

Ernst Mayr Lecture

26. Oktober 2009



Introduction

PAUL SCHMID-HEMPEL

Meine Damen und Herren:

Where, would you think, could you read something like this:

“... [modern] scientific equipment cannot by itself safeguard from errors of thought [by] scientists brought up in capitalist civilizations. Their psychology is limited by class and social prejudices. This law manifests itself... in the tendency of many bourgeois biologists to attribute to objects and phenomena of living and non-living nature qualities, which in reality they do not possess and which ... are but a transfigured reflection of the relations of production in bourgeois society ...”¹ (p.13),

“...the unscientific and warped interpretations of the life of the bee colony by different authors... are attempts... to convince the readers that the existing social system of exploitation is in conformity with nature...” (p.14),

only to conclude:

“... Today, in the epoch of the decline of capitalism when everybody can see that bourgeoisie has reduced society to a state in which no other ties are left between men than those of heartless gain, traits diametrically opposed to those observed by [Bernard] Mandeville² are being discovered in bees. Now bees are described as possessing a happy gift of suppressing individual eccentricity for the common good...” (p.19)

- 1 Khalifman, Iosif Aronovitch (1953): *Bees*. Moscow: Molodaya Gvardia Publishing House; published in English by Foreign Language Publishing House, Moscow.
- 2 Bernard Mandeville (1670–1733) suggesting that bee colonies work because every bee pursues her selfish interest. It is the drive for personal pleasure that keeps the economy going (*Fable of the Bees*, 1724). Named after him, the Mandeville-paradox of economics says that individual benefits must not necessarily be identical with the global (societal) benefits.

Well, the small booklet where these cites are taken from is a guide to honeybee keeping by Iosif Khalifman, published in 1953 and winner of the Stalin Prize 1951. The booklet is still cited every now and then in contemporary beekeeping texts.

It is therefore not always the lion or the eagle – icons of strength and superiority – that are used for a political message. Also, social insects have not only fascinated people for a long time but have been shoehorned for political reasons. This is especially true for the honeybees, the study subject of our speaker, Prof. Robert Page from Arizona State University at Tempe. The desire for a better world has made bees an icon for hard-working individuals that are industrious and sacrifice themselves for the welfare of their society. Never mind that we had shown a number of years ago that honeybees do not work as hard as they could. But how could such a seemingly harmonious animal society not be a role model for human societies whose members forever seem to be entangled in conflict over wealth, fame and influence, and where rivalling societies lead wars against each other?

Herbert Spencer (1820–1903), known for his forays into “Social Darwinism”, actually not only coined the term “survival of the fittest” but also wrote about “super-organic evolution”³ to mean an entity emerging from the ensemble of individuals. But it was the eminent American entomologist, William Morton Wheeler (1865–1937) who applied the term “superorganism” to ants⁴, and so captured the idea of a tightly knit society much better than any before him. In a superorganism the single individuals are thought to be what the single cells are to a “normal” organism, a body. There are clear analogies here. For example, in a social insect colony only some individuals (the queen) reproduce. Similarly, only some cells of the body do reproduce, that is, those of the germ line. This division of the reproductive labour that can be observed in social insects also struck Charles Darwin. In his *Origin of Species* he mentions the neuters (i.e. the sterile workers) of honeybees as a “... special difficulty, which ... appeared ... fatal to the whole theory [of evolution]...”⁵. Clearly minded as he was, he immediately suggested that this difficulty might disappear by remembering that “... selection may be applied to the family, as well as to the individual...”

We now see these problems with much more clarity than in Darwin’s or Wheeler’s days. Hamilton’s theory of kin selection and inclusive fitness gave us a powerful tool for study, showing that co-operation evolves more readily in societies of related individuals. But the “superorganism” has again become the focus of an ongoing dispute

3 Principles of Sociology (1876).

4 Wheeler, W.M. (1911): The ant colony as an organism. In: *J Morphol* 22(2): 307–325.

5 Darwin, C. (1859): On the origin of species.

over what actually is the unit of selection. Is it really the society, more in the spirit of Wheeler and his forerunners – or is it the individual, and how do these things relate to one another? A debate that has also roots in the rise of sociobiology championed by E.O. Wilson in the mid-1970s. As you can see from these remarks, the study of honeybees, and of social insects more generally, show us many interesting questions that are not only fascinating by themselves but also cut deep into our understanding of evolution by natural selection and of the mechanisms that might be responsible for social cooperation. This rich domain of interesting questions is exactly what has fascinated Robert Page for some time.

Robert Page was born in California, a state that is not only famous for its high tech industries but also has an important agricultural sector, especially fruit production. And there we are again back to pollination and bees! For his career, Robert Page studied in California and earned his PhD at UC Davis in 1980. After years of postdoctoral work, he became an Assistant Professor in the Dept. of Entomology at Ohio State University, Columbus (1986–1989). In 1989, he returned to UC Davis as Associate and then Full Professor of Entomology. He chaired the Department of Entomology, where he had graduated earlier, from 1999 to 2004. He also was an external faculty member at the Santa Fe Institute from 2001 to 2005. In 2004, however, Robert Page made an important move and became the Founding Director and Professor of Life Sciences at the newly started School of Life Sciences at Arizona State University in Tempe, where he still is. In his time, many new faculties were hired, the reputation grew, and the School is now a major address for the study of social insects. Currently, he takes a leave from his full duties and is a Fellow of the Wissenschaftskolleg zu Berlin 2009/2010, where he acts as a convener (together with Manfred Laubichler) for a focus group, which studies the evolutionary developmental biology of social insects.

Robert Page has contributed substantially to the understanding of the evolutionary biology of social insects. He employed molecular markers and provided the first quantitative demonstration of low genetic relatedness in a highly eusocial species, an observation contrary to what had been expected from simple applications of kin selection theory. He proposed and experimentally validated a hypothesis for the evolution of polyandry based on genetic sex determination. Furthermore, Robert Page pioneered genetic mapping in social insects by mapping quantitative trait loci for many behavioural and physiological traits. He and his colleagues also isolated and validated the single complementary sex determination (*csd*) gene of the honeybee. Robert Page's more recent research has started where others did not dare to go – this is, with a large and still ongoing selection experiment with honeybees to understand the long-standing problems posed already by Darwin. His recent research has revealed links between reproductive physiology, behaviour and the underlying genes, thus casting new light on the evolution of division of labour and social evolution in honeybees.

Robert Page's work is laid down in some 200 publications. He has been the holder of many grants and has been honoured by a Humboldt Senior Scientist Award in 1995, from where he knows Berlin very well from many stays in this town. I am especially pleased to say that Robert Page has had a number of brilliant students, post-docs and academics in his lab and this is a legacy that no doubt carries over to the next generation.

The 'Spirit of the Hive' and How a Superorganism Evolves

ROBERT E. PAGE, JR.

Darwin's Dilemma

Like every evolutionary biologist alive today, I was influenced by Ernst Mayr because he was at the center of evolutionary biology for the entire second half of the 20th Century. I was fortunate to meet him once when we both spent a few days at the Archbold Biological Station in Lake Placid Florida. I was a new Ph.D. and he was ... well, Ernst Mayr. I felt very privileged then, and especially tonight, giving this lecture. I am also privileged to work on social insects. Social insects have fascinated natural historians and philosophers since Aristotle and continue to fascinate us today with their self-sacrificing altruism, complex nest architecture, untiring industry, and division of labor. However, they presented Charles Darwin with special difficulties for his fledgling theory of evolution by natural selection. How can sterile castes, such as worker honey bees and ants, evolve when they don't normally reproduce? The existence of sterile castes seems to be in direct opposition to a theory that requires differential survival and reproductive success. However, Darwin considered a bigger difficulty to be the observation that the reproductive individuals in colonies are often anatomically differentiated from the sterile workers, showing adaptation of a sterile caste. However, he considered the biggest difficulty to be the anatomical differentiation within the worker caste that is dramatically demonstrated in many species of ants. Darwin waved his arms and invoked selection on families as an explanation, an explanation later shown by W.D. Hamilton, Robert Trivers (last year's Ernst Mayr Lecturer), and others, to be not quite that simple.

But social insects provided additional difficulties for Darwin when he considered the architecture of the honey bee nest (Fig. 1). Darwin had a Cambridge mathematician study the comb of the bee from an engineering perspective of strength and economy and concluded "for the comb of the hive bee, as far as we can see, is absolutely perfect in economizing labour and wax." How could the wax combs be built with such precision to maximize the strength of the comb and at the same time save costly building materials? And, as he pointed out, "this is effected by a crowd of bees



Figure 1
A colony of honey bees engaged in the construction of wax comb. (Photo by Jacob Sahertian)

working in a dark hive”¹. How could they achieve this architectural feat with instincts alone, working without any central control of construction tasks? Darwin experimented with honey bees and demonstrated to his satisfaction that bees could construct combs using just their instincts and local information regarding cell construction, thereby, solving his dilemma of perfection and instincts.

The Nobel Laureate poet, playwright, and author, Maurice Maeterlinck also was fascinated by social insects. In his wonderfully-romantic book *The Life of the Bee*, first published in 1901, he noted that there was no central control of cooperative behavior, thought by many to be the domain of the queen, and noted “She is not the queen in the sense in which men use the word. She issues no orders; she obeys, as meekly as the humblest of her subjects, the masked power, sovereignly wise, that for the present,

1 Darwin, C. (1998): *The Origin of Species by Means of Natural selection or the Preservation of Favored Races in the Struggle for Life*. New York: The Modern Library, p.339.

and till we attempt to locate it, we will term the 'spirit of the hive.'² Here he resorted to a mystical vitalism to explain how colonies full of individuals working in the dark organize into a cooperative whole, and left it for later for someone to identify the "spirit of the hive" and where it resides.

Insect Colonies are Superorganisms

William Morton Wheeler, the early 20th century entomologist and philosopher rejected the vitalism of Maeterlinck but also the strict interpretation of Darwin as an explanation for the existence of social insects, "... the 'struggle for existence', 'survival of the fittest'. 'Nature red in tooth and claw,' ... depicts not more than half the whole truth".³ He believed that Darwinian selection based on competition for survival and reproduction could not build the kind of cooperation he observed in colonies of his beloved ants. In 1911 he wrote an essay "The Ant Colony as an Organism".⁴ He defined an organism as a "complex, definitely coordinated 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". In other words, they are organized for nutrition, reproduction, and defense. He later proposed (1928) the term "superorganism"⁵ in an apparent attempt to set aside social insect evolution from, or expand on, the individual-based Darwinian struggle for existence. However, he did not provide an alternative to natural selection nor a mechanism for the evolution of the superorganism.

The 50 years following Wheeler saw the superorganism used primarily as a metaphor on which to hang analogies. By the early 1960's the superorganism concept was completely irrelevant because, according to Wilson⁶ "The concept offers no techniques, measurements, or even definitions by which the intricate phenomena in genetics, behavior, and physiology can be unraveled. ... Add to this the continuing quest

2 Maeterlinck, M. (1913): *The Life of the Bee*. New York: Dodd, Mead, and Company, pp.38–39.

3 Wheeler, W. M. (1926): *Social Life Among the Insects*. London: Constable and Company Limited, see p. 3.

4 Wheeler, W. M. (1911): The ant colony as an organism. In: *Journal of Morphology* 22: 307–325, see p. 308.

5 Wheeler, W. M. (1928): *The Social Insects*. New York: Harcourt, Brace and Company.

6 Wilson, E. O. (1971): *The Insect Societies*. Cambridge: Harvard University Press, pp. 318–319.

for precise evolutionary, that is, genetic, explanations of the origin of sociality and variations among the species in details of social structure, and one has the exciting modern substitute for the superorganism concept.” However, the superorganism experienced a brief reprieve in the 1980’s with papers by Lumsden,⁷ E.O. Wilson,⁸ Seeley,⁹ and D.S. Wilson and Sober,¹⁰ reviving it as a model for social development (sociogenesis), social homeostasis, and multi-level selection. The conceptual problems of Darwinian selection encountered by Darwin himself and William Morton Wheeler were resolved by the new theory of the superorganism as an entity shaped by group selection acting on colonies and shaping colony-level organizational structures. But, again, the superorganism concept lacked traction, lost interest, and was used by only a few diehard researchers as a model.¹¹

In 2008, the superorganism was re-revived with publication of *The Superorganism: the Beauty, Elegance and Strangeness of Insect Societies* by Hölldobler and Wilson.¹² Like Wheeler, they present the superorganism as a true organism, not a heuristic metaphor. They define a superorganism as “a society, ... that possesses features of organization analogous to the physiological properties of single organisms,” and weave a string of natural history analogies in support of how insect societies evolve and behave like organisms. They present their view of how multi-level selection can shape social structure and address the three major themes presented by Darwin, Maeterlinck, and Wheeler: 1) the evolution of sterility, 2) the evolution of insect castes, and 3) how colonies composed of large numbers of individuals organize themselves into a cooperative social unit without central control (the “spirit of the hive”). From their book it is apparent that in the 150 years since publication of the *Origin of Species* we have solved part of the problem of the evolution of sterility, though the debate continues, we have worked out some of the developmental mechanisms of caste determination, and we

- 7 Lumsden, C. J. (1982): The social regulation of physical caste: the superorganism revived. In: *Journal of Theoretical Biology* 95: 749–781.
- 8 Wilson, E. O. (1985): The principles of caste evolution. In: B. Hölldobler and M. Lindauer (eds.), *Experimental Behavioral Ecology*. Cambridge: Harvard University Press.
- 9 Seeley, T. D. (1989): The honey bee as a superorganism. In: *American Scientist* 77: 546–553.
- 10 Wilson, D. S. and E. Sober (1989): Reviving the superorganism. In: *Journal of Theoretical Biology* 136: 337–356.
- 11 Page, R. E. and S. D. Mitchell (1993): The superorganism: a new perspective or a tired metaphor? In: *Trends in Ecology and Evolution* 8: 265–266.
- 12 Hölldobler, B. and E. O. Wilson (2008): *The Superorganism: the Beauty, Elegance and Strangeness of Insect Societies*. New York: W. W. Norton.



Figure 2
A natural swarm of honey bees. (UC Davis)

have a better understanding of the ways in which colonies self-organize into social units with a division of labor. But, we still know little about how selection on social traits of colonies effects heritable changes that are reflected through different levels of organization such as development, physiology, and behavior of workers, thereby, resulting in changes in superorganism phenotypes – the Darwinian explanation.

How does a superorganism evolve a complex social organization? There is no centralized control of behavior, no social genome on which natural selection can act, and a hierarchy of organizational levels from genes to the society. In the following sections I am going to provide a brief overview of the biology and natural history of honey bees, define the mechanisms behind the mystical “spirit of the hive” of Maeterlinck, and then discuss a 20 year selection program designed to map the effects of colony level selection on a single social trait, a characteristic of the superorganism, across different levels of biological organization.



Figure 3
A natural honey bee nest with exposed combs. (Photo by Kim Fondrk)

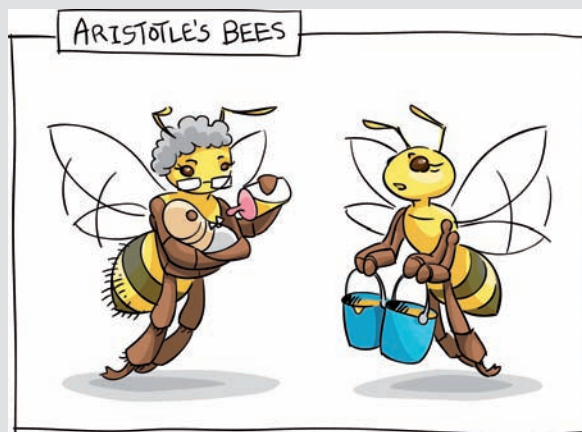
Natural History

A honey bee colony typically consists of 10–40 thousand worker bees who are all female, and depending on the time of year, zero to several hundred males (drones), and a single queen – the mother of the colony (Fig. 2).¹³ The nest is usually constructed within a dark cavity and is composed of vertically oriented, parallel combs made of wax secreted by the workers (Fig. 3). Each comb can contain thousands of individual hexagonal cells on each of the vertical surfaces. The individual cells of the combs serve as vessels for the storage of honey (the carbohydrate food source for bees), pollen (the source of protein), and as individual nurseries for developing eggs, larvae, and pupae. In addition, the comb serves as the social substrate for the colony. The nest has

13 Winston, M. L. (1987): *The Biology of the Honey Bee*. Cambridge: Harvard University Press. An excellent review of honey bee natural history.

an organizational structure that is similar to concentric hemispheres, only expressed in vertical planes, where the innermost hemisphere contains the larvae and pupae (the brood), the next hemisphere above and to the sides of the brood contains the stored pollen, and the upper and outer regions contain honey that is derived from the nectar of flowers. If you remove a comb that is near, but to the side of, the center of the nest it will contain three bands covering both sides: the outer band will be honey, the center band pollen, and the lower central part of the comb will contain the brood. The amount of surplus pollen is regulated by colonies, first shown by Jennifer Fewell and Mark Winston.¹⁴ They added pollen to colonies and then looked at the effects on pollen foraging and pollen intake. Colonies reduced the intake of pollen until they consumed the “surplus” pollen. When pollen was removed from colonies, pollen intake increased until the pollen was restored.

In addition to the social and nest structures, there is also a structured division of labor. Aristotle pointed this out more than 2,300 years ago (see Box 1). As bees age,



Box 1

A cartoon of Aristotle's hypothesis of the effect of age on division of labor in honey bee colonies. He thought that bees became pubescent as they aged, as in humans, therefore, when he observed that foragers were shiny and had less hair than the bees working inside the nest, he concluded that the foragers must be younger, as depicted here. This was incorrect in fact, but correct in concept. There is an age-related division of labor where the older bees forage. Older bees have less hair because the hairs break off and are not replaced. They are “born” with all the hair they will ever have. (Cartoon by Sabine DeViche. Photos by Osman Kaftanoglu)

- 14 Fewell, J. H. and M. L. Winston (1987): Colony state and regulation of pollen foraging in the honey bee, *Apis mellifera* L. In: *Behavioral Ecology and Sociobiology* 30: 387–393.

they progress through changes in their location in the hive and the behavioral tasks they perform. When they first emerge from their cells as adults they engage in cleaning cells in the brood nest. When they are about a week old they feed and care for larvae, followed by tasks associated with nest construction and maintenance, food processing, receiving nectar from foragers, guarding the entrance, etc. Then in about their third or fourth week of life they initiate foraging. As foragers they tend to specialize on collecting pollen or nectar, demonstrated by a bias in the amount of each they return to the nest. Once they initiate foraging they seldom perform any within-nest tasks for the duration of their short lives of 5–6 weeks.

How does a Superorganism get Organized?

One cannot observe a hive of honey bees without getting the feeling that they are engaged in highly coordinated and cooperative behavior. As discussed above, both Darwin and Maeterlinck struggled with how this can occur. It seems as if there must be some kind of central control, but on careful examination none can be found. This led Maeterlinck to call upon the “spirit of the hive”. But what is it? I will show you here that the coordinated behavior long observed and admired emerges from simple algorithms of self organization and requires only that worker honey bees respond to stimuli that they encounter; when they respond they change the amount of stimulus at that location and thereby affect the behavioral probabilities of their nestmates.

Pollen foraging stimuli

Stored pollen inhibits foragers from collecting pollen while young larvae stimulate pollen foraging. Young larvae produce a mixture of chemicals, called brood pheromone, that is secreted onto the surface of their bodies. It is the brood pheromone that releases pollen foraging behavior.¹⁵ Pollen foragers returning from a foraging trip seek out combs with brood and pollen and walk along the margin where they have the opportunity to have contact with the pheromone produced by larvae, and contact stored pollen.¹⁶ Stored pollen that is located in the comb is consumed by nurse bees, so it is likely that returning foragers can assess the need for pollen by contacting emp-

15 Pankiw, T., R. E. Page, and M. Kim Fondrk (1998): Brood pheromone stimulates pollen foraging in honey bees (*Apis mellifera*). In: Behavioral Ecology and Sociobiology 44: 193–198.

16 Dreller, C. and D. R. Tarpy (2000): Perception of the pollen need by foragers in a honeybee colony. In: Animal Behavior 59: 91–96.

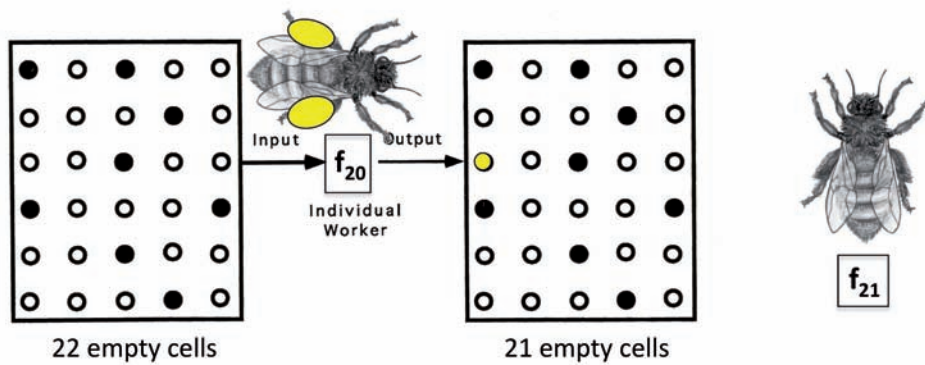
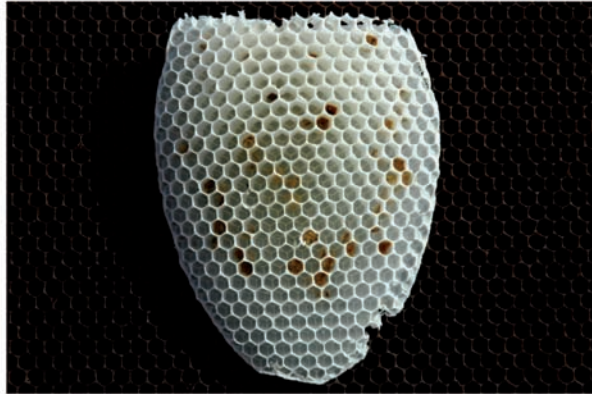


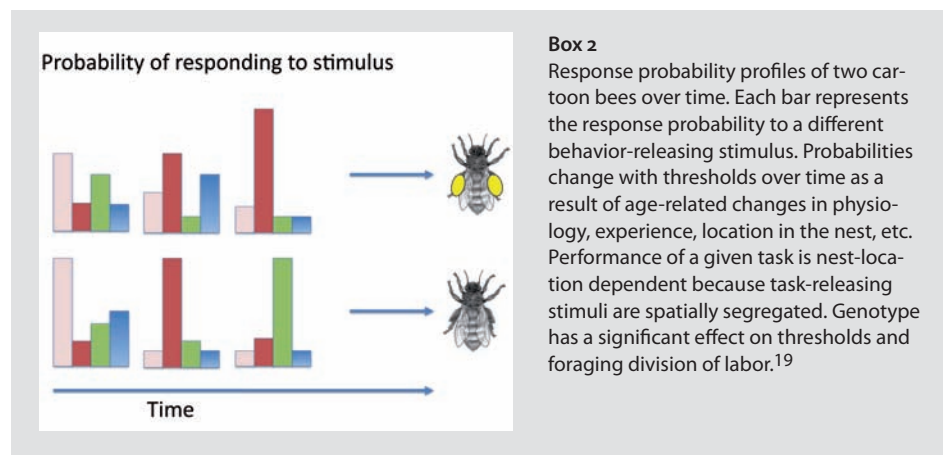
Figure 4
Cartoon showing the response relationship between the stimulus level (empty cells) and behavior, and the correlation between behavior and the stimulus.

ty cells along this margin. Empty cells would indicate that pollen had been consumed and fed to developing larvae. Behavior of a pollen forager is affected by direct contact with the brood and stored pollen. They apparently use information obtained from direct contact to assess “colony need”.¹⁷

¹⁷ Dreller, C, R. E. Page, and M. K. Fondrk (1999): Regulation of pollen foraging in honeybee colonies: effects of young brood, stored pollen, and empty space. In: *Behavioral Ecology and Sociobiology* 45: 227–233.

Self-Organized interactions

Imagine that pollen foragers have response thresholds to empty cells encountered along the brood/pollen boundary. If a forager encounters more empty cells than some value representative of her response threshold, she will leave the hive and collect another load of pollen. If, however, she encounters fewer empty cells, she does not continue to forage for pollen, perhaps she is recruited to nectar or water foraging. This is a very simple view, but not unsupported. Tom Seeley¹⁹ reported unpublished results of Scott Camazine that showed that the number of cells inspected before unloading increased with more stored pollen. In addition, the probability that a pollen forager performed a recruitment dance decreased with more stored pollen, showing that pollen foragers are able to make local pollen stores assessments. Figure 4 shows a cartoon of a returning pollen forager assessing empty cells on a comb. She has a response threshold of 20 empty cells. If she encounters more than 20 empty cells, she will unload and make another foraging trip. If she encounters fewer, she will stop foraging for pollen. The other individual has a threshold of 21 cells. The pollen forager unloads her pollen then makes another trip. By unloading her pollen, she changes the pollen stores stimulus from 22 empty cells to 21, which now is below the pollen foraging response threshold of the other individual. Thus by responding to the stimulus, the number of empty cells, the forager decreases the stimulus by depositing pollen, and



- 18 See for a review Page, R. E. and J. Erber (2002): Levels of behavioral organization and the evolution of division of labor. In: *Naturwissenschaften* 89: 91–106.
- 19 Seeley, T. D. (1995): *The Wisdom of the Hive*. Cambridge: Harvard University Press. This book also provides a description of social regulation of nectar foraging behavior.

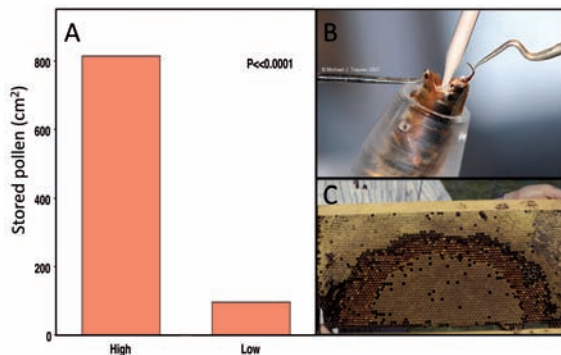


Figure 5
The left panel (A) shows the results of 11 generations of selection for stored pollen. High strain colonies stored on average about 10X more pollen than low strain colonies. Panel B shows the instrumental insemination of a queen honey bee. Panel C is the phenotype that was measured. Note the central band of pollen lying between the capped pupae and the honey.

affects the probability that other individuals will engage in that task. Though this is an over-simplified example, and may not be correct with respect to the specific mechanisms of assessment of the stimuli, this example demonstrates the fundamental basis of self-organized division of labor in the nest.

How Does Complex Social Behavior Evolve?

Selection for pollen hoarding

Response to a stimulus and the correlated change in the stimulus as a result of the behavioral response is the fundamental mechanism of social organization and, therefore, the “spirit of the hive” of Maeterlinck (Box 2). But how does complex social behavior evolve? To answer this question, in 1999 Kim Fondrk and I initiated a large scale breeding program. We selected for a single trait, the amount of surplus pollen stored in the comb, also known as pollen hoarding. Stored pollen is regulated by colonies of bees, therefore, it makes a good social phenotype for a study of colony-level selection. It is the consequence of the activities of thousands of individual workers. Nurse bees consume the protein rich pollen and convert it into glandular secretions that are fed to developing larvae. The larvae are the end point consumers. Thousands of workers engage in collecting and storing the pollen, and in recruiting new foragers.

Our selection program was successful in producing two “strains” of honey bees that differed dramatically in the social trait we selected, pollen hoarding.²⁰ Colonies

20 Page, R. E. and M. K. Fondrk (1995): The effects of colony-level selection on the social organization of honey bee (*Apis mellifera* L.) colonies: colony-level components of pollen hoarding. In: Behavioral Ecology and Sociobiology 36: 135–144.

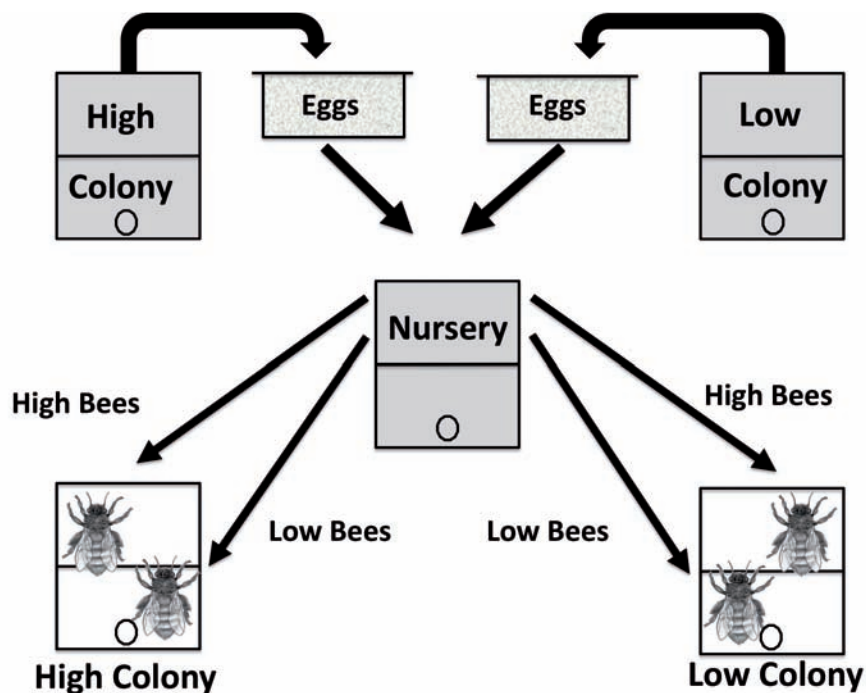


Figure 6

Common garden experiment. Eggs in combs are taken from high and low strain queens and placed into a common nursery colony where they hatch and are cared for by the resident worker bees. About 6 days after hatching, the larvae become pupae and the cells are capped by the bees. Prior to the pupae turning into adults (about 18 days after hatching), the combs are placed in an incubator where the new adults emerge. The new adults are removed from the combs and placed back into high and low strain colonies.

of the high pollen hoarding strain store on average more than 10 times more pollen than colonies of the low strain. We now have completed more than 30 generations of selection. While we conducted the selection experiment we repeatedly asked the question, “what changes have occurred at different levels of organization?” To address this question, we conducted common garden experiments (Fig. 6) where we placed high and low strain workers into hives soon after they emerged as adults and observed their behavior. High and low strain bees were marked so we could determine their origins. We collected them as they returned from foraging trips and determined the age at which they initiated foraging, the weight of nectar collected, the concentration of the nectar, and the weight of their pollen loads.

The phenotypic architecture of pollen hoarding

High strain bees foraged earlier in life (about 12 days in some experiments), collected relatively more pollen, less nectar, nectar of a lower average concentration of sugar, and were more likely to collect water than were the low strain bees. In addition, high strain bees were less likely to return empty from a foraging trip. We expected colony level selection for pollen hoarding to affect pollen and nectar load sizes, but we didn't expect it to affect foraging age, the concentration of nectar collected, water foraging, or the likelihood of being an unsuccessful forager. I had no explanation until Joachim Erber from the Technical University of Berlin joined the effort. Together we asked if pollen and nectar foragers differed in their responses to sugar solutions of different concentrations. We used the proboscis extension response assay (Box 3) to measure the response thresholds of returning foragers. We found that pollen foragers responded more readily to water and to sucrose solutions of lower concentration than did nectar foragers. This was a surprising result that motivated us to test workers from the high and low pollen hoarding strains.



Box 3

Proboscis extension reflex of the honey bee. A droplet of sugar water is touched to the antenna of the bee. If the sugar concentration is sufficiently high the bee will reflexively extend her proboscis as shown with this bee. Using this technique we determine the sucrose response threshold of a bee by presenting her with a series of increasing concentrations. The concentration at which she first responds is her threshold. (Photo by Joachim Erber)

The sucrose responses of returning foragers could have been the result of their foraging activity. However, it could also have been a result of fundamental differences in pollen and nectar foragers that occur prior to initiating foraging that influence their foraging decisions. High and low strain bees differ in their foraging behavior, as discussed above. We tested the sucrose response of high and low strain workers soon (hours to days) after they emerged from their brood cells as adults. This test was independent of foraging behavioral differences because bees don't normally initiate foraging until after their second week of adult life. We found that very young high-strain bees were significantly more responsive to water and to low concentrations of sugar solution than were low-strain bees. Therefore, sucrose responsiveness could be an indicator of fundamental neurological differences between bees that exist very early in adult life and that affect foraging decisions much later. These differences may

be at least partially responsible for the division of labor and be selectable components of the “spirit of the hive”.

If the sensory response system differences we observe are fundamental and affect foraging behavior, then we should be able to test very young bees and predict their foraging behavior 2 or 3 weeks later. Tanya Pankiw tested this hypothesis by taking very young and newly emerged bees from “wild-type” colonies (commercial colonies not derived from the high or low pollen hoarding strains). She tested them for their response thresholds to sucrose, marked them, put them in a commercial hive, and collected them when they returned from foraging trips weeks later.²¹ The results showed that bees that collected water were, on average, the most responsive to water and low concentrations of sucrose followed by bees that collected only pollen, those that collected both, and then those that collected only nectar. Bees that returned empty were those that were the least responsive to sucrose solutions when tested soon after emerging as adults. In addition, she found a significant negative correlation between the concentration of nectar collected by bees and the responsiveness of bees to sucrose. Bees that were the most sensitive to low concentrations of sucrose collected nectar that was more dilute than those that were less sensitive.

We selected for a single trait, the amount of surplus pollen stored in combs. We looked for differences between our strains at different levels of biological organization such as individual foraging behavior and sensory responses. We also looked at the differences in learning and memory and neurobiochemistry. We compared high and low strain workers and we looked for correlations of these traits in wild-type bees to determine if the relationships were specific to our selected strains or represented general properties of the behavioral organization of honey bees (see for review²²). It is interesting how the response to sucrose correlates with such a broad set of behavioral and physiological traits, thus defining a phenotypic architecture associated with foraging behavior that can be changed by colony level selection on stored pollen (Fig. 7).

- 21 Pankiw, T. and R. E. Page (2000): Response thresholds to sucrose predict foraging division of labor in honeybees. In: *Behavioral Ecology and Sociobiology* 47: 265–267. – Pankiw, T., M. Nelson, R. E. Page, and M. K. Fondrk (2004): The communal crop: modulation of sucrose response thresholds of pre-foraging honey bees with incoming nectar quality. In: *Behavioral Ecology and Sociobiology* 55: 286–292.
- 22 Page and Erber 2002 (Fn. 19). – Page, R. E., R. Scheiner, J. Erber, and G. V. Amdam (2006): The development and evolution of division of labor and foraging specialization in a social insect. In: *Current Topics in Developmental Biology* 74: 251–284.

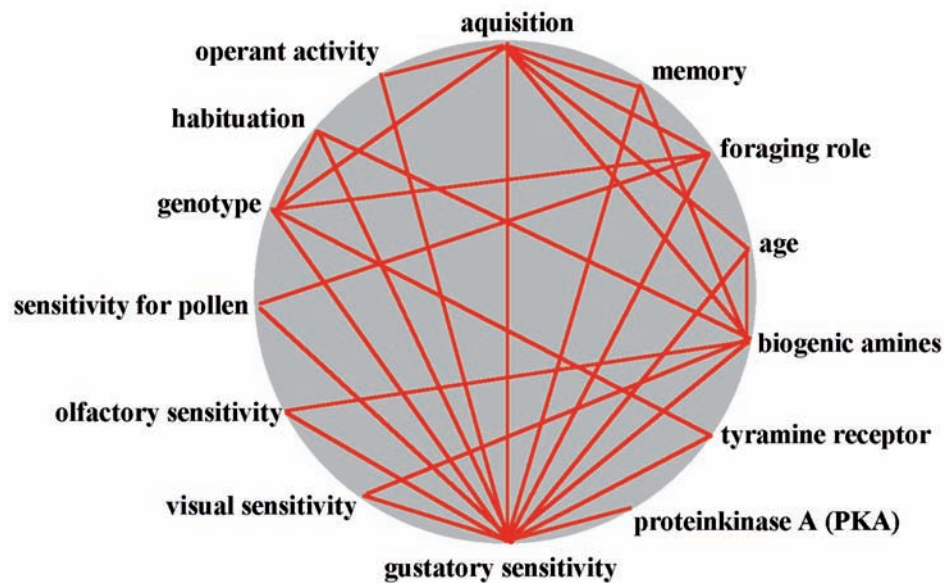


Figure 7

The phenotypic architecture of the pollen hoarding syndrome. Phenotypic traits span levels of biological organization from the genotype to foraging behavior. Lines connect traits that have been demonstrated to be significantly correlated. Studies were performed on high and low strain workers as well as wild-type bees. (See Page and Erber 2002 [Fn. 19] and Page et al. 2006 [Fn. 22] for reviews of the studies that were used for this diagram. The connection between genotype and habituation is based on unpublished data. The original figure was drawn by Joachim Erber.)

The genetic architecture of pollen hoarding

At the individual behavioral level, we have defined a pollen hoarding behavioral syndrome that consists of an early onset of foraging, a bias to collect more pollen and less nectar, and the tendency to collect nectar with lower concentrations of sugar, and water. This syndrome of correlated traits is linked together with a pleiotropic network of genes. Greg Hunt constructed the first genetic map of any social insect and mapped quantitative trait loci that affected differences in quantities of stored pollen between the high and low pollen hoarding strains.²³ We initially identified three quantitative trait loci (mapped regions on chromosomes that contain genes) that explained significant amounts of the variation in stored pollen that we observed between the high-

23 Hunt, G. J., R. E. Page, M. K. Fondrk, and C. J. Dullum (1995): Major quantitative trait loci affecting honey bee foraging behavior. In: *Genetics* 141: 1537–1545.

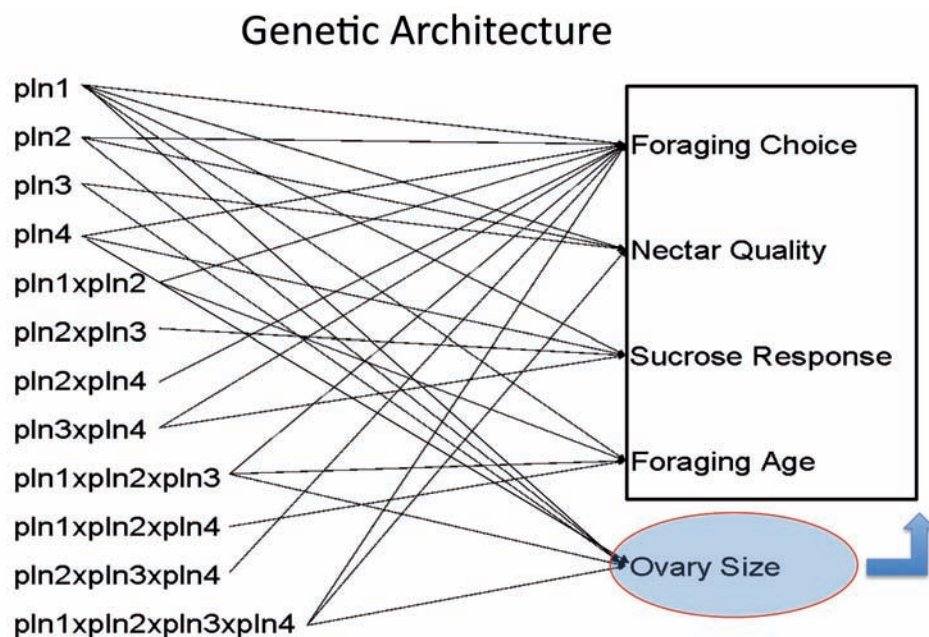


Figure 8
The genetic architecture of the traits associated with the pollen hoarding syndrome, and ovary size (number of ovarioles). Ovary size is believed to be the cause of much of the pleiotropy observed.

and low-pollen-hoarding strains (Fig. 8). Next, with Olav Rueppell²⁴, we mapped the individual foraging and sucrose response traits, found a fourth QTL. We also found that all QTL affected all traits (pleiotropy) and all QTL affected each other (epistasis). The honey bee genome sequence allowed us to look into these regions and seek candidate genes that we are currently testing for effects on foraging behavior. However, our attention has refocused on something that we believe gives us a broad explanation for the complex phenotypic and genetic architectures.

- 24 Rüppell O., T. Pankiw, D. I. Nielsen, M. K. Fondrk, M. Beye, and R. E. Page (2004): The genetic architecture of the behavioral ontogeny of honey bee workers. In: *Genetics* 167:1767–1779. – Rueppell, O., S. Chandra, T. Pankiw, M. K. Fondrk, M. Beye, G. Hunt and R. E. Page (2006): The genetic architecture of sucrose responsiveness in the honey bee (*Apis mellifera* L.). In: *Genetics* 172: 243–251.

The Reproductive Groundplan

Gro Amdam and I suggested that the explanation for the correlations of traits in the pollen hoarding syndrome may be found in the use of a reproductive regulatory network as a mechanism to establish a foraging division of labor in honey bees.²⁵ The network consists of many parts, some with known functions, others parts remain unknown. However, key players that we have focused on, so far, include the ovaries that make ecdysteroid hormones that along with juvenile hormone are thought to act on the fat body of insects and result in the production of vitellogenin, an egg yolk precursor protein. There are certainly many other expanding effects of this network that we are trying to understand, but these give us points of insertion for manipulation of the network so we can study the effects.

We believe that the reproductive network involving these components is ancient in the insects, part of a fundamental reproductive ground plan, operating on the activation and maturation of ovaries, production of egg yolk proteins, and maternal behavior including making a nest, provisioning the nest with protein for their offspring, and laying eggs. It has been shown in many insects that behavior changes with the state of the ovary. We believe that in honey bees, the ancient relationship between ovary and behavior has been co-opted, and is used now as a mechanism for canalizing workers into performing different tasks, and can explain differences in the age of onset of foraging, and foraging behavior.

The ovaries are certainly key players. Honey bees have paired ovaries that consist of ovariole filaments in which eggs are made. Queens have on average more than 150 ovarioles per ovary while workers usually have 2–12. The number of ovarioles is determined during the 5th instar of larval development (about 5–7 days after hatching). At this time workers and queens have the same number of developing ovarioles, however, ovarioles are lost in workers through a process of apoptosis, or programmed cell death. Ovarioles are rescued from apoptosis by juvenile hormone circulating at just the right time. Queens have a bigger spike of juvenile hormone compared to workers, and end up with more ovarioles. When we compare bees from the high and low pollen hoarding strains, we find that high strain workers have more ovarioles, newly

25 See Page et al. 2006 (Fn. 22). – Page, R. E. and G. V. Amdam (2007): The making of a social insect: developmental architectures of social design. In: *Bioessays* 29: 334–343. – Amdam G. V., K. E. Ihle, and R. E. Page (2009): Regulation of honeybee worker (*Apis mellifera*) life histories by vitellogenin. In: Donald W. Pfaff, Arthur P. Arnold, Anne M. Etgen, Susan E. Fahrbach and Robert T. Rubin (eds.) (2009), *Hormones, Brain and Behavior*, 2nd edition, Vol 2. San Diego: Academic Press, pp.1003–1025.

emerged adult bees already have ovaries that are activated, ready to absorb vitellogenin, and have higher titers of circulating vitellogenin compared with low strain bees. In other words, they seem to be in more advanced stages of reproductive readiness. When we look at the amount of circulating juvenile hormone during the 5th larval instar we find what we would expect: high strain bees have higher titers, which may explain why they have more ovarioles. We think of this difference in the titer of juvenile hormone as a developmental signature of colony-level selection from our breeding program. Natural selection should leave similar signatures, as it has with respect to queen and worker ovary development.

Are the results of our comparisons of high- and low-strain bees general results? We studied wild-type bees and found that workers with more ovarioles are more sensitive to sucrose solutions, forage earlier in life, show a bias for collecting pollen, collect nectar with less sugar, are less likely to return empty from a foraging trip, and have higher titers of vitellogenin when they are young.²⁶ These traits fit exactly with those found for the high strain bees and independently verify the relationships between ovariole number and behavior. We additionally confirmed the relationships by genetically mapping quantitative trait loci that affect the pollen hoarding syndrome onto variation for ovariole number.²⁷ The same major QTLs for the behavioral traits mapped onto the anatomical trait suggesting that the behavioral effects are derived from the effects of these QTL on the ovary (Fig. 8).

We can remove the ovaries from one bee and put them into another (Y. Wang, R. Page, and G. Amdam, unpublished). When we do this, we can show that the transplanted (grafted) ovaries live, develop, and respond like the resident ovaries (Fig 9). For experiments, we inject glass beads as a control. They are immunologically inert but require the same surgical procedure. We conducted a study where we grafted ovaries into a test group, beads into the controls, placed the bees into an observation hive, then watched their behavioral transitions. Ovary grafted bees made the transitions through within nest behavior and into foraging faster than the bead controls. This is the same pattern we see in high strain bees versus lows (high strain bees have more ovarioles), and that we have shown for wild-type bees with more ovarioles versus those with fewer.

We can disrupt the reproductive regulatory network by eliminating or greatly re-

26 Amdam, G., A. Csondes, M. K. Fondrk, and R. E. Page (2006): Complex social behaviour derived from maternal reproductive traits. In: *Nature* 439: 76–78. – Tsuruda, J. M., G. V. Amdam, and R. E. Page (2008): Sensory response system of social behavior tied to female reproductive traits. In: *PLoS One* 3: e3397.

27 Wang, Y., G. V. Amdam, M. A. Wallrichs, M. K. Fondrk, O. Kaftanoglu, and R. E. Page (2009): *PDK1* and *HR46* gene homologs tie social behavior to ovary signals. In: *PLoS One* 4: e4899.

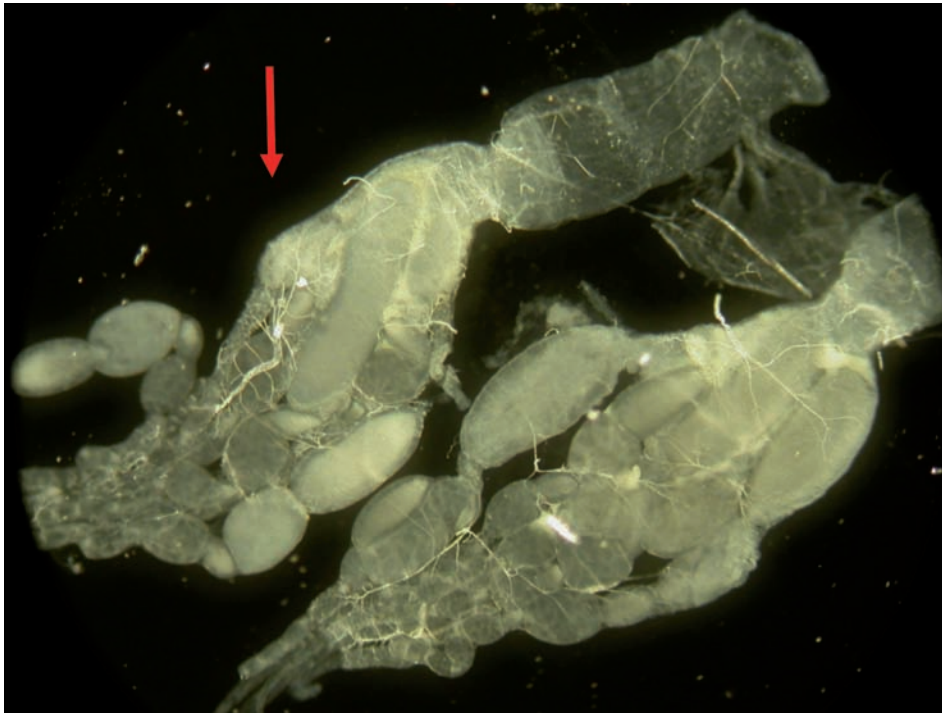


Figure 9

Transplanted worker ovary. The ovary at the top marked with the red arrow was transplanted from one newly emerged worker into another 18 days earlier. The ovary at the bottom is the “resident” ovary. The ovaries are both activated with developing eggs. (Photo by Ying Wang)

ducing the presence of vitellogenin. We inject double stranded RNA, the template used by cells to make proteins from genes. The dsRNA is then taken up by the fat body cells where vitellogenin is normally made, but it blocks the production of the protein. Normal RNA is in a single strand. When we block vitellogenin we affect the behavioral traits associated with the pollen hoarding syndrome: bees are more responsive to sucrose, forage earlier in life, and show a bias for collecting nectar.²⁸

- 28 Nelson, C.M., K.E. Ihle, M.K. Fondrk, R.E. Page, G.V. Amdam (2007): The gene vitellogenin has multiple coordinating effects on social organization. In: *PloS Biology* 5: 673–677.
– Amdam, G. V., K. Norberg, R. E. Page, J. Erber, R. Scheiner (2006): Downregulation of *vitellogenin* gene activity increases the gustatory responsiveness of honey bee workers (*Apis mellifera* L.). In: *Behavioral Brain Research* 169: 201–205.

Conclusion

How does social organization evolve? At least in part, selection on the superorganismal trait of stored pollen changes frequencies of alternative alleles (forms) of genes or gene regulatory networks with broad pleiotropic effects including affects on reproductive signaling networks. Changes in signaling networks affect changes in development that affect the ovaries of workers, that in turn affect sensory physiology and response systems, and thus the behavior and interactions of thousands of individuals. And where does the “spirit of the hive” reside? At least in part, it resides in the ovaries of “a crowd of bees working in a dark hive”.

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