diversity in honey bees and plants [83], and could therefore lead to increases in gene birth. This may also be true for ants, because novel genes tend to have increased GC-content compared to non-novel genes [73••]. Thus, the high levels of recombination associated with eusociality may contribute to the lineage-specific elaboration of eusociality by producing genetic novelty.

Conversely, genomic regions of restricted recombination (i.e., supergenes) have also recently been found to influence key aspects of social organization in fire ants (*Solenopsis invicta*) [84], and it has been suggested that suppression of recombination, resulting in the evolution of supergenes, may play a key role in many social traits [85,86]. The high rates of recombination found among highly eusocial insects may in part explain why there have not been discoveries of supergenes in more species. Additional comparative studies are needed to identify the causal relationships between recombination and social evolution, as well as conditions that favor a relationship between elevated versus suppressed recombination and eusociality.

Understanding the source of genetic novelty and how it influences social traits through evolution will require additional study of the function of novel genes and the reasons they are more likely to arise in certain areas of the genome than others. Further evaluation of the hypothesized secondary role for novel genes in social evolution will require rigorous investigation of novel genes found among closely related species that vary in social behavior in multiple phylogenetic clades.

Workers as a center of genomic novelty

The worker phenotype initially presented a special challenge for Darwin to explain through natural selection, due to its definitive lack of reproductive success [87]. Both Darwin and later theoreticians determined that selection for the worker phenotype must be routed through family members (e.g., queens) that benefit from the help of workers [88]. This model has been formally expanded into a general theory of inclusive fitness (i.e., selection through direct and indirect fitness costs and benefits), and the genetic basis for worker behavior has traditionally been treated as a 'black box' or regarded as an 'allele for altruism' in models stemming from this theory [88]. The post-genomics era has provided an opportunity to open that black box, and doing so has revealed the genomic features that underlie worker phenotypes and the evolutionary processes that act on them. The emerging picture is that workers are a key source of

genomic and phenotypic novelty. Worker-biased genes are more likely to have novel or unknown functions in ants [[7], but see [22]] or lack homology to other known genes when compared to queen-biased genes in *Polistes* wasps [13] and honey bees [71,89], suggesting evolution of the worker phenotype involved the acquisition of new genes. Furthermore, worker-biased genes in honey bees and ants [9,29,60••,67,90] are evolving more rapidly than queen-biased genes [but see [21,22,66]]. Some of this may be because selection is less effective on genes with indirect social effects on colony fitness, such as those coding for worker traits [91], or because conditional expression reduces the effectiveness of purifying selection [64]. There is also evidence for adaptive evolution of genes with worker-biased expression or that code for worker traits [60••,90]. In *P. canadensis* wasps, caste-biased genes do not show signatures of rapid evolution, possibly because genes in this species are probably under antagonistic pleiotropic constraint as division of labor is not as strong in this species, as compared to honey bees, and genes may function in both worker-related and queen-related phenotypes [13,63,92].

Worker-biased novelty may also initially arise as a by-product of the high recombination rates observed among highly eusocial species. In honey bees, worker-biased genes are in high-recombination regions of the genome [67,93], though it is unclear whether this pattern is specific to worker-biased genes or caste-biased genes in both queens and workers [82••]. If this bias is truly specific to worker-biased genes, this localization pattern presents the opportunity for mutational novelty in genes that are more likely to be expressed in workers. It is not clear, however, how these mutations would be subsequently treated by selection, because although selection is more effective in high recombination regions [94], it is weaker under conditional expression [64], as would be the case for worker-biased genes. Rigorous identification of genes underpinning worker traits in more species that vary in social complexity, using comparative transcriptomics, gene-knockdown studies, and transgenic techniques as methods are developed for use in non-model organisms, will provide important material to help to elucidate how selection operates on worker phenotypes and how this influences social evolution. These data can then be integrated with models from inclusive fitness theory to improve understanding of how novel phenotypes arise despite a lack of direct fitness.

Conclusions

Researchers interested in the genomic basis of social evolution face similar challenges of those researching the evolution of other complex traits. The phenotypes that characterize social behavior extend across individuals, developmental stages, tissues, and are highly influenced by the ecological and social environment. The recent increase in availability of genomic resources for closely related species of insects that vary in sociality has created an opportunity to investigate the genomic basis of social evolution through increasingly informative comparisons (Figure 1). The comparative approach is useful for disentangling the genomic underpinnings at each level of phenotypic complexity. An emerging theme of these refined comparisons is that while there may be common features of independent origins and elaborations of eusociality, the details are largely lineage specific [31••,58•]. Numerous studies, across many taxa of social insects provide compelling evidence that changes in the regulation of conserved gene networks have allowed for the decoupling of the reproductive and non-reproductive (maternal care) portions of an ancestral ground plan into social castes. There is further evidence that social evolution is associated with increased complexity of the regulation of gene networks [31••]. The causes and consequences of these changes, however, are less clear. Changes in the expression dynamics of key genes or key regulatory mechanisms may release other components of a shared regulatory network from genetic constraint, allowing for accumulation of genetic variation. Alternatively, genetic variation may accumulate in parts of a regulatory network that are under relaxed selection due to functional redundancy or population factors. Either way, this genetic variation may be a prime source of novel genes, variation in existing genes that underlie worker behavior, and species-specific elaborations of eusociality that characterize ants, honey bees, and stingless bees. Increasing rates of recombination may enhance or inhibit this process, and it is not yet clear what role recombination plays in the origins and maintenance of eusociality. The evolutionary processes leading to the regulatory changes hypothesized to kick off this cascade are also unknown. Evidence that different transcription factors, genes, and components of gene regulatory networks are involved in independent origins of eusociality suggests that the evolutionary processes leading to changes in these elements is also likely to be different in each instance [31••,58•]. Detailed comparisons of homologous gene regulatory networks involved in eusociality across different species (e.g., wing development in ants [32]) may provide a means to elucidate these evolutionary processes.

Outlook on sociogenomics

Box 1 lists outstanding questions generated from the sociogenomic topics reviewed above that require further investigation in several lineages with independent advents of sociality. Acquiring additional genomic resources for the non-hymenopteran eusocial insects will provide a more comprehensive understanding of the mechanisms underlying convergent social evolution (e.g., termites [74], gall aphids [95], and thrips [96]). It will be especially important to study species with different types of social biology. For example, communal living (i.e., nest-sharing without division of labor) and semi-sociality (i.e., nest co-founding

Box 1 Outstanding questions in sociogenomics

- Are conserved genetic pathways repeatedly recruited into functional roles associated with social traits?
- How does network architecture influence caste-biased gene expression, the evolutionary trajectory of these genes, and the potential for phenotypic novelty?
- What is the role of novel genes in the origins and elaborations of eusociality? Do they play an important role in multiple stages of social evolution?
- What is the relative role of adaptive evolution and genome architecture on the origin of novel genes and their integration into existing gene networks?
- Is the worker phenotype the source of evolutionary novelty in eusocial insects? If so, how do relaxed selection and positive directional selection act to shape this phenotype, despite the lack of direct reproductive success by workers? How are these processes influenced by genome architecture?
- How does the evolutionary pathway to communal living and semisociality compare to the origins and elaborations of eusociality?
- How do changes at the molecular level influence fitness at the individual and colony level?

and division of labor among sisters) are presumed to have evolved via alternative routes than the subsociality — to — eusociality route [97], but very little is known about the genomic basis for these lifestyles. We are approaching a horizon of reconciliation between ultimate and proximate explanations for eusociality. Predictive theoretical frameworks allow researchers to identify genes underlying altruism [98], epigenetic [99] and genomic [100,101] signatures of kin and group selection, evidence of genomic imprinting [43,102], and maternal manipulation [103]. As advancing technology enables easier access to genomic resources from species across the social continuum, students of social evolution will face a new challenge of acquiring behavioral, ecological, and physiological data (i.e., 'socio-phenomics') for additional species at a rate that matches the availability of new genomes.

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