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E-Article

Honeybee Social Regulatory Networks Are Shaped by Colony-Level Selection

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abstract: Social interactions pervade all aspects of life in the social insects. Networks of interacting nestmates enable the maintenance of colony homeostasis and regulation of brood development. Artificial colony-level selection on the amount of pollen stored in honeybee colonies has produced high- and low-pollen-hoarding strains that have been used as a model system to study the genetic and physiological basis of differences in forager behavior that contribute to colony-level differences in pollen hoarding. Here we extend this model system using an interacting-phenotypes approach that explicitly studies genetic components arising from social interactions. High- and low-pollen-hoarding-strain larvae were reared in hives with high- or low-strain older larvae and high- or low-strain adult workers. The ovariole number and dry mass of focal individuals depended on interactions between the genotypes of the focal individuals and their brood and adult worker nestmates. These results show that trait expression by individual honeybee workers is modulated by the genotypic composition of the colony, indicating that individual-level phenotypes are properties of the composite "sociogenome." Thus, colony-level selection has produced strains with distinct combinations of socially interacting genes, which make up the social networks that regulate development and expressed phenotypes.

Keywords: developmental program, genotype-by-genotype epistasis, indirect genetic effects, interacting phenotypes, levels of selection, social evolution.

The eusocial insects are exemplars of social evolution and complex social organization. Colonies of some species contain millions of sterile workers and a single reproductively active queen. Communication among colony members enables coordinated division of labor and the strict regulation of colony homeostasis (Wilson 1971; Hölldobler and Wilson 1990). How have these marvels of social evolution been shaped by selection, both within and between colonies? What are the genetic and developmental bases of complex social phenotypes? And, how does the web of social interactions so fundamental to social insect life affect the genetic basis, evolution, and development of social phenotypes? These questions are of broad significance because social insects are well-established models for social evolution and emerging models for the evolution of polyphenisms, aging, and behavior (Evans and Wheeler 1999 2001; Honey Bee Genome Sequencing Consortioum 2006), and the evolution of eusociality is considered to be a major transition in evolution comparable to the evolution of eukaryotes and multicellularity (Maynard Smith and Szathmáry 1995).

The standard approach to characterize the genetic basis of traits is to search for correlations between individuals' genotypes and their phenotypes (Lynch and Walsh 1998). Many studies have used such an approach to elucidate the genetic and molecular basis of social insect phenotypes (reviewed in Robinson et al. 2005; Hunt et al. 2007; Oldroyd and Thompson 2007). However, this approach provides an incomplete picture in social organisms. It ignores major genetic components of "interacting phenotypes" that are influenced by or directly involved in social interactions (Moore et al. 1997). Like all phenotypes, interacting phenotypes are determined by the interplay of genes and the environment. However, with social interactions, this relationship becomes more complicated because the environment an individual experiences is in part provided by social partners. In this case, an individual's phenotype is directly affected by its own genes (direct genetic effects) and indirectly affected by genes expressed in social partners (indirect genetic effects; Moore et al. 1997). These additional genetic components arising from social interactions fundamentally alter the genetic architecture of phenotypes and can strongly influence evolutionary dynamics (Cheverud and Moore 1994; Moore et al. 1997; Wade 1998; Wolf 2003; Bijma et al. 2007).

Because social interactions are an inextricable part of social insect life, there is reason to suspect that interacting phenotypes are ubiquitous in the social insects (Linksvayer and Wade 2005). Traits involved in nestmate communication, such as brood-nurse signaling and response, are

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clearly interacting phenotypes whose evolution is shaped by the coevolution of genes expressed in brood and nurse workers (Linksvayer 2007; also see Wolf and Brodie 1998; Kölliker et al. 2005). Such communication forms the basis of the social regulatory networks of social insect colonies (Hölldobler and Wilson 1990; Seeley 1995). However, all other phenotypes are also potentially affected by these social networks. For example, traits determined during larval development, such as adult body size and reproductive caste, are determined mainly by nutrition, which is regulated by brood-nurse interactions. Similarly, the environment experienced by adults is determined by the network of chemical and physical interactions with nestmate brood and adult workers and queen(s) (Slessor et al. 2005). Thus, many social insect phenotypes, from body size to physiological state to behavior, can meaningfully be described as interacting phenotypes with both direct and indirect genetic components (Linksvayer and Wade 2005). It is not surprising then that although an explicit interacting-phenotypes approach has only recently been applied to social insects (Linksvayer 2006, 2007; see also Bienefeld and Pirchner 1990, 1991), a variety of studies provide evidence for genetic components of the social environment for morphological and behavioral phenotypes, and several indicate that direct genetic effects are mediated by the social environment (Rinderer et al. 1986; Moritz et al. 1987; Oldroyd et al. 1991; Calderone and Page 1992; Guzmán-Novoa and Page 1994; Keller and Ross 1995; Rüppell et al. 2001; Calis et al. 2002; Pankiw et al. 2002; Allsopp et al. 2003; Hunt et al. 2003).

The amount of pollen stored in honeybee hives ("pollen hoarding") is a colony-level trait that is regulated according to current colony needs via feedback mechanisms involving interactions among thousands of foragers, nurses, and brood (reviewed in Seeley 1995; Fewell 2003). Foragers collect pollen, and nurses eat and digest pollen, convert the pollen proteins to proteinaceous glandular sections, and feed these secretions to larvae. Young larvae and their pheromones stimulate pollen foraging (Pankiw et al. 1998), while excess pollen inhibits pollen foraging (Fewell and Winston 1992).

In order to study the genetic basis of pollen hoarding, Page and Fondrk (1995) selected for increased and decreased pollen hoarding in a population of European honeybees derived from commercial stocks (see also Hellmich et al. 1985). By the third generation, colonies of the high-pollen-hoarding strain had approximately six times more pollen than colonies of the low-pollen-hoarding strain, demonstrating that this colony-level trait was heritable and could respond to selection (Page and Fondrk 1995). With subsequent generations of selection, Page and coworkers studied the genetic, behavioral, neural, and physiological differences between high- and low-pollen-hoarding foragers that contribute to differential colony-level regulation of pollen stores (reviewed in Page et al. 2006). Relative to low-pollen-hoarding-strain workers, high-pollen-hoarding-strain workers are biased toward collecting pollen, they collect larger pollen loads (Page and Fondrk 1995; Fewell and Page 2000), and they initiate foraging earlier in life than low-strain bees (Pankiw and Page 2001), among other differences. An underlying cause of these differences in forager behavior may be physiological tuning associated with differences in ovary size (Amdam et al. 2006; see Oldroyd and Beekman 2008 for a criticism of this hypothesis and Amdam and Page 2008 and Tsuruda et al. 2008 for a response). High-strain workers have larger ovaries (more ovarioles) than do low-strain workers. Likewise, unselected wild-type bees with more ovarioles forage earlier in life and collect more pollen than do bees with smaller ovaries (Amdam et al. 2006). These associations between reproductive physiology and foraging behavior in the selected pollen-hoarding strains and in wild-type bees have been proposed to be signatures of an underlying reproductive ground plan common to all insects that has been modified to contribute to the division of labor in honeybee societies (Amdam et al. 2004, 2006). Furthermore, these associations between the traits of individual foragers and colony-level traits suggest that changes in colony-level phenotypes can be produced through genetic modification of lower-level traits (Amdam et al. 2004, 2006).

Here we expand on the model pollen-hoarding system by studying how individual-level phenotypes that are thought to contribute to the colony-level pollen-hoarding phenotype are themselves shaped by social interactions at the colony level. We use an interacting-phenotypes approach that explicitly studies genetic components arising from social interactions. We focus on two important individual-level traits that differ between the artificially selected pollen-hoarding strains: worker ovariole number and body mass. These traits are determined during larval development and are both strongly shaped by the quantity and quality of nutrition provided to larvae by nurse workers, so that a priori we expect indirect genetic effects to be important (see Beekman et al. 2000; Calis et al. 2002; Pankiw et al. 2002).

Methods

Experimental Setup

We used a cross-fostering design in which high/low-pollen-hoarding-strain focal individuals were reared with high/low-strain older brood (i.e., larvae that were approximately 3 days older than the focal individuals) and high/low-strain adult workers, resulting in eight combinations of focal

individuals, older brood, and workers. Three replicates, each with a different pair of high- and low-strain pollen-hoarding colonies matched for size and condition, were conducted March 31–April 27, May 15–June 11, and June 26–July 23, 2006, at the Arizona State University Honey Bee Research Facility in Mesa, Arizona. On the first day, queens were caged on a frame for 3 days and then recaged on a new frame for 3 days. Eggs from the first caging resulted in the older brood, and those produced during the second caging resulted in the younger focal individuals on which phenotypes were measured after adult emergence. Care was taken to ensure that frames used for focal individuals were relatively new and of similar age, because pupal casings accumulate as frames become older, and individual cells become smaller, resulting in smaller adults. After the third day of the second caging, experimental colonies were constructed. Queens from the high- and low-strain colonies were removed and the colonies were split in half. The halves were monitored to ensure that each half contained approximately half of the workers throughout the experiment. Each half received Bee Boost (PheroTech, Delta, British Columbia), a commercial artificial blend of queen mandibular pheromone that is meant to simulate queen presence. We used Bee Boost to make queen effects consistent across treatments, although this does not preclude the possibility that workers and brood from the different strains respond differently to queen pheromone. Each of the four experimental colonies received one empty frame with 75 g of pollen, one frame of honey, a half-frame of either high- or low-strain older brood, and a quarter-frame of high-strain focal brood and a quarter-frame of low-strain focal brood (frame portions were divided based on brood area). We removed all other frames with brood, honey, and pollen from the experimental colonies. Frames with capped brood cells were removed to an incubator. Over the next 3 days, equal amounts (by weight) of newly emerged bees from the two source colonies were introduced into the experimental colonies with matching worker bees. This procedure ensured that all experimental colonies had bees of the youngest age class. One day before emergence of the old brood, the frames containing old brood were removed. One day before emergence of the focal individuals, the frames with high- and low-strain focal individuals were moved into a lab incubator kept at 34°C. As the focal individuals emerged, they were dissected; the crop, ventriculus, and rectum were pierced and any contents expelled; ovaries were removed and placed on microscope slides with cover slips, and the number of ovarioles was counted using a compound microscope with a phase-contrast attachment. Subsequently, each focal individual was dried overnight in a drying oven at 60°C, and dry mass was measured to the nearest 0.1 mg using a Mettler-Toledo AB204-5 microbalance.

Statistical Analysis

Ovariole number and dry mass phenotype data were analyzed with the following mixed model, using the mixed model procedure of SAS:

$$\begin{aligned} y_{ijklm} = {} & \mu + focal_i + worker_j + brood_k + focal_i \\ & \times worker_j + focal_i \times brood_k + worker_j \\ & \times brood_k + focal_i \times worker_j \times brood_k \\ & + replicate_{l(ijk)} + \varepsilon_{ijklm}, \end{aligned}$$

where y_{ijklm} is the observed ovariole number or dry mass of a focal individual; μ is the overall mean; $focal_i$ is the strain of the focal individual (fixed effect); $worker_j$ is the strain of nestmate workers (fixed effect); $brood_k$ is the strain of nestmate older brood (fixed effect); $replicate_{l(ijk)}$ is replicate colony nested within focal, worker, and brood strain (random effect); and ε_{ijklm} is random error. *Focal*, *worker*, and *brood* effects are due primarily to genetic differences between the high- and low-pollen-hoarding strains, assuming that environmentally based differences between strains are negligible. As colonies were matched for size and condition and were kept in the same environment, this assumption is reasonable. *Replicate* effects include random differences between replicate colonies as well as seasonal environmental differences between the replicates, for example, due to differences in temperature or pollen availability. Error variances were not homogenous across focal-worker-brood combinations for both ovariole number and dry mass (Levene's test, $P < .05$), so we used a model with heterogeneous error variances (Kang et al. 2004).

Results

We collected and dissected a total of 979 focal bees: 339, 320, and 320 in the first, second, and third replicates, respectively. Total ovariole number was measured for 944 workers, and dry mass was measured for 972 workers. For the total number of ovarioles of focal individuals, there was a main effect of focal strain and worker strain and an interaction between focal individual strain and nestmate worker strain (table 1; fig. 1). For the dry mass of focal individuals, there was a main effect of focal individual strain and an interaction between nestmate brood and nestmate worker strain (table 2; fig. 2).

Dry mass and total ovariole number were negatively correlated across all focal individuals ($\rho = -0.193$, $P < .001$, $N = 950$) because workers of the high-pollen-hoarding

Table 1: Focal individual ovariole number was affected by focal individual genotype, nestmate worker genotype, and an interaction between focal genotype and worker genotype

Effect	*F* value	*P*
Focal	107.93	<.0001
Brood	1.38	.2560
Worker	7.32	.0148
Focal × brood	.03	.8667
Focal × worker	4.55	.0475
Brood × worker	.21	.6551
Focal × brood × worker	.01	.9429

Note: Type 3 tests of fixed effects from the mixed model are shown. For all effects, df = 1, 17.4; denominator degrees of freedom estimated with the Satterthwaite method (Kang et al. 2004).

strain are smaller but have more ovarioles than low-strain workers. Within the strains, there was no correlation between dry mass and ovariole number (low strain: $\rho = 0.090$, $P = .050$, $N = 474$; high strain: $\rho = -0.078$, $P = .089$, $N = 476$).

Discussion

Worker body size and ovary size differences between the high- and low-pollen-hoarding strains depend on interactions between the genotypes of the focal individuals on which the traits are measured (direct genetic effects) and the genotypes of worker and brood nestmates (indirect genetic effects). These results indicate that the strains have diverged for distinct sets of genes expressed in interacting nestmates that determine worker ovary size and body size. Size and ovariole number are determined during development, and nestmate workers and brood influence the expression of these traits by shaping the developmental environment. Thus, control of the developmental program of focal individuals is shared with brood and worker nestmates, and individual-level traits are properties of the composite "sociogenome" of the colony.

The networks of interactions that determine developmental trajectories and expressed phenotypes can be considered to be social regulatory networks, or social physiology (Seeley 1995), of the colony. Just as colony-level traits such as pollen stores are regulated, social networks also regulate individual-level traits expressed by workers. Thus, our results show that the network of social interactions that shapes development and expressed phenotypes has changed as a result of the colony-level selection program on pollen hoarding. Just as selection shapes physiological networks within organisms, our study shows that selection also shapes regulatory networks of superorganisms (Seeley 1995, 1997).

Genetic Signature of Social Evolution

Social evolution involves the evolution of interacting phenotypes. Like all phenotypes, interacting phenotypes require heritable variation in order to evolve. Unlike other phenotypes, interacting phenotypes have genetic components contributed by social partners, that is, indirect genetic effects (Moore et al. 1997). Variation for these indirect genetic effects is genetic variation for the social environment and can be considered "fuel" for social evolution. Divergence between populations or strains for indirect genetic effects provides a strong signature of social evolution (Linksvayer 2007).

The genotypic interactions affecting worker ovary size and body size differences between the pollen-hoarding strains are a type of intergenomic epistasis arising from social interactions (Wade 1998; Wolf 2000*a*, 2000*b*; Linksvayer 2007). Just as epistasis arising from the physiological interaction of gene products within organisms can contribute to phenotypic differences between lineages (Whitlock et al. 1995; Orr 2001), so can epistasis arising from social interactions between organisms. This epistasis detected between the pollen-hoarding strains indicates that the strains have diverged for distinct sets of coevolved genes expressed in interacting nestmates. Such epistasis has also been found between nurses and brood contributing to worker size differences between three species of acorn ants (Linksvayer 2007). Within a population, such intergenomic epistasis indicates the simultaneous coevo-

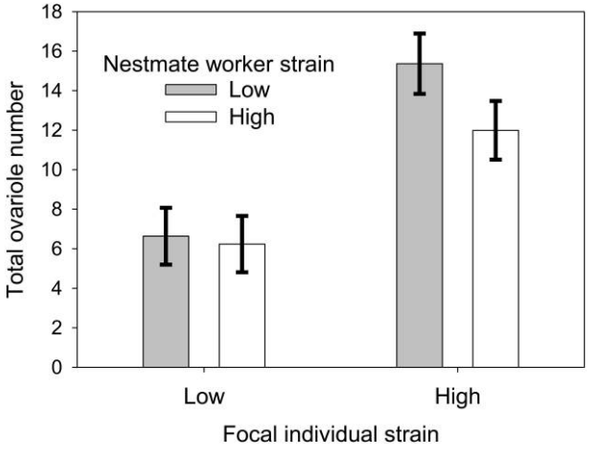

Figure 1: Mean ovariole number of high- and low-pollen-hoarding focal individuals reared with high- and low-strain adult worker nestmates. The ovary size of focal individuals depended on their own genotype but also on the genotype of worker nestmates. High-strain focal individuals were more sensitive to the social environment than low-strain focal individuals, and high-strain individuals reared by low-strain workers had the largest ovaries. Error bars show 95% confidence intervals.

Table 2: Focal individual dry mass was affected by focal individual genotype and an interaction between nestmate brood genotype and nestmate worker genotype

Effect	*F* value	*P*
Focal	17.27	.0007
Brood	.28	.6009
Worker	.00	.9744
Focal × brood	2.14	.1624
Focal × worker	1.82	.1959
Brood × worker	4.63	.0469
Focal × brood × worker	.06	.8121

Note: For all effects, df = 1, 16.1.

lution of interacting phenotypes. For example, epistasis between direct effect and indirect effect genes indicates the simultaneous coevolution of an individual with its social environment (Wade 1998; Wolf 2000*a*, 2000*b*).

Previous authors have suggested that colony-level selection can directly shape the social networks in insect societies that regulate both colony-level and individual-level traits (Lumsden 1982; Wilson 1985; Owen 1989). Furthermore, social insect researchers have long demonstrated how the social physiology of honeybees—the social regulatory networks controlling pollen foraging, nectar foraging, and so on—is adaptive at the colony level (Seeley 1995, 1997). Here we show how a program of artificial selection on a colony-level trait has shaped the social regulatory networks that affect expressed individual-level traits. Colony-level selection on pollen hoarding thus shapes a complex network of interactions, and the epistasis detected in our experiment is a genetic signature of how colony-level selection has tuned this interactive social network.

The interaction between older brood and worker nestmate genotypes is particularly interesting because it is independent of the genotype of focal individuals on which the phenotypes are measured—it involves only components of the social environment and is a pure indirect-by-indirect genetic interaction. This highlights the importance of the genotypic composition of the social environment for shaping development of the focal individuals that experience the environment.

Group (e.g., colony-level) selection acts on genetic components arising from social interactions (Goodnight and Stevens 1997; Wade 1998; Bijma et al. 2007), while individual-level selection can act efficiently only on additive genetic variance for direct effects. As such, it is not surprising that the colony-level selection program has produced strains with divergent socially interacting gene complexes. More broadly, natural colony-level selection has clearly played a prominent role in shaping a variety of honeybee phenotypes (Seeley 1997; Tarpy et al. 2004) and social insect phenotypes in general (Oster and Wilson 1976; Linksvayer and Wade 2005), so that coevolved socially interacting gene complexes may be widespread in social insects.

Possible Mechanistic Basis of Observed Interaction Effects

While we did not investigate the mechanistic details of the social interactions that contributed to genetic differences between pollen-hoarding strains, several strong possibilities exist. Components of brood pheromone produced in the salivary glands of larvae have been shown to affect the quantity and quality of food provisioned by nurse workers as well as elicit various physiological and behavioral responses of workers involved in the collection of pollen, conversion of pollen to brood food, and provisioning to brood (Le Conte et al. 1995, 2006; Pankiw et al. 1998). Direct behavioral interactions between workers and focal individuals—for example, through larval food solicitation behaviors and nurse provisioning responses—may be involved in the social interactions as well. These types of chemical and behavioral social interactions are ubiquitous in social insect colonies and are the foundation of the regulatory networks that affect development and expressed phenotypes (Hölldobler and Wilson 1990; Seeley 1995). These social interactions ultimately determine colony productivity and therefore are shaped by colony-level selection. Recent theory suggests that physiological epistasis is an inescapable property of gene regulatory networks (Gjuvsland et al. 2007), and similarly, intergenomic epis-

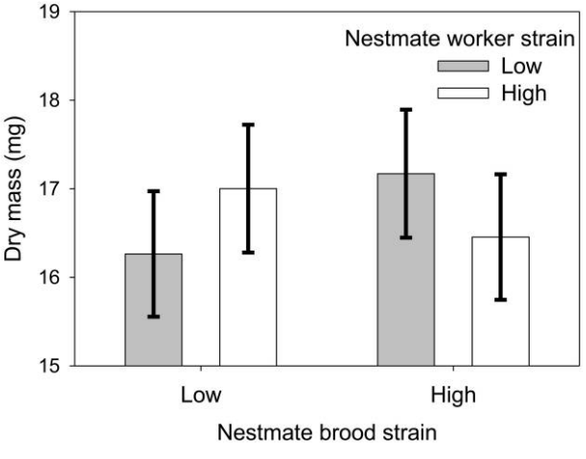

Figure 2: Mean dry mass of focal individuals reared with the four combinations of high- and low-pollen-hoarding-strain adult worker and brood nestmates. The dry mass of focal individuals depended on the genotypes of both worker and older brood nestmates. The largest focal individuals were produced by colonies with mismatched worker and brood nestmate genotypes. Error bars show 95% confidence intervals.

tasis may be a common outcome of the social interactions that regulate development and trait expression.

Besides being due directly to social interactions, differential sensitivity of larval genotypes to the social environment may have contributed to the interaction between worker genotype and focal larval genotype (fig. 1). For example, endocrine feedback loops, which mediate physiological responses to the environment, have been found to be more sensitive in high-strain individuals than in low-strain individuals (Amdam et al. 2007). It appears that physiological networks within individuals are integrated with social networks to produce expressed traits in social insects.

Extension of Model Pollen-Hoarding-Strain System

Previous research with the pollen-hoarding strains has shown that traits of individual foragers—including genotype, physiological tuning, ovary size, neural state, behavior, and so on—contribute to pollen hoarding, our colony-level phenotype of interest (reviewed in Page et al. 2006). These results reveal how modification of lower-level traits can build higher-level colony traits (Amdam et al. 2004, 2006; Page et al. 2006). Results of the current study build on these previous results. We show that the traits of individual foragers are strongly affected by the genotypic composition of the colony, which we interpret as underlying colony social-regulatory networks. Thus, traits measured on individual foragers are actually colony-level properties of the "sociogenome." Modification of individual-level traits can change colony-level phenotypes, but modification of colony-level phenotypes in turn changes individual-level traits. Together, these results demonstrate that colony-level and individual-level phenotypes arise via the integration of social networks of the colony with physiological regulatory mechanisms within individuals. The colony-level selection program has achieved changes in pollen hoarding through integrated changes in the network of social interactions in the hive (social physiology; Seeley 1995) and physiology of individual workers.

Previous studies found that individual foragers with more ovarioles (in high-pollen-strain bees relative to low-pollen-strain bees and in unselected wild-type bees) are more likely to collect pollen (Amdam et al. 2006). We expected that colony-level selection for increased pollen hoarding would have resulted in the developmental integration of direct and indirect effects that maximized worker ovariole number. In contrast to our expectation, we found that high-pollen-hoarding-strain larvae reared by low-pollen-hoarding-strain workers had the most ovarioles per ovary (fig. 1). A possible explanation is that there is an optimal intermediate worker ovariole number that leads to the most pollen collection, such that ever-increasing ovariole number does not always lead to increased pollen foraging. For example, workers with large ovaries are more likely to have activated ovaries (Makert et al. 2006), so that there may be a trade-off between ovariole number and foraging efficiency.

The dry mass of focal individuals was highest when focal individuals were reared with older brood and worker nestmates mismatched for strain (fig. 2). This pattern of worker-by-brood interaction for dry mass is interesting yet difficult to explain because it is not clear how worker size and colony pollen hoarding are related. Older brood nestmates require much more provisioning than younger larvae, and perhaps in cases where the old brood and workers were mismatched for genotype, the older brood monopolized nurse worker provisions so that the younger focal larvae developed into smaller adult workers. Thus, we speculate that for both ovariole number and dry mass, cross-fostering disrupts coadapted complexes of socially interacting genes (see Linksvayer 2007).

Pankiw et al. (2002) studied the effect of the larval and adult rearing environments on the wet mass, responsiveness to sucrose solution, and behavior of foragers from the high- and low-pollen-hoarding strains. Similarly to this study, they found that forager genotype and the composite rearing environment affected forager mass and also responsiveness to sucrose solution and forager behavior. They also found evidence for a genotype × larval rearing environment interaction for forager sucrose responsiveness and behavior (Pankiw et al. 2002).

Why Is an Interacting-Phenotypes Approach Necessary?

Besides Pankiw et al. (2002), various other studies with honeybees and ants have also examined the contribution of the larval rearing environment and adult social environment to morphological and behavioral phenotypic variation (e.g., Rinderer et al. 1986; Moritz and Southwick 1987; Oldroyd et al. 1991; Calderone and Page 1992; Beekman et al. 2000; Pankiw and Page 2001; Calis et al. 2002; Ross and Keller 2002; Allsopp et al. 2003; Beekman and Oldroyd 2003). These studies provide evidence for genetic components to the social environment but do not use a formal framework that explicitly considers social genetic components. Why is a formal framework, as provided by an interacting-phenotypes approach, necessary? What unique insights can an interacting-phenotypes approach achieve?

An interacting-phenotypes approach is a natural extension of formal quantitative genetic theory, and it is necessary to use such a formal approach to evaluate the evolutionary effects of genetic components arising from social interactions (e.g., Griffing 1981; Kirkpatrick and Lande

1989; Moore et al. 1997; Wade 1998; Bijma et al. 2007). This approach has recently been used to study the evolution of social dominance (Moore et al. 2002), sexual selection (Moore and Pizzari 2005), and especially parental care (e.g., Cheverud and Moore 1994; Agrawal et al. 2001; Hunt and Simmons 2002; Rauter and Moore 2002; Lock et al. 2004). Such a formal approach is necessary because direct and indirect variance components must be correctly identified and combined to correctly understand evolutionary dynamics (Moore et al. 1997; Wolf 2003). This is particularly tricky with complex social systems, such as social insects, where indirect genetic effects arise from several sources, for example, queen(s), workers, and brood (Linksvayer 2006). Genetic components arising from social interactions remain cryptic with standard approaches that do not explicitly study them. A general result from previous studies using an interacting-phenotypes approach is that variance for indirect genetic effects often makes substantial contributions to total phenotypic variance in a variety of social organisms (e.g., Cheverud and Moore 1994; Hunt and Simmons 2002; McAdam et al. 2002; Rauter and Moore 2002; Wolf 2003) including an ant species (Linksvayer 2006). As a result, sole focus on variance for direct effects gives incorrect estimates of underlying genetic parameters (e.g., heritabilities, genetic correlations, etc.) and incorrect predictions of evolutionary dynamics (e.g., response to selection; Cheverud and Moore 1994; Moore et al. 1997; Wolf 2003). Furthermore, the evolutionary dynamics of interacting phenotypes can be fundamentally different from the dynamics of standard phenotypes, for example, involving runaway dynamics (Moore et al. 1997; Wade 1998).

Social genetic components also potentially make up a fundamental portion of the full network of genes affecting a trait. Thus, gene discovery approaches designed to identify the genetic or molecular basis of traits (e.g., quantitative trait loci mapping, expression approaches) that do not consider social genetic components will simply overlook significant portions of the genetic architecture (Wolf et al. 2002; Cui et al. 2004; Mutic and Wolf 2007). For example, it is clear that in both solitary and social organisms, networks of genes expressed within developing individuals influence adult body size and other traits. However, in social organisms, additional networks of genes expressed in social partners are also an integral part of the genetic architecture of adult body size and other individual-level traits.

It is obvious that social interactions are important in insect societies, and, as detailed above, a variety of studies provides evidence for genetic components of the social environment. Even so, studies of the genetic basis of social insect traits typically do not consider these social genetic components. For example, in a recent review of the behavioral genetics of honeybees, Oldroyd and Thompson (2007) focus exclusively on genes expressed in individuals that directly affect their physiology or task thresholds (see figs. 1, 7 in Oldroyd and Thompson 2007), and while environmental factors such as the amount of brood pheromone are explicitly recognized, the fact that these "environmental" factors can have genetic components is not. Thus, the significance of social genetic components to social insect evolution has not been widely appreciated.

Conclusions

While standard approaches to study the genetic basis of traits assume a simple one-to-one match between individual genotype and phenotype, these approaches ignore important biological complexity. Social systems are not so simple, and insect societies in particular are well known for their social complexity. In fact, this social complexity is precisely the characteristic that makes social insects such a fascinating study system. Our study shows that social insect phenotypes are built not just by changing genes and gene expression within the focal individuals on which the traits are measured but also by changing networks of socially interacting genes. Thus, a reductionist approach that ignores social genetic components is incomplete, and a broader approach that incorporates social components of the colony is necessary to elucidate the full genetic basis, evolution, and development of social insect phenotypes.

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