Regression of the Time-Keeping Ability in Carabid Beetles by Phylogenetic Adaptation to Cave Conditions

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Z. Naturforsch. **40c**, 438–445 (1985); received September 26, 1984/February 8, 1985

Biological Time Keeping, Non-Stationary Processes, Analysis of Biological Time Series

Locomotor activity patterns of six cavernicolous carabid species representing the course of regressive evolution have been investigated under constant conditions. The analysis establishes new relationships between the circadian structure of the activity patterns observed under constant conditions (LL and DD) and the phylogenetic adaptation to cave conditions. This is due to the mathematical method used for investigating the activity patterns, *i. e.* the Wigner-Ville spectrum that enables the evaluation of nonstationarities. It overcomes the hypothesis of stationarity which was the base of previous examinations. The new method delivers a more refined gradual decrease of the stability of the circadian pattern of activity in cavernicolous beetles than could be achieved by classical periodogram analysis. It is also shown that the locomotion patterns of extremely evolved troglobitic clearly deviate from a purely signal-free (white noise) structure. Possible time-keeping mechanisms that may cause the measured stabilities and regressive lines of possible evolutionary development are discussed.

Introduction

Temporal patterns of locomotor activity may be caused by different time-keeping mechanisms: the best known is the physiological (circadian) clock [1]. A perhaps simpler mechanism to measure intervals of several hours is the control of behaviour by stochastic regularities (*i. e.* by time-invariant or regularly time-dependent transition probabilities) [2]. A further mechanism is comparable to time-measuring by an hour glass [3]. Little known is the phenomenon of outdamping oscillations which have been observed after a transition from LD conditions to constant conditions [4]. It is different from a simple hour glass mechanism, and perhaps more widespread than it is known today. The underlying mechanism of this phenomenon may also control anticipatory behaviour in LD cycles [5]. However, we do not want to exclude the possibility, that basic structures of these different time-keeping mechanisms are identical. For example, outdamping oscillations are mostly interpreted as a consequence of increasing desynchronisation within a population of circadian clocks.

A temporally organized environment strongly favours an adapted, stable organization of the behaviour by time-keeping mechanisms. 4 external complexes of selection may mainly fit the genetics of time-keeping mechanisms of animals to measure the time of several hours:

(1) the benefit to adapt functions of the organisms (locomotion, reproduction, developmental steps) to the temporal changes in the environment;
(2) the necessity to concentrate or to dislocate interactions between individuals (interaction for reproduction or within predator/prey relations);
(3) the necessity to develop seasonally ("photoperiodism");
(4) the necessity to orientate by an astronomical compass.

Moreover, an internal selection factor may be effective:

(5) the necessity to separate metabolic reactions inside the organism (for example reactions of rest and activity metabolism).

Under cave conditions, that means in temporally constant conditions, at least (1) and (4), in many cases (3), too, are futile. The question arises to what extent a constant environment allows the regression of behavioural stability.

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Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen
0341–0382/85/0500–0438 $ 01.30/0

The evolutionary status of recent cavernicolous animals is rather varying. There are troglophilic species which differ only slightly from their epigean relatives. On the other hand, between various troglophilic species, we find forms morphologically extremely adapted to a life under cave conditions. The question arises whether species which can be arranged into different steps of this evolutionary process show a graduation of the time-keeping abilities. In particular, such graduation should allow conclusions to the biological significance of time measuring mechanisms. For example, animals living preferentially in the shadow of cave entrances are exposed to very small daily changes of the environmental parameters. Does such an ecological situation still demand a precise time measuring mechanism like in epigean animals, or behave such species like animals living in the depth of caves?

Previous investigations about the control of locomotory (running) behaviour in cavernicolous animals were mostly restricted on the question, whether the patterns of locomotion are arrhythmic or whether they contain circadian-periodic components of a varying extent. In this paper, we leave this special problem out of consideration. Indeed, our point of view is the *stability* of their temporal structure independent of the kind of pattern (*e.g.* rhythmic, arrhythmic). That means, we then leave open, by what endogenous time-keeping mechanisms, allowing the measuring of events in the range of several hours ("circadian" range), stability is caused.

The applied mathematical procedure, the Wigner-Ville spectrum [6] allows to measure the degree of non-stationarity of signals representing locomotory patterns. As it does not presuppose stationarity, this method exceeds the procedures like the periodogram commonly applied up to now. This is important, since it allows to investigate changes of the temporal structure of the activity patterns without presuming any mechanism.

2. Material and Methods

2.1. Material

Weber [7] reported running activity patterns of several cavernicolous carabids living in different habitats and differing in their evolutionary status. Troglophiles as well as extremely specialized troglobites were investigated. Thus, it can be tested, in which way the circadian system reacts during the course of regressive evolution when external selection pressures become gradually weakened.

(I) Trechini

- *Duvalius exaratus.* Lives in moisty and cool woods near running fresh water under stones and in thick layers of leafs. One subspecies also in a cave. Colour is darkbrown; eyes and extremites have size corresponding to that of epigean carabids. Captured at Carinthia (Austria).
- *Geotrechus orpheus.* Lives only in caves and comparable habitats. It has no eyes and no optical ganglia. Only a little cuticle pigment. Captured at Ariège (France).
- *Aphaenops cerberus* and *pluto. Aphaenops* species are highly adapted to live in caves. They have no eyes, no optical ganglia and no cuticle pigment. Antennae, legs and bristles are strongly prolonged. Since both species do not differ in ecology and behaviour, data of both species are grouped. Captured at Ariège (France).

(II) Sphodrini

- *Ceutosphodrus oblongus.* The usual habitat are caves, especially cave entrances. But the species can also be found under stones in semi-darkness near cave entrances. It even lives in moisty and cool woods without the neighbourhood of caves. Eyes and optical ganglia are nearly reduced. The cuticle does not show metallic gleam, but it is still pigmented. Captured in a cave at Ariège (France).
- *Ceutosphodus navaricus.* Lives in caves and predominantly in cave entrances. Eyes are clearly reduced, but rhobdomes are still present. Colour is brown, antennae and legs are prolonged. Captured at Basses Pyreneés (France).

(III) Pterostichini

- *Typhlochoromus stolzi.* Lives in deep ground and in superficial rock fissures. Eyes are missing. Captured at Friuli (Italy).

2.2. Recording running activity

The animals were tested individually in actographs consisting of two plastic dishes (for the larger species 14 cm ∅, for the smaller ones 8.5 cm ∅). The lower dish was filled with gypsum kept wet by a little bottle filled with water. In the actographs, the animals were offered a darkened hiding place. In order to avoid disturbences, the animals were not fed during the experiments. Running activity was recorded by an IR ray barrier (800 nm). The frequency of passing the barrier was transferred every ten minutes to a tape recorder. For further details see [7].

2.3. *Measuring the stability and precision of time series in locomotory patterns*

Time series of locomotory activity can be considered as realizations of nonstationary processes [8]. This general model includes all special cases like signals in noise. By assuming nonstationarity, we can measure fluctuations of a temporally structured pattern, *i.e.* its degree of non-stationarity.

In order to measure the stability and precision of such a temporally structured pattern we first estimate the Wigner-Ville spectrum of the sampled data. The Wigner-Ville spectrum has been shown to be the unique solution to the problem of assigning a generalized spectrum to a random signal with a time-varying content. This uniqueness property is imposed by the concept of a linear time-frequency dualism (or equivalently by the concept of linear filtering and modulation) together with a proper interpretation of local moments of the generalized spectrum as expected instantaneous frequency and group delay [6, 9].

Under suitable conditions, the natural estimator of the Wigner-Ville spectrum is the pseudo-Wigner estimator recently introduced by Martin and Flandrin [10, 11]. Using this Pseudo-Wigner estimator, the estimated generalized spectral power of neighbouring frequency bands is uncorrelated. Hence, in order to treat the problem of measuring the degree of non-stationarity, we can treat each band of frequencies separatly.

If the random signal happens to be stationary, the spectral power of each band will be constant, but in case of a nonstationary signal, the generalized power will be a piecewise constant function. This allows us to define two measures of non-stationarity. As a measure of stability deviation, we define the weighted relative absolute deviation of the piecewise constant function from the total mean power of the treated spectral band. And as a measure of precision deviation, we introduce the total deviation of the weighted relative mean square error from the constant one. For both measures, the weights are given by the relative temporal length of each segment. Surely, a stationary process will have stability deviation zero and precision deviation zero.

In order to estimate both measures of the degree of non-stationarity we have the problem to estimate the piecewise constant function representing the generalized spectral power of a band of frequencies. This can be solved rather efficiently by a dynamic programming algorithm, the only difficulty resting the estimation of the number of segments. But an Akaike-type criterion – as in the case of determing the order of an AR-model – can be defined in our situation solving the problem [11].

2.4. *Data analysis*

By Table I, an overview is given indicating the analysed data. Data has been sampled using a sampling intervall of $\triangle t = 10$ min. The interesting time-keeping range is located roughly at $(36^{-1}h^{-1}, 12^{-1}h^{-1})$. Hence, data could be considered as oversampled, and in order to reduce the computational burden, data were aggregated by summing up each three neighboured data points. The resulting time series now reflects the activity of half an hour intervalls ($\triangle t = 30$ min). Data was checked for outliners by means of calculating histograms and an appropriate clipping. The program package TIMESDIA [12] was used for this preliminary analysis.

The Wigner-Ville spectra have been calculated by the Pseudo-Wigner estimators. An observation window of 63.5 h and the same amount of zero padding gave sufficient frequency resolution. For an appropriate temporal resolution, we used $T_{\triangle} = 6$ h. For displaying the Wigner-Ville spectra (*cf.* Fig. 1–4), we imposed a temporal smoothing via a rectangular smoothing window of 25.5 h width.

For estimating the stability and precision, we took as test frequency the peak frequency of the marginal spectrum of the estimated Wigner-Ville spectrum. Table II indicates the estimated stability deviation and precision. These results show a rather high variability. Since we do not know the distribution of the estimator and we also do not know the adequacy of a linear scale of the measure, we used a non-parametric test for further analysis. Applying a Kruskall-Wallis test, the exhibited differences turn out to be significant at a niveau $\alpha < 0.01$. This held within the LL- as well as the DD experiments.

3. Results

The examined populations of the tested species (Table I) show a different degree of non-stationarity of the endogenously controlled running activity. Differences are especially indicated by the stability deviation of running activity under continous dim light (LL) and continous darkness (DD) (Table II).

3.1. *Running activity under LL*

Ceutosphodrus oblongus, C. navaricus, and *Duvalius exaratus* show the highest stability of the tested species. They live preferentially under circumstances where they are exposed to daily changes of illumination of small amplitude. The other three species, living preferentially under constant darkness conditions deviate clearly: they exhibit much smaller stabilities. Inspecting Table II, we get the following scaling going from high stability to low one: (Note that in Table II, a low stability deviation means high stability.)

C. oblongus → *C. navaricus* → *D. exaratus* →
A. pluto/cerberus → *T. stolzi* → *G. orpheus*

3.2. *Running activity under DD*

Ceutosphodrus oblongus and *navaricus* have far more stable patterns than the other species. In contrast to the LL-situation *Duvalius exaratus* has changed into the group of species exhibiting small stability. By Table II the following scaling is given from high stability to low one:

C. navaricus → *C. oblongus* → *G. orpheus* →
D. exaratus → *A. pluto/cerberus* → *T. stolzi*

Species	Light conditions*	Intensity of light [lux]	Temperature [°C]	Number of animals	Number of measuring values**
Ceutosphodrus	DD		16–17	14	467
oblongus	LL	10^{-2}	18–19	14	660
Ceutosphodrus	DD		10.0	15	602
navaricus	LL	10^{-2}	10.0	13	901
Duvalius	DD		10.5	14	905
exaratus	LL	1	10.5	14	911
Geotrechus	DD		10.0	6	847
orpheus	LL	10^{-2}	9.0	9	856
Aphaenops	DD		10.0	13	999
cerberus/pluto	LL	10^{-2}	9.5	19	858
Typhlochoromus	DD		10.0	18	962
stolzi	LL	10^{-1}	9.0	14	999

Table I. Analysed experiments on regression of time control of running activity in cavernicolous carabids.

* DD ≙ constant darkness, LL ≙ constant light.
** Δt ≙ 30 min.

Light conditions*	Species	Deviation of stability [%] ± Standard deviation	Precision [%]
LL	*C.o.*	165.6 ± 148.05	112.0 ± 32.67
	C.n.	180.4 ± 193.90	99.7 ± 40.87
	D.e.	223.8 ± 117.16	112.9 ± 36.75
	G.o.	725.8 ± 307.50	71.6 ± 38.07
	A.c./p.	447.7 ± 243.62	101.2 ± 28.35
	T.s.	557.6 ± 207.48	135.8 ± 28.25
DD	*C.o.*	438.1 ± 500.91	104.5 ± 23.09
	C.n.	284.7 ± 260.31	104.3 ± 22.74
	D.e.	582.6 ± 240.49	94.8 ± 50.96
	G.o.	577.4 ± 285.17	108.9 ± 52.40
	A.c./p.	613.4 ± 360.35	112.7 ± 47.75
	T.s.	991.5 ± 1003.17	108.4 ± 37.07

Table II. Estimation of the degree of non-stationarity of the time series of running activity of cavernicolous carabids under constant conditions.

* DD ≙ constant darkness, LL ≙ constant dim light.

3.3. Comparison of results under DD und LL for each species

Stability is smaller during DD than during dim LL in 5 species, only *Geotrechus orpheus* is showing the opposite (Table II). Table III gives the α-niveaus of a test on difference between DD- and LL-stability. *Ceutosphodrus oblongus* and *Duvalius exaratus* exhibit a significant loss of stability during DD (Fig. 1a. 2). In *Ceutosphodrus navaricus*, too, the stability is weakened in DD, however, the difference is not significant. Comprising the estimated values of stability (Table II), we get the following scale of regression for the three species with eyes:

C. navaricus → *C. oblongus* → *D. exaratus*.

For these species, there is no significant difference of their precision (*cf.* Table II).

The three tested species without eyes do not show significant differences of their DD and LL mean stability (Table III), but they behave altogether far more unstable. Considering the precision values, there exist significant differences between the LL values of *Aphaenops, Geotrechus* and *Typhlochoromus*. Under LL *Geotrechus* shows "under precision", *i. e.* long runs with low variability (≙rest

Table III. α-Niveaus indicating rejection of zero-hypothesis "equal stability of the time series under continuous darkness and continuous light".

Species	α
Ceutosphodrus oblongus	0.0741912
Ceutosphodrus navaricus	0.2107979
Duvalius exaratus	0.0000004
Geotrechus orpheus	0.4557206
Aphaenops cerberus/pluto	0.2306573
Typhlochoromus stolzi	0.3282831

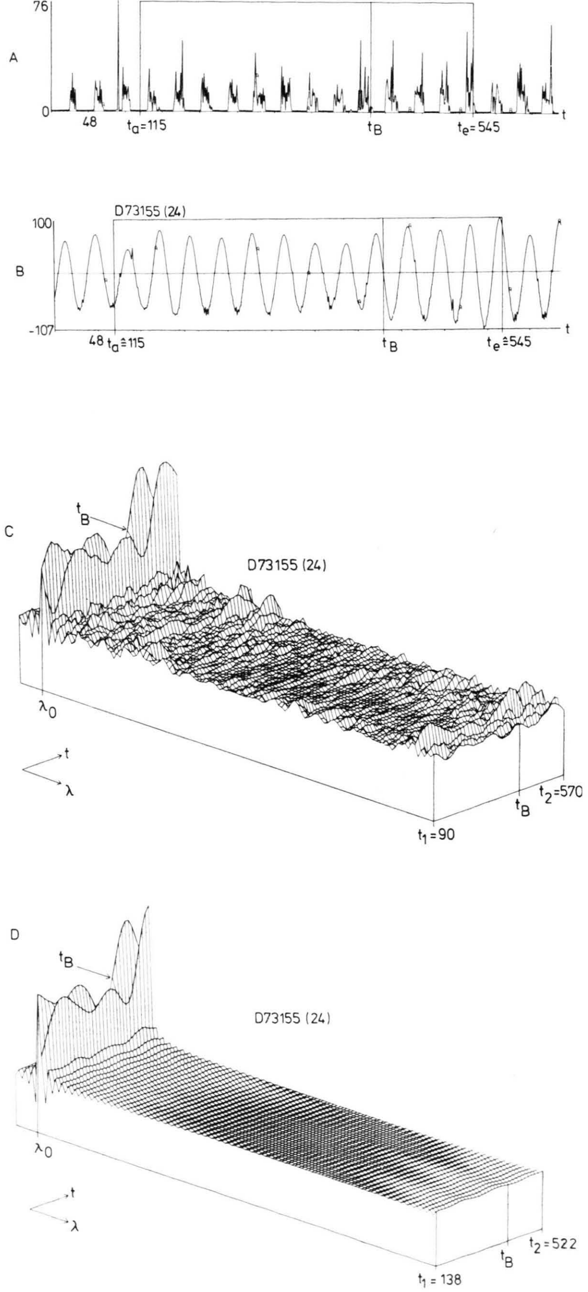

Fig. 1. Analysis of locomotion activity of a *Ceutosphodrus oblongus* under continuous dim light.
A) Time-history plot of the signal, $\Delta t = 0.5$ h;
B) band-pass filtered signal;
C) Wigner-Ville spectrum of the sampled signal estimated by a pseudo-Wigner estimator. The length of the observation window is 63.5 h, and this has been doubled by zero-padding. The rectangular smoothing window has a width of 25.5 h, and a temporal resolution of 6 h has been used;
D) Wigner-Ville-spectrum of the band-pass filtered signal estimated as in C).
t denotes time and λ denotes frequency axis. The ordinates indicate the relative pseudo-power of a spectral component at a given time instant.
t_a and t_e indicate start and end of the segments that have been calculated for estimating the break points t_B of the piecewise constant function best fitting the frequency band centered at λ_o.

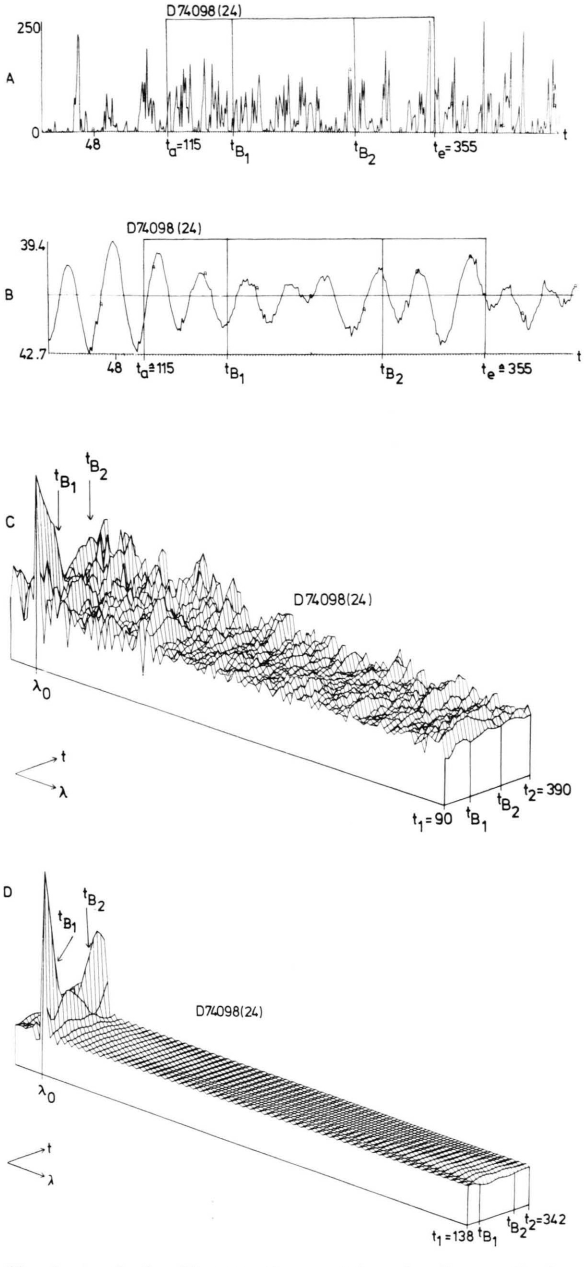

Fig. 2. Analysis of locomotion activity of a *Ceutosphodrus oblongus* under continuous darkness. Compare Fig. 1.

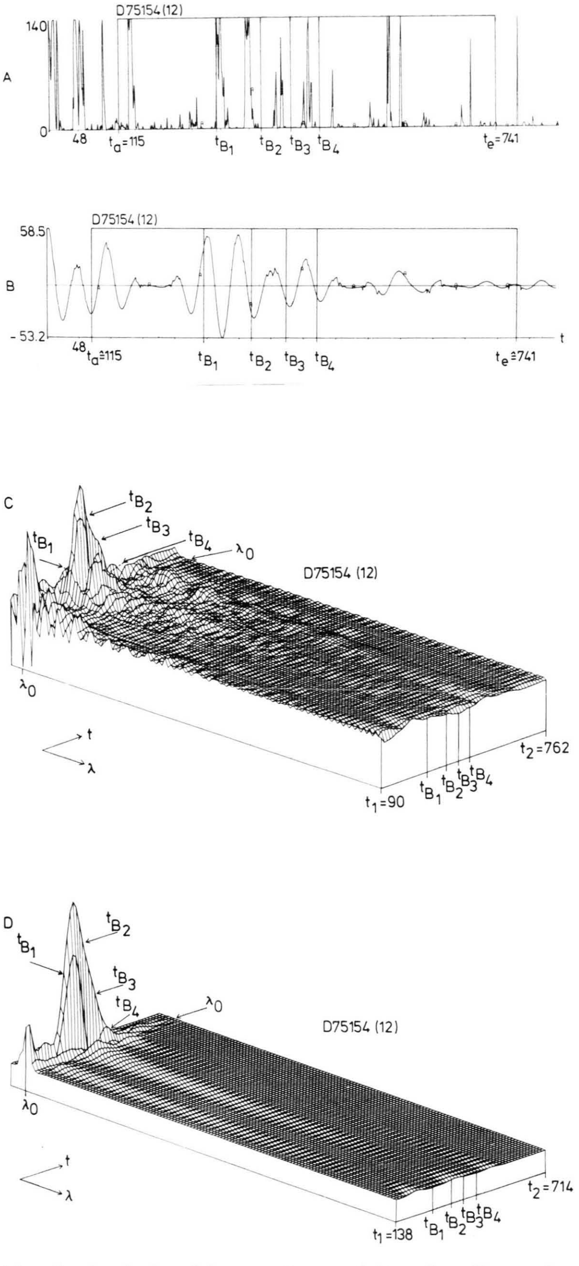

Fig. 3. Analysis of locomotion activity of a *Geotrechus orpheus* under continuous dim light. Compare Fig. 1.

pauses) and short intervals with higher regularities (≙ activity bursts) (Fig. 3). In *Typhlochoromus* an "over precision" can be found, *i. e.* long runs with high activity intersected by short rest pauses (Fig. 4). The precision values of *Typholochromus* and *Geotrechus* are significantly different under DD and LL conditions. These differences may be interpreted as a consequence of a light reaction (possibly irritation), which, however, does not influence the deviation of stability (non-stationarity) (see above). The strength

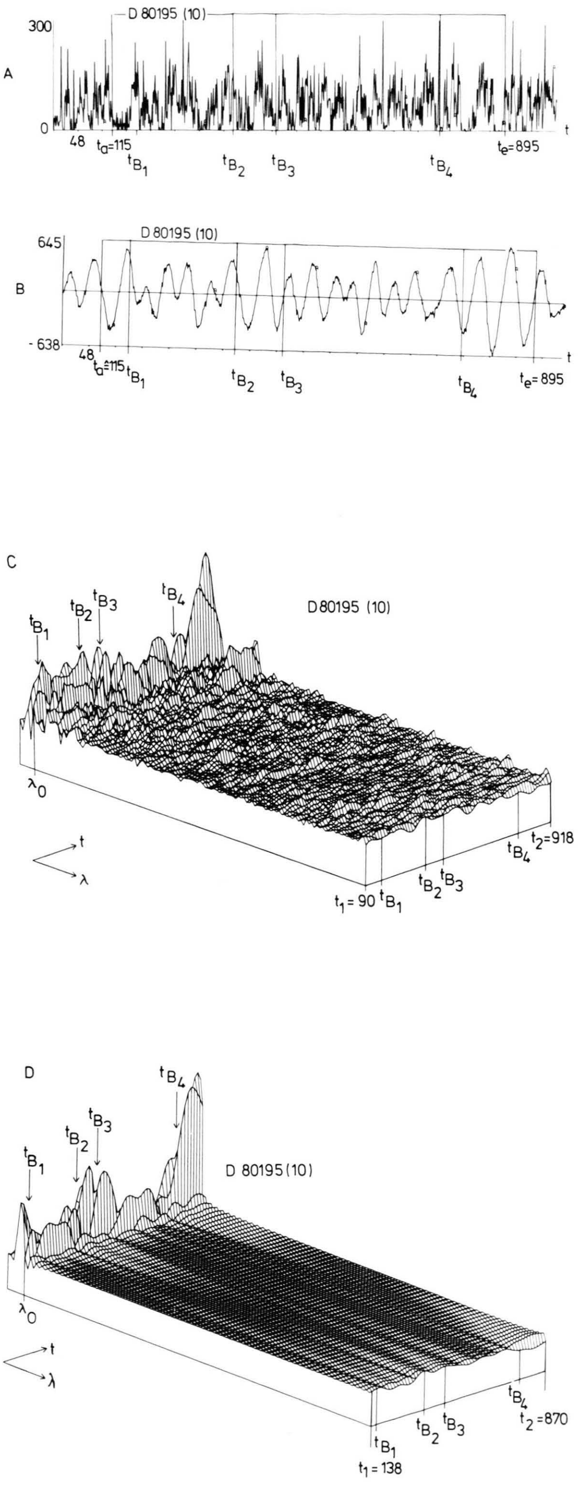

Fig. 4. Analysis of locomotion activity of a *Typhlochoromus stolzi* under continuous dim light. Compare Fig. 1.

of the light reaction seems to be weakened in the following direction:

G. orpheus → *T. stolzi* → *A. pluto/cerberus.*

This result partly corresponds to the results of light/dark experiments. *Typhlochoromus* exhibits corresponding activity rhythms in light/dark cycles, whereas *Geotrechus* and the *Aphaenops* species were not found to react to light/dark cycles (Lamprecht a. Weber [13]).

Discussion

By using the Wigner-Ville spectrum we have got a very refined classification of the stability of locomotory behaviour of cavernicolous animals under constant conditions. In general, the stability decreases as the evolutionary adaptation to cave conditions increases. The troglophilic species, *Ceutosphodrus oblongus* and *Duvalius exaratus,* and the moderately troglophilic species, *Ceutosphodrus navaricus,* living preferentially in cave entrances, exhibit relatively high stabilities, at least under conditions of dim illumination. The time-keeping mechanisms of these species is obviously controlled by the necessity to adapt the functions of the animals to the weak daily changes in the twilight of cave entrances and related habitats. This underlines the important role of daily changing external factors for the genetic fitness of time-keeping mechanisms [14].

On the other hand, our results demonstrate that extremely troglobitic species, for example the *Aphaenops* species, still exhibit well structured locomotion patterns: they clearly deviate from patterns of white noise. In these cases the fitting mechanism may consist of the necessity to separate adverse metabolic reactions inside the organisms (*i. e.* the reactions of activity and rest metabolism). A question that derives from our work is, what time-keeping mechanisms may be effective in the studied cavernicolous beetles. The LL actograms of *Ceutosphodrus oblongus, navaricus* and *Ducalius exaratus* exhibit strong circadian-periodic structures [7]. In these animals, under the condition of a weak illumination, circadian clocks may control the distribution of rest and activity. In the DD actograms of *Ceutosphodrus navaricus* and *Duvalius exaratus* stochastic regularities have been found [7]. In the extremely troglobitic species (*Aphanops cerberus/pluto* and *Geotrechus orpheus*), too, stochastic regularities seem to be effective as a time-keeping mechanism

[13]. That means, in extremely evolved troglobitic species, internal stability is not guarenteed by a regular clock mechanism but by a perhaps simpler time-keeping mechanism based one stochastic regularities.

Typhlochoromus stolzi shows in LD a rhythmic pattern in which activity onset anticipates the L/D transition. This oscillatory pattern damps out in subsequent constant conditions [15]. However, it is unknown, what mechanism causes the relative regularity (the deviation from a white noise-purely arrhythmic process) under constant conditions. Time-invariant or regularly time-dependent transition probabilities (in the sense of [2]) have not been found, however, in some cases positive correlations between the length of the activity bursts and the length of the following rest phase. – *T. stolzi* exhibited another peculiarity: a rather large variability of stability under DD conditions (Table III). High variabilities in morphological characters are a common feature of transit phases of the regressive evolution [16]. This phenomenon may be reflected in the behaviour of this eyeless beetle which is living in the so-called superficial compartment of the underground world, under deep bedded stones, in rock fissures, etc.

We are conscious of the problem to conclude from an observable function (*i.e.* locomotion) on the evolutionary state of the internal time-keeping mechanisms. But, we emphasize that the obtained classification of regression of time-keeping ability has been found without hypothesizing the special underlying mechanisms. Thus, our results show that this question does not play a role when investigating the selection pressure of external factors on behavioural processes like time-keeping of locomotion activity.

[1] E. Bünning, Die physiologische Uhr, 3. Aufl., Springer, Berlin–Heidelberg 1977.
[2] H. Kaiser and U. Lehmann, J. comp. Physiol. **96,** 1–26 (1975).
[3] W. Pflüger, Oecologia (Berl.) **11,** 113–150 (1973).
[4] W. Erckens and W. Martin, Z. Naturforsch. **37a,** 1253–1265 (1982).
[5] J. Aschoff, C. v. Goetz, and K.-I. Honma, Z. Tierpsychol. **63,** 91–111 (1983).
[6] W. Martin, Proc. ICASSP '82 (Paris), IEEE-Press, 1325–1328 (1982).
[7] F. Weber, Mém. Biospéol **7,** 287–312 (1980).
[8] W. Martin, Signal Proc. **3,** 147–155 (1981).
[9] P. Flandrin and W. Martin, 9^e Colloq. Nat. GRETSI, Nizza, 43–49 (1983).
[10] P. Flandrin and W. Martin, ASSP Spectrum Estimation Workshop II (Tampa, Florida), IEEE-Press, 181–185 (1983).
[11] W. Martin and P. Flandrin, In: Signal Processing II (ed. H. Schüßler), 455–458, North Holland, Amsterdam 1983.
[12] W. Martin, U. Kipry, and K. Brinkmann, EDV Biol. u. Med. **8,** 90–94 (1977).
[13] G. Lamprecht and F. Weber, Intern. J. Speleol. **10,** 351–379 (1978).
[14] G. Lamprecht and F. Weber, In: Environmental Adaptation and Evolution (ed. D. Mossakowski a. G. Roth), 151–178, Fischer, Stuttgart–New York 1982.
[15] G. Lamprecht and F. Weber, Mém. Biospéol. in press.
[16] H. Wilkens, N. Peters, and C. Schemmel, Verh. Dtsch. Zool. Ges., pp. 123–140, Fischer, Stuttgart 1979.