Circadian Foraging Rhythms of Bumblebees Monitored by Radio-frequency Identification

Ralph Jürgen Stelzer, Ralf Stanewsky and Lars Chittka

J Biol Rhythms 2010 25: 257

DOI: 10.1177/0748730410371750

Circadian Foraging Rhythms of Bumblebees Monitored by Radio-frequency Identification

Ralph Jürgen Stelzer,[1] Ralf Stanewsky, and Lars Chittka
Queen Mary University of London, School of Biological and Chemical Sciences, Mile End Road, London, UK

Abstract Circadian clocks enable organisms to anticipate changes of environmental conditions. In social insects, the colony as a superorganism has a foraging rhythm aligned to the diurnal patterns of resource availability. Within this colony rhythm, the diurnal patterns of individuals are embedded, and various tasks within the colony are performed at different times by different individuals to best serve the colony as a whole. Recent studies have shown that social cues influence the traits of the circadian clock in social insects, but keeping track of the activity of individual workers is not an easy task. Here the authors use fully automatic radio-frequency identification (RFID) to analyze the circadian rhythms of bumblebee foragers (*Bombus terrestris*) in the normal social context of their nest. They monitored their foraging patterns under different light conditions in the laboratory, including light:dark cycles (LD) as well as constant darkness (DD) and constant light conditions (LL). Their results show that the majority of bumblebee foragers exhibit robust circadian rhythms in LD under laboratory conditions, while they show free-running rhythms both in DD and LL, with free-running periods being significantly shorter in LL conditions. The authors also found that bumblebee workers show an increased level of arrhythmic activity ("death dance") in the hours or days before their death.

Key words *Bombus terrestris*, foraging rhythm, sociality, social cues, RFID, death dance

Circadian rhythms enable organisms to anticipate and to prepare for predictable changes in their environment. These rhythms are generated by endogenous clocks, which cycle with a period of about—but not exactly—24 hours. To entrain them precisely to the 24-hour day, these clocks are synchronized by external cues or zeitgebers, the most prominent being the natural light-dark cycles and associated temperature variations (Dunlap et al., 2004; Fuchikawa and Shimizu, 2007; Glaser and Stanewsky, 2005; Hardin, 2006; Refinetti, 2006; Stanewsky, 2003). The molecular clock is best understood in an insect, the solitary fruit fly *Drosophila melanogaster* (Helfrich-Forster, 2005; Rosato et al., 2006; Stanewsky, 2002). Typically, individuals of such solitary species such as *Drosophila* only have to adjust their own behavior rhythms to daily patterns of daylight and conditions that fluctuate in synchrony with light levels, although recent studies have shown that circadian rhythms in *Drosophila* can also be influenced by social cues (Levine, 2004; Levine et al., 2002b).

Conversely, in colonies of social insects, there exists a high level of division of labor. Different tasks, like foraging or tending the brood, are allocated to different individuals of the colony to best serve the need of the colony as a whole (Robinson, 1992; Wilson, 1971,

1. To whom all correspondence should be addressed: Ralph J. Stelzer, Queen Mary University of London, School of Biological and Chemical Sciences, Mile End Road, London, E1 4NS, United Kingdom; e-mail: r.stelzer@qmul.ac.uk.

JOURNAL OF BIOLOGICAL RHYTHMS, Vol. 25 No. 4, August 2010 257-267
DOI: 10.1177/0748730410371750

2000). The colony as a superorganism has circadian rhythms—for example, a foraging rhythm aligned to the diurnal patterns of resource availability—and within this colony rhythm, the diurnal patterns of individuals are embedded (Frisch and Aschoff, 1987; Frisch and Koeniger, 1994; Moore, 2001).

In honeybees, division of labor is age related (polyethism) and concurrent with the ontogeny of circadian rhythms in individual bees. Workers usually do not forage until they are about 3 weeks of age (Johnson, 2010). Before becoming foragers, the workers only show weak or no rhythms at all while performing within-nest tasks such as cleaning the hive or tending the brood (Crailsheim et al., 1996; Moore et al., 1998). Developing larvae inside the nest need care around the clock. Therefore, having arrhythmic nurses might be an advantage and guarantees that the brood gets the attention it needs at all times. Foragers, on the other side, need a working circadian clock for sun-compass navigation (von Frisch, 1967) and for timing their visits to flowers when their production of nectar and pollen is high (Beling, 1929; Koltermann, 1971; von Frisch, 1967; Wahl, 1932). The age-related change in locomotor rhythmicity of honeybee workers is also associated with changes of their molecular circadian clock, for example, a changed expression of *period* (*per*), a clock gene, whose product is a key player of the central molecular pacemaker in insects and vertebrates (Bloch et al., 2001; Stanewsky, 2003; Toma et al., 2000). However, foragers can switch back to within-nest tasks and to tending the brood around the clock when nurses are removed from the hive (Bloch and Robinson, 2001; Rösch, 1930). On the other hand, if foragers are removed from the hive, young bees will start foraging sooner, accompanied by the development of robust rhythms sooner than normally (Huang and Robinson, 1992; Robinson et al., 1989; Toma et al., 2000).

Thus, social cues can have a major influence on the development and strength of circadian rhythms in social insects (Meshi and Bloch, 2007), and monitoring circadian rhythms in their normal social environment would therefore provide valuable information. But keeping track of the foraging activity—for example, of individual bees around the clock in their normal social context—is experimentally demanding, not only due to the sheer number of individuals in a colony but also because the identification of individual animals poses another major problem, especially in constant darkness during circadian studies. Data collection by human observers or video equipment requires a constant illumination of some sort. This problem can be avoided by using passive radio-chips for fully automatic radio-frequency identification, which has recently become available for studies on social insects (Molet et al., 2008; Robinson et al., 2009; Streit et al., 2003; Sumner et al., 2007). Since radio-technology is used, observations can take place in complete darkness, and the foraging activity of dozens or even hundreds of workers in different nests can be monitored simultaneously over their whole life span. Most important, the tagged workers experience their normal environment inside the nest and perform their behaviors in their normal social context.

In this study, we use radio-frequency identification (RFID) to record and analyze circadian rhythms in the foraging patterns of bumblebees (*Bombus terrestris*), both on the colony and the individual level, under different light conditions in the laboratory (12h:12h light:dark cycle, constant darkness and continuous light). In contrast to honeybees, division of labor in bumblebees is related to the body size of the workers (Alford, 1975; Michener, 1974). Small workers are more likely to stay in the nest and take over within-nest tasks (nurses), while bigger workers have a higher probability of becoming foragers, leaving the nest for the first time at about 3 days of age after eclosion from the pupae (Cumber, 1949; Free, 1955; Garófalo, 1978). Like in honeybees, division of labor in bumblebees is also associated with a remarkable plasticity in the circadian rhythms of foragers and nurses. Observations on free-flying bumblebees have shown that large workers forage with robust diurnal circadian rhythms, while smaller workers perform their within-nest duties with only weak rhythmicity (Yerushalmi et al., 2006). Bumblebee queens are typically singly mated, which means that due to the haplodiploid sex determination system in social insects, workers of a colony (full sisters) have a mean of 75% of their genes in common. Body size variation within a bumblebee nest is largely determined by the amount of food the workers receive as larvae (Plowright and Jay, 1968), and body size, in turn, is a major determinant of worker specialization. Under constant laboratory conditions, small bumblebee workers also show weaker circadian rhythms in their locomotor activity, while larger workers have stronger rhythms and develop them sooner (Yerushalmi et al., 2006). However, the bees tested in that study were individually placed in separate monitoring cages (see Yerushalmi et al., 2006, for details) and thus lacked any social cues from their nest mates. Here, we analyze circadian rhythms of bumblebee

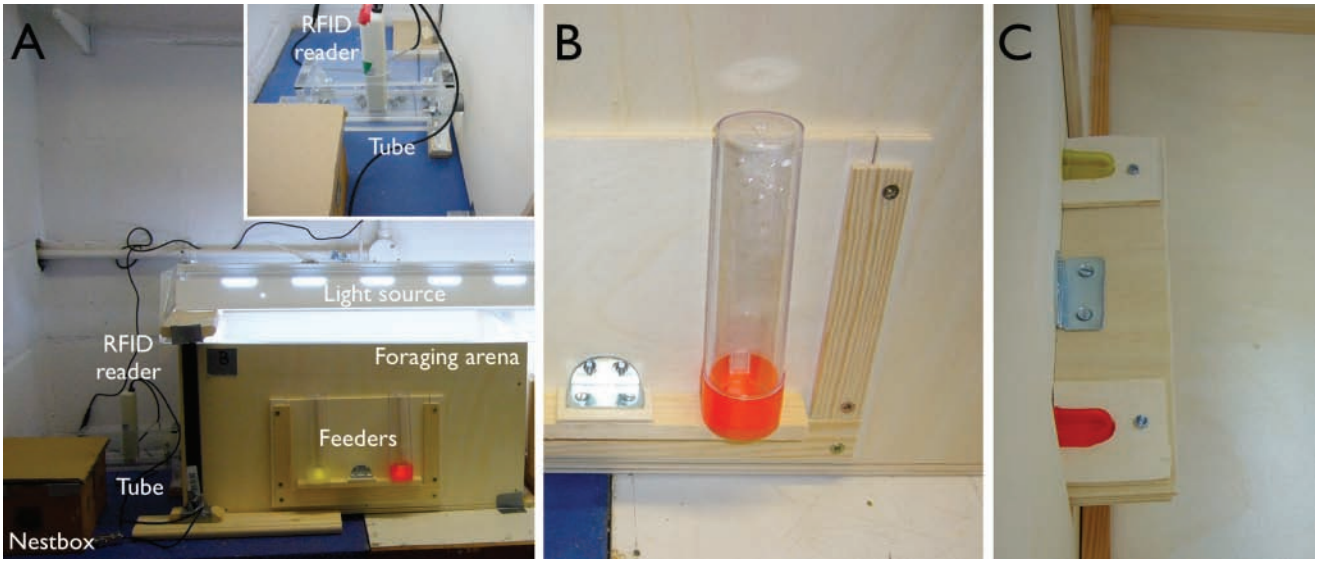

Figure 1. Experimental setup. (A) Nest boxes were connected to the foraging arenas via transparent Plexiglas tubes with an integrated RFID reader that automatically recorded passing radio-tagged workers. (B) Sucrose solution in the arenas was provided by birdcage feeders, which were mounted to the outside wall of the arenas for easy access by the observers. (C) The openings of the feeders extended into the arenas through fitted holes in the wall.

foragers for the first time in their normal social context under laboratory conditions.

MATERIAL AND METHODS

Experimental Colonies and Setup

In total, we tested 13 queenright (i.e., including a queen) *Bombus terrestris* (subspecies *terrestris*) colonies under different light conditions. The experiments were conducted in 4 distinct runs. In the first run, only 1 colony was tested, while in each of the following 3 runs, 4 colonies were tested simultaneously under the same environmental conditions. Five colonies (the first 2 runs) were kept under a 12h:12h light:dark cycle (LD) for the first 7 days of the experiment, followed by another 7 days in constant darkness (DD). The remaining 8 colonies (2 runs) were also kept under LD conditions for the first 7 days, followed by constant light conditions (LL) for the following 7 days. Colonies were obtained from a commercial breeder (Syngenta Bioline Bees, Weert, Netherlands) shortly before they were tested. The colonies contained between 40 and 80 workers upon arrival and made a healthy impression throughout the experiments. The colonies were housed in plywood nest boxes (28 × 16 × 11 cm), which were divided into a nest chamber (rear) and a front chamber where the bees could deposit their waste, and were covered with Plexiglas lids. The nest and the front chamber had 3 and 2, respectively, small mesh-covered holes (ø = 2 cm) in their sidewalls to allow for ventilation inside the box. The boxes were connected to foraging arenas (60 × 40 × 30 cm) via a transparent Plexiglas tunnel with a system of shutters to enable movements of bees into and out of the nest to be controlled by the observer (Fig. 1).

During the experiments, the bees were fed on 50% sucrose solution (v/v) provided ad libitum through standard birdcage feeders/fountains (fountain and feeder 5415, TRIXI Heimtierbedarf GmbH & Co KG, Tarp, Germany) mounted to the outside walls of the arenas (Fig. 1). That way, we were able to refill the feeders without disturbing the bees. The foragers were able to reach the feeders either by flying or by walking up the arena wall. Two of these feeders were attached to every arena. Pollen, which is used as protein source by the bees, especially by developing larvae, was provided directly into the nest box every other day at random times of day to avoid it becoming a temporal stimulus for the bees. Room temperature was automatically recorded once every other minute (run A) or every minute (runs B and C) using a data logger (HOBO® U10-001 Temperature Data Logger, Onset Computer Corporation, Pocasset, MA, USA). The mean room temperatures during the individual runs were as follows: 23.8 °C ± 1.3 °C (run A;

mean daily fluctuations 1.4 °C ± 0.5 °C), 23.5 °C ± 0.7 °C (run B; mean daily fluctuations 1.0 °C ± 0.4 °C), and 23.3 °C ± 1.0 °C (run C; mean daily fluctuations 1.2 °C ± 0.5 °C; all values are mean ± SD). Colonies were placed inside a windowless room. Illumination (where needed) was provided by high-frequency lightning (TMS 24F lamps with HF-B 236 TLD [4.3-KHz] ballasts, Philips, Netherlands), fitted each with 1 Activa daylight fluorescent tube (Osram, Germany) and 1 ultraviolet (UV) blacklight fluorescent tube. Light intensities in the 4 foraging arenas varied between 2168 lux and 2435 lux (measured at the bottom of the foraging arenas using a PCE-L335 Lux Meter, PCE Group, oHG, Meschede, Germany). The occasions when a researcher entered the experimental room, to feed the bees and download the data from the RFID readers (see below), were reduced to an absolute minimum (once a day). The times when the room was entered were chosen randomly. During the DD parts, the experimental room was always entered from another dark room, and work inside the experimental room (replacing the feeders and downloading the data) was done using night vision goggles with an directional infrared (IR) light source (>750 nm), which was only used shortly when it was absolutely necessary, although bumblebees cannot see light above 650 nm (Chittka and Waser, 1997).

Radio-frequency Technology (RFID)

To be able to monitor the complete foraging activity of individual bees, RFID was used (Molet et al., 2008; Streit et al., 2003). Small RFID tags (mic3®-TAG 64 bit RO, iID2000, 13.56 MHz system, 1.0 × 1.6 × 0.5 mm; Microsensys GmbH, Erfurt, Germany) were glued to the dorsal surface of the thorax of the bees (see Figure S1 in the supplementary online material for a picture of tagged workers). An RFID reader (iID2000, 2k6 HEAD; Microsensys GmbH) was integrated into the tunnel close to the nest entrance (Fig. 1). All bees were allowed to leave and enter the nest at will during the experiments. The RFID reader automatically recorded date and time when a tagged worker passed it, as well as the identity of the passing bee. Preliminary tests revealed a high reliability of the RFID system (Molet et al., 2008). The data were downloaded from the RFID readers every other day at different times of the day.

Data Analysis

Level of activity was analyzed in 60-minute bins. The resolution on the individual level was chosen as 1 minute (i.e., for each hour, the number of minutes in which a given tagged bee passed the reader at least once was counted and used as level of activity within that hour). The raw data downloaded from the RFID readers were processed accordingly, using macros (Virtual Basic for Applications [VBA]) developed by the authors in Microsoft Excel. The processed data were then used for the circadian analysis on both the colony and the individual level in Matlab (version R2007a, The Mathworks), using existing functions for *Drosophila* (Landskron et al., 2009; Levine et al., 2002c), which were adjusted to be able to process the bumblebee data. The actual algorithms for the circadian analysis were not changed. For the analysis on the colony level, all available processed data for a given colony were used. On the individual level, only bees that were active during at least 6 consecutive days during each light regime block were used. Actograms and daily average histograms were plotted in Matlab. Statistical significance of circadian rhythms was assessed using the term Rhythmicity Statistics (RS), which provides a numerical accounting of significance (Levine et al., 2002a). The RS value for each individual bee was obtained through autocorrelation analysis performed in Matlab (Levine et al., 2002c). Bees with an RS value of 1 or above were considered rhythmic (see Levine et al., 2002a, for how this cutoff was determined). The free-running periods were calculated using Maximum Entropy Spectral Analysis (MESA) (Levine et al., 2002a, 2002c). Mean period lengths under LL and DD conditions were compared using an independent *t* test, with the colonies as unit of replication.

RESULTS

In LD, the foragers showed a clear diurnal pattern of their foraging activity with the majority of it taking place during the photophase and being lowest during the middle of the scotophase (Fig. 2). In all colonies, the bees anticipated the lights-on change in the circadian morning, indicated by a raised activity starting 1 to 3 hours before the actual light change (Fig. 2). Some colonies also showed activity peaks in the hours following the lights-off change in the evening; however, anticipation of the lights-off change in the evening (i.e., an increasing activity in the hours before the lights-off change) can only been seen in two colonies (B2 and B3), and even in these colonies, it is rather weak (Fig. 2).

On the colony level, the colonies showed a robust rhythm in LD (Fig. 3), while they exhibited free-running rhythms in DD (Fig. 3). In contrast, the

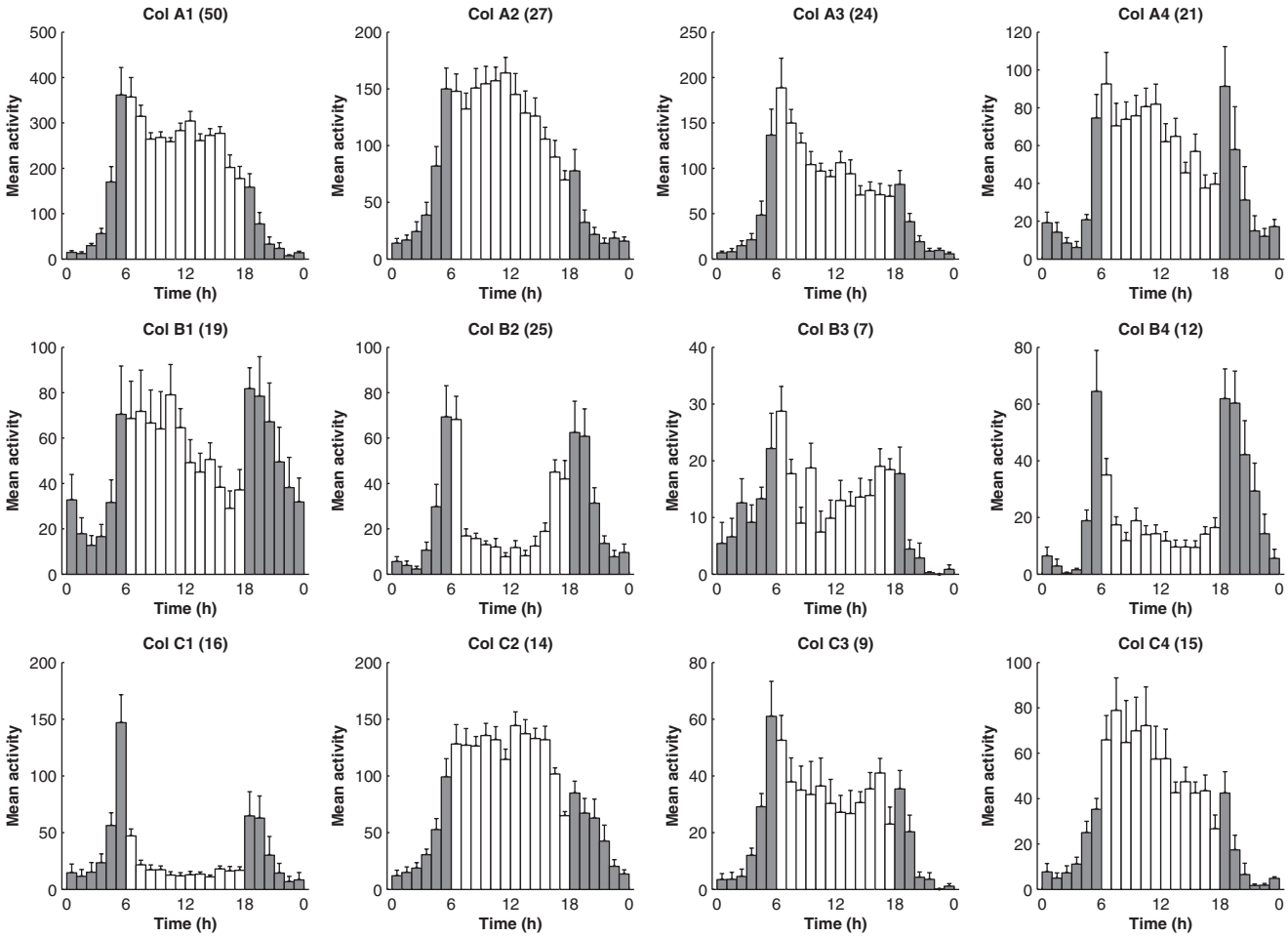

Figure 2. Daily average plots of the colonies tested in the 3 groups for the LD portions of the experiments (7 days). Numbers in brackets indicate the number of tagged workers that could be used for the individual circadian analysis in the colony. Each bar represents an hour of the day, the height of the bars indicates the level of activity, and the white bars indicate when the lights were on.

colonies lose their rhythms completely under constant light conditions (LL), with foraging activity being high around the clock (Fig. 3).

However, the majority of individual bees (57.9% ± 3.9%; mean ± 1 SEM; Fig. 4) stay rhythmic in constant light conditions and show free-running rhythms with a mean period of 22.4 ± 0.1 h (Figs. 3 and 4). The variation in free-running period length of the rhythmic bees in LL and the irregular activity of the rest of the bees under these conditions add up to the constant activity around the clock seen on the colony level under constant light conditions. In the other two light regimes, not all bees were rhythmic either (Fig. 4), but the majority of individual bees showed robust rhythms in LD (68.7% ± 3.6%) and free-running rhythms in DD (69.8% ± 4.6%). The periods of the free-running rhythms of individual bees were significantly longer in DD (24.0 ± 0.2 h) than in LL (22.4 ± 0.1 h; *t* test, $t = -9.335$, $p < 0.001$, $df = 11$; Fig. 4). See Table S1 in the supplementary online material for a summary of the data obtained from each colony.

Foragers did not typically use the whole available photoperiod to forage in LD. Foraging activity of individual foragers was often restricted to a few hours in the morning and in the evening (Fig. 5).

Foragers that died during our experiments often showed an increased arrhythmic activity in the hours or even days before their death (Fig. 5), a phenomenon known as "death dance" in *Drosophila* (Levine et al., 2002c). At first they show normal, rhythmic activity patterns, until their activity level suddenly increases. They then stay active without any rest until they die.

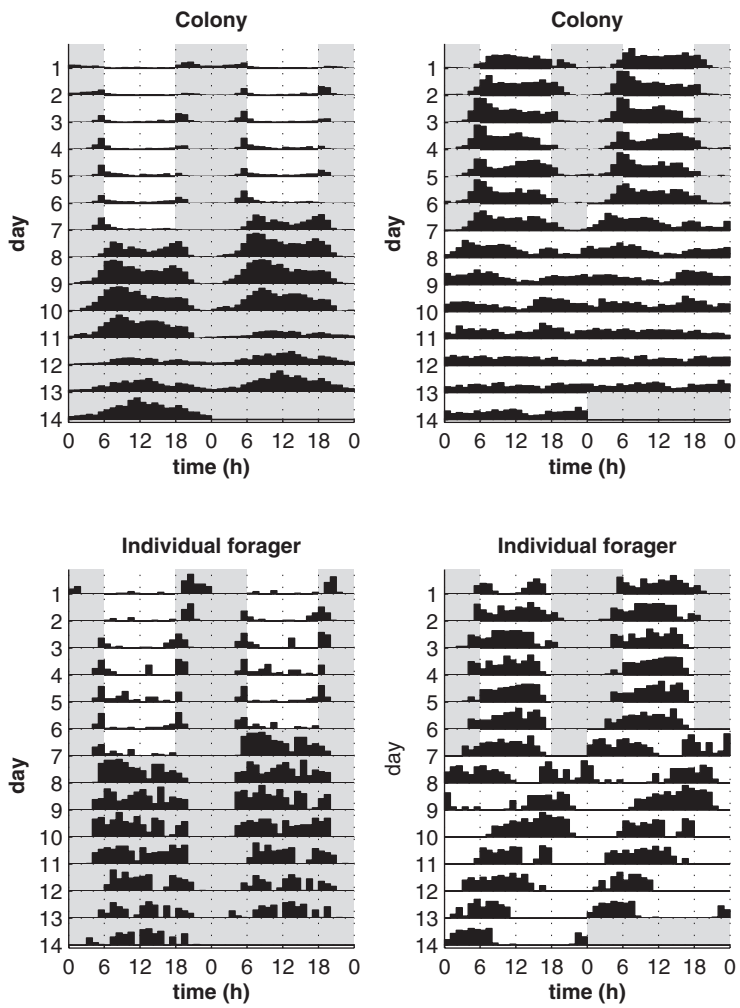

Figure 3. Exemplary double-plotted actograms of 2 colonies (A1 and C1) showing the data of all tagged workers (top line) and of 2 individual foragers (bottom line). The left row shows the data for 1 colony (top) tested in LD followed by DD and for 1 individual bee of that colony (bottom). The colony on the top right was kept in LD followed by LL; the individual forager shown underneath is from that colony. Each line represents 2 consecutive days of the experiment, each bar indicates 1 hour of the day, and the height of the bars indicates the level of foraging activity in that hour. Gray areas indicate when the lights were off. Note that the level of activity increased in DD conditions.

DISCUSSION

Our results show that the majority of bumblebee foragers exhibit robust circadian rhythms in LD under laboratory conditions, while they show free-running rhythms both in DD and LL, with free-running periods being significantly shorter in LL conditions.

As expected for diurnal animals, the majority of the foraging activity was taking place during the photophase in LD conditions. Robust rhythms were found at the colony level, as well as for the majority of individual workers (Figs. 3 and 4). The anticipation of the lights-on change, indicated by increasing activity levels 1 to 3 hours before the light change (Fig. 2), is characteristic for light-entrained circadian rhythms and is well described in *Drosophila* (Hamblen-Coyle et al., 1992). Some colonies also show activity peaks during the first few hours after the lights-off change, which can most likely be explained by the sudden light change in our experiments compared to the gradual change during dawn and dusk in nature since we found no or just very low peaks in the mornings and evenings during field experiments (R. J. Stelzer and L. Chittka, unpublished data). Individual recorded activity levels (i.e., the amount of minutes per hour in which a given forager crossed the RFID reader at least once; see Material and Methods) of up to 30 and more per hour during these peaks found in the laboratory suggest that the foragers repeatedly left the nest and turned back when they arrived at the dark arena. Only 2 of the tested colonies (B2 and B3) showed an anticipation of the lights-off change in the evening (E-anticipation), and even in those colonies, it is not convincing (Fig. 2). Since evening anticipation in *Drosophila* is often more robust than the lights-on anticipation, it seems that—in contrast to fruit flies—bumblebee locomotor behavior is not crepuscular under laboratory LD conditions. However, activity levels in our study were only measured at the colony entrance and not inside the nest, which means that a possible increased activity in the nest around the light changes was not recorded.

In DD, free-running rhythms have been found on both the colony and the individual level (Fig. 3). Free-running rhythms in constant DD conditions are another main characteristic of light-entrained circadian rhythms. Compared to LD, the overall level of activity increased drastically in DD (Fig. 3). Again, commonly found activity levels of 30 and more per

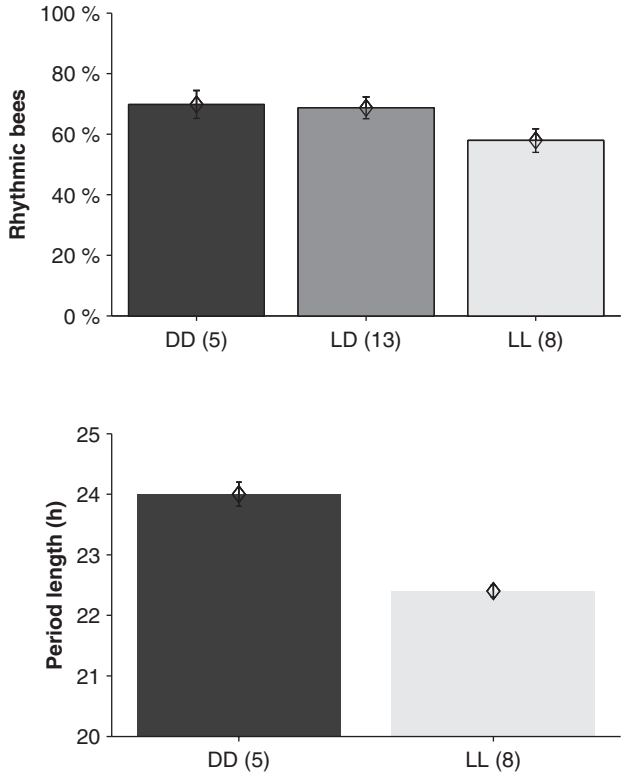

Figure 4. Proportion of rhythmic bees (top) and their measured free-running periods in DD and LL (bottom). All values are means ± SEM. Numbers in brackets indicate the number of tested colonies.

hour for individual bees suggest that they were walking back and forth in the tube, passing the RFID reader every other minute or more, rather than walking into the arena to collect sucrose solution. However, the feeders needed regular refilling, and the honey pots in the colonies were full when the DD experiments were concluded. Thus, the foragers were able to collect enough food in DD. Although being naturally visual foragers, bumblebees are able to exploit food sources in the vicinity of the nest in complete darkness in laboratory conditions by using odor marks and possibly a magnetic compass to navigate (Chittka et al., 1999). The fact that we only found very rare reader crossings during LD field experiments during the night (R. J. Stelzer and L. Chittka, unpublished data)—most likely caused by a guard bee in the entrance tunnel—indicates that bumblebees only show night activity in artificial laboratory conditions and only in constant darkness, since activity levels in the LD experiments were low during the middle of the scotophase.

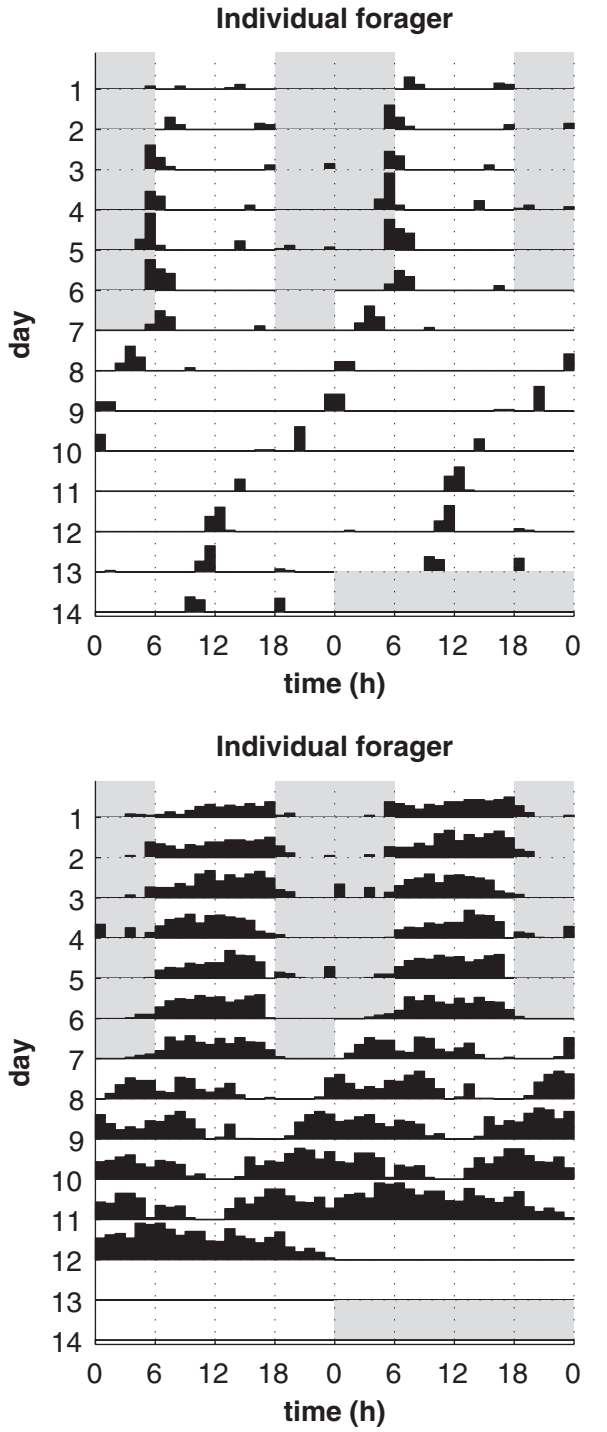

Figure 5. Double-plotted actograms of 2 individual workers. Top: actogram of a worker that was mainly foraging for a few hours in the circadian morning and whose activity free ran with a short period in LL. Bottom: actogram of a worker that died during the experiment. Shortly before death, this individual shows an increased level of arrhythmic activity (death dance).

The majority of foragers also stayed rhythmic under constant light conditions, with significantly shorter free-running periods in LL than in DD. This is in concurrence with Aschoff's rule, which states that the free-running periods of diurnal animals are longer in constant darkness and get shorter the brighter it gets (Aschoff, 1960). However, there are many exceptions to this rule (Aschoff, 1979), honeybees being one of them (Frisch and Aschoff, 1987; Moore and Rankin, 1985; Spangler, 1972)—that is, they show longer free-running periods in LL than in DD. The same is true for *Drosophila*: free-running period increases with increasing LL light intensity, eventually resulting in arrhythmicity (Konopka et al., 1989).

The fact that bumblebees and honeybees stay rhythmic in constant light conditions is surprising in view of the knowledge about the molecular mechanisms of the insect circadian clock gathered so far mostly using *Drosophila*, which become completely arrhythmic in LL (Konopka et al., 1989). Photic entrainment in *Drosophila* is largely accomplished by the blue-light receptor cryptochrome (dCRY), which acts as a circadian photoreceptor (Emery et al., 1998; Emery et al., 2000a; Emery et al., 2000b; Stanewsky et al., 1998). The decoding of the honeybee genome revealed that honeybees do have a cryptochrome gene (*cry*), but in contrast to *Drosophila*, it encodes a vertebrate-like protein (CRY2) (Rubin et al., 2007), which has also been found in other insect species, such as butterflies and mosquitoes (Zhu et al., 2005). In contrast to dCRY, CRY2 is not photosensitive (Yuan et al., 2007) and thus might not function as a circadian photoreceptor but rather as a transcriptional repressor required for clock function. To our knowledge, it has not been tested yet if bumblebees have a *Drosophila*-type CRY, but due to the close relatedness to honeybees and the fact that CRY2 has been found in the North American bumblebee *Bombus impatiens* (Yuan et al., 2007), it can be assumed that *B. terrestris* also lacks a photoreceptor-type CRY. That might explain why honeybees and bumblebees stay rhythmic in constant light—a similar behavior as observed for *Drosophila* carrying mutations in the *cry* gene (Emery et al., 2000a). Moreover, it has been shown that at least some of the *Drosophila* clock neurons that have the potential to drive behavioral rhythms in constant light are distinct from those that normally express Cry (Murad et al., 2007; Nagoshi et al., 2010; K. F. Chen, N. Peschel, and R. Stanewsky, unpublished data). Although very speculative, the lack of a photoreceptor-type Cry in bumblebees may also contribute to the lack of a pronounced evening activity peak during LD conditions (Fig. 2). This is because it has recently been shown that both Cry and the neuropeptide PDF are required to elicit this activity increase before the light turnoff in *Drosophila* (Cusumano et al., 2009; Zhang et al., 2009).

The mean proportion of rhythmic bumblebees in the 3 tested light regimes in our experiments varied between 58% in LL and about 70% in LD and DD (68.7% and 69.8%, respectively). Previous studies have shown that 91% of large, forager-sized bumblebee workers exhibit robust circadian rhythms in their locomotor activity in LD, when tested separated from their colony in monitoring cages, while only 67% of small, nurse-sized workers developed robust rhythms in these conditions (Yerushalmi et al., 2006). But although larger bumblebee workers show a higher probability of becoming foragers, this is only a tendency, and there are many exceptions. Therefore, in view of these findings, the proportions of rhythmic foragers in our experiments are not surprising. Even though the RFID readers in our experiments were placed between the nest and the foraging arena, not all the recorded traffic must have necessarily been caused by foragers since all available workers in the colonies were RFID tagged before the experiments started. Tagged nurses could have left the nest to defecate or to remove dead larvae from the colony or could perceive the foraging arena as part of the nest. The artificial laboratory conditions could also explain the comparatively low proportion of rhythmic foragers and the many workers that were only foraging for a few hours a day, mostly in the circadian morning, keeping a robust or free-running rhythm in doing so (Fig. 5). The bees were fed ad libitum in our experiments, and a complete foraging trip in the arena only takes a few minutes. The honey pots in the colonies, in which the bees store the collected sucrose solution, can be filled relatively swiftly in the morning, and there might be no need for all foragers to collect sucrose solution for the rest of the photoperiod. Under laboratory conditions, a few foragers are enough to keep the food supply in the colony at its maximum most of the time. Field studies using the RFID system revealed a mean of 95% of bumblebee foragers showing circadian rhythms in their foraging activity (R. J. Stelzer and L. Chittka, unpublished data), which is comparable or even higher than proportions found in other field studies (Yerushalmi et al., 2006). Therefore, we conclude that the comparatively low percentage of rhythmic foragers found in our experiments is not due to limitations of the RFID system but to the fact that there is no need for workers to forage constantly under laboratory conditions.

Another interesting finding in our laboratory conditions is the increased arrhythmic activity level shown by foragers 1 to 2 days before they die (Fig. 5). Such "death dances" have also been found in *Drosophila* during circadian studies (Levine et al., 2002c), suggesting that the circadian clock mechanisms, or the translation of clock time into behavioral output, are not working properly anymore, which could lead to the observed restless activity under laboratory conditions. Alternatively, it has been suggested that increased oxidative stress levels in animals close to their death can result in the breakdown of sleep:wake cycles (Koh et al., 2006). Such behavior could benefit the colony in two ways. First, a raised foraging activity increases the influx of nectar and pollen of the colony. Second, the chances are higher that the worker dies outside the colony. Decaying corpses pose a health problem for the colony and are therefore removed from bee and ant colonies (necrophoric behavior) (Ayasse and Paxton, 2002; Robinson and Page, 1988; Visscher, 1983; Wilson, 1971; Yao et al., 2009). A recent study in ants (Heinze and Walter, 2010) has revealed that dying workers actively leave the nest and isolate themselves from their nest mates hours or even days before their death. The observed death dances in bumblebees under laboratory conditions could be a sign of a similar behavior, with the soon-to-die workers constantly trying to separate themselves unsuccessfully from the colony and their nest mates in the small enclosed space of the nest box and foraging arena. However, the finding of the same behavior in the solitary fruit fly suggests a nonsocial cause.

Our study shows that using RFID is a powerful method for collecting data on circadian rhythms in social insects. Since it seems that light enters the molecular clock of bees through a yet unknown pathway, further studies are needed to discover the underlying mechanisms, and RFID might simplify the necessary experiments. One of the next steps will be to take the LL experiments to the field and test the behavior of bumblebees under natural continuous daylight conditions, such as those that prevail north of the Arctic Circle during the summer, when the energy demands of the colonies are higher and thus social cues might stimulate different foraging patterns compared to laboratory conditions.

ACKNOWLEDGMENTS

The authors thank Oscar Ramos-Rodríguez for technical support, Dr Joel Levine and John Schneider for their support regarding the data analysis in Matlab, and Syngenta Bioline Bees for providing bumblebees. Finally, they would like to thank three anonymous referees for their helpful comments and suggestions on an earlier version of this manuscript. This work has been supported by a Westfield Trust Studentship to RS.

NOTE

Supplementary online material for this article is available on the journal's Web site: http://jbr.sagepub.com/supplemental.

REFERENCES

Alford DV (1975) *Bumblebees*. London: Davis-Poynter.

Aschoff J (1960) Exogenous and endogenous components in circadian rhythms. Cold Spring Harb Symp Quant Biol 25:11-28.

Aschoff J (1979) Circadian rhythms: Influences of internal and external factors on the period measured in constand conditions. Z Tierpsychol 49:225-249.

Ayasse M and Paxton R (2002) Brood protection in social insects. In *Chemoecology of Insect Eggs and Egg Deposition*, Hilker M, Meiners T, eds. Berlin: Blackwell.

Beling I (1929) Über das Zeitgedächtnis der Bienen. Z Vergl Physiol 9:259-388.

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

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

Chittka L and Waser NM (1997) Why red flowers are not invisible to bees. Isr J Plant Sci 45:169-183.

Chittka L, Williams NM, Rasmussen H, and Thomson JD (1999) Navigation without vision: Bumblebee orientation in complete darkness. Proc R Soc Lond B 266:45-50.

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.

Cumber RA (1949) The biology of humble-bees, with special reference to the production of the worker caste. Trans R Entomol Soc (Lond) 100:1-45.

Cusumano P, Klarsfeld A, Chélot E, Picot M, Richier B, and Rouyer F (2009) PDF-modulated visual inputs and cryptochrome define diurnal behavior in *Drosophila*. Nat Neurosci 12:1431-1437.

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

Emery P, So WV, Kaneko M, Hall JC, and Rosbash M (1998) CRY, a *Drosophila* clock and light-regulated cryptochrome, is a major contributor to circadian rhythm resetting and photosensitivity. Cell 95:669-679.

Emery P, Stanewsky R, Hall JC, and Rosbash M (2000a) A unique circadian rhythm photoreceptor. Nature 404: 456-457.

Emery P, Stanewsky R, Helfrich-Forster C, Emery-Le M, Hall JC, and Rosbash M (2000b) *Drosophila* CRY is a deep brain circadian photoreceptor. Neuron 26:493-504.

Free JB (1955) The division of labour within bumblebee colonies. Insect Soc 2:195-212.

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.

Garófalo CA (1978) Bionomics of *Bombus* (*Fervidobombus*) *morio*: 2. Body size and length of life of workers. J Apicult Res 17:130-136.

Glaser FT and Stanewsky R (2005) Temperature synchronization of the *Drosophila* circadian clock. Curr Biol 15:1352-1363.

Hamblen-Coyle MJ, Wheeler DA, Rutila JE, Rosbah M, and Hall JC (1992) Behavior of period-altered circadian rhythm mutants of Drosophila in light:dark cycles. J Insect Behav 5:417-446.

Hardin PE (2006) Essential and expendable features of the circadian timekeeping mechanism. Curr Opin Neurobiol 16:686-692.

Heinze J and Walter B (2010) Moribund ants leave their nests to die in social isolation. Curr Biol 20:249-252.

Helfrich-Forster C (2005) Neurobiology of the fruit fly's circadian clock. Genes Brain Behav 4:65-76.

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

Johnson B (2010) Division of labor in honeybees: Form, function, and proximate mechanisms. Behav Ecol Sociobiol 64:305-316.

Koh K, Evans JM, Hendricks JC, and Sehgal A (2006) A *Drosophila* model for age-associated changes in sleep:wake cycles. Proc Natl Acad Sci USA 103: 13843-13847.

Koltermann R (1971) 24-Std-Periodik in der Langzeiterinnerung an Duft- und Farbsignalen bei der Honigbiene. Z Vergl Physiol 75:49-68.

Konopka RJ, Pittendrigh C, and Orr D (1989) Reciprocal behaviour associated with altered homeostasis and photosensitivity of *Drosophila* clock mutants. J Neurogenet 6:1-10.

Landskron J, Chen KF, Wolf E, and Stanewsky R (2009) A role for the PERIOD:PERIOD homodimer in the *Drosphila* circadian clock. PLoS Biol 7:820-835.

Levine JD (2004) Sharing time on the fly. Curr Opin Cell Biol 16:210-216.

Levine JD, Funes P, Dowse HB, and Hall JC (2002a) Advanced analysis of a cryptochrome mutation's effects on the robustness and phase of molecular cycles in isolated peripheral tissues of *Drosophila*. BMC Neurosci 3:5.

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

Levine JD, Funes P, Dowse HB, and Hall JC (2002c) Signal analysis of behavioral and molecular cycles. BMC Neurosci 3:1.

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 Rhythm 22:343-355.

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

Molet M, Chittka L, Stelzer R, Streit S, and Raine NE (2008) Colony nutritional status modulates worker responses to foraging recruitment pheromone in the bumblebee *Bombus terrestris*. Behav Ecol Sociobiol 62:1919-1926.

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

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

Moore D and Rankin MA (1985) Circadian locomotor rhythms in individual honeybees. Physiol Entomol 10:191-197.

Murad A, Emery-Le M, and Emery P (2007) A subset of dorsal neurons modulates circadian behavior and light responses in *Drosophila*. *Neuron* 53:689-701.

Nagoshi E, Sugino K, Kula E, Okazaki E, Tachibana T, Nelson S, and Rosbash M (2010) Dissecting differential gene expression within the circadian neuronal circuit of *Drosophila*. Nat Neurosci 13:60-68.

Plowright RC and Jay SC (1968) Caste differentiation in bumblebees (*Bombus* Latr-Hym): 1. Determination of female size. Insect Soc 15:171-192.

Refinetti R (2006) *Circadian Physiology*. Boca Raton, FL: CRC Press.

Robinson EJH, Richardson T, Sendova-Franks A, Feinerman O, and Franks NR (2009) Radio tagging reveals the roles of corpulence, experience and social information in ant decision making. Behav Ecol Sociobiol 63:627-636.

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

Robinson GE and Page RE (1988) Genetic determination of guarding and undertaking in honey-bee colonies. Nature 333:356-358.

Robinson GE, Page RE, Strambi C, and Strambi A (1989) Hormonal and genetic control of behavioral integration in honey bee colonies. Science 246:109-112.

Rosato E, Tauber E, and Kyriacou CP (2006) Molecular genetics of the fruit-fly circadian clock. Eur J Hum Genet 14:729-738.

Rösch GA (1930) Untersuchungen über die Arbeitsteilung im Bienenstaat, II. Die Tätigkeiten der Arbeitsbienen unter experimentell veränderten Bedingungen. Z Morphol Tiere 12:1-71.

Rubin EB, Shemesh Y, Cohen M, Elgavish S, Robertson HM, and Bloch G (2007) 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. Genome Res 16: 1352-1365.

Spangler HG (1972) Daily activity rhythms of individual worker and drone honey bees Hymenoptera-Apidae. Ann Entomol Soc Am 65:1073-1076.

Stanewsky R (2002) Clock mechanisms in *Drosophila*. Cell Tissue Res 309:11-26.

Stanewsky R (2003) Genetic analysis of the circadian system in *Drosophila melanogaster* and mammals. J Neurobiol 54:111-147.

Stanewsky R, Kaneko M, Emery P, Beretta B, Wager-Smith K, Kay SA, Rosbash M, and Hall JC (1998) The cryb mutation identifies cryptochrome as a circadian photoreceptor in *Drosophila*. Cell 95:681-692.

Streit S, Bock F, Pirk CW, and Tautz J (2003) Automatic life-long monitoring of individual insect behaviour now possible. Zoology 106:169-171.

Sumner S, Lucas E, Barker J, and Isaac N (2007) Radio-tagging technology reveals extreme nest-drifting behavior in a eusocial insect. Curr Biol 17:140-145.

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 Natl Acad Sci USA 97:6914-6919.

Visscher PK (1983) The honey bee way of death: Necrophoric behaviour in *Apis mellifera* colonies. Anim Behav 31: 1070-1076.

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

Wahl O (1932) Neue Untersuchungen über das Zeitgedächtnis der Bienen. Z Vergl Physiol 16:529-589.

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

Wilson EO (2000) *Sociobiology: The New Synthesis*. Cambridge, MA: Belknap Press of Harvard University Press.

Yao M, Rosenfeld J, Attridge S, Sidhu S, Aksenov V, and Rollo C (2009) The ancient chemistry of avoiding risks of predation and disease. Evol Biol 36:267-281.

Yerushalmi S, Bodenhaimer 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.

Zhang L, Lear BC, Seluzicki A, and Allada R (2009) The CRYPTOCHROME photoreceptor gates PDF neuropeptide signaling to set circadian network hierarchy in *Drosophila*. Curr Biol 19:2050-2055.

Zhu H, Yuan Q, Briscoe AD, Froy O, Casselman A, and Reppert SM (2005) The two CRYs of the butterfly. Curr Biol 15:R953-R954.