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REVIEW

The Social Clock of the Honeybee

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Abstract   The honeybee has long been an important model for studying the inter-
play between the circadian clock and complex behaviors. This article reviews
studies further implicating the circadian clock in complex social behaviors in bees.
The article starts by introducing honeybee social behavior and sociality and then
briefly summarizes current findings on the molecular biology and neuroanatomy
of the circadian system of honeybees that point to molecular similarities to the
mammalian clockwork rather than to that of Drosophila. Foraging is a social
behavior in honeybees that relies on the circadian clock for timing visits to flow-
ers, time-compensated sun-compass navigation, and dance communication used
by foragers to recruit nestmates to rewarding flower patches. The circadian clock
is also important for the social organization of honeybee societies. Social factors
influence the ontogeny of circadian rhythms and are important for social synchro-
nization of worker activities. Both queen and worker bees switch between activi-
ties with and without circadian rhythms. In workers this remarkable plasticity is
associated with the division of labor; nurse bees care for the brood around the
clock with similar levels of clock gene expression throughout the day, whereas
foragers have strong behavioral circadian rhythms with oscillating brain clock
gene levels. This plasticity in circadian rhythms is regulated by direct contact with
the brood and is context-specific in that nurse bees that are removed from the hive
exhibit activity with strong behavioral and molecular rhythms. These studies on
the sociochronobiology of honeybees and comparative studies with other social
insects suggest that the evolution of sociality has influenced the characteristics of
the circadian system in honeybees.

Key words   *Apis mellifera*, sun-compass orientation, circadian rhythms, division of labor,
social behavior, evolution

Honey Bee Societies

Whereas most species of bees (order: Hymenoptera)
are solitary or live in small groups, honeybees are *euso-
cial* and show the most extreme form of sociality in the
animal kingdom. Eusociality is commonly defined by
3 traits: (1) cooperative care of young by members of
the same colony; (2) reproductive division of labor, with
more or less sterile individuals working on behalf of
fecund colony members; and (3) an overlap of at least
2 generations of adults in the same colony (Michener,
1974; Wilson, 1971). The honeybee society includes

Figure 1.   Division of labor in honeybees. (A) Nurse bees with their head inside larvae-containing cells. Nurse bees care for the brood around the clock with no apparent circadian rhythms (photograph: Amit Shalev). (B) A forager bee on a flower. Foraging is a social behavior in bees because foragers collect nectar and pollen to feed nestmates rather than for personal consumption or for feeding offspring. Foragers show strong circadian rhythms (photograph: Aviv Bloch). (C) A guard bee inspecting an incoming bee (photograph: Didi Dabush).

several tens of thousands of individuals that use elaborate communication systems to coordinate almost all aspects of their behavior and physiology. The reproductive division of labor is based on the profound morphological and anatomical polymorphism between the highly fecund *queen* and the rest of the females, the *workers*. Workers do not mate and typically do not lay (unfertilized) eggs. The honeybee queen maintains her reproductive dominance by secreting *queen pheromones* that are distributed throughout the entire colony (reviewed in Wilson, 1971, Winston, 1987; Le Conte and Hefetz, 2008).

There is a clear division of labor between workers specializing in different activities (Fig. 1). In honeybees, the division of labor relates to age (reviewed in Lindauer, 1961; Robinson, 1992; Seeley, 1995). Young workers typically specialize in brood care ("nursing," Fig. 1A) and other in-hive activities. Later they perform activities such as comb construction and honey storage, which is followed by a shift to activities outside the nest such as guarding and foraging (Figs. 1B and 1C). Another common division of labor system relates to body size, which is typical of bumblebees. In this system there is considerable morphological polymorphism with up to 9-fold differences in body size between full-sister workers developing in the same colony (Wilson, 1971; Michener, 1974). Large individuals are more likely to perform foraging activities and may start foraging as early as their first day as adults, whereas small bees tend to perform in-nest activities (e.g., Yerushalmi et al., 2006).

The organization and function of complex societies require elaborate communication systems. Information can be conveyed by distinct chemical, tactile, vibratory, visual, or auditory signals and can involve the simultaneous use of several modalities (Wilson, 1971). This complexity of information transfer is exemplified by the symbolic dance communication system of honeybees, which conveys spatial and quality information regarding the location of sites appropriate for nesting or ones that are rich in rewarding flowers (Lindauer, 1961; von Frisch, 1967; Dyer, 2002). The dance that is performed inside the dark hive consists of a series of repeated figure-8 dances with waggling runs in the part connecting the 2 circles. During the waggling run the worker bee emits a burst of sound by buzzing her wings and releases recruitment pheromones (Thom et al., 2007). Direction relative to the sun is encoded in the orientation of the waggling run relative to gravity. Honeybees as well as other social insects also use a broad array of chemical signals (pheromones) to organize the activities of the individuals in a colony (Wilson, 1971; Michener, 1974; Le Conte and Hefetz, 2008).

The Gears of the Honeybee Clock: Anatomical and Molecular Organization of the Brain's Circadian Clock

Molecular and genomics analyses suggest that for some important traits the honeybee clockwork fits better with the mammalian model than with that of Drosophila (Rubin et al., 2006; Weinstock et al., 2006). The honeybee genome does not encode orthologs to *Cry-d* (*Drosophila*-type Cry, also known as *insect Cry1*) or *Timeless1 (Tim1)* genes that are essential for clock function in the central pacemaker of *Drosophila*, but does have orthologs to the mammalian-type protein *Cry-m* (also known as *insect Cry2*) (Rubin et al., 2006). In vitro cell culture assays suggest that the honeybee *Cry-m,* like its mammalian Cry orthologs, is an effective transcriptional repressor but is not sensitive to light and therefore is not likely to fulfill the photic input functions of Drosophila *Cry-d* (Yuan et al., 2007).
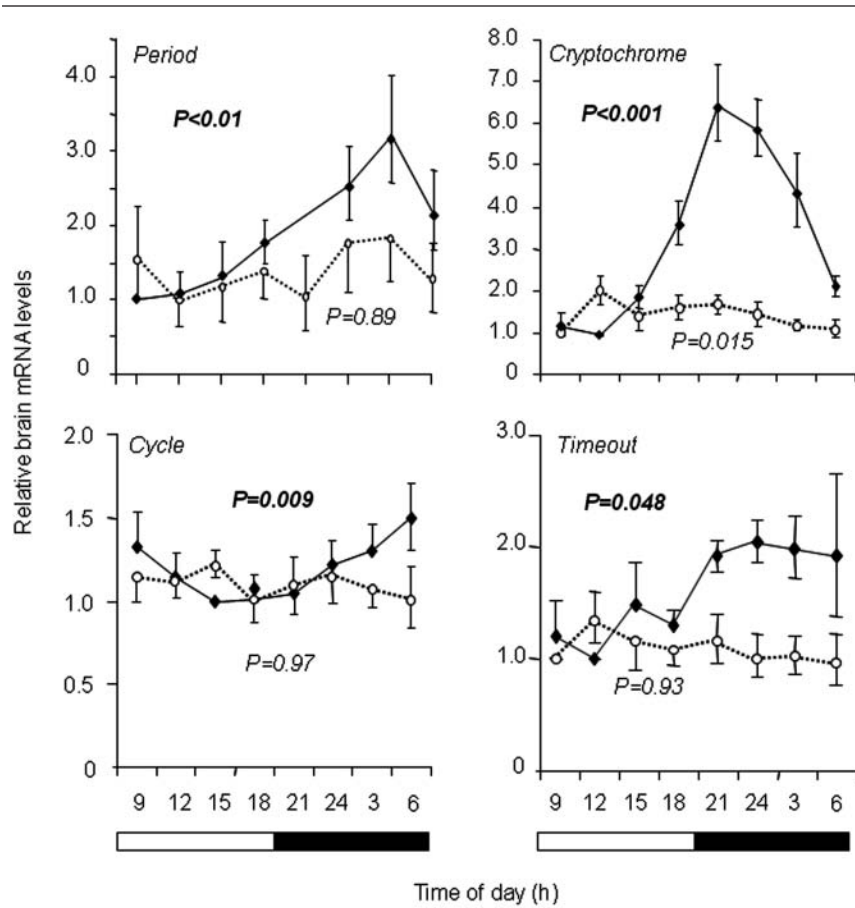
Figure 2. Oscillations in brain mRNA abundance for putative clock genes are attenuated in nurses relative to foragers in LD illumination regime. Foragers (filled diamonds, continuous line) and nurses (open circles, dashed line) were entrained for 7 days in LD and collected in LD. The values were normalized relative to the lowest point (separately for nurses and foragers). The bars at the bottom of plots represent illumination regime: open box = light, filled box = dark. Sample size = 5 to 6 bees per time point. The *p* values above and below the plots show the results of 1-way analyses of variance for foragers and nurses, respectively (data from Shemesh et al., 2007).

The honeybee genome also encodes a single ortholog for the putative clock genes *Per*, *Cyc*, *Clk*, and *Timeout (Tim2)*. There are highly conserved orthologs to *Vrille (Vri)* and *Par Domain Protein 1* (*PDP1*), 2 basic zipper transcription factors that are implicated in the regulation of *Clk* expression in Drosophila. A search at the 3kb upstream of the start codon of either *amClk* or *amCyc* found putative binding sites for VRI and PDP1 consistent with a model in which Vri and PDP1 are involved in the positive limb of the honeybee clock (Rubin et al., 2006; Weinstock et al., 2006).

In foragers and other bees with strong circadian rhythms, brain mRNA levels of *Cry-m* and *Per* consistently oscillate with strong amplitude and a similar phase under both light–dark (LD) and constant darkness (DD) illumination regimes. In contrast to Drosophila, the predicted honeybee CYC protein contains a transactivation domain and its brain transcript levels oscillate at virtually an antiphase to *Per*, as is the case in the mouse. Another difference with regard to Drosophila (and a similarity with the mouse) is that the honeybee CLK ortholog does not appear to contain a transactivation domain, and its transcript levels do not appear to vary over the day (Rubin et al., 2006; Shemesh et al., 2007) (Figs. 2 and 3A). Based on the above findings, the working model for the molecular clockwork in the honeybee assumes that *Per* and *Cry-m* act together in the negative limb of the interlocked feedback loop and *Cyc* is the oscillating factor in the positive limb of the loop (Fig. 3B). Shimizu et al. (2001) further suggested that in both *Apis mellifera* and *Apis cerana* there are 2 alternative splice forms of *Per* and that their ratio varies during the day. Although there is evidence that alternative splicing in *Per* is functional in Drosophila (Majercak et al. 1999, 2004), its role, if any, in the honeybee has yet to be determined.

The photic input pathways to the honeybee clock have not been characterized. The absence of orthologs to *Tim1* and *Cry-d*, and the evidence that *amCry-m* is not sensitive to light, suggest that honeybees use a novel light input pathway. An interesting suggestion is that the recently discovered pteropsins, a family of opsins that are more closely related to vertebrate visual opsins than to invertebrate opsins, are involved in the regulation of circadian rhythms in honeybees and other nondrosophilid insects (Velarde et al., 2005). Another interesting possibility is that *amTim2* is involved in light input pathways in the honeybee. The transcript levels of *amTim2* oscillate under both DD and LD illumination regimes but with a different phase, suggesting that *amTim2* expression is influenced both by light and by the circadian clock (Rubin et al., 2006). The mammalian *Tim2* ortholog is expressed in the retina, and mutations or down regulation of *dmTim2* reduces light resetting of circadian rhythms in Drosophila (Benna et al., 2010).
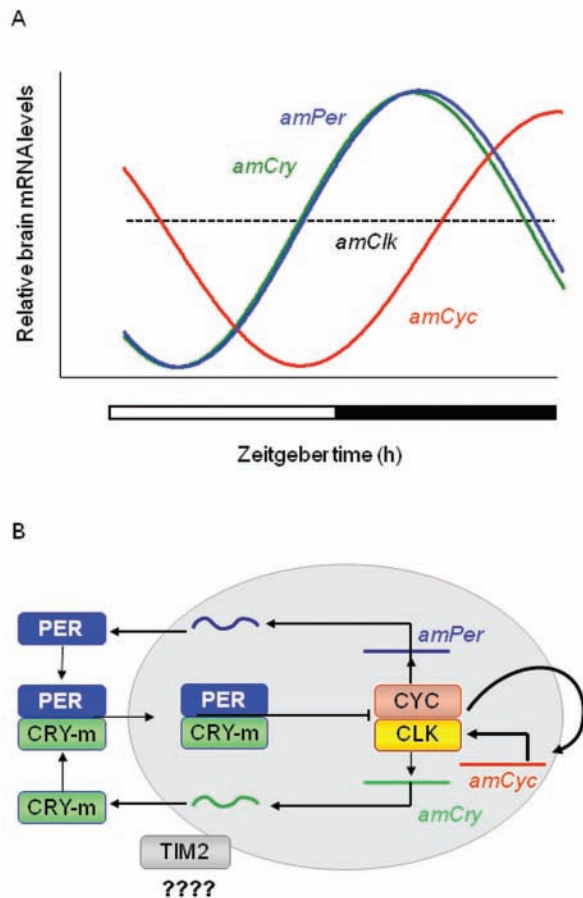
Figure 3. A model for the honeybee molecular clockwork. (A) Schematic representation of the oscillations of clock genes in the honeybee brain in LD and DD illumination regimes. The phase of mRNA cycling is shown for *Period (amPer)*, *Cryptochrome-m (amCry)*, and *Cycle (amCyc)*, for which there is a strong correlation with a cosinus model with about a 24-h cycle. The depicted lines were obtained by fitting a cosine model to values measured for foragers in Rubin et al. (2006). The phase of *amCyc* transcript is almost in antiphase to that of *amPer* and *amCry*. The straight dashed line depicts *Clock (amClk)*, which appears to have similar transcript levels throughout the day. No model is shown for *Timeout (amTim2)*, for which the pattern of mRNA variation over time was influenced by the environment. Amplitudes for the various genes are not to scale. **(B)** A schematic working model for the molecular clockwork in the honeybee brain. The honeybee genome does not contain orthologs for *Timeless (Tim1)* or Drosophila-type *Cry (Cry-d)*. It is thought that the mammalian-type *Cry (Cry-m)* functions together with *Per* in the negative feedback loop of the bee clock (Rubin et al., 2006). The model is based on the known organizational principles of the circadian clockwork in Drosophila and mammals, the structure of the putative honeybee clock proteins, and expression data available for forager honeybees (see Figs. 2 and 3A and text). Gene name abbreviations in capital letters and italic lowercase letters refer to proteins and DNA locus, respectively. The mRNA and protein for each gene are illustrated by similarly colored wavy lines and geometric shapes, respectively.

The anatomical organization of the circadian clock has not been described in detail for the honeybee or for any other social insect. Nevertheless, it is safe to assume that the central pacemaker controlling rhythmic behavior is located in the brain, as in all other insects studied so far (Helfrich-Förster, 2004; Helfrich-Förster et al., 1998; Saunders, 2002). The current picture of the anatomical organization of the honeybee clock is based largely on immunocytochemical studies with antibodies against PER and pigment dispersing factor (PDF) (Bloch et al., 2003; Zavodska et al., 2003). The most consistent PER immunoreactivity (ir) was detected in the cytoplasm of about 8 large cells in the lateral protocerebrum. Immunostaining intensity cycles with elevated levels during the night, consistent with the oscillations in brain *Per* mRNA levels (Bloch et al., 2003). Additional neurons in the optic lobes and other parts of the brain showed nuclear staining.

The Circadian Clock and the Temporal Organization of Social Behavior

The circadian clock and social foraging. Foraging in honeybees (and other social insects) is commonly seen as a social behavior and is termed "social foraging" (Seeley, 1986). By contrast to solitary animals, the honeybee worker does not forage for personal consumption or for feeding its offspring but rather adjusts its foraging behavior to meet the needs of the colony (e.g., Rinderer and Baxter, 1979; Fewell and Winston, 1992; Camazine, 1993). Timing visits to flowers was the first forager circadian behavior to be studied in honeybees (von Buttel-Reepen, 1900; Beling, 1929; Koltermann, 1971; Wahl, 1932). Bees can establish a time-memory after experiencing a single feeding bout, whereas a minimum of 3 to 4 days of restricted feeding are necessary to elicit food anticipatory activity in birds (Lindauer, 1961; Moore and Doherty, 2009; Prabhu and Cheng, 2008). Forager honeybees can learn to arrive at a specified location at any time of the day and can learn as much as 9 time points with intervals of only 45 min between feeder availability. Time memory is an internal circadian rhythm: It free-runs under constant conditions, is entrained by LD cycles, can be phase shifted, and has a narrow range of entrainment (20- to 26-h cycles), similar to other circadian oscillations (reviewed in Moore, 2001). Recent studies have shown that the time of day can be associated with complex cognitive functions. For example, bees can retrieve different memories and learn to perform different tasks at different times of day (Pahl et al., 2007; Zhang et al., 2006). This sophisticated time sense is thought to improve foraging efficiency as it enables bees to collect nectar and pollen at times of maximal

availability, which is different for different flowers (von Frisch, 1967; Willmer and Stone, 2004).

Foraging bees also rely on their circadian clock for time-compensated sun-compass orientation and for correcting their waggle-dance in accordance with the shift in the sun's azimuth. Bees use the sun as a celestial compass; they orient themselves by maintaining a fixed angle to the sun (von Frisch, 1967). Because the sun moves in a predictable path (that varies with latitude and season), the bee "consults" its circadian clock and compensates for the sun's movement over time. Foragers that stay for long periods inside the hive (e.g., due to bad weather) rely on their clock to shift the direction of their waggle dance with a remarkable correlation to the sun's path in the sky (Lindauer, 1961; von Frisch, 1967).

The sophistication of honeybees' time-memory and time-compensated sun-compass navigation raises the question of whether these behaviors have been influenced by social evolution. For example, honeybee colonies exploit large foraging areas and therefore individual foragers need to orient themselves over distances that are much larger than those explored by most solitary bees. The sophistication of the circadian behavior of the honeybee may also be related to their dance communication system that relies on precise temporal information (Dyer and Gould, 1981).

Task-related plasticity in the circadian system. The circadian clock influences complex behaviors in foragers, but not all the bees in the colony forage and not all show overt circadian rhythms. For example, nurse bees that care for the brood inside the hive are active around the clock with no circadian rhythms (Crailsheim et al., 1996; Moore et al., 1998) and have similar levels of clock gene mRNA levels throughout the day (Fig. 2; Toma et al., 2000; Bloch et al., 2001, 2004; Shemesh et al., 2007; Shemash et al., in press). Although foragers are typically older than nest bees, age alone cannot account for these differences in activity rhythms. Nurse bees switch to activity with strong circadian rhythms shortly after transfer to the laboratory, indicating that their lack of circadian rhythmicity in the hive cannot be attributed to the fact that their circadian system is not yet functional (Shemesh et al., 2007; Shemash et al., in press). In colonies with a severe shortage of nurses, some of the old foragers revert to care for the brood and are active around the clock with attenuated oscillations in clock gene mRNA levels, like nurses in normal colonies (Bloch and Robinson 2001; Bloch et al., 2001). Variation in the environment experienced by nurses and foragers (e.g., light and temperature) also fails to explain task-related plasticity in circadian rhythms. Nurses are also

active around the clock when experiencing an LD illumination regime, and foragers show strong circadian rhythms under constant conditions (Moore et al., 1998; Rubin et al., 2006; Shemesh et al., 2007; Shemesh et al., in press). Thus, plasticity in circadian rhythms relates to task and is context specific.

This association between chronobiological plasticity and the division of labor may improve task specialization and colony efficiency. Honeybee larvae are frequently attended to by nurse bees (Huang and Otis, 1991; Heimken et al., 2009); around-the-clock activity may enable nurses to provide better care for the brood. Foraging is limited to daytime and relies on the circadian clock (see above). The hypothesis that task-related plasticity in circadian rhythms is functionally significant is supported by the strong link between division of labor and the expression of circadian rhythms in honeybees and by comparative studies. There is a similar task-related plasticity in the bumblebee *Bombus terrestris*, in which division of labor is based primarily on size rather than age as in honeybees (Yerushalmi et al., 2006), and in ants, whose age-related division of labor evolved independently of that of honeybees (Ingram et al., 2009; Jong and Lee, 2008). In the bumblebee, large workers that are more likely to perform foraging activity emerge from the pupae with stronger circadian rhythms (Yerushalmi et al., 2006), and more cells in their brain are immunostained with an antiserum for the putative circadian neuropeptide PDF (Weiss et al., 2009).

Little is known about activity rhythms in workers engaged in tasks other than nursing or foraging. Observations in colonies freely foraging in the field reveal an ontogeny of diel rhythmicity. Many workers carrying out pre-foraging activities such as food storing show a 24-h activity cycle, with longer unbroken bouts of rest during the night than during the day (Moore et al., 1998; Klein et al., 2008). However, since these studies were conducted in the field it is impossible to determine whether the rhythms are internal (circadian) or influenced by daily environmental changes (diurnal). Studies in small groups under constant laboratory conditions suggest there are circadian rhythms in the aggressive behavior of "guard bees" (Troen et al., 2008). Guards are middle-aged workers that typically patrol the hive entrance, inspect incomers, and attack any suspected foe (Fig. 1C). It is difficult, however, to determine to what extent these differences stem from genuine circadian rhythms in aggressive behavior or from variation in arousal state or activity levels that may increase the probability of an aggressive encounter during the day. It is also unknown whether aggressiveness varies during the day in field colonies.

The circadian behavior of bees is context-dependent. The same nurse bee that is active around the clock while in a DD or LD illuminated hive switches to activity with robust circadian rhythms in locomotor activity shortly after transfer to an individual cage in a constant laboratory environment. Nurse bees that were transferred from the hive to a cage with about 30 other workers built up molecular oscillations in clock gene expression that were evident after ~16 h but not after 8 h in the cage (Shemesh et al., in press). These observations suggest that the circadian system of nurses is capable of generating normal circadian rhythms, but these are not expressed in the social context of the hive.

The naturally occurring chronobiological plasticity of the bee contrasts with evidence of increased pathologies and deterioration in performance in animals induced to be active with no circadian rhythms (Dunlap et al., 2004). How can the circadian system of the bee allow it to switch between activities with and without circadian rhythms? Plasticity in circadian rhythms can result from changes in the mechanism for rhythm generation, by uncoupling the central pacemaker from downstream mechanisms controlling behavior, or from external influences on behavior that override (mask) the influence of the internal clock (Fig. 4). Modifications in rhythm generation can involve several levels of clock regulation that may act individually or in concert to produce modified rhythms in behavior and physiology (Fig. 4, A-C). Studies on the molecular bases of task-related chronobiological plasticity in honeybees have shown that the brain transcript levels of *Per*, *Cry-m*, *Tim2*, and *Cyc* typically vary with circadian rhythms in foragers but are attenuated in nurses (Fig. 2). Foragers are typically older than nurses, but social manipulations uncoupling age and task indicate that the temporal pattern of clock gene expression is linked more strongly to task than to age (Toma et al., 2000; Bloch et al., 2001, 2003, 2004; Shemesh et al., 2007). Expression analyses with microarrays spotted with oligo probes for all the predicted or known honeybee transcripts further suggest that the number of oscillating brain transcripts in foragers is about 3 to 4 times (depending on the threshold set for determining oscillation) higher than in sister nurses from the same colonies (Rodriguez-Zas, Robinson, and Bloch, unpublished data). The lack of observed oscillations in clock gene expression in nurses is not an artifact in which individual nurse bees, each with a cycling clock but a different phase, are pooled together, since clock gene expression also does not cycle in nurse bees that were synchronized by a potent LD illumination regime (Shemesh et al., 2007; Shemesh et al., in press). These findings run counter to the masking and uncoupling hypotheses that assume that the circadian clockwork in nurse bees generates robust circadian oscillations that are not expressed as overt behavioral rhythms. Rather, the attenuated cycling in nurses suggests that plasticity in circadian rhythms is mediated by a reorganization of the molecular clockwork (Fig. 4). Recent studies further suggest that the lack of oscillations in nurses in the hive cannot be explained by the hypothesis that the molecular feedback loop in their clock cells is fixed at a certain point from which cycling starts up again when the nurse bee is removed from the hive. The onset of circadian rhythms in locomotor activity for nurses that were removed from the hive to the lab was correlated with the subjective morning in the hive rather than with the time removed from the hive (Shemesh et al., in press). Thus, it seems that at least some cells in the brain continue measuring time even when nurses are active around the clock with no oscillation in clock gene expression. Additional studies, including measurement of clock gene expression in identified pacemakers, are needed to better understand the molecular and neuronal bases for task-related plasticity in circadian rhythms in honeybees.

What in the worker environment modulates the switch between activity with and without circadian rhythms? The first culprit to suspect is the brood because brood care is the main activity of nurses, which are active around the clock. However, it should be noted that the transition from nursing to foraging, which is associated with plasticity in circadian rhythms, is also influenced by interactions with the queen and old workers (e.g., Huang and Robinson, 1992, 1996; Pankiw et al., 1998). Recent studies indicate that nurses are active around the clock when placed in a cage with brood but no queen or older workers. By contrast, full-sister bees of a similar age that are caged on a broodless comb inside or outside the hive are more active during the day and show robust oscillations in brain clock gene mRNA levels (Shemesh et al., in press). These observations suggest that the signals are not volatile odorants or factors in the microenvironment of the hive because these were probably similar inside and outside the mesh wall in the in-hive caging experiment. Visual signals are also not very likely because the hive is typically dark. The
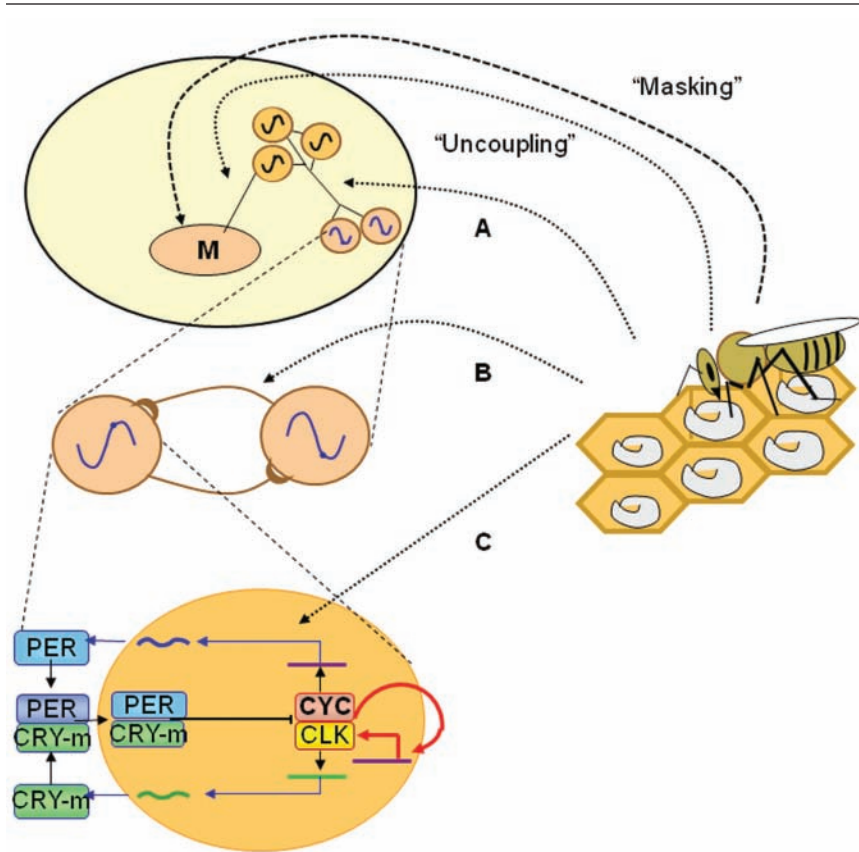
Figure 4.   Multilevel regulation of circadian rhythms and their possible social modulation. *Left bottom:* Circadian rhythms are generated autonomously within pacemaker cells. The panel shows the working model for a pacemaker in the honeybee brain (see Fig. 3B for details). *Left middle:* Interactions between cells in the same cluster are needed to generate circadian rhythms in behavior. *Left top:* Pacemaker cells and other neuronal circuits interact to produce overt circadian rhythms. The circadian network is composed of clusters of coupled pacemaker cells (circles containing sinusoidal wave lines). The large oval shape depicts the central nervous system. M = motor controlling center. *Right:* Direct interactions between nurse bees and the brood may influence circadian rhythms in several ways. Masking (upper dashed arrow) = modulation of motor controlling centers without affecting the circadian system; Uncoupling = modulating the interaction between the circadian system and motor controlling centers; A = modulating the interaction between clusters of pacemakers; B = modulating the interaction between pacemakers within the same cluster; C = modulating the circadian circuit within pacemaker cells.

Social influences of the ontogeny of circadian rhythms. The eclosion of most solitary insects studied so far, including solitary wasps (Fantinou et al., 1998; Fleury et al., 2000; there are no data for solitary bees), is gated by the circadian system such that it occurs at a specific time of day; the newly emerged adults almost instantly show circadian rhythms in locomotor activity (Saunders, 2002). In contrast, young honeybee workers (reviewed in Moore, 2001) and bumblebee workers and queens (*B. terrestris*; Yerushalmi et al., 2006; Eban-Rothschild et al., submitted) typically show no circadian rhythms during their first days as adults. The ontogeny of circadian rhythms is endogenous because it occurs under constant conditions, and rhythms free-run with a period of about 24 h. In honeybees, the ontogeny of overt circadian rhythms is associated with a development of molecular oscillations and an overall increase in brain *Per* mRNA levels (Bloch et al., 2001, 2003, 2004; Shimizu et al., 2001; Toma et al., 2000). Interestingly, despite their around-the-clock activity, newly emerged bees show a sleep-like behavior similar to that of foragers, but sleep bouts are distributed throughout the day rather than being consolidated to night time as in foragers (Eban-Rothschild and Bloch, 2008). Perhaps the posteclosion ontogeny of circadian rhythms is more common in social than solitary species because adult social insects emerge into a protected environment.

modulation of circadian rhythms appears to be mediated by direct contact with the brood, but the precise identity of these signals is yet to be determined.

The presence of brood also modulates plasticity in circadian rhythms in *B. terrestris* queens in which a nest-founding queen cares alone for the first batch of larvae (Eban-Rothschild et al., submitted). These findings are consistent with the hypothesis that around the clock nursing behavior in workers evolved from maternal behavior in the solitary ancestors of social bees (Bloch and Grozinger, in press).

Several internal signals influencing the transition from nursing to foraging activity in honeybees have been studied for their influence on the ontogeny of circadian rhythms. Manipulating neuroendocrine signaling by juvenile hormone (JH), octopamine (OA), and cyclic GMP, 3 factors that advance the age of first foraging (reviewed in Bloch et al., 2009), did not affect the age at onset of circadian rhythms in locomotor

activity (Ben-Shahar et al., 2003; Bloch et al., 2002; Bloch and Meshi, 2007). However, treatment with OA or an OA antagonist, as well as the removal of the corpora allata (the only source of JH in bees), appeared to affect brain *Per* mRNA levels. It is possible that JH or OA influences noncircadian functions of *Per* or *Per*-expressing cells involved in circadian processes other than locomotor activity.

External and specifically social factors appear to influence the ontogeny of circadian rhythms in young bees. For example, Meshi and Bloch (2007) developed an automatic video tracking system capable of high-resolution monitoring of locomotor activity in a complex social environment and showed that the onset of circadian rhythms occurred earlier in young bees housed with old foragers than in sister bees housed with a similar number of young bees. This influence of the old bees can be attributed to social masking or to a genuine influence of the development of internal circadian rhythms. The latter hypothesis is supported by studies in which bees experiencing different social environments were transferred to be monitored individually in the lab. In these studies the appearance of circadian rhythms occurred earlier in bees developing for 48 h inside the hive (even if separated from other bees by a double mesh cage) compared to same-age full-sister bees that spent a similar period outside the hive (Eban-Rothschild and Bloch, unpublished data). Finally, studies with both honeybees and bumblebees suggest that the ontogeny of circadian rhythms may be influenced by environmental and seasonal variables that affect pre-adult development (Bloch et al., 2006; Yerushalmi et al., 2006).

Social synchronization of worker activity. Some of the best evidence for social influences on the animal circadian system comes from studies of social synchronization. However, social entrainment has not been observed in all studies, including in experiments with social or group-leaving species (e.g., Frisch and Koeniger, 1994; Gattermann and Weinandy, 1997; Levine et al., 2002; Rajaratnam and Redman, 1999; Southwick and Moritz, 1987). In insect societies, social synchronization appears to be one of the mechanisms to improve coordination among individuals. For example, nectar receivers (typically ~2 to 3 weeks of age) that stay inside the hive need to coordinate their activity with that of nectar foragers (Crailsheim et al., 1999), and this can be done through social synchronization. It has been suggested that both workers and the queen function as time givers in honeybee societies (Moritz and Sakofski, 1991; Southwick and Moritz,

1987; Frisch and Koeniger, 1994). Bees are synchronized even if restricted to the inner part of the hive and hence deprived of any light and flight experience; their brain *Per* mRNA levels cycle with circadian rhythms and have higher levels at night, as is typical of foragers (Bloch et al., 2004). Social synchronization leads to an emerging colony level circadian rhythm. Colony rhythms, like individual animals, have a stable free-running period in a constant environment and their phase can be shifted by changes in environmental factors such as light, temperature, and feeding cycles (Frisch and Aschoff, 1987; Frisch and Koeniger, 1994; Kefuss and Nye, 1970; Moore, 2001).

Little is known about the signals mediating social synchronization in honeybees. Southwick and Moritz (1987) found that bees need physical contact to achieve a synchronized group rhythm. Moritz and Kryger (1994) later reported that rhythms in temperature and oxygen consumption were partially synchronized between 2 groups of workers even when separated by a solid Plexiglas partition. Synchronization improved in experiments in which the Plexiglas division was punched with holes, suggesting that both direct contact (that may include food exchange and contact pheromones) and indirect influences (e.g., temperature and volatile pheromones) may be involved in social entrainment. Social synchronization may be the product of a self-organized process in which the activity of some bees changed the environment (e.g., temperature, CO_2 concentration [Anderson and Wilkins, 1989], comb vibrations) and by that entrained the clock of other bees. Temperature is an attractive time giver for honeybees or other cavity or underground nesting social insects (Moritz and Kryger, 1994). However, typical-size colonies are tightly thermoregulated (Winston, 1987), and in controlled experiments minimal temperature oscillations of 6 to 10 °C are needed to entrain circadian rhythms in honeybees (Moore and Rankin, 1993; Fuchikawa and Shimizu, 2007). The role of chemical signals needs to be further explored because pheromones modulate almost all aspects of life in insect societies (Wilson, 1971; Hölldobler and Wilson, 1990), and the olfactory system is implicated in the social entrainment of both rodents and flies (Goel and Lee, 1997; Levine et al., 2002; Krupp et al., 2008).

Concluding Remarks

The honeybee provides an excellent system with which to study the circadian clock and complex behavior

in the real world. The studies reviewed above show that the circadian system of honeybees is very sensitive to social influences and shows remarkable plasticity. These characteristics of the bee clock may have been shaped by the social evolution of bees and the need to forage over long distances to collect food for large colonies. Socially modulated plasticity in circadian rhythms may improve the temporal organization of social behavior and overall colony efficiency. The hypothesis that social evolution influenced the circadian system is important and has implications that go far beyond the social biology of bees. However, to establish that the remarkable functions of the circadian system in honeybees are indeed linked to their social life, and not to some other aspects of their ecology or phylogeny, it is necessary to study related species showing diverse life histories. Studies on social ants and bumblebees have provided a good start by showing a similar association between division of labor and plasticity in circadian rhythms. Functionality can be also investigated by manipulating a system (the experimental approach). For example, the finding that bees induced to revert from foraging to nursing activities switched back to activity with no circadian rhythms lends credence to the hypothesis that plasticity in circadian rhythms is associated with the division of labor (Bloch and Robinson, 2001).

To understand the interplay between the circadian system and complex behaviors such as dance communication, sun-compass navigation, and the division of labor at the proximate level, it is necessary to better understand the basic chronobiology of bees. There are many gaps in our knowledge of the molecular biology of the circadian clockwork in honeybees, and the neuroanatomical characterization of the circadian system is only at its very initial stages. It is also important to study peripheral clocks in bees; recent studies suggest that peripheral clocks may influence complex behaviors such as orientation and pheromonal communication in insects (Merlin et al., 2009; Krupp et al., 2008).

The hope is that future studies will succeed in characterizing the specific social signals and the sensory modalities by which the social environment modulates the circadian behavior of bees. Honeybees are well suited for this line of inquiry because of the rich knowledge on their sociobiology and communication systems. These studies may provide a working model to explore how the behavior and physiology of individuals are temporally coordinated to create emerging colony level behavior.

REFERENCES

Anderson CM and Wilkins MB (1989) Phase resetting of the circadian-rhythm of carbon-dioxide assimilation in bryophyllum leaves in relation to their malate content following brief exposure to high and low-temperatures, darkness and 5-percent carbon-dioxide. Planta 180:61-73.

Beling I (1929) Über das Zeitgedächtnis der Bienen. Zeitschrift für Vergleichende Physiologie 9:259-388.

Ben Shahar Y, Leung HT, Pak WL, Sokolowski MB, and Robinson GE (2003) cGMP-dependent changes in phototaxis: A possible role for the foraging gene in honeybee division of labor. J Exp Biol 206:2507-2515.

Benna C, Bonaccorsi S, Wulbeck C, Helfrich-Forster C, Gatti M, Kyriacou CP, Costa R, and Sandrelli F (2010) Drosophila timeless2 is required for chromosome stability and circadian photoreception. Curr Biol 20:346-352.

Bloch G and Robinson GE (2001) Reversal of honeybee behavioural rhythms. Nature 410:1048.

Bloch G and Meshi A (2007) Influences of octopamine and juvenile hormone on locomotor behavior and *period* gene expression in the honeybee, *Apis mellifera*. J Comp Physiol A 193:181-199.

Bloch G and Grozinger CM (in press) Social pathways, their origins, and modifications along the evolution of sociality in bees. Phil Trans Royal Soc B.

Bloch G, Toma DP, and Robinson GE (2001) Behavioral rhythmicity, age, division of labor and period expression in the honeybee brain. J Biol Rhythms 16:444-456.

Bloch G, Sullivan JP, and Robinson GE (2002) Juvenile hormone and circadian locomotor activity in the honeybee *Apis mellifera*. J Insect Physiol 48:1123-1131.

Bloch G, Solomon SM, Robinson GE, and Fahrbach SE (2003) Patterns of PERIOD and pigment-dispersing hormone immunoreactivity in the brain of the European honeybee (*Apis mellifera*): Age- and time-related plasticity. J Comp Neurol 464:269-284.

Bloch G, Rubinstein CD, and Robinson GE (2004) *period* expression in the honeybee brain is developmentally regulated and not affected by light, flight experience, or colony type. Insect Biochem Mol Biol 34:879-891.

Bloch G, Shemesh Y, and Robinson GE (2006) Seasonal and task-related variation in free running activity rhythms in honey bees (*Apis mellifera*). Insectes Soc 53:115-118.

Bloch G, Shpigler H, Wheeler DE, and Robinson GE (2009) Endocrine influences on the organization of insect societies. Volume II. Non-mammalian hormone-behavior systems, non-mammalian invertebrates. In *Hormones,*

Brain and Behavior, Pfaff D, Arnold A, Etgen A, Fahrbach SE, and Rubin R, eds., pp 1027-1068, San Diego, CA, Academic Press.

Camazine S (1993) The regulation of pollen foraging by honey-bees—How foragers assess the colony need for pollen. Behav Ecol Sociobiol 32:265-272.

Crailsheim K, Hrassnigg N, and Stabentheiner A (1996) Diurnal behavioural differences in forager and nurse honey bees (*Apis mellifera carnica* Pollm). Apidologie 27:235-244.

Crailsheim K, Riessberger U, Blaschon B, Nowogrodzki R, and Hrassnigg N (1999) Short-term effects of simulated bad weather conditions upon the behaviour of food-storer honeybees during day and night (*Apis mellifera carnica* Pollmann). Apidologie 30:299-310.

Dunlap JC, Loros JJ, and DeCoursey PJ (2004) *Chronobiology: Biological Timekeeping.* Sunderland, MA: Sinauer Associates Inc.

Dyer FC (2002) The biology of the dance language. Annu Rev Entomol 47:917-949.

Dyer FC and Gould JL (1981) Honey bee orientation—A backup system for cloudy days. Science 214:1041-1042.

Eban-Rothschild AD and Bloch G (2008) Differences in the sleep architecture of forager and young honeybees (*Apis mellifera*). J Exp Biol 211:2408-2416.

Eban-Rothschild AD, Belluci S, and Bloch G. Maternity-related plasticity in circadian rhythms of bumble bee (*Bombus terrestris*) queens. Submitted.

Fantinou AA, Alexandri MP, and Tsitsipis JA (1998) Adult emergence rhythm of the egg-parasitoid *Telenomus busseolae*. BioControl 43:141-151.

Fewell JH and Winston ML (1992) Colony state and regulation of pollen foraging in the honey-bee, *Apis-mellifera* L. Behav Ecol Sociobiol 30:387-393.

Fleury F, Allemand R, Vavre F, Fouillet P, and Bouletreau M (2000) Adaptive significance of a circadian clock: Temporal segregation of activities reduces intrinsic competitive inferiority in Drosophila parasitoids. Proc Biol Sci 267:1005-1010.

Frisch B and Aschoff J (1987) Circadian rhythms in honeybees: Entrainment by feeding cycles. Physiol Entomol 12:41-49.

Frisch B and Koeniger N (1994) Social synchronization of the activity rhythms of honeybees within a colony. Behav Ecol Sociobiol 35:91-98.

Fuchikawa T and Shimizu I (2007) Effects of temperature on circadian rhythm in the Japanese honeybee, *Apis cerana japonica*. J Insect Physiol 53:1179-1187.

Gattermann R and Weinandy R (1997) Lack of social entrainment of circadian activity rhythms in the solitary golden hamster and in the highly social Mongolian gerbil. Biol Rhythm Res 28:85-93.

Goel N and Lee TM (1997) Olfactory bulbectomy impedes social but not photic reentrainment of circadian rhythms in female *Octodon degus*. J Biol Rhythms 12:362-370.

Heimken C, Aumeier P, and Kirchner WH (2009) Mechanisms of food provisioning of honeybee larvae by worker bees. J Exp Biol 212:1032-1035.

Helfrich-Förster C (2004) The circadian clock in the brain: A structural and functional comparison between mammals and insects. J Comp Physiol A 190:601-613.

Helfrich-Förster C, Stengl M, and Homberg U (1998) Organization of the circadian system in insects. Chronobiol Internat 15:567-594.

Hölldobler B and Wilson EO (1990) *The Ants*. Cambridge (MA): Belknap Press.

Huang ZY and Otis GW (1991) Inspection and feeding of larvae by worker honey-bees (hymenoptera, apidae)—Effect of starvation and food quantity. J Insect Behav 4:305-317.

Huang ZY and Robinson GE (1992) Honeybee colony integration: Worker–worker interactions mediate hormonally regulated plasticity in division of labor. Proc Nat Acad Sci U S A 89:11726-11729.

Huang ZY and Robinson GE (1996) Regulation of honey bee division of labor by colony age demography. Behav Ecol Sociobiol 39:147-158.

Ingram K, Krummey S, and LeRoux M (2009) Expression patterns of a circadian clock gene are associated with age-related polyethism in harvester ants, *Pogonomyrmex occidentalis*. BMC Ecology 9:7.

Jong JJ and Lee HJ (2008) Differential expression of circadian locomotor rhythms among castes of the gray-black spiny ant, *Polyrhachis dives* (Hymenoptera: Formicidae). Sociobiology 52:167-184.

Kefuss JA and Nye WP (1970) The influence of photoperiod on the flight activity of honeybees. J Apic Res 9:133-139.

Klein BA, Olzsowy KM, Klein A, Saunders KM, and Seeley TD (2008) Caste-dependent sleep of worker honey bees. J Exp Biol 211:3028-3040.

Koltermann R (1971) 24-Std-periodik in der langzeiterinnerung an duft- und farbsignale bei der honigbiene. Zeitschrift für Vergleichende Physiologie 75:49-68.

Krupp JJ, Kent C, Billeter JC, Azanchi R, So AK, Schonfeld JA, Smith BP, Lucas C, and Levine JD (2008) Social experience modifies pheromone expression and mating behavior in male *Drosophila melanogaster*. Curr Biol 18:1373-1383.

Le Conte Y and Hefetz A (2008) Primer pheromones in social hymenoptera. Ann Rev Entomol 53:523-542.

Levine JD, Funes P, Dowse HB, and Hall JC (2002) Resetting the circadian clock by social experience in *Drosophila melanogaster*. Science 298:2010-2012.

Lindauer M (1961) *Communication among Social Bees*. Cambridge, MA, Harvard University Press.

Majercak J, Sidote D, Hardin PE, and Edery I (1999) How a circadian clock adapts to seasonal decreases in temperature and day length. Neuron 24:219-230.

Majercak J, Chen WF, and Edery I (2004) Splicing of the *period* gene 3′-terminal intron is regulated by light, circadian clock factors, and phospholipase C. Mol Cel Biol 24:3359-3372.

Merlin C, Gegear RJ, and Reppert SM. 2009. Antennal circadian clocks coordinate sun compass orientation in monarch butterflies. Science 325:1700-1704.

Meshi A and Bloch G (2007) Monitoring circadian rhythms of individual honey bees in a social environment reveals social influences on postembryonic ontogeny of activity rhythms. J Biol Rhythms 22:343-355.

Michener CD (1974) *The Social Behavior of the Bees*. Cambridge (MA): Belknap Press of Harvard University Press.

Moore D (2001) Honeybee circadian clocks: Behavioral control from individual workers to whole-colony rhythms. J Insect Physiol 47:843-857.

Moore D and Rankin MA (1993) Light and temperature entrainment of a locomotor rhythm in honeybees. Physiol Entomol 18:271-278.

Moore D and Doherty P (2009) Acquisition of a time-memory in forager honey bees. J Comp Physiol A 195:741-751.

Moore D, Angel JE, Cheeseman IM, Fahrbach SE, and Robinson GE (1998) Timekeeping in the honeybee colony: Integration of circadian rhythms and division of labor. Behav Ecol Sociobiol 43:147-160.

Moritz RFA and Sakofski F (1991) The role of the queen in circadian rhythms of honeybees (*Apis mellifera* L.). Behav Ecol Sociobiol 29:361-365.

Moritz RFA and Kryger P (1994) Self-organization of circadian rhythms in groups of honeybees (*Apis mellifera* L.). Behav Ecol Sociobiol 34:211-215.

Pahl M, Zhu H, Pix W, Tautz J, and Zhang SW (2007) Circadian timed episodic-like memory—A bee knows what to do when, and also where. J Exp Biol 210:3559-3567.

Pankiw T, Huang ZY, Winston ML, and Robinson GE (1998) Queen mandibular gland pheromone influences worker honey bee (*Apis mellifera* l.) foraging ontogeny and juvenile hormone titers. J Insect Physiol 44:685-692.

Prabhu C and Cheng, K. (2008) One day is all it takes: Circadian modulation of the retrieval of colour memories in honeybees. Behav Ecol Sociobiol 63:11-22.

Rajaratnam SMW and Redman JR (1999) Social contact synchronizes free-running activity rhythms of diurnal palm squirrels. Physiol Behav 66:21-26.

Rinderer TE and Baxter JR (1979) Honey bee hoarding behavior—Effects of previous stimulation by empty comb. Anim Behav 27:426-428.

Robinson GE (1992) Regulation of division of labor in insect societies. Annu Rev Entomol 37:637-665.

Rubin EB, Shemesh Y, Cohen M, Elgavish S, Robertson HM, and Bloch G (2006) Molecular and phylogenetic analyses reveal mammalian-like clockwork in the honey bee (*Apis mellifera*) and shed new light on the molecular evolution of the circadian clock. Gen Res 16:1352-1365.

Saunders DS (2002) *Insect Clocks*. Amsterdam, Elsevier.

Seeley TD (1986) Social foraging by honeybees—How colonies allocate foragers among patches of flowers. Behav Ecol Sociobiol 19:343-354.

Seeley TD (1995) *The Wisdom of the Hive*. Cambridge (MA): Harvard University Press.

Shemesh Y, Cohen M, and Bloch G (2007) Natural plasticity in circadian rhythms is mediated by reorganization in the molecular clockwork in honeybees. FASEB J 21:2304-2311.

Shemesh Y, Eban-Rothschild AD, Cohen M, and Bloch G. Molecular dynamics and social regulation of context-dependent plasticity in the circadian clockwork of the honey bee. J Neurosci. In press.

Shimizu I, Kawai Y, Taniguchi M, and Aoki S (2001) Circadian rhythm and cDNA cloning of the clock gene *period* in the honeybee *Apis cerana japonica*. Zool Sci 18:779-789.

Southwick EE and Moritz RFA (1987) Social synchronization of circadian rhythms of metabolism in honeybees (*Apis mellifera*). Physiol Entomol 12:209-212.

Thom C, Gilley DC, Hooper J, and Esch HE (2007) The scent of the waggle dance. PLoS Biol 5(9):e228. doi:10.1371/journal.pbio.0050228.

Toma DP, Bloch G, Moore D, and Robinson GE (2000) Changes in *period* mRNA levels in the brain and division of labor in honey bee colonies. Proc Nat Acad Sci U S A 97:6914-6919.

Troen H, Dubrovsky I, Tamir R, and Bloch G (2008) Temporal variation in group aggressiveness of honeybee (*Apis mellifera*) guards. Apidologie 39:283-291.

Velarde RA, Sauer CD, Walden KKO, Fahrbach SE, and Robertson HM (2005) Pteropsin: A vertebrate-like non-visual opsin expressed in the honey bee brain. Insect Biochem Mol Biol 35:1367-1377.

von Buttel-Reepen HB (1900) Sind die Bienen Reflexmaschinen? Biol Zentralbl 20:177-192.

von Frisch K (1967) *The Dance Language and Orientation of Bees*. Cambridge (MA): Harvard University Press.

Wahl O (1932) Neue Untersuchungen ueber das Zeitgedaechtnis der Bienen. Zeitschrift fuer Vergleichende Physiologie 16:529-589.

Weinstock GM, Robinson GE, Gibbs RA, et al. (2006) Insights into social insects from the genome of the honeybee *Apis mellifera*. Nature 443:931-949.

Weiss R, Dov A, Fahrbach SE, and Bloch G (2009) Body size-related variation in pigment dispersing factor-immunoreactivity in the brain of the bumblebee *Bombus terrestris* (Hymenoptera, Apidae). J Insect Physiol 55:479-487.

Willmer PG and Stone GN (2004) Behavioral, ecological, and physiological determinants of the activity patterns of bees. Adv Study Behav 34:347-466.

Wilson EO (1971) *The Insect Societies*. 3rd ed. Cambridge (MA): Belknap Press of Harvard University Press.

Winston ML (1987) *The Biology of the Honey Bee*. Cambridge (MA): Harvard University Press.

Yerushalmi S, Bodenheimer S, and Bloch G (2006) Developmentally determined attenuation in circadian rhythms links chronobiology to social organization in bees. J Exp Biol 209:1044-1051.

Yuan Q, Metterville D, Briscoe AD, and Reppert SM (2007) Insect cryptochromes: Gene duplication and loss define diverse ways to construct insect circadian clocks. Mol Biol Evol 24:948-955.

Zavodska R, Sauman I, and Sehnal F (2003) Distribution of PER protein, pigment-dispersing hormone, prothoracicotropic hormone, and eclosion hormone in the cephalic nervous system of insects. J Biol Rhythms 18:106-122.

Zhang SW, Schwarz S, Pahl M, Zhu H, and Tautz J (2006) Honeybee memory: A honeybee knows what to do and when. J Exp Biol 209:4420-4428.