SHORT COMMUNICATION

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Circadian consequences of social organization in the ant species *Camponotus compressus*

Abstract The locomotor activity rhythm of different castes of the ant species *Camponotus compressus* was monitored individually under laboratory light/dark (LD) cycles, and under continuous darkness (DD). The colony of this ant species comprises two sexual castes, the queens and the males, and three worker castes, namely the major, media, and minor workers. The virgin males and virgin queens display rhythmic activity patterns, but the mated queens were arrhythmic while laying eggs, with the rhythmicity resuming soon after egg-laying. Under the LD regime, major workers showed nocturnal patterns, while about 75% of the media workers displayed nocturnal patterns and about 25% showed diurnal patterns. Under the DD regime, most major workers exhibited circadian rhythm of activity with a single steady state, whereas media workers displayed two types of activity patterns, with activity patterns changing after 6–9 days in DD (turn-arounds). The pre-turn-around τ of the ants that showed nocturnal activity patterns during LD entrainment was <24 h after release into DD, which then became >24 h, after 6–9 days. On the other hand, the pre-turn-around τ of those ants that exhibited diurnal patterns during LD entrainment was first >24 h after release into DD, and then became <24 h, after 6–9 days. The activity of the minor workers neither entrained to LD cycles nor showed any sign of free-run in DD. It appears that the circadian clocks of the ant species *C. compressus* are flexible, and may perhaps depend upon the tasks assigned to them in the colony.

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Introduction

Many biological rhythms, when monitored under constant conditions in the laboratory, occur with a "free-running period" (τ) of approximately 24 h (Dunlap et al. 2003). Although the τ of such circadian rhythm is often regarded as an inherent property of the organism's clock, there is evidence that τ can vary in response to environmental time cues, often reflecting a residual effect of the previously experienced environmental condition (Saunders 2002; Sharma 2003a). Given the fact that the temporal niche adopted by an organism depends upon its τ, changes in τ might be particularly important for organisms living in periodic environments (Sharma 2003a).

Social insect colonies normally face various challenges, such as changes in colony sizes, time of the year, food availability, predation pressure, and climatic conditions. The survival of the colonies under such demanding conditions requires a number of tasks to be performed simultaneously, and social insects seem to have evolved a division of labor to meet such challenges (Bourke and Franks 1995). In honeybees, newly emerged workers (during first 3–5 days after eclosion) remain in the nest, and their activity, oxygen consumption, and temperature regulation are arrhythmic when assayed under periodic field conditions, as well as in periodic and constant laboratory conditions (Bloch et al. 2001). The older bees act as foragers, and exhibit a circadian rhythm of locomotor activity (Toma et al. 2000; Bloch et al. 2001).

In several ant species, a significant proportion of old workers remain in the nest and some newly emerged ones rapidly develop to become foragers (Sendova-Franks and Franks 1993). Thus young foragers and old nurses may be found simultaneously in the same colony (Sendova-Franks and Franks 1993, 1994). Calabi (1988) and Gordon (1989) demonstrated that a few species of ants have the capability for behavioral flexibility and task switching. In addition, in a number of ant genera, division of labor is associated with physical polymorphism (Hölldobler and Wilson 1990). In several species of ants, winged sexual castes mate during or following a flight at a species-specific time

of the day and the inseminated females lose their wings and start new colonies (McCluskey 1992). The above studies suggest that division of labor in ant colonies is flexible and probably much more complex than in honeybee colonies. Notwithstanding the importance of circadian clocks for social insects in facilitating social organization, the consequences of social organization and division of labor on circadian rhythms have never been investigated in any social insect other than honeybees. Although, circadian locomotor activity rhythms have been documented in individual workers of wood ants, our understanding of the consequences of flexibility in division of labor on the circadian clocks of ants is still in its infancy (North 1987, 1993).

In this paper we report the results of our experiments aimed at investigating the circadian timing systems of several castes of the ant species *Camponotus compressus*. The locomotor activity rhythms of different castes were monitored in LD and DD regimes in the laboratory to understand whether or not circadian rhythms of different castes are manifested due to the tasks in the colony.

Materials and methods

Different castes of ants of the species *C. compressus* belonging to the family Formicidae of the order Hymenoptera (Hölldobler and Wilson 1990) were collected from four different colonies in the campus of the Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore ($13°00'$N, $77°32'$E). The age of these ants was not known and therefore caste identifications were done solely based on morphological and behavioral features. This species of ants is polymorphic; the major, media, and minor workers were 14–18, 11–16, and 6–8 mm long, respectively, while the queens were 18–24 mm long and the males were 10–13 mm long. The queens of this species of ants are egg-producing machines and the males are involved only in mating, after which they die. The minor workers, besides occasionally foraging, are also involved in nursing and in attending to other nest-related chores. The media workers are task generalists, are more active, and perform tasks similar to major and minor workers; although the majority of the major workers are foragers, some of them (guards) additionally defend the colony against any external danger (Hölldobler and Wilson 1990). Those major workers that act as guards have significantly bigger heads and mandibles than other major workers. The ant colonies were maintained in the laboratory under 24-h (12:12 h) LD cycles in plastic tubs with plaster of Paris at the bottom and covered with an acrylic sheet. Several connected shallow compartments were made on the plaster of Paris surface, covered by glass plates to create narrow chamber-like spaces resembling their natural nests. Food (honey solution) and water were provided in glass vials closed with cotton plugs, and occasionally freshly killed grasshoppers were offered. The temperature was maintained at $24±1°C$ and the relative humidity varied between 70 and 90%. The activity data for each ant was recorded individually using a computerized activity monitoring system (Sharma 2003b). The activity was monitored for the first 15 days under LD cycles ("lights-on" at 0800 hours and "lights-off" at 2000 hours), and then in DD, in cubicles illuminated constantly with dim red light of $\lambda>650$ nm.

The periodicity of the locomotor activity rhythm was estimated by subjecting the primary data to Fourier spectral analysis (Sharma et al. 2004b). The τ of the locomotor activity rhythm in the two steady states of the media workers was subjected to mixed-model analysis of variance (ANOVA). Post-hoc multiple comparisons of τ were done using Tukey's test. The τ values of the locomotor ac-

tivity rhythm of the virgin and mated queens were compared using Student's *t*-test.

Results

The fraction of workers that survived isolation was greatest for major workers, followed by media and minor workers. Almost all rhythmic major workers showed nocturnal activity patterns in the LD regime (Fig. 1a), whereas about 75% of media workers displayed nocturnal activity patterns, and about 25% of them showed diurnal activity patterns (Fig. 2a, b). Most major workers (34 out of 46) exhibited circadian locomotor activity rhythm in DD, with an average τ of $23.98±0.62$ h (mean ± SD) (Fig. 1a). A few major workers (6 out of 46) exhibited a greater-than-average level of activity, and did not display any rhythmicity of activity either in LD or in DD regimes (Fig. 1b). All 16 media workers exhibited a cir-
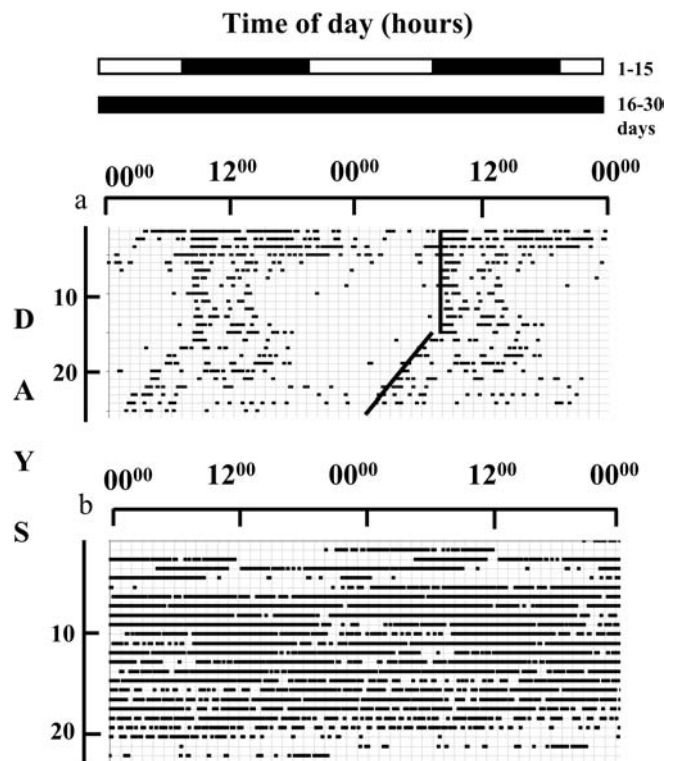

Fig. 1 The locomotor activity record of a rhythmic (forager: **a**) and an arrhythmic major worker (soldier: **b**) (out of 46 that survived isolation), assayed for the first 15 days in LD cycles of 12:12 h (lights-off at 0800 hours and on at 2000 hours) and then under continuous darkness (DD). The *bars* and *blots* represent activity and their *absence* denotes rest. The *time* of the day is plotted along the abscissa and the *days* along the ordinate in chronological order. The *lines* drawn through the onsets are drawn to give a visual impression of the approximate trend the locomotor activity rhythm takes during the free-running and entrained conditions. The locomotor activity rhythms of the forager (**a**) entrained to LD cycles, with activity beginning with "lights-off" and free-run in DD. The free-running period (τ) of this ant was 23.27 h. The locomotor activity rhythms of the guard (**b**) neither entrained to LD cycles nor did it show any sign of free-run in DD
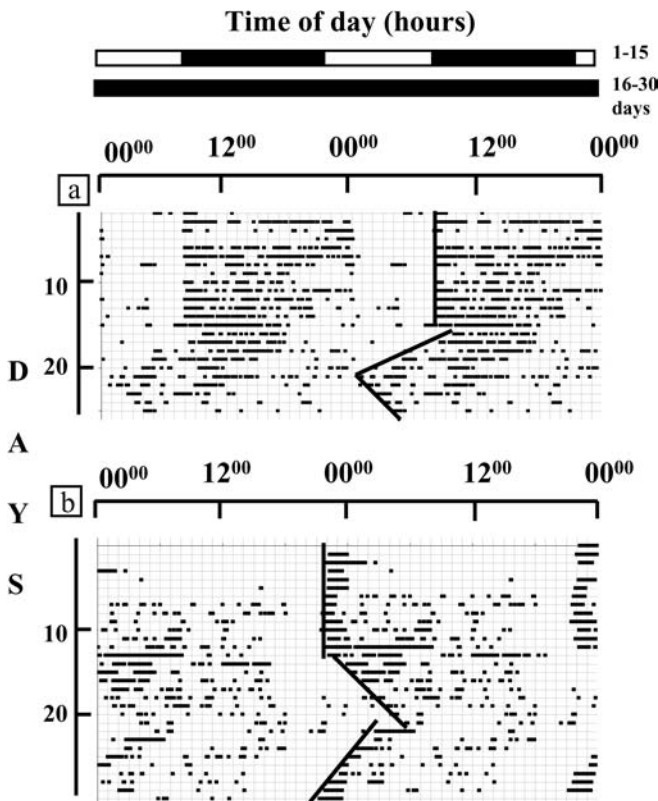
Fig. 2 The locomotor activity records of a type-1 (**a**) and type-2 (**b**) media worker (out of 16 that survived isolation), maintained for the first 15 days in LD cycles of 12:12 h. The locomotor activity rhythm of the ants entrained to LD cycles and free-ran under DD displaying a free-running period (τ) of 22.33 h for the first 6 days and of 25.22 h for the remaining 5 days (**a**), and of 24.83 h for the first 7 days and 23.34 h for the remaining 9 days (**b**). Other details are as in Fig. 1

Fig. 3 The locomotor activity records of a virgin male (out of five that survived isolation) and a virgin queen (out of nine that survived isolation) maintained for the first 10 days in 24-h light/dark (*LD*; 12:12 h) cycles. The locomotor activity was recorded in continuous darkness (*DD*) of the laboratory after day 11. The locomotor activity rhythm of the ants displayed a free-running period (τ) of 23.19 and 23.42 h, respectively. Other details are as in Fig. 1

cadian rhythm of locomotor activity in DD, displaying two different activity patterns ("turn-arounds"); the τ of activity rhythm in 10 out of 16 ants lengthened (type 1), and shortened in 6 out of 16 ants (type 2). The τ of the type-1 ants that exhibited nocturnal activity patterns in LD cycles was first 23.32±0.26 h (mean ± SD) on release into DD and then lengthened after 6–9 days to 24.56±0.20 h (mean ± SD) (Fig. 2a). The τ of type-2 ants that exhibited diurnal activity patterns in LD cycles was first 24.32±0.46 h (mean ± SD) on release into DD and then shortened after 6–9 days to 23.47±0.11 h (mean ± SD) (Fig. 2b). ANOVA on the τ values revealed a significant effect of type of pattern on τ ($F_{1,31}=13.11$, $P<0.0001$), and a significant interaction between types of pattern and the steady state τ values ($F_{1,31}=26.38$, $P<0.0001$). Post-hoc comparisons using Tukey's test showed that the τ values of the type-1 and type-2 ants were significantly different between the pre- and post-turn-around steady states ($P<0.001$). No sign of circadian rhythmicity in the locomotor activity was seen in the minor workers.

Only five out of nine virgin males survived in isolation for more than 6 days. The mating flights of virgin males
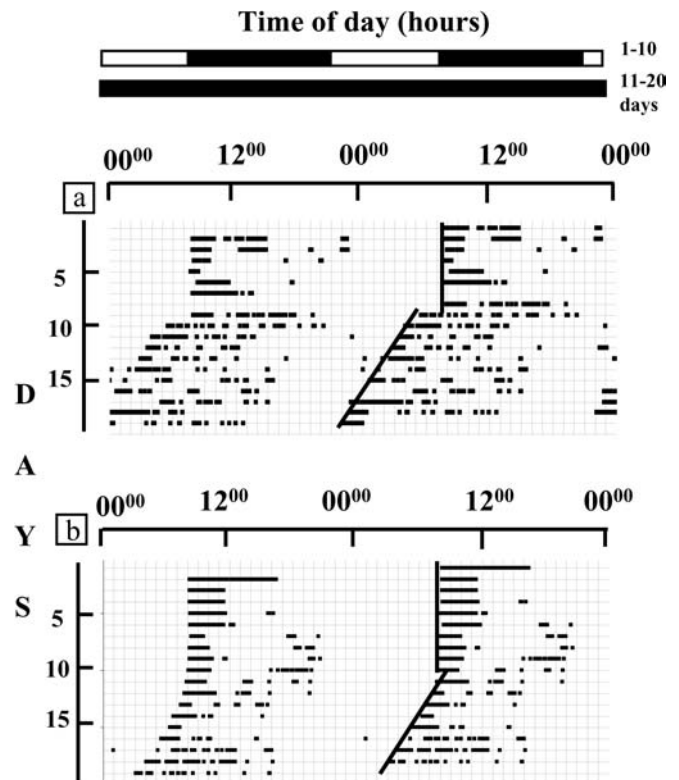
and virgin queens were possibly synchronized, because these ants were captured from the mating sites at the same time (Fig. 3a, b). The locomotor activity rhythm of the two reproductive castes, i.e., virgin males and virgin queens, free-ran in DD with an average τ of 23.72±0.17 and 23.60±0.48 h, respectively. The τ of the virgin males and the virgin queens did not differ significantly ($P>0.05$), suggesting similarity in the circadian clocks between the two reproductive castes. On the other hand, the locomotor activity of the mated queens was arrhythmic for the duration of egg laying; robust rhythmicity appeared soon after the egg-laying phase was over (data not shown).

Discussion

The major workers (guards) in the *C. compressus* colony remained active a round the clock, both under LD as well as DD regimes, whereas the major workers (foragers) who probably need to precisely anticipate periodic events in nature, displayed locomotor activity rhythm that synchronized to 24-h LD cycles, and free-ran with a near 24-h period. The media workers, the generalists, displayed both nocturnal and diurnal patterns under LD re-

gime (Sharma et al. 2004a). According to Aschoff's rule, the τ of nocturnal animals in DD is generally <24 h and that of diurnal animals is generally >24 h (Saunders 2002). Therefore, the ants that display nocturnal patterns in LD cycles should have τ <24 h, and those that exhibit diurnal patterns should have τ >24 h. This was indeed the case; nocturnal ants did exhibit locomotor activity rhythm with τ <24 h, and diurnal ants displayed it with τ >24 h (Fig. 2a, b). The 6–9 day patterns in the circadian locomotor activity of the media workers, wherein the activity rhythm changed from one steady state to another, indicates that media workers are perhaps involved in shift-work schedules in their colony, with a change in shift every 6–9 days (Sharma et al. 2004a). On the other hand, it is possible that most media workers are nocturnal and a few are diurnal, and they remain so throughout their life. It is conceivable that there are considerable inter-individual differences in activity patterns. Alternatively it is also possible that such turn-arounds in patterns may simply be a general property of insect circadian clocks under constant conditions. Such random and unpredictable spontaneous changes in circadian period have already been reported for a variety of animals (Saunders 2002). However, in our study, the switching over of τ from <24 to >24 h and vice versa adheres to a rigid pattern and is not irregularly spontaneous. Thus such changes in activity pattern indicate a temporal distribution of foraging behavior in media workers in the *C. compressus* colony; a well-designed strategy of the media workers, perhaps to meet the challenges arising due to their task as generalists.

The lack of circadian rhythmicity in the minor workers is consistent with their role in the colony, as they mostly perform nest work, and seldom go out foraging. However in contrast to *C. compressus* the minor (nurse) workers of other *Camponotus* ant species display circadian rhythms in brood-tending activities (Roces and Núñez 1996). It is even more interesting that the locomotor activity of the guard ants and the minor workers neither entrained to LD cycles, even in a "masking mode", nor displayed free-running activity patterns. This suggests that the locomotor activity in these castes, in addition to lacking circadian rhythmicity, may even be refractory to LD cycles, doubly ensuring constant vigil and incessant activity.

The virgin males and the virgin queens of the ant species *Camponotus compresses* also exhibit a robust activity rhythm with comparable phase and period. Thus our results not only confirm previous observations on virgin queens of a few species of ants (McCluskey 1992), but additionally suggest that virgin queens exhibit rhythmic activity even when maintained in isolation under constant conditions of the laboratory. Previous reports claim that, in sharp contrast to the virgin queens, the mated queens do not show any rhythmicity of activity (McCluskey 1992). In one of our previous experiments we had reported that during such arrhythmicity the mated queens laid eggs, and displayed robust circadian rhythmicity in some other phenomena such as brood care, and the circadian rhythm of locomotor activity reappeared as soon as the egg-laying phase was over (Sharma et al. 2004b). It appears that the apparent loss of circadian rhythms might not be due to arrhythmic circadian clocks, but due to loss of coupling between activity and the circadian clocks (Sharma et al. 2004b).

The results of our experiments suggest that independent endogenous circadian rhythms, as well as caste-bound differences at the level of individuals, exist in ants. The results suggest a simple but interesting possibility that the workers of *C. compressus* adopt different phase and/or period of circadian clocks to ensure that some ants are always working to meet the needs of the colony. If so, this would be an interesting difference between ants and honeybees. For example a labile circadian clock could provide an additional advantage to the media workers changing tasks and in temporally "scanning" the natural environment, as it were. The ecological and behavioral significance of this phenomenon eludes our present understanding and warrants more extensive field and laboratory studies.

Although our data clearly indicate that activity patterns of the media workers show both nocturnal and diurnal patterns under LD cycles, and undergo changes during laboratory free-runs, we do not know whether such phenomena occur under colony conditions in nature. What we know is that the media workers are normally seen outside their colonies during the night, and occasionally they can also be spotted, although in fewer numbers, during the day (S.R. Lone, personal observation). Our study appears to be the only such investigation, the results of which imply that there are significant circadian consequences to the intricate social evolution and organization of ant societies. More extensive studies are in progress in our laboratory to confirm many of the novel features enumerated in this paper, to understand the precise role of circadian pacemakers in the behavioral integration of different castes of ants.

References

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

Bourke AFG, Franks NR (1995) Social evolution in ants. Princeton University Press, Princeton, N.J., pp 400–443

Calabi P (1988) Behavioural flexibility in Hymenoptera: a re-examination of the concept of caste. In: Trager JC (ed) Advances in myrmecology. EJ Brill, Leiden, The Netherlands, pp 237–258

Dunlap JC, Loros JJ, DeCoursey PJ (2003) Chronobiology. Sinauer Associates, Sunderland, Mass.

Gordon DM (1989) Dynamics of task switching in harvester ants. Anim Behav 38:194–204

Hölldobler B, Wilson EO (1990) The ants. Springer, Berlin Heidelberg New York

McCluskey ES (1992) Periodicity and diversity in ant mating flights. Comp Biochem Physiol 103A:241–243

North RD (1987) Circadian rhythm of locomotor activity in individual workers of the wood ant *Formica rufa*. Physiol Entomol 12:445–454

North RD (1993) Entrainment of the circadian rhythms of locomotor activity in wood ants by temperature. Anim Behav 45:393–397

Roces F, Nuñez JA (1996) A circadian rhythm of thermal preference in the ant *Camponotus mus*: masking and entrainment by temperature cycles. Physiol. Entomol. 21:138-142

Saunders DS (2002) Insect clocks. Elsevier, Amsterdam, pp 10–13

Sendova-Franks AB, Franks NR (1993) Task allocation in ant colonies within variable environments: a study of temporal polyethism: experimental. Bull Math Biol 55:75–96

Sendova-Franks AB, Franks NR (1994) Social resilience in individual worker ants and its role in division of labour. Proc R Soc Lond B 256:305–309

Sharma VK (2003a) Period responses to Zeitgeber signals stabilize circadian clocks during entrainment. Chronobiol Int 20:389–404

Sharma VK (2003b) A simple computer-aided device for monitoring activity of small mammals and insects. Biol Rhythm Res 34:3–12

Sharma VK, Lone SR, Goel A, Chandrashekaran MK (2004a) Lability in the circadian activity rhythms of the medium workers of the ant *Camponotus compressus*. Chronobiol Int 21:297–308

Sharma VK, Lone SR, Goel A (2004b) Clocks for sex: loss of circadian rhythms in ants after mating? Naturwissenschaften DOI: 10.1007/s00114-004-0526-8

Toma DP, Bloch G, Moore D, Robinson GE (2000) Changes in *period* mRNA levels in the brain and division of labor in honeybee colonies. Proc Natl Acad Sci USA 97:6914–6919