Developmental Evolution in Social Insects: Regulatory Networks From Genes to Societies

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ABSTRACT

The evolution and development of complex phenotypes in social insect colonies, such as queen–worker dimorphism or division of labor, can, in our opinion, only be fully understood within an expanded mechanistic framework of *Developmental Evolution*. Conversely, social insects offer a fertile research area in which fundamental questions of *Developmental Evolution* can be addressed empirically. We review the concept of gene regulatory networks (GRNs) that aims to fully describe the battery of interacting genomic modules that are differentially expressed during the development of individual organisms. We discuss how distinct types of network models have been used to study different levels of biological organization in social insects, from GRNs to social networks. We propose that these hierarchical networks spanning different organizational levels from genes to societies should be integrated and incorporated into full GRN models to elucidate the evolutionary and developmental mechanisms underlying social insect phenotypes. Finally, we discuss prospects and approaches to achieve such an integration. *J. Exp. Zool. (Mol. Dev. Evol.) 318:159–169, 2012.* © 2012 Wiley Periodicals, Inc.

A major goal of *Developmental Evolution* is to elucidate the developmental mechanisms underlying patterns and processes of phenotypic evolution and to contribute to a mechanistic understanding of the origin of evolutionary innovations including body plan evolution (Wagner et al., 2000; Davidson, 2006; Davidson and Erwin, 2006; Erwin and Davidson, 2009). *Developmental Evolution* and related research programs in *Evolutionary Developmental Biology* (Evo-Devo) typically employ a comparative approach, studying phenotypic variation between widely divergent lineages (Laubichler, 2007). A relatively new frontier for Evo-Devo is consideration of phenotypic variation within populations, which is more relevant to microevolutionary processes (Stern, 2000; Moczek and Nijhout, 2003). Such a focus is a critical step toward integrating mechanisms generating phenotypic variation with the evolutionary processes that most directly shape variation within populations. Phenotypic variation has traditionally been the focus of *Evolutionary Genetics*, which focuses on within-population evolutionary processes but has treated the mechanistic details of how genetic variation gives rise to phenotypic variation through development as a black box (Pigliucci and Schlichting, '97). A *Developmental Evolution* approach focused on understanding the mechanistic underpinnings of phenotypic variation within populations has great potential to lead to a more complete synthesis of development and evolution.

The question then becomes how to integrate these two traditionally separate fields? Here, we suggest that the concept of *Gene Regulatory Networks (GRNs)* provides the most detailed theoretical and experimental framework for understanding the evolutionary implications of developmental systems (e.g., von Dassow et al., 2000; Wilkins, 2002; Davidson, 2006; Davidson

and Erwin, 2006; Davidson and Levine, 2006; Wagner, 2007; Wilkins, 2007; Erwin and Davidson, 2009). GRNs provide a mechanistic explanation of differentiation during development based on the causal connections between genomic elements that control gene expression on spatial and temporal scales. They have first been proposed as a theoretical concept by Britten and Davidson ('69, '71) and are today known in great detail and in a variety of different organisms and developmental processes. A GRN describes the connections and regulatory logic between a hierarchy of regulatory elements and target genes (so-called differentiation gene batteries). Increasingly research into the specific architecture of GRNs suggests the existence of certain recurring network motifs that fulfill specific regulatory functions, such as the double negative gate (Davidson, 2006; Davidson and Levine, 2008; Davidson, 2010). GRNs also provide quantitative and testable models about both the connections within the network and the phenotypic consequences of disruptions of the network (Sharp and Reinitz, '98; Jaeger et al., 2004; Davidson, 2006; Janssens et al., 2006; Erwin and Davidson, 2009). A more inclusive description of expanded regulatory networks across levels, from genome through phenotype, should therefore enable us to predict the phenotypic consequences of evolutionary changes in specific elements of the network and provide us with a developmental understanding of phenotypic transformations more generally (Erwin and Davidson, 2009).

Most current research on GRNs focuses on the genetic elements of these networks located within the genome of the organism of interest (including transcription binding sites, enhancers, silencers, etc.), and has treated other forms of developmental signals (such as cell–cell signaling) as input into the network. In theory, the full range of biological organization from genes to organisms to social groups to ecological communities can be incorporated into expanded regulatory network models, providing a more integrated and complete description of how phenotypes are constructed. For elements located outside the genome of the focal organism to be evolutionarily relevant, the only requirement is that they are reliably inherited and show variation; such a criterion set allows consideration of a broad range of additional factors that input into the GRNs.

In the case of social organisms, we argue that these regulatory network approaches can be scaled up to include the social environment within which social phenotypes develop. For example, in social insects, the relevant developmental context for a socially determined phenotype, such as the development of female eggs into queens versus workers, also includes the behavior of brood caring workers who, by differentially feeding brood, mediate input signals that function as switches between different developmental trajectories (Wheeler, '86; Linksvayer et al., 2011). Effects of social environments have been incorporated into evolutionary genetic models as indirect genetic effects, also known as associative effects (e.g., Griffing, '81; Moore et al.,

'97; Linksvayer, 2006; Bijma et al., 2007). Maternal effects, a special type of indirect effect, have been especially well studied and have been shown to broadly impact development, trait expression, and evolution (Cowley and Atchley, '92; Mousseau and Fox, '98; Wade, '98; Hunt and Simmons, 2002). Recent advances have enabled the mapping of variation for genes with indirect effects to variation in specific traits (Wolf et al., 2002; Cui et al., 2004; Biscarini et al., 2010; Wolf et al., 2011); similar approaches extended to expression studies of social behaviors should facilitate the identification of gene networks with social effects on the development of individuals. Thus, it is feasible that mechanistic models of GRNs can be expanded to include these social effects.

It is revealing to note that some of the best studied developmental GRNs are actually initiated by maternal effects. For example, dorsal–ventral and anterior–posterior patterning of the *Drosophila* embryo is controlled by the distribution of maternal transcription factors that are derived from maternal genes, such as Dorsal and Bicoid (Levine and Davidson, 2005; Stathopoulos and Levine, 2005). In the same way, social insect development is socially controlled, albeit in a much more conspicuous fashion (Wheeler, '86; Linksvayer et al., 2011). In between these two extremes are social influences on development in other social organisms. For example, in some dung beetle *Onthophagus* species, an emerging model for *Developmental Evolution*, whether a male has large horns or is hornless depends on the size of the dung ball provisioned by parents (Hunt and Simmons, 2000, 2002), and both parental provisioning and the larval developmental response have been shown to have genetic components (Hunt and Simmons, 2002; Moczek and Nijhout, 2002). Thus, social/trans-generational inputs as regulators of initial development are widespread and may be ubiquitous.

Here, we argue that the social insects provide particularly valuable opportunities as a model system for *Developmental Evolution*, and conversely, that the evolution and development of the complex social phenotypes found within the social insects can be fully understood only through the mechanistic framework of *Developmental Evolution* (see also Toth and Robinson, 2007; Yang, 2007; Khila and Abouheif, 2008; Smith et al., 2008; Khila and Abouheif, 2010). While we focus below on studying social organisms within an expanded GRN framework, inputs arising from interspecific interactions could, in theory, also be incorporated.

SOCIAL INSECTS AS A MODEL SYSTEM FOR DEVELOPMENTAL EVOLUTION

The eusocial insects are often described as the pinnacles of social evolution (Wilson, '75; Hölldobler and Wilson, '90). Colonies of some highly derived species contain millions of sterile workers that act as foragers, nurses, and soldiers; and a single reproductively active queen that can live for decades and is a veritable egg-laying machine. This reproductive division of labor

between queens and workers, manifested as varying degrees of queen–worker dimorphism, is a fundamental characteristic of eusociality. Additionally, in some lineages, workers are polymorphic, with a further division of labor based on worker morphology. The evolutionary origin and subsequent elaboration of these aspects of the female caste system, including the question of whether female larvae develop into queens or variably sized workers, has been a core research focus of sociobiology for several reasons. The evolution of a sterile worker caste is an extreme example of reproductive altruism, and as a result, social insects have become well-established model systems for studying key concepts of social evolution (Bourke and Franks, '95). The origin of a worker caste, that is, the origin of eusociality, also represents the evolutionary origin of an additional level of biological organization, and is seen as a major evolutionary transition, on par with the evolution of eukaryotes and multicellularity (Maynard Smith and Szathmary, '95). Finally, female caste determination itself has received growing attention as a model for the evolution and development of polyphenisms, when a single genotype can produce two or more distinct phenotypes, depending on the developmental environment (Evans and Wheeler, 2001).

Below, we describe how studying the evolution and development of social insect phenotypes, in particular female caste phenotypes, can provide new insights into the evolution and development of complex phenotypes. We emphasize several properties of social insects that make them excellent models:

(1) Regulatory networks affecting the evolution and development of social insect phenotypes have a hierarchical structure. GRNs governing individual development and trait expression are influenced by colony-level social regulatory networks based on interindividual interactions, and vice versa. Social regulatory networks may have similar network characteristics (e.g., topology and motifs) as GRNs, and furthermore, social interactions can have similar evolutionary implications as physiological interactions between genes (Wade, '98; Wolf, 2000; Linksvayer, 2007; Linksvayer et al., 2009). However, social interactions can be experimentally deconstructed more readily than interactions between genomic and developmental components within individuals, so that experimental dissection of social regulatory networks has the potential to elucidate other types of networks. Detailed analysis of eusocial systems may also reveal commonalities with other major evolutionary transitions, including the evolution of multicellularity and of eukaryotes.

(2) Social insects are remarkably diverse in terms of social system, morphology, and degree of queen–worker dimorphism and worker polymorphism. Because of this variation, a comparative approach has tremendous potential for identifying the genetic and developmental basis of key social phenotypes such as division of labor, caste determination, and also to determine how associated morphological novelties evolve.

(3) Social insect research has traditionally had a population-level focus, and thus can easily fit into a *Developmental Evolution* research program focused on how the evolution of development affects the evolutionary process. Emerging tools, including genomic, transcriptomic, proteomic, epigenetic, and associated systems bioinformatic resources, also ensure that studies of the mechanistic basis of social insect phenotypes will be possible.

Here, we emphasize that in order to fully understand the evolution of social insect phenotypes, and for social insects to achieve their potential as a model of *Developmental Evolution*, an integrative hierarchical regulatory network perspective is necessary both for theoretical and empirical research. We proceed by describing examples of research programs that have focused largely on specific components of the social, physiological, and GRNs involved in social insect evolution and development. Finally, we review research that has begun to integrate these components, and introduce our vision for research using an integrative hierarchical network approach.

GRNs IN SOCIAL INSECTS

Regulatory networks are often visualized as a directed graph composed of nodes representing genes and edges that indicate regulatory logic, for example how a transcription factor regulates expression of target genes (e.g., see Fig. 1).

The work of Abouheif and Wray (2002) on the genetic regulation of winglessness in ant workers of three different ant subfamilies provides an excellent example of using a traditional Evo-Devo comparative approach to study the regulatory gene networks underlying an important social phenotype. One of the characteristic differentiations between workers and queens in ant societies is that new adult queens typically have functional wings that they use to fly to mating swarms, and subsequently discard after mating and dispersing. In contrast, workers do not produce wings during any phase of their life cycle (Hölldobler and Wilson, '90). Wingless workers probably evolved once very early in the evolution of ants. Ants evolved approximately 115–125 million years ago (Brady et al., 2006) and wingless ant workers have been described from 90 million years old amber fossils from New Jersey (Grimaldi et al., '97; Grimaldi and Agosti, 2000). Nevertheless, Abouheif and Wray (2002) found that in different ant subfamilies, different genes in the wing developmental pathway are suppressed during worker development. This result highlights the need for a GRN perspective because the functional consequences of changes in the different genomic elements contributing to the same wingless worker phenotype across ant taxa can only be understood in light of the whole GRN underlying wing development (Nahmad et al., 2008).
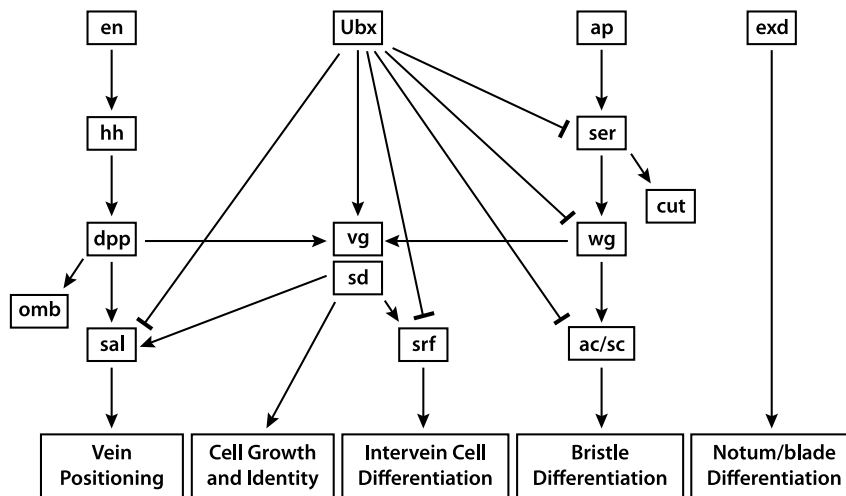
Figure 1. Gene regulatory network (GRN) involved in wing patterning during the late larval stage in *Drosophila* and ants, modified from Abouheif and Wray (2002). Gene products (indicated by abbreviated gene name) interact and activate downstream genes involved in growth, differentiation, and morphogenesis of the wing during metamorphosis. Arrowheads indicate activation and bars indicate repression.

Abouheif and Wray (2002) found that the ant wing developmental pathway shows strong homology with that of *Drosophila*, showing that, at least in some instances, the GRNs underlying evolutionarily novel traits in social insect, such as the emergence of a wingless caste of workers, are based on GRNs conserved in other insects. Thus, there is potential to use tools and knowledge accumulated for model organisms such as *Drosophila* to study the molecular mechanisms underlying evolutionary changes in morphology, anatomy, and behavior unique to social insects, while at the same time exploiting the wide phenotypic diversity in social traits found in social insects. However, using GRN models constructed for solitary model organisms to study social insect traits also has important limitations because the evolution of complex insect societies likely involves the evolution of novel genes and gene functions, in particular genes involved in communication and the functioning of social regulatory networks, that is the "social physiology" of the superorganism (Johnson and Linksvayer, 2010; Johnson and Tsutsui, 2011). It is these social regulatory networks, introduced in the next section, that we propose must be integrated with the GRNs described in solitary model organisms.
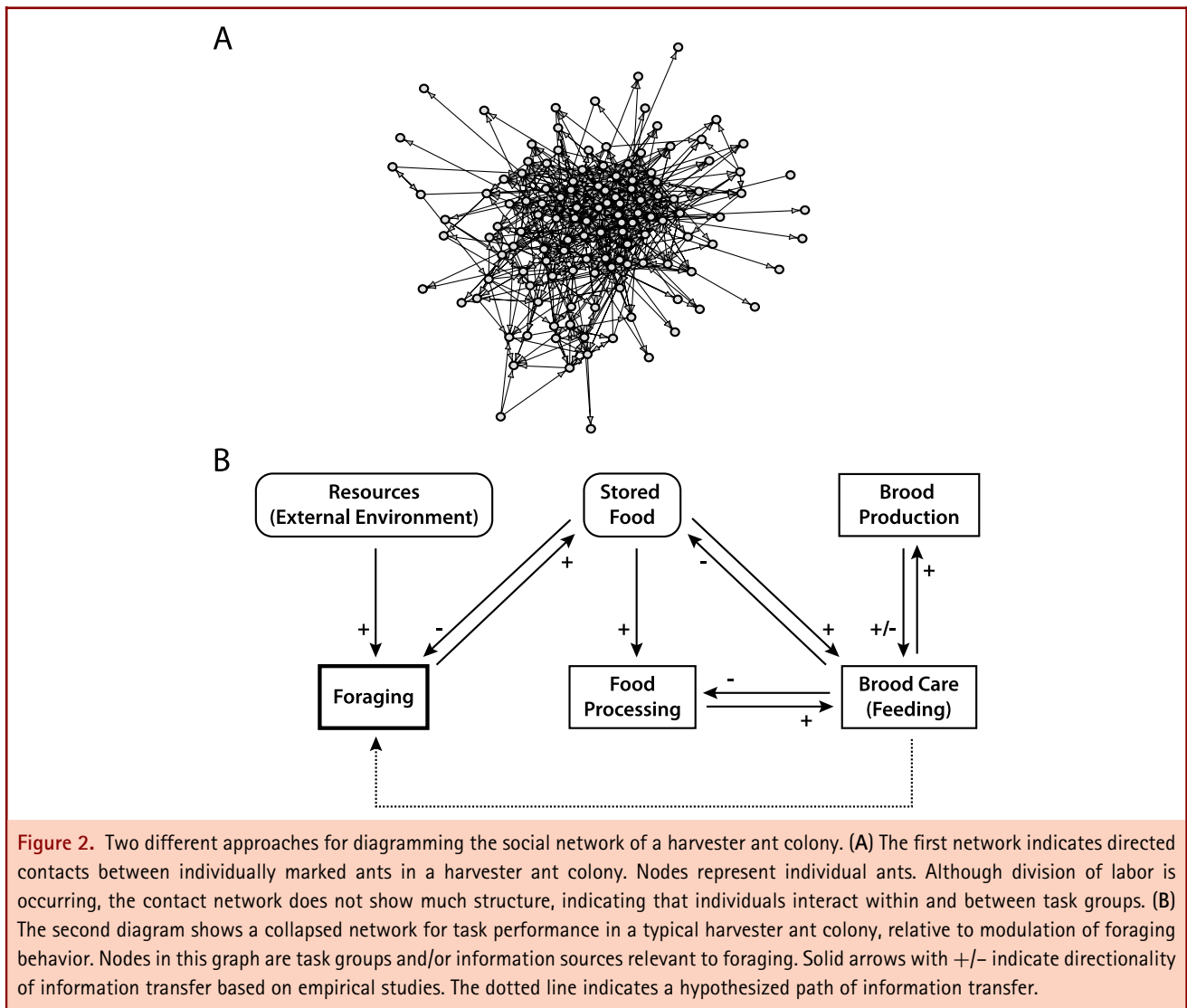
SOCIAL REGULATORY NETWORKS IN SOCIAL INSECTS

GRNs can be described as networks in which different kinds of gene and gene product components interact in a coordinated fashion to produce one or more clearly defined developmental outcomes (Milo et al., 2004). In this sense, they are usually described as being part of an integrated and more or less closed system, with nodes identified by function. Social networks, made up of interacting individuals within a social system, are not generally described in a similar way. In contrast to gene or metabolic regulatory network graphs in which nodes are categorized by functional type, nodes within social networks are the individuals themselves, with edges indicating interindividual contact (Travers and Milgram, '69; Pool and Kochen, '78; Newman, 2003; Goh et al., 2006) (Fig. 2A).

Social network analyses have recently and rapidly expanded from human social networks to other biological systems, including social vertebrates and insects (e.g., Krause et al., 2007; Croft et al., 2008). Social networks generally diagram proximity or association among individual group members; individuals may cluster in part by sex and age and do so outside of a specific set of functional activities. Such an association network can be a relevant approach to examining social networks in both humans and animal social systems in which interactions among group members serve the goals of the individuals themselves; the network structure is shaped by the social requirements and strategies of the individual nodes.

A eusocial insect colony can be diagrammed as a network of individuals (e.g., Naug, 2009), but it can also be graphed around the tasks performed by the workers of the colony (Fig. 2B). In this case, it becomes a system of functional units—task groups—in which the behaviors of sets of individuals provide utility for the network as a whole. In this way, the eusocial colony becomes similar to gene regulatory and metabolic networks. As an example, in network diagrams of division of labor within an ant or honeybee colony, instead of representing each individual worker as a node, we can collapse workers into functional categories based on which task they perform (Fewell, 2003). We can then graph task performance similarly to a

Figure 2. Two different approaches for diagramming the social network of a harvester ant colony. **(A)** The first network indicates directed contacts between individually marked ants in a harvester ant colony. Nodes represent individual ants. Although division of labor is occurring, the contact network does not show much structure, indicating that individuals interact within and between task groups. **(B)** The second diagram shows a collapsed network for task performance in a typical harvester ant colony, relative to modulation of foraging behavior. Nodes in this graph are task groups and/or information sources relevant to foraging. Solid arrows with +/− indicate directionality of information transfer based on empirical studies. The dotted line indicates a hypothesized path of information transfer.

regulatory network, in which communication across task groups serves to up- or downregulate the performance of any given task (Fig. 2B). This approach generates a network structure useful for generating hypotheses on regulation of specific tasks, just as a GRN generates hypotheses about the transcriptional regulation of specific genes. The graph also generates the associated hypothesis that connectivity between task groups is as important in regulation of division of labor within the colony as connectivity within task groups. For example, regulation of foraging in a harvester ant colony as shown in Figure 2 should rely heavily on information transfer gained from seed storage areas as well as seed processing by other workers, information may also be transmitted from brood care, either through direct signaling or via use of seeds. As with GRNs, the division of labor within a colony represents a relatively closed and self-regulatory system with a set of functions and outcomes that are relevant to the group as a whole (Johnson and Linksvayer, 2010).

In the case of social insects, social regulatory networks can thus be described by models that are isomorphic to those used to characterize GRNs, raising the empirical question of how similar these two kinds of networks are in structure, and whether they are governed by similar organizing principles (Milo et al., 2004). There are differences in the structure of GRNs and social networks composed of individuals and their interactions (Milo et al., 2004), but the similarity between GRNs and social regulatory networks based on interactions between functional groups is less clear. Importantly, from an expanded regulatory network perspective of *Developmental Evolution*, these two levels of regulatory networks are closely integrated.

INTEGRATING SOCIAL AND GRNs IN SOCIAL INSECTS

Above we have briefly described how network approaches have been used to study social insects at different levels of biological organization. We suggest that studies across these different levels of regulatory networks can be used to inform each other and, furthermore, that in complex systems composed of multiple hierarchically structured levels, it is necessary to explicitly study the whole system, (i.e., all regulatory levels and their interactions). In the case of social insects, this approach has particular methodological consequences, because it is easier to experimentally manipulate networks at the level of social behavior and explore how these controlled interactions intersect with the regulatory logic of development. Such manipulations allow us to study evolutionary transitions in social behavior experimentally and also, once the details of the underlying regulatory networks (behavioral, physiological, developmental, genomic) have been uncovered, to provide a mechanistic explanation of the origin of these phenotypic innovations.

Just as it is difficult to understand multicellular organisms by focusing only on single cells, it is similarly difficult to understand other hierarchically organized systems by exclusively studying the lowest level. By combining approaches focused on different levels of organization, we can have a richer understanding of the genetic and developmental underpinnings of complex phenotypes. For social insects, we stress that the social network, which regulates lower levels, must be incorporated into models of the genetic basis of complex social phenotypes for a more complete mechanistic understanding of developmental evolution.

Research into the genetic, physiological, and social basis of division of labor in the honeybee *Apis mellifera* provides perhaps the best studied example of the evolution and development of complex social insect phenotypes (Robinson et al., '89; Huang and Robinson, '92; Hunt et al., '95; Seeley, '97; Fewell and Page, 2000; Pankiw et al., 2001; Robinson, 2002; Whitfield et al., 2003, 2006; Amdam et al., 2004;, 2006; Ruppell et al., 2004; Page et al., 2006, 2009). Specific GRNs have been shown to affect physiological tuning and behavior of individual foragers (Amdam et al., 2007; Nelson et al., 2007; Ament et al., 2008; Ihle et al., 2009), and GRN models of forager behavior are beginning to be constructed (Ament et al., 2010). The importance of the colony social environment (determined by factors such as quantity of brood, quantity of stored food resources, and genotypic composition of netsmates) in determining the current physiological state and behavior of individual foragers has also been well documented (Calderone and Page, '92; Fewell and Winston, '92; Huang and Robinson, '92; Guzmán-Novoa and Page, '94; Seeley, '97; Huang et al., '98; Pankiw et al., 2001; Leoncini et al., 2004). The social environment also strongly shapes larval development and subsequently expressed adult phenotypes. Workers strictly regulate the microclimate and nutritional environment of developing brood and influence the developmental trajectory of larvae, including whether female larvae develop into workers or queens) (Linksvayer et al., 2011). We believe that elucidating the GRNs for colony members together with the social regulatory networks that form the connections among colony members will lead to a more complete understanding of the genetic basis and evolution of division of labor.

The production of queens versus workers in honeybee colonies has emerged as a relatively well-characterized socially regulated developmental genetic process, and provides a further excellent example of how an integrative GRN approach can be used to elucidate the evolution and development of complex social phenotypes. Female larvae develop into queens or workers mainly based on the nutritional environment they experience during development, that is, nurse workers feed queen-destined larvae large quantities of protein-rich food while worker-destined larvae are fed smaller quantities. Recently, researchers have begun to characterize cascades of genes that are expressed differentially in queen- and worker-destined larvae in response to these different nutritional environments (Evans and Wheeler, '99, 2000; Wheeler et al., 2006; Barchuk et al., 2007; Patel et al., 2007). While social genetic influences on caste development have so far been little studied, the social factors involved in the regulation of queen production in colonies are well known (Winston, '87), and in some cases, genes expressed in queens and workers that affect the social environment of developing larvae are already known. For example, queens produce a signal (queen mandibular pheromone) that inhibits production of new queens, and a suite of genes in worker brains are differentially expressed in response to queen mandibular pheromone (Grozinger et al., 2003), presumably affecting aspects of nurse worker rearing behavior (Fig. 3). Similarly, the major royal jelly protein genes, a major component of the royal jelly glandular secretions fed to queen-destined larvae (Drapeau et al., 2006), may be upregulated in nurse workers in the absence of the queen signal. Thus, genes expressed in queens and nurse workers affect the social environment experienced by developing larvae, and indirectly regulate larval development into queens or workers. Overall, GRNs within queens, nurse workers, and larvae determine the physiological state of each individual, and behavioral and pheromonal social interactions act as environmental inputs into each other's GRNs (see Fig. 3). Incorporation of the composite socially interacting GRNs of the sociogenome will lead to a more complete understanding of the evolution and development of social traits (Linksvayer et al., 2009; Johnson and Linksvayer, 2010).

PROSPECTS FOR AN INTEGRATIVE REGULATORY NETWORK APPROACH

Above we have introduced GRNs and social regulatory networks and suggested that these regulatory networks can be, and indeed must be integrated in order to understand the genetic basis and evolution of social insect phenotypes. What approaches are
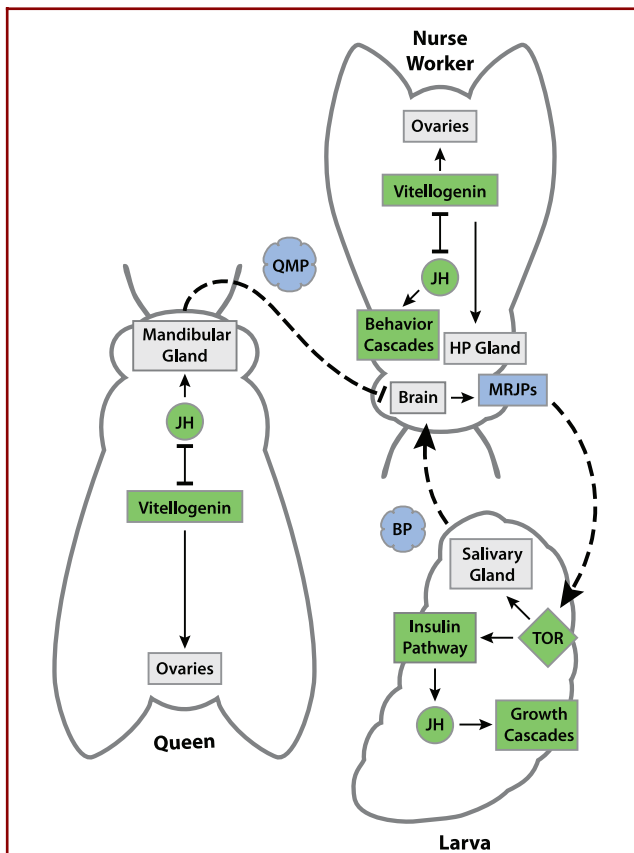
Figure 3. Hypothetical diagram of a few of the physiological and social factors making up the hierarchical regulatory network expressed in developing honeybee larvae, nurse workers, and the colony queen, that influences whether larvae develop into queens or workers. Components of GRNs affecting individual physiology are shown in green, connected by solid lines, while social regulatory factors are shown in blue with dotted lines indicating inputs into lower level GRNs. Important tissues and exocrine glands are shown in gray. Nurse nutritional inputs (major royal jelly proteins, MRJP, expressed in the hypopharyngeal glands, HP) affect larval nutritional pathways (TOR and insulin signaling pathways); the endocrine system (including juvenile hormone, JH) mediates effects of nutritional state on growth and differentiation, including whether larvae develop as new queens or workers; larval nutritional condition is signaled to nurse workers by brood pheromone (BP) synthesized in the salivary glands, which acts through the nurse worker neurosensory system and upregulates queen-rearing behavior, and likely transcription of MRJP genes; Nurse worker physiological and behavioral state is mediated by the nurse endocrine system and the egg yolk protein, vitellogenin; vitellogenin uptake occurs in worker ovaries and HP glands; Nurse queen-rearing behavior is inhibited by a queen signal produced in the queen mandibular gland (queen mandibular pheromone, QMP) that signals queen reproductive state.

available to construct GRNs and social regulatory networks, and what are the prospects for integrating these networks?

There are a variety of approaches to build models of GRNs with different degrees of sophistication, ranging from lists of network parts and models of network topology to quantitative dynamic models (Schlitt and Brazma, 2007). While well-characterized GRNs in a handful of model organisms are the result of years of research by multiple labs, new high-throughput technologies are beginning to make building GRN models more feasible for nonmodel organisms. Whole-genome microarrays and RNA sequencing simplify gathering expression data, the first step in building a transcriptional network model. Groups of genes that are coexpressed in various conditions can be hypothesized to share regulatory elements. Further examination of these groups of coexpressed genes for shared regulatory elements can provide further evidence for shared transcription factors (Schlitt and Brazma, 2007; Wilczynski and Furlong, 2010; Kim and Park, 2011). Other high-throughput technologies used to identify regulatory networks include chromatin immunoprecipitation (ChIP), a method of identifying DNA fragments that bind to a protein of interest (e.g., a transcription factor), followed by genomic tiling arrays (ChIP-chip) or sequencing (ChIP-seq) (Kim and Park, 2011). Ideally, all nodes and edges in a GRN model can subsequently be experimentally verified, for example by mutating nodes or by experimentally manipulating levels expression. Finally, an array of computational approaches are being developed to use data from various sources to reverse engineer network models, with the goal of building dynamic GRN models that make quantitative predictions (Reinitz, '99; Meir et al., 2002; Perkins et al., 2006; Schlitt and Brazma, 2007; He et al., 2009; Huang et al., 2009; Kim and Park, 2011). Despite these advances that are potentially broadly applicable to characterize GRNs in nonmodel organisms, there are currently difficulties at each step, and typically only well-interconnected portions of networks (i.e., modules) have been modeled in detail (Wilczynski and Furlong, 2010).

Identifying social networks have analogous approaches and goals, as well as difficulties as building models of GRNs. First, the network parts must be identified, then relationships between parts must be deduced, and finally quantitative models can be constructed. As described above, social network models typically use individuals as nodes, so that constructing a social network often involves observing interactions (often defined by specific types of physical contact or proximity) among groups of marked individuals (Krause et al., 2007; Croft et al., 2008). Current limitations include technologies to mark and track individuals and computational approaches to build interaction networks. However, recent advances in video recognition, tracking technology, and software ensure that constructing well-characterized social regulatory networks involving many individuals will become feasible (Krause et al., 2011). Above we described how contact networks can be collapsed to form social networks based on

functional groups (Fig. 2). In particular, for very large societies, we believe that it will often be more useful to study how interactions among functionally defined groups relate to the GRNs within group members.

How can social regulatory networks and GRNs be integrated? Because social insect societies are built of functionally differentiated individuals or castes, one straightforward approach would be to first characterize GRNs separately for each functional group, or caste. Subsequently, additional knowledge about social regulation via interactions among functional castes can be used to build a network model for how outputs from the GRNs of one caste can be inputs for the GRNs of a different caste. For example, when modeling the development of honeybee female larvae into either queens or workers that we introduced above, knowledge about social interactions between queens, nurse workers, and developing brood can be used to hypothesize that glandular secretions are inputs into the nutrient sensing networks of larvae that activate growth and differentiation gene batteries of the GRN, and the quantity and quality of glandular secretions (likely determined in part by the transcriptional activity of the major royal jelly protein genes) in turn depends on the physiological status of the nurse (Fig. 3). An integrated hierarchical GRN model may be directly studied by studying global patterns of gene (co)expression across all types of individuals (e.g., the nurses, brood, and queen shown in Figure 3). Alternatively, instead of starting with functional groups or castes, patterns of gene expression could be studied at the colony level (i.e., pooling across colony members) in the same way that studies of gene expression often pool cell or tissue types. However, information about which social partners contributed which transcripts and how social interactions between social partners affected transcription and ultimately the observed phenotypes would be lost, just as information about the contribution of gene products to whole-organism patterns or the signaling between cells or tissues is lost when cells and tissues are pooled. Subsequently, experimental manipulation can be used to verify the various edges and nodes in the composite GRN.

While characterizing any aspect of the full regulatory networks underlying the expression of social insect phenotypes is a daunting task, we believe that recognizing the importance of studying the full hierarchical GRN is an important first step.

CONCLUSIONS

We have reviewed how a regulatory network approach can be used to study the evolution and development of phenotypes at different levels of biological organization in social insects. We presented evidence indicating that higher level social regulatory networks provide inputs into lower level physiological and GRNs controlling development and behavior in individual organisms. Complex phenotypes in social insects are thus the result of hierarchically structured regulatory networks integrating genomic,

physiological, and behavioral components. Until these networks are explicitly modeled and studied as an integrated whole, we will have an incomplete picture of the full set of regulatory interactions between genomic, cellular, physiological, and behavioral factors involved in the development and evolution of social phenotypes.

Because higher level social networks likely share properties with lower level networks, but are much easier to manipulate and study, organisms with well-developed social regulatory networks, such as social insects, can be used as model systems to elucidate the evolution of networks and the emergence of complexity. Furthermore, the ability to experimentally explore different levels of regulatory networks (from the genomic to the social) and their interactions makes social insects an excellent model for the development of a comprehensive mechanistic theory of *Developmental Evolution* that combines a detailed description of a hierarchical regulatory network determining the development of individual phenotypes with a multilevel selection framework for a more complete understanding of phenotypic evolution.

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