TRENDS in Genetics

Evolution of social behavior – insights from the honey bee

Controlling insect immune genes
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Evolution of *cis*-regulatory sequences in *Drosophila*

Evo-devo and the evolution of social behavior

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The integration of evolutionary biology with developmental genetics into the hybrid field of 'evo-devo' resulted in major advances in understanding multicellular development and morphological evolution. Here we show how insights from evo-devo can be applied to study the evolution of social behavior. We develop this idea by reviewing studies that suggest that molecular pathways controlling feeding behavior and reproduction in solitary insects are part of a 'genetic toolkit' underlying the evolution of a particularly complex form of social behavior, division of labor among workers in honeybee colonies. The evo-devo approach, coupled with advances in genomics for non-model genetic organisms, including the recent sequencing of the honeybee genome, promises to advance our understanding of the evolution of social behavior.

From evo-devo to the evolution of social behavior

A major goal in biology is to understand the evolution of complex traits, such as the development of multicellular body plans and animal social behavior. Studies of the evolution of development, or 'evo-devo', have greatly improved our understanding of morphological evolution in animals [1]. The use of genetics and molecular biology has enabled evo-devo to identify conserved molecular pathways that regulate development in diverse species. This information has helped elucidate how evolution acts on some of these pathways to generate morphological diversity. We believe a similar approach to behavior will yield insights of comparable import for social evolution.

Social insects are important for studying the evolution of social behavior because they display the most extreme form of social organization in the animal world, eusociality (see Glossary). Research on social insect behavior is now significantly enhanced by the availability of the first sequenced genome for a eusocial species, the Western honeybee *Apis mellifera* [2].

We begin by describing guiding principles from evo-devo that can be applied to study the evolution of social behavior. We then review research with honeybees that uses genetics, molecular biology and genomics to explore the idea that information from the behavior and physiology of solitary insects can be used to identify candidate genes and molecular pathways for social behavior. This research focuses on two complex forms of socially regulated behavior that are part of the division of labor in the colony: the transition of worker bees from hive work to foraging behavior (age polyethism), and the specialization of foragers on pollen or nectar collection (see Figure I in Box 1). Findings to date reveal that genes involved in solitary behavior have been used to generate social behavior.

Insights from evo-devo

Results from evo-devo provide three main insights that can be applied to studies of the evolution of social behavior [1]. The first pertains to the modular organization of body plans; diversity in form arises from adaptive specialization of segments or appendages. Similarly, complex behavior in vertebrates and invertebrates can be viewed as being built from simpler 'behavioral modules', and the deconstruction of behavior into component phenotypes (or endophenotypes) facilitates studies of its mechanistic basis [3]. The application of this insight has already elucidated roles for two genes, *Amfor* and *Ammvl*, in the regulation of honeybee social behavior (see following section).

Second, although changes in coding sequences can be important for the evolution of novel traits, many traits

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Glossary

Age polyethism: age-related differences in behavior, a common system of division of labor in colonies of eusocial insects in which young workers perform nest work and old workers perform foraging.
Communal: describes species that nest in aggregations but do not display any of the defining traits of eusociality.
Endophenotypes: discrete and easily measurable component phenotypes that act in concert with other component phenotypes to give rise to a complex phenotypic trait.
Eusociality: describes species that exhibit: (i) cooperative care of brood; (ii) overlapping generations of individuals sharing a nest; and (iii) a reproductive division of labor where one or a few individuals monopolize reproduction. One or a few queens are responsible for the reproductive output of a colony whereas the more numerous workers (dozens to millions) engage in little or no personal reproduction and instead perform tasks necessary for the growth and development of their colony.
Genetic architecture: the full range of genetic effects on a trait, which includes complex epistatic and pleiotropic effects and the context dependence of the genes affecting the trait.
Hymenoptera: the order of insects that includes all wasps, bees and ants. The other 'classic' social insects are termites, in the order Isoptera. Eusociality is otherwise limited to scattered species in a few other insect orders, a few other species of invertebrates, and one group of mammals, the naked mole rats.
Semisocial: describes species that display cooperative brood care and reproductive castes, but no generational overlap.
Subsocial: describes species that exhibit parental care and in which offspring can assist the parents with care of subsequent broods.
Symplesiomorphy: shared traits that are the result of recent common ancestry.

Box 1. Division of labor among worker honeybees

In many insect societies there is a division of labor among workers, in addition to the one between queen and worker (Figure I). A worker performs tasks in the nest when young and then forages outside the nest when it gets older. The mechanisms underlying this form of behavioral maturation, known as 'age polyethism', have been extensively studied in the honeybee, *Apis mellifera* [3,66].

Adult worker honeybees perform a series of tasks in the hive when they are young (such as brood care or 'nursing') and at ∼2–3 weeks of age shift to foraging for nectar and pollen outside the hive for the remainder of their ∼5–7-week lives. Age polyethism involves changes in endocrine activity, metabolism, nutritional status, circadian clock activity, brain chemistry and brain structure [3,14,66]. Age polyethism is also associated with differences in the expression of thousands of genes in the brain [67], some of which are associated with known neural, physiological or metabolic changes [26].

The actual act of foraging seems similar between honeybees and solitary bees, but the motivations are different. Worker honeybees forage for their whole colony, which consists primarily of siblings. By contrast, a solitary bee forages for itself or its offspring. Worker honeybees determine the needs of their colony in addition to their own and in some cases communicate among nestmates to coordinate their foraging activities.

Because the onset age of foraging depends on the needs of the colony, the pace of behavioral maturation in honeybees is not rigid [66]. Foraging onset is socially regulated; pheromones, colony age demography, and other social cues delay or accelerate the onset of foraging [68]. These social influences are known to affect physiological factors including juvenile hormone (JH) (Box 3) [66] and vitellogenin [33], and molecular pathways associated with the *foraging* (*for*) and *malvolio* (*mvl*) genes [18,24], which are among the presumably many genes that have a role in behavioral maturation in honeybees [67].

Variation in the pace of honeybee behavioral maturation also has a genotypic component [32,69]. Numerous studies have shown that bees of certain genotypes mature faster and make the transition from working in the hive to foraging at a younger age than do workers from other genotypes [32,69]. This genotypic variation can be seen in comparisons of different *Apis mellifera* subspecies [26,70], lines of bees created by artificial selection [32], and different patrilines within honeybee colonies that arise as a result of polyandrous mating by the queen [71].

There is also genotypic variation for intrinsic physiological factors and response to social inputs that influence honeybee behavioral maturation [26], but there seems to be no obligate link between genotypic variation in the age at onset of foraging and variation in specific physiological or social factors studied to date [32]. Together, these observations suggest there are several distinct mechanisms on which natural selection can act to alter the speed of behavioral maturation in honeybees. One implication of this suggestion is that variation in the rate of behavioral maturation can be influenced by the actions of different networks of genes in bees with different genotypes (see ref. [72] for a theoretical treatment of this idea).
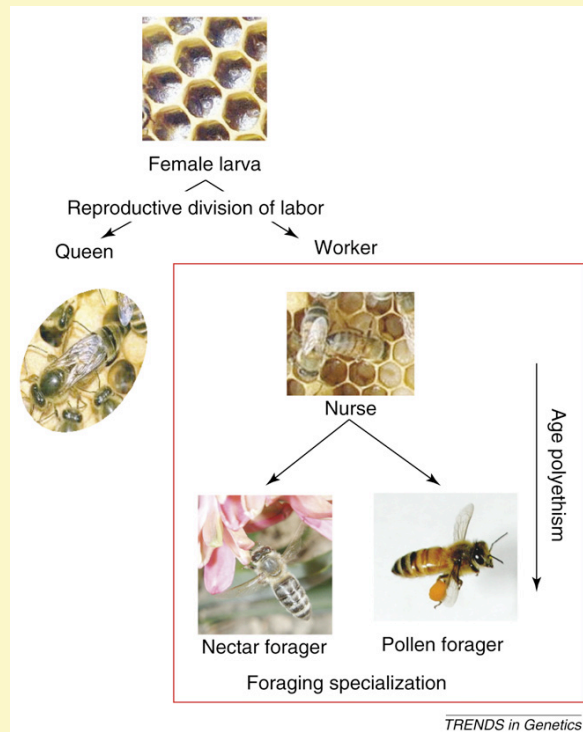

Figure I. There are two types of division of labor in honeybee colonies: a reproductive division of labor between queens and workers, and a division of labor among workers for tasks related to colony growth and development. During early larval development, the quality and quantity of food fed to a female larva determines whether it will develop into an adult queen or worker. Among workers, there is an age-related division of labor ('age polyethism'); key tasks include feeding the brood – performed by young 'nurse' bees – and foraging for nectar and pollen – performed by older bees. Among foragers, individuals tend to specialize on the collection of pollen or nectar. This review focuses on division of labor among worker honeybees (red square). Photos courtesy of P-O. Gustafsson (larvae), C.W. Whitfield (nurse bee) and Z-Y. Huang (nectar forager and pollen forager).

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arise from changes in gene expression [4,5]. Changes in gene expression – which affect timing, location or overall levels of mRNA abundance – are receiving increasing attention for behavioral evolution [6]. A focus on gene expression might be particularly important for analyses of behavior in social insects [7,8]. Just as cells within an organism develop into distinct cell types on the basis of differential gene expression, individuals within a social insect colony develop into 'castes' (queens and workers) that differ in both behavior and (sometimes) morphology [9]; this also typically occurs on the basis of differential gene expression [3]. Several lines of research with honeybees (reviewed in the following two sections) suggest that changes in gene regulation are important in the evolution of social behavior.

The third insight from evo-devo relates to the idea of a genetic 'toolkit' for development that is greatly conserved at the molecular level across diverse taxa (e.g. homeobox 'Hox' genes [10] and body form, and paired box 'Pax' genes and eye development [11]). The genetic toolkit for development is thought to consist of a set of genes with specialized functions, especially transcription factors [1]. Similarly, there are now several cases of specific genes, pathways or networks with conserved roles across species that are important in behavior (Box 2). The research reviewed below suggests two toolkits for honeybee social behavior – one based on genes related to feeding in the distantly related *Drosophila melanogaster*, and another based on a proposed genetic architecture for reproduction in insects.

From solitary to social: molecular pathways related to food gathering

Many basic forms of behavior, such as food gathering or foraging, seem similar between solitary and social bees – both collect pollen and nectar from flowers. However, there

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Box 2. Conservation and convergence in social evolution

There are now several examples in the study of animal behavior that demonstrate conserved mechanisms across vertebrates and invertebrates. For example, cyclic AMP-dependent protein kinase-related proteins are involved in learning and memory in worms, insects and mammals [49]; serotonin has a role in aggression in fish and crustaceans [50,51]; and genes encoding cyclic GMP-protein kinases affect foraging patterns in worms, flies, bees and ants [73]. Are the observed similarities in mechanisms controlling behavior the product of conservation or convergent evolution?

Because the phenotypes of ancestral taxa are rarely known, it is difficult to distinguish between conservation and convergence even with a well-resolved phylogeny. Consider the case of eye evolution [74,75]. It was long thought that vertebrate and invertebrate eyes were the products of convergent evolution, but molecular genetic analyses have sparked a new and illuminating debate on whether eyes have evolved repeatedly or once from an ancestral light-sensitive structure.

Similar issues will be raised as the evo-devo approach penetrates deeper into behavioral biology. For example, we know that there have been multiple independent origins of eusociality in insects [9]. Age polyethism (Box 1), present in ants, bees, wasps and even the distantly related termites [76], therefore seems to be the result of convergent evolution (Figure I). Within the order Hymenoptera, however, West-Eberhard suggested that age polyethism is derived from a shared ancestral trait or symplesiomorphy: age-related cycles of behavioral change and ovary development in solitary species [30]. There are both solitary and social species of bees and wasps (all ants are social). The solitary lineages that gave rise to eusocial species are all characterized by maternal females that make a nest and then protect and provision their offspring [52]. According to the 'ovarian ground plan' idea of West-Eberhard, the behavioral phases exhibited during solitary insect life cycles, that is, the egg-laying reproductive phase and the subsequent 'maternal' foraging-provisioning nonreproductive phase, became so distinct that they eventually occurred in separate organisms, queens and workers [30]. Then some elements of age polyethism in derived species of social insects are proposed to be linked to the ancestral ovarian cycle in the following way.

Young workers tend to stay in the nest and, although sterile, have some potential to lay unfertilized eggs; thus their behavior could be evolutionarily related to that of the egg-laying solitary wasp. Older workers, on the other hand, forage outside of the nest and generally have extremely low reproductive potential, suggesting a link to the nonreproductive phase. Results presented in the main text (see 'From solitary to social: molecular pathways related to reproduction') support this idea. These results, combined with those presented in the section headed 'From solitary to social: molecular pathways related to food gathering', suggest that if age polyethism evolved in evolutionarily distant lineages by convergent evolution, it did so with the involvement of common 'genetic toolkits' (Figure I).
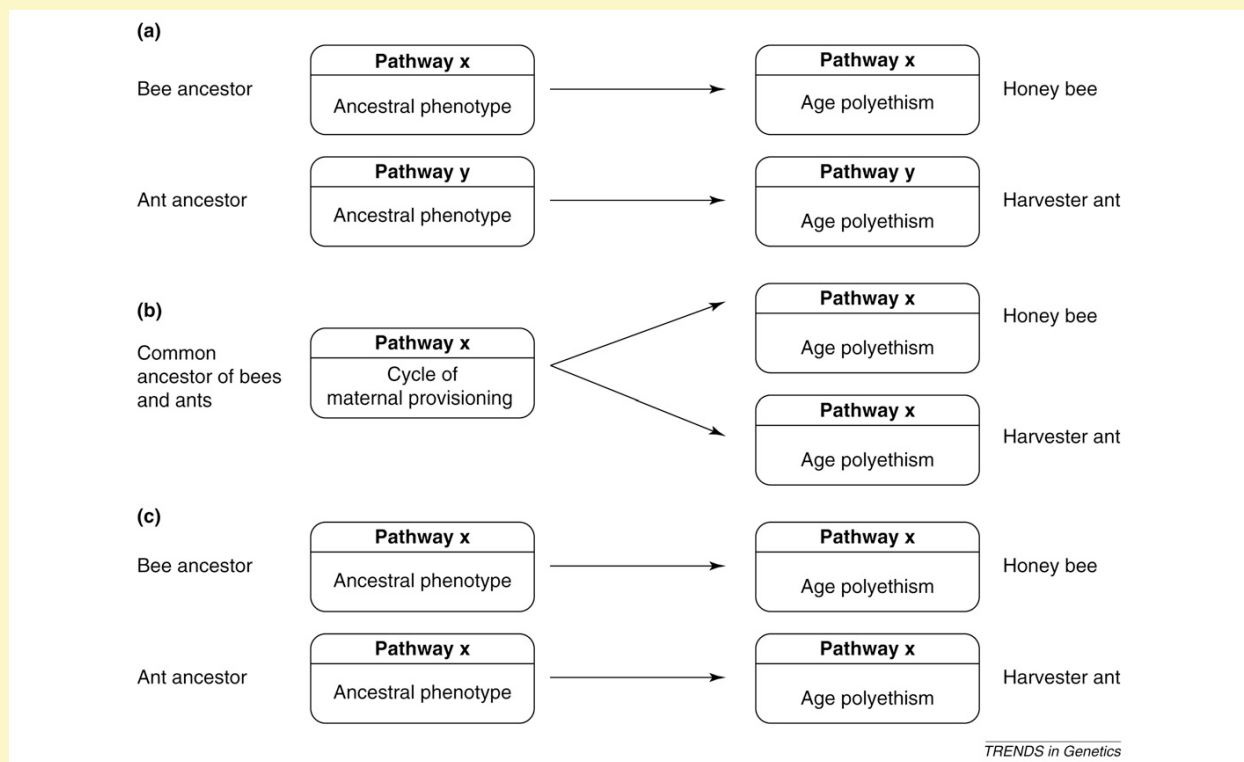

Figure I. Different mechanistic scenarios for the evolution of age polyethism. For illustrative purposes we use harvester ants (*Pogonomyrmex barbatus*) and honeybees, two distantly related taxa that evolved eusociality from distinct wasp ancestors [9]. **(a)** No conservation of mechanism, convergent evolution of phenotype. Under this scenario, age polyethism arose independently in the lineages giving rise to honeybees and harvester ants, and the regulatory mechanisms are expected to differ in the two lineages (pathways 'x' and 'y'). Mechanisms x and y were probably pre-existing in each lineage, but involved in regulating different, ancestral phenotypes. **(b)** Conservation of mechanism, parallel evolution of phenotype. A precursor phenotype in the solitary common ancestor of both bees and ants is proposed, consisting of cycles of egg-laying and maternal foraging and provisioning behavior. Pathway x is hypothesized to have a role in both maternal behavior and age polyethism. This scenario is the context for the 'ovarian ground plan' (Box 3). **(c)** Convergent evolution of mechanism, convergent evolution of phenotype. Age polyethism antecedents arose independently in ancestral forms of both the bee and ant lineages (convergent phenotypic evolution). In both lineages, pathway x genes were independently co-opted for the regulation of age polyethism.

is a fundamental difference: the behavior of a social bee is adapted to increase the fitness of its colony rather than its own personal fitness. This gives rise to important differences in when and how foraging is performed (Box 1). Despite these differences, recent results (discussed below) suggest that genes associated with food gathering and eating in solitary insects are involved in social foraging in honeybees.

Physiological studies also support mechanistic connections between food-gathering behavior in solitary species and the age at which worker honeybees shift from working in the hive to collecting food for their colony. Nutritional depletion of a honeybee colony causes bees to begin to forage precociously [12], and changes in individual abdominal lipid stores are among the causative factors [13,14]. Decreases in abdominal lipid [13] and the yolk-storage protein vitellogenin (Vg) [15] are correlated with the age-related transition from hive work to foraging (Box 1). Experimental lipid depletion [14] or RNAi (RNA interference) knockdown of the vitellogenin gene Vg [16] cause a precocious onset of foraging. These studies demonstrate that differences in nutritional state in individual bees trigger them to forage, even though the food they collect is donated to the colony, rather than used solely for their own sustenance.

Studies involving cyclic-GMP (cGMP) signaling pathways have revealed strong conservation in the molecular underpinnings of food-gathering behavior. In Drosophila melanogaster, the foraging gene (for) encodes a cGMP-dependent protein kinase (protein kinase G; PKG), and naturally occurring allelic variation in this gene results in two phenotypes, 'sitters' and 'rovers' [17]. Although D. melanogaster lives most of its life in solitary fashion, the behavioral variation associated with these allelic differences suggested an interesting parallel with the food-gathering behavior of the honeybee. As stated above, honeybees exhibit age-related changes in behavior; for example, young bees stay in the hive (analogous to the fly 'sitters') but as they age, they 'rove' outside the hive in search of food.

Amfor, an ortholog of the Drosophila for gene, was found to regulate this age-related change in behavior in honeybees. Levels of Amfor mRNA in the brain are greater in foragers than in bees working in the hive, and experimentally activating PKG causes precocious foraging [18]. Amfor expression is socially regulated in honeybees: levels of Amfor mRNA are also elevated in the brains of young bees induced to forage early owing to a lack of older bees [18]. cGMP signaling also affects feeding arousal in Caenorhabditis elegans [19] and the harvester ant Pogonomyrmex barbatus [20], demonstrating the evolutionary lability of pathways involving PKG signaling (Box 2). Thus, evolutionary changes in gene regulation are important in generating variation in social behavior [18].

Examination of PKG in several species suggests that molecular pathways governing nutritional state and feeding behavior in solitary animals represent a 'toolkit' for the evolution of division of labor in social insects. This idea is further supported by studies of malvolio (mvl) and neuropeptide Y (NPY). mvl encodes a manganese transporter, and a mutation at this locus in D. melanogaster causes a loss of responsiveness to sucrose; this deficit is eliminated by treatment with manganese [21]. Sucrose responsiveness is a behavioral module of foraging in honeybees [22], and quantitative trait locus (QTL) analysis showed that the genetic architecture of sucrose responsiveness and onset age of foraging are related [23]. These results suggested that mvl also was a good candidate gene for the regulation of division of labor in honeybees.

The mRNA levels of the honeybee ortholog Ammvl were greater in foraging workers than in bees working in the hive, and manganese treatment not only increased sucrose responsiveness but also caused an earlier onset of foraging [24]. Food-deprived bees also have increased sucrose responsiveness [25], suggesting important associations between foraging behavior, nutritional state, sucrose responsiveness and brain expression of Ammvl (and probably other genes [26]).

NPY (neuropeptide Y) acts as a hunger signal, regulating food intake in vertebrates, and is part of a well-conserved pathway for feeding-related behaviors in both vertebrates and invertebrates [27]. The D. melanogaster ortholog, neuropeptide F, influences several feeding-related behaviors, including food aversion, increased movement and cooperative burrowing [28]. In C. elegans, a variation in npr-1, which encodes a putative receptor for an NPY-like molecule, causes naturally occurring variation in feeding behavior [29]. Strains of nematodes with the 215 V allele feed alone whereas strains with the 215F allele aggregate during feeding. Preliminary results from microarray studies suggest that brain expression of genes related to NPF signaling and the related insulin pathway is also important in governing division of labor in honeybee colonies*.

These findings suggest that some well-conserved molecular pathways that influence food-gathering behavior in solitary insects such as D. melanogaster also regulate the division of labor in social insect societies. According to this idea, when an insect with a solitary lifestyle is hungry, certain molecular pathways are activated that motivate it to search for food; but when some of the same pathways are activated in a worker honeybee, they hasten the transition from working in the hive to foraging for the entire colony. An important issue for the future is to address why certain feeding-related pathways, or components of these pathways, are more evolutionarily labile than others.

From solitary to social: molecular pathways related to reproduction

Reproductive behavior also differs fundamentally between social insects and solitary insects; workers are partially or completely inhibited from reproducing (see Figure I in Box 1). Nevertheless, recent evidence suggests that genes that govern insect reproduction represent another 'toolkit' that can be used in the evolution of worker behavior in social insects.

It has been proposed that a 'ground plan' involving reproductive physiology and behavior in solitary insects

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was acted on by natural selection to produce social insect castes (see Figure I in Box 2) [30]. The result is that maternal provisioning behavior was separated from reproductive behavior. This idea is similar to the notion that parental behavior in vertebrates was acted upon by evolution to produce other forms of sociality [31]. This idea predicts changes in the expression of so far unknown genes influencing reproductive and maternal behavior [8]; recent work suggests that such changes in gene regulation have occurred during social evolution (A. Toth *et al.*, unpublished).

Evidence supporting the general idea that selection has acted on reproductive systems to shape worker behavior has emerged from a convergence of studies on Vg with studies using lines of honeybees that have been selected for increased and decreased amounts of pollen stored in the hive [32,33]. Lines of honeybees were originally selected to identify QTLs that affect the tendency of a forager to specialize on collecting either pollen or nectar [32,33]. These lines also differ in several other aspects of behavior, including responsiveness to sucrose and tactile stimuli, and age at onset of foraging. The genetic architecture of this suite of traits has been shown to involve four major QTLs that have complex pleiotropic and epistatic interactions [34]. Recently, worker bees from these lines were found to differ in aspects of reproductive development, for example, ovary size and circulating levels of Vg [32]. Workers from the high-pollen foraging line have more ovarioles and greater levels of circulating Vg than do workers from the low-pollen foraging line [35].

These results have led to the suggestion that, although worker honeybees are typically sterile, the genetic architecture involved in insect reproduction has been co-opted during social evolution to regulate worker honeybee foraging behavior [35]. Correlations between foraging specialization, Vg titers and ovariole numbers, and the finding that *Vg* knockdown with RNAi causes bees to collect larger nectar loads, all support this hypothesis [16]. It will be important to see whether other genes related to insect reproduction are also involved in regulating honeybee foraging specialization; the QTL studies mentioned above clearly indicate that this trait is polygenic.

Vg is also involved in controlling the pace at which worker bees shift from hive work to foraging. Vg circulating levels decline with worker age, and treatment with *Vg* dsRNA led to reduced levels of Vg and an early onset of foraging [16]. These results are consistent with the idea that pathways involved in insect reproduction have been shaped by social evolution to regulate worker honeybee foraging behavior. However, the RNAi results seem to conflict with results from the high- and low- pollen hoarding lines. Relative to the low-pollen hoarding line, bees from the high-pollen foraging line have greater Vg blood titers before the onset of foraging [36] but an earlier age at onset of foraging [16]. Genotypic differences in temporal patterns of Vg production have been proposed to account for the different results [33]; this suggestion awaits experimental verification.

It is not known whether these novel functions for Vg reflect evolutionary changes in *Vg* gene number, coding sequence or gene expression. With the evidence to date

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Box 3. Co-opting an endocrine system for social evolution

JH functions as a gonadotropin in most solitary and many social insects, and stimulates egg development and vitellogenesis [39]. However, in honeybees it does not act as a gonadotropin, and in workers it regulates division of labor, in particular the age at onset of foraging.

JH titers are generally lower in nurse bees than in foragers; they remain low in bees experimentally induced to become overage nurses, increase prematurely in precocious foragers, and drop in foragers that are forced to revert to nursing [77]. Removal of the glands that produce JH delays the onset of foraging [78] and reduces the metabolic rate, making it more difficult to sustain normal flight [79]. Hormone treatment causes precocious foraging and also causes massive changes in brain gene expression, producing a brain expression profile that strongly resembles the profile of a forager bee [26].

In most insects there is a positive relationship between JH and Vg; increasing titers of JH cause an increase in Vg synthesis and uptake [80]. In honeybees, however, a stimulatory effect of JH on Vg synthesis has been found only in pupal stages [81]; during adulthood Vg is negatively regulated by JH [82]. JH and Vg are thought to function as mutual repressors in the hive bee-to-forager transition [83]. This idea is supported by results showing that decreasing Vg titers by means of Vg RNAi leads to an increase in JH titer [84] and early onset of foraging in workers [16]. Both JH and Vg are key regulators of honeybee behavioral maturation, and both components of a key insect endocrine system seem to have evolved novel roles in the greatly derived system of honeybee division of labor. Similarly, hormone receptors in vertebrates have recently been shown to be evolutionarily labile [85]. However, JH does act as a gonadotropin in the fire ant, *Solenopsis invicta*, a species with a system of division of labor comparable to that of the honeybee [80]. This suggests that JH could have different roles in social organization in the multiple independent evolutions of eusociality that occurred in the Hymenoptera.

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suggesting that it is the same Vg protein that functions as a yolk protein in queen honeybees, it currently seems more likely to be a change in the temporal or spatial regulation (or both) of *Vg*. Changes in *Vg* regulation are probably linked with evolutionary changes in the function of juvenile hormone (JH) (Box 3).

Vg is a good example of a gene evolving novel roles, while retaining its ancestral function. Another example involves genes encoding hexamerins, implicated in the regulation of caste differences in termites [37] and social wasps (J.H. Hunt *et al.*, unpublished), and the genes encoding royal jelly proteins, which are used for brood feeding in honeybees [38]. Similar to Vg, hexamerins act as storage proteins and might affect JH titers by binding directly to this hormone [39]. Additional studies of these genes should help in understanding how novel functions arise through changes in gene regulation, gene duplication or other means.

Overall, these findings suggest that molecular pathways that govern reproductive status and behavior in solitary insects represent another 'toolkit' that can be used in the evolution of division of labor in social insects.

Towards a genetic toolkit for division of labor?

The two lines of study reviewed here highlight two putative 'genetic toolkits' for division of labor among workers – one involving genes associated with feeding and the other involving genes associated with reproduction. We suggest these two lines of research will converge on insulin

signaling. The insulin–insulin-like growth factor signaling pathway is a key integrative pathway regulating nutrition, fertility, aging and other important biological processes throughout animals [40].

This signaling pathway is attracting increased interest in the study of insect polyphenisms [41], including roles in worker–queen differentiation [42,86] and worker behavior [43] in honeybees. Studies with *C. elegans* suggest there could be a mechanistic link between PKG and NPF signaling for feeding [44], and connections between NPF and insulin signaling in the brain are well known [45]. Similarly, recent advances in insect molecular endocrinology have revealed connections between insulin signaling and JH [46], and there might be functional links between the insulin pathway and vitellogenin in *C. elegans* [47] and honeybees [48]. Exploring the potential for this convergence, especially regarding the role of insulin signaling in social behavior, is an important direction for future research.

Unresolved issues
This review illustrates the value of taking an evo-devo approach to the study of social behavior. The genetic toolkit for development is hypothesized to consist of a small set of genes with strongly specialized functions [1]; we present evidence for two possible genetic toolkits for honeybee social behavior. Important questions for the future are whether this basic evo-devo concept will apply broadly to social behavior, and if so, will there be an underlying core of 'Hox-type' genes regulating neural and behavioral plasticity, or will different toolkits correspond to different kinds of behavior? These questions require broad analyses of many genes and molecular pathways, in addition to the focus on specific genes that characterize most of the research reviewed here.

We believe the evo-devo approach is broadly applicable to the study of behavior. Studies of learning and memory [49] and aggression [50,51] have demonstrated deep conservation of molecular pathways involved in behavior, even across invertebrates and vertebrates. It has long been thought that components of solitary behavior have been co-opted during social evolution to sculpt more complex behavioral systems [9,52]. Thanks to advances in genomics, the idea that common molecular pathways underlie both solitary and social behavior is now finally being tested, and the first results with the honeybee, reviewed here, are encouraging. But this success leads to a challenging question: how can molecular pathways involved in behavior be conserved even when species show major differences in brain structure and the overall organization of the nervous system? In this regard, applying the concepts of evo-devo to behavior will be harder than for development because behavior is produced by a specialized structure, the brain. A special challenge for understanding the evolution of social behavior is how to integrate two fundamental lines of study in neuroscience: identifying the molecular pathways involved in producing a behavior and the neural circuits that they act upon. These issues also pertain to studies of the evolution of social behavior in vertebrates [31], which also are amenable to the approaches outlined here.

Identifying pathways involved in the evolution of a social behavior provides insight into its mechanistic origins, but this is distinct from identifying the forces of selection acting on these pathways during social evolution. For example, the evolution of age polyethism (see Figure I in Box 2) has been explained by invoking colony-level selection (increased efficiency of task allocation [53]) and individual-level selection (young workers remain on the nest as 'hopeful reproductives' and only commence foraging after their reproductive potential declines [54]). However, neither argument predicts specific mechanisms. After important underlying pathways for a particular social behavior are well characterized, it might be possible to explore how they have been shaped by the selective forces hypothesized to underlie the evolution of social behavior (i.e. kin selection, colony-level selection, multilevel selection, and various ecological factors [55–57]).

For studies on the evolution of social behavior to progress as successfully as evo-devo has, comparisons of the spatial and temporal regulation of relevant genes in the brain across species are necessary. For age polyethism, fly vs bee comparisons are not sufficient. The honeybee is greatly derived, making it hard to discern direct links to solitary behavior. Likewise, *D. melanogaster* does not exhibit maternal care, which is widely seen in solitary Hymenoptera (bees, ants and wasps), and is thought to underlie the evolution of more derived forms of social behavior in the insect societies.

Different animals exhibit a wide range of communal, semisocial and subsocial intermediate forms of social organization [9]. Some lineages (e.g. sweat bees and bumble bees) contain both solitary and eusocial taxa. This diversity – from solitary to eusociality – provides an excellent framework for comparative analyses of the mechanisms and evolution of eusociality itself, and the division of labor among workers. Such comparative analyses require phylogenetic information both across and within families, which is an area of active research in the hymenopteran insects [58,59]. Recent improvements in sequencing technology [60] and the sequencing of the honeybee genome [2] make it possible to develop extensive comparative sociogenomic analyses within the bees and other social insects [61]. This approach – taking full advantage of multiple independent evolutions of eusociality [9] and the tremendous diversity of forms of social organization in insect colonies – will enable discovery of major conserved pathways used in social evolution and in particular the fundamental reproductive division of labor in the insect societies, the foundation for other more derived traits such as age polyethism (see Figure I in Box 2).

Concluding remarks
The field of evo-devo has been successful in discovering molecular mechanisms underlying the development of certain morphological traits and then using that information as a foundation to study the evolution of novel versions of that trait. However, except for a few cases [62,63], evo-devo studies lack a strong ecological context, in part because of a focus on genetically tractable taxa, rather than those that are best positioned taxonomically to address evolutionary questions [64]. By contrast, much is

known about how ecological factors influence the evolution of many forms of behavior [65]. Insights from behavioral ecology, when integrated with our increasing knowledge about genes and molecular pathways involved in behavioral evolution, will provide the material for a synthesis that might prove to be even richer than what is currently seen in evo-devo. A full integration of knowledge about the molecular and neural basis of behavior, in addition to its ecological context and phylogenetic history, can provide a strong framework for studying the evolution of social behavior, and the selective forces that contribute to it.

Acknowledgements

References

1 Carroll, S.B. *et al.* (2005) *From DNA to Diversity: Molecular Genetics and the Evolution of Animal Design,* Blackwell Publishing
2 Honey Bee Genome Sequencing Consortium (2006) Insights into social insects from the genome of the honey bee *Apis mellifera*. *Nature* 443, 931–948
3 Robinson, G.E. *et al.* (2005) Sociogenomics: social life in molecular terms. *Nat. Rev. Genet.* 6, 257–270
4 Colosimo, P.F. *et al.* (2005) Widespread parallel evolution in sticklebacks by repeated fixation of ectodysplasin alleles. *Science* 307, 1928–1933
5 Wray, G.A. (2007) The evolutionary significance of cis-regulatory mutations. *Nat. Rev. Genet.* 8, 206–216
6 Khaitovich, P. *et al.* (2006) Positive selection on gene expression in the human brain. *Curr. Biol.* 16, R356–R358
7 Robinson, G.E. and Ben-Shahar, Y. (2002) Social behavior and comparative genomics: new genes or new gene regulation? *Genes Brain Behav.* 1, 197–203
8 Linksvayer, T.A. and Wade, M.J. (2005) The evolutionary origin and elaboration of sociality in the aculeate Hymenoptera: Maternal effects, sib-social effects, and heterochrony. *Q. Rev. Biol.* 80, 317–336
9 Wilson, E.O. (1971) *The Insect Societies,* Belknap Press of Harvard University Press
10 Gellon, G. and McGinnis, W. (1998) Shaping animal body plans in development and evolution by modulation of Hox expression patterns. *Bioessays* 20, 116–125
11 Pichaud, F. and Desplan, C. (2002) *Pax* genes and eye organogenesis. *Curr. Opin. Genet. Dev.* 12, 430–434
12 Schulz, D.J. *et al.* (1998) Effects of colony food shortage on behavioral development in honey bees. *Behav. Ecol. Sociobiol.* 42, 295–303
13 Toth, A.L. and Robinson, G.E. (2005) Worker nutrition and division of labour in honeybees. *Anim. Behav.* 69, 427–435
14 Toth, A.L. *et al.* (2005) Nutritional status influences socially regulated foraging ontogeny in honey bees. *J. Exp. Biol.* 208, 4641–4649
15 Bitondi, M.M.G. and Simoes, Z.L.P. (1996) The relationship between level of pollen in the diet, vitellogenin and juvenile hormone titres in Africanized *Apis mellifera* workers. *J. Apicult. Res.* 35, 27–36
16 Nelson, C.M. *et al.* (2007) The gene vitellogenin has multiple coordinating effects on social organization. *PLoS Biol.* 5, e62
17 Osborne, K.A. *et al.* (1997) Natural behavior polymorphism due to a cGMP-dependent protein kinase of *Drosophila*. *Science* 277, 834–836
18 Ben-Shahar, Y. *et al.* (2002) Influence of gene action across different time scales on behavior. *Science* 296, 741–744
19 Fujiwara, M. *et al.* (2002) Regulation of body size and behavioral state of *C. elegans* by sensory perception and the EGL-4 cGMP-dependent protein kinase. *Neuron* 36, 1091–1102

20 Ingram, K.K. *et al.* (2005) Task-specific expression of the foraging gene in harvester ants. *Mol. Ecol.* 14, 813–818
21 Rodrigues, V. *et al.* (1995) Malvolio, the *Drosophila* homolog of house Nramp-1 (Bcg), is expressed in macrophages and in the nervous system and is required for normal taste behavior. *EMBO J.* 14, 3007–3020
22 Pankiw, T. and Page, R.E. (1999) The effect of genotype, age, sex, and caste on response thresholds to sucrose and foraging behavior of honey bees (*Apis mellifera* L.). *J. Comp. Physiol. [A]* 185, 207–213
23 Rueppell, O. *et al.* (2006) The genetic architecture of sucrose responsiveness in the honeybee (*Apis mellifera* L.). *Genetics* 172, 243–251
24 Ben-Shahar, Y. *et al.* (2004) Phenotypic deconstruction reveals involvement of manganese transporter malvolio in honey bee division of labor. *J. Exp. Biol.* 207, 3281–3288
25 Ben-Shahar, Y. and Robinson, G.E. (2001) Satiation differentially affects performance in a learning assay by nurse and forager honey bees. *J. Comp. Physiol. [A]* 187, 891–899
26 Whitfield, C.W. *et al.* (2006) Genomic dissection of behavioral maturation in the honey bee. *Proc. Natl. Acad. Sci. U. S. A.* 103, 16068–16075
27 Konturek, S.J. *et al.* (2004) Brain-gut axis and its role in the control of food intake. *J. Physiol. Pharmacol.* 55, 137–154
28 Wu, Q. *et al.* (2003) Developmental control of foraging and social behavior by the *Drosophila* neuropeptide Y-like system. *Neuron* 39, 147–161
29 de Bono, M. and Bargmann, C.I. (1998) Natural variation in a neuropeptide Y receptor homolog modifies social behavior and food response in *C. elegans*. *Cell* 94, 679–689
30 West-Eberhard, M.J. (1996) Wasp societies as microcosms for the study of development and evolution. In *Natural History and Evolution of Paper Wasps* (Turillazzi, S. and West-Eberhard, M.J., eds), pp. 290–317, Oxford University Press
31 Keverne, E.B. and Curley, J.P. (2004) Vasopressin, oxytocin and social behaviour. *Curr. Opin. Neurobiol.* 14, 777–783
32 Page, R.E. *et al.* (2006) The development and evolution of division of labor and foraging specialization in a social insect (*Apis mellifera* L.). *Curr. Top. Dev. Biol.* 74, 253–286
33 Page, R.E. and Amdam, G.V. (2007) The making of a social insect: developmental architectures of social design. *Bioessays* 29, 334–343
34 Ruppell, O. *et al.* (2004) Pleiotropy, epistasis and new QTL: the genetic architecture of honey bee foraging behavior. *J. Hered.* 95, 481–491
35 Amdam, G.V. *et al.* (2006) Complex social behaviour derived from maternal reproductive traits. *Nature* 439, 76–78
36 Amdam, G.V. *et al.* (2004) Reproductive ground plan may mediate colony-level selection effects on individual foraging behavior in honey bees. *Proc. Natl. Acad. Sci. U. S. A.* 101, 11350–11355
37 Zhou, X. *et al.* (2006) Social exploitation of hexamerin: RNAi reveals a major caste-regulatory factor in termites. *Proc. Natl. Acad. Sci. U. S. A.* 103, 4499–4504
38 Drapeau, M.D. *et al.* (2006) Evolution of the Yellow/Major Royal Jelly Protein family and the emergence of social behavior in honey bees. *Genome Res.* 16, 1385–1394
39 Hartfelder, K. (2000) Insect juvenile hormone: from 'status quo' to high society. *Braz. J. Med. Biol. Res.* 33, 157–177
40 Nassel, D.R. (2002) Neuropeptides in the nervous system of *Drosophila* and other insects: multiple roles as neuromodulators and neurohormones. *Prog. Neurobiol.* 68, 1–84
41 Emlen, D.J. *et al.* (2006) Insulin signaling and limb-patterning: candidate pathways for the origin and evolutionary diversification of beetle 'horns'. *Heredity* 97, 179–191
42 Wheeler, D.E. *et al.* (2006) Expression of insulin pathway genes during the period of caste determination in the honey bee, *Apis mellifera*. *Insect Mol. Biol.* 15, 597–602
43 Hunt, G.J. *et al.* (2007) Behavioral genomics of honey bee foraging and nest defense. *Naturwissenschaften* 94, 247–267
44 Cheung, B.H.H. *et al.* (2004) Soluble guanylate cyclases act in neurons exposed to the body fluid to promote *C. elegans* aggregation behavior. *Curr. Biol.* 14, 1105–1111
45 Williams, G. *et al.* (2004) Anabolic neuropeptides. *Physiol. Behav.* 81, 211–222
46 Atar, M. *et al.* (2003) The endocrine regulation of aging by insulin-like signals. *Science* 299, 1346–1351

47 Nakamura, A. *et al.* (1999) Vitellogenin-6 is a major carbonylated protein in aged nematode, *Caenorhabditis elegans*. *Biochem. Biophys. Res. Commun.* 264, 580–583

48 Seehuus, S.C. *et al.* (2006) Reproductive protein protects functionally sterile honey bee workers from oxidative stress. *Proc. Natl. Acad. Sci. U. S. A.* 103, 962–967

49 Kandel, E.R. (2006) *In Search of Memory: the Emergence of a New Science of Mind,* W.W. Norton and Company

50 Suomi, S.J. (2003) Gene-environment interactions and the neurobiology of social conflict. *Ann. N. Y. Acad. Sci.* 1008, 132–139

51 Kravitz, E.A. (2000) Serotonin and aggression: insights gained from a lobster model system and speculations on the role of amine neurons in a complex behavior. *J. Comp. Physiol. [A]* 186, 221–238

52 Evans, H.E. and West-Eberhard, M.J. (1970) *The Wasps,* University of Michigan Press

53 Oster, G.F. and Wilson, E.O. (1978) Caste and ecology in the social insects. *Monogr. Popul. Biol.* 12, 1–352

54 Monnin, T. and Ratnieks, F.L.W. (1999) Reproduction versus work in queenless ants: when to join a hierarchy of hopeful reproductives? *Behav. Ecol. Sociobiol.* 46, 413–422

55 Wilson, E.O. and Holldobler, B. (2005) Eusociality: Origin and consequences. *Proc. Natl. Acad. Sci. U. S. A.* 102, 13367–13371

56 Crozier, R.H. and Pamilo, P. (1996) *Evolution of Social Insect Colonies: Sex Allocation and Kin Selection*, Oxford Series in Ecology and Evolution, Oxford University Press

57 Lehmann, L. and Keller, L. (2006) The evolution of cooperation and altruism – a general framework and a classification of models. *J. Evol. Biol.* 19, 1365–1376

58 Cameron, S.A. and Mardulyn, P. (2001) Multiple molecular data sets suggest independent origins of highly eusocial behavior in bees (Hymenoptera: Apinae). *Syst. Biol.* 50, 194–214

59 Danforth, B.N. *et al.* (2003) Phylogeny of eusocial Lasioglossum reveals multiple losses of eusociality within a primitively eusocial clade of bees (Hymenoptera: Halictidae). *Syst. Biol.* 52, 23–36

60 Margulies, M. *et al.* (2005) Genome sequencing in microfabricated high-density picolitre reactors. *Nature* 437, 376–380

61 Wang, J. *et al.* (2007) An annotated cDNA library and microarray for large scale gene expression studies in the ant *Solenopsis invicta*. *Genome Biol.* 8, R9

62 Kingsley, D.M. *et al.* (2004) New genomic tools for molecular studies of evolutionary change in threespine sticklebacks. *Behaviour* 141, 1331–1344

63 Grant, P.R. *et al.* (2006) A developing paradigm for the development of bird beaks. *Biol. J. Linn. Soc.* 88, 17–22

64 Jenner, R.A. and Willis, M.A. (2007) The choice of model organisms in evo-devo. *Nat. Rev. Genet.* 8, 311–319

65 Krebs, J.R. and Davies, N.B. (1997) *Behavioural Ecology: An Evolutionary Approach,* Blackwell Science

66 Robinson, G.E. (2002) Genomics and integrative analyses of division of labor in honeybee colonies. *Am. Nat.* 160, S160–S172

67 Whitfield, C.W. *et al.* (2003) Gene expression profiles in the brain predict behavior in individual honey bees. *Science* 302, 296–299

68 Leoncini, I. *et al.* (2004) Regulation of behavioral maturation by a primer pheromone produced by adult worker honey bees. *Proc. Natl. Acad. Sci. U. S. A.* 101, 17559–17564

69 Giray, T. *et al.* (2000) Genetic variation in worker temporal polyethism and colony defensiveness in the honey bee, *Apis mellifera*. *Behav. Ecol.* 11, 44–55

70 Brillet, C. *et al.* (2002) Racial differences in division of labor in colonies of the honey bee (*Apis mellifera*). *Ethology* 108, 115–126

71 Page, R.E. and Robinson, G.E. (1991) The genetics of division of labor in honey bee colonies. *Adv. Insect Physiol.* 23, 117–169

72 Greenspan, R.J. (2001) Opinion – The flexible genome. *Nat. Rev. Genet.* 2, 383–387

73 Fitzpatrick, M.J. and Sokolowski, M.B. (2004) In search of food: Exploring the evolutionary link between cGMP-dependent protein kinase (PKG) and behaviour. *Integr. Comp. Biol.* 44, 28–36

74 Kozmik, Z. (2005) Pax genes in eye development and evolution. *Curr. Opin. Genet. Dev.* 15, 430–438

75 Fernald, R.D. (2006) Casting a genetic light on the evolution of eyes. *Science* 313, 1914–1918

76 Konate, S. *et al.* (2000) Colour variation and polyethism of the soldier caste in the termite *Macrotermes bellicosus*. *Entomol. Exp. Appl.* 94, 51–55

77 Huang, Z.Y. and Robinson, G.E. (1996) Regulation of honey bee division of labor by colony age demography. *Behav. Ecol. Sociobiol.* 39, 147–158

78 Sullivan, J.P. *et al.* (2000) Juvenile hormone paces behavioral development in the adult worker honey bee. *Horm. Behav.* 37, 1–14

79 Sullivan, J.P. *et al.* (2003) Juvenile hormone and division of labor in honey bee colonies: effects of allatectomy on flight behavior and metabolism. *J. Exp. Biol.* 206, 2287–2296

80 Robinson, G.E. and Vargo, E.L. (1997) Juvenile hormone in adult eusocial Hymenoptera: gonadotropin and behavioral pacemaker. *Arch. Insect Biochem. Physiol.* 35, 559–583

81 Barchuk, A.R. *et al.* (2004) *Apis mellifera* ultraspiracle: cDNA sequence and rapid up-regulation by juvenile hormone. *Insect Mol. Biol.* 13, 459–467

82 Bloch, G. *et al.* (2002) Endocrine influences on the organization of insect societies. In *Hormones, Brain and Behavior* (Pfaff, D. *et al.*, eds), pp. 195–235, Academic Press

83 Amdam, G.V. and Omholt, S.W. (2003) The hive bee to forager transition in honeybee colonies: the double repressor hypothesis. *J. Theor. Biol.* 223, 451–464

84 Guidugli, K.R. *et al.* (2005) Vitellogenin regulates hormonal dynamics in the worker caste of a eusocial insect. *FEBS Lett.* 579, 4961–4965

85 Bridgham, J.T. *et al.* (2006) Evolution of hormone-receptor complexity by molecular exploitation. *Science* 312, 97–101

86 Patel, A. *et al.* The making of a queen: TOR pathway is a key player in diphenic caste development. *PLos ONE* (in press)