

Genetic and genomic analyses of the division of labour in insect societies

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Abstract | Division of labour — individuals specializing in different activities — features prominently in the spectacular success of the social insects. Until recently, genetic and genomic analyses of division of labour were limited to just a few species. However, research on an ever-increasing number of species has provided new insight, from which we highlight two results. First, heritable influences on division of labour are more pervasive than previously imagined. Second, different forms of division of labour, in lineages in which eusociality has arisen independently, have evolved through changes in the regulation of highly conserved molecular pathways associated with several basic life-history traits, including nutrition, metabolism and reproduction.

Division of labour

A social system in which individuals specialize in specific occupations. In insect societies, queens mostly reproduce, whereas workers engage in all tasks related to colony growth and development. Young workers tend to work in the nest, whereas older individuals forage outside the nest.

It is often difficult to trace the evolution of complexity¹, but this is not so for the division of labour in social insects. The evolution of the reproductive queen and non-reproductive worker castes, which is the fundamental novelty of the social insects, laid the groundwork for the evolution of a behavioural division of labour among workers. In some lineages of social insects this was followed by the evolution of systems of division of labour based on sometimes striking differences in worker morphology, behaviour, or both² (FIG. 1).

Some of the social insects — the termites, ants, bees and wasps — are the most well known eusocial animals. Although rare, eusociality is highly successful — in some tropical habitats ants and termites are the dominant life forms on a biomass basis, despite their tiny individual size³. Social insects exhibit remarkable diversity in development, behaviour, social organization and ecology, all of which contribute to variation in the extent to which labour is divided. Some species display remarkably complex social traits, such as agriculture, warfare and intricate communication systems (FIG. 2), and many of these species also exhibit extreme forms of phenotypic plasticity, with colony members (potentially derived from the same genome) that differ in size 500-fold (FIG. 1). Efforts to solve the compelling evolutionary puzzle of social insect altruism, which is tied to the evolution of all of these traits, have led to the development of many of the most influential theories of social evolution (BOX 1).

Division of labour in social insects provides an excellent model system for sociogenomics⁴ (BOX 2). Recent reviews have focused on the adaptive significance of

genetic diversity among workers for workforce efficiency⁵ and how genomics can be used to study queen and worker caste evolution⁶. Here we provide the first synthesis of results on both the hereditary and molecular mechanistic basis of division of labour, which has led to the emergence of two main insights.

First, hereditary effects on division of labour are more pervasive than previously imagined — even for queen determination, which has long been thought to be primarily environmentally determined. These effects raise the spectre of reproductive conflict, but it seems that mechanisms have evolved in many species to minimize conflict while still preserving genetic diversity and its potential benefits to colony life. The genes that underlie hereditary effects on division of labour have yet to be identified, but the findings reviewed here provide an excellent starting point for such investigations.

The second insight is that the same set of highly conserved molecular pathways is involved in the regulation of different forms of division of labour, even across distantly related social insect taxa that evolved eusociality independently. Some of the pathways are related to the fundamental processes of nutrition, metabolism and reproduction, supporting the idea that life-history traits of solitary insects that are related to these processes could have served as evolutionary precursors to eusociality.

These studies also demonstrate how research on division of labour in social insects has the potential to address broad issues in development, evolution and behaviour, such as the adaptive roles of genotypic diversity in groups and the evolution of complex traits.

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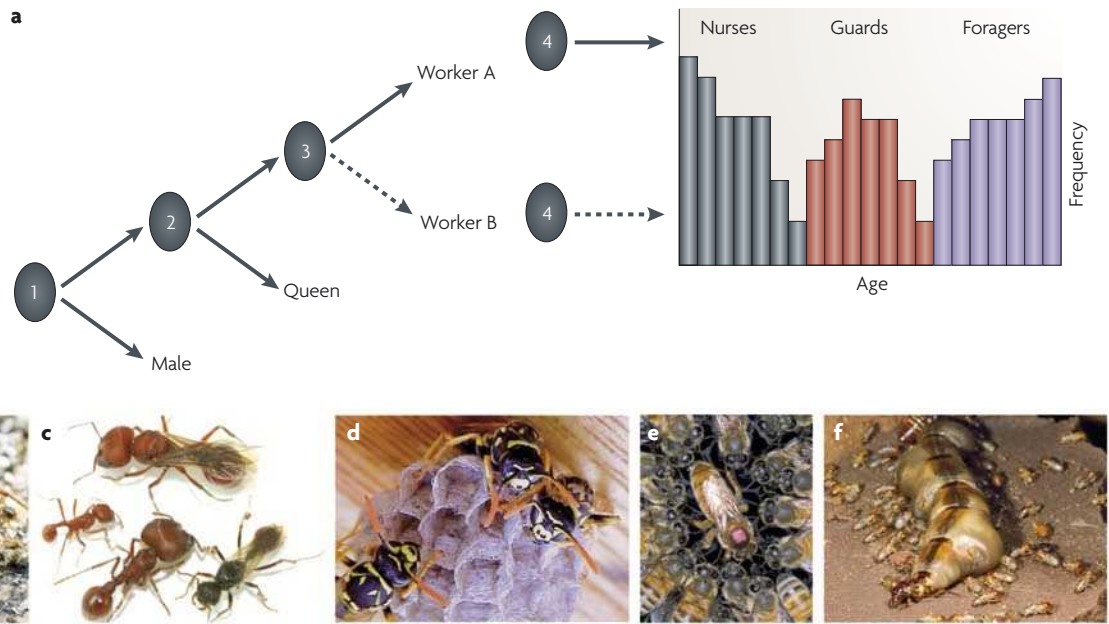


Figure 1 | Caste and division of labour in insect societies. **a** | Castes in insect societies represent an extreme example of phenotypic plasticity. Males differentiate from females on the basis of genetic differences (**1**). Diploid termites have XY sex determination. In some haplodiploid Hymenoptera, sex is determined by a single locus, in others many loci are implicated¹⁰⁹. In the honeybee, sex is controlled by *complementary sex determination (csd)*¹¹⁰, the first sex-determination gene identified for a social insect. *csd* was identified by positional cloning, facilitated by the extremely high recombination rate of the honeybee genome³⁴. Males are homo- or hemizygous at *csd*; females are heterozygous. *csd* probably evolved from another gene that has recently been shown to have a crucial part in the honeybee sex-determination cascade — *feminizer (fem)*, which shows homology to *transformer*, the principal sex-determining gene in *Drosophila melanogaster*¹¹¹. The functions of *csd* and *fem* were demonstrated by RNAi^{110,111}. Comparative sequence analysis with other bees suggests that *csd* is a recent innovation in the honeybee lineage. (**2**) The primary division of labour in an insect society is between reproductive and non-reproductive individuals; in most species of social insect this involves queens and workers, respectively. In the largest and most complex (advanced eusocial) insect societies, queens and workers differentiate from each other early in larval or nymphal development. In primitively eusocial societies, colonies are smaller and queen–worker status may be decided during adulthood (with some caste bias during larval development now known in some species), leading to the formation of adult dominance hierarchies. Workers (**3**) perform all the jobs necessary for colony growth and development. In termites and some ants there are morphologically distinct worker castes; this differentiation occurs later in larval or nymphal development than queen–worker differentiation. In most insect societies with worker–worker division of labour, specialization (**4**) occurs through age polyethism; workers progress through a series of tasks as they get older, usually beginning with nest activities and culminating in defence and foraging duties outside⁷. Although worker behavioural differentiation probably evolved before morphological differentiation, it occurs later during development, in adulthood. **b–f** | Examples of morphological diversity among social insect castes **b** | ‘Major’ and ‘minor’ workers of *Pheidologeton maurader* ants; the major is 500 times heavier than the minor. **c** | Clockwise from top, reproductive female, male, and minor worker of the Florida harvester ant, *Pogonomyrmex badius*. **d** | *Polistes dominulus* wasps cooperating to build a nest. **e** | Queen *Apis mellifera* honeybee surrounded by workers. **f** | *Macrotermes* termite queen, highly modified for reproduction, surrounded by workers. Part **b** is reproduced, with permission, from www.myrmecos.net © Alex Wild (2004). Part **c** is reproduced courtesy of Adrian A. Smith, Arizona State University. Part **d** is reproduced courtesy of Elizabeth A. Tibbetts, University of Michigan. Part **e** is reproduced courtesy of Scott Bauer, USDA Agricultural Research Service, www.forestryimages.org. Part **f** is reproduced, with permission, from REF. 113 © (2008) Encyclopaedia Britannica Online.

Queen

Individual(s) that produces most or all of the offspring in a social insect colony.

Worker

Individual that performs all tasks related to the growth and development of a social insect colony; engage in little, if any, personal reproduction.

Caste

Term used to describe a group of individuals in social insect colonies that specializes to some extent in specific occupations as a result of division of labour. Social insect castes can be associated with differences in age, anatomy and morphology.

Eusocial

Traditionally defined as social species that show three features: extreme asymmetries in reproduction, with some individuals reproducing a great deal and others little or not at all; overlapping generations of adults in the nest; and cooperative care of offspring.

Age polyethism

Change in behaviour with age; a term used primarily to describe behavioural maturation in some species of social insect workers. Results in age-related division of labour at the colony level.

Genetic variation and division of labour

Insect colonies have long been dubbed superorganisms, in part because of their intricate systems of division of labour⁷. Consistent with this metaphor was the long-standing belief that all individuals are genetically totipotent, and environmental factors determine caste^{3,7}. However, although cells within an organism are effectively clones of one another, individuals in an insect society are not. High recombination rates⁸, multiple mating⁹ and multiple queens³, which have evolved many times in some social insect lineages¹⁰, all lead to

high levels of genetic variation among colony members. In fact, several species of social insect have among the highest reported degrees of polyandry and recombination rates of all animals^{8,9}.

To what extent do the genetic differences between individual social insects predispose them to different caste roles? The following sections review how this genetic variation affects four aspects of division of labour: queen–worker caste determination; queen–queen specialization; worker–worker specialization; and caste-ratio flexibility.

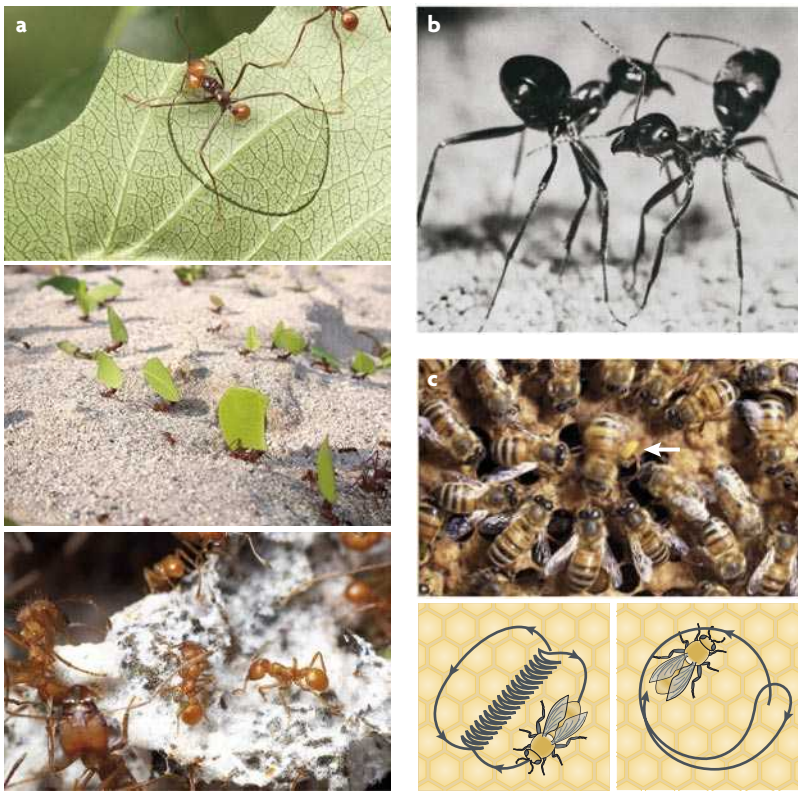


Figure 2 | Fruits of sociality. Division of labour among workers enables some of the most spectacular traits of insect societies, including the following. **a** | Agriculture: *Atta* ants cutting (top) and transporting (middle) pieces of leaf back to their nest, where they then use the pieces as a substrate to grow fungus (bottom). This system features a crop fungus, a weed fungus (*Escovopsis*) and a bacterial fungicide¹¹². **b** | Warfare: honeypot ants (*Myrmecocystus*) face-off in territorial tournaments. Each worker assesses her size against ants from another colony; colonies then somehow integrate these individual comparisons to determine whether they will mount an attack or prepare to defend against an attack³. **c** | Symbolic communication: the photo depicts a pollen-laden honeybee forager (arrow) ‘dancing’ to recruits. Drawings depict the round and waggle dances of honeybees. Right: round dance, which alerts recruits to nearby resources, but does not specify resource location. Left: waggle dance; waggles (depicted as a wavy line) convey information on distance, and angle of dance conveys information about direction. Once workers were limited to serve mostly as non-reproductive helpers, their traits could be shaped by natural selection in these and other ways to increase colony fitness. The photos in part **a** are reproduced, with permission, from www.myrmecos.net © Alex Wild (2003–2007). Part **b** is reproduced, with permission, from REF. 114 © American Philosophical Society (1979). Part **c** photo is reproduced, with permission, from Nature Picture Library © 2002–2008. Part **c** figure is modified from REF. 115.

Superorganism

A metaphor for a colony of social insects that highlights three key characteristics: first, the colony functions as a single, highly integrated unit; second, natural selection acts on the colony; and third, a relative lack of competition among individuals.

Polyandry

Multiple mating by females.

Queen–worker caste determination

There is extensive literature demonstrating environmental influences on queen–worker caste determination. However, early findings for stingless bees suggested heritable influences, and numerous recent studies of other taxa have come to the same conclusion (TABLE 1). In these cases, individuals of some genotypes are more likely to develop into queens than individuals of other genotypes (genetic caste determination).

Genetic caste determination in some *Pogonomyrmex* harvester ants¹¹ and *Solenopsis* fire ants¹² involves social hybridogenesis, or more pointedly ‘reciprocal parasitism’. Microsatellite, allozyme and random amplified

polymorphic DNA (RAPD) markers revealed the presence of two genetically distinct lineages within *Pogonomyrmex* colonies; queens are homozygous and belong to one or the other of these lineages, whereas workers are a cross between the two¹¹. Eggs fertilized by sperm of males that share the queen’s lineage produce queens, and eggs fertilized by sperm from the other lineage produce workers (which do not reproduce). There is thus practically no gene flow between the lineages, and both gain fitness by producing reproductive individuals — just not in the same colony. Both lineages are therefore completely dependent on one another, and queens obligately mate with males of each. Nuclear or nuclear–cytoplasmic incompatibilities between lineages are proposed to prevent queen production in ‘hybrid’ matings, but whether they act developmentally or behaviourally is unclear¹³.

The spread of this system in *Pogonomyrmex* may involve co-evolution between alleles that bias queen production and those that repress it, with the evolution of queen-biasing, or ‘queen’, alleles driving the evolution of the repressor alleles. According to this idea, dependent lineages are at an equilibrium state, in which both biasing and repressor alleles are fixed within lineages¹³. Although hybridization between *Pogonomyrmex barbatus* and *Pogonomyrmex rugosus* has been implicated in the evolution of this system, recent evidence¹⁴ suggests it arose first in one species, and then spread to the other. Maternal effects also influence queen–worker caste determination in harvester ants¹⁵.

In several ant species, queens facultatively fertilize eggs to produce workers, but produce new queens by thelytokous parthenogenesis¹⁶. Alleles found in workers, but not in queens, must therefore be maintained in the population by their presence in males, which in the case of the little fire ant, *Wasmannia auropunctata*, has led to an almost complete separation of male and female (queen) gene pools¹⁷. The genetic mechanisms underlying this novel form of parthenogenesis are not known.

The above examples deal with hymenopteran social insects, so they all involve haplodiploid systems. Recently, a case of genetic caste determination was reported for a termite¹⁸. In *Reticulitermes speratus*, queens that produce workers do not produce progeny capable of reproduction, which are known as reproductives. Reproductives are only produced when the queen is absent; then some workers become reproductively active, mate, and produce new kings and queens, but they do not produce workers. Breeding studies suggest that this system is controlled by a single sex-linked locus with two alleles. The alleles have sex-specific effects; males with a particular allele develop into reproductive individuals, whereas females with the same allele develop into workers.

According to kin selection theory, non-reproducing individuals in an insect colony gain indirect fitness through the reproduction of their relatives. Therefore, alleles that increase an individual’s probability of becoming a queen, rather than a worker, in effect parasitize other alleles in the colony because they contribute less to colony growth and development, and more to reproduction. Natural selection might be expected to favour

Box 1 | It's all about the colony

The presence of worker castes marks a major transition in social evolution¹. When workers sacrifice reproductive opportunities, it is the colony as a whole that is the target of natural selection. The selective forces involved in the evolution of eusociality are once again a topic of debate; there is support for roles for kin selection⁸⁷ and for colony-level selection⁸⁸, and there are analyses that suggest that these are one and the same^{67,89}.

Social insects are social 'extremists' — they coordinate almost all of their activities with other colony members⁹⁰. The social environment of an insect colony exerts potent influences on division of labour, especially in advanced eusocial insect societies (FIGS 1, 2). Cues and signals provided by the nest, brood, and other individuals provide valuable information about colony needs and conditions outside the nest. In addition to governing 'normal' patterns of colony growth and development, this information allows colonies to respond to changing circumstances. These responses involve developmental plasticity, which results in changes in the production of members of different castes, and behavioural plasticity among adult workers, which results in changes in task specialization.

Some social influences on division of labour are manifest by the distribution of food in a colony. For example, workers in many insect societies are often smaller than queens, and are too poorly nourished during development to form fully functional reproductive systems⁷. Nutritional effects on development and behaviour occur through endocrine systems, the molecular basis of which are the target of ongoing investigation. Most of this research has targeted the honeybee *Apis mellifera*, because molecular and genomic resources were developed first for this species and, at present, it is the only social insect with a sequenced genome. But similar resources are being developed for a range of other species, and this Review highlights some of the emerging findings.

Many communication channels in insect societies are chemical. Social insects use a large number of pheromones to coordinate and allocate labour⁹¹, the effects of which are beginning to be understood in molecular terms. For example, in honeybees, queen mandibular pheromone inhibits the transition from working in the hive to foraging⁹², and also causes changes in brain gene expression that are consistent with this effect; genes upregulated in the brains of foragers tend to be downregulated by this pheromone⁹³. Changes in brain gene expression are even induced by releaser pheromones, which cause rapid, non-permanent changes in behaviour⁹⁴.

queen alleles, but if too many were to accumulate there would be too few workers to maintain colony function. The termite system outlined above minimizes intra-colonial conflict because workers cannot reproduce in the presence of the queen and the queen is incapable of producing individuals that develop into reproductives¹⁸. More generally, frequency-dependent selection might maintain queen alleles if they are rare, or alleles that only weakly influence queen determination might be maintained at a higher frequency without causing large fitness losses to nest-mates. In some studies of ants and honeybees, it is the rare genotypes in a colony that show a queen bias^{19,20}. Other studies, however, have observed a queen bias for the most common patriline²¹ or no relationship between queen bias and patriline frequency²².

Some eusocial species have completely lost the queen caste, and colonies are composed of reproductively capable workers²³. Workers are thus no longer technically 'workers' and must have the capacity for reproduction either through mating ('gamergates' in ants) or via thelytokous parthenogenesis. Complete loss of the reproductive caste has occurred in several species of ant²³, and in a particular strain of the Cape honeybee, *Apis mellifera capensis*²⁴. The reproducing 'pseudo-queen' workers of the Cape honeybee strain are resistant to social suppression of reproduction (by queen pheromones and worker policing) and reproduce freely. Remarkably, just

a single clone of this strain has parasitized thousands of colonies of *Apis mellifera scutellata* across southern Africa²⁴. Breeding studies suggest that parthenogenesis in this strain is determined by a recessive allele at a single locus. Linkage mapping has located a candidate region in the genome that contains a particularly promising transcription factor gene²⁵.

The genetics of the *capensis* strain do not seem to be similar to that of the 'anarchist' honeybee, a mutant strain in which workers show a high probability of developing their ovaries and laying unfertilized (male-destined) eggs in the presence of a queen. Inheritance patterns for the anarchistic phenotype suggest it is controlled by a small number of genes²⁶. However, microarray analysis of the anarchistic strain has so far only implicated genes suspected of being well downstream of the mutation(s)²⁶.

Queen-queen specialization

Variation in queen behaviour occurs in many species of ant, bee and wasp. For example, in both the wasp *Polistes dominulus*²⁷ and the ant *Acromyrmex versicolor*²⁸, unrelated queens establish nests together and then create a division of labour whereby one individual specializes more on foraging and the other on brood care. A genetic component to these types of difference in queen behaviour has not yet been found, but a suggestive finding has been made for *A. versicolor* workers. Workers from different matrilineal lines specialize either on foraging or on brood (and fungal garden) care²⁹, just as unrelated queens do during colony establishment.

Morphological or physiological variation among queens in some ant species can also have a genetic component (TABLE 1). For example, the fire ant *Solenopsis invicta* produces two types of queen. Heavy queens head colonies alone, whereas queens of lighter weight head multi-queen colonies, with anywhere from two to hundreds of queens per colony. Identified in *S. invicta*, *general protein 9 (Gp-9)* is the first gene, or genetic marker tightly linked to other causal genes³⁰, to be implicated in the regulation of queen number, a key aspect of colony organization. Variation at the *Gp-9* locus is associated with variation in queen physiology, queen behaviour and colony social structure³⁰. Workers with a *b* allele (with the genotype *Bb*; *b* is a recessive lethal) will accept additional queens with the *b* allele, but will kill any queens with a *BB* genotype. In monogynous colonies, *BB* workers will not tolerate any other queens, regardless of genotype. *Gp-9* is thought to encode a putative odorant-binding protein, so its alleles might have a differential effect on the production and perception of queen-recognition pheromones. These differences also have profound influences on the ecological impact of this species. *Solenopsis* colonies with multiple queens are much more invasive than colonies with a single queen³⁰. *Gp-9* is the only gene so far implicated in queen-queen specialization.

Worker-worker specialization

Genetic variation in insect colonies has strong effects on the division of labour among workers. This variation often biases the jobs that workers perform, such as

Social hybridogenesis

Only hybrid matings can produce workers; non-hybrid matings produce queens. It is similar to hybridogenesis in solitary species, whereby hybrid matings produce viable hybrid offspring, but the germ line is purely parental.

Thelytokous parthenogenesis

Production of females from unfertilized eggs (asexual reproduction).

Haplodiploid

A genetic system in which females develop from fertilized, diploid eggs and males develop from unfertilized, haploid eggs. In Hymenoptera (ants, bees and wasps), sex determination can occur through a single locus (although multiple loci exist in some species); heterozygotes are female and hemizygotes are male.

Box 2 | Social insects and sociogenomics

The goal of sociogenomics is to achieve a comprehensive understanding of social life in molecular terms: how it evolved, how it is governed, and how it influences aspects of genome structure, genome activity and organismal function⁴. Social insects provide excellent models that figure prominently in sociogenomics for the following reasons:

- Eusociality arose independently numerous times (current estimates range greatly, but at least 13 times), providing 'natural experiments' for addressing evolutionary hypotheses. It arose in at least five orders, primarily in the Hymenoptera (ants, bees and wasps)^{95–97}. Although it arose just once in the ants, morphological specialization among the worker caste has evolved independently about 18 times in this group of >10,000 species^{2,97}. Eusociality evolved once in the termites, and there have been multiple independent evolutions of soldier castes in aphids⁹⁸. Eusociality has also evolved sporadically in crustacea (snapping shrimp)⁹⁹ and mammals (naked mole rats)¹⁰⁰. Hymenoptera exhibit many forms of sociality, covering the range between solitary and eusocial lifestyles⁷.
- Hymenoptera are haplodiploid — that is, males arise from haploid eggs and females from diploid eggs. Males participate little, if at all, in the day-to-day functioning of haplodiploid insect colonies⁷, although in diploid societies, such as those of termites, both sexes work. The hymenopterian haplodiploid genetic system facilitates genetic linkage mapping for complex phenotypes. In addition, on the basis of the few species analysed thus far, social Hymenoptera have among the highest reported recombination rates⁸.
- The sequencing of the honeybee genome¹⁰¹ and the development of genomic resources for honeybees and other species (for example, ESTs, microarrays and RNAi; TABLES 2,3) provide powerful tools for molecular dissections of sociality.
- Insect colonies of many species can be easily manipulated, in some cases both in the laboratory and the field; this provides unrivaled opportunities to alter the social environment in precise and repeatable ways and then trace the effects at molecular and higher levels of biological organization.
- Social insects present outstanding opportunities for studies of genome evolution because of great variation in genetic properties between species, including chromosome number and genome size^{102,103}.

guarding the nest entrance or foraging for specific types of resource. These behavioural effects, first discovered in honeybees³¹, are now known to be widespread among the social insects⁵. The best-studied case involves inherited differences in the tendency of worker honeybees to collect either nectar or pollen; QTLs for these differences have been identified and efforts are underway to find the underlying genes³². Genetic variation also affects morphologically based task specialization among workers in several ant species (TABLE 1).

Genetic influences on worker–worker task specialization are better known than genetic influences on queen–worker caste determination. This might simply be because the former has been studied more extensively than the latter. Another possibility is that inherited differences in worker–worker task specialization are more widespread because they do not lead to the kinds of conflicts of interest described above for queen–worker caste determination. In fact, research shows that inherited differences in worker–worker task specialization benefit colonies by increasing the efficiency of division of labour or increased colony homeostasis⁵. An illustrative example of this was seen in two sets of honeybee colonies that were created by instrumental insemination: one headed by queens inseminated with semen from 15 unrelated males and the other headed by queens inseminated with semen from just a single male³³. Controlling for

differences in sperm number, brood viability, and disease effects, the genetically diverse colonies had greater productivity (measured in terms of comb building, brood rearing and foraging), which, in turn, increased colony fitness (measured in terms of colony survival and numbers of reproductives).

Research on inherited differences in worker–worker specialization^{5,33} demonstrates a link between intracolony genetic diversity and workforce efficiency. However, it is not known which evolved first, genetic diversity or genetic differences in task performance. High recombination rates might break up allele combinations that bias queen–worker caste determination, allowing colonies to reap the benefits of workforce efficiency while minimizing the costs of intracolony conflicts of interest. Preliminary evidence that recombination rates are higher in social insects with high within-nest genetic diversity (due to polyandry) supports this idea¹³. But high recombination rates are also favoured for haplodiploid sex determination and are even higher in the vicinity of the sex-determination gene *complementary sex determiner (csd)* locus³⁴ (FIG. 1). Further complicating the picture, genetic diversity generated through recombination not only affects workforce efficiency, but might also promote disease and parasite resistance and decrease variance in relatedness within colonies, which could act to decrease nepotism and thus increase colony stability⁸. Perhaps the precise evolutionary relationship between genetic variation and workforce efficiency can be deduced by comparative analyses that draw upon the many independent evolutions of eusociality in social insects (BOX 2; TABLE 1).

Caste-ratio flexibility

If individuals are totipotent with respect to caste, then colonies can regulate caste ratios on the basis of environmental need². However, genetic influences can, in theory, constrain a colony's ability to adjust caste ratios in a flexible manner. This constraint would be most severe for types of division of labour that involve morphologically distinct castes, because individuals in these castes are probably more constrained behaviourally than individuals in age-based castes.

Genetic influences might result in the production of queens before a colony has attained sufficient size or acquired sufficient resources to reproduce successfully^{2,3}, which would be wasteful. Yet evidence from *Melipona* stingless bees, *Pogonomyrmex* ants and *R. speratus* termites suggests that genetic influences on queen–worker caste determination do indeed constrain caste ratios. The numbers of workers and queens produced in these colonies are in accord with genetic predictions based on known genotype frequencies, regardless of colony size or season^{18,35,36}.

If genetic caste determination can lead to maladaptive caste ratios, then why does it exist? This evolutionary puzzle needs to be addressed in future studies. Perhaps individuals that are younger than the age at which caste determination occurs might be able to influence their own caste fate by changing how they allocate nutrition to somatic versus reproductive

Kin selection

W. D. Hamilton's theory to explain the evolution of the hallmark of social life: altruistic cooperation (performing acts that are costly to the benefactor but that benefit others). For example, by helping a relative, an individual increases its fitness by increasing the number of copies of its genes in the population.

Colony-level selection

A special form of group (family) selection, first described by Charles Darwin, to explain the evolution of sterile workers in insect societies.

development. This idea is consistent with recent findings that genes implicated in caste determination are involved in nutrition and metabolism, as discussed below. Results of social manipulations support the idea that adult–immature interactions provide the means for environmental factors to overcome genetic caste determination³⁷. In *Melipona*, experimentally induced

changes in food availability or endocrine status can also alter caste ratios³⁸. Furthermore, nutrition, social environment and genetics do not act uniformly across castes within a colony, and there is genetic variation for sensitivity to these factors^{20,39}. Together, these results suggest that a colony's worker force might be able to overwhelm genetic biases in queen production.

Table 1 | **Social insects known to have heritable variation in various forms of division of labour**

Type of division of labour	Species	Order	Family*	Refs
Queen–queen				
Adult morphology	<i>Solenopsis invicta</i> [†]	Hymenoptera	Formicidae	30
	<i>Solenopsis geminata</i>	Hymenoptera	Formicidae	116
	<i>Monomorium</i> spA	Hymenoptera	Formicidae	116
	<i>Ectatomma ruidum</i> , <i>Ectatomma tuberculatum</i>	Hymenoptera	Formicidae	118
	<i>Lasius niger</i>	Hymenoptera	Formicidae	119
	<i>Formica truncorum</i>	Hymenoptera	Formicidae	120
	<i>Myrmecina graminicola</i>	Hymenoptera	Formicidae	121
	<i>Harpagoxenus sublaevis</i>	Hymenoptera	Formicidae	122
<i>Pogonomyrmex badius</i>	Hymenoptera	Formicidae	39	
Queen–worker				
Adult morphology	<i>Acromyrmex echinator</i>	Hymenoptera	Formicidae	20
	<i>Pogonomyrmex rugosus</i> , <i>Pogonomyrmex barbatus</i>	Hymenoptera	Formicidae	11,13
	<i>Pogonomyrmex badius</i>	Hymenoptera	Formicidae	39
	<i>Solenopsis geminata</i> x <i>xyloni</i>	Hymenoptera	Formicidae	12
	<i>Wasmannia auropunctata</i> [§]	Hymenoptera	Formicidae	17
	<i>Vollenhovia emeryi</i> [§]	Hymenoptera	Formicidae	123
	<i>Cataglyphis cursor</i> [§]	Hymenoptera	Formicidae	16
	<i>Melipona</i> spp.	Hymenoptera	Apidae	35,38
	<i>Apis mellifera capensis</i> [§]	Hymenoptera	Apidae	24,25
	<i>Apis mellifera</i>	Hymenoptera	Apidae	19,22,26
	<i>Reticulitermes speratus</i>	Isoptera	Rhinotermitidae	18
	<i>Pemphigus obesinymphae</i> [§]	Homoptera	Pemphigidae	124
	Worker–worker			
Adult morphology	<i>Acromyrmex echinator</i>	Hymenoptera	Formicidae	37,125
	<i>Camponotus consobrinus</i>	Hymenoptera	Formicidae	126
	<i>Pogonomyrmex badius</i>	Hymenoptera	Formicidae	39,127
	<i>Solenopsis invicta</i>	Hymenoptera	Formicidae	30
	<i>Eciton burchelli</i>	Hymenoptera	Formicidae	128
	<i>Formica selysi</i>	Hymenoptera	Formicidae	129
Adult behaviour	<i>Leptomyrmex rudis</i>	Hymenoptera	Formicidae	130
	<i>Acromyrmex versicolor</i>	Hymenoptera	Formicidae	29
	<i>Formica argentea</i>	Hymenoptera	Formicidae	131
	<i>Polybia aequatorialis</i>	Hymenoptera	Vespidae	132
	<i>Apis mellifera</i>	Hymenoptera	Apidae	5,33,133,131
	<i>Mastotermes darwiniensis</i>	Isoptera	Mastotermitidae	134

*The common names for these families are: Formicidae, ants; Apidae, bees; Rhinotermitidae, termites; Pemphigidae, aphids; Vespidae, wasps; Mastotermitidae, termites. [†]*S. invicta* queens differ in terms of their physiology (for example, mass) rather than morphology *per se*. [§]Denotes involvement of thelytokous parthenogenesis.

Tip of the iceberg?

Most of these findings have been reported quite recently, suggesting that hereditary influences on caste determination are more widespread than previously thought⁴⁰. The findings reviewed above suggest that genotyping social insects in diverse contexts will continue to be fruitful, and that more discoveries of heritable effects on various aspects of social organization are probable. Social insects are likely to emerge as important models with which to study interactions between nature and nurture at the molecular level⁴¹ — that is, how variation in genotype and the environment interact to affect gene expression, which, in turn, influences other aspects of phenotypic plasticity. However, this will require the identification of the genes that underlie the heritable effects described above, which has not yet happened.

Genes and life-history trait precursors

Various evolutionary forces have been implicated in the evolution of the different forms of division of labour that characterize eusociality (BOX 1). Recent molecular analyses are now helping to elucidate the mechanistic routes to eusociality, a topic that has not received as much attention. Comparative ethology and natural history have long suggested that certain life-history traits might act as evolutionary precursors to eusociality. Genetic and genomic analyses now make it possible to explore these ideas in new ways. In contrast to the studies discussed in the previous section, this research has relied mainly on studies of gene expression, using candidate genes⁴² and microarrays to look for changes in expression that correlate with morphological or behavioural differences among colony members (TABLES 2, 3). More traditional genetic approaches have been used sparingly³² because of limitations in our ability to efficiently breed many social insect species and to maintain large numbers of mutant or selected lines. Here we discuss several lines of research that support the idea that deeply conserved pathways associated with several basic life-history traits contributed to the evolution of different types of division of labour: nutrition and food-gathering behaviour, maternal care, diapause, and reproduction. The results reviewed in the following sections illustrate the promise of coupling molecular analyses with the rich body of natural history and theory that exists on the evolution of eusociality.

Nutrition and food-gathering behaviour

Nutrition has long been known to have a potent influence on queen–worker division of labour (BOX 1). Female honeybee larvae that receive a rich diet develop into queens, rather than workers, through changes in gene expression that are orchestrated by juvenile hormone (JH) and insulin/insulin-like growth factor-1 (IGF1)-like signalling (IIS) pathways. Developing queens show upregulation of several IIS genes⁴³, and knockdown of the bee orthologue of the IIS gene *target of rapamycin (tor)* by RNAi blocks queen development⁴⁴.

Further molecular evidence for the role of nutrition in queen–worker division of labour comes from studies of genes encoding storage proteins such as hexamerin. Developing queens show high whole-body levels of hexamerin mRNA or protein in several species of bee and wasp (TABLE 2). In *Reticulitermes flavipes* termites, soldiers show higher expression of *hexamerin*, and *hexamerin* RNAi knockdown blocks soldier production^{45,46}. Higher expression of other metabolic and storage-protein genes is also seen in queen–destined larvae in diverse species of bee, ant and wasp (TABLE 2). Considering the increased growth rate and body size of developing queens, it is likely that some of these differences are a consequence, rather than a cause, of differences between these two castes.

Queen–worker differences in gene expression persist into adulthood: studies in bees and wasps (TABLE 2) again highlight genes related to metabolism and nutrition, among other categories. Adult queens continue to enjoy a nutritional advantage relative to workers, so many of these differences are probably also a consequence, rather than a cause, of differences between these two castes. In honeybees, approximately 2,000 genes have been shown to be differentially expressed in the brains of queens compared with workers⁴⁷, illustrating massive caste-specific gene regulation.

Nutrition also affects worker–worker division of labour. In honeybees, foragers have diminished lipid and protein reserves relative to nest workers⁴⁸, and treatments that reduce these reserves, including RNAi knockdown of the storage-protein gene *vitellogenin (Vg)*⁴⁹, lead to precocious foraging. Pathway analyses of honeybee microarray data revealed that nest workers and foragers differ in the expression of metabolism genes in the brain⁵⁰. These differences might be related to the extensive changes in brain structure and brain chemistry that occur during maturation from hive worker to forager⁵¹.

The relationship between nutrition and worker behaviour is complex; workers cannot be simply acting on a ‘hunger signal’ because they eat before leaving the nest in search of food. Additional evidence of this complexity comes from a recent study in honeybees⁵⁰ that reported upregulation of IIS genes in foragers (in the brain and abdomen) relative to hive bees, and a delay in the shift from working in the hive to foraging induced by inhibition of the TOR pathway. High IIS-gene expression is thus associated with low nutrient status in adult worker bees, which is contrary to the norm⁵², and the opposite of what is found during caste determination^{43,53}. These results suggest evolutionary changes in the way IIS pathways are regulated. Measurements of brain IIS-gene expression from *Polistes metricus* wasps⁵⁴ suggest that the involvement of this pathway in social insect division of labour might be widespread. Other nutritionally responsive genes implicated in the regulation of worker behaviour are *foraging* and *malvolio*^{4,32,55–59}.

In a striking case of convergent evolution, there also is evidence for nutritional influences on division of labour in termites. Nutrition-related genes, including

Diapause
Dormancy owing to unfavourable environmental conditions.

Storage proteins
Circulating lipoproteins used by insects to store nutrients.

those encoding vitellogenin and proteins involved in fatty acid biosynthesis, are associated with differences between worker and reproductive castes in *R. flavipes*⁶⁰, as in the Hymenoptera. Also as in the Hymenoptera (see below), JH has an important role in caste determination in *R. flavipes*, and acts in part through hexameric storage proteins, which can bind JH and are likely to modulate

JH availability and thus caste determination⁶¹. Various cytochrome P450 genes, some of which are upregulated in workers compared with queens in several bee and ant species (TABLE 3), are also differentially expressed between soldiers (which specialize on nest defence) and workers in three termite species⁶². Similarly, the mitochondrial gene *coxIII* is differentially expressed in

Table 2 | A summary of molecular analyses of queen–queen and queen–worker division of labour in the social insects

Type of division of labour	Species (common name of group)	Genes/pathways*	Biological processes/molecular functions	Method	Refs
Queen–queen					
Gyne–foundress–queen	<i>Polistes metricus</i> (wasp)	Various (20, for example insulin pathway)	Growth/energy allocation	qPCR	54
Winged–wingless queen	<i>Solenopsis invicta</i> (ant)	Various (40, for example vitellogenin, yellow)	Yolk protein, nutrient transport, antibacterial peptides	qPCR, SSH, NB	136
Queen–worker					
Queen–worker (larval)	<i>Apis mellifera</i> (bee)	Various (7, for example hexamerin)	Storage proteins, fatty-acid binding, oxidoreductase	SSH, NB	137
Queen–worker (larval)	<i>Apis mellifera</i> (bee)	Translation-initiation factor <i>AmlF-2_m</i> , cytochrome oxidase 1, cytochrome c	Mitochondrial enzymes	qPCR, DD	18
Queen–worker (larval, pupal and adult)	<i>Apis mellifera</i> (bee)	major royal jelly protein genes (7), yellow genes (7)	Production of brood food, sex-specific reproductive maturation	NB, EST, PCR	139
Queen–worker (larval)	<i>Apis mellifera</i> (bee)	Insulin pathway (4)	Growth/energy allocation	qPCR	43
Queen–worker (larval)	<i>Apis mellifera</i> (bee)	Various (240)	Cellular localization, development, physiometabolism	MA	140
Queen–worker (adult)	<i>Apis mellifera</i> (bee)	vitellogenin, insulin pathway (3)	Yolk protein, growth/energy allocation	qPCR	72
Queen–worker (adult)	<i>Apis mellifera</i> (bee)	Various (~2,000)	Oxidoreductases, peptidases, mitochondrial proteins, immune response	MA	47
Queen–worker (larval)	<i>Apis mellifera</i> (bee)	target of rapamycin, chico [†] (insulin pathway)	Growth and energy allocation	qPCR, RNAi	44
Queen–worker (larval)	<i>Bombus terrestris</i> (bee)	Various (~60)	Storage proteins, metabolic enzymes, mitochondrial proteins	SSH, NB	141
Neotenic reproductive–worker	<i>Cryptotermes secundus</i> (termite)	Various (5, for example vitellogenin)	Yolk protein, cytochrome P450 enzymes, guanylate cyclase, juvenile hormone esterase	qPCR, RDA	142
Queen–worker (adult)	<i>Lasius niger</i> (ant)	Various (16, for example vitellogenin)	Odorant binding, metabolic enzymes, nucleic-acid binding	NB, DD, MA	143
Queen–worker (adult)	<i>Melipona quadrifasciata</i> (bee)	Various (~300)	Transcriptional regulation, cytochrome P450 enzymes, protein biosynthesis, fatty-acid synthesis	qPCR, RDA	105
Queen–worker (adult)	<i>Polistes canadensis</i> (wasp)	Various (43, for example vitellogenin, hexamerin)	Yolk protein, storage proteins, metabolic enzyme, binding protein	SSH, NB	144
Queen–worker (adult)	<i>Polistes metricus</i> (wasp)	Various (20, for example insulin pathway)	Growth/energy allocation	qPCR	54
Queen–worker (larval)	<i>Polistes metricus</i> (wasp)	hexamerin	Storage proteins	PAGE, LC-MS	68
Nymph–reproductive	<i>Reticulitermes flavipes</i> (termite)	Various (34, for example vitellogenin, hexamerin)	Yolk protein, storage proteins, fatty-acid biosynthesis, apoptosis inhibition	MCA, qPCR	60
Queen–worker (adult)	<i>Solenopsis invicta</i> (ant)	Cytochrome P450 genes <i>CYP4AB1</i> and <i>CYP4AB2</i> , general protein 9	Cytochrome P450 enzymes	SSH, NB	145
Queen–worker (larval)	<i>Vespa squamosa</i> (wasp)	Various (52, for example hexamerin)	Storage proteins, odorant binding, protein metabolism	LIB	53

*Numbers in parentheses represent the number of genes for which differential expression was found. [†]Gene for which a causal association has been demonstrated. DD, RNA-differential display; LC-MS, liquid chromatography-mass spectrometry; LIB, cDNA libraries; MA, microarray; MCA, cDNA macroarray; NB, Northern blots; PAGE, SDS-polyacrylamide gel electrophoresis (densitometry); PCR, polymerase chain reaction; qPCR, quantitative PCR; RDA, representational difference analysis; SSH, suppression subtractive hybridization.

the brains of *Reticulitermes santonensis* workers and soldiers⁶³; *coxIII* and other *cox* genes have been implicated in honeybee queen–worker differentiation (TABLE 2). These similarities exist despite more than 400 million years of evolution between termites and hymenopteran social insects, and major differences in patterns of caste development⁷.

Termites also display several unique mechanisms of caste development. In order to digest wood, termites have gut symbionts and also possess endogenous cellulose-digesting enzymes, some of which are differentially expressed between soldiers and workers⁶⁴. And two novel genes have been implicated in the extreme morphological specializations of termite soldier heads⁶⁵.

The studies summarized above demonstrate that several of the same genes and pathways influence both queen–worker and worker–worker division of labour. These results suggest that pathways governing nutritional state and feeding behaviour in solitary animals represent a multifaceted genetic ‘tool kit’ for the evolution of division of labour⁴⁸.

Maternal care

Several authors⁴⁸ have proposed that the essence of eusociality — sibling care by workers — evolved from maternal care. In the Hymenoptera, maternal care involves brood-provisioning by foraging for food followed by active feeding of young. According to this idea,

Table 3 | A summary of molecular analyses of worker–worker division of labour in the social insects

Type of division of labour	Species (common name of group)	Genes/pathways*	Biological processes/ molecular functions	Method	Refs
Nest work–foraging	<i>Apis mellifera</i> (bee)	<i>amylase</i> and <i>glucose oxidase</i>	Carbohydrate metabolism	NB, ENZ	146
Nest work–foraging	<i>Apis mellifera</i> (bee)	<i>period</i>	Circadian rhythms	qPCR, NB	147
Nest work–foraging	<i>Apis mellifera</i> (bee)	<i>acetylcholinesterase</i>	Hydrolysis of acetylcholine (neurotransmitter)	qPCR	148
Nest work–foraging	<i>Apis mellifera</i> (bee)	<i>foraging</i> [†]	Protein kinase	qPCR, PHARM	55
Newly emerged–foraging	<i>Apis mellifera</i> (bee)	<i>inositol 1,4,5-trisphosphate 3-kinase</i>	Second messenger signalling	DD RT-PCR	149
Nest work–foraging	<i>Apis mellifera</i> (bee)	Various (~2,500, for example <i>mitogen-activated protein kinase</i> , <i>failed axon connections</i> , <i>glycogen phosphorylase</i> , <i>mrjp1</i> , <i>mrjp5</i>)	Energy pathways, mitochondrial genes	MA	83,84
Response to queen pheromone	<i>Apis mellifera</i> (bee)	Various (~2,000, for example <i>kruppel homologue 1</i>)	Transcription factors	MA	93
Pollen foraging–nectar foraging	<i>Apis mellifera</i> (bee)	<i>vitellogenin</i> [†]	Yolk protein	qPCR, RNAi	70
Nest work–foraging	<i>Apis mellifera</i> (bee)	<i>malvolio</i> [†]	Manganese transporter	qPCR, PHARM	59
Sterile worker–egg laying worker	<i>Apis mellifera</i> (bee)	<i>mrjp2</i> , <i>mrjp7</i> , <i>NCP2-like</i> (Nieman-pick type C2-like)	Production of brood food	MA	150
Nest work–foraging	<i>Apis mellifera</i> (bee)	Various (17, for example <i>enolase</i> , <i>mjrp</i> , <i>CuZn superoxide dismutase</i>)	Major royal jelly protein, ATP synthases/ATPases, glycolytic enzymes, antioxidant enzymes	LC-MS	151
Nest work–foraging	<i>Apis mellifera</i> (bee)	NPY pathway (3) and insulin/target of rapamycin (TOR) pathway (4) [†]	Feeding behaviour, growth/energy allocation	qPCR, PHARM	50
Soldier–worker	<i>Hodotermopsis sjostedi</i> , <i>Nasutitermes takasagoensis</i> (termite)	<i>endo-beta-1,4,glucanase</i>	Cellulose digestion	qPCR, ENZ	64
Soldier–worker	<i>Nasutitermes takasagoensis</i> (termite)	<i>ntsp-1</i>	Secretory carrier protein	NB	65
Nest work–foraging	<i>Pogonomyrmex barbatus</i> (ant)	<i>foraging</i>	Protein kinase	qPCR	56
Soldier–worker	<i>Reticulitermes flavipes</i> (termite)	<i>hexamerin</i> [†]	Storage proteins	qPCR, RNAi	45
Soldier–worker	<i>Reticulitermes flavipes</i> (termite)	CYP4 genes (7)	Cytochrome P450 enzymes	qPCR	62
Soldier–worker	<i>Reticulitermes santonensis</i> (termite)	<i>coxIII</i>	Cytochrome oxidase	RT-PCR	63
Soldier–worker nymph	<i>Tuberaphis styraci</i> (aphid)	<i>cathepsin B</i>	Venom protease	SUB	152

*Numbers in parentheses represent the number of genes for which differential expression was found. [†]Gene for which a causal association has been demonstrated. DD, RNA-differential display; ENZ, enzyme assay; LC-MS, liquid chromatography–mass spectrometry; MA, Microarray; *mrjp*, major royal jelly protein; NB, Northern blots, PHARM, pharmacological manipulation; qPCR, quantitative PCR; RT-PCR, reverse transcription PCR; SUB, cDNA subtraction.

two principal behaviours exhibited by solitary female Hymenoptera, reproduction (egg-laying) and maternal care (brood-provisioning), became uncoupled during the early stages of social evolution⁶⁶ and these behaviours eventually occurred in separate castes, queens and workers, respectively. A molecular dimension was added to this idea with the prediction that sibling care and maternal care behaviours should be regulated by similar patterns of gene expression⁶⁷. This was tested in *Polistes metricus* wasps, a species that is 'primitively' eusocial (FIG. 1), and thus well suited to evolutionary analyses. As predicted, expression of 32 genes in the brains of workers was more similar to maternal females (foundresses) than to females not showing maternal care (queens and gynes)⁵⁴.

Diapause

Another life-history trait that might be involved in the evolution of eusociality in some lineages is diapause. Just as food availability during larval development determines whether individuals of some solitary insect species will develop into adults or enter diapause and develop into adults the following season, a similar process might regulate whether a larval social wasp develops into a non-reproductive or delays development and then becomes a reproductive⁶⁸. For example, *P. metricus* gynes are better-nourished than workers, and they enter a quiescent period before developing into reproductives the next season⁶⁸. It has been proposed that the molecular machinery underlying diapause in solitary insects is involved in caste determination in social wasps. Molecular-level support for this idea comes from measurements of storage proteins known to mediate diapause, including the above-mentioned hexamerin⁶⁸. Gyne-destined larvae had higher levels of hexamerin expression than worker-destined larvae. It remains to be seen whether these signatures simply reflect overwintering adaptations or have a more fundamental role in social evolution; further studies are needed to determine whether they also cause reproductive differences and track the evolution of queen and worker castes in other species.

Reproduction: ovarian physiology

It has been proposed that a suite of behavioural and physiological traits that were originally associated with reproduction were co-opted to control aspects of worker division of labour^{58,69}. This could only have occurred after the uncoupling of maternal care and reproduction described above — that is, after the evolution of the worker caste. The main body of evidence for this idea comprises findings showing that vitellogenin (best known as an egg yolk protein) has taken on novel functions in honeybee division of labour^{70–72}, especially in the division of labour for pollen and nectar foraging. Strains that were artificially selected to collect high or low amounts of pollen show differences in reproductive capacity⁷³. Even though worker bees are typically sterile, individuals from the high-pollen-foraging strain have more ovarioles and higher levels of circulating vitellogenin than do workers from the low-pollen-foraging strain. These correlations are supported by the finding that Vg RNAi caused bees to collect larger nectar loads

and also caused precocious foraging⁴⁹. These strains were found to differ in several other aspects of behaviour, including responsiveness to sucrose and age at onset of foraging. The genetic architecture of this suite of traits involves four major QTLs that have complex pleiotropic and epistatic interactions^{32,57}. However, using a reciprocal approach, strains of honeybees that were selected for differences in reproductive capacity failed to show the predicted differences in task specialization⁷⁴. These findings suggest that the links between ovariole number and foraging behaviour are not always detectable or present, and more research is needed to determine how strong and general this relationship is in honeybees.

Reproduction: endocrine physiology

Hormones are important for integrating developmental, physiological and behavioural responses to the environment. There are several examples of hormones adapted for particular functions taking on new roles to facilitate the evolution of new forms of behaviour⁴. Juvenile hormone (JH) is well known as an insect gonadotropin that stimulates ovary development. Thus, it is not surprising that JH affects queen–worker differentiation in various social insects⁷⁵. Juvenile hormone also seems to have taken on a second role in regulating behavioural differences in workers, with a causal influence on differences in behaviour in honeybees⁷⁶, swarm-founding wasps⁷⁷ and paper wasps⁷⁸. These effects are mediated, at least in honeybees, by a negative feedback regulatory loop involving both JH and vitellogenin⁷⁹. In addition, genotypic differences in JH titres during honeybee larval development are associated with genotypic differences in worker foraging behaviour⁸⁰, suggesting that organizational effects of hormones on adult behaviour occur in insects as in vertebrates⁸¹.

One idea suggests that during the evolution of sterile workers, JH became 'freed' from its reproductive function, which allowed it take on novel roles in regulating worker behavioural specialization^{75,82}. However, the fact that JH might affect worker behaviour in primitively eusocial paper wasps supports an alternative hypothesis that JH had roles in both behaviour and reproductive physiology in solitary ancestors⁷⁸. Comparative studies of the molecular pathways associated with JH regulation of worker behaviour could help distinguish between these two hypotheses.

Molecular pathways associated with JH regulation of worker behaviour are starting to be identified in honeybees. An earlier microarray study revealed that as many as one-third of the genes expressed in the brain are differentially expressed between workers engaged in brood care (nurses) and those that forage outside the hive⁸³. Additional microarray analysis revealed that many of the nurse–forager differences in brain gene expression are regulated by JH, which is also known to have diverse effects on metabolism and neural plasticity⁸⁴. Differences in division of labour in two honeybee subspecies were correlated with differences in JH and JH-target-gene expression, suggesting that this endocrine system mediates the subspecies genetic differences in behaviour⁸⁴. Although JH is important, it is likely that

Gynes

In insect societies, females that are destined to become queens.

Box 3 | First fruits from the first social insect genome sequence**The honeybee genome sequence and division of labour**

The availability of the honeybee (*Apis mellifera*) genome sequence¹⁰¹ provides an excellent resource for social insect genetics and genomics, and has yielded several insights relevant to division of labour. The *A. mellifera* genome contains almost double the number of olfactory receptor genes found in that of *Drosophila*, consistent with the honeybee's heavy reliance on chemical communication for division of labour (BOX 1). The honeybee genome also contains markedly fewer innate immunity genes than that of *Drosophila*. This finding might reflect the anti-pathogen benefits attributed to high colony genetic variability¹⁰⁴, together with a reliance on behavioural mechanisms to combat disease. The honeybee genome project also found evidence for at least 500 transcriptionally validated genes with no known orthologues in other organisms¹⁰¹, providing an important counterpoint to the emphasis on conserved genes in this Review. Novel or rapidly evolving genes such as *complementary sex determination* (*csd*) and genes encoding royal jelly proteins (brood food)¹⁰¹ could also be important to our understanding of the mechanisms and evolution of division of labour.

Cis-regulation and division of labour

Microarray analyses of honeybees have revealed that many genes encoding transcription factors show expression changes that are associated with division of labour^{84,93,105}. There have been two scans of the honeybee genome¹⁰¹, one for regulatory motifs associated with genes differentially expressed during queen–worker caste determination¹⁰⁶ and the other in the adult brain in association with worker–worker division of labour¹⁰⁷. Both found that the promoter regions for these genes are enriched for binding sites for certain transcription factors. These results provide a starting point for experimental elucidation of gene regulatory networks. They also suggest that social insects can contribute to the discovery of *cis*-regulatory codes for social regulation of development and behaviour.

Epigenetics and division of labour

The sequencing of the honeybee genome led to the discovery of a fully functional methylation system¹⁰¹, the first such finding for an insect, and caste determination has been found to involve epigenetic regulation¹⁰⁸. Worker development is associated with increased DNA methylation; larvae reared *in vitro* with RNAi knockdown of *DNA methyl transferase 3* show greatly increased probabilities of developing into queens. DNA methylation might serve as a developmental switch to regulate the expression of many downstream genes involved in determining caste fate, including the insulin/insulin-like growth factor 1-like (IIS)-related genes discussed in the main text^{43,44}.

there are several pathways that affect the division of labour in various species of honeybee⁸⁵ (TABLE 3).

Future directions

Genetic and genomic analyses have led to exciting insight into division of labour in social insects: how genetic variation affects it and which molecular pathways are involved in its regulation. These findings highlight the importance of social insects for the goal of forging a comprehensive molecular understanding of social life. They also are beginning to contribute to our understanding of several important issues in biology, including: the functional significance of genetic variation in both populations and cooperative groups; the effects of genetic variation on competition and cooperation within a group; the mechanisms by which extreme phenotypic plasticity evolves; and the roles of conservation and convergence of molecular mechanisms in the evolution of development and behaviour. For example, we now know that, despite its potential to cause more conflict among colony members, genetic diversity within colonies seems to improve colony functioning. We have also learned that extreme phenotypic plasticity can result from regulatory changes in genes related to basic life-history traits. And despite numerous examples of convergent evolution in social organization across the social insects, there is a general trend for the same pathways to be used in different lineages to generate diversity in social form.

Achieving the goal of a deeper understanding of the molecular basis of social life requires the ability to establish causal relationships between genes and division of labour. It is likely that the use of RNAi, an approach that is already proving to be effective, will intensify in work with

social insects (TABLES 2,3). New developments in genomics also hold great promise, especially the innovative new methods that aim to radically shrink sequencing costs (BOX 3). Comparative analyses will benefit immensely from the imminent availability of genome sequences for three non-social parasitic *Nasonia* wasp species. In addition, we predict that there will be whole genome sequences for 10–20 social insect species and their relatives within the next 10 years. These could be strategically chosen to span the full range of sociality — from solitary to eusocial — to provide powerful resources to study the mechanisms and evolution of division of labour.

The marriage of genetic and genomic analyses of social insects, which have thus far mostly been studied separately, will broaden our understanding of both the evolutionary and mechanistic processes underlying division of labour. It is therefore important to integrate genetic methods used to study the effects of heritable variation on division of labour (for example, SNPs⁸⁶, QTLs, breeding and selection) with molecular analyses of division of labour. An early success of this united approach in social insects concerns *csd*, which was identified with the help of a genetic approach and is now being used to develop a deep mechanistic understanding of the process of sex determination in honeybees (FIG. 1). Honeybee lines selected for foraging specializations³² or parthenogenesis²⁵ hold similar promise. This union of genetics and molecular biology, aided immensely by techniques in functional genomics (and proteomics), is likely to reveal how selection has moulded highly conserved pathways of development and neural plasticity to generate the remarkable diversity and complexity of division of labour in the insect societies.

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FURTHER INFORMATION

Andrew Suarez's homepage: <http://www.life.uiuc.edu/suarez>
Gene Robinson's homepage: <http://www.life.uiuc.edu/robinson>
Amy Toth's homepage: <https://netfiles.uiuc.edu/amytoth/www/Site/Professional%20information.html>
BeeBase: <https://secure.csl.gov.uk/beebase>
BeeSpace: <http://www.igb.uiuc.edu/beespace>
ALL LINKS ARE ACTIVE IN THE ONLINE PDF