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The Molecular Substrates of Insect Eusociality

Sarah Kocher¹ and Callum Kingwell^{1,2}

¹Department of Ecology and Evolutionary Biology, Lewis-Sigler Institute for Integrative Biology, and Howard Hughes Medical Institute, Princeton University, Princeton, New Jersey, USA; email: skocher@princeton.edu

²Smithsonian Tropical Research Institute, Ancon, Panama

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Abstract

The evolution of eusociality in Hymenoptera—encompassing bees, ants, and wasps—is characterized by multiple gains and losses of social living, making this group a prime model to understand the mechanisms that underlie social behavior and social complexity. Our review synthesizes insights into the evolutionary history and molecular basis of eusociality. We examine new evidence for key evolutionary hypotheses and molecular pathways that regulate social behaviors, highlighting convergent evolution on a shared molecular toolkit that includes the insulin/insulin-like growth factor signaling (IIS) and target of rapamycin (TOR) pathways, juvenile hormone and ecdysteroid signaling, and epigenetic regulation. We emphasize how the crosstalk among these nutrient-sensing and endocrine signaling pathways enables social insects to integrate external environmental stimuli, including social cues, with internal physiology and behavior. We argue that examining these pathways as an integrated regulatory circuit and exploring how the regulatory architecture of this circuit evolves alongside eusociality can open the door to understanding the origin of the complex life histories and behaviors of this group.



INTRODUCTION

Social interactions play crucial roles in helping animals find and secure mates and successfully raise offspring, and they can often include cooperation among group members to carry out these tasks collectively. In a handful of species, these cooperative interactions are taken to an extreme, and some group members will forgo their own reproduction in order to help raise the offspring of others. The social insects epitomize these extreme forms of cooperation: Their eusocial colonies are composed of overlapping generations of individuals that engage in cooperative brood care and contain a mix of reproductive individuals and nonreproductive helpers (7, 86).

The origins of eusocial societies from their solitary ancestors present an evolutionary conundrum. Given that natural selection acts on fitness differentials among individuals, how can a nonreproductive helper evolve? Evolutionary theory has given us powerful insights into this process. We now understand that helpers can gain fitness benefits from raising genetic relatives (47) and that competition among colonies can create selection differentials upon which natural selection can act (16).

There is a similar interest in understanding the molecular mechanisms that can facilitate the evolution of eusociality and generate the dramatic polyphenisms observed among colony members. What molecular and neurobiological changes enable such extreme forms of cooperation? How is a single genome capable of producing both a large, long-lived social insect queen and a smaller, nonreproductive worker with a short lifespan? Unlike the progress made in evolutionary theory, clear answers to these questions are currently lacking, but the past few decades have provided ample insights into these processes, and several common features of the molecular underpinnings of social evolution are beginning to come into focus.

A key feature of eusocial insects is their ability to produce a diverse range of phenotypes from a single genome, including long-lived, reproductive queens and shorter-lived, nonreproductive workers (142). Nestmates can vary drastically in behavior, physiology, and morphological features, including changes in size and growth rates. In many eusocial species, discrete castes (i.e., polyphenisms) are established during development, though there are also species where the behavioral and physiological variation is highly plastic throughout their lifetime. Such phenotypic differences are shaped by the integration of environmental factors, including nutritional and social stimuli, with molecular mechanisms that can modify gene expression (63).

Here, we focus on the insights we have gained through studies of the social insects belonging to the order Hymenoptera (bees, ants, and wasps). Within this group, social behavior has been gained and lost multiple times, providing ample opportunities to better understand the mechanisms that shape the evolution of eusociality (69). We begin with a discussion of the evolutionary history of eusociality within the Hymenoptera, and then we outline numerous proximate evolutionary hypotheses that have been proposed to help explain the origins and elaboration of this complex trait. Finally, we review our current understanding of the molecular underpinnings of eusociality within this group.

BEHAVIORS ARE HIGHLY VARIABLE AMONG SOCIAL INSECTS

Across the Hymenoptera, there is ample variation in social behavior that can help provide insights into the mechanisms that shape these traits (69). Although some of the better-known social insect species are indeed eusocial, most species within this group lead solitary lives where they live alone and reproduce independently. Subsocial care is also common; in these species, parents and offspring have an extended period of overlap within the nest, and parents often engage in extended interactions with their offspring throughout development. Yet other lineages have

	SOLITARY A single female establishes a nest and provisions her own offspring. There is no extended care or generational overlap. 	COMMUNAL Multiple females share a nest, but each female reproduces and cares for her offspring independently. 	SUBSOCIAL A single individual founds a nest and cares for offspring, but there are continued interactions between parent and offspring. 	SIMPLE All individuals are capable of mating and reproduction, but a primary individual lays the eggs and reproduces. Nonreproductive offspring engage in alloparental care and nest maintenance. 	COMPLEX Individuals divide labor among specialized castes: One or a few are primary egg layers while the majority tend to brood care and nest maintenance. Colonies tend to be larger, and caste is determined during development. 
Offspring provisioning	✓	✓	✓	✓	✓
Multiple reproductives share nest	✗	✓	✗	✓	✓
Overlapping generations	✗	✓	✓	✓	✓
Cooperative brood care	✗	✗	✗	✓	✓
Reproductive division of labor	✗	✗	✗	✓	✓
Totipotent adult workers	✗	✗	✗	✓	✗
Obligate worker sterility	✗	✗	✗	✗	✓
Developmental caste determination	✗	✗	✗	✗	✓
Morphological variation among workers	✗	✗	✗	✗	✓
Superorganismal	✗	✗	✗	✗	✓

Figure 1

Overview of social variation within the Hymenoptera. Social insects exhibit a wide range of behaviors, from solitary to eusocial. Here, we highlight some of the key terms that can be used to distinguish among different life history strategies, but we note that insect life histories and social behaviors are complex and highly variable, and attempts to classify behaviors into simple categories nearly always result in some oversimplifications (49, 106). Some simple eusocial species exhibit facultative eusociality: Within these lineages, individuals are capable of both eusocial and noneusocial reproduction. However, there are no known examples of facultative, complex eusociality. In these societies, neither the queen nor worker caste can survive and reproduce independently, and thus they are interdependent on each other for reproductive success. This is why complex eusocial lineages are often referred to as a superorganism (14). Here, we elect to use the terms simple and complex to describe variation in eusociality; historically, these have also been described as primitive and advanced eusociality [ca. Michener (86)]. A black check indicates that a trait is present within each behavioral form, a light gray check indicates that the trait is variable within a particular behavioral classification, and an x indicates trait absence. Bee illustrations created by Wenfei Tong.

evolved various forms of communal living where multiple, independent females share a nest but continue to provision and reproduce by themselves (Figure 1).

Among all eusocial lineages, there is generational overlap among colony members and a reproductive division of labor where one or a few individuals are solely responsible for reproduction while other nonreproductive colony members help care for these offspring (7).

For the scope of this review, we use the terminology of simple and complex eusociality to describe variation among eusocial lineages (Figure 1), but there are numerous descriptions and definitions of eusociality beyond the framework we present here (for further discussion, see 14, 69, 106, 131). We also note that the binning of eusociality into various categories such as simple and

complex can provide a useful framework for discussion, but these categories do not fully encompass the multidimensionality of eusocial forms (49). No one-size-fits-all term can capture the subtleties and complexities represented by all taxa (49, 106).

Simple eusocial species tend to form smaller colonies and include species such as paper wasps, stenogastrine wasps, and some carpenter bees and sweat bees (66, 86, 141). In these colonies, nonreproductive workers are capable of mating and reproduction, and new colonies are usually founded by a single foundress who then produces worker offspring. The division of labor is flexible, influenced by social interactions (107) and nutritional status (63), and reversions to solitary life are not uncommon, especially in some bee lineages (119). As a result, lineages exhibiting simple eusociality can be obligate (i.e., all individuals within a species exhibit this behavior) or facultative (i.e., some individuals within a species are capable of multiple behavioral strategies). By contrast, complex eusocial societies, exemplified by honey bees, ants, and some wasps, have large colonies and limited ability for adult workers to take on reproductive roles within the group (86). These obligate eusocial colonies function as superorganisms (14), with a high degree of interdependence among members and fixed roles for reproductive and nonreproductive individuals.

THE EVOLUTION OF EUSOCIALITY

Eusociality has arisen multiple times throughout the evolutionary history of bees, ants, and wasps (15, 19, 37, 101) (**Figure 2**). There have been at least two origins within vespid wasps, a

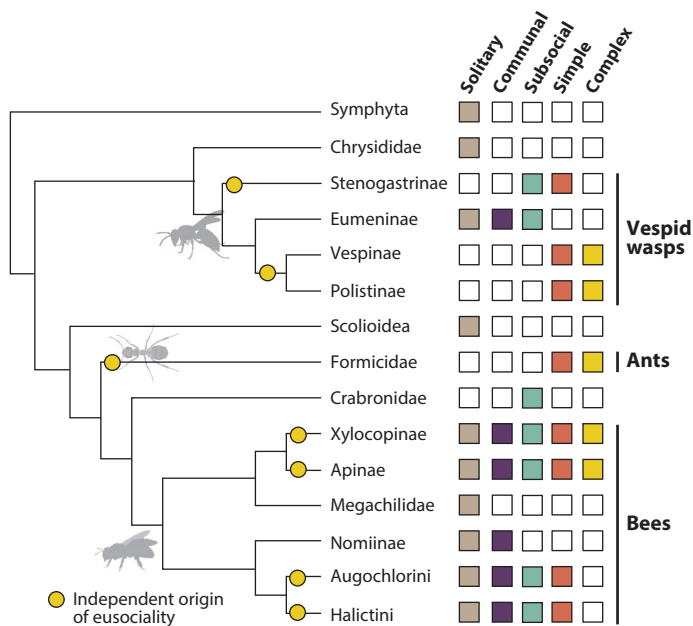


Figure 2

Origins of eusociality in social insects. Within the social insects, eusociality has arisen at least seven times: twice in vespid wasps (Vespidae); once in ants (Formicidae); at least once in carpenter bees (Xylocopinae); once in corbiculate bees (Apinae), which include bumble bees, orchid bees, stingless bees, and honey bees; and twice in the halictid bees, or sweat bees (once each in Augochlorini and Halictini; 15, 19, 37, 101). In each of these lineages, a range of social behaviors has been documented within and between species, facilitating comparisons between social and nonsocial lineages to examine the molecular causes and consequences of eusociality. Figure adapted from images created with BioRender.com.

single origin shared by all ants, and at least four independent transitions from solitary to eusocial living within bees (**Figure 2**). As discussed below, the multiple independent origins can likely be explained by flexible molecular pathways present in a common ancestor that underlie these transitions.

Phylogenetic evidence indicates that these transitions occur first as a shift from solitary to simple societies and that complex eusocial lineages evolve secondarily. In other words, complex eusocial societies have evolved from simple eusocial ancestors. Moreover, the evolution of complex eusociality with developmentally determined castes appears to be evolutionarily irreversible (14). Thus, there are no known reversals from complex eusociality to a solitary life history.

Proximate Evolutionary Hypotheses

Numerous hypotheses have been developed to explain the proximate mechanisms that underlie social evolution. A common theme is that modifications to the timing and coordination of molecular pathways expressed in a solitary common ancestor can be co-opted to regulate phenotypic variation across social castes.

Ground plans and related hypotheses. The ovarian ground plan hypothesis is a foundational concept in the evolution of eusociality. It posits that the molecular pathways governing ovarian and behavioral cycles in a solitary ancestor were parallelized to enable division of labor among queens (ovarian cycle) and workers (provisioning cycle) in simple eusocial societies (141) (**Figure 3**). Subsequent elaborations include the reproductive ground plan hypothesis, which proposes that these ancestral cycles were further co-opted to regulate nursing and foraging behaviors in honey bees (1), and the diapause ground plan hypothesis, which suggests that worker- and queen-destined castes in eusocial Hymenoptera derive from the molecular and physiological mechanisms regulating diapause cycles in solitary ancestors (52).

More generalized versions of these ground plans have also been proposed. The maternal heterochrony hypothesis predicts that a shift in the timing of the expression of genes underlying maternal care (and not just reproductive and provisioning cycles) leads to the early expression of care-related behaviors and physiologies in workers (81). The genetic toolkit hypothesis predicts that an evolutionarily conserved set of genes underlies the origins and elaborations of eusociality in multiple eusocial lineages. It argues that the same or similar molecular pathways have repeatedly been co-opted from solitary ancestors across independent origins of eusociality and that these toolkits shift in the timing and expression to facilitate the generation of queen and worker castes from a shared genome (131).

Evidence is rapidly accumulating in support of ground plan hypotheses and a molecular toolkit that underlies caste differences and their evolution in ants (23, 104, 111, 140), bees (57, 65, 66, 120), wasps (31, 132, 147), and even termites (70, 79; further reviewed in 131). Together, these studies point to a common set of pathways that have been modified throughout social evolution in each of these major insect groups.

Gene regulation and novel genes. Another set of hypotheses is focused on which specific DNA sequence changes are associated with transitions in eusociality. While changes can occur (and likely have occurred) at each of these transitional stages, some researchers have made specific predictions about which types of mutations are the most relevant to social evolution (for an overview, see 131). These include predictions that novel, taxon-restricted genes (such as gene duplications) may have arisen to facilitate the appearance of new, social-specific traits because the new genes are less evolutionarily constrained. Alternatively, others have argued that it is primarily changes in gene regulation that play a more important role in the origins of eusociality (66).

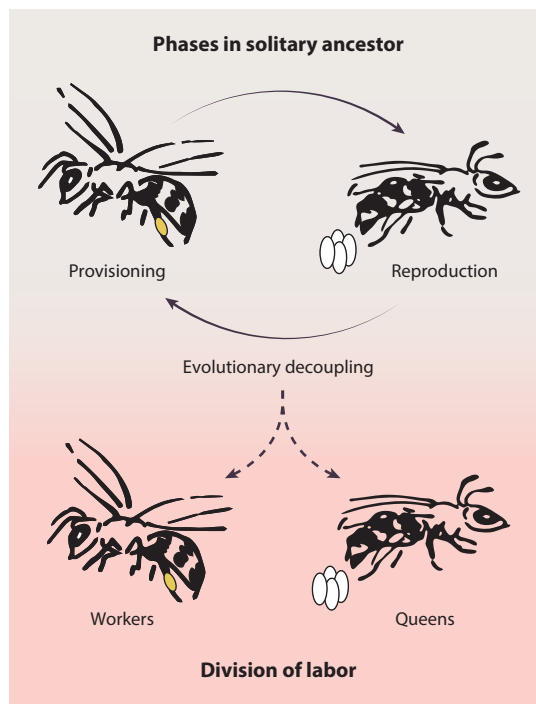


Figure 3

Ground plan hypotheses predict a decoupling of ancestral pathways and parallel expression to enable a division of labor across social insect castes. Molecular pathways that govern behavioral and physiological traits related to provisioning and egg-laying in a solitary ancestor (whose life cycle alternates between these two phases) are decoupled throughout evolution to enable parallel expression of these pathways in queens and workers throughout the course of eusocial evolution. Bee illustrations created by Wenfei Tong.

Indirect genetic effects. Indirect genetic effects (IGEs) occur when genes expressed in one individual impact traits in others. IGEs are predicted to play a particularly important role in mediating the strength of selection on genes that underlie social traits (81). Evidence for IGEs in social behavior is growing. A genome-wide association study in honey bees identified IGEs underlying colony-level aggression (4), and others have identified rapid rates of molecular evolution on genes associated with social components of caste determination in honey bees (138). Similar studies in ants have also identified IGEs from ant caretakers that impact reproductive allocation (80). IGEs can also be context dependent; studies of the clonal raider ant *Ooceraea biroi* identified IGEs from caregivers that vary by age and colony size, influencing the development rates, survival, body size, and caste fate of developing larvae (102).

Genetic accommodation and plasticity first. In addition, the genetic accommodation and plasticity-first hypotheses predict that new phenotypes initially emerge through phenotypic plasticity. Over time, genetic mutations will accumulate to stabilize different developmental trajectories into alternative phenotypes (for an overview of these ideas, see 78). Plastic expression of eusocial behavior can help buffer lineages against fitness costs when its expression is not advantageous, while allowing time for genetic adaptations favorable to eusociality to arise (142).

Testing these concepts is particularly challenging, but correlative evidence from genomic studies in social insects suggests that such a process is plausible. For example, molecular pathways

associated with developmental plasticity in the socially flexible sweat bee *Megalopta genalis* are also associated with differences in social behavior in this species. Moreover, genetic variation among socially distinct individuals impacts transcription factor binding probability in regulatory regions, providing a potential mechanism by which the changes underlying social flexibility and plasticity can become encoded in the genome (65).

Examining the Toolkit

Though the specific genes may vary by lineage, there is striking convergence on the molecular pathways that govern nutrient sensing [via the insulin/insulin-like growth factor signaling (IIS) and target of rapamycin (TOR) pathways], endocrine regulation [especially juvenile hormone (JH)], epigenetic regulation, and fecundity across all of these groups (23, 31, 57, 79, 104, 111, 120, 132, 140). Throughout the course of social evolution, the timing and expression patterns of many of these pathways have become decoupled, and genes within these pathways often become differentially expressed between castes (70, 140), though specific changes vary across evolutionary lineages (31, 79, 140, 147).

Below, we outline our current understanding of the molecular biology underlying each of these pathways and present evidence from different eusocial lineages that support their role in social evolution. We then highlight how the intricate links across these different pathways form a nutrient–endocrine regulatory circuit that has served as a major substrate for the evolution of eusociality in social insects.

Nutrient Sensing and the Insulin Signaling Pathway

The IIS and TOR signaling pathways are the major nutrient-sensing pathways in insects and regulate growth in response to nutrition (6, 71). In many insects, nutrient-rich environments cause the insulin-producing cells (IPCs) in the brain to secrete insulin-like peptides (ILPs) that bind to insulin receptors (IRs) and activate the IIS pathway in the periphery. Starvation represses both the synthesis and secretion of the ILPs (21, 53). The IIS pathway stimulates the TOR pathway via ILP binding to the IRs on cell membranes, but the TOR pathway can also be activated directly by free amino acids that bind to an amino acid transporter on the cellular membrane (35).

Interestingly, the spatial and temporal patterns of IIS/TOR signaling are diverse and varied across tissues and across species. For example, ILPs can be produced by several tissues in *Drosophila*, including the midgut, imaginal discs, and salivary glands, as well as in the glia of the central nervous system (24, 127). ILP production also varies across species [different species produce different numbers of ILPs (71)], but all ILPs are thought to bind IRs and mediate growth (71). Similarly, while flies and lepidopterans possess only one IR, honey bees and ants have two (26, 82). Organs can also differ in their sensitivity to nutrition and to IIS/TOR signaling, helping to regulate organ-specific growth rates (71).

Nutritional effects on social behavior. Behavioral variation in social insects is tightly linked to their nutritional state. Nutritional differences during development underlie caste differentiation into queens and workers in many complex eusocial species, and nutrition can also regulate variation among specialized worker castes (reviewed in 63, 71). In simple eusocial lineages where all colony members are morphologically identical, differences in larval and adult nutrition are frequently correlated with task allocation and reproductive division of labor. Significant effects of larval diet have been associated with reproductive development in newly eclosed adult paper wasps (33, 59), and protein restrictions on foundress-destined larvae lead instead to adults with worker-like traits (59). In the small carpenter bee *Ceratina calcarata*, the first daughter to emerge is small and often remains as a guard in the natal nest rather than dispersing to found her own

nest (74). These dwarf eldest daughters are provisioned by mothers with smaller pollen masses composed of a different set of floral resources compared to those provided to their reproductive sisters (74). Similarly, poorer nutritional resources have been associated with the worker caste in several sweat bee species (17, 108). In many complex eusocial lineages with developmental caste determination, protein-rich diets are often fed to queen-destined larvae, including, famously, the protein-rich royal jelly fed to honey bee queens. This effect has been tentatively linked to the presence of the protein Royalactin in honey bee royal jelly; larval consumption of Royalactin increases body size and JH (61).

IIS/TOR and social evolution. Changes in the expression patterns of the IIS signaling pathway are correlated with nutritional and physiological differences found across castes of the buff-tailed bumblebee *Bombus terrestris*. In this species, the genes encoding ILP, insulin-like growth factor 1 (IGF-1), and several hexamerin storage proteins are upregulated in reproductive queens compared to nonreproductive workers and unmated or diapausing queens (25, 54). Moreover, the genes encoding both of the insulin receptors InR-1 and InR-2 are expressed at lower levels in reproductively active queens, consistent with an activated IIS signaling system (54). By contrast, the regulatory relationships between IIS, nutrition, and reproduction vary greatly across developmental stages and castes in the honey bee. In early developmental stages, the relationships between IIS, TOR, and endocrine signaling are similar to those observed in most insects; however, in late-instar larvae, IRs and other members of IIS signaling are downregulated in queen-destined larvae in spite of access to the high-protein diet fed to developing queens (26, 29, 143, 146). Moreover, knock-down of *TOR* and *insulin receptor substrate (IRS)* expression is sufficient to cause queen-destined larvae to develop worker-like traits (97, 146). Similarly, in a study that included seven ant species, *ilp2* was the only gene with higher brain expression in queens compared to workers in all seven species (23). Other studies have identified shared gene regulatory networks that underlie caste differences, which include expression differences in members of the insulin signaling pathway (104). Together, these studies highlight how changes in IIS/TOR signaling play an important role in the evolution of eusociality across a wide range of social insects.

Endocrine Signaling

The endocrine signaling system plays a central role in regulating insect growth, metabolism, development, and reproduction (89). Insects have two different types of endocrine organs: glandular tissues specialized in the synthesis and secretion of hormones and groups of specialized neurons in the central nervous system that produce neurohormones. The glandular tissues include the prothoracic glands that secrete ecdysteroids and the corpora allata (CA), which secrete JH. The release of these hormones is controlled by the neurosecretory cells in the central nervous system. These cells produce small polypeptides known as neurohormones, and they are distributed throughout the central nervous system but are especially abundant in the brain. As a result, the nervous system is the primary regulator of hormone production (89).

Ecdysteroids. Ecdysteroids are steroid hormones that regulate molting, metamorphosis, and embryogenesis and are ligands that activate their receptors as transcription factors (89). They are synthesized in the prothoracic gland from dietary cholesterol or related plant sterols (73). Cholesterol is converted to the steroid ecdysone through a series of enzymatic steps encoded by the Halloween gene group (96). Ecdysone release into the hemolymph occurs in pulses during larval development to regulate metamorphosis and is converted into its active form, 20-Hydroxyecdysone (20E), in the periphery. During metamorphosis, the prothoracic glands degenerate and ecdysteroid synthesis is taken over by gonads in the adult (113). Two peptide hormones released from the brain control ecdysone secretion and release: prothoracicotropic hormone (PTTH) and the ILPs

(96). Interestingly, the genes encoding PTTH appear to have been convergently lost in eusocial bees, suggesting that an increased social influence on early development may be associated with a diminished role for PTTH in these lineages (27).

An ecdysone importer (EcI) is required for 20E to cross the cell membrane (92). Once inside the cell, 20E binds to a nuclear hormone receptor, the ecdysone receptor (EcR). In some insects, the retinoid X receptor (RXR) acts as the coreceptor, but, in at least Diptera and Lepidoptera, a modified RXR receptor, ultraspiracle (USP), acts as the coreceptor. The EcR/USP heterodimer binds specific DNA sequences [the ecdysone response element (EcRE)] (123). In the absence of 20E, the heterodimer can act as a corepressor and maintain a closed chromatin state; when 20E is present, the complex can act as a coactivator (134).

Juvenile hormones. JHs are sesquiterpene hormones synthesized in the CA. JH is generally thought to maintain the larval form throughout development and is therefore sometimes referred to as the status quo hormone (56). JH3 is the form found in all insects, and it is also the most common JH in Hymenoptera. ILPs may regulate JH biosynthesis, but it remains unclear whether this is via direct or indirect action (134).

The molecular response to JH is less well understood than ecdysone, largely because the discovery of the JH receptor came later than that of the EcR (134). The JH receptor (8, 42, 145) Methoprene-tolerant (Met) is bound to heat shock protein 83 (Hsp83) in the cytoplasm (134). Upon binding of JH, Met moves to the nucleus and heterodimerizes with its coactivator, the bHLH-PAS (basic helix-loop-helix/ Period-ARNT-Single-minded) protein Taiman (Tai) (150). Then, the Met/Tai heterodimer binds to JH response elements (JHREs) in the genome to activate transcription of JH response genes, including *Krüppel homolog-1* (*Kr-h1*) (55, 56). Kr-h1 is a zinc-finger transcription factor that regulates the gene expression of many downstream targets.

Insect endocrine systems are highly labile. The insect endocrine system appears to be extremely evolutionarily labile; the specific roles and functions of ecdysteroids and JHs can vary substantially across different lineages. In most insects, JH facilitates the onset of reproductive maturation (113). In almost all simple eusocial species studied thus far, JH retains its ancestral, positive relationship with reproductive maturation; titers of JH are positively correlated with ovarian development in bumblebees (11), most paper wasps (12, 112, 121, 130), and sweat bees (10, 125). By contrast, the gonadotropic effects of JH are frequently lost in the complex eusocial ants and honey bees (48).

In social insects, juvenile hormone and 20-Hydroxyecdysone also regulate caste-specific behaviors. The endocrine system influences many different aspects of caste, reproduction, and behavior in social insects, both during development and in adulthood (46). Endocrine signaling has repeatedly been linked to reproductive division of labor and/or task allocation, and JH levels during development can regulate caste determination and differentiation in many social insect species (48). JH also influences caste-specific behaviors in adults. In the Indian jumping ant *Harpegnathos saltator*, Kr-h1 binds to and represses a different set of genes in the brain in a hormone-dependent manner. When JH levels are high, Kr-h1 binds to and represses the expression of genes associated with the reproductive caste (gamergates). By contrast, when 20E levels are high, Kr-h1 binds to and represses genes that are highly expressed in worker brains. Moreover, RNA interference (RNAi) knockdown of *kr-h1* in gamergates leads to the expression of worker-like behaviors, while knockdown in workers leads to the expression of gamergate-associated traits. Thus, hormone induction of Kr-h1 acts to stabilize caste identity and behavior in a context-dependent manner (43). This provides an example of the evolutionary decoupling that is central to ground plan hypotheses: The hormonal systems used to regulate reproductive maturation in solitary ancestors have been co-opted to regulate caste differentiation and social behaviors in eusocial lineages.

Vitellogenins

Insect vitellogenin (*Vg*) genes encode the major egg yolk protein precursors. *Vgs* belong to a superfamily of large lipid transfer proteins (5). *Vgs* are synthesized by the fat body and secreted into the hemolymph, and then a receptor-mediated endocytosis facilitates uptake and storage of *Vgs* in competent oocytes (136). Posttranscriptional processing of *Vgs* enables them to carry carbohydrates, lipids, and other nutrients to the ovaries. Once in the egg yolk, *Vg* is stored as vitellin (*Vn*) and acts as the main nutritional reserve for developing embryos (136).

Hormones regulate *Vg* transcription directly via activating a hormone-receptor complex to a hormone response element that is typically located in the promoter region (136). Not surprisingly, the precise hormones that regulate *Vg* can vary across species: Many insects rely solely on JH for *Vg* regulation, while others (Diptera) require both JH and ecdysteroids. Lepidopterans require other hormones in addition to JH and ecdysteroids to regulate their reproduction (136). In most insects, JH facilitates the onset of reproductive maturation by regulating *Vg* synthesis in the fat body and its uptake by developing oocytes (113). When JH is detected in the fat body, it triggers the transcription of *Vg*, which encodes the precursor of the yolk protein (136).

Throughout the course of evolution, *Vg* has acquired additional roles beyond embryonic development. The best examples come from honey bees, where *Vg* levels can influence the behavior of worker bees by affecting the timing of their transition from in-hive duties such as nursing to outside tasks such as foraging (1). Moreover, high *Vg* in workers suppresses their reproductive capabilities, while high levels of *Vg* are correlated with increased lifespan and reduced oxidative stress in queen honey bees (26). Thus, *Vg* has acquired new functions associated with behavioral maturation and the division of labor in this complex eusocial species.

Neuromodulators

Changes in the brain underlie changes in behavior. Connections between individual neurons or the excitatory state of particular neurons can be affected by neurotransmitters and neuromodulators. Neurotransmitters typically have rapid, local, and specific effects on target neurons that are mediated by ionotropic receptor molecules. Neuromodulators have more widespread and prolonged effects that simultaneously influence the activity of multiple neurons or circuits; these longer-lasting effects are mediated by metabotropic receptor molecules (G-proteins) and different cellular signaling pathways (126). For example, neuromodulators can alter neuronal activity by changing membrane potentials or altering the sensitivity of neurons to neurotransmitters; they can regulate neurotransmitter release and fine-tune the strength of synaptic transmission; and they can modulate receptor sensitivity to induce long-term changes in synaptic efficacy (126). In insects, numerous neuromodulators have been identified, and many more are rapidly being discovered. They can include compounds such as neuropeptides, biogenic amines, and even gases like nitric oxide or carbon dioxide (88).

Neuromodulators play a role in the development and plasticity of the insect nervous system and can affect how neural circuits are formed and how they change in response to environmental stimuli (126). They can also coordinate complex behaviors such as feeding, mating, aggression, and social interactions by modulating circuit activity and allowing insects to modify behavior based on internal states or external stimuli. It has been proposed that, much like decoupling observed in the social insect endocrine systems, the roles and functions of neuromodulators that regulate reproductive traits in solitary ancestors have been co-opted and modified throughout social insect evolution to regulate traits now linked to social behaviors (114).

Biogenic amines. Some of the best-studied biogenic amines (BGAs), including octopamine, dopamine, and serotonin, can act as fast neurotransmitters or as slower, long-lasting

neuromodulators. These chemicals are highly conserved throughout evolution, potentially predating the origin of the nervous system (34). Among social insects, BGAs have gained novel functions to modulate social behavior and the regulation of social organization (62). Dopamine is associated with mating and increased receptivity in solitary insects as well as reproductive state in many social hymenopterans (114). Variation in octopamine and dopamine has been linked to reproductive and dominance status in several species, though the specific nature of these relationships varies across species. For example, dopamine levels vary according to reproductive states in honey bees (115), bumblebees (117), *H. saltator* ants (98), and *Polistes* paper wasps (116, 149). In some queenless ants, octopamine levels show similar trends: Variation in octopamine levels correlate with social rank (28, 91). However, it appears that, overall, octopamine levels are more likely to be correlated with nonreproductive social behaviors (114). Octopamine and serotonin levels increase aggression in solitary and some social insects, and octopamine has also been implicated in nestmate recognition, potentially by increasing sensitivity to pheromone cues (62).

Neuropeptides. The oxytocin/vasopressin neuromodulators are well known in vertebrates for their regulation of mammalian social behavior. The insect homolog, inotocin, is present in some social insects but has apparently been lost in corbiculate bees and in *Drosophila melanogaster* (32). Within ants, it appears to regulate nursing and foraging task allocation, but these effects are age and context dependent (23).

Studies focusing on neuropeptides as modulators of social behavior are rapidly expanding. For example, several insect neuropeptides have been associated with caste specificity in ants, but these neuropeptides are largely understudied in other social insects. One example is the neuropeptide corazonin. Corazonin is an ortholog of the gonadotropin-releasing hormone that regulates reproduction in vertebrates (44), and it is associated with metabolism and stress response in *D. melanogaster*. In ants, higher levels of corazonin are associated with workers versus reproductives and with foraging (44) and hunting behavior (94). In addition, the neuropeptide tachykinin modulates aggression in *Drosophila* (3), and tachykinin levels have been associated with age-related task transitions in the desert ant *Cataglyphis fortis* (118). The identification and investigation of novel insect neuropeptides are fruitful areas for future studies.

Juvenile hormone as a neuromodulator. Recent studies have identified an important role for the insect blood–brain barrier (BBB) in modulating the uptake of JH into the brain (57, 58). The carpenter ant *Camponotus floridanus* juvenile hormone esterase (CfJhe) has lost the ability to be efficiently secreted from cells, as is the case in *Drosophila*. As a result, CfJhe is retained and functions intracellularly within the BBB to regulate JH3 entry into the brain. RNAi-mediated knockdown of CfJhe expression leads to an increase in the levels of JH3 in the brain and, subsequently, an increase in foraging activity in major workers (58). Regulation of JH transport to the brain may be a convergent mechanism regulating social behaviors in ants and in bees. A comparative genomic study across sweat bees also identified convergent and complementary signatures of selection on two pleiotropic genes that regulate JH binding and transport, *hexamerin 110* (*hex110*) and *apolipoprotein* (*apolpp*) (57). ApoLpp is expressed in a subset of glial cells that comprise the insect BBB, and the protein can cross the *Drosophila* BBB with cargo intact (18).

Epigenetics

Epigenetic mechanisms help to regulate gene expression and can dynamically respond to the environment. DNA methylation, histone posttranslational modifications, and noncoding RNAs represent chromatin-modulatory mechanisms that can influence multiple aspects of social insect biology, including reproduction, behavior, caste determination, and aging.



Chromatin regulatory factors generally do not bind directly to DNA and thus are recruited by DNA-binding transcription factors to provide genome location information. Transcription and chromatin factors can be activated by environmental perturbations, and the gene expression changes generated often persist even after the environmental signals abate. Thus, the cumulative epigenome has a key role in maintaining environmentally induced changes in transcriptional state (122).

DNA methylation and eusociality. While there is no clear association between total DNA methylation levels and eusociality (38), epigenetic factors have often been linked to caste determination during development. For example, DNA methylation plays an important role in caste determination in honey bees: Higher methylation levels are associated with the worker phenotype. Knockdown of the *DNA methyltransferase 3* (*dnmt3*) biases developing larvae toward queen-like traits even in the absence of the nutrient-rich royal jelly (72). Similar mechanisms have been identified in the termite *Zootermopsis nevadensis*, in which queens and workers differ in DNA methylation patterns (39). If and how DNA methylation impacts caste development likely vary widely across the Hymenoptera, but a growing body of research suggests that DNA methylation may indeed play a role in caste determination in a number of social insect species (122). However, the mechanisms of DNA methylation remain to be determined because, first, DNA methylation occurs in gene bodies in social insects rather than in DNA regulatory regions as in mammals and, second, higher methylation levels are correlated with higher gene expression in social insects rather than lower expression as in mammals (126).

Histone modifications and caste determination. Histone modifications also play a role in caste differentiation in ants and honey bees. In the carpenter ant *C. floridanus*, one particular histone acetylation site (histone H3K27ac), which is associated with transcriptional activation, varies among major and minor worker castes correlating with caste-specific gene expression (40). Injection of a histone deacetylase inhibitor (HDACi) into the brain induces behavioral reprogramming that increases foraging rates in the nonforaging majors. The HDACi increases total histone acetylation levels and specifically upregulates a neuronal chromatin corepressor coREST (corepressor for element-1-silencing transcription factor) along with HDAC1. These chromatin repressors in turn decrease expression of the JH-degrading enzyme, juvenile hormone esterase (Jhe), leading to a rise in JH that is associated with increased foraging activity (40). Interestingly, in foraging minors, expression of the DNA-binding transcription factor *tramtrack* in late pupal stages recruits coREST immediately after eclosion to repress the *Jhe* gene, leading to high levels of JH (41). Thus, epigenetic factors can modulate complex and caste-specific behavioral programs within this species. Similar mechanisms are likely at play in numerous social insect species. For example, honey bee royal jelly contains HDACis that may impact the chromatin states of developing larvae toward queen development (128).

The Nutrition and Endocrine Signaling Systems Interact to Form a Regulatory Circuit

Decades of work in model organisms have significantly advanced our understanding of how insects integrate environmental signals into appropriate behavioral and physiological responses (113). The IIS/TOR pathways sense nutrient availability, including carbohydrates and amino acids. These pathways then directly affect the production of juvenile hormones and ecdysteroids. JH and 20E regulate numerous physiological processes, including development, reproductive physiology, neural processes, innate immunity, oxidative stress resistance, and lifespan (89).

The IIS/TOR and endocrine signaling systems are intimately linked, forming a regulatory circuit that couples resource intake with context-appropriate phenotypic expression. Insulin

signaling mediated through IRs increases the expression of JH-biosynthetic enzymes in the CA, increasing JH titer and fat body Vg production (135). TOR signaling in response to protein intake similarly promotes JH release by promoting biosynthetic activity in the CA (151). Inhibition of either IIS or TOR signaling decreases JH-biosynthetic enzyme expression and JH titer, indicating that these two signaling pathways work in tandem to adjust JH release and reproductive investment (100, 151). Ecdysteroidogenesis during postembryonic development is also regulated by IIS/TOR (reviewed in 148). In turn, JH and 20E can also regulate the IIS/TOR pathway and modulate the sensitivity of cells to ILPs (56), creating a finely tuned regulatory circuit.

Neuromodulators and Epigenetic Mechanisms Modulate the Nutrition–Endocrine Regulatory Circuit

The nutrition–endocrine axis is tightly regulated by the central nervous system (**Figure 4**). Neurosecretory cells in the brain respond to environmental input and release signaling molecules, including ILPs, BGAs, allatostatins, and corazonin, that can directly regulate the release of JH and ecdysone signaling in the retrocerebral complex (RCC) (88). The RCC includes both the corpora cardiaca (CC) and CA; the CC release prothoracicotropic hormone that activates ecdysone biosynthesis. The CA are the glands that biosynthesize and release JH into the insect circulatory system. Thus, this neural circuit represents one mechanism through which the integration of environmental cues and endocrine signaling can be accomplished.

Endocrine signals are involved in remodeling the nervous system throughout insect metamorphosis (83) and also in early adult life (77). It is likely that similar mechanisms have been co-opted to modulate the caste-specific behaviors and neurobiology found among the social insects. Both JH and 20E cross the insect BBB and can induce downstream effects in the brain (57, 58, 93). As discussed above, JH and 20E modify the epigenetic and transcriptional profiles of neurons and glial cells in the brain. In ants (58), and potentially also in bees (57), genetic changes have occurred throughout evolution to proteins that influence the ability of these hormones to cross the BBB, ultimately regulating hormone levels in the brain and serving as an important modulator of social behaviors.

Social Signals Can Both Respond to and Hijack the Nutrition–Endocrine Regulatory Circuit

Social signaling mechanisms in eusocial insect colonies include behavioral dominance interactions, trophallactic transmission of metabolites, and pheromonal communication. The nutrition–endocrine regulatory circuit is targeted by and modulates these social signaling channels, guiding the behavioral and physiological development of individual colony members. In many insects, environmental cues such as temperature or day length influence the nutrition–endocrine regulatory circuit to steer development. In eusocial lineages, social signals can similarly be co-opted to serve as regulators (68).

Endocrine regulation of pheromone production. Across many ant, bee, wasp, and termite species exhibiting both simple and complex eusociality, chemical signals produced by the queen can regulate reproductive and behavioral development of colony members. The production of these queen pheromones (124) is often regulated by the endocrine system; the application of JH or its analogs to nonreproductive individuals has frequently been shown to induce a shift in chemical profiles toward those typical of a reproductive individual [e.g., wasps (67, 90, 103), ants (84), termites (20), and bees (2)]. Hormones likely induce differential pheromone biosynthesis between castes by altering gene expression patterns in target tissues. For example, in the ant *H. saltator*, the transition of workers to reproductive gamergates is associated with a rapid increase in the

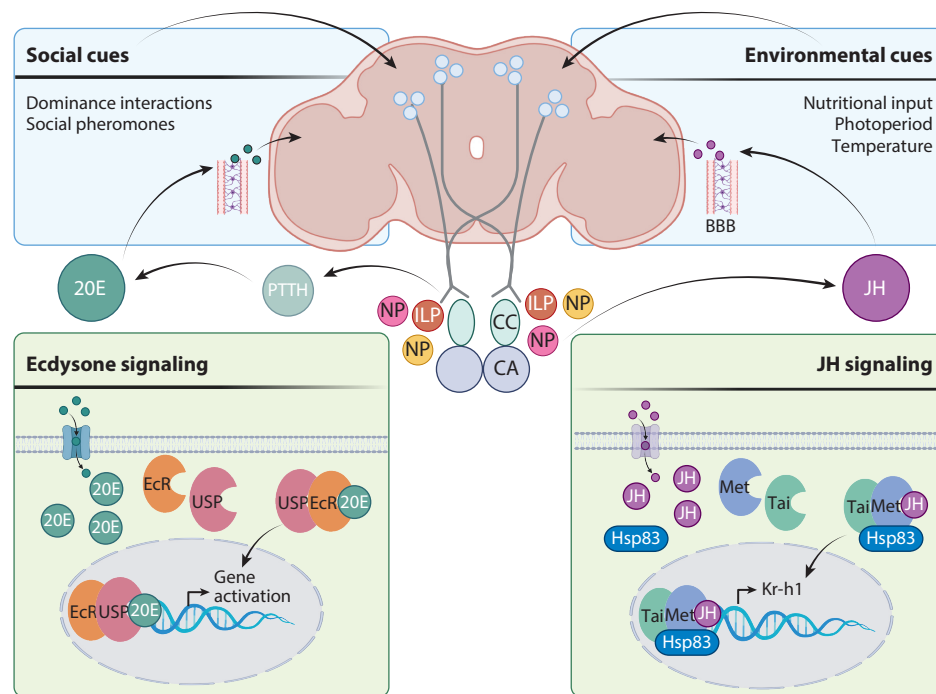


Figure 4

The nutrition–endocrine regulatory circuit. The brain integrates information from external social and environmental cues. Neurosecretory cells receive this information and produce neuropeptides (NPs), including insulin-like peptides (ILPs). These peptides are released at the retrocerebral complex and regulate biosynthesis and release of products from the corpora cardiaca (CC) and corpora allata (CA). The CC indirectly regulate the biosynthesis of the precursor to 20-Hydroxyecdysone (20E) via prothoracicotropic hormone (PTTH). The CA regulate the biosynthesis and release of juvenile hormone (JH). 20E is imported into cells via the ecdysone importer, where it binds the ecdysone receptor (EcR) and heterodimerizes with ultraspiracle (USP). This complex is then transported to the nucleus to activate downstream gene expression. It is unknown whether JH diffuses or is actively imported into cells, but once in the cytoplasm, it is bound by its receptor, Methoprene-tolerant (Met). Met heterodimerizes with Taiman (Tai) and is imported into the nucleus via Heat shock protein 83 (Hsp83). There, it activates the transcription factor, Krüppel homolog-1 (Kr-h1). 20E and JH can also act directly on the brain, and their entry is regulated by the insect blood–brain barrier (BBB) (58). These signaling pathways are reviewed in References 6, 56, 71, 113, and 134. Figure adapted from images created with BioRender.com.

cuticular abundance of queen-specific cuticular hydrocarbons (CHCs). During this time, there are wide-ranging shifts in gene expression, including changes in cytochrome P450, elongase, and desaturase gene families (13). While members of these gene families are involved in CHC biosynthesis in some solitary insects, directly linking individual genes' expression changes with specific CHC components in eusocial insects is challenging because the genetic underpinnings of CHC biosynthesis are not yet well characterized (51). Interestingly, JH levels do not differ between reproductive castes in *H. saltator*, suggesting that this hormone may not be a driver of biosynthetic gene expression during the worker-to-reproductive transition (99). A more likely candidate is ecdysone, which is released by the ovaries at high levels during this physiological transition (94).

Pheromonal regulation of the nutrition–endocrine regulatory circuit. Queen and other social pheromones can, in turn, regulate the nutrition–endocrine circuit. For example, the honey

bee queen pheromone can famously influence worker JH and ecdysteroid titers (60, 109, 133), epigenetic state (22), gene expression (50), and BGAs (9), and it also impacts the regulation of conserved developmental pathways [Notch signaling (30)]. In *O. biroi*, *ilp2* expression is inhibited by the presence of developing larvae (23). The ILP2 peptide promotes ovarian development, and this intergenerational signaling can thereby serve to regulate the cycling of nonreproductive (brood care) and reproductive phases of colony development. Interestingly, adult sensitivity to larval inhibition of *ilp2* is linked to nutritional state: More fecund workers are more resistant. Moreover, in *Pheidole* ants, exposure of the developing brood to soldier inhibitory pheromone (SIP) inhibits the development of degenerative imaginal wing discs that establish allometric scaling that defines the soldier body plan. SIP reduces sensitivity to a nutrition-linked JH pulse in the last larval instar that typically acts as an internal trigger to stimulate larvae to develop as soldiers (144), thus biasing larvae toward development as minor workers (105). Notably, SIPs have convergently evolved in termites and similarly interact with JH signaling (reviewed in 87).

The social stomach. In ants, passing of ingested material from one individual to another via trophallaxis produces a social stomach for the colony. These social fluids contain an astonishingly complex mix of metabolites that impact caste development, including Vg, microRNAs, carrier proteins, JH, and Jhe (75, 76). Phylogenetic comparative analysis of JHE in trophallactic fluid suggests that the secretion of these metabolites in the fluid evolved under positive selection and is not incidental (75). The protein makeup of trophallactic fluid also changes in predictive fashion with colony age, aligning with colony metabolic needs, suggesting a role for trophallactic metabolites as superorganismal hormones (45).

Dominance interactions. Physical interactions can also impact nutrition–endocrine signaling. Aggression-mediated dominance hierarchies in *Polistes* paper wasps can reduce JH titers in low-ranking, cohoused females in comparison to isolated wasps, and gene expression diverges within a few hours (129, 137). In the bumblebee *Bombus impatiens*, direct contact with a live, behaving queen is required to suppress ovarian development in workers; neither indirect contact with queen volatiles nor direct contact with queen CHCs represses ovarian development in this species (85, 95). A particularly interesting avenue for future comparison is offered by the finding that JH titers are not influenced by social context in solitary species that have been examined (64).

Sensory perception. Finally, sensory perception and behavioral response to intercaste pheromonal signals have also been linked to nutritional and endocrine (JH) status. This provides an additional molecular substrate to fine-tune the behavioral phenotypes of individuals making up a social colony. In the honey bee, workers deprived of nutritional resources during larval development show increased attraction (retinue response) to queen mandibular pheromone (QMP) upon reaching adulthood. By contrast, deprivation during the adult stage decreases workers' affinity for QMP (139). The functional relevance of these divergent behavioral responses to QMP from a fitness perspective remains unclear, but presumably they allow individuals to optimally direct their efforts based on their current nutrient status (for example, directing energy toward foraging instead of retinue behavior when nutrient stores are low). In *H. saltator*, workers transitioning to become reproductive gamergates show a decreased response to reproductive CHCs; in this case, the difference in sensitivity occurs at the peripheral level within the OSNs of the antennal sensillae (36). The molecular mechanism by which physiological (e.g., nutritional) state modulates the processing of pheromonal signals via endocrine signals has yet to be characterized in a social insect.



Modifications to the Nutrition–Endocrine Regulatory Circuit Underlie Social Evolution

The regulatory architecture of the nutrition–endocrine circuit has often been modified throughout insect social evolution (110) to enable differential regulation of hormonal responses across castes and the decoupling of reproductive traits and parental care. The onboarding of social cues into this nutrition–endocrine regulatory circuit appears to be an important component of social evolution. This circuit is also considered to play a major role in governing insect lifespan and mediating the trade-offs between fecundity and longevity (110), and it is now being studied as an integrative network in aging research (70). Similar approaches are likely to yield great success in the study of social behavior and its evolution. In both cases, a carefully designed phylogenetic comparison of social insects that encompasses the full spectrum of behavioral variation should provide major insights into how this regulatory circuit has been modified and fine-tuned throughout the course of social insect evolution.

CONCLUSIONS

Bees, ants, and wasps have repeatedly evolved eusociality. The resulting behavioral variation encompassed by this group provides an ideal system to better understand the molecular mechanisms that facilitated the origins and elaborations of social life in this diverse group of insects. Ground plan hypotheses provide a foundational concept for understanding the molecular and physiological toolkit that enables the reproductive division of labor central to eusocial life. Insulin signaling and endocrine pathways are key players in the evolution of social complexity in insects, and they are intertwined with additional epigenetic factors and neuromodulators that can dynamically regulate their action. This network is largely modulated by nutrition and other abiotic cues in solitary insects, but social insects have also integrated social cues into this circuit to enable them to dynamically respond to the social environment. A deeper understanding of the nutrition–endocrine regulatory circuit and how it has been modified across independent gains and losses of eusociality will greatly improve our knowledge of the molecular mechanisms that underlie the development and evolution of diverse insect social behaviors.

SUMMARY POINTS

1. Eusociality has arisen multiple times within the social insects, generating substantial variation within and among species that is ideal for gaining insights into the mechanisms that shape the evolution of this trait.
2. Emerging evidence largely fits predictions of ground plan and toolkit hypotheses, but independently evolving lineages also harbor unique molecular changes associated with the elaboration of eusociality on their branch.
3. Nutritional and endocrine pathways—and the neuropeptides and epigenetic mechanisms that modulate their activity—are a major substrate for social evolution. These pathways are deeply intertwined and have repeatedly been co-opted to shape social traits, from the evolution of dominance hierarchies in simple eusocial societies to the development of specialized worker castes in complex eusociality.
4. The nutrition–endocrine regulatory circuit is tightly linked to social evolution and enables the integration of external environmental and social cues with internal physiology and behavior.

5. Investigations of the nutrition–endocrine regulatory circuit and how it has been modified along independent evolutionary trajectories is likely to yield fruitful insights into the molecular mechanisms that shape social behavior and its evolution.

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