Polyphasic Wake/Sleep Episodes in the Fire Ant, *Solenopsis Invicta*

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Abstract Sleep is a well-studied biological process in vertebrates, particularly birds and mammals. Less is know about sleep in solitary and social invertebrates, particularly the ants. This paper reports a study of light/dark periods on worker activity as well as sleep location, posture and the wake/sleep cycles of fire ant workers and queens located in an artificial nest chamber. Workers slept in one of three locations: on the ceiling, against the chamber wall or in the center of the chamber floor. Workers on the ceiling or against the chamber wall slept for longer periods than those at the center of the chamber floor where most grooming and feeding activity occurred. When sleeping, queens huddled together. Their close contact generated synchronized wake/sleep cycles with each other. Sleep posture was distinctly different than wake posture. During deep sleep, queens and workers folded their antennae and were non-responsive to contact by other ants. Another indicator of deep sleep was rapid antennal movement (RAM sleep). Sleep episodes were polyphasic. Queens averaged ~92 sleep episodes per day, each episode lasting ~6 min, for a total of ~9.4 h of sleep per day. Workers averaged ~253 sleep episodes lasting 1.1 min each for a total of ~4.8 h of sleep per day. Activity episodes were unaffected by light/dark periods. Workers were hypervigilant with an average of

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80% of the labor force completing grooming, feeding or excavation tasks at any given time. These findings reinforce the parental exploitation hypothesis—sterile workers are a caste of disposable, short-lived helpers whose vigilance and hyperactivty increases the queen's fitness by buffering her and her fertile offspring from environmental stresses.

Introduction

The function of sleep remains unknown despite decades of vigorous research (Greenspan et al. 2001; Stickgold 2005). A number of hypotheses have been proposed, but none has gained general acceptance. Pioneering research on sleep with cockroaches uncovered remarkable similarities to sleep in mammals (Tobler 1983). Sleep research on the behavior and neural mechanisms of fruit flies extended our knowledge of sleep in insects. Fruit flies choose a particular place and position to sleep; they twitch in a manner similar to REM sleep and they are non-responsive to their surroundings when sleeping (Hendricks et al. 2000; Shaw et al. 2000; Nitz et al. 2002). The genes that influence sleep patterns in fruit flies are similar to those in mammals (Huber et al. 2004; Hendricks and Sehgal 2004; Cirelli et al. 2005). Pharmacological products that regulate sleep in mammals, birds and fish have similar affects on sleep in fruit flies (Paredes et al. 2006; Roth II et al. 2006, Zhdanova 2006; Zimmerman et al. 2006). Converging evidence supports the hypothesis that neurons and neural processes are universal across species—vertebrates and invertebrates alike (Hildebrand and Shepherd 1997; Griffith and Rosbash 2008; Vyazovskiy et al. 2008). Thus, behavioral differences among species lie in the circuitry of the nervous system—how neurons are wired together—rather than the form and function of individual neurons.

Research into the neural mechanisms of sleep in one species can enlighten us on the neural mechanisms of sleep in other species (Roth II et al. 2006). Studies of sleep among caste members in the social insects can extend our understanding of intra- and inter-specific variability of sleep/wake cycles day-to-day and over evolutionary time. In this respect, social insects, particularly ants with their distinctive caste system, are excellent systems of study. This paper reports a study of the affect of light on worker activity levels as well as a description of sleep location, posture and wake/sleep episodes for queens and workers in the fire ant, *Solenopsis invicta*.

The success of ants worldwide is largely a result of the ability of workers to quickly switch back and forth from individual to group tasks based on need. Although worker and queen activity has been widely studied (Hölldobler and Wilson 1990; Tschinkel 2006), little is known about worker or queen inactivity. Because fire ants are subterranean dwellers and thus irregularly exposed to photoperiods, we hypothesized that inactivity in individuals would be polyphasic and asynchronous, depending more on tasks at hand then on light/dark periods. As hypothesized, worker inactivity cycles were polyphasic and asynchronous. Unexpectedly, we found that queen activity/inactivity cycles were synchronous with other queens.

Table 1 Proportion of the Time that Zero, One, Two or Three Queens were Inactive at the Same Time

X	0	1	2	3
Prob(X = k)	0.2046	0.4279	0.2982	0.0693

Methods

Polygyne Fire Ant Colonies Dozens of polygyne fire ant colonies were collected from sites in Brazos Valley in Texas, U.S.A. Colonies were separated from the soil in the usual the drip-floatation method (Banks et al. 1981) and transferred to large plastic bins containing artificial nests. Colonies were maintained in the plastic bins on shelves in an insectary at 27°C and ~50% relative humidity with a 14:10 h (light: dark) photoperiod. Colonies were provisioned with water *ad libitum* and a daily diet of 25% honey water and yellow mealworms (*Tenebrio molitor*). These conditions are standard for successfully maintaining fire ant colonies which grow rapidly regardless of whether the insectary is light or dark. Ants adjust to changes in light within seconds (Cassill and Tschinkel 1999).

Sampling For our study on sleep cycles of fire ant workers and queens, a single colony was selected from the laboratory-maintained colonies. This colony contained a large ratio of brood (eggs, larvae and pupae) relative to workers, indicating the presence of healthy queens. To simulate the small groupings of fire ants found in field nest chambers (Cassill et al. 2002), 3 queens, 30 workers and 30 large larvae where placed in one artificial chamber (15 mm × 10 mm × 5 mm deep with an exit tunnel). A glass cover slip was placed over the chamber and tunnel, allowing us to videotape the entire group except the occasional scout when it left the chamber to forage for food. The artificial nest was placed in a small plastic bin 15.0 cm in diameter and 5.0 cm deep. This colony was video taped continuously for 4.7 days.

Queen Inactivity/Sleep Data on the activity/inactivity of the three queens was collected from the videotapes. A computerized event recorder was used to record queen activity/inactivity. Queens were selected, one at a time, for behavioral analysis. Active and inactive durations were recorded in seconds along with the time of the change in behavior. From these data, the frequency, duration and synchrony of each queen's active and inactive periods were analyzed. In addition, the posture and location of queens was noted every hour over the 4.7 day period (sample size = 113).

Worker Inactivity/Sleep Sleep data for the 30 workers was collected from one randomly selected 24-hour period of the study. Data was recorded only on workers

Table 2 Observations of the Three Queens Included 2,291 Activity/Inactivity Configurations

Number active queens	0	1	2	3
Observed frequencies	489	868	653	281

Table 3 Expected Frequency of the Activity/Inactivity Periods of Three Queens

Number active queens	0	1	2	3
Expected frequencies	469	980	683	159

who were inactive on the chamber floor or ceiling, not on workers who were active (sample size = 287 data points).

To determine worker activity levels, one worker was selected randomly and observed for 8 h (1 videotape). The videotape was paused every 10 min and the number of contacts made during the 10-minute episode by this worker with queens, larvae or other workers was recorded (sample size = 48 counts).

To test for photosensitivity of worker activity rhythms, an experiment was designed with eight treatments: 12 AM-light; 12 AM-dark; 6 AM-light; 6 AM-dark; 12 PM-light; 12 PM-dark; 6 PM-light and 6 PM-dark. At the designated time, 20 workers were placed in a clear plastic vial (100 mm × 25 mm) with damp sand. Ants become stressed when above ground and will immediately excavate a subterranean chamber. After 6 h, vials were frozen. The following day, the length and width of tunnels excavated in each vial during the six-hrs by the workers were measured. Workers dig along the inside edge of clear plastic vials, thus shafts and chambers are visible to the eye and easily measure with a flexible plastic metric ruler. Five replicates from five stock colonies were created per treatment for a 5 × 8 experimental design.

Data Analysis Data were summarized and analyzed using JMP IN statistical software (Sall and Lehman 2005). Duration data were not normally distributed; thus, data were log transformed and analyzed using ANOVA or t-tests. Means were transformed back to integers for the figures. Frequency of activity per 8-hr period among queens was normally distributed;

To quantify the obvious synchrony of queen activity, the proportion of inactivity time for a single queen was estimated by the ratio (inactivity time)/(total time). The most conservative estimate of inactivity (i.e. the one that yields the highest probability for a queen to be inactive) was 0.5893. Assuming no synchrony between the queens, we have the following probability distribution for the number of simultaneously active queens (Tables 1, 2, 3).

Results

Queen Activity/Inactivity

Indicators of wake-active episodes in queens took three forms: antennal movement, head movement, or body movement in the form of walking. When walking or moving their heads, queens extended their antennae with the scape forward of the eyes and scape and funiculi at an obtuse angle to each other (Fig. 1a). In addition, the mandibles and glossae (tongue) were partially extended. Queens that ingested fluids from a worker or from a larva's anal slit, held their antennae with the scape
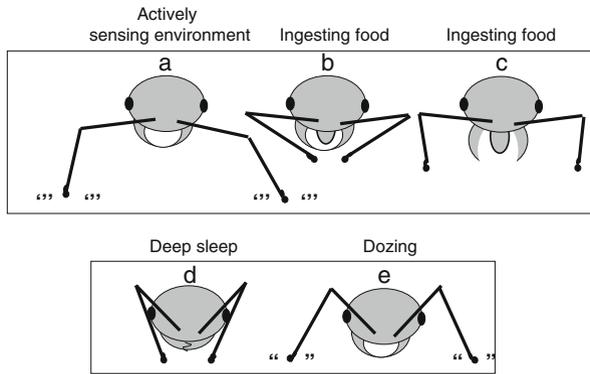
Fig. 1 Differences in the angles of a queen's antennae during active and inactive phases. The geometry of a fire ant's antennae, mandibles and glossae (tongue segments) varied with wake and sleep episodes. During deep sleep, the antennae, mandibles and glossae where retracted. Frequently, during periods of deep sleep, the folded antennae of queens quivered continuously in a "rapid antennal movement" (RAM) resembling rapid eye movement (REM) in vertebrates. During dozing episodes, the antennal scapes (the first and elongated segments) were retracted above the eyes, but the funiculi were extended. In addition, mandibles were partially open such that the tips of the teeth were touching, but not overlapping. During wake episodes, the antennae were extended such that the scapes and funiculi were low and in front of the eyes. Mandibles and glossae were partially or fully extended depending on whether the queen was ingesting or donating nutrients.

and funiculi at acute angles and with the scape extended in front of the eyes (Fig. 1b). The tips of the antennae poked and probed the worker glossae or the larva's anal slit. The mandibular teeth tips were touching but not overlapping and the glossae were partially extended. Queens that regurgitated food to a worker or another queen extended their antennae with the scape in front of the eyes, forming a right angle with the funiculi (Fig. 1c). Mandibles were open and the glossae fully extended.

Indicators of queen sleep episodes took two forms: "deep sleep" or "dozing". During deep sleep, antennae (scape and funiculi) where completed retracted as were glossae and mandibles (Fig. 1d) and queens were unresponsive to contact by workers. Frequently, during periods of deep sleep, the folded antennae of queens quivered in a "rapid antennal movement" (RAM) that might be an analog to the rapid eye movement (REM) in vertebrates.

Dozing differed from deep sleep in that antennal were partially extended, with the scape and funiculi at right angles to each other; in addition, mandibles were partially open such that the tips of the teeth were touching, but not overlapping (Fig. 1e). Dozing queens were more likely to respond to contact by workers or other queens with antennal movements.

The duration of wake-active episodes for queens was significantly longer (2.5 times longer) than the duration of sleep episodes (Wilcoxon Test: Chi-square = 28.6; $p<$ 0.0001). In total, queens slept a mean total of 9.4 h/day and were active the remaining 14.6 h/day.

Queen sleep/activity episodes were synchronized with each other (Fig. 2). The Chi-square goodness-of-fit test for synchronized cycles of queen activity was significant (Tables 2, 3; Chi-square = 109.29; $p<0.001$). The mechanism of synchrony was tactile. Queens huddled together with overlapping body parts when inactive (Fig. 3). Increased activity of one queen was transmitted through contact
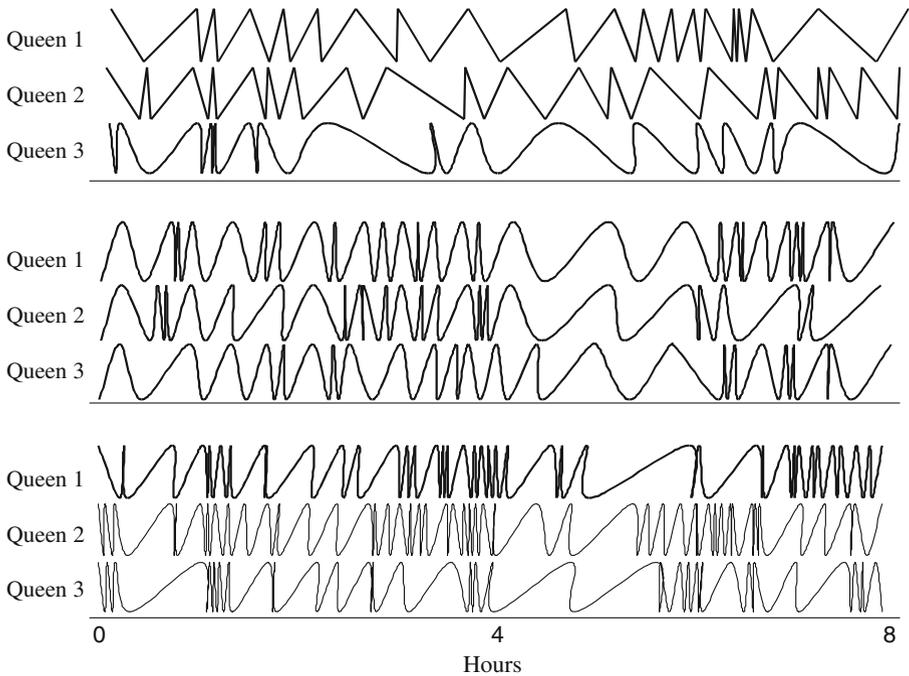
Fig. 2 Lines represent wake/sleep episodes for each of three polygyne queens residing in the same chamber. Sleep episodes were demarcated from high point to low point (\ ). Wake episodes were demarcated from low point to high point (/). This graphic display comparing wake/sleep episodes of three queens suggested a high degree of activity synchrony among the queens which was confirmed by analysis (Friedman test).
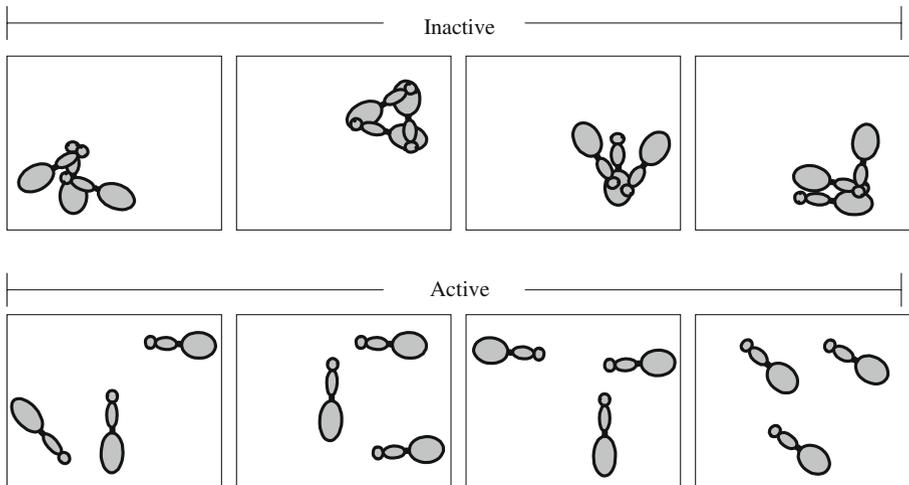

Fig. 3 Queen location in chamber during wake and sleep episodes. Queens huddled together during sleep episodes. When active, queens were close, but not touching each other. Caveat: Queen body size is not proportional to chamber size.
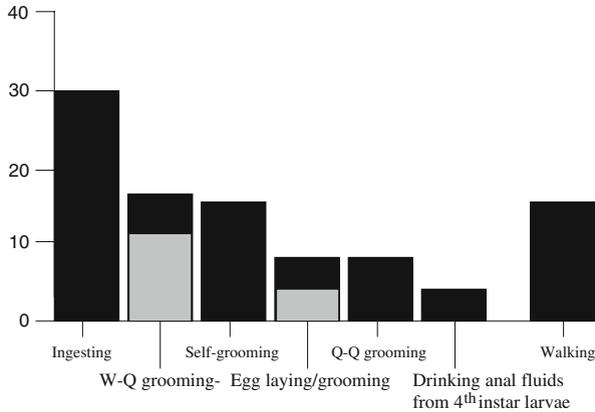
Fig. 4 Type and frequency of queen activities during a 4.7 day period. When queens were active, 29.0% of their interactions were feedings by workers; 16.7% of their time was spent grooming workers or being groomed by workers (11.7% gray bar; 5% black bar respectively); 16.4% of their time was spent self-grooming; 9.2% of their time was spent in interactions with other queens; 8.1% of their time was spent laying eggs (black bar) and grooming eggs (gray bar); 4.3% of their time was spent drinking anal fluids from 4th instar larvae. The remaining 16.3% of their time was spent walking around the brood chamber.

with the other queens, much like a cue ball activating billiard balls, and the queens moved apart from each other. When queens were active, half (50.4%) of their interactions involved grooming (Fig. 4). The remaining activities included feeding and walking about the brood chamber.

Worker Activity/Inactivity Workers averaged 253.0 sleep episodes a day for a total sleep time of 4.8 h/day. The duration of sleep episodes did not vary significantly with the time of day (6 AM, 2 PM, 10 PM, ANOVA: $F_{2,140} = 0.82$; $p = 0.441$. However, the duration of sleep episodes did vary significantly with worker location in the chamber (ANOVA: $F_{2,140} = 40.05$, $p < 0.0001$). Workers on the chamber floor were inactive for a mean 32.8 s; workers on the glass ceiling were inactive for a mean 82.1 s; workers on the side of the chamber were inactive for a mean 108.7 s.

On average, 20% of workers were asleep at any given time; stated another way, 80% of the labor force was wake and active at any given time. The number of sleeping workers varied by time of day (ANOVA: $F_{2,284} = 26.270$; $p < 0.0001$). Significantly fewer workers were asleep in the hours 6 AM–2 PM than in the hours 2 PM–10 PM (Tukey-Kramer HSD: $q = 0.46$; $p < 0.0001$). Significantly fewer workers were asleep in the afternoon than at night (Tukey-Kramer HSD: $q = 0.01664$; $p = 0.029$). Of the sleeping workers, 53.8% were on the chamber floor; 27.3% were hanging from the glass ceiling, and 18.9% were huddled against the chamber wall.

Our analysis of one individual worker over an eight-hour period found that its activity cycles were idiosyncratic with bursts of high activity interspersed with low activity episodes (Fig. 5). This individual worker contacted other workers significantly more often than it contacted queens or larvae (ANOVA: $F_{2,141} = 112.02$; $p < 0.001$). The worker was significantly more active during the second 4-hr period than the first 4-hr period (t-Test: $t_{46} = 18.84$; $p < 0.001$) suggesting that activity levels are idiosyncratic and vary over time because of and apart from group activity.
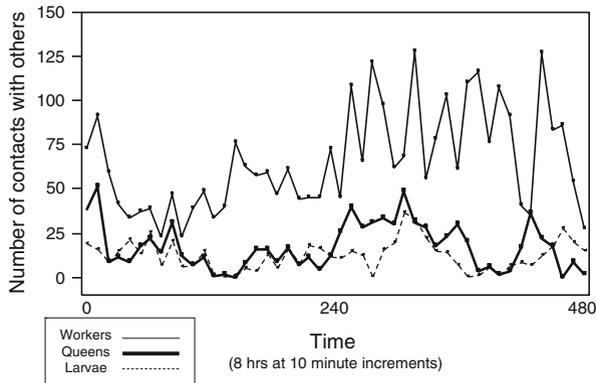
Fig. 5 Type and frequency of contact by one worker with other colony members. During one 8-hour period, this one worker contacted other individuals a total of 4,601 times. This worker contacted other workers 3,149 times, larvae 610 times and queens 842 times. Two features of this worker's activity were apparent. (1) During any given 10-min period, this worker's activity was never zero, supporting the idea of workers are hyperviligant sentinels protecting the queen. The level of its activity was greater during the last 4 h than the first 4-hrs of the observation period. These data suggest that, as a group, workers are vigilant 24 h a day, while individuals have extended periods in which they are less vigilant than at other times.

Significantly longer tunnels were excavated in lighted versus dark conditions (ANOVA: $F_{1,39}=-3.28$; $p=0.002$). Time of day had no affect on tunnel length (ANOVA: $F_{4,36}=0.96$; $p=0.424$). These data suggest that ants react to local stimuli rather than innate biorhythms.

Discussion

Our study on sleep in fire ants reinforces the idea that sleep location, sleep posture and sleep episodes in invertebrates such as cockroaches (Tobler 1983), Drosophila (Hendricks et al. 2000; Shaw 2003; van Swinderen et al. 2004), scorpions (Tobler and Stalder 1987) and the honey bee (Kaiser 2004) share many of the fundamental components that define mammalian sleep (Campbell and Tobler 1984, 1989), avian sleep (Amlaner and Ball 1994; Paredes et al. 2006) and fish sleep (Tobler and Borbély 1985; Yokogawa et al. 2007). Fire ant workers slept in one of three locations, on the ceiling, against the chamber wall or in the center of the chamber floor. Workers were disturbed more frequently by active workers and slept for shorter episodes when on the floor near the center of the chamber. During deep sleep, queens and workers folded their antennae and were non-responsive to contact by other ants. Another indicator of deep sleep was rapid antennal movement (RAM sleep) which might be analogous to REM sleep in vertebrates, suggesting that deep sleep is a feature of all organisms, vertebrates and invertebrates, with a central nervous system (reviewed in Hobson 2005).

The wake/sleep episodes were polyphasic and synchronous for queens. Queens averaged over 9 h of sleep per day, occurring in dozens of sleep episodes lasting 6 min each. Queens sleep together and moved slightly apart only when they were active. Wake/sleep episodes were polyphasic and asynchronous in workers. Workers

averaged 5 h of sleep per day, occurring in hundreds of sleep episodes lasting 1 min each.

The relatively lengthy live span and sleep time for fire ant queens (6 years; 9 h/day) relative to their sterile offspring (1 month to 1 year; 5 h/day) complements the findings in Drosophila in which flies that sleep less die younger (Cirelli et al. 2005). A comparative study across ant species correlating the relationship between longevity and sleep between the fertile and sterile castes would extend the findings on Drosophila.

As a result of their arrhythmic, polyphasic wake/sleep episodes, 80% of the worker labor force was available every hour of every day to complete tasks needing attention. Experiments investigating the effects of sleep deficits on division of labor could enlighten us on the importance of power naps for effective decision-making (Tietzel and Lack 2002).

Workers were polyphasic, excavating sand round the clock regardless of time of day or light/dark periods. Polyphasic activity is found in the honey bee nest building and the naked mole rat nest excavation (Toma et al. 2000). Perhaps animals that spend the majority of their lives in dark habitats lose sensitivity to photoperiods and lack the circadian rhythm present in their ancient surface-dwelling ancestors (Ishay et al 2005).

Ultimately, from the queen's perspective, a hyper-vigilant labor force reduces her exposure to risk and thus increases her longevity. A hyperactive labor force also increases a queen's reproductive efficiency. With 80% of worker offspring awake at any given time, no task is ever left untended for longer than a few seconds (Cassill and Tschinkel 1995, 1999). These findings reinforce the parental exploitation hypothesis for the evolution of sterile offspring (Ghiselin 1974; Cassill 2006). Workers are a disposable caste (Cassill 2002) that buffer queens from environmental stresses, increasing the queen's survival and ability to produce fertile offspring over a lifetime that can extend to 45 years (Tschinkel 1988; Hölldobler and Wilson 1990).

In conclusion, our study on sleep in fire ants provides a baseline for studying the function of sleep in a highly social organism with a caste system of short-lived and long-lived siblings. The study was largely descriptive, providing data on sleep posture and cycles for fire ant queens and workers residing within the same chamber with a 1:1 ratio of workers to larvae. Future studies under different conditions will reveal whether age, size or idiosyncratic factors influence the number and duration of sleep/wake episodes. Experiments measuring changes in the number or duration of sleep episodes among a fertility caste of queens and workers as they age, and under various levels of sleep disruption could add to our understanding of the form and function of sleep (Lima et al. 2005).

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