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Prokaryotic and eukaryotic unicellular chronomics

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Abstract

An impeccable time series, published in 1930, consisting of hourly observations on colony advance in a fluid culture of *E. coli*, was analyzed by a periodogram and power spectrum in 1961. While the original senior author had emphasized specifically periodicity with no estimate of period length, he welcomed further analyses. After consulting his technician, he knew of no environmental periodicity related to human schedules other than an hourly photography. A periodogram analysis in 1961 showed a 20.75-h period. It was emphasized that "... the circadian period disclosed is not of exactly 24-h length." Confirmations notwithstanding, a committee ruled out microbial circadian rhythms based on grounds that could have led to a different conclusion, namely first, the inability of some committee members to see (presumably by eyeballing) the rhythms in their own data, and second, what hardly follows, that there were "too many analyses" in the published papers. Our point in dealing with microbes and humans is that analyses are indispensable for quantification and for discovering a biologically novel spectrum of cyclicities, matching physical ones. The scope of circadian organization estimated in 1961 has become broader, including about 7-day, about half-yearly, about-yearly and ex-yearly and decadal periodisms, among others. Microbial circadians have become a field of their own with eyeballing, yet time-microscopy can quantify characteristics with their uncertainties and can assess broad chronomes (time structures) with features beyond circadians. As yet only suggestive differences between eukaryotes and prokaryotes further broaden the perspective and may lead to life's sites of origin and to new temporal aspects of life's development as a chronomic tree by eventual rhythm dating in ontogeny and phylogeny.

Keywords

Prokaryotic; Eukaryotic; Unicellular chronomics; Desynchronization; Free-running; *E. coli*; Circadian

1. Introduction

Circadian variation is now found in a single prokaryote [1]. Moreover, Mihalcescu et al.'s [1] curve-fitting software allows focus on any extra-circadian variation that need not be

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relegated to the noise term. While the authors' technical feat is a major step forward in itself, it could also serve as a model for the study of mechanisms underlying the transition from circadians to periodisms with other, e.g. lower frequencies and from rhythms in individuals to those in populations, notably since in their study [1], the cells divide faster than in the length of a circadian cycle. This reminds us that humans procreate and die faster than the length of an about 500-year cycle (corresponding to the rule of reciprocal cycles in and around us [2]) not only in the emergence of outstanding physicians, historians and poets [3] and in tree rings, but also in cave temperatures recorded in stalagmites [4]. Should it become possible to extend the observation span and/or analyses beyond that in the figures published in [1], a further comparison of broader-than-circadian chronomes in eukaryotes vs. prokaryotes would eventually become possible [5,6]. Before humans came about—before hominization [7]—there had to be a cell, and a heretofore mostly spatial cellular system promptly acquired a chronomic system in time, much broader than circadian, what we attempt to demonstrate herein [4,5] (cf. [8,9]).

We see about 7-day variations—circaseptans—with an amplitude larger than circadian in the electrical potential of a single cell under conditions of release into continuous light after standardization in light (L) and darkness (D) alternating every 12h, LD12:12. We need not repeat another restriction of focus, namely that of ignoring circadian rhythms in prokaryotes, a story of historical interest that also constitutes a lesson for the future, namely the need for time-microscopy to complement reliance on the unaided eye—"eyeballing"—at all stages of an analysis. Indeed, Mihalcescu et al. [1] derive characteristics of an average oscillation as a first approximation that has "not only a consistent period and phase but also a constant amplitude" [1]. In a properly enthusiastic editorial, again with focus only on circadians, Johnson [10] writes: "Have you ever heard of a bacterium with jet lag? The idea might seem absurd: 20 years ago, experts in the field would have thought so too". Not *all* experts! We used lighting regimen shifts (a simulated jet lag) to plan studies and find earlier and writing up 15 years ago that figurative jet lags prolonged the survival of the enucleated *Acetabularia* [11]. Jet lag was simulated by lighting regimen shifts to which a unicell without a nucleus was exposed at different intervals. Indeed, we dealt with an enucleated and hence anucleate eukaryote and hence with a simulated prokaryote.

2. Tunnel vision: restricted circadian focus

We are now dealing with a change similar to that from time-macroscopy to time-microscopy: to a transition like that from alchemy to Dmitri Ivanovich Mendeleyev's periodic table. As to a decreed absence of circadians in bacteria, Johnson is quite right: a committee formed by opinion leaders in 1975, at a meeting in Berlin, published the view that circadians are a property of eukaryotes only [12]. At the time, one of us (F.H.) promptly wrote to each committee member, asking why they ignored time series analyses [8] on impeccable data on *E. coli* [13], shown in Figure 1a, subsequently viewed in a broader context [9,14]. A committee member, who eventually also used time-microscopy [15,16], kindly responded, writing that the "consensus" had been that there were "too many analyses" in the 1961 publication [8] and again too much analysis in confirmatory follow-up studies [17,18]. He explained further that the consensus was also in keeping with negative unpublished results by several symposium participants (who then relied, apparently mainly if not exclusively, on data inspection by the naked eye, and hence missed the now widely confirmed microbial circadians in their own data!).

With a little bit of thought, the conclusion could have been the opposite: analyses by some objective criteria are the more needed the higher the noise term. It is wise to look for bacterial effects as a full-blown disease, but microscopes are useful to diagnose the mearily, the earlier the better [19], and electron microscopes are better still when they are needed to

resolve an infectious agent. To carry this analogy to 1961 [8], all the available tools were used. The then-fashionable, overly conservative power spectra were accompanied by a periodogram with results obvious to the naked eye of those who take the time to look up original publications rather than relying on hear-say. To their credit, the symposium's organizers there after extended their focus time-microscopically to about 7-day (circaseptan) variation, documenting their open mind [20–22] and we are grateful to both of them, with one of them becoming a very close associate in research [11,21–26] as well as a close personal friend.

The late visionary Jürgen Aschoff, also a scholar of history ([27] (cf. [6]), dedicated to him and to Pittendrigh [28], who eventually asked, received and published an acrophase chart), may have anticipated what would develop: Aschoff replied in a personal letter (in German) asking something like “Do you wish to take a cord for posterity?” The answer was in the affirmative, as is being done in this paper. Indeed, albeit with a lag of another decade [29], microbial circadian variation became an active field of investigation [29–34], culminating in the recent, partly time-microscopic demonstration of circadian rhythmicity in a single prokaryote [1].

It seemed important to set the record straight since a recent review, after citing the 1961 paper [8], reads [35]: “However, in their studies [8, my addition], the diagnostic features of the circadian rhythm were not tested, except that the oscillation in the growth rate of *Klebsiella* sp. was described to be ~24-h” (*sic*). The periodogram published in 1961 on *E. coli* (not *Klebsiella*) showed a peak with a period much shorter (by over 3 h) than the 24-h length and, even without a confidence interval (CI), not given at the time, the values of amplitude had dropped to nearly one-half of the peak, at a trial period of exactly 24 h, as shown in the figure of the original publication, which gave the period length as 20:75 h. The committee's decision was based on an allergy against time-microscopy, a curable disease when diagnosed early, given one has time to desensitize, a purpose of this paper. The next generation of scholars may evaluate Fig. 1a in the light of Fig. 1b–d. It is important to make things as simple as possible (and eyeballing superficially seems to be simple) until it is realized that some bacterial and other rhythms are invisible to the naked eye and sole reliance upon inspection may encounter series that qualify as rogues and have to be ignored, something hardly acceptable in dealing with a patient, nor recommended when it leads to selection in basic science. The qualification attributed to Einstein “Make everything as simple as possible *but not simpler*” applies to all who do not eventually wish to be in the position of Molière's M. Jourdain:

“M. Jourdain: Par ma foi! il y a plus de quarante ans que je dis de la prose sans que j'en susse rien. et je vous suis le plus obligé du monde de m'avoir appris cela.” (My goodness! Here it is 40 years I've spoken prose [chronobiology and chronomics: attempting to resolve time-varying phase relations at multiple frequencies] without realizing it, and I'm most grateful to you in all the world for telling me so) (Molière: *Le Bourgeois gentilhomme*, Act II. Scene V).

3. From circadians and circaseptans to the decade and transyears

While Mihalcescu et al. [1], based on their data, report only circadians, it may be useful to revisit the data of 1930 [13] and to add to their (too many) but only global analyses in 1961 [8] and to follow-ups showing added oscillations with lower frequency [9,14] to examine whether they also have “not only a consistent period and phase, but also a constant amplitude” [1]. As far as the *E. coli* data are concerned, a quantification of any changes in period, amplitude and phase, to say nothing of waveform, cannot be done by eyeballing in Fig. 1a or by a global spectral analysis, included in Fig. 1b, which suggested that the

periodicities noted first by Rogers are free-running [8]. Fig. 1b also shows with the small 95% CI of the global period estimate, further separate estimates from a serial section carried out for consecutive 48-h data intervals, as shown in Fig. 1c, d. The 95% CIs, by their non-overlap, are in keeping with the impression that the period lengthens from the beginning to near the end of this relatively short record. According to Fig. 1c, d, we may be dealing with a gradual change of the period that could have been a trend toward exactly 24-h length, whether pulled by human schedules and/or by changes in subtle factors such as those in the magnetic field. But in the absence of calendar dates of Rogers' study, a comparison with the changes in the antipodal geomagnetic index aa, that would have been available for the span of his experiments, cannot be carried out.

A lesson to be derived from the foregoing is that (with the clock-hour) the calendar date and site, and thus the temporal and spatial locations of measurements, are also best recorded [36,37], as is done routinely by some investigators [38,39]. Organisms are systems open to their magnetic as well as photic, thermic and other physical environments [2,40]. It seems possible that the changes in circadian amplitude and period represent an infradian modulation of these characteristics, requiring both more data and appropriate (never "too many") analyses. What seems certain is that in the cyanobacteria studied by Mihalcescu et al. [1], the circadian predominates in amplitude over any infradian, but the complete absence of infradians, and a constant amplitude along with constant period and phase awaits parameter tests [41]. There is unfortunately hardly a single circaseptan cycle covered in earlier data published by Kondo et al. [34], but an about 7-day component, with a non-zero-amplitude by cosinor [9], is also obvious to the naked eye, Fig. 1e.

Whenever one encounters, in a biological variable, signatures of both non-photic and photic environmental cycles such as those of the non-photic (geophysical) week and the photic day, amplitude ratios can be computed. This ratio is much smaller than unity for the results on cyanobacteria in Fig. 1e, as it would probably be in the figures of [1]. The same is seen by analyses of data on a eukaryotic unicell kept in LD12:12, Fig. 2a. The non-photic/photic amplitude ratio is again smaller than unity. The week's amplitude is smaller than that of the day. The opposite, however, is seen when *Acetabularia* maintained (standardized) in LD 12:12 is released into continuous light (LL), Fig. 2. In LL only, the non-photic/photic amplitude ratio is larger than unity, Fig. 2b–e.

Unicellular time structure can be viewed in a still wider perspective of chronomes, consisting of 1. trends, 2. a broader-than-circadian and circaseptan intermodulating spectrum of cycles and 3. probabilistic and any other chaos, the latter evaluable usually in data denser than those discussed herein [6,42–45]. Whatever biological variable is investigated, sampling is best planned in the light of what has been mapped and made known about the then-entire available spectrum of rhythms so that once the task of long-term mapping has been done, its essentials can be spotchecked by everybody working in the one domain. This consideration is important for studies planned along the scales of years and decades, Figs. 2f and 3.

It is the more important to cite that sectoring—genetic changes, probable mutations—of cultures of air bacteria and staphylococci studied by one of us (PF) over two decades is characterized by an about 10-year cycle [46,47], corresponding in length to the (non-photic) Schwabe cycle in Wolf's relative sunspot numbers ([48], Fig. 3a–d). This series was then reanalyzed in the light of another recent discovery, namely the near-transyears and the far-transyears, with periods longer than a calendar year [49], the far-transyears corresponding at least in their length to about 1.3- or 1.6-year oscillations, the latter recorded via satellites by physicists in the changes in speed of the solar wind [50,51], the particles ejected from the surface of the sun and the near-transyears found in the solar wind by ourselves [49]. Such

far-transyears were also found in several physiological time series from eukaryotic unicells to humans. In some cases, the transyearly amplitudes were larger than the amplitude at a trial period of the precise calendar year, a finding that also applied to the eukaryotic unicell *Acetabularia* for its circadian characteristics determined in different studies during standardization in LD12:12 and combined into a time series covering 14 years, as summarized in Fig. 3 of [4]. The spectral peaks of transyears in microbial sectoring in the circannual region all have very small amplitudes, if any. They are smaller than the amplitude of the also-modest about-yearly component in the same series, while the transyearly/yearly amplitude ratio in *Acetabularia* is larger than unity and the transyears are more prominent than the calendar year.

It seems pertinent that infradians in unicells, whether prokaryotes or eukaryotes, include about-yearly variations that differ in transyearly/yearly amplitude ratios between prokaryotic and eukaryotic unicells and may tell us about the different sites of life's origin exposed to the daily and yearly alteration of L and D on the surface of the earth or not exposed at the bottom of the sea, in the core of the earth or on other planets for the case of panspermia. Ratios of amplitudes of non-photoc and photoc cycles may reflect the absence or presence of early exposure to daily and yearly changes in visible light. These ratios constitute an interesting aspect in the phylogeny of chronomes [5] that is repeated in early human [52,53], other mammalian [54,55] and still other [56] ontogeny.

It seems useful at all frequencies characterizing a variable and of course, above all for circadians to produce phase maps of internal cycles once a synchronizer such as the lighting (or restricted feeding) regimen is mapped, as is the case for *Euglena gracilis* Klebs in LD12:12, Fig. 4 [57]. For other organisms such as *Gonyaulax polyedra* (or *Lingulodinium polyedrum*), melatonin has been mapped [58]. Comparative amplitude ratios of the amplitudes of photoc cycles such as the day and the year vs. non-photoc, probably partly geomagnetic ones, such as the week, the transyear or decades, await further study.

In the case of microbiological circadecadals that may or may not be Schwabe-cycle-synchronized, the mapping of periods has thus begun. Superposed epochs have also been used (Fig. 3d). Systematic mapping in unicellular chronomics awaits, beyond superposed epochs, also subtraction and replacement approaches that have proved useful in the study of relations between solar and human circaseptans in heart rate [59].

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E ANTICIPATED CIRCASEPTAN MODULATION OF CIRCADIAN LUMINESCENCE RHYTHM OF CYANOBACTERIA*

* Revealed by nonlinear least squares rhythmometry on data of Kondo et al. (1993) collected at 30° C in light, except for 5-min darkness every 30 min; Caveat from brevity of data series.

** 1 mV corresponds to light emission of about 10^8 photons/sec.

Fig. 1.

Fig. 1a. Timeplot of the 1930 data of Rogers and Greenbank [13], a noted bacteriologist described by a Cosmos Club (Washington, DC) Vignette of December 1967 as “the bright star in the [US Department of Agriculture] scientific horizon before World War II”. In 1961, the data on growth rate (actually colony advance) of *E. coli* assessed at hourly intervals for slightly over 6 days were taken off the graph published by Rogers and Greenbank in 1930. Some regularity in the time series is apparent from the spikes in colony growth occurring at intervals shorter than 24 h, as seen by reference to the vertical dashed lines drawn at daily intervals, yet eyeballing can hardly serve for an estimate of a period and certainly forestalls any evaluation of uncertainties. Rogers and Greenbank wrote: Fig. 1, which is representative of a number of experiments, shows that there is a considerable degree of periodicity to the alternation of rapid and slow growth. In considering this graph, it should be kept in mind that the curve represents not extent of growth, but rate of advance of growth for definite time periods. The number of bacteria in the different periods could not be determined. © Halberg. Fig. 1b. By 1961, Rogers’ data on growth rate of *E. coli* (Fig. 1a) had been analyzed by a global periodogram and power spectra to find what the naked eye may miss, i.e. what was then interpreted as a free-running circadian rhythm, with a period of about 20:75 h by Halberg and Conner [8]. An infradian modulation was subsequently reported. Herein, a result of linear-nonlinear rhythmometry summarizes period estimates for 2-day sections as well as for all data with their uncertainties. The spectral peak at a frequency slightly higher than 7 cycles per week (or 1 cycle per day) confirms the visual impression of the presence of

a circadian variation with a period slightly shorter than 24 h, presumably free-running. This about 21-h component accounts for 14.4% of the overall variance and is found to be statistically significant by cosinor ($P < 0.001$). Nonlinearly, the period is resolved as averaging 20:09 h (95% CI extending from 19.8–21.9 h), as shown by the horizontal bar above the spectral peak. The peak's amplitude averages 52.6% of the MESOR (chronome-adjusted mean), with a 95% CI extending from 19.4% to 85.7%, not overlapping zero (thereby rejecting the assumption of no rhythm). Additional nonlinear analyses applied to a moving 2-day interval (nonlinear chronomic serial section) indicate that this period is not fixed (see Fig. 1c). The 95% CIs of the shortest and longest periods are also shown as horizontal bars drawn at the corresponding amplitudes. © Halberg.

Fig. 1c. Periods and corresponding 95% CIs characterizing the data shown in Fig. 1a are here estimated for intervals of 2 days progressively moved throughout the time series by 6 h. An interval of 2 days was selected to have at least one replication to secure a more reliable estimate of the period while also allowing the investigation of any change in period length with time. Separate, non-overlapping 1-day intervals analyzed similarly yield acceptable similar estimates. The period is seen to lengthen as a function of time. As a first approximation, the dash-dotted line is in keeping with a linear increase. Being only of the order of about 18 h at the beginning of the series, it approaches 24 h by the end of the first week of monitoring. With the qualification that consecutive estimates are not independent, the lengthening of the period has an ordering $P < 0.001$ ($r = 0.900$). The fit of a second-order polynomial (solid line) suggests that the period levels off. In this model, the quadratic term significantly reduces the residual variance (ordering $P < 0.02$) beyond the linear term. The results could be interpreted as an originally free-running circadian component that may be pulled by (if not become synchronized with) the 24-h day, but there is a possible alternative, shown in Fig. 1d. © Halberg.

Fig. 1d. Amplitude estimates and 95% CIs corresponding to the changing periods (in Fig. 1c) are shown herein. With the same qualification that consecutive amplitude estimates are not independent, further linear-nonlinear analyses of the amplitude sequence indicate the presence of an infradian component with a period of about 5 days (dashed curve). The “circadian” amplitude is quite large (of the order of 50% of the MESOR), being larger in the middle of the series than at the start. The change in amplitude could be in keeping with a synchronization of the originally free-running component to a 24-h cycle, but in that case there should have been a more or less continuous increase until the end of the record. Results from the nonlinear analysis are in keeping with the alternative hypothesis of an infradian modulation of the circadian amplitude with a period of 4.96 days (95% CI: 3.64–6.28 days). It should be noted that the data were collected under presumably constant conditions, except for the hourly illumination of the colony for taking the measurements. In his nineties, Lore Rogers (the senior author) first wrote in response to an inquiry that he could not recall the conditions of the study. Later he wrote to report that his technician and co-author G.R. Greenbank (in Florida) had sent him a Christmas card. Rogers subsequently learned from Greenbank about the lack of any known external 24- or 20:75- h cycle (the latter the average cycle length in a global analysis). © Halberg.

Fig. 1e. The brevity of the time series is a grave limitation but an apparent decreasing trend at the outset is reversed toward the end of the series and a cosinor shows the predicted circaseptan component superposed on the circadian component in the luminescence of cyanobacteria, a slight modulation only analyses can exclude in the data of Mihalcescu et al. [1]. The issues of 55 years ago, revolving around any endogenicity of circadians, reemerge in biology [6] (cf. [61]) around the often very wobbly and transient circaseptans. But discussing data covering only one cycle remains the equivalent of taking a heartbeat for one second. It has grave limitations, but can tell us that the subject is still alive (read: that there is a rhythm). © Halberg.

A OXYGEN PRODUCTION by ACETABULARIA

ON REGIMENS of ALTERNATING LIGHT and DARKNESS (LD 12:12)

and ON CONTINUOUS LIGHT (LL):

RELATIVE PROMINENCE of CIRCASEPTAN (CS)
VERSUS CIRCADIAN (CD) AMPLITUDE *

* Mean and 95% confidence interval. ** In LD 95% confidence limits of CD periods 23.7 - 24.2 h, of CS periods 137 - 185 h. *** In LL 95% confidence limits of CD periods 23.0 - 24.7 h, of CS periods 171 - 210 h. \$ In 2 cells CS periodicity was not statistically significant.

B SIGNAL-AVERAGED ELECTRICAL POTENTIAL
of *ACETABULARIA ACETABULUM* *;
TIME-MACROSCOPIC VIEW

* 20 single cells; Total number of observations: 38,578; experimental span: 376 days. Metaanalyzed data of Dr Sigrid Berger, Dr Luebbo von Lindern and the late Dr Hans-Georg Schweiger.

C LARGER *ABOUT-WEEKLY* THAN *ABOUT-DAILY* CYCLE
in ELECTRICAL POTENTIAL of a UNICELLULAR ALGA
ACETABULARIA ACETABULUM EVOLVED 500 MILLION YEARS AGO *

* Nonlinear spectral analysis on signal-averaged data from 20 single cells, released (zero time) into continuous light (LL), after prior standardization in light and darkness alternating at 12-hour intervals (LD12:12) for up to one week. Total number of observations: 38,578; experimental span: 376 days. Note a more prominent amplitude (A) for a component with a period near a week, than the As of the about daily and about half-weekly components (all free-running). The circaseptan A is equated to 100 and the other As are expressed as percentage of the circaseptan. Metaanalyzed data of Dr. Sigrid Berger, Dr. Luebbo von Lindern and the late Dr. Hans-Georg Schweiger.

D

DIFFERENT TIMING
of FREE-RUNNING CIRCASEPTAN COMPONENT
in OXYGEN PRODUCTION (OP), CHLOROPLAST
MIGRATION (CM) and ELECTRICAL POTENTIAL (EP)
in *ACETABULARIA ACETABULUM* *

* Released into continuous light (LL) after prior standardization in light and darkness alternating at 12-hour intervals; period of 162.13 hours is average of presumably free-running periods of 160.7, 162.2 and 163.5 hours, for OP, CM and EP, respectively. Signal-averaged values of 20 - 24 single cells, each series detrended by 2nd or 3rd order polynomial, depending on length of series (< or > 14 days). Data from Dr. Sigrid Berger, Dr. Luebbö von Lindern and the late Dr. Hans-Georg Schweiger.

E SOLAR CYCLE MODULATION OF CIRCASEPTAN BAND IN SOLAR MAGNETIC FIELD INTENSITY ?

* PR: Percentage rhythm; darker shading indicates larger PR, with absence of shading (white) associated with ordering P-value from zero-amplitude test > 0.05. Solid horizontal line corresponds to spectral peak of 6.7 days, differing from exactly 7 days (dotted line). NOTE PROMINENCE OF AN ABOUT BUT NOT PRECISE 7-DAY COMPONENT AT OR AFTER SOLAR MAXIMA (SM).

* Relative prominence gauged by Ar of oxygen production by *Acetabularia* (as in the neonatal human circulation, not shown) is above unity, whereas it is below unity for sectoring of colonies of air bacteria and *Staphylococci*. Trans-year(s) defined as (one or several) spectral peak(s), each showing a 95% confidence interval lying between (and not overlapping) precisely 1 and 2 years.

Fig. 2.

Fig. 2a. Comparison of two components in the partial spectral element in the chronome of *Acetabularia* with periods of 24 (CD) and 168 (CS) hours in 12-h alternating light and darkness (LD) vs. continuous light (LL). Oxygen production in *Acetabularia* undergoes about 24-h and about 7-day rhythms. When assessed at fixed periods by fitting precise 168 and 24-h cosine curves to oxygen production data of *Acetabularia*, a large circadian is found in LD and is greatly reduced in LL vs. a small circaseptan in LD, which increases somewhat in LL. A summary of oxygen production at best-fitting periods reveals in LD the dominance of the circadian as compared to the circaseptan component and hence the circaseptan-to-circadian amplitude ratio is much smaller than unity. By contrast the relative prominence of the circaseptan to the circadian variation in LL results in an amplitude ratio slightly larger

than unity or a much larger such ratio after release into LL, a manifestation after a single stimulus that carries no 7-day information, as seen in Fig. 2b. © Halberg.

Fig. 2b. Signal averaging of the electrical potential after release into continuous light reveals the prominence in this variable of the circaseptan vs. the circadian component, with an even less prominent circasemiseptan variation. © Halberg.

Fig. 2C. The naked eye sees on the left that the about 7-day (circaseptan) component is more prominent than the circadian rhythm in the electrical potential of *Acetabularia acetabulum* after release into continuous light, seen with eyeballing on the left (as in Fig. 2b, but now with a fitted model). Amplitude ratios are shown on the right. A spectral component with 95% CIs not overlapping a precise week, a hint of endogenicity, is present early in phylogeny, in a unicellular eukaryotic alga's oxygen production as well as in its electrical potential, as it was also in *E. coli* [8]. The differences in relative prominence are shown on the right. The ratio of the amplitude, e.g. of the week over that of the day can be the gauge. This ratio is above unity in the one eukaryotic unicell studied over 14 years from this viewpoint (*Acetabularia*). Edited data from the same source as those here analyzed are in the public domain, thanks to Dr. Lübbo von Lindern [62]. © Halberg.

Fig. 2d. Differences in phase among the circaseptan components of three variables of *Acetabularia* that in continuous light free-run with very similar frequencies, but with differences in phase at the average frequency. © Halberg.

Fig. 2e. Any heliomagnetic week, when transiently present, may be Schwabe solar cycle stage-dependent and relatively small in amplitude. It is isolated only after filtering. By contrast, the geomagnetic near-weekly component confirmed by physicists [63–65] is also highly variable but less inconsistent [66,67]. © Halberg.

Fig. 2f. The amplitude ratio gauges not only the prominence of a week vs. that of a day, but also that of a transyear vs. a calendar-yearly component. The transyear dominates over the biological equivalent of a calendar year in *Acetabularia*, whether one analyzes a time series consisting of circadian MESORs (chronome-adjusted means), circadian amplitudes or acrophases. By contrast, in a large mix of “air bacteria”, there is only a minuscule transyear. For most staphylococcal cultures as well, the amplitude at a trial period of a calendar year, while small, is still larger than that at a trial period of a transyear; hence the ratio is smaller than unity, except for two colonies [5,8] that exhibit cisyears, defined as components with 95% CIs between 0.5 and 1.0 year (not shown) [46,47].

Staphylococci may be phylogenetically older than *Acetabularia*, but they show a ratio below unity. Did they originate in a photic environment while *Acetabularia* developed in a non-photoc one? Many questions remain: first whether we can generalize from the few, decade-long series available, and hence can only note that each series for any one variable as yet is unique to our knowledge. If we proceed, there is a second hurdle to pass, namely the problem whether *Acetabularia* and staphylococci have changed since they developed. This may be the case. Moreover, any change need not have been in the same direction, yet another qualification. Moreover, a change could also have occurred in solar wind behavior, but, as proposed by von Steiger, this may not have been the case [68]. If so, the foregoing line of questioning holds. In the interim, we can also try, again with foregoing qualifications, to draw inferences based on amplitude ratios concerning the very long-ago behavior of the solar wind. Did this wind oscillate, with an about 1.3-year period, more prominently after as compared to before the time (~500 million years ago) [69] when unicellular eukaryotes like *Acetabularia* developed? If so, physics can benefit from biology. In any event, with (and because of) all of the foregoing reservations, we advocate the collection of further microbial time series that should become available in a few decades if we start as soon as possible to keep track officially and systematically of the outcome, e.g. of controls in microbial sensitivity tests to antibiotics [60]. © Halberg.

A

ASTROPHYSICAL INFLUENCE ON SECTORING IN COLONIES OF MICROORGANISMS?*

Bacterial Sectoring (—) and Solar Activity, Gauged by Wolf Numbers (—)

Bacterial Sectoring (—) and Solar Activity, Gauged by Wolf Numbers (—)

** In colonies of air bacteria (left) and staphylococcus aureus strain Nm (right)

The figure consists of two vertically stacked time-series plots sharing a common x-axis representing the calendar year from 1970 to 1992.

The top plot displays the Kp index as a percentage of its mean. The y-axis is labeled "Kp (% of Mean)" and ranges from 0 to 350 in increments of 50. The data is represented by a dense, noisy line fluctuating around a mean value of 2.45, which is indicated by a horizontal line and text within the plot area.

The bottom plot displays the Equatorial Dst Index as a percentage of its mean. The y-axis is labeled "- Dst (% of Mean)" and ranges from -2 to 13 in increments of 3. The data is represented by a dense, noisy line fluctuating around a mean value of 17.96, which is indicated by a horizontal line and text within the plot area.

* Showing cross-spectral coherence with Dst (0.647; ordering $P = 0.005$; $\text{ndf} = 20$) at period of 18.6 days).

**C HINTS (NO MORE) FROM SIMILAR BACTERIAL
and ENVIRONMENTAL PERIOD LENGTHS****

* Genetic changes, e.g. mutations, chromosome duplication or plasmid loss ground by sectoring data of Dr Piero Faresco. * Best-fitting by non-linear least squares rhythmically as a function of the lengthening time series and/or the addition of a different population. ** Both Air bacteria and *Staphylococcus*. 1.16m/3000 counts in Rpm. 22.50% = Wolf relative sunset number. 111.11 = 0.01 = Equatorial geomagnetic disturbance. 4 Kp = Planetary geomagnetic disturbance. ## as = Antipodal geomagnetic index. ## in view of period within non-overlapping 95% confidence intervals do not necessarily indicate statistically significant differences, they serve for caution by their distances (above) or proximity (below), and are requiring follow-up by parameter tests and/or subtraction and addition

* Percent of mutations and/or other genetic changes, daily data.

Parameters of the fitted curve: MESOR = 12.3 %,

Period (y)	Amplitude (%)	Acrophase (degrees)
9.445	5.37	-245.7
1.026	2.84	-223.4

Fig. 3.

Fig. 3a. Bacterial sectoring shown with darker shading undergoes circadecadal cycles, obvious to eyeballing, out of phase with the lighter-shaded, long-known Schwabe cycle of relative sunspot numbers (Wolf numbers). But a time relation of these two cycles cannot be quantified by the naked eye. Original data of Dr. Piero Faraone. © Halberg.

Fig. 3b. When data on the geomagnetic indices—the planetary K_p and the equatorial Dst—are added for comparison with the microbial data, the naked eye again fails to quantify

relations, yet there is cross-spectral coherence between sectoring and geomagnetics. Top: original data of Piero Faraone [46,47]. © Halberg.

Fig. 3c. Chart of reciprocal candidate periods: time series analysis shows different results in the length of the cycles resolved when air bacteria are analyzed separately or all data from air bacteria (including staphylococci) and from only staphylococci are pooled. Similar periods characterize air bacteria and the equatorial index Dst. When all microbial data are pooled, more of the variables show similar periods. But the similarity of periods, useful as a hint, is no evidence of mechanisms [46,47]. © Halberg.

Fig. 3d. A spectral analysis, in the tabulation below, quantifies several components that the naked eye can try to discern, including a candidate near-transyear, which, however, turns out to be a broad peak, covering the precise 1-year trial period useful as a hint, is no evidence of mechanisms [46,47]. © Halberg.

Fig. 3e. Another approach by superposed epochs of 3-day sectoring data of air bacteria in relation to 28 storms. Each epoch covers a span of 10 days before and 10 days after the day of a storm. The storm is defined by the peak of the highest daily average of eight 3-h $K_p = 5.625$ (computed as the sum of 3-h measurements totaling $= 45/8 = 5.625$). A decrease of sectoring on day 3 (days 2–4) and an increase in sectoring on day 9 (days 8–10) is seen. The *P*-value from a one-way analysis of variance (ANOVA) shown on the top left of this figure suggests that a storm has statistically significant effects. Much more and stronger evidence not yet available is required for a causal relation [46,47]. © Halberg.

Circadian Acrophase Chart of *Euglena gracilis*

Fig. 4.

Phase charts like the one here shown remain to be carried out for other organisms and chronomic systems other than circadian. © Halberg.

Fig. 5. Circadian rhythm of melatonin in *L. polyedrum* (= *G. polyedra*), illustrates the use of a multiple cosine fit for the eventual determination of an ortho-phase chart, preferably on log-transformed data (in the case of distributions with tails). © Halberg.