The making of a social insect: developmental architectures of social design

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Summary

We marvel at the social complexity of insects, marked by anatomically and behaviorally distinguishable castes, division of labor and specialization—but how do such systems evolve? Insect societies are composed of individuals, each undergoing its own developmental process and each containing its own genetic information and experiencing its own developmental and experiential environment. Yet societies appear to function as if the colonies themselves are individuals with novel "social genes" and novel social developmental processes. We propose an alternative hypothesis. The origins of complex social behavior, from which insect societies emerge, are derived from ancestral developmental programs. These programs originated in ancient solitary insects and required little evolutionary remodeling. We present evidence from behavioral assays, selective breeding, genetic mapping, functional genomics and endocrinology, and comparative anatomy and physiology. These insights explain how complex social behavior can evolve from heterochronic changes in reproductive signaling systems that govern ubiquitous and ancient relationships between behavior and ovarian development.

Introduction

Honey bees live in societies consisting of thousands of individuals.(1) Within these societies are three anatomically distinct groups: queens, workers and drones. Queens and workers belong to different female castes; drones are males. Queens and workers develop from fertilized eggs that are totipotent with respect to caste development; drones develop from unfertilized, haploid eggs. Workers and queens differ in many anatomical characters including size (queens are roughly twice as large as workers), development of the ovaries (queens have much larger ovaries), the structure of the hind pair of legs (workers have a special set of hairs used for holding pollen, the pollen basket), and the structure of the sting (workers have a barbed sting while the sting of queens is smooth).(2) Drones differ from both workers and queens. They are about the same size as a queen but lack the sting of the two female castes, lack the pollen basket of workers, and have large eyes that converge at the top of the head (see Winston,(1) Table 3.2, p 40). Male and female differentiation results from a primary signaling protein product derived from the *complementary sex determining gene* (*csd*) activating the female developmental cascade. Queen and worker development diverge as a consequence of nutritional signals activating queen or worker developmental genetic cascades.(3,4)

Associated with honey bee sexes and castes are different ontogenies and repertoires of behavior that play out as different life histories. These are downstream consequences of the sex-and caste-determining signals. Drones emerge as adults, undergo a period of maturation where they

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sit on combs, are fed by workers and feed from open cells of honey. When they are about 12–13 days old, they are sexually mature with sperm in their seminal vesicles and they initiate mating flights.(5) If not mated, mature drones return to the nest and sit on combs until the next day. They do not perform any social tasks. Queens emerge from their cells as adults and engage in dominance struggles with other virgin queens. If successful, they stay in the nest until they are 5–14 days old when they initiate a series of orientation and mating flights. After successfully mating, they return to the nest where after an additional 2–3 days they begin egg laying, their primary behavioral activity for life.(6) Queens appear to have lost most of the maternal behavioral repertoire characteristic of solitary and primitively social insect females, while gaining a greatly increased egg-laying capacity and an ability to pheromonally regulate worker ovary development and behavior. Workers emerge as adults and immediately begin performing social tasks within the nest (e.g. cleaning cells, feeding larvae). After a few days, they initiate orientation flights and then begin foraging about their 3rd week of life. Once workers initiate foraging, they seldom perform within-nest tasks.(7) Understanding the developmental processes behind these life-cycle events of worker bees is key to understanding the evolution of complex social behavior.

Division of labor and specialization

Honey bee workers demonstrate a division of labor that is physiologically based and where, under most conditions, bees of different ages perform different tasks. Younger bees perform tasks within the nest such as feeding larvae, constructing and maintaining the nest and processing honey, while older bees forage. This division of labor is further divided into specialists who on average perform some tasks more frequently than do other individuals. For example, foragers specialize on collecting pollen, a protein source, or nectar, a source of carbohydrate. This is best observed as the ratio of the two substances collected, some bees collect relatively more pollen, others more nectar.(8)

The onset of foraging in honey bees is linked to hormones generally involved in larval development and adult reproductive maturation in insects. Many studies have shown correlation of increasing levels of juvenile hormone (JH), a hormone frequently involved in ovary development, with onset of foraging behavior. JH titres in honey bee workers normally increase with age. Foraging worker bees have higher hemolymph (blood) tires of JH than do bees working in the nest. Treatment of young bees with methoprene, a JH analogue, results in them initiating foraging earlier in life.(9) JH is produced in the *corpora allata*, paired secretory glands located close to the brain. Removal of the *corpora allata* results in bees that are able to initiate foraging although they are delayed in their foraging onset.(10,11) These results demonstrate an effect of JH on age at onset of foraging but also show that JH is not necessary for initiation and maintenance of foraging behavior. Therefore, it is likely that foraging onset in worker bees is governed by redundant control mechanisms.

JH and vitellogenin interact in honey bees by reciprocally inhibiting each other.(10,12) Vitellogenin is produced in the fat body and secreted into the hemolymph as a yolk precursor and storage protein,(13) but also acts remotely on JH production in the *corpora allata*,(12,14) *Vitellogenin* mRNA expression can be silenced by injection of double-stranded RNA (dsRNA) into the hemolymph of newly emerged bees.(15) When vitellogenin is knocked down by this RNA interference (RNAi), bees forage earlier in life and collect more nectar.(16) Thus, vitellogenin appears to have a dual function with respect to division of labor and foraging specialization (Fig. 1). Topical application of methoprene decreases vitellogenin while dsRNA knockdown of vitellogenin increases JH. Thus, reducing vitellogenin and increasing JH in newly emerged bees, factors involved in endocrine signaling and ovariole follicle development in reproductive insect females, affects honey bee division of labor and foraging specialization. Amdam and Omholt(10) proposed that the interaction of vitellogenin and JH produces a steep

regulatory switch that results in a rapid transition of bees from working within the nest to foraging. Gene expression studies have shown that roughly 39 percent of the genes expressed in the brain differ between foragers and nest bees,(17) presumably a downstream result of this regulatory switch.

The pollen-hoarding syndrome

It is clear that JH and vitellogenin are parts of a reproductive regulatory network that now governs division of labor in facultatively sterile worker honey bees.(18,19) But what are the origins of foraging specialization? To answer this question Page and Fondrk(20) conducted a two-way selection program for the amount of surplus pollen stored in the combs of honey bee colonies. This trait has been called "pollen hoarding". Bees store surplus pollen in cells of combs located near the brood (eggs, larvae and pupae), at the center of the nest. Parents (queens and drones) each generation were derived from colonies with low and high stores, respectively, to produce the high and low pollen-hoarding strains. Each generation, only the single phenotypic trait was used for selection. Pollen hoarding is a complex social phenotype involving the interactions of thousands of individuals engaged in foraging, recruitment to forage resources, consumption of pollen from cells, feeding of larvae and consumption of brood food by larvae.

Correlated responses to selection for individual behavior and physiology were studied during selection for the pollen-hoarding phenotype. These studies reveal a complex phenotypic architecture associated with pollen hoarding that spans individual behavior, sensory response systems (including learning and memory), reproductive anatomy, neurobiochemistry and hormonal signaling.

Individual behavior

Workers from the high pollen-hoarding strain forage earlier in life than do workers from the low strain(21,22) Pankiw and Page(22) introduced newly emerged high- and low-strain bees into a common hive occupied by commercial bees that were not part of the pollen-hoarding selection program. Their study showed that high-strain bees foraged significantly earlier in life. High-strain foragers collected larger loads of pollen and smaller loads of nectar. Several "common garden" experiments have also shown that high-strain bees are more likely to collect water(23) and will collect nectar with lower concentrations of sugar,(22,23) though nectar concentration results are not consistent in all experiments (c.f. Ref. 26).

Sensory-response systems

High-strain bees respond more to water and to lower concentrations of sucrose solution when tested using the proboscis extension response (PER).(23,25-29) Bees are placed into small tubes that restrain their movements. The head of the bee extrudes from the tube. A droplet of solution is touched to the tip of each antenna. If the concentration of the solution is sufficient, the bee reflexively extends its proboscis. The sucrose-response thresholds of individual bees can be determined by presenting sucrose solutions in an increasing series. High-strain bees on average have lower response thresholds to sucrose than do low-strain bees. The responses of high- and low-strain bees to water and sucrose solution complement the foraging results presented above where high-strain bees tend to collect more dilute nectar, and water.

High- and low-strain bees also differ in performance on learning assays.(25,28) Tactile and olfactory stimuli can be paired with the unconditioned response of the proboscis extension reflex in a classical conditioning assay when a sucrose reward is presented at the tip of the proboscis when it is extended. Bees can readily be trained to respond to specific odors or to specific tactile patterns presented to the antennae. After even a single trial many bees learn to

associate the tactile pattern or odor with the reward and extend their proboscis in the presence of the conditioned stimulus alone. The number of paired, rewarded trials needed before a bee responds with the unconditioned stimulus (the sucrose presented to the antenna) provides a measure of the rate at which the bee learns to associate the stimulus with the reward (acquisition). High-strain bees require fewer paired trials before responding to the conditioned stimulus (Fig. 2). Subsequent studies have shown that differences in acquisition rates are a result of differences in perception of the value of the reward used during conditioning, which is related to their response thresholds to sucrose.(30) High- and low-strain bees that have the same responses to sucrose have the same rates of acquisition.(25)

Studies of "wild-type bees" (bees of commercial origins that were not specifically selected for pollen hoarding) confirm that the sucrose response of a honey bee is a general neural property that is intimately linked to behavior. Pankiw and Page(26) marked newly emerged wild-type workers and placed them into a common host hive. Bees were collected from the combs when they were less than a week old and tested for their response thresholds to sucrose solutions. The bees were individually tagged and placed back into the hive until they initiated foraging. The hive entrance was observed daily, all tagged foragers were collected, and foraging loads were determined. Bees that were more responsive to low concentrations of sucrose and pure water, were more likely to collect water and pollen. There was also a significant correlation between the response thresholds of nectar foragers and the sugar concentration of nectar collected. Bees that responded to lower concentrations collected nectar with lower concentrations of sugar. These results have been independently repeated by testing adult workers less than 4 hours old and show again a fundamental relationship between sensory responses measured by PER and behavior, and additionally the age of initiation of foraging. (31,32) Classical conditioning of wild-type bees likewise shows the same patterns observed between bees of the high and low pollen-hoarding strains. Scheiner et al.(25,28) tested wild-type bees for their PER response to sucrose solutions. Bees were then conditioned to tactile or olfactory stimuli. Bees with similar responses to sucrose had similar rates of acquisition demonstrating that the results found for high- and low-strain bees represent very general principles of learning.

Collectively these studies demonstrate broad general features of the neural system that can be observed as responses to sucrose sensory input that affect broad patterns of behavior from learning to division of labor and specialization. The origins of these relationships must precede the evolution of the worker caste and division of labor because queens and drones of the high and low strains differ in sucrose responses in the same way as the workers even though they no longer forage. In addition, drones of the high-strain initiate mating flights (perhaps homologous with onset of foraging in workers) earlier than those of the low strain.(33) This correlation of drone and worker onset of flight behavior was also observed by Giray and Robinson(34) for wild-type bees.

Reproductive anatomy and physiology

Workers from the high pollen-hoarding strain have higher titers of vitellogenin and JH when they emerge as adults.(18,35) High-strain bees have larger ovaries that contain more ovarioles (individual filaments that contain developing eggs) than do bees from the low pollen-hoarding strain.(19) When high-strain bees emerge as new adults, significantly more individuals have ovaries that are activated, prepared to assimilate vitellogenic proteins. In the absence of a queen, worker ovaries become vitellogenic and they lay unfertilized eggs that develop into males. High-strain bees develop faster into egg-laying workers than do bees from the low pollen-hoarding strain. Wild-type bees with more ovarioles are more likely to have activated ovaries, (36) forage earlier in life, and collect more pollen and nectar of lower concentration than do bees with smaller ovaries.(19) Wild-type bees with large ovaries also are more sensitive to

sucrose and have higher vitellogenin transcription levels (Tsuruda, Amdam and Page, unpublished data). These results link reproductive physiology to sensory-response systems, division of labor and foraging specialization.

Neurobiochemistry

c-AMP signaling has been implicated in both sucrose sensitivity and learning performance. Wild-type bees that are more sensitive to sucrose stimulation have higher levels of c-AMP activated kinase (PKA) than less-sensitive bees. Activated PKA is important for activation of protein kinase C (PKC), which has been shown to be involved in memory formation.(37) Newly emerged high-strain bees have higher titres of both PKA and PKC in the central brain than the less-responsive bees from the low pollen-hoarding strain.(38) Tyramine is a neuromodulator that is also involved in c-AMP signaling. Recent studies have shown its effects on sucrose responses in wild-type honey bees.(39) High-strain bees have more tyramine receptor mRNA in the central brain when they emerge as adults (Humphries et al. unpublished). This could at least partially explain differences between the high and low strains in responsiveness to sucrose which is evident within 4 hours of emerging as adults. Tyramine is also found at elevated levels in egg-laying wild-type bees,(40) indicating a link between high levels of tyramine receptor RNA and enhanced reproductive physiology of high-strain workers.

Hormonal signaling

In insects in general, interplay between ovarian signaling and JH governs shifts between behavioral and sensory states in the reproductive cycle, for example, by controlling the shift from nectar to blood-host foraging in female *Culex nigripalpus* mosquitoes,(41) the shift from feeding and sexual behavior to fasting and parental activity in the earwig *Labidura riparia*, (42) the initiation of oviposition in crickets,(43) the initiation of sexual behavior of male *Agrotis ipsilon* moths,(44) and shifts between periods of intense flight activity and reproductive behavior in several taxa.(45) Morphogenic endocrine factors associated with reproduction affect adult behavior by regulating the growth and central processing of sensory and motor neurons,(46-50) resulting in synchronized changes in sensory perception, locomotor activity and reproductive physiology.(48,51) Similar regulatory principles can underlie the differences in behavior, sensory perception, reproductive physiology and neurobiochemistry between the selected pollen-hoarding strains.(18)

QTL mapping

Breeding, behavioral, genetic and physiological studies have revealed a set of traits expressed at different levels of biological organization that are all associated with division of labor and foraging specialization. These associations define a pollen-hoarding behavioral syndrome. (52-54) Quantitative trait locus (QTL) mapping studies of the high and low pollen-hoarding strains have revealed four major QTL (*Pln1–Pln4*) that demonstrate pleiotropic effects and explain much of the pollen-hoarding syndrome.(8,27,52,53) Each QTL affects multiple traits associated with pollen and nectar foraging. They also interact with one another in complex ways, demonstrating that the genetic architecture is highly epistatic. All individual QTL and most interactions among them affect pollen and nectar load sizes. All QTL also affect the sugar concentration of nectar collected by foragers. *Pln1* appears to be central because it has demonstrated direct effects on all traits. The level of interaction of these QTL and the set of traits involved in the pollen-hoarding syndrome suggests that these QTL are involved in signaling cascades with broad overlap and broad effects.

The combination of these QTL studies and the recently completed honey bee genome sequence and annotation (www.hgsc.bcm.tmc.edu/projects/honeybee) provide informed candidate genes for the genetic basis for variation in foraging behavior. JH and ecdysteroids interact just

prior to adult emergence and signal the initiation of vitellogenin expression.(55) Vitellogenin and JH interact in adults and affect sensory responsiveness and the onset of foraging.(12,14, 16) In solitary insects, endocrine cascades involving JH and ecdysteroids have pleiotropic effects on sensory tuning, yolk protein production, and ovarian physiology (see above), suggesting a link between the observed effects in the social honey bee and solitary ancestors. These endocrine cascades appear to be regulated upstream by the insulin/insulin-like signaling (IIS) pathway.(56-60) IIS pathway regulation would be consistent also with the highly interactive genetic architecture uncovered by QTL studies. A sweep of the genome sequence spanning the 97% confidence intervals of the mapped QTL reveals 113 predicted peptides and supports the hypothesis that IIS signaling underlies the pollen-hoarding syndrome. The QTL regions are characterized by a disproportional density of IIS components(61) (Fig. 3).

Our hypothesis that the pollen-hoarding syndrome is governed by signaling through the IIS pathway is supported by recent QTL studies in *Drosophila*. Orgogozo et al.(62) mapped QTL responsible for ovariole number differences between *D. simulans* and *D. sechellia*. Their best QTL based on a 2-LOD support interval contained the insulin receptor gene (InR) and P13K. The InR substrate (IRS) is located in our QTL *Pln4* and PI3K-II, which is probably also involved in IIS signaling, is located in QTL *Pln3* (Fig. 3). Furthermore, *D. melanogaster* mutant studies have previously shown that the IIS pathway affects ovariole number,(63) a trait strongly correlated with the pollen-hoarding syndrome of honey bee workers.(9)

The evolution of division of labor and specialization

In the following sections, we provide plausible hypotheses for the evolution of division of labor and foraging specialization in honey bees and its progenitors. The Apidae is a diverse family of insects spanning all levels of social organization from solitary to highly social, like the honey bee.(64) It is doubtful that our hypotheses cover the diversity and complexities of social evolution in all of these groups, but we believe they capture fundamental principles and suggest future directions for broader research on the emergence of sociality using solitary, primitively social and highly social species of bees.

The reproductive ground plan hypothesis was proposed by Amdam and Page to explain the evolution of division of labor and foraging specialization in honey bees.(18) Like the ovarian ground plan framework proposed by West-Eberhard for social wasps,(65,66) the reproductive ground plan hypothesis links individual behavior and the social structure of honey bee colonies to ovary development and vitellogenin production. The first step was a heterochronous shift in the timing of reproductive hormonal signaling events from the mature adult stage into the late pupal stages.(67) This shift turned on the production of vitellogenin prior to emergence (55) and further caused behavioral traits interlinked with reproductive maturity to be expressed in young adult bees. Vitellogenic females bypassed the phases of dispersal, diapause and aestivation that characterized the ancestral pre-reproductive period. Instead, they expressed maternal reproductive behavior toward siblings.(19)

Many female insects preferentially forage for nectar or protein during different stages of ovary development. Nonreproductive females tend to forage for nectar as a carbohydrate source for self maintenance. When they become reproductively active, they tend to seek protein that is used to make eggs and provision their young.(18) Worker honey bees, which are facultatively sterile and seldom lay eggs, still have functional ovaries that can become activated and lay eggs in the absence of a queen and young larvae.(68) We have presented data above strongly linking ovary development in workers to the onset of foraging and nectar versus pollen foraging. Bees with more ovarioles tend to forage earlier in life and collect pollen, a source of protein. In addition, bees with more ovarioles have ovaries in higher states of activation(18, 36) and have higher levels of vitellogenin mRNA when they emerge as adults. Bees with a

vitellogenin knockdown phenotype show foraging preferences similar to bees with reduced numbers of ovarioles.(16) Insects with pre-vitellogenic ovaries need to forage for protein in order to produce eggs, unless they have storage proteins carried over from the pupal stage. Though data are sparse for primitively social bees, in at least some cases ovary state and behavior have been shown to be closely linked.(64,69) Therefore, it is reasonable to hypothesize that the evolutionarily ancient relationships of ovary development and behavior have been maintained and used, with some remodeling, by honey bees to socially structure colonies with respect to division of labor and foraging specialization.

The evolution of development of reduced ovaries in worker honey bees

Honey bees have distinct worker and queen castes. The queen caste probably evolved in response to selection favoring longer-lived individuals with higher reproductive capacity. (70) Divergent phenotypes such as these are believed to be derived from originally phenotypically plastic traits using pre-existing physiological and endocrine developmental systems.(71) The main characters distinguishing a honey bee queen from a worker are her larger size and a distended abdomen that accommodates her large ovaries normally containing about 325 ovarioles (Laidlaw,(72) p. 4). Workers normally have fewer than 10 ovarioles, but can normally range up to about 20.(2,73)

Queen and worker differentiation is controlled nutritionally (Winston,(1) pp. 66–68). Royal jelly is fed to both queen and worker larvae initially, but prior to the 4th day of feeding the sugar and protein composition changes and queens receive more food. Significant variation in ovariole number occurs when workers are fed in the laboratory on diets that vary in sugar and protein content, and when fed varying quantities of food, revealing phenotypic plasticity not normally observed when bees are reared in the hive under natural conditions. There is a direct relationship in honey bees between quantity of food consumed by larvae, adult size and number of ovarioles (Kaftanoglu and Page, unpublished data). In one study, larvae allowed *ad lib* access to food in the laboratory weighed on average 115 mg and had 11 ovarioles when they "emerged" as adults compared with 95 mg and 8 ovarioles for bees reared on a quantitatively restricted diet.

Significant genetic variation for ovary development also exists between and within populations of honey bees. Africanized honey bees (AHB) sampled from colonies in a population in Mesa, Arizona, USA have on average 8.3 ovarioles (summing over both ovaries) while European honey bees (EHB) derived from colonies of various US commercial sources maintained in Mesa, AZ have 6.0. These differences are statistically significant (Kaftanoglu and Page, unpublished data). Queens and drones were raised from the AHB colony with the most ovarioles and the EHB colony with the fewest. Crossing the AHB drones and queens resulted in some colonies with inbred workers with significantly more ovarioles than either parental colony. The same was observed with AHB backcrosses of hybrid queens, revealing what appears to be a rare recessive allele for ovariole number (Fig. 4). EHB inbred crosses and backcrosses did not demonstrate this extreme ovary phenotype.

According to Michener(64) (pp. 10–11), the ancestral number of ovarioles is four per ovary for the Apidae. An apparent increase in ovarioles took place in the lineage giving rise to honey bees. The current worker phenotype with reduced ovaries probably evolved from a phenotype where individuals had more ovarioles. Specifically, a restriction of JH titre that triggers programmed cell death (apoptosis) of ovarian tissue has been added to the larval development program of workers.(74,75) Capella and Hartfelder(75) showed that ovariole number is determined in both queens and workers during the 5th (last) larval instar. Prior to the 5th instar, germ cell clusters form bipotential primordia. During the 5th instar, queens and workers diverge in development with cell proliferation continuing in queens leading to developed ovarioles and

apoptosis occurring in most ovarioles of worker larvae. The result is queens with many ovarioles and workers with few. Ovarioles can be "rescued" from apoptosis by application of JH during the early 5th instar. Queens have naturally higher tires of JH at this time than do the worker larvae.(74)

The primitively social progenitor of the queen honey bee was functionally more like the worker today than the queen, as is seen in the primitively social bees.(64) Unlike the queen today, she needed to construct and defend a nest and forage for resources to rear her young. Primitively eusocial queens are usually produced seasonally and often differ from workers in size and reproductive potential, a direct consequence of food availability, as seen in the bumble bees. (64,76) They establish nests on their own and must perform all of the tasks associated with nest construction, defense and foraging for pollen and nectar. In the honey bee, an increase in ovariole number probably evolved prior to the evolution of the anatomical queen phenotype in response to selection favoring an increase in egg-laying capacity while maintaining flight mobility. Honey bee queens can lay more than 2,000 eggs per day, more than 1 per minute (Michener,(64) p. 58). But, they must be able to reduce the size of their abdomen quickly in order to go with reproductive swarms or to abscond. Having more, short ovarioles may allow them to "dump" and resorb eggs more quickly and be able to fly. Queens of the divergently specialized stingless bees have significantly fewer ovarioles (8–15 per ovary(77)) contained in an elongated, greatly expanded abdomen. Unlike honey bee queens, once they begin egg laying, they never again fly.

In the honey bee worker, ovary size is linked to larval nutrition (Kaftanoglu and Page, unpublished; Hoover et al.(78)), ovary activation is linked to ovary size, and reproductive behavior is linked to ovary size and activation.(19,36) Nutrition, ovary size, activation and reproductive behavior are also linked in at least some primitively social bees (Michener,(64) pp. 85–94). In annual colonies, reproductive individuals are usually produced at the end of the season when the worker/brood ratio is highest and larvae receive the greatest amounts of food. With perennial colonies, control of worker nutrition would help control the unseasonal production of queens and to limit worker ovary development, restricting them from reproductive roles. Honey bee larvae are fed progressively. Bees raised in colonies by their worker siblings are much more uniform in size and ovariole number than those raised in the laboratory on less-well-regulated diets and feeding regimes (Kaftanoglu and Page, unpublished data). Larvae on *ad lib* diets are larger in size and have more ovarioles than their sib-fed nestmates, supporting our hypothesis that nurse bees restrict the diets of larvae destined to be workers, thereby influencing their ovary development. *Ad lib* feeding reveals variation in worker phenotypes that has remained hidden by worker social regulation of feeding and is a footprint of the evolution of the extant worker phenotype.

Once honey bees evolved a mechanism that nutritionally regulated the production of distinct reproductive phenotypes, ovarian developmental control could evolve semi-independently in queens and workers. Dominant, caste-specifically expressed alleles resulting in reduced ovaries may have been favored initially, further suppressing worker reproduction. Our crosses of AHB revealed residual recessive alleles for bigger, queen-like ovaries (Fig. 4). Their effects on the worker ovary phenotype are large, resulting in an average number of ovarioles per ovary four times those of individuals with the dominant genotype. Thus the control of ovary development that was under the behavioral control of worker nurse bees was likely assimilated by dominant alleles affecting downstream regulatory networks that interact with the nutritional signals. We were able to generate extreme ovary phenotypes in worker honey bees by varying their nutrition and by constructing genetic crosses, demonstrating that altering both the environment and the genotype results in similar changes of phenotype, an example of gene–environment interchangeability as proposed by West-Eberhard(79) (pp. 116–129). Nutrition during the larval stage, thereby, not only affects queen versus worker developmental fate, it

also affects the number of ovarioles in workers. These associations point to nutritional controls downstream of the major caste-determining switch—controls that first were regulated behaviorally by nurse bees, and subsequently assimilated genetically into the developmental program of workers.

Conclusion

Behavioral, genetic and physiological studies of selected strains of honey bees spanning more than 20 years have revealed a complex architecture of phenotypic, genetic, anatomical and physiological traits independently confirmed in wild-type bees (Fig. 5). This architecture demonstrates that the social behavior of honey bees is derived from ancient developmental programs involved in reproductive anatomy, maturation and behavior. Neither novel genes nor novel functions of genes were necessary to achieve eusociality with a division of labor and foraging specialists. The initial step of eusociality, females staying at the maternal nest and rearing their siblings, could have been achieved by a shift in the timing of common endocrine signals involved in reproductive maturation. Along with reproductive maturation comes vitellogenin production with vitellogenin titer acting in concert with juvenile hormone to serve as a pacesetter for behavioral development and the onset of foraging. We propose that worker phenotypes were "enforced" initially by larval dietary restriction then genetically assimilated by allelic substitutions at genes involved in translating the nutritional signals into ovary developmental programs. The emergence of bifurcating developmental pathways that produced "novel" worker and queen phenotypes via nutritional control enabled evolution of genetically reinforced specializations forming a new social structure. This scenario likely exemplifies a fundamental process through which developmental change leads to evolutionary change.[79] Consequently, foraging specialization is derived directly from the ubiquitous and ancient associations of behavior with the reproductive states of insects.

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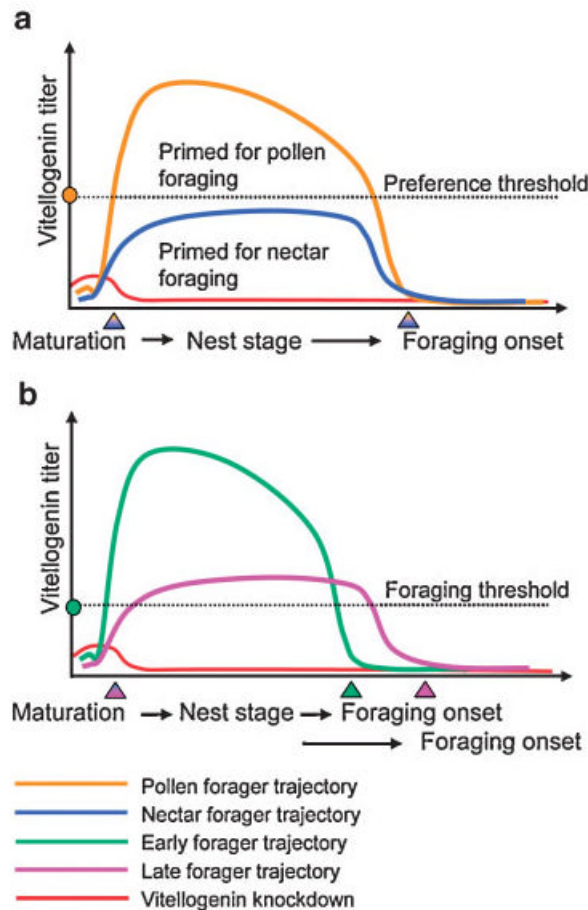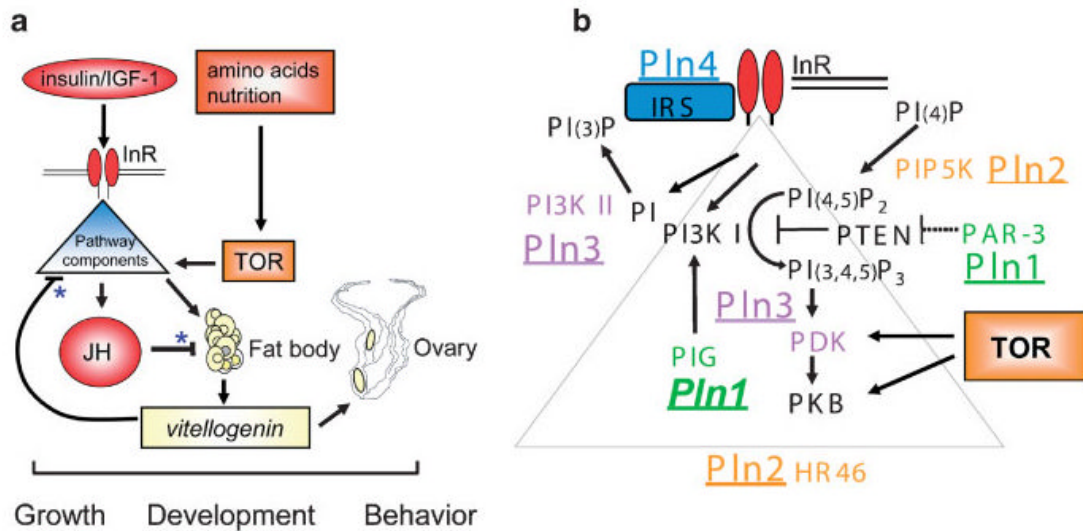
Figure 1.
The ancestral yolk protein vitellogenin has a dual role in regulation of honey bee social behavior.(16) After a maturation phase of about 4 days when young bees are unable to forage, the hemolymph vitellogenin level acts as a primer of foraging preference. **a:** Bees with vitellogenin titers over a Preference threshold will primarily collect pollen later in life. Bees with vitellogenin levels under this same threshold are primed to collect nectar as foragers. In addition, vitellogenin is a suppressor of the transition from nest tasks to foraging activity. **b:** Bees that early in life experience a drop in the vitellogenin level below the Foraging threshold, also will initiate foraging at younger ages. In sum, this model (**a,b**) explains the behavior of workers with a *vitellogenin* gene knockdown phenotype, which forage precociously and preferentially collect nectar.(16) It also explains the behavior of selected pollen-hoarding strains. High-strain bees have high levels of vitellogenin soon after emergence but titers drop rapidly early in life. Low-strain bees, conversely, have lower vitellogenin levels that stay constant for a longer time (Amdam and Hartfelder, unpublished data). Accordingly, high-strain bees forage early and primarily collect pollen, while low-strain bees forage late and preferentially collect nectar.

Proboscis Extension Reflex

Tactile Learning
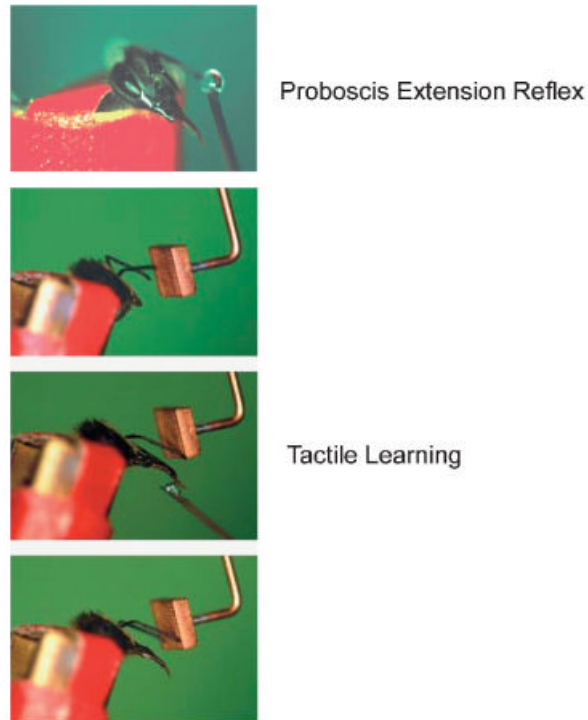
Figure 2.
The top panel demonstrates the proboscis extension reflex of a restrained worker honey bee.
A droplet of sucrose is touched to the antenna eliciting the extension of the proboscis. The
bottom three panels demonstrate conditioning a worker honey bee to a tactile stimulus. The
bee's eyes are occluded with paint. A droplet of sucrose was applied to the antenna eliciting
the extension of the proboscis. A droplet of sucrose was presented to the tip of the proboscis
as a reward. After conditioning, the bee responds to the tactile stimulus by extending the
proboscis (photos by J. Erber).

Figure 3.
Variation the insulin/insulin-like (IIS) pathway, including cross talk with target of rapamycin (TOR) signaling, is a possible explanation for the different life history syndromes of high and low pollen-hoarding strain bees. **a:** In general, insulin-like peptides (insulin/IGF-1) bind to the insulin receptor (InR). The resulting signal is transmitted via components of second messenger pathways, and effects on fat body synthesis of yolk peptides/protein (vitellogenin), ovarian maturation state, organismal growth, development, and behavior emerge via pleiotropic cascades that involve systemic hormones (e.g. juvenile hormone, JH). Note that the feedback relationship(10) between vitellogenin and JH (blue asterisks) in honey bees is uncommon in insects,(12) and may emerge via vitellogenin-mediated regulation of IIS(63) Signal transmission can be conditional on nutrients (amino acids) and/or energy status, through cross talk with the nutrient sensing TOR pathway. **b:** Explicitly, the genomic regions associated with the pollen-hoarding behavioral and physiological syndrome (QTL *Pln1–4*) are characterized by a density of IIS/TOR pathway components that is much higher than expected by chance alone (see Hunt et al.63). Abbreviations: PI, phosphoinositol; PIP, phosphoinositol phosphate; IRS, insulin receptor substrate; PI3K, phosphoinositide-3 kinase (class I or II); PIP5K, 1-phosphatydylinositol-4-phosphate 5-kinase; PIG-P, phosphatidyl-inositolglycan-peptide; PDK1, 3-phosphoinositide-dependent kinase 1; PKB, protein kinase B; HR46, honeybee ortholog of Dmel/HR46; PTEN, phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase.
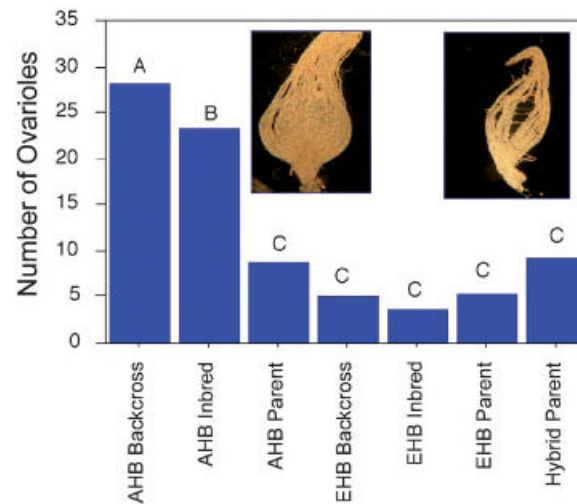
Figure 4.
Queens were raised from the AHB parent and mated to drones from the AHB parent to produce four AHB inbred colonies. EHB parent queens were raised and mated to EHB parent drones to produce four EHB inbred colonies. An EHB queen was raised from the EHB parent colony and mated to a drone from the AHB parent colony to produce the hybrid parent colony. Queens were raised from the hybrid parent colony and mated to drones from the AHB parent to produce seven AHB backcross colonies and the EHB parent to produce 5 EHB backcross colonies. 25 workers were sampled from each colony, dissected and ovarioles counted. Data from different colonies within genetic crosses were pooled for analyses. Bars with different letters are statistically different at P < 0.0001, except for A and B P = 0.0125.
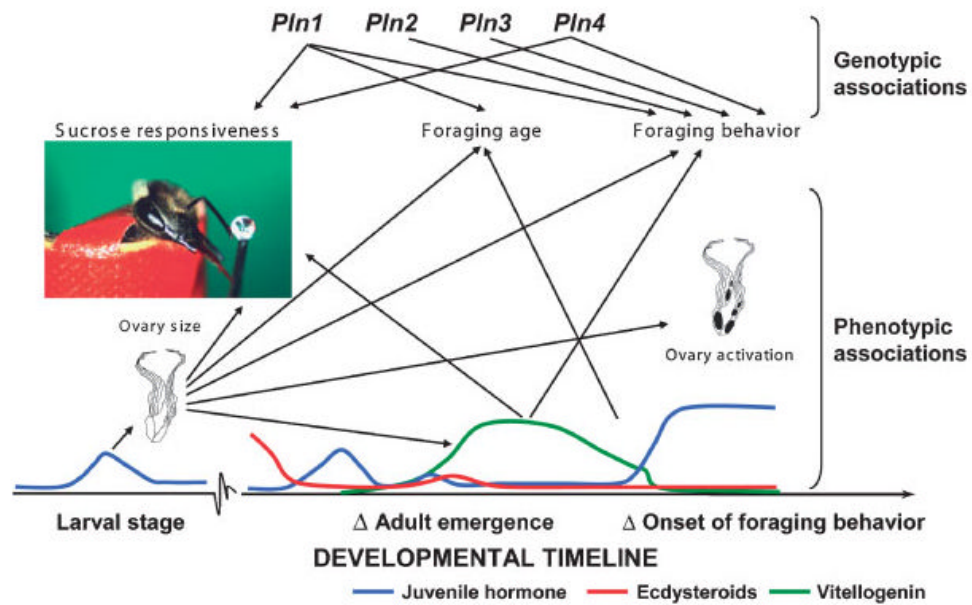
Figure 5.
Two-way selection for pollen hoarding revealed a complex phenotypic, hormonal and genetic architecture affecting division of labor and foraging specialization in honey bees. Four pollen-hoarding QTLs were mapped that affected sensory response systems, behavioral development (age of foraging onset) and foraging behavior. High- and low-strain bees differed also in the size of their ovaries, and titres of vitellogenin and juvenile hormone soon after emerging as adults. Sucrose responsiveness correlates with vitellogenin titres and ovary size, while ovary size correlates with foraging onset, and foraging behavior, etc.