

# Thinking outside the Embryo: The Superorganism as a Model for EvoDevo Studies

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## Abstract

Traditional model systems such as fly, mouse, and chick have formed the foundation of the EvoDevo research program. These animal systems have provided a wealth of information on the patterns and mechanisms of developmental change over large phylogenetic scales. However, the almost exclusive focus on individual embryos as model organisms has also limited the field's ability to address the central roles that natural selection and life history adaptation play in the evolution of developmental systems. Likewise, focus on this small set of "unitary" model organisms may also constrain the explanatory reach and theoretical robustness of EvoDevo if we consider that many core developmental processes like homeostatic regulation, ontogenetic differentiation, and norms of reaction are also manifest at other levels of biological organization, such as colonial organisms. The study of social insect systems can help bridge these considerable gaps in EvoDevo's current approach. Because social insect colonies span the fine line between simple associations of "unitary" organisms and full-fledged "superorganisms," these systems help elucidate what kinds of processes fundamentally characterize developmental systems and their evolution. What is more, the traits of social insect colonies show high degrees of population-level variability that can be linked directly to selective factors in the environment, making them ideal systems in which to more fully integrate EcoEvoDevo approaches.

## Keywords

EvoDevo, eusocial insects, evolution and development, evolvability, kin selection, life history, superorganism

*Development, as we have shown, is in essence the interaction between parts, for it is in this way that the spacing and patterning of an individual is achieved. But if the individual merges into an association of individuals, then there will be an extension of the principle of development to include these larger associations, for here also the colony of the society is integrated and unified by the interaction between parts.*

*If in evolution there has been an increase in size and an increase in the compounding of living units (into colonies, multicellular organisms and societies) then there must also have been a corresponding extension of development.*

—John Tyler Bonner (1958)

In 1958 Bonner wrote a slim compendium of three lectures that can be considered one of the earlier synthetic treatments of the topic we now broadly call EvoDevo: “The abstraction I hold before you is the development of living organisms. The novel view that we shall take of this well-worn and battered subject is its evolution.” While it is questionable whether evolutionary considerations of development were completely novel at that point, Bonner’s approach did significantly diverge from most approaches that preceded or have followed since. This is because the organisms that formed the basis of Bonner’s analysis included no mouse embryo, no frog embryo, not even a *Drosophila* or a chick. Instead, the creatures that were the foundation of his discussion were slime molds, *Volvox*, and ant colonies. These choices may seem off-the-mark for neglecting the standard model systems that characterize the field of EvoDevo today. However, I will argue that Bonner’s choice of colonial organisms—and social insects in particular—reflected a prescience rather than ignorance about the vital and central questions of EvoDevo studies.

Writing in the late 1950s, Bonner described the limitations of a “causal embryology” that focused solely on proximate mechanisms of development. In contrast, his framing asked readers at that time to consider the following two goals: (1) to understand the evolutionary origins of developmental systems explicitly in the context of adaptation and natural selection, and (2) to distill the most basic, unifying principles of developmental systems in evolutionary context. It would seem that the goals of contemporary EvoDevo are very much the same. As Rudy Raff (1996) has articulated it, “Our goal is to define a simple set of principles by which internal processes might function and by which they interact with the external process of selection.”

It could be argued, however, that EvoDevo remains in its infancy in addressing the two goals that both Bonner and Raff set out—either in gaining an understanding of the role of natural selection, or in extending the explanatory power of its “set of principles.” A key obstacle in both cases has been the limitation of model systems themselves. Organisms such as the fruit fly *D. melanogaster*, nematode *C. elegans*, or mouse

*M. musculus* were not chosen for their particular significance in understanding evolution by natural selection. Rather, they became model organisms because of characteristics that made them good laboratory subjects, and subsequently bred in a way that has left them largely insensitive to environmental effects (Gilbert 2001). The almost exclusive focus on the embryonic stages of model organisms has also severely limited what kinds of traits, and hence adaptations, we can ask about. The origin of structures such as limbs, petals, and body segments are “adaptive” to the extent that they have persisted in groups that greatly radiated during evolutionary history. However, these are usually not traits that exhibit the kind of population-level variation necessary for straightforward assessment of function and selective advantage in a microevolutionary context. Such concerns have encouraged greater interest in EcoDevo or Eco-EvoDevo approaches (Gilbert 2001; Gilbert and Bolker 2003; Blute 2006).

There is no question that traditional model systems have provided an important and foundational understanding of developmental processes that are broadly employed in a phylogenetic sense, such as HOX genes and their role in major transitions of body form (Arthur 2002). But the fact that all traditional model systems are “unitary organisms” may also limit the explanatory reach and theoretical robustness of EvoDevo research when we consider how many core developmental processes such as homeostatic regulation, ontogenetic differentiation, and phenotypic plasticity are also manifest at additional levels of biological organization, including a variety of colonial organisms. It is precisely because many of these colonial entities span the fine line between what we think of as simple associations of unitary organisms on the one hand, and full-fledged colonial “superorganisms” on the other that these systems are particularly relevant. Their very organization raises critical conceptual and empirical questions about what kinds of processes fundamentally characterize developmental systems and their evolution.

Bonner wrote that the goal of his lectures was “to explore the many implications of the idea that with the association of individuals into groups of various sorts including colonies and societies there has been an extension of the principles of individual development to include the development of the whole association.” Indeed, on the 50th anniversary of his book many of the questions and most of the details of how developmental principles extend beyond unitary individuals within an EvoDevo framework remain open, and tantalizingly so. What follows is a brief overview of the ways that two groups within the eusocial hymenoptera, the ants and bees, have become rich systems for exploring the interactions between genetics, development, adaptation, and ecological life history. By way of a few examples I want to explore how core developmental processes manifest themselves in the form of behavioral regulation on the colony level; the significance of kin selection in

the developmental character of social insect colonies; and the novel and integrated kinds of analysis that these colonial models offer an understanding of how developmental systems connect with life history adaptation.

### Unpacking the Superorganism: Colonies as Developmental Entities

Our understanding of development is conditioned by the assumption that the individual organism is the canonical unit of analysis. Although it is well recognized that what constitutes an “individual” in a biological sense is a complex question with its own long history (Huxley 1926; Brandon and Burian 1984; Buss 1987; Gould and Lloyd 1999), almost inevitably what we mean by “an individual” in developmental biology is the ontogeny arising from a single zygote or embryo. The idea that social insect colonies are a kind of individual—a “superorganism”—was first articulated by the eminent ant biologist William Morton Wheeler (Wheeler 1911; but see Laurent 1990). The intention on Wheeler’s part was not merely to draw an analogy between social insect colonies and unitary organisms (Mitchell 1995). Rather, his claim was that such colonies were *equivalent* to conventional, unitary organisms in all of its essential characteristics:

An organism is a complete, definitely co-ordinated and therefore individualized system of activities, which are primarily directed to obtaining and assimilating substances from an environment, to producing other similar systems, known as offspring, and to protecting the system itself and usually also its offspring from disturbances emanating from the environment. (Wheeler 1911: 5)

While the notion of the superorganism continues to be highly controversial, many have argued for the utility of the concept for examining the biology of many kinds of colonial organisms, and especially social insects (Lumsden 1982; Seeley 1989; Wilson and Sober 1989). To be clear, the claim is not that viewing cooperative colonial associations as superorganisms is the exclusive, or necessarily even most important, level of analysis in all cases. Instead, the claim is a pluralistic one—that shifting focus to include “a higher level of self” (Queller and Strassman 2002) can help provide fundamental insights into how developmental, homeostatic, and adaptive processes operate in evolution.

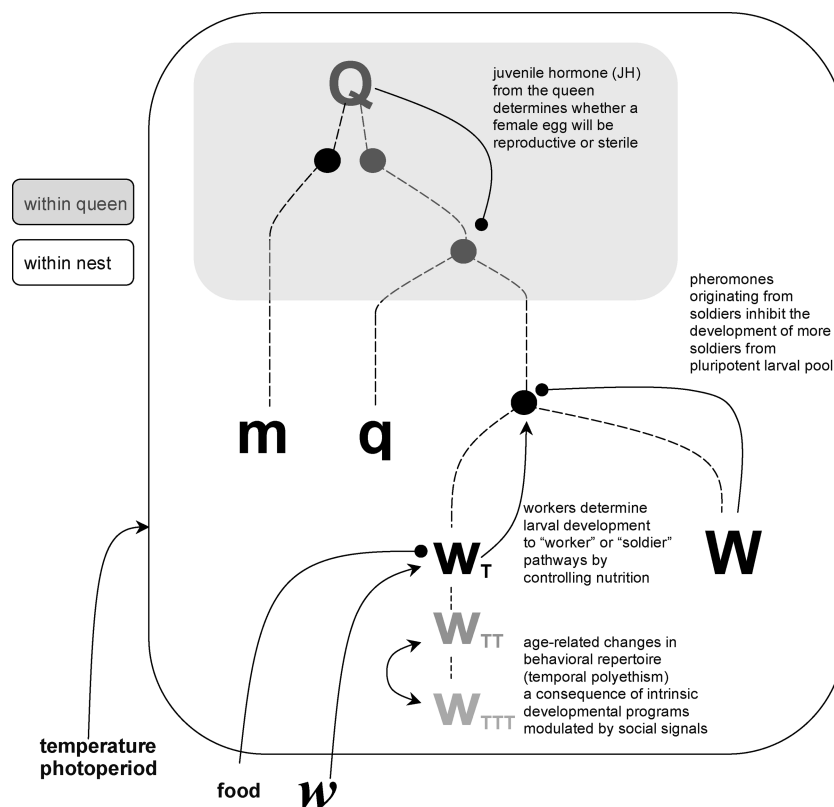
Wheeler was also perhaps the first to recognize that social insect colonies are not only ecological units of organismal function and reproduction, but developmental units as well. It is well known that just as typical unitary organisms show internal differentiation of the germ line, the soma, and specialized tissues of the soma, “eusocial” insects colonies also exhibit their own distinct reproductive castes (queens and males) as well as nonreproductive workers that form distinct specialized subcastes within the colony. Specialization alone is not suffi-

cient to establish the developmental nature of eusocial insect colonies—after all, a typical factory with its division of labor is not a developmental unit in the sense of an organic whole. However “division of labor” has been an important organizing concept for those seeking to quantitatively formalize the dynamics of social insect colonies (Oster and Wilson 1978; Lumsden 1982; Hasegawa 1997).

One such example is Adaptive Demography Theory (Wilson 1971; Oster and Wilson 1978), which focuses on division of labor through a system-optimality approach, while taking into account the colonial phenotype as a developmental process, rather than as a product. Like Wilson’s conception of “sociogenesis” (1985) and Tschinkel’s notion of “sociometry” (1991), adaptive demography models recognize that eusocial insect colonies are developmental entities, made up of various interacting components (individual castes and their members) that have their own ontogenetic patterns of growth and differentiation. These components are regulated by a complex array of signaling and feedback mechanisms that are both inherent to the colony as well as sensitive to modulation by environmental cues (Hölldobler and Carlin 1987; Hölldobler 1995, 1999; Anderson and McShea 2001).

Figure 1 illustrates some of these developmental mechanisms currently known for one system, ants of the genus *Pheidole*. The various types of individuals that make up a colony arise through different pathways in polyphenic development. A fertilized egg’s developmental trajectory as a reproductive or nonreproductive is determined early by signals of juvenile hormone (JH) controlled by the queen during egg-laying (Wheeler and Nijhout 1981). Once an egg takes on a worker fate, further developmental decisions are mediated through a combination of signaling pathways present within the colony. For example, the decision to become a minor worker, or instead a morphologically and behaviorally distinct major worker/soldier, depends on the “threshold size” of larvae at a critical time late in their development. Below the threshold size the larva becomes a minor worker; above the threshold size it develops into a soldier (Wheeler and Nijhout 1981).

Significantly, this aspect of a colony’s development and phenotype can be both up and down regulated—minor workers control the amount of food that growing larvae receive and thus larval size, while soldiers produce inhibitory pheromones that suppress the differentiation of larvae through the soldier developmental pathway (Figure 1). When a colony faces outside competition, minors can use the cues of contact with foreign colonies to increase major worker/soldier production (Passera et al. 1996), likely through increased feeding. Likewise, if a colony has too high a proportion of specialized major workers, the concentration of their inhibitory pheromones suppress further production of this caste (Wheeler and Nijhout 1984). Since these caste proportions fundamentally affect colony function (Brown and Traniello 1998; Yang et al.



**Figure 1.**

Social insect colonies have characteristic patterns of growth and differentiation just as unitary organisms do. Shown here are the developmental pathways leading to different castes in *Pheidole* ants. The division of the germ line from the somatic worker line is followed by a further differentiation into “worker” and “soldier” sterile castes through a polyphenic threshold mechanism. The proportion of these worker types is regulated by nutritive and pheromonal signals sensitive to environmental conditions, such as competition. Workers go through further ontogenetic change in behavioral repertoire known as “temporal polyethism” that are mediated by changes in hormones, neurotransmitter levels, and neural connectivity in the brain. Various environmental factors also influence colony development directly and operate as cues that initiate seasonal life history changes in colony growth and division of labor. Q=egg laying queen, m=male reproductive, q=unfertilized queen, w=worker (“T” subscript denotes temporal changes in relative number of tasks an ant can undertake), W=major worker/soldier. ↓ Arrows represent stimulatory inputs, ⬇ sticks represent inhibitory inputs.

2004), the two signaling pathways play a crucial role in the homeostatic and adaptive regulation of colony phenotype. In addition to biotic cues from the environment, abiotic factors such as temperature and photoperiod also function as cues and modulators in the coordination of colony-level homeostasis (Southwick 1983; Cassill and Tschinkel 2000; Stabentheiner et al. 2003) as well as seasonal life history changes in growth and division of labor (Kipyatkov 1979; Heinrich 1981; Tschinkel 1993, 1998; Yang 2006).

In addition to morphological castes, age-related changes in task specialization, known as temporal polyethism, or simply “temporal castes” are important (Hölldobler and Wilson 1990; Robinson 1992) (Figure 1). Despite what is already a large literature, recent studies continue to uncover fine-grained details of how these ontogenetic patterns unfold by means of intrinsic developmental “programs” that are further shaped and tuned through social cues (Calderone and Page 1996; Beshers et al. 2001). In the present case of *Pheidole* ants, for example, researchers have found that older workers develop increased responsiveness to olfactory cues

of both food and brood, while younger workers only possess sensitivity to brood signals (Seid and Traniello 2006). Older workers consequently perform a broader repertoire of tasks and respond to changing colony conditions needs more effectively.

While this kind of behavioral data is a mainstay of behavioral ecology and social insect research, these developmental changes in repertoire and plasticity are now beginning to be correlated to processes on the cellular and molecular levels. For example, Seid and Traniello (2005) have found increases in dopamine and serotonin levels associated with ontogenetic changes in the task repertoire of *Pheidole*, as well as associated changes in the neural architecture of their brains (Seid et al. 2005). Worker response thresholds and repertoire in turn impact colony phenotype and adaptivity in a number of key ways (de Biseau and Pasteels 2000; Beshers and Fewell 2001; Theraulaz et al. 2002). These new molecular approaches are thus opening up unprecedented levels of detail for integrating developmental mechanisms with the phenotypes across multiple levels of organization, from neuron to colony.

## Kin Selection and Reproductive Conflict: A Basis for Colony Regulatory Networks

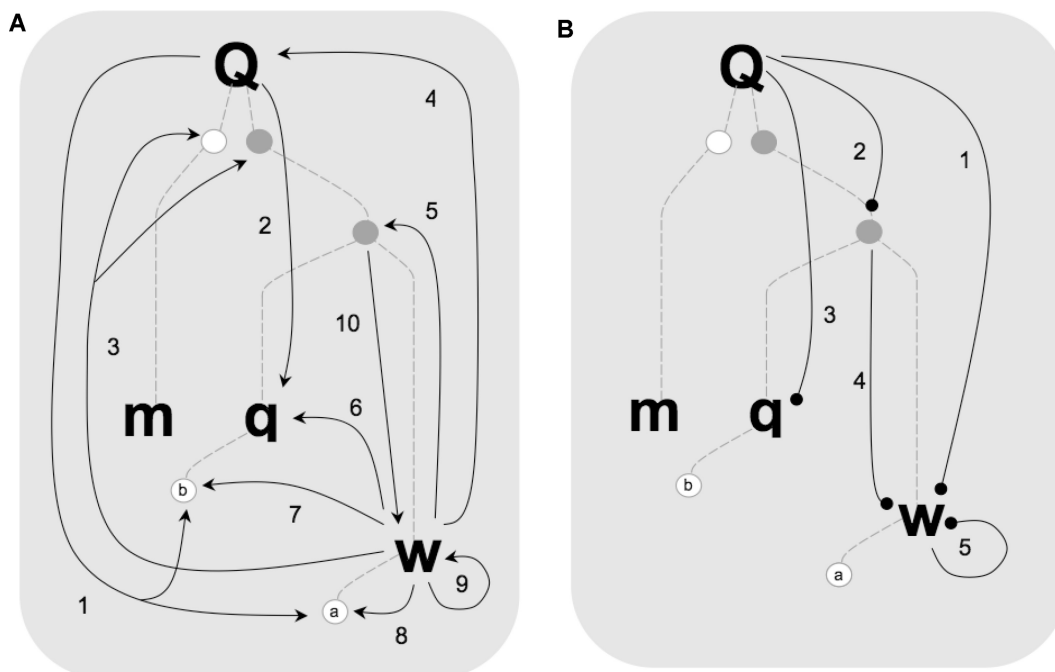
A central component to understanding how the developmental systems of social insect colonies evolve in response to natural selection is the nature of reproductive conflict that exists within them. Kin selection is often thought of as a “gene-level” account of social behavior that stresses reproductive conflict over cooperation. To the extent that this is true, it does not necessarily follow that colony-level analyses and kin selection are by definition inconsistent with each other. Nor is it true that the superorganism concept is only explicable in a group selection formulation of colony traits. While some recent discussions may appear to position kin selection in opposition to, or exclusive of, higher-level accounts (Wilson 2005; Foster et al. 2006; Wilson and Hölldobler 2006), there is in fact no necessary conflict between kin selectionist and superorganismal approaches to analyzing eusocial colony dynamics (Ratnieks and Reeve 1992).

In fact, rather than undermining the concept of colonies as superorganisms, reproductive conflict and kin selection are in large part responsible for the developmental nature of colonial entities, be they multicellular entities (Michod et al. 2003; Michod and Herron 2006), or superorganismic ones (Ratnieks and Reeve 1992). Indeed, for social insect colonies it is because—and not in spite—of genetic heterogeneity and potential reproductive conflict that a wide variety of regulative and homeostatic mechanisms have evolved that maintain functional cohesiveness (Figure 2).

Conflict in reproductive optima between queen and workers, workers and workers, as well as potential rival queens have all been extensively studied, leading to a wealth of data on regulatory mechanisms that have evolved as a consequence of kin selection (Mehdiabadi et al. 2003; Ratnieks et al. 2006). These mechanisms exhibit both remarkable commonalities and divergences across ant and bees, which together have experienced at least eight independent origins of the advanced sociality characteristic of superorganisms (Wilson 1974; Grimaldi and Engel 2005). Such regulatory diversity can be seen in the conflict of queen versus worker reproduction. Queens of both ants and bees produce signals that inhibit worker reproduction and also produce chemicals that uniquely mark their eggs as distinct from any eggs surreptitiously laid by workers (the latter of which are then destroyed by workers that “police” the brood). However this signaling is accomplished in very different ways: In the ant *Camponotus floridanus* one chemical signal deposited on the egg by the queen functions both to mark the egg as well as inhibit worker fertility (Endler et al. 2004), while the honeybee *Apis mellifera* achieves its regulatory ends by employing two different pheromones produced from two different organs (Hoover et al. 2003; Martin et al. 2005).

What makes such pheromonal systems examples of *regulatory* signaling rather than simply mechanisms of individual defense or aggression? To be sure, these kinds of queen chemical cues have traditionally been discussed in kin-selectionist and inclusive-fitness accounts as a matter of queen-worker “conflict” and queen “control” over worker reproduction. However, there is a growing recognition that such mechanisms may in fact be better understood as honest signals that act to coordinate colony development and maximize the colony’s overall reproductive potential (Keller and Nonacs 1993; Ratnieks et al. 2006). The distinction is more than semantic; the two perspectives are both operationalizable and testable in ways that have important consequences for understanding social insects colonies as superorganisms amenable to developmental analysis. Study of a phenomenon called *reproductive sharing* serves well as an example: Is this apparent sharing of reproductive rights within a colony the result of “incomplete control” by a dominant queen over subordinate members, or instead evidence for “reproductive concessions” that a dominant gives to subordinates in exchange for their cooperation? Under kin selection theory, these two alternate hypotheses can be formulated and tested quantitatively. The work of Reeve et al. (1998) found support for the reproductive concessions model, indicating that in fact complex mechanisms of intracolony regulation—not lack of dominance control by an individual queen—is likely to account for reproductive sharing.

Taking a superorganismic view, phenomena previously considered as the ken of behavioral ecology can now be understood to illuminate more fundamental questions about the evolution of regulative networks (Figure 2). If we continue with the example of reproductive coordination, it may be interesting to ask what kind of signals—chemical signals based on the hydrocarbon profiles of individuals, or behavioral signals based on dominance interactions—are employed to regulate reproductive and other forms of specialization in a colony (Monnin and Peeters 1999). This is because systems based on chemical signals may not have the same degree of feedback, and thus regulative control, compared to behavioral signals that can be mediated more quickly by the nervous system. On the other hand, chemical signals may have a further reach in the colonial body, depending on whether they are volatile, and hence operating under dynamics akin to a diffusible morphogen, or nonvolatile and thus spatially localized in their effects. If both chemical and behavioral signals are employed, how may they interact to affect the stability of the colony as it develops, or indeed the stability of the developmental system itself as it continues to evolve? Certain stabilizing signaling mechanisms among colony members may in fact have the consequence of generating instability in other parts of the system (Reeve et al. 1998), whereas other mechanisms may enable canalization. Here, not surprisingly, we find that considerations on the colony level mirror those of interest on the cell and genetic



**Figure 2.**

Kin selection is a key factor in the evolution of the superorganismal character of social insect colonies. A number of regulative and homeostatic mechanisms have evolved to maintain the functional cohesiveness of colonies given potential conflict over reproduction among its members. Shown in black are some of the better-known mechanisms of regulation and feedback that mediate conflict and integrate functionality, as compiled from a variety of social insect taxa spanning ants, bees, and some wasps. **A** shows mechanisms based on physical interactions between members, including biting, licking, food exchange (trophallaxis), physical dominance, or execution. **B** shows examples of regulation and signaling achieved by means of pheromones, cuticular hydrocarbons, and other chemical signals that often play inhibitory roles. Dotted gray lines represent the ontogenetic developmental trajectories of different colony members (see Figure 1). Q = egg laying queen, m = male reproductive, q = unfertilized queen, w = worker, a = worker laid egg, b = queen laid eggs (a and b are both unfertilized eggs and hence male). ↓ Arrows represent stimulatory inputs, ↓ sticks represent inhibitory inputs. (A) Keys for regulative processes via behavioral signals and actions: (1) “Queen policing” and destruction of eggs laid by workers or other reproductives (Kikuta and Tsuji 1999). (2) Dominance behaviors between reproductives (Reeve et al. 1998; Monnin and Peeters 1999). (3) Worker culling of queen laid eggs due to sex ratio conflict with queen (Passera et al. 2001), or with female larvae (Bourke and Ratnieks 1999). (4) Worker feeding and care of queen regulates her reproductive state. (5) Regulation of larval worker caste fate by worker control of nutrition (Wheeler and Nijhout 1981; Passera et al. 1996). (6) Execution of supernumery queens by workers (Ross and Keller 1998). (7) “Worker policing” and destruction of eggs laid by nondominant queen (Ratnieks et al. 2006). (8) “Worker policing” and destruction of eggs laid by other workers (Ratnieks and Visscher 1989). (9) Worker-worker antennal signals, acoustic signals, and other physical—interactions (Hölldobler 1999, Gobin and Ito 2003). (10) Larval signaling to nurse workers (Huang et al. 1989; Creemers et al. 2003). (B) Keys for regulative processes via chemical cues and signals: (1) Queen hormonal control over reproductive caste fate of a female egg (Wheeler and Nijhout 1981, 1984; Passera et al. 2001); Queen pheromonal modulation of worker sensitivity to pheromonal signals (Crozier 2002; Vander Meer and Alonso 2002); Reproductive/non-reproductive discriminatory cues (Dietemann et al. 2003). (2) Queen behavioral signal pheromonal control over worker fertility (Keller and Nonacs 1993; Hoover 2003; Endler 2004). (3) Queen pheromonal control over the fertility of other reproductives (Krieger and Ross 2002; Crozier 2002). (4) Distinctive pheromonal marking of queen eggs (Endler 2004). (5) Reproductive/non-reproductive discriminatory cues (Dietemann et al. 2003); Worker-worker chemical signals, including trail and various recruitment pheromones (reviewed by Hölldobler 1999). (For general reviews of regulatory signaling and behaviors see: Reeve and Ratnieks 1992; Vander Meer et al. 1998; Hölldobler 1999; Anderson and McShea 2001; Ratnieks et al. 2006.)

levels regarding the evolution of robustness and stability in developmental systems (Nijhout and Paulsen 1997; Gerhart and Kirschner 1998; Nijhout et al. 2003).

At this point it is important to note that of the many potential conflicts in a colony that kin selection predicts (based on reproductive optima), only some become “actual” to the extent that they result in the destruction of colony members. The fact that we can even make this distinction is conceptually significant. Again, this is a consequence of the fact that kin selection and inclusive fitness theory provide a set of predictions about reproductive conflicts, and therefore also predictions on how the developmental mechanisms that govern the colony life history traits may evolve to maintain the functional cohesiveness of the superorganism.

The work of Bourke and Ratnieks (1999) on potential conflict between workers and the larval females they care for illustrates this nicely. Under kin selection, female larva may prefer to develop into a reproductive rather than a worker, while the workers may prefer that larvae become only other workers. One of the critical features influencing whether this potential/predicted conflict becomes an actualized conflict is the degree of self-determination female larvae have over their own growth and development. If the fate of a larva is determined “blastogenically” (by hormonal signals imparted on the egg at the time of laying), then the possibility for potential conflict to become an actualized conflict is low, since the larva’s possible future as a reproductive is completely out of her hands. However, if the female’s fate is determined “trophogenically”

(by the quality of food she eats during growth), then the degree of control that a larva has over its own food intake becomes critical. The model and its predictions were tested in *Melipona* bees and a high degree of self-determination was found in larvae because of their autonomous, trophogenic development in sealed, food-provisioned cells. As expected, actual conflict is realized in this system and many larvae develop into queens in excess of the optima, which in turn induces workers to kill these queens in a kind of colony-level apoptosis. A lack of inhibition and regulative control in one aspect of colony development is therefore counteracted by other predicted mechanisms of regulation at other points in the developmental system. These case studies illustrate how important kin selection and differences in reproductive interest are in shaping the evolution of development on the colony level. The utility of kin selection's theoretical framework for predicting and testing the conditions under which regulatory mechanisms evolve in the superorganismic context is evident.

### Dissection and Integration: EcoEvoDevo in Social Insect Systems

Having set this general context for colony development, two specific questions still remain: How mechanisms at different levels of developmental organization (gene to colony) causally integrate, and also how the evolution of these developmental mechanisms directly function as life history adaptations. It is here that social insects are powerful and potentially unmatched model systems for EcoEvoDevo.

In contrast to the highly canalized and proto-functional nature of embryonic phenotypes, the traits of social insect colonies show a high degree of variation on the macroevolutionary scale (due to the wide range in sociality exhibited across taxa), as well as on the microevolutionary scale (due to population-level variability on the species level). In both cases, life history traits as adaptations provide a crucial entry that links development directly to selective factors in the environment.

Some of the most innovative work on the macroevolutionary front is that of Gro Amdam and collaborators, who are teasing apart the molecular and hormonal signaling pathways that underlie the development of castes and their distinct phenotypes in unprecedented detail. This work brings a new understanding of how regulatory architectures can be co-opted and augmented in evolution to produce highly articulated and coordinated developmental systems (Nelson et al. 2007; Patel et al. 2007). In the honeybee, *Apis mellifera*, for example, Amdam has shown how the “reproductive ground plan” of ancestral solitary bees has likely been co-opted to serve as a detailed developmental template for adaptations of the colony-level, such as the division of labor found in worker foraging (Amdam et al. 2004). By providing an understanding of the

genetic circuitry, hormonal feedback, and the subsequent effects on behavioral ecology, this research is not only able to elucidate the specifics of EcoEvoDevo on a mechanistic level, but also develop a theoretical framework for how a whole other level of developmental organization—that of the superorganismic colony—may have come about over macroevolutionary time (Amdam et al. 2003, 2004, 2006). Likewise, Abouheif and Wray's (2002) research on the evolution of the genetic regulatory networks of wing polymorphism across the ants, caste-specific gene expression studies in bees (Evans and Wheeler 1999; Pereboom et al. 2005; Cristino et al. 2006), as well as certain “sociogenomic” approaches (Robinson et al. 2005) all point to how adaptive life history traits can be used to explore evolutionary change in developmental systems over both multiple levels of organization and large time scales.

Population-level variation in social insect life histories, on the other hand, provides an unprecedented avenue for examining the microevolutionary dynamics that concern EcoEvoDevo. An interesting example is that of African and European honeybees. Colonies of the African population appear to have a selective advantage in neotropical environments over their European counterparts due to the African subspecies' comparatively higher rates of growth and colony reproduction. What specific differences in colony developmental mechanisms account for these life history differences? Fewell and Bertram (2002) found strong evidence that genetically based differences in worker pollen preference and pollen foraging rates were likely driving the differences in colony growth rates (Fewell and Bertram 2002). Evidence suggests this adaptive divergence in colony growth may be traced to genetic differences in developmental timing that affect foraging preferences (Hunt et al. 1995; Pankiw and Page 2001; Fewell and Bertram 2002).

In many model organisms this would very well be the end of the story. But the ability to experimentally create chimeric and functionally viable African and European “pseudomutant” colonies (Wilson 1985) allowed a further layer of analysis into the role both internal and external environmental factors play in the phenotype of colony growth: Fewell and Bertram discovered that the overall colony-rearing environment also played a significant role in inducing earlier foraging behavior and thus preference for pollen. This increased pollen intake itself induced workers to increase brood care, therefore up-regulating an inducible sensitivity of in-hive workers that further contributed to colony growth. Like in any complex organism, we see specific changes in superorganismal regulatory networks can have profound effects on multiple levels of developmental organization.

One final and compelling case in which we see the potential of social insect systems for tracing such changes and their adaptive consequences is the single-queen (monogyne) and multiple-queen (polygyne) colony forms in the fire ant, *Solenopsis invicta*. As profound as this life history difference

turns out to be, it is known to hinge on an allelic variation in one gene, *Gp-9*, which appears to code for a pheromone-binding protein (Ross and Keller 1998). The current model for how this difference in pheromone sensitivity affects colony organization and development reveals the richness of understanding social insect systems may provide, as the following schematic summary shows: Polygyne females, both workers and queens, are heterozygous at the *Gp-9* allele, which is thought to result in a high-sensitivity form of the pheromone receptor in these females. It is hypothesized that this sensitivity has an inhibitory feedback effect on polygyne queens, suppressing their own body sizes, fertility, and decreasing their own level of pheromone production (Crozier 2002; Krieger and Ross 2002). Consequently, the highly sensitive workers of polygyne colonies do not detect the presence of multiple polygyne-form queens, thus permitting a multi-queen colonial form to exist (Ross and Keller 1998; Crozier 2002; Krieger and Ross 2002). By contrast, monogyne queens in their monogyne colonies produce high levels of pheromone. Monogyne-form workers have a low intrinsic sensitivity to queen pheromone, however pheromone from the monogyne queen of the colony appears to act as a modulatory “primer” on monogyne workers, increasing their sensitivity to queen pheromone beyond their inherent threshold (Crozier 2002; Vander Meer and Alonso 2002). Because of this, only the most fertile queen producing the most pheromone is allowed to survive; all other queens from inside or outside of the colony are killed by workers (Ross and Keller 1998; Krieger and Ross 2002; Crozier 2002).

The evolution of the *Gp-9* allele therefore appears to have cascading consequences throughout the superorganism, including the emergence of various and novel feedback and signaling circuits. This enabled the evolution of the polygyne queen life history strategy from a monogyne ancestor in direct response to selection in high population-density environments created by their invasive spread. In this way, the fire ant system not only provides a view onto the how novel and homeostatic regulatory networks can emerge from small genetic changes, but just as importantly addresses the often elusive goal of connecting the evolution of developmental systems directly to its ecological consequences.

### Connecting Dots: Realizing EvoDevo Through Social Insects

Study by study, the growing potential of social insects as model systems for broadening and deepening the research program of EvoDevo becomes evident. More than just analogy, we see that the superorganismal phenotypes of social insect colonies and the complex, multilevel developmental systems that they employ are fertile ground for integrating concepts across levels of biological organization. The patterning of individual larvae contributes to the patterning of caste structure, divi-

sion of labor, and up through the regulatory processes that pattern the colonial phenotype as a whole. This holds equally true for other social insects beyond the ants and bees, such as wasp systems (Hunt et al. 2007; Patel et al. 2007), as well as the extensive research in non-hymenopteran social insects, the termites (Miura et al. 1999; Miura 2005; Zhou et al. 2006). As a completely independent experiment in the evolution of sociality, the termites present an excellent opportunity to explore and test hypotheses regarding the evolution of developmental in colonial insects (Miura 2004) as well as a comparative tool for elucidating the overall contribution that haplo-diploidy and kin selection have played in colony regulatory mechanisms.

While social insects offer a lens through which to integrate the “micro” and “macro” as well as the genetic and the ecological, the analysis of colony-level processes also provides a unique opportunity to explore the limits of processes thought to be fundamental in the evolution of developmental systems (Yang et al. 2004). Taking Gerhart and Kirschner’s (1997, 1998) work on evolvability as a point of departure, areas of conceptual resonance between cell-genetic processes and colony-level processes are wide open to further synthesis. Is it true, for example, that at all levels of developmental organization regulatory processes governing “core processes” such as metabolism, reproduction, and so forth, are largely inhibitory in nature, with activation achieved through inhibition of inhibitors (Gerhart and Kirschner 1997)? How exactly does the architecture of regulatory processes compare across organizational levels, and how do properties like robustness, redundancy, compartmentalization, and flexibility manifest themselves when considered on the superorganismal level? Because the hymenopteran insects exhibit the full range of social structures—from completely solitary to completely eusocial—and over eight independent originations of colonial eusociality, there is ample opportunity to examine basic questions concerning the emergence and the diversification of complex multicomponent organisms and “evolutionary transitions in individuality” (Michod et al. 2003).

The combination of robust and exploratory processes, regulatory linkage together with phenotypic plasticity, and successful conflict/cooperation mediation are all thought to be responsible for the evolvable success of the metazoans (Bonner 1958; Gerhart and Kirschner 1997; Michod and Herron 2006). The success of eusocial hymenopteran colonies may be yet another instance of such developmental evolvability that deserves further study and articulation. The recent advances in the genomics, developmental biology, and behavioral ecology of social insects are now providing the practical basis for hitherto distinct disciplinary approaches to productively merge, and make this articulation possible. Social insect biologists have long fruitfully applied developmental models of activation, inhibition, signal transduction, feedback, and canalization to understand social insect dynamics (Calderone and

Page 1996, reviewed by Anderson and McShea 2001; Beshers et al. 2001). My argument here is that EvoDevo is in a place to both greatly contribute and greatly gain from embracing the superorganism concept in its research, with social insects as model systems. As a nexus for various research methods, EvoDevo can provide an additional framework for synthesizing the variety of approaches taken in social insect studies. In exchange, EvoDevo can utilize social insects to tackle questions of ecology, life history, and adaptation more directly while also testing and extending its fundamental concepts to all levels of biological organization. By thinking beyond the embryo and embracing its Bonnerian roots, EvoDevo is in the position to learn much from the wisdom of the hive.

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