Latitude Dependent Circadian Rhythms of Carabid Beetles

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Comparative studies of circadian activity rhythms were performed with three stocks of the species Pterostichus rhaeticus Heer (from Southern Europe, Central Europe, and the Subarctic) and with one Central European stock of the sibling species Pterostichus nigrita Paykull. The behaviour was investigated in various LD conditions, constant conditions including LL with changes of the illumination intensity and constant darkness with light pulses. The data were analysed with the program system “Timesdia” containing various techniques like power spectrum estimation, periodogram analysis and complex demodulation. This allowed to reveal the time course of rhythms and to determine the number of phase jumps as a measure for precision. With the help of these methods, the activity patterns are classified in different types of behaviour: synchronization, temporarily synchronization, aperiodic behaviour, free run, temporarily free run, relative entrainment, and relative coordination. Besides graduated differences between the populations in south-to-north direction (e.g. in the tendency to rhythmic behaviour) the analysis revealed splitting of rhythms into two or even three components of different frequencies and transitions from rhythmic to aperiodic behaviour or vice versa after arbitrarily chosen light pulses in constant darkness. This behaviour cannot be explained by a system which is composed of one single limit cycle oscillator, but the results rather support a multi-oscillator model.

Introduction

Animals usually show many adaptations to latitude in terms of physiology and behaviour. Since the circadian system is adapted to environmental conditions [1, 2] one has to suppose that circadian rhythms are also effected by a parameter like geographic latitude. Results from the entrainment of circadian rhythms of birds [3] support such an idea. In populations of Carabid beetles Thiele already found considerable differences in the time control system of gonad maturation in adaptation to different geographic regions [4, 5].

We therefore started a characterization of the circadian activity rhythms of Carabid beetles from different geographic latitudes. For the comparative study, four different stocks of Carabids were used: a Subarctic, a Central European and a South European one of the species Pterostichus rhaeticus Heer as well as a Central European stock of the sibling species Pterostichus nigrita Paykull. Furthermore, this selection allows to compare the rhythmic organization of the sibling species P. rhaeticus and P. nigrita in respect to the adaptation to environmental conditions.

Appropriate experiments — especially constant darkness with light pulses — are designed to determine the complexity of the control systems (one-, two- or multi-oscillator systems). Differences in behaviour may be attributed to different model parameters. But this also requires the choice of appropriate methods of analysis. For characterizing the variability in behaviour, a distinction between “true” synchronization and relative entrainment, for example, is necessary. Only thereby comparative statements concerning the entrainment in different populations are justified. For these purposes, one has to apply methods which inform about the temporal course of the rhythms, let recognize their liabilities, and therefore allow to define the precision of observed periodicities. For an exact qualitative and quantitative characterization of behaviour, the application of the computer program system “Timesdia” [6—12] proved to be useful.

Thus, we represent a new effort to characterize the rhythmic organization of insect populations in their whole variability.
Material and Methods

Material

The experimental animals were reared in the laboratory. The following stocks were used:

1. *P. rhaeticus* from South Europe: Istria, Yugoslavia (45°35' N).
2. *P. rhaeticus* from Central Europe: Cologne (51° N).
4. *P. nigrita* from Central Europe: Lower Rhine region (51°45' N).

Until recently the three stocks of *P. rhaeticus* Heer were appointed to the species *P. nigrita* Paykull [5, 13, 14]. But it has been shown that one has to distinguish between the sibling species *P. rhaeticus* and *P. nigrita* [15, 16] which differ in their karyotypes and the shape of the genitals.

Experimental conditions

The temperature was kept constant at 20 °C (±1 °C). The artificial light was produced by fluorescent tubes of the type "white". Light intensity was controlled by a fullwave single-phase rectifier circuit driven by a programmable twelve bit up-down counter and a network of some difference and reference amplifiers. The activity of the beetles was measured by IR-light barriers (maximum intensity at 978 nm). A pulse-counting printer recorded the impulses resulting from the animals crossing the barriers, the values were printed out every half hour. If possible, under the same experimental conditions there were tested 20 animals of each stock (10 females and 10 males), at least however 10 beetles (5 females and 5 males).

Characterization of activity patterns under external excitement by light-dark cycles

Exciting circadian rhythms is a common procedure to evaluate properties of the circadian system [2, 17]. In this study, we only consider excitement by light-dark cycles. (To the effect of temperature cycles see Fig. 1. Analysis of a periodic time series (animal of the species *P. rhaeticus* from Istria under Zeitgeber conditions LD 20/4).

a. The original data. Ordinate: locomotor activity (number of impulses per 30 min). Abscissa: time (days).

b. The log-power spectrum. One can recognize a peak in the region of 24 h and divisors (B = bandwidth, C = confidence-interval).

c. The periodogram. Ordinate: intensity, i.e., the extent q to which the time series contains a periodic component p with 0 ≤ q(p) ≤ 1. Abscissa: period (h). The periodogram shows a striking peak at 24 h as well as further maxima at divisors of 24 h and their multiples (for example 12 h, 16 h). Peaks outside the shaded area are significant at the 5%-level.


e. The average signal. Ordinate: locomotor activity (impulses). Abscissa: time of day (h). Variation of the points is given by 90% t-confidence regions. The bar above indicates the L:D ratio (hatched area = darkness). Evidently the animal was night active with a maximum soon after the onset of darkness.
Leyk, Thiele, Neumann [18]. One of the first who applied excitement by light-dark cycles to carabids where Weber and Lamprecht [19].

In order to characterize and compare the obtained activity patterns of the stocks, they were classified in different types of behaviour after an exhaustive time series analysis by using the program system TIMESDIA. Fig. 1 demonstrates this procedure. It shows the analysis of the data of a South European animal (P. rhaeticus) kept under a light-dark cycle with a L:D ratio of 20:4. The power spectrum (Fig. 1b) of the sampled data (Fig. 1a) shows a peak in the regions of 24 h and divisors. The periodogram (Fig. 1c) indicates the improved estimation of the period of the activity rhythm which was 24 h like that of the Zeitgeber. (There are always further peaks at multiples and divisors of the period due to the shape of the signal.) In order to get information about the temporal stability of the rhythm one can check the complex demodulated version of the data (Fig. 1d). The constant phase indicates a precise 24-hour rhythm without phase jumps which was kept during the whole time course of the experiment. The average signal (Fig. 1e) provides informations about the shape of the rhythm, the phase relation to the Zeitgeber, and the duration of activity time. Evidently this animal has been active during the short night with a maximum soon after the onset of darkness.

Besides rhythmic behaviour, the beetles showed various intermediate reactions between both extrema: complete synchronization and complete arhythmic behaviour. Such an intermediate case is demonstrated by the phase diagram of the complex demodulated data in Fig. 2. Besides sections with a stable phase other ones with an irregular course are striking. We called this type “temporarily synchronization”, and we used the following definitions:

synchronization (S): 70% ≤ duration of synchronization ≤ 100% of the experimental time (cf. Fig. 1);
temporary synchronization (tS): 30% ≤ duration of synchronization < 70% of the experimental time (cf. Fig. 2);
arhythmic behaviour (A): duration of synchronization < 30% of the experimental time (Fig. 3).

Furthermore other types of behaviour could be observed under Zeitgeber conditions, that was relative entrainment and relative coordination (see also Wever [20]):

relative entrainment (RE): The Zeitgeber period is dominant, i.e. only one periodicity with the period of the Zeitgeber or a strong periodicity and additionally a weaker circadian peak can be detected by the power spectrum and the periodogram. The complex demodulation always reveals a wave-like course of the amplitude and the phase (see Fig. 4);
relative coordination (RC): The circadian period is
dominant, i.e. a strong circadian periodicity and additionally a peak caused by the Zeitgeber can be detected by the use of the power spectrum and the periodogram. The typical course of the phase of the complex demodulated time series is characterized by a shifting which gets more slowly during some sections (Fig. 5).

Fig. 4. Analysis of the data of a Central European animal (P. rhaeticus, Cologne stock) kept under a LD cycle with $T = 10 \, \text{h}$ ($L:D = 1:1$). In these conditions the beetle showed relative entrainment with $T = 10 \, \text{h}$.

a. The original data and the LD cycle. Ordinate: amount of activity (number of impulses per 30 min) or illumination intensity (lux). Abscissa: time (days).

b. The log-power spectrum. One can recognize a peak in the region of 10 h ($B =$ bandwidth, $C =$ confidence-interval).

c. The complex demodulation. One can recognize a wave-like course of the amplitude (above) and the phase (below). Furthermore the amplitude is damping down.

Fig. 5. The analysis of the data of a Central European animal (P. nigrita, Rees). The beetle shows relative coordination.

a. The original data. Ordinate: amount of activity (number of impulses per 30 min). Abscissa: time (days).

b. The log-power spectrum. A significant peak in the region of 24 h can be recognized ($B =$ bandwidth, $C =$ confidence-interval).

c. The autocorrelation. There is a periodicity shorter than 24 h. It consists of two nearly completely fused waves.

d. The periodogram. Ordinate: intensity, i.e., the extent to which the time series contains a periodic component with $0 \leq q(p) \leq 1$. Abscissa: period (h). There is a circadian peak (at 22.6 h) as well as a smaller one at the Zeitgeber period (24 h) (further peaks at divisors of the two periods and at multiples of divisors).

e. The complex demodulation at 24 h. The figure shows the phase diagram. Caused by the circadian component at 22.6 h, the phase is drifting away and is only retained by the Zeitgeber from time to time.
Characterization of activity patterns under constant conditions

There could be observed free running rhythms, arhythmic behaviour and transitions between these types. We used the following definitions:

free running rhythms (Fr): with a period deviating from that of the Zeitgeber and an essentially linear course of the phase (after complex demodulation; the duration of the free running state \( \geq 70\% \) of the experimental time, Fig. 6);

temporary free running rhythms (tFr): Free run during parts of the experiment (30\% \leq \text{the duration of the free running state} < 70\% \text{ of the experimental time}). (This type of behaviour is not illustrated by a figure, for the time series analysis shows analogous results as for the temporary synchronization. Only the period is different.)

(For arhythmic behaviour results are similar to those shown in Fig. 3.)

Results

Excitement by light-dark cycles with \( T < 24 \text{ hours} \)

In both species the activity rhythms were only entrained in the region of \( T = 24 \) until \( T = 19 \text{ h} \) (primary range of entrainment) and in LD cycles with \( T = 12 \text{ h} \) and \( T = 10 \text{ h} \), which represent a secondary range of entrainment (Fig. 7). Within this region only entrainment with a period of \( 2T \) (frequency-demultiplication FD) occurred. One might have expected a further range of entrainment at \( T = 8 \text{ h} \), for 8 is also a divisor of 24. But frequency-demultiplication could only be observed at one South European beetle, which showed synchronization with a period of \( T = 24 \text{ h} \). Otherwise in this LD cycle often relative coordination occurred.

A comparison of the behaviour of the three stocks of \( P. \text{ rhaeticus} \) in the entrainment experiments reveals differences in the lower limits of entrainment. Whereas in the 19-hour day most of the South European animals (70\%) were still synchronized, in the stock from Cologne, entrainment could only be found in 40\% of the animals. In the Subarctic stock even none of the beetles showed synchronization under these conditions.

Similar tendencies could be observed in the secondary range of entrainment. In summary the limits of the ranges of entrainment appeared to be shifted to shorter days within the species \( P. \text{ rhaeticus} \) in the following order: Lapland \( \rightarrow \) Cologne \( \rightarrow \) Istria.

Concerning the sibling species \( P. \text{ nigrita} \) Rees the lower limits of entrainment were already reached in the 19-hour day and in the 10-hour day (only 20\% entrainment each). In comparison to the South European stock of \( P. \text{ rhaeticus} \) in \( P. \text{ nigrita} \) (Rees) the lower limits of entrainment appear to be shifted to longer days.

At the limits of entrainment and outside often there could be recognized relative entrainment. In shorter days the circadian component became more
important but undisturbed free running rhythms scarcely occurred.

In the secondary range of entrainment and its limits, relative entrainment with the frequency of the Zeitgeber, with half of the Zeitgeber frequency and combinations of both possibilities occurred. The phase relation of synchronized animals changed within the ranges of entrainment systematically, the maximum of activity shifted in shorter days towards later points of the dark intervals.

**Constant conditions with light pulses and steps**

During LL with stepwise changing illumination (reduction of one or two powers of ten) various alterations in behaviour appeared.

All stocks of both species reacted sensitively to changes in the light conditions. Transitions to other types of behaviour (for instance free run to arhythmicity or vice versa), period changes and splitting of the rhythms into two or in two cases even three components with different frequencies could be observed. Fig. 8 demonstrates the behaviour of a South European animal (*P. rhaeticus*) after reduction of the illumination intensity from 250 to 2 lux when the free running rhythm splitted into three periodicities. If the same periodicity was kept after the alteration of the light intensity, generally at least a phase jump occurred. The data from *P. nigrita* (Rees) and from the Central European and South European stock of *P. rhaeticus* indicate the tendency that changes in behaviour after reduction of the light intensity result in more rhythmic behaviour. Towards DD the percentages of free running behaviour increased in these stocks (Fig. 9).

Similar to changes in the light intensity in LL the animals reacted to arbitrary chosen light pulses during constant darkness with fundamental changes in behaviour. Even transitions from rhythmic to arhythmic activity patterns could be observed in all populations. Such a case is demonstrated by Fig. 10. Before the first light pulse a free running rhythm with a period of 23.2 h was detectable but afterwards the activity became arhythmic.
Fig. 8. Analysis of the data of a South European animal (P. rhaeticus) kept under LL with variation of the light intensity. After reduction of the illumination intensity from 250 to 2 lux, there occurred a splitting of the free running rhythm in the way that 3 periodicities with different frequencies existed simultaneously.

a. The periodogram. It shows 3 peaks in the circadian region (at 22.3, 23.7, 24.2 h).

b. The periodogram after the periodicity with $\tau = 23.7$ h had been extracted, the two others are still detectable.

c. The periodogram after successive extraction of the periodicities with $\tau = 23.7$ h and $\tau = 22.3$ h, the third periodicity is still detectable. The periodograms shown in part b and c of this figure underline the independence of the three overt rhythms.

was given a second light pulse (see arrow in Fig. 10). Thereafter a free running behaviour could be observed again but with a different period as indicated by the essentially linear but not constant course of phase.

Although changes in behaviour as reactions to reduction of the illumination intensity or to light pulses during constant darkness appeared in all stocks, the sensitivity to perturbations was different. It increased within the species P. rhaeticus in the order

Fig. 9. The behaviour under LL conditions with stepwise changing illumination intensity.

a and b. P. nigrina (Rees) and P. rhaeticus (Cologne). The illumination intensity was reduced stepwise in the following order: 250 lux $\pm$ 10% $\rightarrow$ 21 lux $\pm$ 10% $\rightarrow$ 2.5 lux $\pm$ 10% $\rightarrow$ 0.2 lux $\pm$ 0.1 lux $\rightarrow$ DD. The figure shows the percentages of the types of behaviour.

c. The stocks from Istria and Lapland (P. rhaeticus). The illumination intensity was reduced from 250 lux $\pm$ 10% to 2.5 lux $\pm$ 10%. The figure shows the percentages of the types of behaviour.

Fig. 10. The behaviour of a Central European animal of the species P. nigrina (Rees) kept under DD with light pulses (duration = 2.5 h, 2000 $\pm$ 100 lux).

a. The original data. Ordinate: amount of activity (number of impulses per half-hours-intervals). Abscissa: time (days).

b. The phase diagram of the complex demodulated data (centre of the band at 23.2 h). The rhythm first free running with a period of 23.2 h (nearly constant phase in the first segment) was interrupted by the first pulse (see arrow). Within the interval between the two light pulses, the beetle behaved arrhythmically (irregular course of phase), but after the second pulse (see arrow) the activity pattern got periodically again, however with another period than before the first pulse (extensively linear but not constant phase, $\tau = 23.8$ h).
Istria → Cologne → Lapland. In this direction there could be observed fundamental changes in behaviour. Fig. 11 demonstrates these tendencies for the DD experiments with light pulses. The sensitivity of *P. nigrita* (Rees) can be classified as intermediate between the stocks from Istria and Cologne of *P. rhaeticus*. The Subarctic beetles exhibited the most labile rhythmicity (Fig. 11).

**Experiments under “natural” light-dark conditions and constant conditions**

An excitement with $T = 24$ h reflects “natural” light-dark conditions as the period-length of the Zeitgeber is concerned. Results point to the same direction as those shown in Fig. 7. The percentages of animals with rhythmic behaviour found in the Subarctic stock were smaller than in all other populations. The rhythms were less precise. Besides, the considerable individual variability is striking. Within the species *P. rhaeticus* the tendency to entrainment under Zeitgeber conditions and to rhythmic behaviour in general as well as the precision of the rhythms decrease in the following order (south-to-north direction): Istria → Cologne → Lapland. These tendencies could also be observed during constant conditions (LL and DD). A comparison of the stocks of the sibling species exhibited many similarities to *P. rhaeticus* from Istria and also parallels between the Central European animals of both species but important differences to the Subarctic stock of *P. rhaeticus*. (For a more extensive evaluation of these experiments we confer to Leyk, Thiele, Neumann [18].)

**Discussion**

**LD cycles and constant conditions**

In all stocks at least a part of the beetles showed free running rhythms under constant conditions. The self-sustainment of the circadian system is proved by the limited regions of entrainment and the appearance of free running rhythms and relative coordination under Zeitgeber conditions. In the tendency to rhythmic behaviour and in the precision of the rhythms, however, graduated differences between the stocks could be detected and interpreted with respect to deviating environmental conditions in the different geographic regions. For the Subarctic beetles one can suppose a regressive evolution of the circadian system as adaptation to an environment without strong Zeitgeber (for more detailed discussion see Leyk, Thiele, Neumann [18]).

**LD cycles with $T < 24$ hours**

As to expect from self-sustained oscillators (Wever [21], Meyer and Guicking [22]) entrainment with a considerable systematic alteration of the phase relation could only be observed within certain ranges. Similar to the behaviour of an oscillator of the van-der-Pol type which has been simulated by Wever [20] first relative entrainment and then — farther away from the ranges of entrainment — relative coordination appeared. Phenomena like relative coordination and frequency-demultiplication were also observed in other species [23–31].
Corresponding to a statement of Aschoff and Pohl [32] the ranges of entrainment should be considerable larger in insects than in vertebrates. For the lower limit of the primary range of entrainment, a comparison of our results of these Carabid beetles with the data of vertebrates [33−35] does not confirm this tendency.

In LD cycles with shorter periods (12, 10, 8 h) there occurred in some cases relative entrainment with the periods \( T \) and \( 2T \) synchronously. Similar to observations on van-der-Pol oscillators [36, 37] concerning the coexistence of different periodic or almost periodic oscillations (for instance harmonics and subharmonics of the forcing oscillation) in LD cycles with shorter periods (12, 10, 8 h) there occurred in some cases relative entrainment with the periods \( T \) and \( 2T \) simultaneously.

In summary from these entrainment experiments, one can conclude that the observed phenomena are in accordance with the behaviour of a self-sustained oscillator under Zeitgeber conditions.

**Constant conditions with light pulses and steps**

Supposing a one-oscillator model — that means an oscillator which is defined by 2 state variables combined in a nonlinear differential equation —, one has to expect the behaviour of a limit cycle oscillator [38]. Such systems often were investigated by the help of phase-response curves [38]. A periodic phase response curve surrounds at least one singularity point which can be stable or unstable. If by an appropriate disturbance — for instance a light pulse during constant conditions — the system gets to this point or nearby, the oscillator can be stopped. The striking result from the experiments with the Carabid beetles under DD conditions with light pulses is that even an arbitrarily chosen light pulse can cause a transition from rhythmic to arhythmic behaviour and vice versa beneath other fundamental changes in behaviour. In respect of these observations it makes no sense to try and get phase response curves for these Carabid beetles. In summary the results of these experiments lead to the conclusion that the behaviour of these beetles cannot be explained by one stable limit-cycle oscillator. Hence it appears not suitable to interpret the observed arhythmic behaviour as reactions to pulses in the sense of reaching the singularity point as has been done for other species [39−43].

The splitting of the rhythm into two components of different frequencies which could be observed in some cases during constant conditions including those with changing light intensity speaks in favour of a time control system containing at least two oscillators [44−52]. Many of the results got for *P. rhaeticaus* and *P. nigrita* can be explained by a two-oscillator model (besides others, the abnormal values of \( T \) like 11.2, 13.4, 14.6 h as observed in constant conditions; Kawato, pers. communication). But for some phenomena there result complications. One of these is the appearance of a periodicity with a period of about 6 h which could be detected in LL after a change of the illumination intensity. This possibly needs four oscillations for explanation (Kawato, pers. communication). Another one is the splitting of the rhythm into three components with deviating periods illustrated by Fig. 8 which could be observed in two cases (*P. nigrita* from Rees and *P. rhaeticaus* from Istria) in LL after reduction of the light intensity.

**Conclusion**

Our results emphasize that a detailed time series analysis is inevitable. Without getting information about the temporal course of detected rhythms, an exact classification of the behaviour is impossible. Our methods, however, allow to characterize the behaviour of different stocks qualitatively and quantitatively especially in respect of the rhythmic state including the precision of rhythms and alterations in behaviour. Even periodicities with different frequencies which occur simultaneously can be detected. Hence statements about the complexity of the time control systems become possible. The results support a system consisting of a population of weakly coupled oscillators. (Such a system has also been proposed for other insects, for instance by Christensen and Lewis for the New Zealand weta *Hemideina thoracica* [53].) According to Pavlidis [54] certain observations which could be made at *P. rhaeticaus* and *P. nigrita* especially speak in favour of a multisynchronator system with weak coupling of the units. Only by such a system, all phenomena which have been detected at these beetles can be explained. In this context, the results about splitting of the rhythms as well as the spontaneous disappearance and reappearance of periodicity as observed in a few cases are of importance. According to Pavlidis this lability of the free run period and the arhythmic state caused in pulse experiments support this model. By such
population of coupled oscillators even the striking phenomena which have been observed at the beetles can be explained. For instance the synchronization is strained in the absence of a Zeitgeber and the presence of noise, but rhythmicity may reappear. This lability of the synchronous state can explain the spontaneous disappearance and reappearance of rhythmicity. Besides total synchronization of the units, synchronization in groups 180 degrees apart is also a possible stable state. Thus the unusual periods like 11.2, 14.6 or even 6.2 h can be interpreted.

A critical stimulus may increase the relative spread of phases among different units and thus the observation of arhythmic states is possible.

The synchronization of all units can be done in different ways and the apparent period may deviate. Thereby an observation made in LD cycles with periods smaller than 24 h can be explained. Correlation could not be obtained between entrainment of activity at the lower ranges of entrainment (primary and secondary region) and the periods of the free running rhythms exhibited by some of those animals in constant darkness. If one assumes that the observed free run period is only one of several possible values, the observation of such a correlation (small \( \tau \) and still entrainment of the rhythm at the lower range of entrainment) could be only by accident.

In respect of the differences between the investigated stocks of Carabid beetles, one ought consider the fact that all phenomena like free run, relative entrainment, arhythmic state after light pulses, splitting etc. could be observed in all populations of both species only in deviating extent. Hence it suggests itself to look at qualitative differences of certain parameters instead of fundamental deviations in the systems. Besides, one has to take into account that three of the stocks belong to the same species and the fourth one to the near related sibling species. Assuming that the coupling strength between the units is different in the populations the differences in behaviour can be explained. To interpret the greater lability of the rhythms in the Subarctic stock the oscillators should be coupled more weakly. Then the increasing tendency to rhythmic behaviour and decreasing lability of the periodicities in the order Lapland \( \rightarrow \) Cologne \( \rightarrow \) Istria within the species \( P. \) rhaeticus can be attributed to quantitative differences in a model parameter, the coupling strength. A comparison of the sibling species \( P. \) nigrita (Rees) with the populations of \( P. \) rhaeticus reveals many parallels to the South European one of \( P. \) rhaeticus. Therefore one might suppose a similar time control system for the strong related species \( P. \) nigrita (Rees) with a coupling of the oscillators resembling that of the Istria stock. But considering the differences in the ranges of entrainment there exist obviously still differences in the circadian systems in spite of many parallels in adaptation to similar photoperiodic conditions. These differences might be caused by slightly different oscillators or by different dependence of the coupling strength from the Zeitgeber which is mainly manifested in Zeitgeber period deviating from 24 h.

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