Circadian Rhythms in Male Ants of Five Diverse Species

Abstract. *When kept at constant temperature and subjected to a daily cycle of light and darkness, males of* Paraponera clavata, Iridomyrmex humilis, Solenopsis saevissima, Veromessor andrei, *and* Camponotus clarithorax *were active at different hours. With* Solenopsis *and* Veromessor *the active hours relative to the laboratory dawn corresponded with the hours of mating flight in the field. In four of the five species the rise preceding maximum activity began before change to light or to darkness. This endogenous behavior, widespread in males, is of interest because timing in the worker caste has been questioned.*

The hour of the mating flight of an ant is characteristic of the species; such factors as temperature and light intensity affect this timing (*1, 2*). That an internal "clock" also may play a part is indicated by the observation that *Veromessor andrei* (Mayr) males, which fly in the field soon after dawn, are active in a constant-temperature laboratory principally near the laboratory dawn, with a significant rise in activity before the end of darkness (*3, 4*); this rhythm persists in constant darkness. In the field a clock may prompt the ants to reach the nest exit in time to perceive the environmental cue of dawn, thus synchronizing flights and increasing the chance of finding mates from other nests; such a clock could also initiate physiological preparation of the ants hours ahead of flight. Observed on the same days as *Veromessor* were males of *Iridomyrmex humilis* (Mayr), the Argentine ant; they leave their nest units during only the last 2 hours of the light period, in either fluctuating or constant temperature, at a time of unchanging light. This rhythm also persists in constant darkness.

I now report the rhythmic activity and differing relations in phase to the light-dark cycle for males of *Solenopsis saevissima* (Fr. Smith), the fire ant, and for males and workers of *Camponotus clarithorax* Emery (*5*). The ants were kept under fluorescent light from 0800 to 2000 hours; light intensity on the ants was about 400 lux. The mechanism that turned-off the white light turned-on a clear ruby light; there was no detectable change in temperature with change in the light cycle. The red light was left on continuously during the constant darkness which followed the several days of alternating light and darkness. One group each of *Camponotus* males, *Camponotus* workers, and *Solenopsis* males was placed in a circle of clear plastic tubing that included at one point a tiny, clear, plastic chamber with cotton-plugged test tubes of water and of sugar water inverted through its roof. Chamber, with tubes, was covered with an orange plexiglass box to simulate the darkness of a natural nest (*4*). A polystyrene tunnel formed another part of the circle; an adjacent capacitance-operated relay recorded the number of times ants passed through the tunnel.

Figure 1 shows activity rhythms in alternating light and darkness, and Fig. 2 summarizes these rhythms by considering records of successive days as replicates. *Solenopsis* was most active during the middle and late part of the light period. The machine count was corroborated by simultaneous observations (see curve above histogram). The significance level of the beginning of rise in activity during the light is indicated in Fig. 2. When the white lights were left off, a good peak appeared at the usual hour the first day; the counter failed at this time, making it impossible to verify persistence.

The peak in activity of *Camponotus* males was at the beginning of the light period, with but few counts between midday and the following morning. Workers were counted simultaneously in an identical assembly; their time of activity was at night, opposite to that of the males. The record for both castes through 5 immediately subsequent days of constant darkness (see Fig. 1 for first day) showed no persistence of rhythm, in contrast with observations of *Iridomyrmex* and *Veromessor* in which persistence had been obvious (*4*); this difference may correlate with the greater sharpness of maxima for the latter two species.

The plaster nest unit used to confirm the rhythm of *Iridomyrmex* males (*4*) included queen, brood, workers, and another generation of males—portion of the colony reported on in (*4*). In fluctuating room conditions the males left the nest only at the end of the day (Fig. 3*a*). Now, through the transparent roof of the nest, activity within was observed to continue through the daylight hours in marked contrast with the nonappearance of males outside.

Figure 3 compares the rhythms of the males of five species, those already mentioned and *Paraponera clavata* (Fabr.). Activity of *Paraponera* (*6*) was recorded under the same conditions as activities of *Solenopsis* and *Camponotus*. These five species represent four of the nine subfamilies of ants. They include tropical (*Paraponera*), temperate (*Veromessor*), nocturnal (*Paraponera*), and diurnal (*Veromes-*

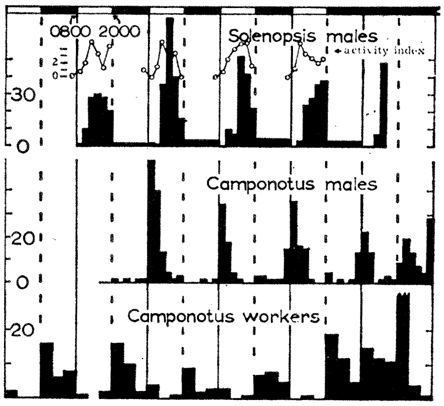

Fig. 1. Activity rhythms in constant temperature (26° ± 1.0°C); 12-hour light alternating with 12-hour darkness, followed by constant darkness, as shown by light-regime bar; machine counts of ants passing through tunnel during 2 hours (males) or 4 hours (workers); five ants in each set. *Solenopsis*: 18 (0800 hours) to 22 (1600 hours) October 1959; rate for night derived from cumulative total for night; activity index (one point for each ant moving, two points if active) refers also to a replicate group of males. *Camponotus*: workers, 19 (0800 hours) to 20 (1200 hours) December (when disturbed); workers and males, 21 (1600 hours) to 26 (0800 hours) December 1959.

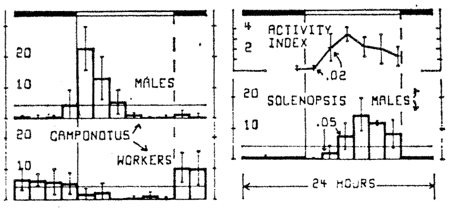

Fig. 2. Statistical summary of rhythms in alternating light and darkness (light-regime bars at top). Histograms: ordinate, mean percentage of daily total, based on successive days of Fig. 1 as replicates [each replicate percentage was hourly fraction (averaged from 2 hours) of the 24-hour total centered on that 2-hour interval]; horizontal line at 4 percent (100 percent = 24 hours) is average mean; *Solenopsis*, 18 (0800 hours) to 22 (0800 hours) October; *Camponotus* (males), 21 (1600 hours) to 25 (0800 hours) December; (workers) 18 (0800 hours) to 20 (0800 hours), 21 (2000 hours) to 25 (0800 hours) December. Activity-index ordinate, mean based on replicate days and groups of Fig. 1. Vertical lines represent 90-percent confidence intervals; *.02* and *.05*, based on one-tailed *t* tests, indicate significance of rise in activity during light.

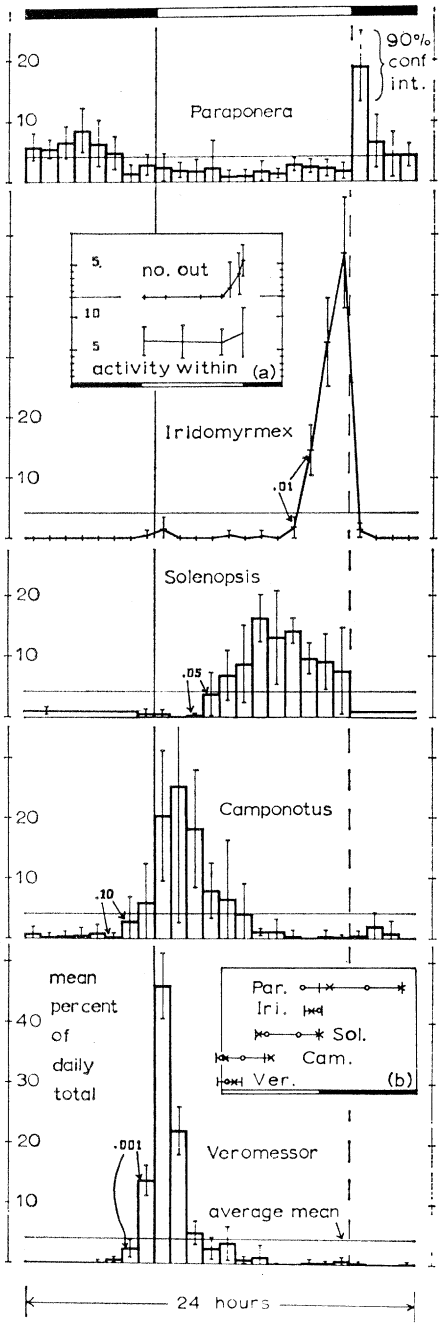

Fig. 3. Comparison of male rhythms in constant temperature; 12-hour light : 12-hour darkness (light-regime bar at top) except for *Iridomyrmex* and *Veromessor* (14:10 hours; their "dawns" and "dusks" were matched with those of other genera by shifting two zero hours from midday to midnight part of graph). Hourly means based on replicate days listed (each replicate percentage was hourly fraction of the 24-hour total centered on that hour); *Iridomyrmex*, spot count of number out of nest; others, cumulative machine counts. *Paraponera*, 4–9 February 1960; *Iridomyrmex*, 28 September to 3 October 1957; *Solenopsis*, 18–22 October 1959; *Camponotus*, 21–25 December 1959; *Veromessor*, 29 September to 6 October 1957 (*Iridomyrmex* and *Veromessor* data from ref. *10*). *P* values, from one-tailed *t* tests between adjacent hours shown, indicate significance of increase at beginning of rise in activity during light (*Iridomyrmex* and *Solenopsis*) or darkness (*Camponotus* and *Veromessor*). Inset *a*: *Iridomyrmex* (day part of cycles, room conditions, December 1959), comparison of number out of nest (spot count) and activity index within nest unit (number per minute passing under wire across roof, plus number running). Inset *b*: from same data as main part of figure (shown from dawn to dawn instead of midnight to midnight): o--o, 95-percent confidence interval of highest count of day; x--x, ditto for second-highest count; |--|, time when hourly means were above average mean.

sor) ants; ants with a single queen (*Veromessor*) or with many queens (*Iridomyrmex*); ants having a mating flight (*Veromessor*) or mating in the nest (*Iridomyrmex*). This wide representation suggests that endogenous circadian behavior is a general characteristic of at least the male caste of ants. Such a characteristic should be expected, since such rhythms have been demonstrated in many other organisms (*7*); it is significant here because neither Reichle nor Dobrzański (*8*) has been able to confirm Grabensberger's (*9*) claim of a circadian "time memory" in worker ants similar to that of bees (*10*).

The peak for each species is defined in Fig. 3*b* by a 95-percent confidence interval based on locations of the highest counts on successive days. This interval nearly coincides with the time interval, which includes all the hourly means that are above the average mean; the time interval is shown by vertical lines in Fig. 3*b*.

The maxima of *Veromessor* and *Iridomyrmex* appear to be narrowest; they are equally narrow even when based on only the first 3 days, the smallest number of replicates available for any of the other ants. *Veromessor* males were otherwise quiet during the 24 hours (*11*) when in a counting assembly like that used for *Camponotus* and *Solenopsis*; they may be active at additional hours within the nest when part of a normal colony, as was *Iridomyrmex*.

The periods of maximum activity of the various species occurred at different hours. Evidence that this difference in timing represents a real difference between species is the fact that all ants were studied under similar conditions—and in the following instances simultaneously: *Iridomyrmex* and *Veromessor* (*3*), and *Iridomyrmex* and *Paraponera* (*6*) (the fact that *Iridomyrmex* rhythm was the same in both instances serves to compare *Paraponera* and *Veromessor*).

Table 1. Number of *Iridomyrmex* males outside nest unit at beginning of rise preceding maximum activity. Mean of two counts, 1 minute apart; light on, 0600–2000 hours; no observations 3 and 4 May; data from ref. *11*. Difference between counts at 1840 and 1910 hours indicated by *t* value of 5.35, $P < .001$ (one-tailed test).

Hr	April			May				
	28	29	30	1	2	5	6	7
1840	1	0	0	0	0	0	3	3½
1910	2	1	1	3	1½	3½	5½	5

These relations of activity to light phase suggest that the light cycle may participate in flight timing, as has been found in *Veromessor* males. Flight of *Solenopsis saevissima* repeatedly occurred "close to the middle of the day" in Alabama and occurred at "early midday" in Argentina (*12*). Individual *Paraponera* females were attracted to artificial lights at night in the Canal Zone; the only males seen were at a nest exit soon after dark (6). *Iridomyrmex humilis* males are found at lights at night (*13*); Warter (*14*) saw wing fluttering by males after they had climbed grass stems in the evening. Thus in two or more species the time of peak activity in constant temperature corresponds with time of flight in the field, where factors such as fluctuation in temperature are also important (*2*). Perhaps an internal clock times flight to a species-characteristic point in the light-dark cycle, when flight occurs if environmental conditions are suitable. Where the hour, or the very practice, of mating flight is uncertain [as in *Iridomyrmex* (*13*, p. 12)], the timing of activity in the laboratory suggests the best hour to search for flights in the field.

Evidence for the endogenous nature of the clock in *Veromessor* and *Iridomyrmex* males is summarized in (*4*). In four of the five species on which I now report, the rise in activity preceding maximum began *during* light or darkness rather than at dawn or dusk (Fig. 3). The abrupt change in *Iridomyrmex* is shown by the increase in number of ants out of the nest before the lights went off; increase was sufficient within 30 minutes to be significant at the .001 level (Table 1). Thus one may suspect that the behavior reported reflects underlying temporal organization of the animal (see *15*).

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3 August 1965

Organ Weights in Primates and Other Mammals

Abstract. *In mammals the weight of the heart, kidney, lungs, and other organs can be related to total body weight through power laws (allometry). Weights of primate organs are analyzed by this technique. Allometric coefficients and size-independent organ-weight or body-form ratios may be used to compare primates, including humans, and other mammals.*

About 1895–1900 it was observed that the relative brain size of anthropoids was best assessed by use of parameters obtained from a log-log plot of brain weight against body weight. This analytical technique subsequently came to be known as "allometry," and was much advanced by a book by Julian Huxley (*1*). Huxley showed that the plotting of many gross body measurements against body weight or a reference body length yields straight lines on log-log paper. A massive collection of allometric data on organ weights and body-size parameters was provided by Brody (*2*). Subsequently it was demonstrated (*3, 4*) that the allometric technique could also be applied to body minerals and fluids, many physiological parameters, muscle masses, a variety of skeletal measurements, and other parameters.

This report presents allometric data (Table 1) on the weight of the principal organs (heart, lungs, liver, kidneys, adrenals, thyroid, pituitary, spleen, pancreas, and brain) of primates and "mammals" (which here designates mammals other than primates). Statistical analysis reveals that the allometric prediction equations are reliable and that the coefficients in these equations appear to be statistically indistinguishable for the primate and mammalian groups, except in the case of the brain. It is also pointed out that one may form size-independent ratios of organ weights which constitute dimensionless organismal "shape factors."

The weight data for mammalian organs were obtained from Brody (*2*, chap. 17). He includes all necessary statistical measures except for total numbers of animals, but these were large, as is seen from the graphs of his regression lines. Brody does not give satisfactory prediction equations for the pituitary, pancreas, or spleen of mammals. Data on organ weights, from an extensive collection of such data by Crile and Quiring (*5*), were used to obtain the parameters shown for the spleen and pituitary in mammals in Table 1; there are not enough published data on the pancreas to enable allometric coefficients to be obtained for mammals. The mammalian prediction equations are known to be valid in the size range from mice (25 g) to steers (500 to 1000 kg), and may even apply to elephants and whales.

Data on weights of primate organs were obtained from published reports of Hrdlicka (*6*), Crile and Quiring (*5*), Kennard and Willner (*7*), and Herrmann *et al.* (*8*). New data collected at the Oregon Regional Primate Research Center during the last 4 years [Knezevic (*9*), Malinow (*10*)] were also included. There were 321 data points found for primate hearts, with lesser numbers for other organs. As shown in Figs. 1 and 2, the size range of primates covered is from tree shrews (less than 10 g) to humans (70 kg), with limited data on the gorilla (over 100 kg). The points in Figs. 1 and 2 identify authors of data, not kinds of animals, and include a considerable range of body sizes for each kind of primate named, for example, weights of 2 to 10 kg for macaque monkeys. However, most of the primate data are from animals which are of at least "young adult" age. The prediction curves are for organ weights in mature animals of various sizes, and not for organ size during growth and development as such. Data on howler monkeys, collected by Malinow (*10*) at this Center, do include some for fairly young animals, but they fit the general prediction formulas in a satisfactory manner.

The organ weights were analyzed against body weight by a regression analysis, with a SDS-920 computer (*11*). Statistical formulas for fitting the allometric equations $y = a\ x^b$ are provided by Brody (*2*, pp. 398–401 and other pages). In this report, organ weight (y) is given uniformly in grams; body weight (x), in kilograms. Allometric parameters were obtained by a least-squares fit after conversion to $\log_{10}$ values. The a parameter is the value of the ordinate at a body weight of 1 kg. In view of the log-log transformation, the most useful measure of precision for the a value is its standard error, given as a percentage of the mean value. The parameter $S_{a\%}$ is this statistic for a, and represents the 67-percent confidence limit; 95-percent confidence limits are given by twice $S_{a\%}$. The log-log plot slope value is given directly as b; S_b is the standard error of the slope in absolute slope units.

An indication of the validity of the entire plot is given by the value of the correlation coefficient r and the standard deviation of data points from the prediction line, as indicated by the standard error of the estimate (S) in Table 1. For all cases shown in this table, correlations were significant at the .01 level of confidence. There has been considerable discussion of the best techniques for fitting allometric data (*12*), but the cited statistical parameters appear to be adequate.

Table 1 and Figs. 1 and 2 show that the mammalian and primate prediction equations are almost identical, with the major exception of the brain. In the case of the heart, lungs, kidneys, pituitary, and spleen the 1-kg intercept (a) and slope (b) parameters lie within one standard error of each other, while for the liver, adrenals, and thyroid the a and b values are at worst within two standard errors of each other.

This conclusion is confirmed by Figs. 1 and 2, which compare Brody's previously published lines for mam-