the color center and its response to ionizing radiation is at least partially understood. If anomalous RICHs in other silicate minerals such as micas do not develop in fundamentally different ways (albeit the nature of the color centers can involve defects other than Al), then many of the special conditions and special alpha energies invoked to account for Po and giant halos in mica seem no longer necessary. Giant RICHs can grow by hole diffusion. The apparent absence of ring structures readily associated with U in so-called Po halos might be the result of the destruction of color centers by excessive alpha dose near the inclusions.

Mica is chemically and structurally far more complex than quartz, and there is little understanding of its radiation-induced color centers and carrier behavior. We strongly suspect, however, that the sizes and structure of giant and Po RICHs in mica also are artifacts of radiation-induced conductivity and that their explanation requires neither unknown radioactivity nor an abandonment of current concepts of geologic time.


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# Hormonal and Genetic Control of Behavioral Integration in Honey Bee Colonies


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**The ability of insect colonies to adjust the division of labor among workers in response to changing environmental and colony conditions, coupled with research showing genetic effects on the division of labor in honey bee colonies, led to an investigation of the role of genetics and the environment in the integration of worker behavior. Measurements of juvenile hormone (JH) titers and allozyme analyses of worker honey bees suggest that two processes are involved in colony-level regulation of division of labor: (i) plasticity in age-dependent behavior is a consequence of modulation of JH titers by extrinsic factors, and (ii) stimuli that can affect JH titers and age-dependent behavior do elicit variable responses among genetically distinct subpopulations of workers within a colony. These results provide a new perspective on the developmental plasticity of insect colonies and support the emerging view that colony genetic structure affects behavioral organization.**


ADVANCED INSECT COLONIES HAVE long been likened to "superorganisms" (*1*), a metaphor most apt for traits of colonies that are a consequence of cooperation among individual colony members. One such trait is colony development, which is a consequence of the integration of worker behavior. Results of experimental perturbations (*2, 3*) suggest that insect colonies cope with constant variation in age demography (*4, 5*) and resource availability (*5, 6*) via a process of developmental plasticity that involves ongoing adjustments in the proportions of individual workers engaged in various tasks. The coordination of worker responses to changing environmental conditions is poorly understood. Moreover, the recent discovery (*7–9*) of genetic influences on the division of labor among workers raises new questions about the role of genetics and the environment in integrating activity in insect societies. Regulation of developmental plasticity is a central issue in the study of all biological systems, and comparisons of processes in insect colonies and individual multicellular organisms may provide insights of general significance (*10*).

We describe four experiments that probe the hormonal and genetic basis of developmental plasticity in honey bee colonies. The first two experiments demonstrate that changes in colony age structure can affect age-dependent titers of juvenile hormone (JH) that are associated with changes in worker age-dependent behavior (age polyethism). In the third experiment, treatment with a JH analog affected age polyethism, further supporting the hypothesis that extrinsic factors influence the behavior of worker bees via their effects on JH. The fourth experiment demonstrates genotypic differences in the behavioral responses of workers to altered colony age demography.

JH, a major insect developmental hormone (*11*), is involved in the control of age polyethism in adult worker honey bees (*12–16*). Hemolymph levels of JH increase with worker age (*13, 17*). Low titers are associated with behavior in the nest such as brood


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**Table 1.** Mean JH titers ± SE for honey bees experiencing experimentally induced changes in demography in single-cohort colonies ($n$ = number of worker groups, 4 to 16 workers per group; $n$ = 5, except $n$ = 4 for colony 4440-3 normally aged nurses; $n$ = 6 for colony 4450-3 precocious foragers, normally aged foragers, external control nurses, and external control foragers). External controls ($n$ = 500) were taken from the same source colonies used to establish single-cohort colonies, marked at 1 day of age, reintroduced to their respective colonies, and sampled as nurses at 7 to 10 days of age and foragers at 21 to 24 days of age. $P$ values shown are results of $t$ tests (for samples with unequal variances).

| Colony | JH titers (picomoles of JH III per 100 μl of hemolymph) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Normally aged nurses | | Precocious foragers | | Overaged nurses | | Normally aged foragers | External control nurses | | External control foragers |
| 4440-3 | 5.4 ± 2.4 | $P < 0.01$ | 27.3 ± 6.3 | | 2.8 ± 0.8 | $P < 0.01$ | 17.4 ± 2.9 | 5.8 ± 1.3 | $P < 0.01$ | 36.0 ± 8.3 |
| 4450-3 | 2.7 ± 0.9 | $P < 0.01$ | 72.6 ± 10.5 | | 8.6 ± 1.9 | $P < 0.05$ | 24.8 ± 6.2 | 2.9 ± 0.7 | $P < 0.01$ | 35.5 ± 6.1 |

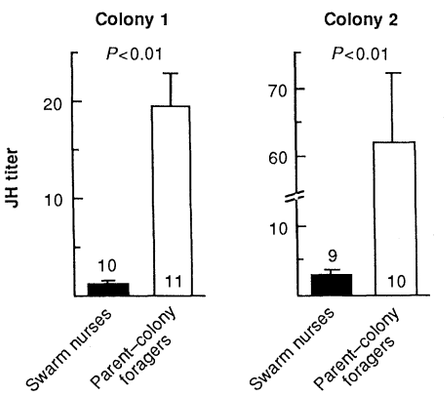


**Fig. 1.** Mean JH titers (picomoles of JH III per 100 μl of hemolymph) ± SE for honey bees experiencing naturally induced changes in demography associated with colony fission. All bees were 20 to 30 days old when sampled (*20*). $P$ values based on results of $t$ tests (for samples with unequal variances). Number of worker groups sampled indicated on graph (4 to 16 workers per group).

care ("nursing") during the first 1 to 3 weeks of the worker bee's ~6-week adult life, whereas a higher titer at about 3 weeks of age induces foraging. We tested the hypothesis (*16*) that the JH titer in bees is sensitive to colony state, providing a mechanism for plasticity in age-based division of labor. Workers were exposed to conditions that uncoupled the usually tightly linked factors of worker age and behavioral status for two tasks: nursing and foraging. An association between behavioral status and JH titer, independent of age, would provide support for the hypothesis that JH acts as a colony integrating mechanism.

Two colonies, each initially consisting of 2000 bees, 1 to 3 days old, were established to induce division of labor independent of worker age (*2, 18*). Within 1 week these "single-cohort colonies" contained some bees that tended brood and others that foraged precociously. The emergence of new adults was prevented by replacing combs of developing pupae with combs of eggs and young larvae from other colonies; the aging experimental colonies then contained "overaged" nurses and "normally aged" foragers. We also exploited demographic changes occurring naturally during colony fission that result in similarly skewed age distributions. A reproductive swarm of honey bees contains workers of all ages (*19*) but develops an age structure dominated by older individuals soon after establishing itself in a new nest site, because new adults do not emerge until 3 weeks after the first eggs are laid. We predicted that as the worker population in a newly swarm-founded colony aged, some of its youngest workers would continue to care for brood as overaged nurses, while similarly aged individuals in a swarm's parent colony would switch from nursing to foraging during this same time period, as a consequence of a continual emergence of young bees.

Groups of nurses and foragers were collected from single-cohort colonies when they were 7 to 10 and 21 to 24 days old. Overaged nurses and normally aged foragers, 20 to 30 days of age, were also collected from two swarms and their respective parent colonies (*20*). A pooled total of 40 to 50 μl of hemolymph was collected from each group for JH radioimmunoassay (*21*).

In each single-cohort colony, pooled samples of foragers had significantly higher hemolymph levels of JH than pooled samples of nurse bees, at both ages (Table 1) (*22*). Similarly, circulating levels of JH were significantly lower for nurse bees from each swarm than for foragers from each parent colony, despite the fact that both groups of workers were similarly aged (Fig. 1). JH titers were also low for unmarked resident nurse bees (>30 days old) in swarm colony two and for 8- to 10-day-old nurse bees in each parent colony (*23*). Hormonal differences between nurses and foragers were evident despite intercolony variability in JH titers at both ages (Table 1 and Fig. 1). The reason for this variability is not known.

In the third experiment, JH analog treatment affected age polyethism in a single-cohort colony (*24*). Bees treated with methoprene at 1 day of age began foraging at younger ages than acetone-treated bees: 62 of 75 (83%) treated bees began foraging by 10 days of age, compared with 13 of 80 (16%) controls ($P < 0.001$, $G = 74.5$, 1 df). Similar results have been reported recently (*25*) and previous studies have demonstrated that treatment with JH, JH mimic (*14*), or JH analog (*15, 16*) also induces precocious foraging in typical colonies with mixed age structures. These results, coupled with those of the first two experiments, support the hypothesis that environmental modulation of endogenous JH titers affects worker behavior. Cues associated with colony age demography probably affect neurohormones (*26*) that modulate the intrinsic rise in JH and accelerate or retard worker behavioral development.

Results of the fourth experiment demonstrate that genotypic differences in behavior among colony members affect colony integration. Because of polyandry (*27–29*) and sperm mixing (*29, 30*), honey bee colonies are composed of numerous subfamilies of workers, each subfamily descending from the colony's queen and one of her mates. We collected precocious foragers, overaged nurses, and normally aged foragers and nurses, as in the first experiment, and used allozyme analysis to determine their subfamily membership (*31*). Significant differences in subfamily frequencies were detected in samples of normally aged nurses and precocious foragers in seven out of nine colonies, and in samples of overaged nurses and normally aged foragers in four out of six colonies (Fig. 2), demonstrating genetic variability for individual behavioral plasticity. Intracolonial genetic differences in both the rate of worker behavioral development (*7*) and the likelihood of performing certain tasks (*8, 9*) have been reported previously. Our results suggest that there is another, previously unrecognized, genetic component of the division of labor among work-

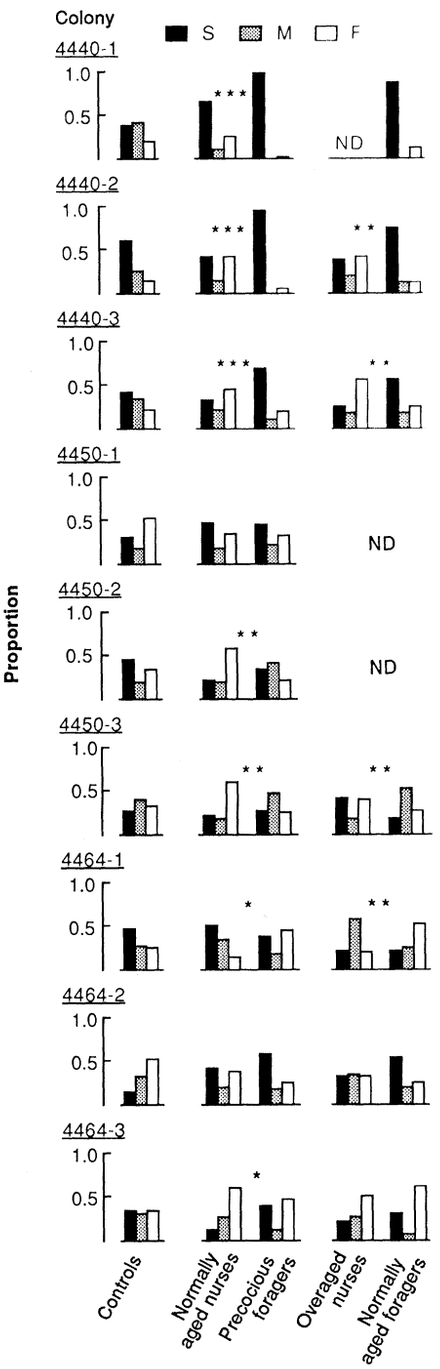


**Fig. 2.** Genotypic composition of samples of 7- to 10-day-old and 21- to 24-day-old nurses and foragers from single-cohort colonies of honey bees composed of electrophoretically distinguishable subfamilies. Relative proportions of each of three subfamilies (S, M, and F) indicated by differently shaded bars. Results of comparisons between similarly aged nurses and foragers indicated on graphs: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ [based on G tests for heterogeneity of actual frequencies (*34*)]. "Control" bees were collected randomly at 1 day of age when colonies were established to estimate subfamily frequencies. Significant ($P < 0.05$) differences in the subfamily composition of samples of control bees versus 7- to 10-day-old foragers: colonies 4440-1, 4440-2, 4440-3, and 4464-2; for control bees versus 7- to 10-day-old nurses: colonies 4440-1, 4440-2, 4450-3, 4464-2, and 4464-3. In all cases $n = 40$, except $n = 39$ for colony 4440-3 overaged nurses. ND (no data), a consequence of colonies destroyed by intercolony robbing.

ers: sensitivity to changing colony conditions.

The experimental colonies had unusually simple age structures. However, effects of colony genetic structure on other aspects of division of labor have been found in colonies with bees of mixed ages (*7–9*). In addition, although the experimental colonies consisted of three subfamilies whereas colonies derived from naturally mated queens may be composed of up to 17 (*28*), genetic differences in worker behavior have also been found in colonies with naturally mated queens (*32*). These findings suggest that genetic variation in behavior similar to what we report also exists in more natural contexts.

Our results reveal both similarities and a fundamental difference between the regulation of developmental plasticity in insect colonies and in multicellular organisms. In a metazoan, biochemical signals that induce differentiation act variably on distinct subpopulations of cells (*33*), but in a honey bee colony, extrinsic stimuli that modulate the JH titer and behavioral development act variably on distinct subpopulations of workers. However, differences in cell-type differentiation within an organism are a consequence of the environment because cells usually possess identical genotypes, whereas differences in the behavioral differentiation of workers within a colony are influenced by both their environment and genotype.

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21. We removed 1 to 12 μl (5 μl in most cases) of hemolymph from each worker. JH analyses were performed in Marseille, France, according to C. Strambi, A. Strambi, M. de Reggi, and M. Delaage [in *Biosynthesis, Metabolism and Mode of Action of Invertebrate Hormones*, J. Hoffmann and M. Porchet, Eds. (Springer-Verlag, Berlin, 1984), pp. 355–362]. Other results obtained with this technique agree with titers determined by gas chromatography/mass spectrometry (GC/MS) [C. A. D. de Kort, A. B. Koopmanschap, C. Strambi, A. Strambi, *Insect Biochem.* **15**, 771 (1985)]; our previous results with honey bees (*17*) are also consistent with measurements made with the *Galleria* bioassay (*13*) and a GC/MS assay [H. Hagenguth and H. Rembold, *Z. Naturforsch.* **33**, 847 (1978)]. Samples were coded, allowing blind analyses. After separation with high-performance liquid chromatography, the immunoreactive product was found to have the same retention time as JH III diol, demonstrating that JH III was the only JH homolog in bee hemolymph. This result agrees with (*17*); K. H. Trautmann, P. Masner, A. Schuler, M. Suchi, H. K. Wipf, *Z. Naturforsch.* **29c**, 161 (1974); and H. Hagenguth and H. Rembold, above.
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23. Mean (± SE) picomoles of JH III per 100 μl of hemolymph for groups of young nurses from parent colony one: 3.0 ± 0.9, $n = 11$ groups (4 to 16 bees per group); from parent colony two: 1.4 ± 0.4, $n = 9$; for unmarked resident nurses from swarm colony two: 6.1 ± 2.1, $n = 5$.
24. The colony was established as in (*18*), except 200

randomly selected workers were individually marked with colored, numbered plastic tags. One hundred tagged bees were treated topically with 200 μg of (*RS*)-methoprene (isopropyl (2*E*, 4*E*)-11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate) dissolved in 5 μl of acetone, and 100 were treated with acetone alone. Although recent evidence [G. D. Prestwich, *Science* **237**, 999 (1987)] suggests that there are different receptor sites for JH homologs and analogs in the tissue of at least one insect species (*Manduca sexta*), methoprene has demonstrated JH-like activity in many species, at the molecular [G. R. Wyatt, K. E. Cook, H. Firko, T. S. Dhadialla, *Insect Biochem.* **17**, 1071 (1987)], physiological, and behavioral levels [G. B. Staal, *Annu. Rev. Entomol.* **20**, 417 (1975)]. The efficacy of methoprene as a JH analog in honey bees is well established (*15, 16*). We quantified the incidence of precocious foraging among the two groups of tagged bees during daily 1-hour observation periods at the colony entrance, when bees were 5 to 10 days old. A census of tagged bees was also taken early in the morning of day 11, before the onset of foraging.

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31. Allozymes of malate dehydrogenase-1 (*Mdh*) that occur naturally in populations of honey bees (*29*) were used as subfamily markers, as in (*9*). Three unrelated virgin queens were instrumentally inseminated [H. H. Laidlaw, *Instrumental Insemination of Honey Bee Queens* (Dadant and Sons, Hamilton, IL, 1977)] with the semen of three unrelated drones. Each drone carried a different *Mdh* allozyme, designated "slow" (S), "medium" (M), and "fast" (F) [E. P. B. Contel, M. A. Mestriner, E. Martins, *Biochem. Genet.* **15**, 859 (1977)]. Three single-cohort colonies were established (*18*) from the progeny of each queen. Use of allozyme markers allowed blind behavioral observations. Individuals sampled for JH titer determinations were among those used for allozyme analysis (with polyacrylamide gel electrophoresis).
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# Dispersed Polaron Simulations of Electron Transfer in Photosynthetic Reaction Centers

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**A microscopic method for simulating quantum mechanical, nuclear tunneling effects in biological electron transfer reactions is presented and applied to several electron transfer steps in photosynthetic bacterial reaction centers. In this "dispersed polaron" method the fluctuations of the protein and the electron carriers are projected as effective normal modes onto an appropriate reaction coordinate and used to evaluate the quantum mechanical rate constant. The simulations, based on the crystallographic structure of the reaction center from *Rhodopseudomonas viridis*, focus on electron transfer from a bacteriopheophytin to a quinone and the subsequent back-reaction. The rates of both of these reactions are almost independent of temperature or even increase with decreasing temperature. The simulations reproduce this unusual temperature dependence in a qualitative way, without the use of adjustable parameters for the protein's Franck-Condon factors. The observed dependence of the back-reaction on the free energy of the reaction also is reproduced, including the special behavior in the "inverted region."**

THE RECENT ELUCIDATION OF THE crystal structure of photosynthetic bacterial reaction centers (*1–3*) has made it possible to explore microscopic aspects of biological electron transfer (ET) theory. Although a semiclassical trajectory simulation of the initial ET reaction has been reported (*4*), no attempts have been made to use a microscopic simulation approach to account for the unusual temperature dependence of the ET reactions or to treat nuclear tunneling effects. We report such a study for the charge-transfer step $P^+H^-Q \rightarrow P^+HQ^-$, where P is the primary electron donor in the reaction center (a bacteriochlorophyll dimer), H is a bacteriopheophytin that accepts an electron from P when the reaction center is excited, and Q is the secondary electron acceptor (ubiquinone in *Rhodobacter sphaeroides* or menaquinone in *Rhodopseudomonas viridis*). We also have studied some aspects of the slower back-reaction $P^+HQ^- \rightarrow PHQ$.

The kinetics of the ET reaction $P^+H^-Q \rightarrow P^+HQ^-$ and of the reaction from $Q^-$ back to $P^+$ have been measured over a wide range of temperatures (*5–9*). In *Rb. sphaeroides*, both reactions are characterized by an intriguing decrease in rate constant with increasing temperature. In *Rps. viridis*, the rates are almost independent of temperature. The dependence of the kinetics on the free energies of the reactions also has been studied, by varying the nature of the quinone or by applying external electrical fields (*7–9*). The back-reaction is particularly interesting in this regard, because the change in free energy is so large as to place the reaction in the "inverted region" (*10–12*) where the rate constant depends strongly on quantum mechanical (QM) nuclear tunneling.

Previous discussions of the dependence of the rate constants on temperature or on the free energy change have consisted largely of fitting the data to theoretical expressions with adjustable phenomenological parameters (*10*). Although these treatments have provided considerable insight into the factors that influence electron reactions in proteins,they have not been based on the actual structure of the protein. Thus, it has been possible to fit the experimental data in different ways by making various assumptions about the type and numbers of vibrational modes that are coupled to the reaction. The availability of the crystal structure makes it possible to evaluate the key parameters directly from the structure by using microscopic simulations.

In the present work we examine the above reactions, using the "dispersed polaron" version (*13, 14*) of our semiclassical trajectory approach (*4, 12, 13*). The dispersed polaron method extends the semiclassical surface-crossing method to the evaluation of QM tunneling effects. Thus we can simulate the temperature dependence of the rate constants and the special behavior in the inverted region, using the actual microscopic properties of the protein and the chromophores.

Electron transfer between a weakly interacting donor and acceptor (such as $H^-$ and Q) can occur only when the potential energy surfaces of the reactant and product states

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