Rhythms in organisms
Observing, experimenting, recording and analyzing

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In memory of my teachers Erwin Bünning, Colin S. Pittendrigh and Jürgen Aschoff,
Tübingen, Mai 2004

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Observing ....
Photography of my grandchildren,
courtesy Dirk Engelmann

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Preface

Many processes in organisms are rhythmic. Rhythms with periods of about 24 hours are found already in the lowest organisms such as *cyanobacteria*. In unicellulars, fungi, plants and animals including humans they are widespread. Our knowledge of such rhythmic processes is increasing constantly by new observations and experimental results. But the underlying mechanisms are not yet clarified completely in a single case and are therefore a challenge for scientists. This new area of *'Chronobiology'* is not only of interest to scientists. Even laymen, pupils and students find it intriguing. Furthermore, they can observe these rhythms in organisms and perform experiments, because simple procedures are often sufficient.

In the natural sciences it is important to learn scientific methods and to practice them. I believe, that this fascinating research area of chronobiology is useful for it. In order to get familiar with the field and the methods used in it we have published a few years ago a workbook (Engelmann and Klemke (34)). It was written to facilitate the observation of and experimenting with rhythms in biological systems. For many of the proposed experiments special equipment was necessary. This can nowadays be replaced by computers. They are much more versatile and also usable for analyzing the data. Often they are cheaper than the special equipment used before. I have therefore written this book in 1996 and 1999 (German and English versions). It served originally to instruct biology students of the University of Tübingen attending courses in chronobiology. It might, however, be of interest also to students of other universities and to teachers at high schools, pupils and others. In 2002 and 2004 the books were revised and made available in the Internet.

I hope that this book rises interest for this fascinating new area of life sciences. It should help to study rhythms more closely. If it furthermore mediates a little bit of the excitement and enthusiasm which results from solving problems in science, its aim is fulfilled. Another book has been published here which serves as an introduction into the field of chronobiology (*Rhythms of life; an introduction using selected topics and examples*).

To satisfy the curiosity of young people is unfortunately nowadays not much promoted, because schools stick often too tightly to the curriculum and offer too much matter of teaching. This leads easily to only secondarily motivated students for whom the scores are the main aim of going to school.[1] It was the great pedagogue Pestalozzi who once stated:

> All unsere Erziehung ist nicht einen Heller wert,
> wenn Mut und Freude dabei verloren gingen[2]

[1] this seems to change now after the Pisa study

[2] All our education is not worth a penny if courage and joy are lost.

Introduction

> Alle Schöpfung schwingt im Reigen,
> Freude heisst ihr hohes Lied.
> Nur der Mensch will sich nicht beugen,
> jagt nach fremdem Glück sich müd.
> Freunde, sucht den Sinn der Dinge,
> dass auch Freude euch durchdringe
>
> *J.W. v. Goethe*[3]

All complicated systems tend to oscillate. Machines, bridges, electronic equipment are examples. The time it takes for one cycle ('period length'[4]) is the result of the properties of the system. It usually does not correspond to the period length of a rhythmic event in the physical environment.

Organisms as another example of complicated systems are also prone to oscillate. This ability is found at all levels of organization. Plants and animals have adapted them self during the course of evolution to the time structure of their environment. This is periodic: The 24 hour day-night rhythm (*daily rhythms*), the 24.8, 12.4 and monthly rhythm (*tidal and lunar rhythms*) brought about by the orbit of the moon, or 12 months rhythms (*annual rhythms, photoperiodism*) caused by the orbit of the earth around the sun. Corresponding rhythms are found in numerous organisms.

This book consists of three parts. In the first part we describe *methods and equipment*. They are the basis for planning, execution and analysis of experiments. Many of the proposed experiments can be done by using a computer in connection with a video camera and a frame grabber (also called digitizer)[5]. In this way the experimental data can be recorded and analyzed. The system and the programs are mentioned briefly. They are described more detailed in a handbook (Engelmann (28)) and in a book (Engelmann (29)). A system to record the locomotor activity of animals with infrared light beams is also described. In a special chapter we mention the importance of modeling, which allows the simulation of biological rhythms. We will also get a glimpse of the different methods to analyze rhythms ('time series analysis').

In the second part *experiments* are proposed. Some short term oscillations which are not adjustments to external periodicities (*'ultradian rhythms'*) will be described: a chemical oscillator, the gravitropic pendulum, a transpiration rhythm and the lateral leaflet movement of the Indian telegraph plant. We will then take a few of the numerous *circadian rhythms* which evolved as adaptations to the 24 hour time struc-

[3] The whole creation swings in round dance
Its high song is joy.
Only man does not want to bow,
hunts after foreign luck.
Friends, look for the sense of things,
that joy might penetrate you.

[4] how oscillations are characterized is described on page 45 and illustrated in figure 3.1

[5] if you do not understand terms, check in the glossary at the end of the book

ture of the earth and which are especially suited for experimentation. For instance the leaf- and petal movement and rhythmic fragrance in plants, the rhythmic change in shape of a marine amoeba, rhythmic events in animals and man. The importance of the 24 hour periodicity has in the last years been studied intensively in respect to shift work, flights crossing time zones, and in certain diseases. The influences are meanwhile well known in the public. Less well known is the participation of the 24 hour rhythmicity in the measurement of the day length. Using this biological calendar many organisms, especially from temperate and higher latitudes, are able to determine the time of the year reliably. This has been pointed out already in the thirties of the last century by Bünning. The field of *photoperiodism* has in the meantime grown considerably and is of much practical importance. There are also numerous interesting studies on *tidal and lunar rhythms* as adaptations of many marine organisms to the coastal conditions of the oceans. However, we will not propose experiments for this field in this book. For information, a few movies can be used at school (see page 122).

The third part of this book is devoted to different ways of *learning and teaching* the field of rhythms in organisms. Simple experiments without expensive equipment are suited for schools and the interested layman (see page 111). Teaching aids, teaching proposals, and didactic considerations are found from page 109 onward. You will find also hints for obtaining, rearing and keeping experimental organisms, sources for buying equipment and laboratory material (page 121).

The best way to learn something in life is by doing:

Verba docent

exempla trahunt[6]

A few technical remarks: This book was for some years in the process of evolving. It was used by students who participated in a course covering the area of chronobiology. It was available in the Internet from 1996 onward and in 1999 it was 'polished up'. Especially most of the figures were improved. For diagrams I used 'Scandata´ in order to obtain data from curves in figures from publications. They were produced with 'Techplot´. Both programs are from Dittrich, Braunschweig. Vector graphics are made with 'Killustrator´ and 'xfig' under the operation system 'Linux´. Text was applied to bitmap figures with the same program. The book text was written with 'Lyx´. It uses Latex in the background. This program was also used under 'Linux´. The figures are inserted as eps- or ps-files and the book stored under ps- or pdf-format. In 2002 the book was somewhat improved, especially the quality of the pdf-file. In 2004 and February 2006 I have gone through this book again and removed errors and exchanged some of the illustrations with better onces.

[6] words teach, examples bring you forward

Part I

Methods and instrumentation

1 Scientific work

> The formulation of a problem is often
> more important than its solution
> *A. Einstein*

Overview:
In scientific work certain rules are used which have proofen to be useful. The method of multiple working hypotheses will be explained. We will learn how to put forward and test critically hypotheses and how to plan, execute and analyze experiments by using the telegraph plant Desmodium motorium *as an example. The significance of communication in science will be treated. Controversies and their function in science will be mentioned briefly. Finally some unsolved problems in the area of chronobiology are pointed out to motivate you for own studies.*

1.1 How research work is done

1.1.1 Introduction

Textbooks give often the wrong impression, that nature is well understood by man. They stress the collected body of knowledge and those problems which have been solved, whereas the unknown is often not even mentioned. As soon as one starts to study more closely a special area of natural science it becomes, however, very obvious that many things are unknown, not studied or not understood.

In the area of chronobiology this is especially obvious. It is a relative young branch of biology which grows rapidly and where much knowledge has been accumulated in the last years. In spite of it interesting studies can be done with simple methods and without intensive literature studies.

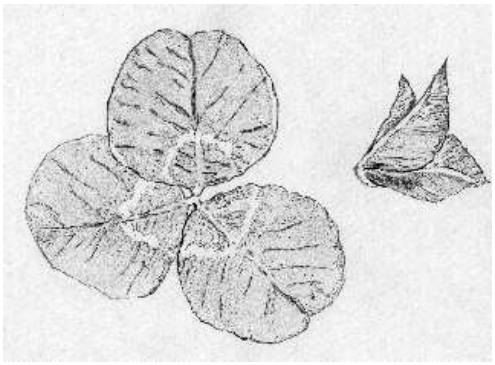


Figure 1.1: *Clover in day (left) and night position (right). Top view*

In the first part we will get to know some general methods of scientific work. They can be used in your own research. Scientific work consists to a substantial part of putting forward and answering questions. Questions arise, for instance, by observing. If we walk in the evening through a meadow, the three-parted leaflets of clover are differently positioned as compared to the day time. They are folded together in a vertical position, whereas during the day they are spread more horizontal (figure 1.1). How does this leaf movement work? We try to find answers (so called hypotheses) and to test them experimentally.

Or we observe in the Persian Violet *Exacum affine (Gentianaceae)* an intensive fragrance in early afternoon. In the morning and evening the odor is less intense (fig-

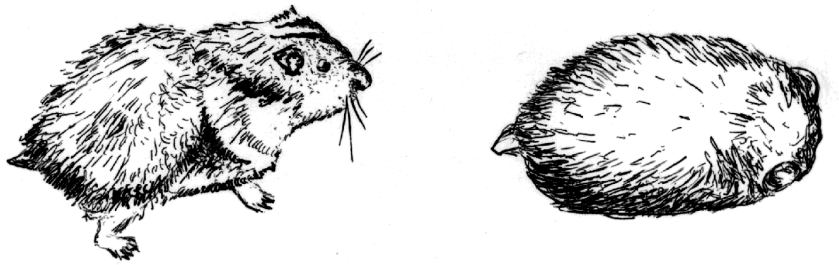


Figure 1.3: *Golden hamsters are night active (left) and rest during the day*

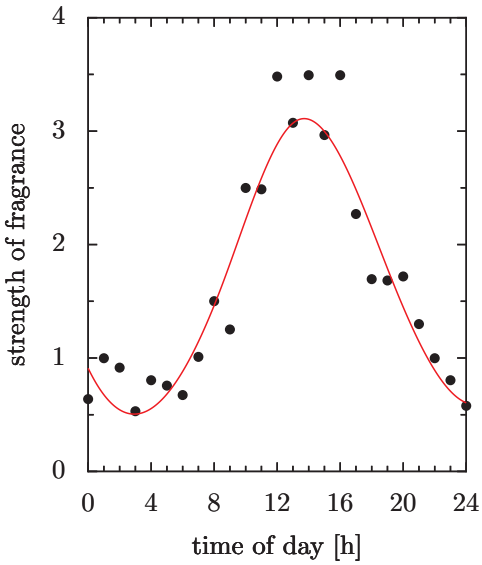


Figure 1.2: *Fragrance intensity of flowers of the Persian Violet* Exacum affine *as a function of day time, estimated by three different persons. Strongest odor in the early afternoon. Mean values*

ure 1.2).

Golden hamsters are night active and rest during the day (figure 1.3). Is this a direct consequence of the light-dark-cycle? Or would the animals show a rhythmic change between activity and rest also under constant light conditions (for instance under very weak light)?

Such observations lead often to questions, which can be answered tentatively by hypotheses and be tested by experiments:

phenomenon ⇒ problem ⇒ hypothesis ⇒ experimental test

1.1.2 Method of multiple hypotheses and strong inference

According to Chamberlain (20) and Platt (92) scientific work is favored by the consequent use of the *method of multiple hypotheses* and *strong inference*. The method consists of the following steps:

1. Stating alternative hypotheses
2. Planning of crucial experiment(s) to disprove hypotheses
3. Execution of the experiment(s), in order to obtain a clear result
4. Repeat 1 to 3, if necessary with new hypotheses

The method of multiple working hypotheses was put forward in 1890 by Chamberlain, a geologist, in a lecture of a meeting of the Society of Western Naturalists. It avoids the danger of the method of simple working hypothesis, were one clinches with motherly affection to one owns hypothesis.

Proposal: Read the reprint of the original article of Chamberlain *'The method of multiple working hypotheses'* (Chamberlain (20)). Platt in 1964 has taken up this method and recommended it as especially useful for scientific work. He calls this strategy *strong inference*. The different steps have been mentioned already and we would advice strongly to read this article by Platt (92).

Using the way of scientific work in the 'Laboratory of Molecular Biology' in Cambridge as an example, Platt demonstrates, how this method, if used systematically, leads rapidly to progress in solving problems. This is a problem oriented instead of a method oriented procedure. Each day the newest results are written on the blackboard, what went wrong, how errors can be avoided, and how the hypotheses can be tested critically by experiments. New experiments and controls to test the hypotheses are proposed.

It is always advisable to use the simplest system, which does still show the characteristics one is interested in. It is more important to ask which experiment *disproves* a hypothesis than to ask how to *proof* it. And: 'Use the simplest system which still shows the properties which you want to study'.

Scientific work is comparable with the work of a detective in solving a crime. By observation or by oral or written information he is confronted with a problem. He tries to solve the 'case' by finding all kinds of possible explanations ('hypotheses'). Neither an able detective nor a good scientist would follow up just one single hypothesis.

If you play the game 'mastermind', where you have to find out the sequence of differently colored pins, it would be unwise to begin with a fixed idea of the correct solution, before you have collected enough informations. This game shows incidently, what is also true for science, that '*negative* results', meaning our guess was wrong, lead normally more rapidly to a solution as compared to a partly correct result. If, for instance, in our game out of four differently colored pins none is correct, we know that only one or two of the remaining colors had been set.

To disprove hypotheses is a better strategy as compared to proofing it. In a strict sense, to proof something means only 'correct with high probability'.

> *'Each conclusion, which is not an exclusion, is uncertain and must be tested again (Lederberg)'.*
>
> *'A theory, which can not be vitally hit, can not live (Bacon)'.*
>
> *'Hypotheses, which cannot be disproved, are meaningless (Bacon)'.*

Proposal: If you don't know the game 'mastermind', play it with somebody.

Of course testing just one hypothesis does also help to advance our knowledge. However this method is less economic. The alternative hypotheses are then usually put forward by other researchers (see section 'Controversies in science', page 38). In this case, crucial experiments are also needed to decide between the different hypotheses, to disprove some and leave some as candidates for further testing.

Proposal: Read the article by Popper (93) *'Von den Quellen unseres Wissens und unserer Unwissenheit'* and a detective story (DuMont's Criminal-Rätsel (Wheatley and Links (116)). In this detective story the solution of the 'case' is reserved in closed pages. Use the method of multiple hypotheses in trying to solve the case.

1.1.3 Testing hypotheses, analyzing and interpreting data

Besides putting forward hypotheses, important steps of scientific work are:

- The planning and execution of experiments.
- The use of certain methods of observing, measuring, and recording.
- The graphic display and analysis of results and their interpretation.
- A protocol serves as the basis of a later publication (Wilson (118)).

We will exercise these steps in different examples.

1.1.3.1 Formulation of hypotheses: Drinking duck

Using the drinking duck[1] (figure 1.4), we will see how questions arise from observations and how they will lead to hypotheses.

Instruction: Fill the beaker with tap water up to the rim and dip the beak of the drinking duck into the water, until the felt of the head is wet. Bring duck back in its original position and observe it. Note down your observations. Which questions arise? Which hypotheses could answer your questions? How could you experimentally test your hypotheses? If the problem is too complicated, it usually helps to subdivide it into smaller problems. They are more easily solvable and might finally lead to the solution of the main problem.

[1] sometimes available in drug stores and gas stations

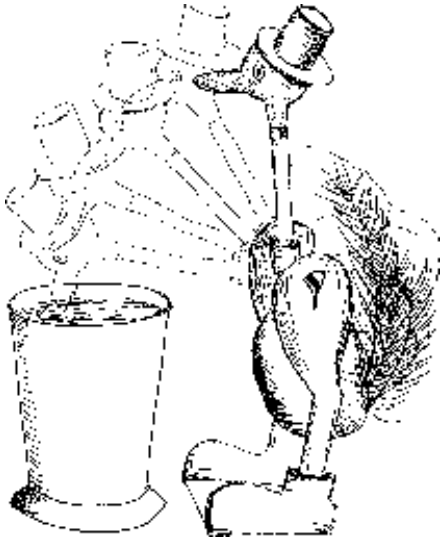


Figure 1.4: *The drinking duck continuously dips its beak into the water beaker*

1.1.3.2 Analysis and interpretation of data: Movement of the lateral leaflet of *Desmodium motorium*

The analysis and interpretation of data shall be demonstrated by using *Desmodium motorium*, the Indian telegraph plant. The small lateral leaflets of this plant move rhythmically. Look at the movie '*Desmodium motorium (Fabaceae)* - Gyration' (Url and Bolhar-Nordenkampf (110)) and observe the movement on the plant directly (figure 1.5).

The oscillation is quite regular. We want to determine the duration of one cycle of the up- and down movement (figure 1.6). Sequential periods are not exactly the same. We have to measure therefore several cycles and to calculate a mean value of the period. We should also find a measure of the variability of the period. For recording the time we use a stop watch. What is the best way to determine period length?

Period length is the time between identical

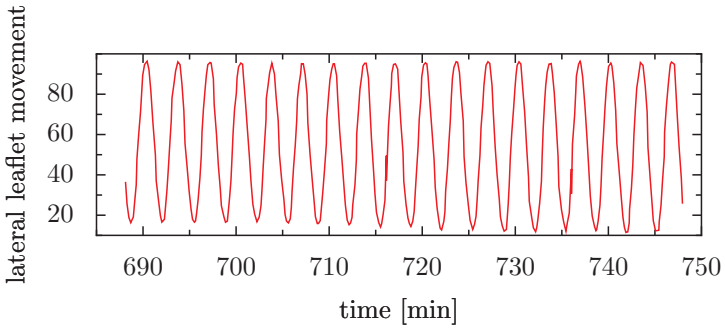


Figure 1.6: *Time course of the Desmodium motorium lateral leaflet movement*

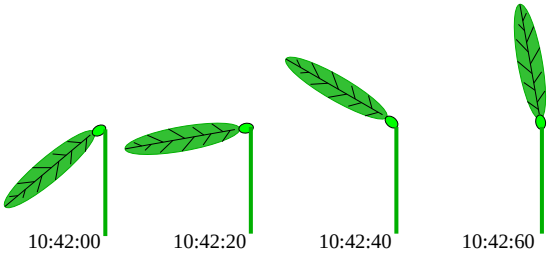


Figure 1.5: *Example illustrations for lateral leaflet movements of* Desmodium motorium*. 20 second intervals between pictures*

phases of the oscillation: for instance the time between a maximum and the following one, or between minima, or between the point of inflections (figure3.1). Which phase (=fixed time in a cycle) would you use and why? In a special chapter (page 45) we will become familiar with methods in which period length is determined by using all the recorded data and not just one special phase point.

Here you should for the time being restrict yourself to a very simple method. Determine the period length of the lateral leaflet movement according to your considerations. Measure as exactly as possible the times of the phase points of the cycles which you decided to use. Use the stopwatch, and note down the times. Determine the periods from the differences of the phase points you have chosen. Calculate a mean value according to the following equation:

$$\overline{x} = \sum x_i / n$$

whereby $\overline{x}$ the mean value, x_i the individual values, and n the number of oscillations. $\sum$ is the sum of all the individual values (see table 1.1, table 1.2).

Why did we not determine the time between the first maximum, and the last maximum and divided it by the number of oscillations? The reason is, that we want to determine the variability of the period. In order to calculate the variability, we need the individual period lengths. Variability is caused by biological and methodological reasons. As a measure of the variability we will use the standard deviation (there are other measures of variability).

The standard deviation SD is

$$SD = \sqrt{\sum (\overline{x} - x_i)^2 / (n-1)}$$

where $\overline{x}$ is the mean value, x_i the individual values, and n the number of periods. The differences of the individual period lengths from the average period length $\overline{x}$ is determined. This value is squared. Then the sum of all squared differences is formed. Finally this value is divided by $n-1$ and the square root taken.

With the help of the standard deviation we can find out which phase is the most suitable one to determine the period length. This phase should have the lowest variability, that is, the smallest standard deviation.

Table 1.1: *Time of maxima, minima and points of inflections of* Desmodium *lateral movements*

No	maximum	minimum	point of inflection up	point of inflection down
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				

Table 1.2: *Time and standard deviation (SD) of maxima, minima and points of inflection (PI) of the* Desmodium *lateral leaflet movement*

time result	standard deviation result
$x_{max} =$	$SD_{max} =$
$x_{min} =$	$SD_{min} =$
$x_{PI\nearrow} =$	$SD_{PI\nearrow} =$
$x_{PI\searrow} =$	$SD_{PI\searrow} =$

Find out whether the maximum, the minimum or the point of inflection to the minimum or to the maximum is the most suitable one (table 1.2).

The standard deviation can also be used to test whether the mean is significantly different from another mean (if, for instance, the period length was determined at another temperature, see page 28).

For this purpose the standard error SE is calculated from the standard deviation SD using the following formula:

$$SE = SD/\sqrt{n}$$

If the means are by 2 or more standard errors apart from each other, the difference is highly significant.

How accurate is the calculated period length? The largest possible error is half of the smallest unit of measurement. If, for instance, we have determined the time to one tenth of a second, it would not be significant to give more than 2 digits behind the decimal point. The last significant digit can be underlined, e.g. 3.7$\underline{5}$ minutes.

How do we interpret our evaluations? We have found, that under our conditions the mean period length of the lateral leaflet movement was 3.75 minutes. The standard deviation is 0.35 minutes. The variability is therefore about 10% of the mean. The standard error is 0.1. We should remember this value for the experiments, where we will measure period length at different temperatures (see page 28). We will then be able to determine whether the means of the periods are significantly different.

1.1.3.3 Planning, execution and analysis of an experiment

To plan, execute and analyze an experiment is one of the main occupations of a scientist. It is also one of the most difficult one. Especially analyzing the results and drawing conclusions from it are full of possible errors and pitfalls. A nice and witty book on it has been written by Beck-Bornhold and Duben (9). Phantasy and creativity are needed as well as carefulness, exactness, wariness and a critical proceeding to plan en experiment. If we want to explain a problem or a phenomenon or how something functions, we put up one or more hypotheses. With an experiment we critically test these hypotheses. In the following a few basic principles are listed (Wilson (118)).

- Try to understand the problem as well as possible: This is the most important prerequisite for the planning of an experiment.
- Analyze the problem: After having understood the problem sufficiently well, bring it in its simplest form or divide it in parts, which are more easily solvable.
- Forming hypotheses: To solve a problem, we put forward hypotheses. This step is much easier, if the theory and the basis of the situation is known.
- Crucial experiments: To decide, which of the hypotheses have to be rejected, crucial experiments are planned and performed. This is a step which is usually more difficult than anticipated, because results are often interpretable in different ways. If this step is not done by the individual researcher, others will collect arguments for or against the different hypotheses. Even they can be partly controversial.
- Aim of an experiment: Experiments serve to test hypotheses. You should therefore ask yourself again and again before performing an experiment, what do I want to test. Ask yourself in each state of the experiment why you do it and whether the next

step will tell you, what you want to know.

- Variables: Scientific work is based on the assumption, that under the same circumstances the same events will occur. For certain events to occur *variables* are the essential conditions. If we observe the process of bringing water in a kettle to boiling, the essential conditions are: the temperature of the fire, the atmospheric pressure, the heat conductivity of the kettle, the pureness of the water. From this the time of boiling can be deduced. Nobody would be astonished if this calculated event will then occur.

- Controls: Besides controllable variables there are unknown or uncontrollable variables. If we want to understand the influence of these variables, experiments have to be performed with controls. Controls are subjected to the same treatments as the experimental objects except the variable under studies. Controls have to be comparable.

- Samples: The selection of samples is an important part of the planning of experiments. The population, from which the sample was drawn, must be well enough defined in respect to the aim of the study. The sample size should be adequate. Experimental difficulties can often be reduced by a clever choice of the experimental object.[2]

[2]The introduction of *Drosophila* as a research animal by Morgan is a good example. The short generation time of this animal has enabled the rapid progress made.

1.1.3.4 Example for the solution of a problem: Does the period length of the lateral leaflet movement rhythm of *Desmodium motorium* depend on the temperature of the surrounding?

How would you build a recording device to record the lateral leaflet movement rhythm of *Desmodium motorium*? A temperature controlled box is used, in which temperature can be kept constant at a certain value (see page 124 *'Building an air conditioned box'*). An electronic thermometer serves to measure the temperature.

Plan an experiment in which the dependency of period length of the leaflet movement rhythm from the temperature is studied. Execute the experiment and use the analysis method explained in the foregoing section to determine mean values, standard deviation and standard error. Plot the period length as a function of the temperature. Add the standard errors as vertical bars to the means. Interpret the results and write a report of your experiments (see page 32). How large is the Q_{10}-value?[3] It is calculated from

$$Q_{10} = (\tau_1 * \tau_2)^{10/(t_2 - t_1)}$$

where τ_1 is the period length at temperature t_1 and τ_2 the period length at temperature t_2. Is, for instance, τ_1 10 minutes at a temperature of $t_1 = 30^0C$ and τ_2 20 minutes at a temperature of $t_2 = 20^0C$, the $Q_{10} = (20/10)^{10/10} = 2$.

Some hints and helps: For growing the plants see the section on the telegraph plant *Desmodium motorium* in the chapter 'Ultradian rhythms' on page 59. For recording the movement you can use the setup shown in figure 1.7 (Koukkari et al.

[3]The Q_{10} value is a measure of the temperature dependency of a process

Table 1.3: *Table for entering the recorded values of the lateral leaflet movement of* Desmodium

time (sec)	15°	25°	35°	time (sec)	15°	25°	35°
0				0			
15				15			
30				30			
45				45			
60				60			
75				75			
90				90			
105				105			
120				120			
135				135			
150				150			
165				165			
180				180			
195				195			
210				210			
225				225			
240				240			
255				255			
270				270			
285				285			
300				300			
315				315			
330				330			
345				345			
360				360			
375				375			
390				390			
405				405			
420				420			
435				435			
450				450			
465				465			
480				480			
495				495			
510				510			
525				525			

(71)). It is easily constructed. Or you use the digitizing method described on page 69. Cut off with a razor blade a leaf with lateral leaflets which are moving well. Put it immediately in a small hole in a polyurethan disc floating on distilled water in a small vial. Transfer to the air conditioned box. If you use the Koukkari method, stick (with a tiny amount of water soluble glue) a delicate thread or a human hair to the tip of the leaflet and to one end of the thin wire balance. Cut off the second lateral leaflet and the terminal leaflet. Cover the cut surface with a tiny amount of vaseline. The

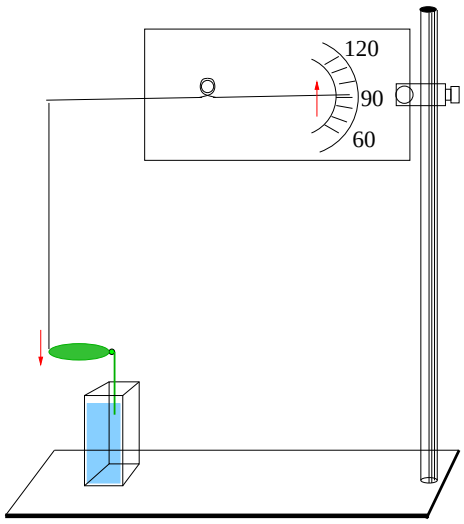


Figure 1.7: *Recording of the lateral leaflet movement of* Desmodium motorium *according to (Koukkari et al. (71)). At the tip of the lateral leaflet a delicate thread is fastened with a tiny amount of a water soluble glue. The other end of the thread is connected to a balance made of a thin wire (cyan) winded once around a bolt (black circle). The pointer of the balance shows the angle of the leaflet (here about 88°)*

measurement is better done by two persons: One calls the times at which the phase is reached which serves as the reference phase. The other person reads the time from a running stop watch and notes it down. The angles are entered into a table every 15 seconds (see table 1.3). These data are the basis for a graphical display of the values as a function of time (figure 1.8).

1.1.4 Experimental protocol

A written protocol is an indispensable part of scientific research work and certain rules have been found to be of much help (see for instance Wilson (118), page 130ff.):

- A laboratory book, preferentially DIN A4 sized, with name, address, date of the experiments and numbered pages is recomended. The first 8 to 10 pages are reserved for the contents to facilitate finding the entrances (figure 1.9). It should always be available in the laboratory (figure 1.10).

- Entrances should be made during the experiment (figure 1.9). Do not use loose pages. Everything which is needed later to write a report or a publication should be noted down. Enter date, time of day, initials (in case more than one person enters notes).

- Entrances and remarks concerning place, time, instruments, books, papers, diagrams and persons should be detailed enough to be still understandable even after years. One should be able to document with the help of the protocol each figure, description, and inference of a publication.

- Observations, numerical results, independent variables such as temperature, illumination conditions, kind of medium, are recorded.

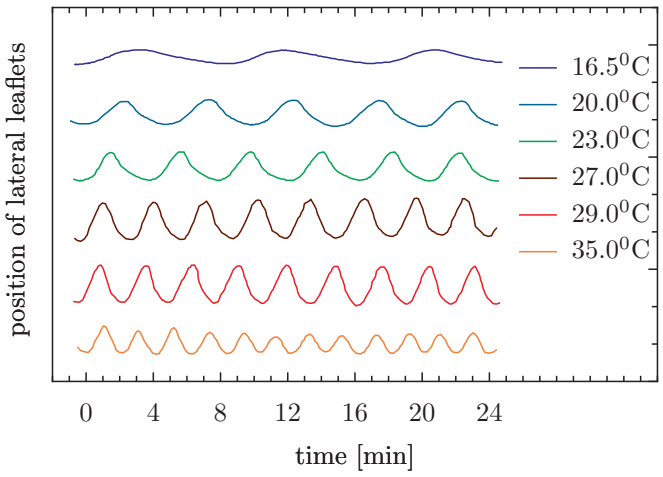
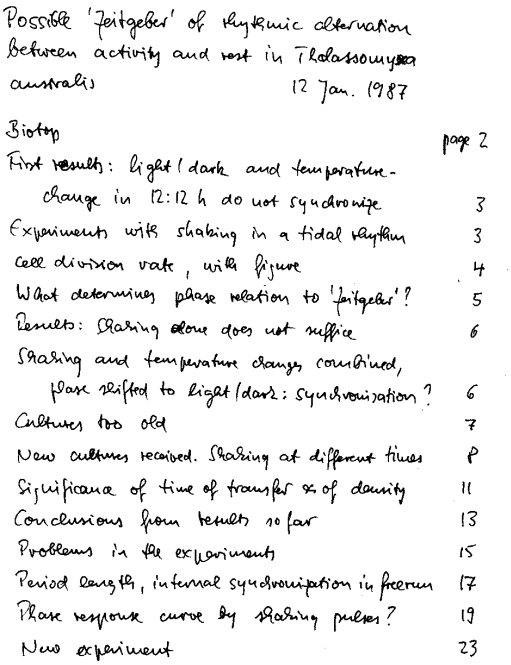


Figure 1.8: *Dependency of period length of the lateral leaflet movement of* Desmodium motorium *from the temperature*

- The data should be entered in the original form and not changed or transformed. For numbers use tables. Indicate the units.
- Figures, sketches, drawings, and tables should be entered directly into the laboratory book or on millimeter paper which is then glued onto a page[4].
- Enter events also which you do not understand at the time, or 'bad' experiments or experiments which went wrong. They can at least serve as a warning what one should avoid to do.
- Later entrances should be done in a different color, dated and signed.
- Which apparatuses and instruments were used? Indicate where descriptions, layouts and instructions of the instrument are found. If changes are made, they should be indicated immediately in the laboratory book. Calibrations, changes and improvements should be remarked and dated. Keep originals of wiring diagram, instructions, construction plans in a separate place. Write the reference number of the instrument also on the instructions and wiring diagrams.
- Chemicals should be noted with name of maker, grade, and eventual treatments.
- Photographs, films, records, spectra and so on should be identified via symbols which refer to the laboratory book and page number. In the laboratory book additional data and notes can there be found. WEII122c would for instance mean, laboratory book number II of WE, page 122, 3rd entrance of this page. In this way no additional explanations of the way of coding must be given.
- A well designed ordering system for films, photographs, records, diagrams, circuits, drawings, copies and the like is especially difficult for either very small or very large numbers of items.

[4] if necessary cut out a page of the book to compensate for thickness

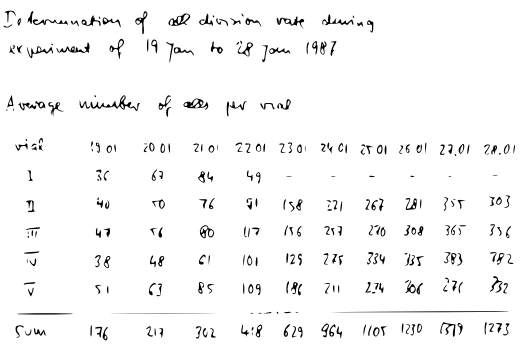
- Indicate the aim of the experiment and give a summary of the conclusions.



Possible 'Zeitgeber' of rhythmic alternation between activity and rest in Thalassomyia australis 12 Jan. 1987

Biotop page 2

Figure 1.9: *Example from a laboratory book: page of content*



Determination of cell division rate during experiment of 19 Jan to 28 Jan 1987

Average number of cells per vial

vial	19.01	20.01	21.01	22.01	23.01	24.01	25.01	26.01	27.01	28.01
I	35	69	84	49	-	-	-	-	-	-
II	40	50	76	91	158	221	267	281	357	303
III	47	56	80	117	156	257	270	308	365	356
IV	38	48	61	101	125	225	334	355	383	382
V	51	63	85	109	186	211	234	306	271	332
Sum	176	217	362	468	629	964	1105	1230	1379	1273

Figure 1.10: *Example from a laboratory book: page of content*

1.2 Communication in science

1.2.1 Introduction

This section is mainly for students, for whom it is an important part of scientific work.

1.2.2 How scientists report their results

A scientist who has finished a research work usually reports the results. He would first explain it to his colleagues, who will constructively criticize methods and execution and propose alternatives to interpret the results. He would then give a talk to a larger group of scientists. This could be a colloquium of the department or a meeting (see the following invitation in table 1.4).

The proposals and critique will be taken into account when writing the publication. The manuscript will be sent or given to colleagues and their opinion and comments on content, style, analysis and interpretation appreciated, before it is submitted to a journal. Usually the editor of the journal sends the manuscript to one or two referees. They use certain rules for their judgment. If the opinion of the two referees is too diverse, the editor has to send it to a third one or decide himself, whether the manuscript is accepted. In most cases the paper is send back to the author with proposals for improvements of style and content. The author will revise the manuscript. It might take a long time from finishing the manuscript until acceptance by a journal. Therefore often short communications are published before the final publication, which have less stringent demands. Interesting new developments and results are reported and commented on

KOLLOQUIENREIHE DES SONDERFORSCHUNGSBEREICHS 45

Vortragender: Prof. Dr. C. S. Pittendrigh
Stanford Univ., USA

Thema: Circadian rhythmicity:
An evolutionist´s view

Ort: Zoologisches Institut Frankfurt
Siesmayerstr. 70
Kleiner Hörsaal

Zeit: Donnerstag, den 17. Dezember 1987
18 Uhr 30

Gäste sind herzlich willkommen

gez. Prof. Dr. G. Fleissner

Beachten Sie bitte auch das Seminar mit Prof. Pittendrigh am darauffolgenden Vormittag über Photoperiodismus:

Redner: Prof. Dr. C. S. Pittendrigh
Thema: Evolutionary adjustment of critical daylength to change in latitude
Zeit: Freitag, den 18.12.87, 9 Uhr 30

Ort: Zoologisches Insttitut Frankfurt, Sitzungszimmer (2. Stock)
Siesmayerstr. 70
Frankfurt, den 1. Dezember 1987

Table 1.4: *Invitation to a lecture*

in special journals such as 'Science News' and others.

1.2.3 Scientific publication: An example

How does such a publication look like, how is it structured and how does one read it?

Proposal: Read the publication of Sulzman et al. (108): '*Neurospora* rhythm in space: a reexamination of the endogenous--exogenous question' from the journal 'Science'.

Read afterward the paper of Mergenhagen (82) in the journal 'Die Naturwissenschaften'. Mark the different sections according to the scheme in the preceding paper. If you want a quick information about the content of the paper, the following procedure is proposed:

Title interesting?⇒abstract⇒figures⇒ introduction⇒discussion⇒result

First decide by reading the title, whether it is worthwhile for you to look at the publication more closely. Then read the abstract or summary. If it seems to be of interest to you, look at the figures and tables and their legends, perhaps also at the introduction and discussion. Important papers must of course be read carefully also in the part containing the results. Sometimes one might be interested in a method or a special point of the discussion only. In this case you would start right away on the particular place.

1.2.4 Writing your own scientific article

The best method to find a good style in publishing a paper is to read scientific articles. In doing so you can learn from good and bad examples. There are also books on it such as Kolle (69), Ebel et al. (27), Silyn-Roberts (105).

Task: Write a paper on the experiments you did where you studied how the temperature influences the *Desmodium*-leaflet rhythm. Use the usual structure of an article. Make a sketch of the experimental setup, a table for the data and a graph showing the dependency of period length on temperature. Do not forget to include a list of references at the end of the article (see also *literature search*).

1.2.5 Literature search

Before we plan a scientific research project we would like to know whether the solution of the problem has perhaps already been found and published. Or, if not, we would at least like to know, whether other people have thought of the problem and what they found out about the background of my studies. Six hours work in the library might save you 6 month work in the laboratory.

Encyclopedias are the most general and useful sources of information if you want to become familiar with a research field. There exist furthermore literature guides for certain fields with basic references, reviews, and journals with abstracts such as Smith et al. (107). Handbooks are also useful to become familiar with a new field (Aschoff (6), Haupt and Feinleib (50)).

Using textbooks you can familiarize yourself with the background, monographs help you to become acquainted with details of certain fields. Subject catalogues of libraries, catalogues of books in bookstores are helpful in this respect. To decide whether a certain book is interesting for you, you can consult reviews on books ('Reviews'). An abstract- and index-journal is Thomson Scientific (109). Instructions are

included in the half-monthly editions.

Quite useful is also the 'Science Citation Index' for Scientific Information (42) (figure 1.11): We know an important, but older paper of the subject we are interested in, and we want to find more recent literature. This publication indicates all authors citing this particular article as a reference in their publication. It is likely that such a paper is also concerned with the same or a similar topic. With the help of the 'Science Citation Index' it is therefore possible to find more recent publications to a topic. The most recent papers can be found in the latest issues of scientific journals. The following journals are specialized in the field of chronobiology (figure 1.12):

- Chronobiologia
- Chronobiology International
- Journal of Biological Rhythms
- Biological Rhythm Research

Most papers in the field of chronobiology are, however, published in numerous journals of quite diverse areas: Plant physiology, animal physiology, microbiology, genetics, behavioral sciences, medicine.

In the 'Current Contents' the pages of contents of journals of the biosciences are reproduced and they contain an index of subjects and authors. In it one can find the wanted information quickly. Under *circadian rhythms*, for example, you find on page 229, 4th column in number 14, volume **34** (April 8, 1991):

127 57
127 41

The first number is the page in the particular issue of 'Current Contents', the second number is the page of the corresponding article in the particular journal (in the case shown a paper by Queiroz-Claret and

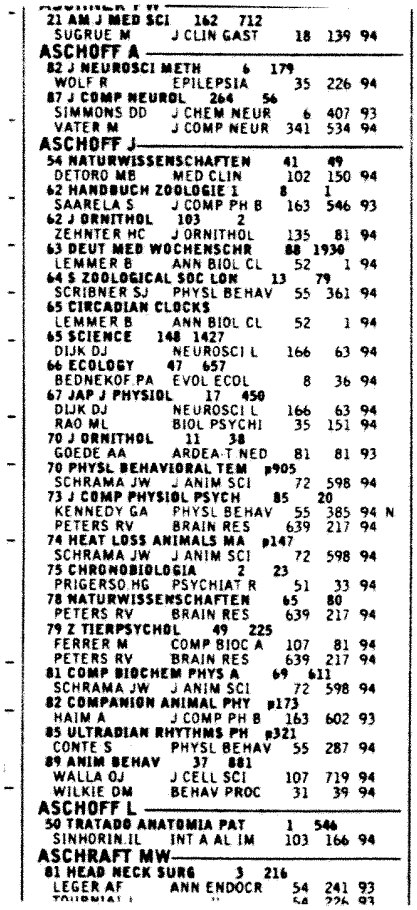

21 AM J MED SCI 162 712
SUGRUE M J CLIN GAST 18 139 94
ASCHOFF A
82 J NEUROSCI METH 6 179
WOLF R EPILEPSIA 35 226 94
87 J COMP NEUROL 264 56
SIMMONS DD J CHEM NEUR 6 407 93
VATER M J COMP NEUR 341 534 94
ASCHOFF J
54 NATURWISSENSCHAFTEN 41 49
DETORO MB MED CLIN 102 150 94
62 HANDBUCH ZOOLOGIE 1 8 1
SAARELA S J COMP PH B 163 546 93
62 J ORNITHOL 103 2
ZEHNTER HC J ORNITHOL 135 81 94
63 DEUT MED WOCHENSCHR 88 1930
LEMMER B ANN BIOL CL 52 1 94
64 S ZOOLOGICAL SOC LON 13 79
SCRIBNER SJ PHYSL BEHAV 55 361 94
65 CIRCADIAN CLOCKS
LEMMER B ANN BIOL CL 52 1 94
65 SCIENCE 148 1427
DIJK DJ NEUROSCI L 166 63 94
66 ECOLOGY 47 657
BEDNEKOF PA EVOL ECOL 8 36 94
67 JAP J PHYSIOL 17 450
DIJK DJ NEUROSCI L 166 63 94
RAO ML BIOL PSYCHI 35 151 94
70 J ORNITHOL 11 38
GOEDE AA ARDEA-T NED 81 81 93
70 PHYSL BEHAVIORAL TEM p905
SCHRAMA JW J ANIM SCI 72 598 94
73 J COMP PHYSIOL PSYCH 85 20
KENNEDY GA PHYSL BEHAV 55 385 94 N
PETERS RV BRAIN RES 639 217 94
74 HEAT LOSS ANIMALS MA p147
SCHRAMA JW J ANIM SCI 72 598 94
75 CHRONOBIOLOGIA 2 23
PRIGERSO HG PSYCHIAT R 51 33 94
78 NATURWISSENSCHAFTEN 65 80
PETERS RV BRAIN RES 639 217 94
79 Z TIERPSYCHOL 49 225
FERRER M COMP BIOC A 107 81 94
PETERS RV BRAIN RES 639 217 94
81 COMP BIOCHEM PHYS A 69 611
SCHRAMA JW J ANIM SCI 72 598 94
82 COMPANION ANIMAL PHY p173
HAIM A J COMP PH B 163 602 93
85 ULTRADIAN RHYTHMS PH p321
CONTE S PHYSL BEHAV 55 287 94
89 ANIM BEHAV 37 881
WALLA OJ J CELL SCI 107 719 94
WILKIE DM BEHAV PROC 31 39 94
ASCHOFF L
50 TRATADO ANATOMIA PAT 1 546
SINHORIN IL INT A AL IM 103 166 94
ASCHRAFT MW
81 HEAD NECK SURG 3 216
LEGER AF ANN ENDOCR 54 241 93

Figure 1.11: *Example from 'Science Citation Index'. The publication of J. Aschoff in 'Zeitschrift für Tierpsychologie 49, page 225 of 1979 was cited by M. Ferrer in Comp. Bioch. A. 107, page 81 (1994) and of R.V. Peters, Brain Res. 639, page 217 (1994)*

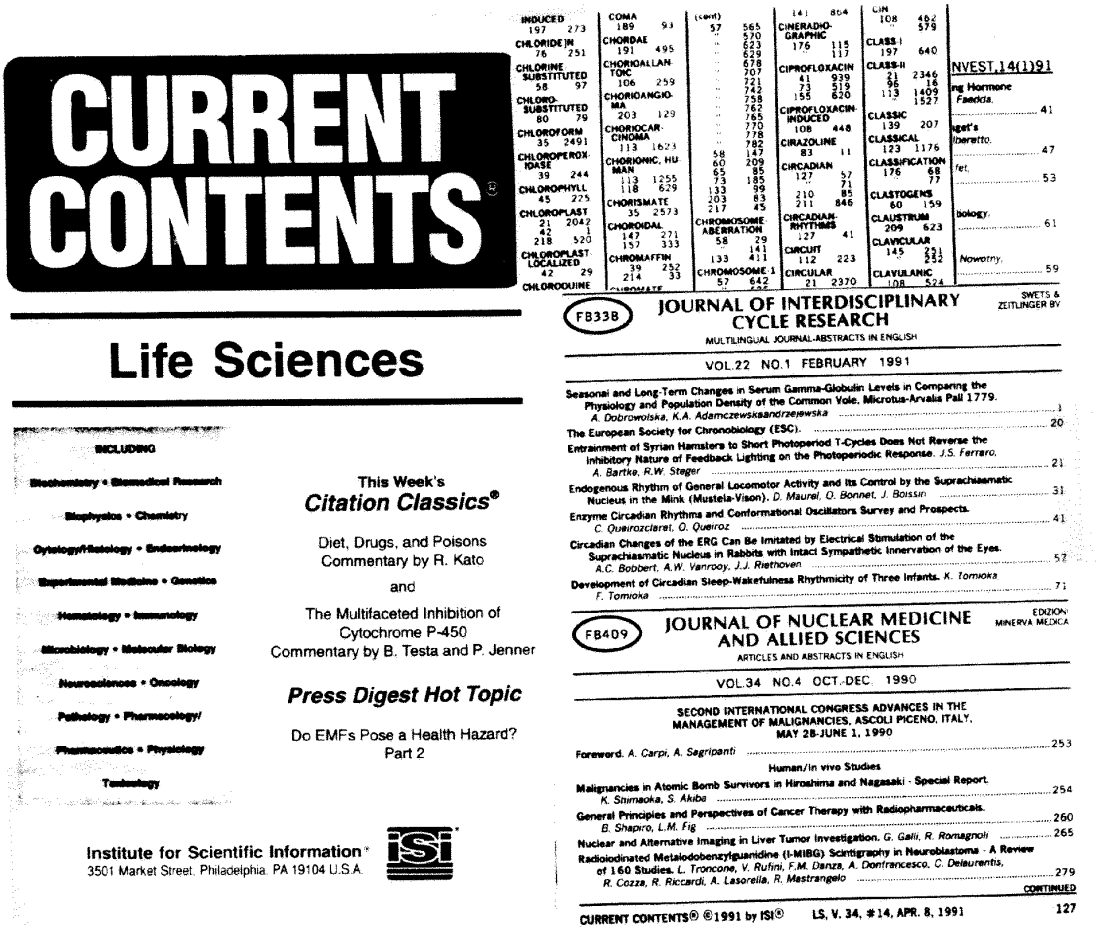


Figure 1.13: *Cover page of 'Current Contents' (left), a page of the key index (top right) and an example of the page of contents of a journal (bottom right). Under 'circadian rhythms' top right one finds 127 41. On page 127 (bottom right) the page with the contents of 'Journal of Interdisciplinary Cycle Research' is found. On page 41 is an article on circadian rhythms by Queiroz-Claret and Queiroz*

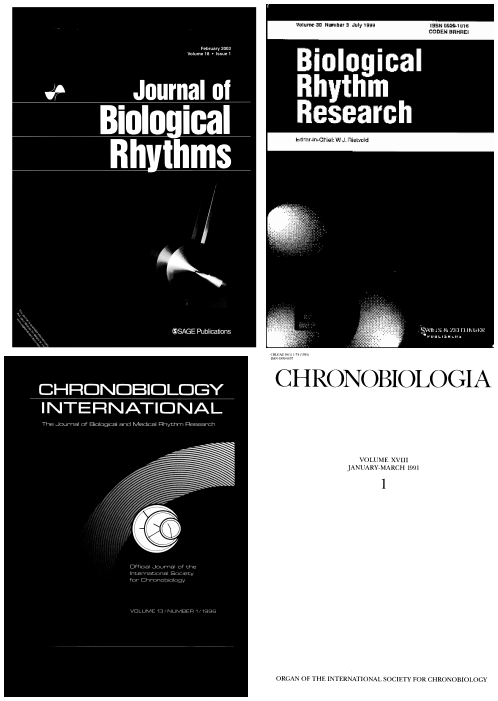


Figure 1.12: *Title pages of journals specialized in chronobiological papers: Journal of Biological Rhythms, Biological Rhythm Research (before: Journal of Interdisciplinary Cycle Research), Chronobiology International, Chronobiologia (not published anymore)*

Queiroz). The 'Current Contents' are also available on diskettes and can be used with a comfortable program allowing to search the literature for key words, authors, journals, and to produce files and printouts of the relevant literature.

It is often helpful if one knows names of scientists working in the particular field (see Science Citation Index). You can write to those people or ask them. But you should have gotten first a founded theoretical and general background in the field. Sometimes it is also advisable to browse through areas which are not directly connected with your field of interest. In this way you might, for instance, find solutions for methodological problems.

> 'Wise selection rather than all-inclusive coverage is the key to library work'.

If you are planning to start your own literature file, you could make use of modern data bases and data base systems. They are available through the university libraries and in the Internet, but also from publishers.[5] With their help it is easy to use reprint collections and to find papers of certain authors, according to certain key words, or according to e.g. the year of publication. Printed literature can be read with a scanner into a computer and made machine readable with an optical character recognition program (OCR).

Proposal: Use the different methods mentioned above to find the more important publications of the last two years for a chronobiological topic of your choice. Make a literature list.

[5] Medline (http://www.biomednet.com), Biological Abstracts, Swets and Zeitlinger with the contents of more than 14000 journals (http://www.swetsnet.nl/direct)

1.3 Controversies in science

The method of multiple hypotheses and 'strong inference' is not always used by a particular research worker, perhaps because he does not know it or because in a special case (where the problem is too complex to allow meaningful alternatives) the method is not useful. In these cases alternative hypotheses are put up at another level. Other scientists might disagree with the proposals and inferences and it comes to scientific controversies. One such controversy from the field of rhythm research is given in the following:

Proposal: Read the book of Brown et al. (15): *The biological clock: Two views.* In this book two controversial views are discussed, how circadian rhythms arise. Brown denies, that they are the result of internal clocks of the organisms and claims, they are the direct result of external factors which are changing in a 24 hour pace. To exclude the light-dark-cycle or a temperature cycle, as is commonly done in research work on circadian rhythms, is according to Brown not sufficient. Instead, the rhythmic alternation of other factors should be prevented, which is, however, often impossible (magnetic fields, high altitude radiation etc.). Hastings, on the other hand, claims, that circadian rhythms are the expression of an internal clock inside the organism. It continues to run even under stringent constant conditions of the environment. This view is supported nowadays by most scientists working in the field. Describe the two views in your own words. How do the two authors explain free-run? Collect the arguments for the two hypotheses and note down the weak points of each. Try to find crucial experiments which can be used to test the hypotheses critically. Read in this context the following papers, which have been published after the book had appeared (Hamner et al. (47), Mergenhagen (82), Mergenhagen and Mergenhagen (83)).

1.4 Unsolved problems

We have stressed already in the introduction, that many problems in the natural sciences are unsolved and that we are far from understanding the nature around us. One of the main goals of science is, to reduce ignorance and superstition of mankind.

Unsolved problems are numerous in the field of chronobiology. The highest priority in the list of unsolved problems is the search for the mechanism of biological rhythms. This question has not yet been answered satisfactorily at the physiological safe at the molecular level. However, molecular studies with clock mutants in *Cyanobacteria*, *Drosophila*, *Neurospora* and mammals have brought about new results and views (see Engelmann (31)).

Important in this respect are not only the methods, but also the systems studied. It is recommendable to use a minimal system, which shows the property to be studied, but exhibits only few phenomena which might disturb the studies. In trying to unravel the mechanism of circadian rhythms a prokaryote would be more suitable as compared to a eukaryote: The former is much more primitive in respect to structure and function. The genetic structures are extremely simple, consisting of a ring-shaped chromosome without nucleus. Organelles and compartments are absent. New molecular genetic methods can be used (Porter (94)). It was therefore a big leap forward when circadian rhythms were found in eukaryotes (Kondo and Ishiura (70)).

Another minimal system are specialized eucaryotic cells such as the red blood cells

of mammals. They do not possess a nucleic acid metabolism, have no mitochondria, no respiration, no protein synthesis and are specialized to transfer O_2. Finding a circadian rhythm in such a system would be an important step to understand the underlying mechanisms. A number of proposed models would be rejected if a circadian rhythm exists in red blood cells. And indeed a circadian rhythm of activities of different enzymes in red blood cells has been described (Ashkenazi et al. (8)). However, attempts to repeat these results were unsuccessful in a number of laboratories (Mabood et al. (76), Ohm-Schrader et al. (87)). See also the preceding subsection *controversies in science* (page 38).

Finally, a circadian rhythm of dry seeds of beans was described (Bryant (16)). This is another minimal system, since besides a very low respiration no other metabolic processes are found. This result has not been confirmed, although the experiments are simple and the results of paramount importance.

We have studied the lateral leaflet movement of *Desmodium motorium* and it would be interesting to understand the underlying mechanism. This is, however, a difficult problem and not solvable in the course of a laboratory exercise. In such a case it is advisable to divide the problem in smaller ones and try to clarify these. In the particular case of the lateral leaflet movement it would be recommendable to divide the problem into two questions: The question, what *mechanism* is responsible for the movement, and secondly, how the movement is *controlled rhythmically*. For the first question, the pulvinus and the turgor mechanism as the basis of the movement is of importance. For the second question of the timing, however, the kinetics of the movement, the temperature dependence of the rhythm, and the influence of inhibitors of various processes such as glycolysis or protein synthesis are of significance.

Proposal: Try to find a limited problem which can be solved during a laboratory course. Further unsolved problems can be found from the examples for rhythms in the second part of the book. For instance: which extra-retinal photoreceptors are responsible for the synchronization of the locomotor activity of flies (see '*Activity rhythms in animals*', page 83).

2 Recording methods

Overview:
Out of numerous recording methods we present two, which are quite versatile and which have stand the praxis. One method serves to record movements and uses a video camera connected to a computer via a frame grabber. With special programs the images are analyzed. Furthermore an infrared light beam method is explained, with which the locomotor activity of animals is recorded.

2.1 Video recording and analysis of rhythm

2.1.1 Introduction

Many rhythmic events in plants, animals and also in unicellulars express them self as movements. They are easily observable. With a video camera coupled via a frame-grabber to a computer and imaging methods such movements are recorded, displayed and analyzed.

2.1.2 Recording principle

The recording principle is shown in figure 2.1. The rhythmic leaf movement of a plant is used as an example. Images of the object are taken in predetermined sequences and, via a frame grabber, transferred to a computer and stored. The position of a leaf is determined by a program which automatically recognizes the tip of the leaf. It saves the values of the position of the tip as x and y values. They can then be graphically displayed as a function of time. The period length of the oscillation can be determined by time series analysis programs.

2.1.3 Recording

Since a handbook (Engelmann (29)) exists for imaging, we will limit ourself here to the essentials. The video camera is connected via a cable to a frame-grabber of a PC (see also figure 2.1).

The particular imaging program needs Linux as the operating system. The programs are available on the Internet and explained (Engelmann (29)). After starting the program, the object has to be put in focus (with the lens of the video camera) and the right size must be chosen (by adjusting the distance between camera and object). A suitable illumination is important. To record in weak safe-light or darkness, infrared light is used. It can be produced with infrared light emitting diodes (LED). The object to be recorded should be clearly visible and not blocked partly by other structures. It is unimportant, whether the object is brighter or darker than the background. However, the lighting conditions should stay constant in time and space. Afterward the sampling rate of the digitizing and the recording interval have to be set.

Different kinds of data can be used for the analysis of the movement: For instance, the x- and/or y-coordinates of the center or the tip of the leaf, or the number of pixels (as a measure for the size of the object). The parameter setting and a commentary can be stored in files. After a file name has been entered, the recording can be started.

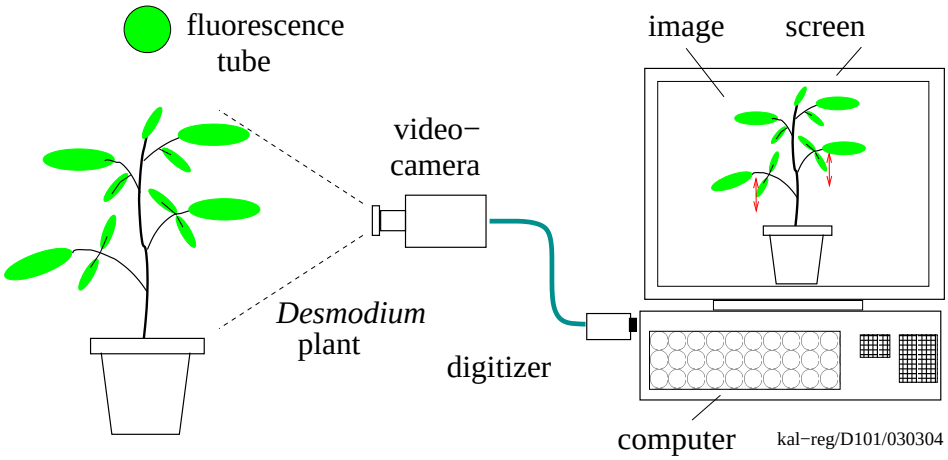


Figure 2.1: *Recording of leaf movement with imaging method. The moving object (here: leaflets of* Desmodium gyrans*) is recorded by a video camera, digitized by a frame grabber of a computer and the images displayed on the monitor screen and stored on the harddisk for further analysis*

During recording the data can be displayed graphically on screen as a function of time.

2.2 Recording of locomotor activity of animals using light beams

2.2.1 Introduction

The locomotor activity of many animals and mobile unicellulars is controlled by an endogenous clock. These movements can be recorded also by the imaging system just described. Examples are given in Engelmann (28). However, in the case of fast moving objects this system is not recommendable. We mention therefore an infrared light-beam system which is described in a handbook (Engelmann and Hellrung (33)) in detail (see figure 8.6).

2.2.2 Recording principle

Individual animals are kept in translucent dishes and supplied with water and food. An infrared light beam crosses vertically a small part of the dish and transmits a signal, if the animal interrupts the light beam.

The system allows to record activity of up to 288 individual animals. It is fast (each animal will be monitored every 25 msec) and quite sensitive (it was originally developed for activity recording of *Drosophila* flies). It functions reliably also at varying light conditions and temperatures of the surrounding.

The activity of the animals can be displayed in form of an actogram: It is determined, whether in a certain time span (for instance 4 minutes) the animal has interrupted the light beam for a certain number (selectable). Or, alternatively, it is recorded, how often the light beams are interrupted in a predetermined time span (for instance one hour).

With the system the environmental con-

ditions such as light intensity and temperature can be monitored additionaly. It can also be used to control temperature, light and other events.

The data are stored in a peripheric processor unit (kind of minicomputer) via multiplexer (kind of inquiry mill, by which the data of different recording places pass the same data line) and are stored in a buffer connected to it. They are transferred hourly to the storage of a host computer. A radio transmitter based clock serves as a time reference. The processor unit is buffered by a battery. In this way no data are lost in case of a power failure.

The data are available in a certain format, for which a plotting program exists. Furthermore the data can be transformed with a program (HELLRODA) into other formats. With special programs (see next chapter) the data can be analyzed.

This system was constructed and the programs written by Winfried Hellrung[1]. The HELLRODA data transfer program was written by Joachim Schuster.

[1] address: Buchenweg 27, D72820 Sonnenbühl(Germany)

3 Display and analysis of time series

Overview:
If measurements of time variable parameters are performed and the data stored, we obtain so called time series. An short overview on time series analysis procedures and -programs is given. Using two examples from the field of biological rhythms some of these procedures will be demonstrated.

3.1 Introduction

If we want to study rhythms, we need not only to obtain data by using recording methods. We need also procedures to display and analyze the data. First elementary terms will be explained. then the following questions will be dealt with:

1. How are periodicities displayed and found? (Section 'elementary terms' and 'graphic display of time series, page 45)

2. How do we remove noise from the recorded data and how do we take care of trends in the curves? (Section 'smoothing', page 47 and 'trend removal', page 48)

3. What is the period length of the oscillation, which procedures are used to determine them? (Section 'time series analysis methods', page 48).

This chapter is for students only, who need the informations for analyzing experimental results.

3.2 Elementary terms

Signals with a rhythmic component are characterized by at least three items:

- Amplitude as the difference between maximum and minimum of an oscillation.

- Period length as the time span between two following maxima or minima (or other identical phase points).

- Phase relation to an arbitrarily chosen reference point such as the maximum of an oscillation.[1]

With these parameters an oscillation can be characterized (see figure 3.1). Furthermore, the form of the oscillation is important: It can be sinus like or like a rectangle, a sawtooth or all kinds of intermediates. An oscillation might possess a trend (upward or downward) and can be noisy (figure 3.1, right curve). A time series could also consist of several oscillations which might superimpose each other.

3.3 Grafic display of time series

Almost always the first step in analyzing a time series is the graphical display of the experimental results. In this way one can

[1] For this purpose the oscillation is normalized to an evident measure, e.g. to 360^0 or 2π circumference of the unit circle or to 24 hours circadian time. 180^0, π or 12 CT as phase references would all mean, that half of the oscillation has passed.

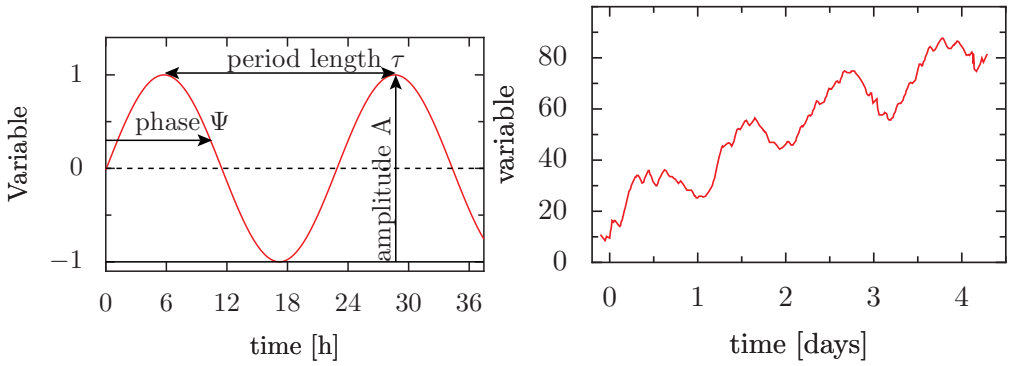


Figure 3.1: Left: *Explanation of amplitude, period length and phase of an oscillation. Right: Superposition of an oscillation by trend and noise*

find out whether trends (oscillation does not deviate more or less symmetrically from a mean value, but tends to higher or to lower values with time) or noise superimposes the oscillation. The graphical display shows furthermore, whether one or more periodicities exist at all, which form the oscillation has, whether it is damped and whether the period is constant. The graphical display allows to decide, whether a time series analysis of the data is worthwhile. If the period length of the oscillation can be determined already from the graphical display, the following procedures are not always necessary, especially since they are time consuming. This is especially true for data which are not machine readable and have to be entered into the computer by hand, and also, if they are not available in regular time intervals (so called non-equidistant sampling).

An example for the graphical display of a time series is shown in figure 1.6. The lateral leaflet movement of the Indian telegraph plant *Desmodium motorium* was plotted as a function of time (see page 24 and legend for details). The period length is in the minute range. The oscillations are quite regular and the amplitudes large. The noise of the recorded data is small. Therefore the period length can be estimated directly without using special time series analysis procedures.

If several outputs are measured simultaneously as in figure 3.2, the mutual phase relationship can be determined in a graphical way: The time series is not plotted in the usual way as a function of time, but one output as the function of the other output. The time information is still present, if for each pair of data a dot is plotted in the coordinate system and if the dots are subsequently entered as a function of time. With equidistant sampling the distance of sequential dots correspond to a certain time interval. If amplitude and phase position of the two recorded outputs are stationary (they do not change with time), the later oscillations will lie on top of each other. If they damp out, the curve circles into the center. If the periods are varying, complicated figures will be seen.

An example for the interpretation of such a phase diagram is given in figure 3.2. The curves show results of measurements on lateral leaflets of *Desmodium motorium* (Antkowiak (4)). The leaflet position was recorded simultaneously with the electrical potential in the pulvinus of the leaflet. The potential varies between -10 and -110 mV. The phase plot of leaflet position against potential shows first of all, that both outputs oscillate with the same period. Furthermore the amplitude is stable. A strong change of the potential to negative values occurs in the upper leaflet position. During this so called hyper-polarization the leaflet stays in its upper position. It is not

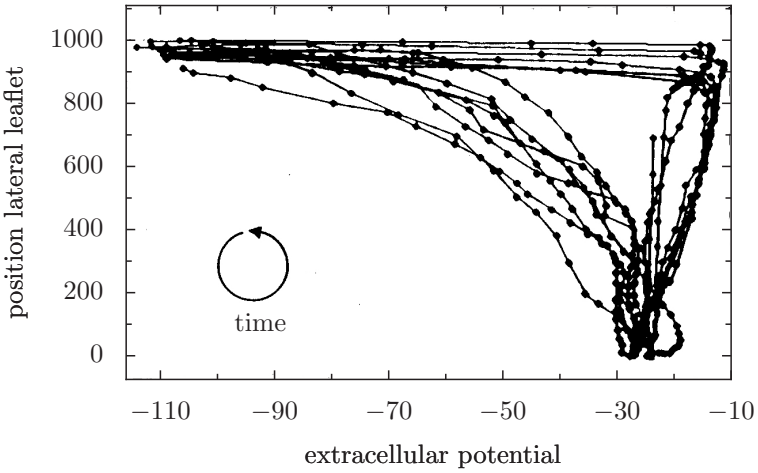


Figure 3.2: *From the lateral leaflet of* Desmodium motorium *the position and the electric potential in the pulvinus were recorded simultaneously and the former plotted against the latter*

before the re-polarization occurs that the downward movement begin indicating, that the hyper-polarization leads the downward movement. The upward movement begins not before the potential has reached small negative values. During the upward movement the potential stays rather constant. From the varying distances of sequential dots one can furthermore conclude: The hyper-polarization in the upper leaflet position and the following re-polarization during the downward movement are fast events: Between two sequential dots large distances in the x direction are covered. The distance of the dots in the y direction informs about the time the leaflet movement takes. With the help of this kind of display possible causal relations between recorded values can be checked. In the example given it can be deduced, that the average potential of the pulvinus of the leaflet is closer connected with the oscillator than the leaflet movement, because the sudden hyper-polarization leads the downward leaflet movement. It could furthermore be, that there is a causal relation between the two items (which can, however, not be deduced from the plot).

An other example is shown in figure 3.3. It is from a publication of Gorton et al. (44). The width of the stomata of isolated guard cells from the epidermis of *Vicia faba* was determined every hour under the microscope. The values were plotted as a function of time (for the basis of stomatal movement see page 64). It is difficult to recognize directly an oscillation from the curve. It is therefore adequate to use certain procedures which will be demonstrated in the following.

3.4 Smoothing

A close look at the upper curve in figure 3.3 reveals a step-like course. However, noise is quite pronounced. Therefore the values were first smoothened. For this purpose a smoothing average was performed with the values. Of five sequential values the average is formed and this value is stored instead of the third original value. The 'smoothing window', consisting of five data, is now moved to a later record and the procedure repeated. In this way we get a mean value for the original fourth value. After all values have thus been averaged, the new time series is shortened by the two

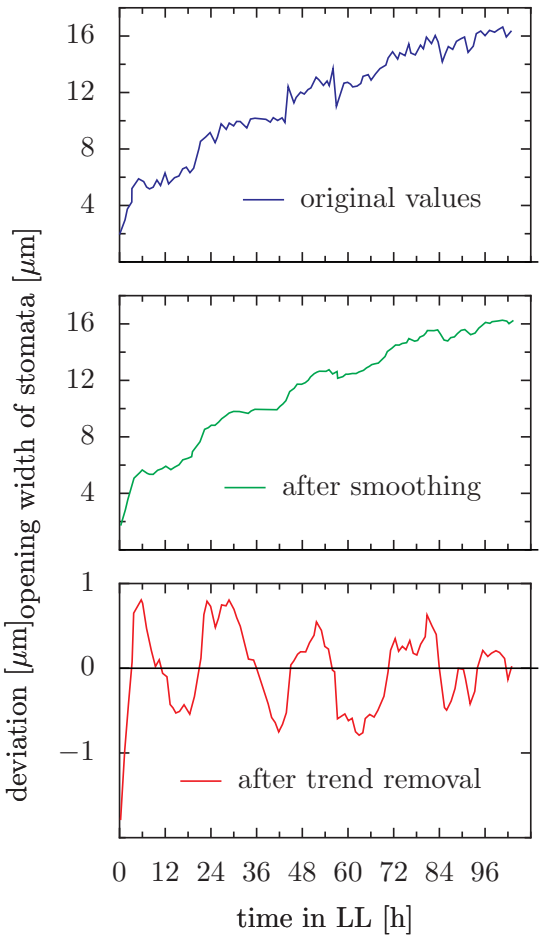


Figure 3.3: *Mean values of stomatal width of 5 isolated guard cell systems of* Vicia faba*. Upper curve: Original values. Center: Curve after smoothing average was applied. Bottom: Curve after trend removal*

first and last values, but the deviations of the curve are reduced (center curve in figure 3.3). The smoothing improves with the length of the smoothing window (but at the same time more data are lost at the begin and end)[2].

We see now the step-like course of the curve of the stomata data better (figure 3.3, center curve), but there is a strong upward trend.

3.5 Trend removal:

A trend usually disturbs the analysis of an existing rhythm and should be removed. Afterward it can usually be better decided, whether a rhythm is pertinent.

A simple method of trend removal is to fit the data with a so called polynomial curve. This fitted curve shows a minimal sum of the quadratic deviations from the smoothed individual values (a characteristic of a good fit). The deviations are shown in the lower curve of figure 3.3 and a rhythmic course is now clearly visible. Trend and noise are removed. The new data can now be analyzed for periodicities. How this is done will be described in the following.

3.6 Time series analysis procedures

3.6.1 RUN-test

The RUN-test is a simple and quick procedure to recognize periodicities. It is a

[2] There are further procedures for smoothing, in which the values of a smoothing window are weighted; the center value could for instance obtain a high weight, the neighboring values a smaller weight, and the peripheral values a still smaller weight. Digital filters weight with certain functions (page 49)

distribution-free test, that is, no special requirements are made in respect to the distribution of the data. After an eventually needed trend removal the mean value of all data of the time series is determined. The values, which are larger than the mean, are replaced by a +, and the values, which are smaller, with a −. In the next step the length of so-called 'runs' is determined. A 'run' is the sequence of + or − signs. If the time series is characterized by noise only, the lengths of the runs will be randomly distributed. If however a rhythm is present, the runs will fluctuate around the length of half the period.

For further analysis we use three procedures. They serve to determine the period length of a rhythm which has beforehand been shown to exist by using the RUN-test or by simple observation.

3.6.2 Frequency folding

In this procedure the time series is cut into pieces which correspond roughly to the length of the period. The pieces are plotted underneath each other (see figure 3.4).

Usually one can now easily recognize whether corresponding phase points of the individual periods lie below each other: In this case the period length was correctly estimated. Otherwise the period was too short or too long. If so, the period can be estimated anew or a line is laid through the maximum of each period (see figure 3.5): Its slope reflects the period length. This procedure allows also to recognize changes in period length. In this case the periods can be determined for the different sections separately. This procedure is especially well suited for the determination of period lengths in actograms (see figure 3.5).

3.6.3 Digital filter

In the case of digital filtering the time series is transformed by a digital algorithm. In this way period lengths can be determined from rhythms which would otherwise, due to noise, be less well analyzable (see figure 3.1).

3.6.4 Maximum-entropy-spectral analysis

The relatively new procedure of maximum-entropy-spectral-analysis (MESA) is especially well suited to determine even short time series reliably.

3.6.5 Signal average

If the period length was determined, one might like to obtain an average curve of a period (see figure 3.6). For this purpose the signal-average-method of De Prins and Cornelissen (24) is useful. It superimposes the individual pieces which correspond to a period and determines the mean values and their standard deviations. This method can also be used to analyze 'residues'. If a periodicity was determined, other oscillations might still lurk in the data set which one obtains after the analyzed oscillation was removed ('residues'). They can now be more easily analyzed as compared to the situation where they were hidden below the main oscillation. This procedure is also useful for separating signals from noise.

3.6.6 Actogram display

By measuring the locomotor activity of animals, the distribution of events in time is obtained. It is usually displayed in the form of an actogram. The data are often reduced to yes/no events: the animal is either active or inactive. To obtain an actogram,

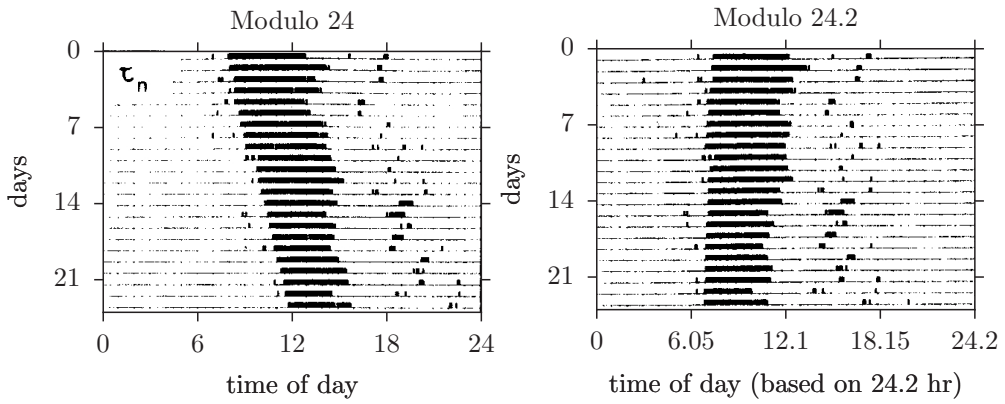


Figure 3.4: *Method of frequency folding: The activity stripes which were originally cut into 24 hour pieces (left) are in the right figure cut into pieces in such a way that activity between the 7th and 24th period lies now below each other. The x-axis corresponds now to the period length in this time span (about 24.5 hours) and not 24 hours anymore*

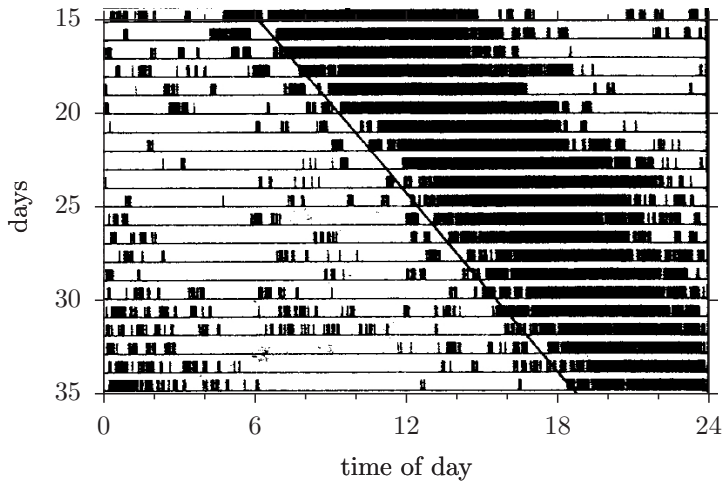


Figure 3.5: *Period determination with slope of a fitting line: The period length is 24.6 hours (as can be estimated also by checking the position of the fitting line on the 25th day (12o'clock) and on the 35th day (18o'clock): The difference is 6 hours, divided by 10 (35-25) gives 0.6 (*$24 + 0.6 = 24.6$ *hrs)*

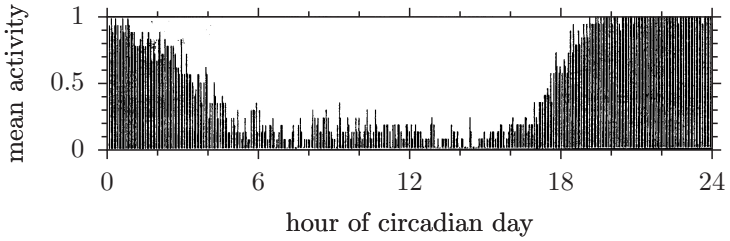


Figure 3.6: *Signal average method: The activity from day 7 to 24 of the right figure of 3.4 was summed up and a mean circadian day (24.6 hour period) was obtained. The smallest activity bin was 4 minutes*

the time series is cut into 24 hour pieces and lined up underneath each other (see figure 3.5). Similar to the frequency folding method these time spans can be chosen also in such a length, that they correspond to the period length (see figure 3.6, right part). If the corresponding values of the individual (subjective) days are vertically summed up, an 'average day' is obtained. This average day can be compared with the corresponding average day of another time span which might have received another treatment (see figure 3.6 and the preceding section).

With this method superimposed oscillations with frequencies close together can be differentiated. This is much more difficult to do with other methods. For this purpose a frequency folding is performed using the period of one of the oscillations. The superimposed oscillation is now more easily recognizable from the figure.

3.6.7 TIMESDIA

A program package for the analysis and simulation of time series was developed by W. Martin in Bonn. It is available on large computers (for instance VAX, IBM) and (so far only) on a HP-computer. A detailed documentation is available (Martin et al. (79)). The data must be equidistant.

The program allows in dialogues to enter and graphically display data sets (up to 9 time series at the same time). Two time series can be analyzed simultaneously. The data can be manipulated (copied, deleted, supplemented, concatenated, selected, listed), characterized (maxima, minima, mean value, variance, standard deviation, standard error, variability, third moment, skewness) and analyzed.

The analysis methods offered include complex demodulation (a band pass technique which allows to analyze non-stationary periods[3]). Other analysis methods can be used such as periodogram analysis, spectral analysis, autocorrelation, Fourrier analysis, different filter techniques. Details are found in the description of the TIMESDIA program.

For literature concerning time series analysis see Martin et al. (79), Randall (95), (Halfmann) and for programs see page 45.

3.6.8 MATLAB

Matlab (stands for MATrix LABoratory) is a powerful and professional program for math work. It contains also routines which

[3] the period does not stay constant, but changes with time

are useful for time series analyses (including fast Fourrier transforms, correlations, various filters and so on). Many engineers and scientists use this program and thus many programs are available for free. Unlike most programs you can look at any of the functions MATLAB comes with and modify it as you wish.

Manipulations of the data are very easy and allow one to sit down and ask 'what if ?' and have an answer often within minutes. Even very complex plots can be generated quickly.

MATLAB is available for Macintosh, Windows, and Unix (X-Windows such as Linux). Programs are highly portable. Educational discounts are available and many universities already have the program. References: Hanselman and Littlefield (48); Marchand (77). See also the web page, http://www.mathworks.com/.

4 Working with models

Overview:
Models play an important role in the study of biological rhythms. They allow to simulate the behavior in time. The results of simulations are tested with experiments and the model has to be improved, if it does not meet the experimental results. We refer here to some model programs and bring examples for rhythmic events.

4.1 Introduction

If man wants to understand his surrounding, be it the physical, social or economic one, he uses models. This is, because the real systems are much too complex for analysis. Only by simplification we have the chance to understand it better. This holds also for rhythmic processes. Dynamical models are especially suited in this case. They consist of state variables (indicators of the state of the system), of combinations of state variables and of parameters of the environment which affect the system. If these parts of the system are known, the behavior can be simulated (Bossel (12)).

To generate complex dynamical systems is quite elaborate. Fortunately procedure have been developed and programs are on the market which allow even the layman to construct dynamical models and to work with them. We will describe some of these programs and bring model examples from the field of biological rhythms with which oscillations can be simulated.

4.2 Model construction and simulation with MODUS

The 'Deutsches Institut für Fernstudien (DIFF)' in Tübingen has developed the program 'MODUS' for PC's[1] (Walser and Wedekind (113)). With this program dynamical models can be constructed and used for simulations. Dynamical systems react time dependent. If their characteristic parameters are plotted against time, it is easy to recognize the behavior of the system. In figure 4.1 an oscillating system is shown.

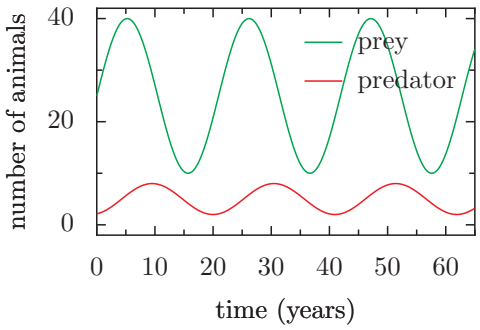


Figure 4.1: *Oscillations in a predator-prey-system simulated as a model. Prey: red, predator: green curve*

With Modus certain symbols can be connected with each other and used to represent dynamical systems as a graph on the computer-screen. The structure diagrams thus formed are then converted to corresponding equations by the program. Different methods exist. MODUS uses a no-

[1] supplied by the 'CoMet Verlag für Unterrichtssoftware, Duisburg'

tation originally going back to Hering and et al. (53). It is based on the system-dynamics method introduced by Forrester (43) and allows to describe interacting dynamical systems.

The following symbols are used by MODUS: State- and changing variables, functions, constants and connections (in the form of arrows). One can also construct model components which can be connected with each other. If you want to work with the model, you should obtain the program and the detailed description from the publisher. In eight steps you are introduced into the handling of the program. On the program disks are also a number of examples, among others the predator-prey model describing oscillations between populations of predator and prey.

4.3 Model construction and simulation with other programs

The DSP-program was developed by an audio-technician, M. Schick, and runs on the ATARI computer. Supply source see subsection 122.

It allows simulations of digital audio-technique, control technique and non-learning neuronal networks. It can, however, also be used to build dynamical systems in biology, which oscillate.

You should get acquainted with the program by reading the instructions which come with the disk. In the creation mode, processes are created which are connected with each other as signal flows. Processes might have inputs and outputs. There are signal sources, signal transforming units and signal sinks (stores). Subsystems allow a hierarchical abstraction (block structure).

4.4 Model examples for rhythms

We will get to know in the following some examples of models for biological rhythms. These examples are realizable with MODUS or with other programs.

4.4.1 Feedback model for biological rhythms

Johnsson and Karlsson developed a feedback model which simulates for instance the gravitropic pendulum movement (see page 62 and Andersen and Johnsson (3)), but also circadian phenomena (Johnsson and Karlsson (62)). It was used by Lewis (72) to simulate the circadian rhythm of locomotor activity of night active insects. The model consists of functional units which are shown together with their connections in figure 4.2. One can study with this model the influence of a changed environmental temperature, of light-dark-cycles or of different light intensities. In this way the reaction of the model to these environmental conditions can be checked and compared with the data obtained experimentally.

You might try the following situations:

- Night active insect in 12:12 hour light-dark-cycle
- Free-run (that is the rhythm without zeitgeber, namely at constant environmental conditions) in continuous darkness.
- Effect of a light pulse given at different circadian times CT.
- Phase response curve towards a one hour light pulse: You plot the results of a simulation as a function of the

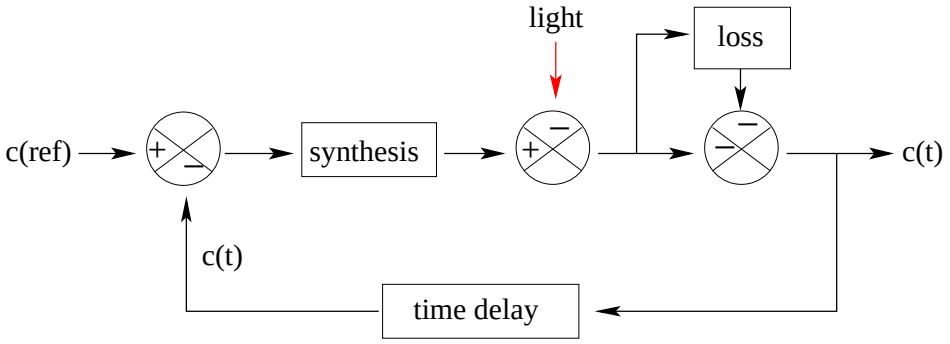


Figure 4.2: *Functional diagram of the feedback model to simulate the locomotor activity rhythm of a night active insect, after Lewis (73). The c(t) value oscillates in a circadian way if synthesis function, loss function and time delay are chosen adequately. Light influences the system by destroying the synthesis product of the system*

time of illumination (advance in relation to the control above the zero line, delay below the zero line)

- Free-run at different environmental temperatures.

Compare your results with those from the literature, which were found by Lewis using weta, a cricket from New Zealand (Christensen and Lewis (21)).

A slightly different feedback model simulates the lateral leaflet movement of *Desmodium motorium*. In contrast to the model explained before it shows a strong temperature dependency of the period. It is therefore useful to simulate rhythms as found in the lateral leaflet movement of *Desmodium motorium*. Try to determine the different period lengths at different environmental temperatures and calculate the Q_{10} value.

Further proposals: Compare your results with your experimentally obtained data.

4.4.2 Predator-prey model

Population rhythms occur, if a predator decimates the prey population. However, at a lower prey population density the predator has a reduced propagation rate. In the model of Lotka-Volterra these relations are described mathematically. It is assumed, that the death rate of the prey depends on the number of predators. The larger the predator population, the more prey animals will fall a victim. On the other hand, the predators are better off if many prey animals are available.

In the time diagram (figure 4.1) the number of predators and prey are plotted on the y axis. The x axis is a time scale with divisions in time units (generations).

The Lotka-Volterra-model is also a feedback model: The prey population has a positive effect on the size of the predator population, whereas the latter has a negative (inhibiting) effect on the size of the prey population. Oscillations as shown in figure 4.1 result. The predator population which so to speak growths on the prey population as a substrate, lags the prey population by 90^0. This is shown in a phase

diagram in which the number of predators is plotted on the y axis and the number of prey animals on the x axis (figure 4.3). If the parameters are adequately chosen, both populations move on a closed path around the equilibrium point.

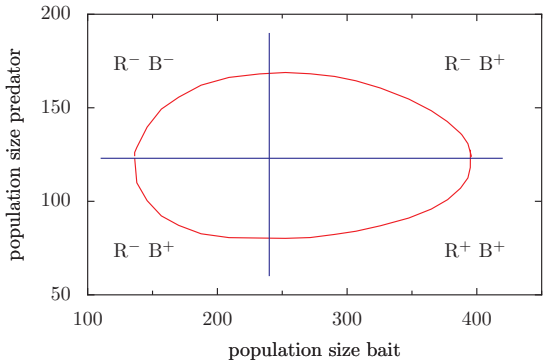


Figure 4.3: *Phase diagram of oscillations of a predator-prey system. R: predator, B: prey, +: increasing, -: decreasing population. In the right lower part the predator population and the prey population increase, in the right upper part the predator population increases and the prey population decreases, in the left upper part the predator- and prey population decreases, and in the left lower part the predator population decreases and the prey population increases*

In nature such cycles were observed in snow hare and lynx in Canada. But things are more complicated than the model suggests, since it was found that the population of snow hare oscillates even in areas were the lynx has died out long ago.

4.4.3 Model building and simulation with a model of Diez-Noguera

A special model to simulate rhythms in animals was developed by Diez-Noguera for the PC[2]. With its help examples of rhythmic locomotor activity patterns were simulated in rats (Diez-Noguera (25)) and flies (Helfrich-Förster and Diez-Noguera (52)). It uses coupled oscillators the properties of which are slightly different from each other. This situation is common in organisms.

[2]Dr. Diez-Noguera, Group de Cronobiologia, Laboratori de Fisiologia, Facultad de Farmacia, Av. Diagonal 643, SP 08028 BARCELONA (SPANIEN)

Part II

Examples for observing rhythms and experimenting

5 Ultradian rhythms

Overview:
Examples of rhythmical events with periods in the minute and hour-range are presented. They are: chemical reactions, reactions of seedlings after stimulation by gravity, transpiration due to movements of stomata, and leaf movements brought about by special joints.

5.1 Introduction

Many biological oscillations are ultradian: Their period length is shorter than the one of circadian oscillations. Typically the period is in the minute- to hour- range. Chemical oscillations can serve as a model for this type of oscillations. The sequence of reactions in some of them are quite well known. They will be described in the next section.

In the third section of this chapter it will be shown how dark grown seedlings show pendulum-like oscillations after stimulation by gravity. Recording will be explained and experiments proposed.

The transpiration of grasses via their stomata can occur rhythmically, as reported in the fourth section. The water loss via the leaves is recorded and it is studied, how the oscillation depends on light intensity.

The lateral leaflets of the telegraph plant *Desmodium motorium* move relatively fast up- and down. These movements occur as the result of volume changes of motor cells in special joints. Recording of these movements and proposals for experiments are described in the fifth section.

5.2 Chemical oscillator

As an example of a chemical oscillator the Belousov-Zhabotinsky-reaction is demonstrated. The rate of most chemical reactions depends on the temperature. We will therefore try to find out whether the period length of this chemical oscillator is also temperature dependent. This system shows besides oscillations in time also those in space, which manifest them self as a pattern in chemical activity.

5.2.1 Background

For an introduction see Field (39), Becker and Leschik (10), Winfree (119). The Belousov-Zhabotinsky-reaction was found by Belousov in 1958 and studied in detail by Zhabotinsky (121).

According to the laws of thermodynamics all spontaneous chemical changes in a homogeneous and closed system are paralleled by a decrease in free enthalpy of this system. Oscillations would therefore be impossible. However, under certain conditions the concentrations of intermediates can oscillate around the expected values of the stationary state while approaching equilibrium (figure 5.1). Oscillations in time and space occur. A prerequisite is, that the system is not yet in an equilibrium and that it contains a feedback. The Belousov-Zhabotinsky-reaction consists of two main reactions A and B. They interact

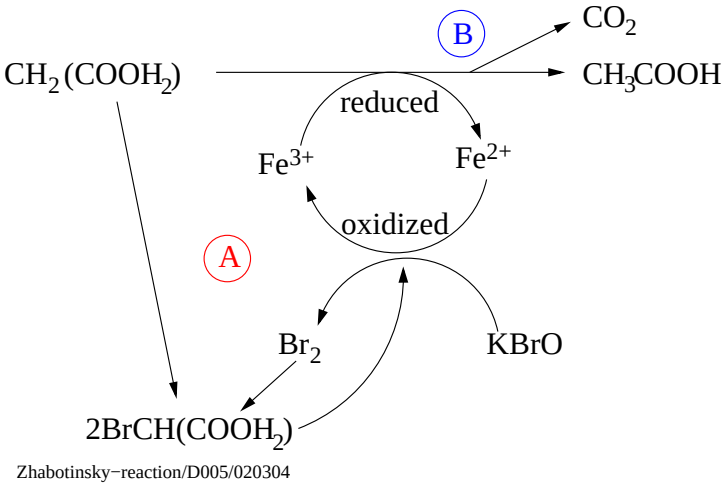


Figure 5.2: *Reaction scheme of the Belousov-Zhabotinsky-reaction with the two underlying reactions A and B*

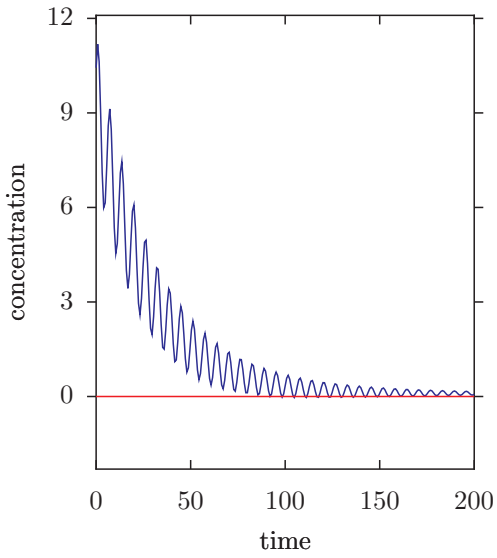


Figure 5.1: *Occurrence of chemical oscillations in a closed system before reaching equilibrium. After Degn 1972*

only marginally, because the reactions of A consist of ions and molecules with paired electron spins (singlets), and B consists of reactions of radicals. Whether A or B dominates, depends on the concentration of the Br^- ions: If the concentration of Br^- ions is high, A dominates, if the concentration of Br^- is low, B dominates. A uses up Br^-; therefore reaction B is induced. B produces Br^-; therefore reaction A is induced (figure 5.2). A: Bromide and bromate form together with malonic acid bromemalonic acid. The ferroin is first blue, due to the three valent iron (bromate oxidizes Fe^{2+} to Fe^{3+}). However, due to bromate being removed, Fe^{2+} is formed and the ferroin turns red.

B: Brommalonate is concentrated enough to reduce Fe^{3+} to Fe^{2+}. Acetic acid, CO_2 (gas bubbles!) and bromide are formed. The latter inhibits reaction A. This in turn inhibits the formation of brome malonate, and reaction B is inhibited. The cycle can start anew.

(A) (bromation of malonic acid)

$$2Br^- + (BrO_3)^- + 3H^+ +$$

$3CH_2(COOH)_2 \rightarrow 3BrCH(COOH)_2 + H_2O$

(B) (contains an auto-catalytic part $HBrO_2 \rightarrow 2HBrO_2$)
$(BrO_3)^- + 4Ce^{3+} + CH_2(COOH)_2 + 5H^+ \rightarrow 4Ce^{4+} + BrCH(COOH)_2 + 3H_2O$

5.2.2 Demonstration

The rhythmic change in color of the Belousov-Zhabotinsky oscillation is demonstrated in the following. The recipe of the necessary solutions are given and shown, how to observe the wave pattern of chemical activity. As an alternative, one of two movies can be shown which deal with chemical oscillators (Hock and Bolze (54), Hock and Bolze (55)).

5.2.2.1 Instruction

- Pour 6 ml of solution 1 in a test tube. Add 0.5 ml of solution 2 and 1 ml of solution 3 under the hood.
- Close the test tube with parafilm and shake solution.
- Add 3 drops of solution 4 after the yellow color of brome has disappeared completely and mix by shaking.
- Observe the change in color between blue and red which begins soon afterwards and determine the period length of the oscillation with a stop watch.

Preparation of solutions: The following four stock solutions are needed:

- Solution 1: Pour carefully 2 ml concentrated sulfuric acid to 50 ml distilled water using a Peleus ball (attention, always add the sulfuric acid to the water, never the other way round! Danger of spatter!) and add 5 g sodium bromate. After solving fill up to 70 ml.
- Solution 2: Solve 1 g NaBr in 10 ml distilled water.
- Solution 3: Solve 1 g malonic acid in 10 ml distilled water.
- Solution 4: 0.025 molar ferroin (ferroin-indicator phenanthroline-ferrosulfate Merck No. 9161).
- All solutions can be kept for a long time in closed bottles.

5.2.2.2 Wave patterns of chemical activity

Pour the described solution in a clean plastic Petri dish (about 10 cm diameter) to a hight of 5 mm. A pattern of waves forms (figure 5.3). A trace of a detergent facilitates the wetting of the dish. The same chemical processes as described before are also responsible for the occurrence of these patterns. However, diffusion of the brome ions due to the lack of mixing plays now a role.

5.2.3 Experiment: Temperature dependence of the period length

It should be determined, whether the period length (time between color changes from blue to red) depends on the temperature of the solution. This should help you to plan and execute a simple experiment. Therefore only some general proposals are made here and some hints are given, how to obtain the necessary informations. It is advisable to work through the chapter 'Scientific work'.

Use the following procedure:

1. What is the aim of the experiment?

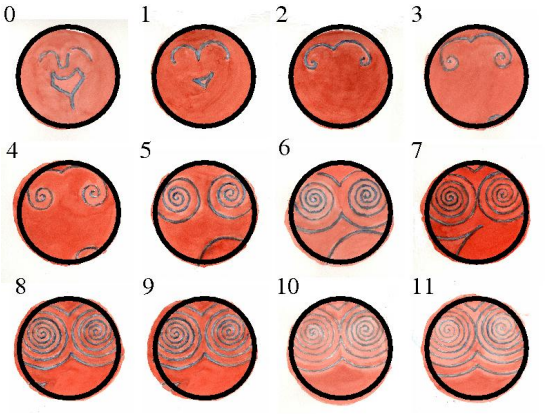


Figure 5.3: *Belousov-Zhabotinsky-reaction as wave pattern in a Petri dish. The solution is red at the begin. By a disturbance at a certain position brome ions are used up and bromate oxidizes Fe^{2+} (red) to Fe^{3+} (blue). Brome ions diffuse into the oxidized (blue) area. The red area turns into a blue ring. After Fe^{3+} has been reduced by brome malonate, the area turns red again*

2. What are the prerequisites (chemicals, glassware, equipment and instructions for handling)
3. What and how to measure? (Temperature: how to set it? Which range? Which step width between temperatures? Oscillations: How do I determine the average period length? What measure of variability do I use?)
4. Analysis
5. Write a report

After having determined the period length at the different temperatures the mean period length and the standard error are plotted against temperature in a diagram. Read in the glossary about the Arrhenius plot and transform the values correspondingly. Determine the Q_{10} value and interpret the results. For the graphic display you can use a plot program. What one should take into account in writing a report is described on page 32.

5.2.3.1 Difficulties and sources of errors

The system oscillates only at temperatures up to 40^0C. At this temperature the oscillation stops after 3 minutes. At room temperature the oscillations can be followed for about 4.5 hours. The concentrations should be kept accurately except the $KBrO_3$ concentration (can be varied to twice the amount) and the Ferroin-concentration (can be varied eightfold). Traces of chloride ions (for instance at the fingers) can inhibit the reaction.

5.3 Gravitropic pendulum

Dark grown seedlings may show a pendulum movement after stimulation by gravity. They will be studied using the morning

glory (*Pharbitis nil*). The oscillations will be recorded with a video recording system. The period length and latency time is analyzed as a function of environmental temperature and hypocotyl length using different programs.

5.3.1 Background

Gravity is used by many plants as a reference for orientation in space: Shoots grow often opposite to, main roots in the direction of the gravity vector. The physiological mechanisms of the balance system of plants are neither on a cellular nor on a molecular basis completely understood. The same is true for the balance system of man and animals. Experiments in space have offered new possibilities to study the function of the balance system of man in the absence of gravity. This is also the case for plants (Wilkins (117), Johnsson (59), Johnsson and Heathcote (61)).

Bending movements controlled by gravity are found in almost all higher plants. Cereals which have been laid down by wind grow upward again. Seedlings of sun flowers, morning glory and other plants which have been horizontally placed do the same. The physiological basis of this gravitropic reaction has been reviewed by Volkmann and Sievers (111) and by Iversen (58). The sedimentation of amyloplasts (organells with starch grains) leads to an asymmetrical distribution and a stimulation of the endoplasmic reticulum. How this occurs is not yet understood in detail. According to Pickardt (91), however, gravity is supposed to influence cell structures as a whole and induce stress reactions which lead to the gravitropic reaction. For more discussion and a more recent view see Engelmann (32).

After gravitropic stimulation gradients of indolyl acetic acid (IAA), K^+ and Ca^{2+} are formed (Pickardt (91), Firn and Digby (40)). The intensity of the reaction depends on the strength and direction of the stimulus. Often the reaction to a geotropic stimulus occurs in form of a damped oscillation. There are, however, also endogenous, self-sustaining oscillations, e.g. in the case of pendulum movements. Such 'circumnutations' of sunflower seedlings were modelled by Israelsson and Johnsson (57). According to the model, the movement was completely dependent on gravity. However, a space-lab experiment in 1983 (space-lab 1) showed that under micro-gravity conditions oscillations were still occurring. They are, however, less regular (Brown (14)). Already Darwin (23) regarded circumnutations as autonomous movements, which occurred independent of external factors. A model for the gravitropic pendulum must, therefore, assume the existence of an internal oscillator *and* an additional influence by gravity.

5.3.2 Material

Seeds of the morning glory *Pharbitis*[1] are treated for 45 minutes with concentrated H_2SO_4 in an Erlenmeyer flask and shaken once in a while (Attention! Danger of spatter). This treatment causes swelling of the seed husks. After the treatment dispose the acid in a sink *filled with much water* and add quickly much water to the seeds. The short, strong increase in temperature leads to a uniform germination of the seeds. Use running water over night to wash off the acid. Place a net on top of the beaker to prevent the seeds from floating off. On the next day the seeds are planted individually about 15 mm deep in sand in a plastic vial

[1] available from the Marutane Trading Co. in Kyoto, Japan

(2-3 cm diameter) and kept moist. At 30-32^{0}C the seeds germinate after 20-30 hours. They are kept in green 'safe light' (physiologically ineffective)[2] until the hypocotyls are about 50-60 mm long. This takes at room temperature about 4 to 6 days.

5.3.3 Induction of the gravitropic pendulum movement

The vials with the seedlings are put into a horizontal position under safe-light conditions for 20 minutes and brought back again in the vertical position. The seedlings start to bend after a latency period into the direction in which they would have grown if left horizontally. After some time bending in this direction comes to a halt, and the plants grow now in the opposite direction. Like a pendulum this oscillating movement continues for some time (see figure 5.4).

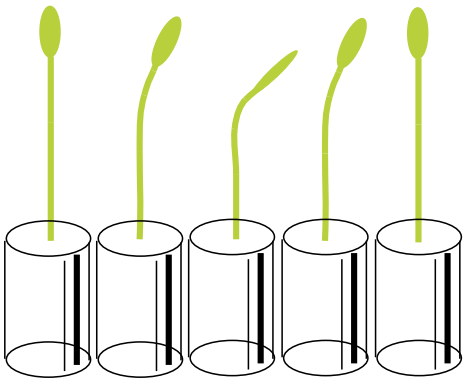


Figure 5.4: *Gravitropic pendulum movement of the dark grown hypocotyl of the morning glory. From left to right: 30, 45, 60, 70, 80 minutes after onset of 30 minute gravitropic stimulation*

[2] green fluorescence tubes Philips TL40W/17 covered with green foil no. 39 Cinemoid

5.3.4 Recording of the pendulum movement, graphical display and analysis

Images of the hypocotyl are taken with a video camera and transferred to a computer equipped with a frame grabber. The position of the hypocotyl is determined by imaging analysis. The data are graphically displayed and analyzed on the monitor screen (see figure 5.5). Function and use of the imaging system and the analysis programs are described in a handbook (Engelmann (29)). It is available at the Internet (Engelmann (28)). The period length is determined by digital filtering. It has already been described in the first part.

Besides digital filtering other procedures can be used to analyze pendulum oscillations which have also been described in the first part of the book (periodogram analysis, maximum entropy spectral analysis, page 48).

5.3.5 Proposals for experiments

- How does period length depend on environmental temperature
- How does period length depend on the length of the hypocotyl
- How does period length depend on the water conditions of the root system

5.4 Transpiration rhythm

The transpiration of grasses via stomata may occur rhythmically. Water loss of the leaves is recorded and it is studied, how the oscillation depends on the light intensity.

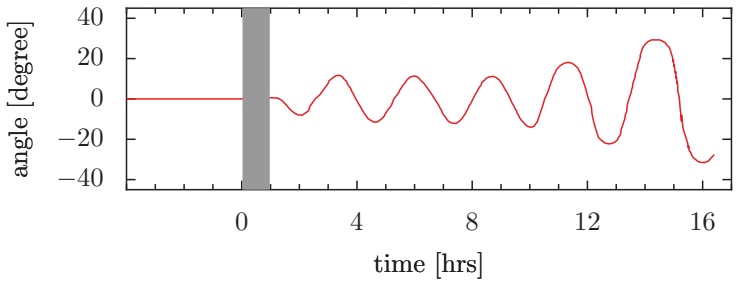


Figure 5.5: *Time course of the gravitropic pendulum movement of a hypocotyl of a morning glory after a 30 minute gravitropic stimulus*

5.4.1 Background

Land plants need CO_2 from the air for photosynthesis. However, parallel to the uptake of CO_2 water is lost, which might lead to difficulties in the water balance of the plant especially under dry conditions. Therefore during evolution special mechanisms were developed which help to solve the dilemma of a plant between hunger and thirst: A cuticula on the outer part of the epidermis cells which has a low permeability for water (and, unfortunately, at the same time for CO_2), and special cell structures, the stomata, for the controlled uptake of CO_2 and water transpiration (figure 5.6).

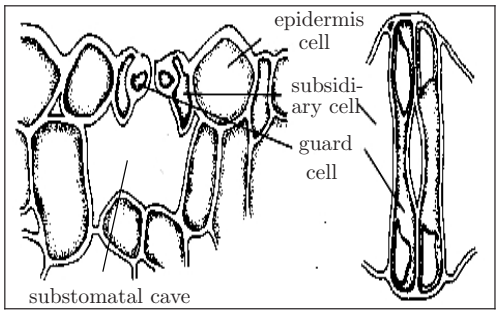


Figure 5.6: Stomatal apparatus in the leaf of an oat plant. Left: cross section, right: view from above. Next to the guard cells lie subsidiary cells, which neighbor epidermis cells. Sub-stomatal chamber as part of the intracellular system

The transpiration of plants via the stomata depends on the light conditions, the water status, the CO_2-concentration in the leaf tissue, on temperature and other conditions. The stomata open and close often rhythmically and synchronously in the entire plant, although the external conditions are kept constant. This results in oscillations of both, transpiration and water uptake in the roots (figure 5.7).

Several feedback loops control the water state. This allows oscillations to occur. Deviations from the normal water state change also the water conditions in the subsidiary cells and with some time delay in the guard cells. High

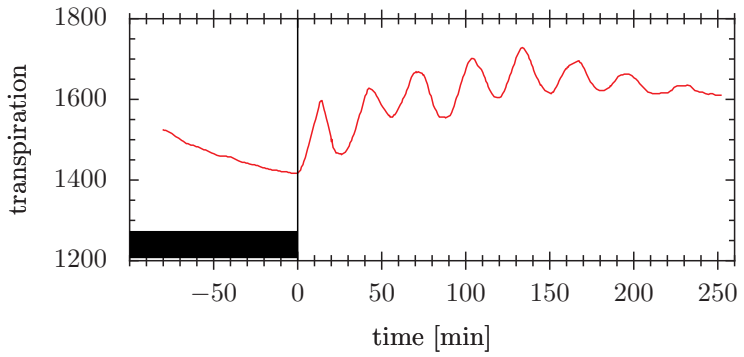


Figure 5.7: *Rhythmic transpiration in an oat leaf. Until time 0 the plant was kept in darkness. At time 0 the plant was illuminated with white light. Transpiration in relative units*

water content of the guard cells induces opening, low water content closing of the stomata (Cowan (22), Johnsson (60) and figure 5.8).

Ultradian rhythms in transpiration have been observed in grasses under certain conditions (see page 68 and Johnsson and Heathcote (61), Johnsson (60)). To record transpiration we will use a humidity sensor. It monitors the humidity of the air after passing the oat seedling.

5.4.2 Material and recording method

Rearing of oat seedlings: Oat grains are soaked over night on a sieve placed on a vial filled with water and brought to germinate. When the first leaf has ruptured the coleoptile (protecting sheath of the primary leaf) and has completely unfolded itself, a seedling is carefully removed with all its roots from the sieve. It is used for recording (figure 5.9).

5.4.2.1 Recording principle

The leaf is mounted in a recording cuvette. Dry air is pumped through the cuvette with an aquarium pump. The air will be moistened by the plant to different degrees, depending on the amount of transpiration. The air passes a tube and reaches the humidity sensor. The electrical signal of the sensor is proportional to the humidity. It is amplified and recorded with a voltage recorder (figure 5.9). Alternatively the data can be transferred directly to a computer via an A/D converter, stored and analyzed.

5.4.2.2 Cuvette

The cuvette is made of acrylglass as shown in figure 5.9. It consists of a front and rear part, an entrance and exit tube for the air, and a small slit in the lower portion where the leaf is sealed with a mold[3].

5.4.2.3 Humidity sensor

The humidity is measured with a humidity sensor (Vaisala, Finnland, see page 124). It consists of a condenser with a dielectric polymer between the condenser plates which is sensitive to humidity. Changes in humidity change the dielectric constant and therefore also the voltage between the condenser plates.

[3] for instance plasticine

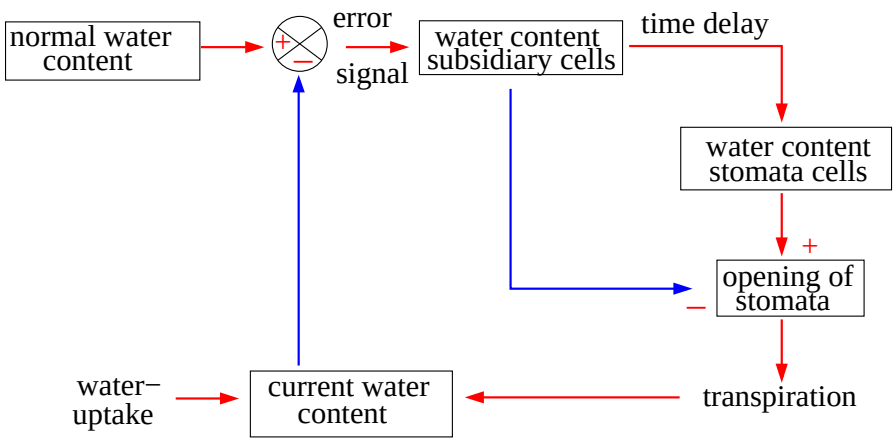


Figure 5.8: *How oscillations of transpiration are created in a leaf of oat: Scheme of the feedback loop. The current status of the water content is compared with the desired one (in the circle with + and - signs). In the case of a difference an error signal is produced, which leads to a change in the stomatal opening (closing it if the water state is low, opening it, if high). Time delays are important for oscillations to occur*

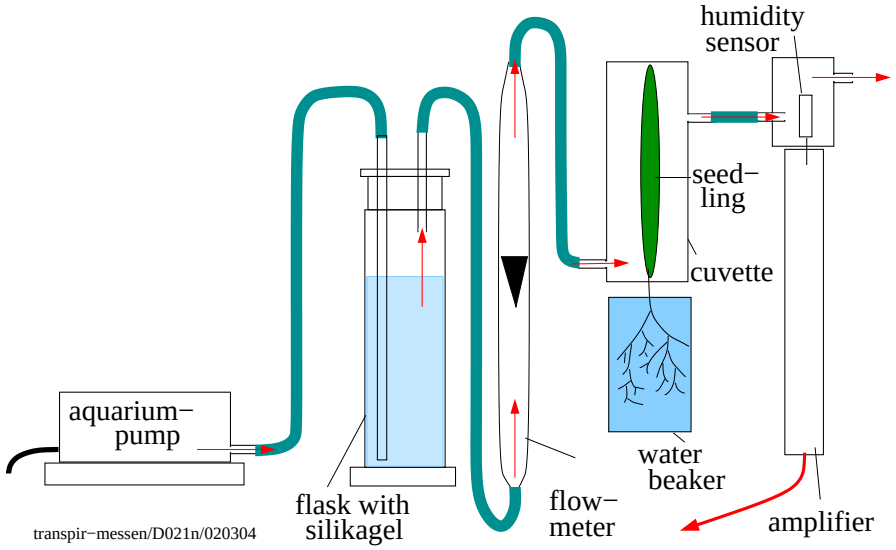


Figure 5.9: *Oat seedlings with primary leaf in cuvette, to record transpiration. Below cuvette glass vial for roots. Aquarium pump presses air through the two water bottles containing dry silica gel. Dry air enters cuvette and is moistened by the transpiration of the oat leaf. Humidity electronically measured, signal amplified and recorded (red arrow at bottom) via a voltage recorder*

5.4.3 Setup of recording system

The cuvette to record transpiration is fixed to a support. The primary leaf of the plant is carefully inserted into the slit of the cuvette which had been filled with a mold. It seals the plant stem tightly from the outer air. The front cover of the cuvette is screwed to the back part with 4 bolts (see figure 5.9). Air is pumped through the cuvette by an aquarium pump via tubes through washing bottles. They are filled with dry (blue) silica gel, which absorbs all humidity from the entering air. A flow meter just before the cuvette controls the air flow. From the cuvette the air reaches the humidity sensor via a tube. The stalk of the sensor contains a converter, which produces an electrical signal. This is amplified and recorded by a voltage recorder. The plant in the cuvette is illuminated by a slide projector. If the distance of the light source to the plant is changed and/or grey filters inserted, the light intensity can be adjusted.

5.4.4 Recording

Getting started for recording

- Mount plant and close cuvette.
- Switch on aquarium pump and control air stream with control knob or squeezer, until flow is 0.5 liter/minute

Setup of recorder

- Set transport speed of recording paper to 120mm/h
- Connect amplifier with recorder.
- Set sensitivity to 50mV[4]

[4]If the offset of the recorder is not sufficient, use counter-voltage, to reduce the signal from the amplifier. This allows to switch to a smaller voltage range

5.4.4.1 Experimental procedure

- Measure transpiration in darkness, until the recorded value has stabilized.
- Switch on projector and illuminate with 5500 lux for 12 minutes. Reduce light intensity to 2300 lux. At this level oscillations of transpiration should occur. Record with the recorder for 4 hours. Period length should be about 30 minutes at 27^0C.

5.4.5 Analysis and graphical display

In order to analyze the data by digital filtering or another time series analysis procedure, the data have to be entered by hand from the recording paper to a computer. A more convenient way is to digitize the data with an A/D converter and to transfer the data to a computer. The curve can also be scanned and converted into data with a special program (e.g. Scandata). Plot programs (e.g. Techplot) are used to display the data as a function of time (see subsection 13.1.1) and time series analysis programs to determine the period length (see chapter 3).

Alternatively, the position of the pointer of a voltage recording instrument is determined by imaging (Engelmann (29)) as a function of time.

Besides the digital filter procedure other programs are available for the analysis of the transpiration rhythm. They have been described already in the first part of the book (page 49).

5.4.6 Proposals for experiments

1. Period length as a function of the ambient temperature
2. Transpiration rhythm as a function

of the light intensity (Brogardh and Johnsson (13))

3. Transpiration rhythm in other grasses (Johnsson et al. (63))

5.5 Recording movements in *Desmodium*

The telegraph plant *Desmodium motorium* shows a fast up- and down-movement of the lateral leaflets. They are based on volume changes of motor cells in special joins. This rhythm is recorded with an imaging system.

5.5.1 Background

Most people are not aware of the fact, that quite a number of higher plants display active movements. If corn has been laid down by wind or rain, it soon begins to grow upward again. But leaves can move too, as can easily be seen in *Leguminosae*: Bean plants, peas, clover and *Robinia* move during the night the leaves or leaflets in a vertical position, during the day they are horizontal. The movement is too slow to be recognized at once. But comparing plants in the day and night position shows the difference very clearly. In some plants much faster movements are found which are more obvious. The best known example is the rapid closing of the leaflets of the sensitive plant *Mimosa*. Not less obvious, but less well known is the up- and down movement of the lateral leaflets of *Desmodium motorium*, the Indian telegraph plant.

5.5.2 Observing the lateral leaflet movement

The lateral leaflets of *Desmodium motorium* show vertical or circular movements. This plant is therefore called 'Telegraph plant' in India, because the leaflets seem to send messages between each other. Watch this movement carefully (figure 1.5). We will use these movements to practice the analysis and interpretation of data. An imaging system is used to record thes movements (see page 41). To analyze the data we use a program for digital filtering (see page 49). How such a curve looks like is shown in figure 1.6.

5.5.3 Experiment

Lithium ions slow down many circadian rhythms. We try to find out whether this applies also to the ultradian rhythm of *Desmodium motorium*. How would you carry out the experiment? Which concentration would you use, how long would you record, what kind of controls would you run?

6 Circadian rhythms in plants

Overview:
Circadian (daily) rhythms are widespread among higher plants. As an example we will study the leaflet movements of Oxalis regnellii *and the petal movements of* Kalanchoe blossfeldiana*. The movements are recorded with a video camera and a computer. Period length can be determined using special analysis programs. Other rhythmic events in plants (fragrance rhythm, acid metabolism) are mentioned.*

6.1 Introduction

One of the most obvious daily rhythms in plants was described already by Androsthenes on the march to India with Alexander the Great: The leaflets of *Tamarindus indica* are folded together during the night and spread out horizontally during the day. These nyctinastic movements are widespread not only among the *Fabaceae*, but also in other plant families such as *Oxalidaceae* and *Maranthaceae*. The movements are brought about by turgor changes in special joints called pulvini (singular: pulvinus). In other cases such as tomatoes and other *Solanaceae,* or in cotton and in *Arabidopsis*, movement is caused by alternating growth of the upper and lower part of the leaves (Haupt (49), Haupt and Feinleib (50)). In Madurai (South India) 62 plants of a small botanical garden have been observed to show leaf movements (Chandrashekaran, unpublished).

There are several hypotheses trying to explain the significance of these nyctinastic leaf movements. According to Darwin (23) the heat loss to the surrounding is reduced in cool nights, if the leaves are in a vertical rather than a horizontal position (see also Enright (36)). According to Bünning and Moser (19) the vertical position reduces the amount of moon light absorbed. This prevents the plant from reacting photoperiodically at the wrong time of the year (for the significance of photoperiodism see page 101). According to Karve et al. (64) the *Fabaceae* are able to grow very close together because of their nitrogen fixation capability. However, the resulting dense canopy of the leaves would not allow sufficient red light to reach the axles of the leaves in the evening and morning. This is, however, necessary for the induction of flowers. The vertical position of the leaves in the morning and evening would solve this problem.

Some flowers show also daily movements, such as the Crassulacean plant *Kalanchoe blossfeldiana* from Madagaskar (figure 6.1). Recording this movement and experimenting with it are described in this chapter. Other flowers show a conspicuous fragrance rhythm, such as *Cestrum nocturnum* (strongest odor during the night, Altenburger and Matile (2), Matile and Altenburger (80), Overland (89)) or the Persian Violet *Exacum affine* (strongest odor in the early afternoon, figure 1.2).

The metabolism of plants is also often controlled by a circadian clock. The CAM (Crassulacean Acid Metabolism) in *Crassulacea* acidifies the cell vacuoles during the

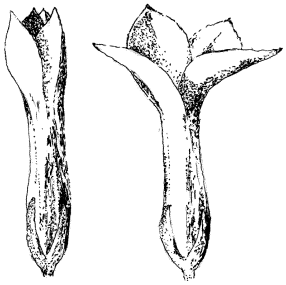


Figure 6.1: Kalanchoe-*flowers in a closed (left) and in an open state (right)*

night and alkalizes it during the day (figure 6.2, Kluge and Ting (67)).

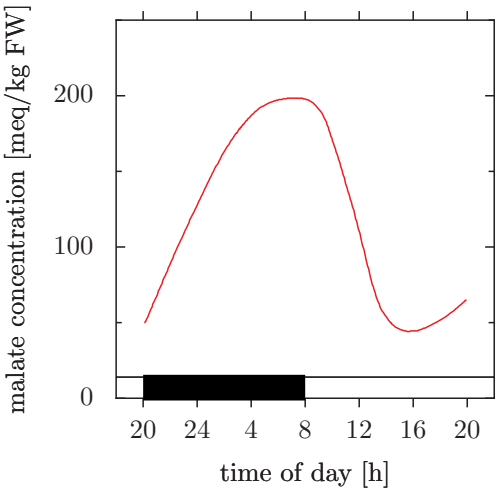


Figure 6.2: *pH rhythm of the cell sap of a CAM plant: acidification of the cell vacuoles during the night and alkalisation during the day (malate concentration plotted against time of day)*

Under constant conditions these rhythms continue. However, the rhythm is now not any longer exactly 24 hours, but circa 24 hours, in the case of *Kalanchoe* for instance 22 hours. This is a clear indication, that these periodic events are controlled by a circadian clock. Such movements are easily observable and we will use an imaging system again for automatic recording (figure 2.1).

These rhythms can be influenced by light-, temperature-, and chemical pulses. Results of such experiments might help to understand the underlying mechanism.

6.2 Leaf movement of *Oxalis regnellii*

To demonstrate circadian leaf movements we use the leaves of the south American wood sorrel *Oxalis regnellii*. The three-parted leaflets are folded together during the night and are downward directed. During the day they are spread out horizontally (figure 6.3).

6.2.1 Rearing the plants

The plants are kept in many botanical gardens and easy to rear. The vegetative propagation from rhizom bulbs is the easiest. Put a bulb in a broad flower pot containing peat rich soil and keep well watered. The light intensity should not be too high. In case of pathogenic symptoms it helps to cut off all leaves. The plants start soon to develop new leaves.

6.2.2 Preparation for recording

The leaves are cut off the plant and the petiole put in a plastic straw which is mounted in the center of a polyurethan disk floating on water in a cuvette (figure 6.3).

With a video camera the cuvettes are recorded from above. The camera is connected via a frame-grabber to a computer. With imaging software as described before (page 41) the movements can be recorded and analyzed. The setup for the recording is shown in figure 6.4. For illumination green fluorescence tubes (Philips TL

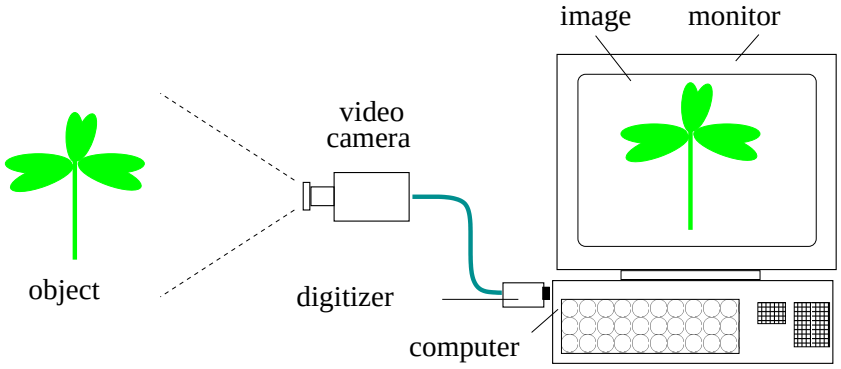


Figure 6.4: *Recording of* Oxalis-*leaf movement with video-digitizing and a computer*

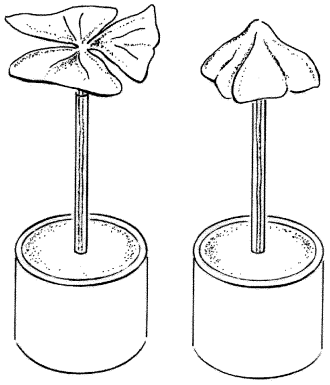


Figure 6.3: *Cuvette with* Oxalis *leaf in a plastic straw, which is mounted in a polyurethan disk. Left day-, right night position*

20W/15) are used. The optimal light intensity is $1.4\,Wm^{-2}$.

6.2.3 Analysis

The data are displayed as a time series with a plot program. Period length is determined by digital filtering or other analysis methods (page 48).

6.2.4 Experiments

A characteristic of circadian rhythms is that the periodic changes continue even under constant conditions. We record such a 'free-run (see next section). To understand the anatomical basis of leaf movements we make cross-sections through the pulvini and study them microscopically.

6.2.4.1 Free-run

The leaves are cut at the end of a light-dark-cycle and mounted in cuvettes. They are then transferred into a recording chamber and exposed to continuous green light. Recording with an imaging system is started and run for 7 to 10 days. Every 20 minutes an image is taken.

The period lengths are determined by using adequate programs. Under continuous

light the period length should not be exactly 24 hours anymore as was the case in a light-dark-cycle, but deviate from it. This proofs the circadian nature of this oscillation.

6.2.4.2 Anatomy of the pulvinus

Cross sections through a pulvinus shows the structure and position of the motor cells (cells with large volumes) between the epidermis and the central core of phloem, xylem and strengthening tissue. In the leaf stalks, on the other hand, the conducting vessels and strengthening tissue are more at the periphery. Such a structure would not allow movement (figure 6.5).

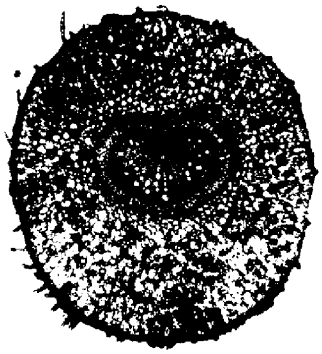


Figure 6.5: *Cross section through the pulvinus of* Oxalis regnellii *with conducting vessels and strengthening tissue in central position and motor cells peripheric to it. Outer cell layer is epidermis with hairs*

6.2.4.3 Leaf movements in other plants

You could study other plants with rhythmic leaf movements.

Proposals: Check different species of clover, bean, wood sorrel, *Maranthaceae*.

6.3 Flower clock *Kalanchoe*

The petals of the flowers of the Crassulacea *Kalanchoe blossfeldiana* exhibit a diurnal movement. They are open during the day and closed during the night (figure 6.1).

This rhythmic movement continues even during constant conditions of temperature and continuous weak green light. The time span from one maximal opening to the next is now, however, only 22 hours and not 24 hours. This movement is the result of a volume change of the upper epidermis and mesophyll cells (figure 6.6), which in itself is caused by changes in the osmotic value of the cell vacuoles (figure 6.7).

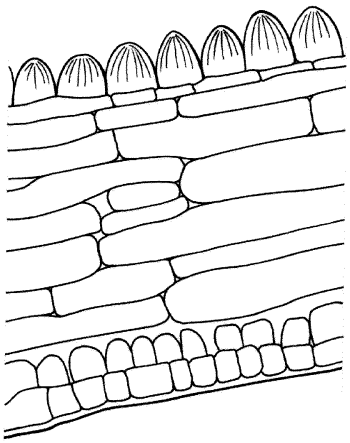


Figure 6.6: *Microscopic picture of a cross-section through a* Kalanchoe *petal with upper (papilla like) and lower epidermis and inter-spaced mesophyll cells*

6.3.1 Material and methods

Plants are induced to flower by short day treatment (11 hours light, 13 hours darkness per day) for some months. After the flowers are formed and have started to open and close daily, they are transfered into a 12:12 hour light-dark-cycle (end of light for

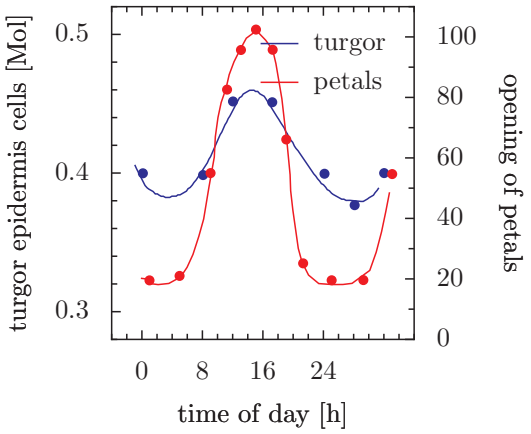


Figure 6.7: *Diurnal changes of the osmotic pressure of the upper epidermis cells (blue) and petal movement (red) of* Kalanchoe

instance 10:00 in the morning) at, for instance, 22^0C. We record with an imaging system (page 41). The flowers are broken off with a pair of tweezers from the plant shortly before the end of the light period (in our case 10:00 o'clock) and mounted in a polyurethan plate (figure 6.8). The plate floats on top of a 0.2 molar sucrose solution in a rectangular plastic dish[1].

The dishes with the flowers are illuminated from above by two green fluorescence tubes (Philips TL 40W/15)[2]and two grey foils (Cinemoid Foil No. 60). The light intensity at the level of the petals should be about 35 lux, because at higher intensities the rhythm of the petals is damped.

6.3.2 Recording and analysis

The imaging program is started on the computer. It is sufficient to sample images every hour for 7 days. Add daily some distilled water to the cuvettes to replace the lost water.

To analyze the results a time series analysis program such as digital filtering is used. It has been described before (page 48). It filters the sampled data digitally and determines the period lengths of the petal movement rhythm of each individual flower (figure 6.9).

Calculate the mean of the periods of the individual flowers of the different groups and plot the results graphically.

6.3.3 Experiments

Three experiments are proposed: In the first two the effect of Li^+-ions and of TEA (tetraethylammonium chloride, a potassium channel blocker) on the period length of the petal movement rhythm is studied. In the third experiment the turgor changes in the motor cells of the petals are determined. Turgor changes are the basis of the petal movement.

6.3.3.1 Effect of Li^+ on *Kalanchoe* clock

Kalanchoe-flowers can be used to study the effect of substances on the circadian rhythm. In this way the underlying mechanism might be better understood. A 5mM Li^+-solution for instance slows the clock by about 2 hours. Use 1 and 5 mM and a control, which contains only sugar solution. Li^+ might work via the phosphoinositol-cycle.

Perform the experiment and evaluate it. Write a report of the results by using your protocol (see page 32).

[1] Fa. Stereo Optik, Mainstr. 13, D63128 DIETZENBACH, Tel. 06074 27222

[2] the tubes might additionally be covered with a green colored foil (Cinemoid Foil No. 39, Strand Electric).

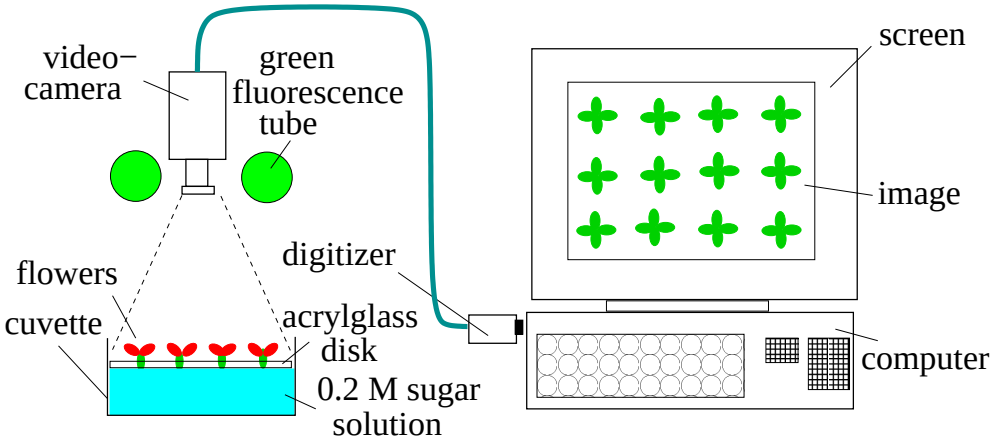


Figure 6.8: *Cuvette with* Kalanchoe *flowers. Acrylglass disk with holes for the flower stalks, 0.2 M sugar solution in cuvette. Illumination with two green fluorescence tubes and additional green foil. Recording with video camera and digitizing of the signal with a frame-grabber. Images are stored on a hard disk of a computer. Flowers are shown on the monitor*

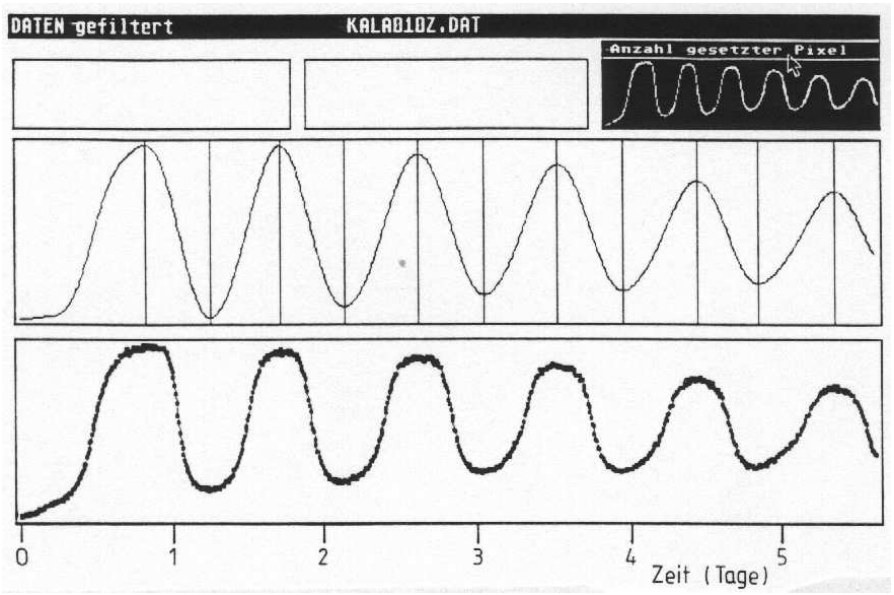


Figure 6.9: *Original curve and digitally filtered curve of* Kalanchoe *petal movement*

6.3.3.2 Effect of the potassium channel blocker tetraethylammoniumchlorid on the petal movement of *Kalanchoe*

During the leaf movement transport of ions through cell membranes plays an important role and K^+-channels are participating. Tetraethylammoniumchlorid (TEA) inhibits K^+-channels and can therefore be used to check, whether such channels are indeed parts of the rhythmic petal movement of *Kalanchoe*.

Use a control group (first cuvette) and three experimental groups with 0.5, 1 and 5 millimolar concentrations of TEA in 0.2 molar sucrose solution. Record for 7 days and analyze the data as described already. Plot the period lengths against the different concentrations used. Compare also other characteristics of the rhythmic movement such as damping and the opening width at the end of the experiment. What are your conclusions about the possible effect of TEA?

Read the article of Satter et al. (98), Ruge and Hampp (97), Mayer and Hampp (81) on the basics and hypotheses of sleep movements in plants.

6.3.3.3 Turgor changes as the basis of the petal movement of *Kalanchoe*

The red colored upper epidermis of petals is peeled off with a fine pair of tweezers. It is transferred into a mannitol solution (0.3 to 0.5 molar) on a microscope slide and covered with a cover slip. After 2 to 5 minutes the number of plasmolyzed cells is determined under the microscope. This is repeated every 3 hours with epidermis pieces of other flowers. To avoid night work you can keep some of the plants 1 to 2 weeks before under an inverted 12:12 hour light-dark cycle. The 12 hour light period is during the night and darkness prevails during the day. In this way epidermis pieces of the normal and inverted groups which are observed for measurements are at each time physiologically 12 hours apart.

6.3.3.4 Further proposals for experiments

Quite a number of plants show a fragrance rhythm of their flowers (Altenburger and Matile (2), Matile and Altenburger (80), Overland (89)). This is often an adaptation to the pollination by insects. Study different plants which are in blossom and try to find out how one can check for a rhythmic sensitivity of the human nose which would pretend a rhythm in fragrance although there is none.

7 Clocks in cells

Overview:
Circadian rhythms are not only found in multicellular organisms, but also in unicellulars and in cells of tissue and organs. Circadian rhythms have been described lately even in prokaryots (in Cyanobacteria*). As an example for rhythms in unicellulars the somewhat special case of* Thalassomyxa australis *is presented. Here, in contrast to the normal circadian rhythms, the period length depends on temperature and synchronization by zeitgeber is unusual.*

7.1 The ancient clock of *Thalassomyxa australis*

The marine naked amoeba *Thalassomyxa australis* was detected by Grell in 1983 at the west coast of Australia (Grell (46)). It changes its form between a pancake like, inactive phase and a moving phase, in which a network of plasmodial extrusions are formed, by which the animal moves around and takes up food (see movie Grell (45)). The change between the inactive phase ρ and the active phase α occurs rhythmically and the period length depends, in contrast to normal circadian rhythms, on the temperature (Silyn-Roberts et al. (106)). At temperatures of 20^0C the animals spend about 2/3 of the time in the resting phase and 1/3 in the active phase. The period length is about 28 hours. As food organisms unicellular marine algae can be used. *Amphiprora*, the green alga *Dunaliella* and a marine *Chlorella* species are useful.

7.1.1 Phenomenon

Look at the movie 'The change in form of *Thalassomyxa australis*'. At the end of the movie a scene is shown in which concentric bands are formed. Play back this part and look at it again. Try to understand how these rings are formed and write down your ideas. How could one determine the time between two neighboring rings of deposition? Do you know other rhythmic events which manifest them self in a similar way? Comment on it.

7.1.2 Rearing, methods of observing

Thalassomyxa and food organisms are kept at the Department of Zoology at the University of Tübingen. The amoebae are reared in seawater at 20^0C and under weak light in Petri dishes. Diatoms (for instance *Amphiprora*) serve as food. Usually the diatoms divide fast enough and only rarely they have to be added to the culture. The cultures can, however, die out after some time. Renew the seawater in time or transfer the amoeba to new dishes by putting microscope slides in the dishes. Once the amoebae have settled to the new slides, they can be transferred to new dishes.

The animals can be observed under the microscope at $100*$ magnification. The inactive phase is readily distinguished from the active phase. If the dishes are checked every 2 to 3 hours, the changes in form can be recorded as a function of time (figure 7.2). With an imaging system (Engelmann (28), Engelmann (29)) the process can be

recorded and reproduced as a time lapse movie.

7.1.3 Proposals for experiments

Four experiments are proposed. They are described in the following. Use the hints that are given earlier in this book on how to plan experiments and to write protocols for experiments.

7.1.3.1 Study 1: Synchronization of *Thalassomyxa australis*

To synchronize biological clocks with the environment, 'Zeitgeber' (time giver, time cues) are used. The most important Zeitgeber to synchronize circadian rhythms is the daily 24 hour change of light and dark. How would you try to find out whether the change in form in *Thalassomyxa australis* is synchronized by the light-dark-cycle? Do the experiment, evaluate the data and write a report (see page 32). Which kind of other Zeitgeber could synchronize *Thalassomyxa australis*? How would you test your hypotheses?

7.1.3.2 Study 2: Dependency of change in form from temperature in *Thalassomyxa australis*

Circadian rhythms as well as common clocks run at different temperatures of the environment at the same speed. Organisms in very constant environmental conditions such as some parts of the tropics or the sea with uniform water temperatures do not need to have temperature independent clocks. However, even under these circumstances the dependency of period length from temperature is low, although not quite as low as in organisms from higher latitudes of the earth with more pronounced daily and annual changes in temperature. Ultradian rhythms (period length in the minute- and hour-range), on the other hand, are usually strongly dependent on temperature (see the chapter *Ultradian rhythms*, page 59ff.). There are, however, also temperature compensated ultradian rhythms known. How would you test whether the change of form in *Thalassomyxa australis* is, as other circadian rhythms, independent of the environmental temperature? Plan an experiment, execute it and analyze the results. Write a report (see page 32).

7.1.3.3 Study 3: Synchronous culture of *Thalassomyxa australis*

If you want to study the biochemical and physiological processes during the change in form of *Thalassomyxa australis*, a method is needed to synchronize amoebae mutually. The first study has shown, that a light-dark-cycle does not synchronize the culture. There is, however, a very simple method to produce a synchronous culture. It is done by selecting amoebae in the same phase, which then stay in phase for many days because their period length is very similar (or because they mutually synchronize each other?[1]). The following method is recommend: Pour seawater out of a densely populated dish, drop a jet of seawater out of a pipette from 4 cm heights onto the amoebae on the bottom of the dish by spiraling the pipette over the whole dish. Shake vividly and pour all amoebae out which have lost contact to the surface of the dish (figure 7.1). The animals which were washed off are the once in active phase. The animals in the resting phase are not detached from the dish by this treatment. Control under the microscope whether indeed all animals were washed off and repeat

[1] see study 4.

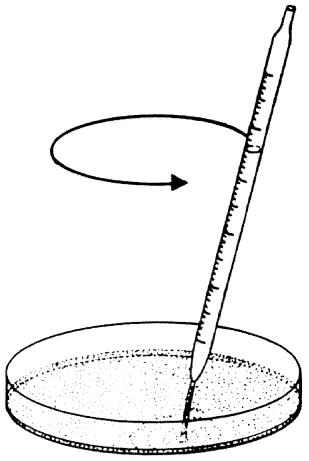


Figure 7.1: *Production of synchronized cultures out of a population of* Thalassomyxa australis *by selection of same phase stage. To wash away the active amoebae a jet of seawater is used. Animals in the resting stage stick to the dish*

this procedure, if necessary. Add seawater to the dish, wait some hours and repeat the treatment. The amoebae collected then are all in a very similar phase, since they have gone from the resting phase into the active phase almost at the same time. They can be used as a synchronous culture.

7.1.3.4 Study 4: Do *Thalassomyxa australis* mutually influence each other in their rhythmic change of form?

If the collection of amoebae of the same phase is repeated after 12 hours, one can use the two cultures to test whether the cells are mutually influencing each other. Plan an experiment and the necessary evaluation, conduct it and write a report (see page 32).

One can run also a **computer model** which simulates such a mixing experiment of amoebae in different phases with different numbers of amoebae at the begin. It is assumed that there is *no* mutual influence. An advantage of such a simulation is to get in a short time results of such different 'experiments'. One can then look for those results which allow a clear decision. For instance, the optimal phase of the two cultures to be mixed and the number of cells in each can be found in this way. Intuitively one would probably mix a culture with a second one which is phase shifted by 180^0 and would expect a new oscillation which results from the superposition of the oscillations of the two original cultures, and one would probably take equal amounts of amoebae from both cultures. If there is, however, a mutual influence of the two cultures, this combination is not recommendable, because the influence of culture 1 on 2 is the same as vice versa. It would be better to use a smaller number in e.g. culture 2 and to use a phase which differs from the phase of culture one by 90^0, because then culture 2 would after some time lock to culture 1.

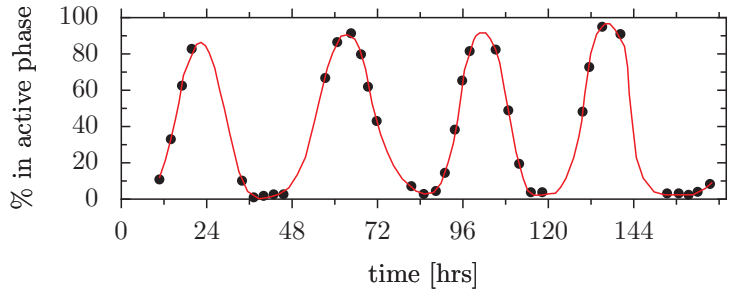


Figure 7.2: *Percentage of* Thalassomyxa *amoebae in active phase as a function of time. Vertical bars label days*

8 Circadian rhythms in animals and man

Overview:
*Locomotor activity rhythms are are wide spread among animals. As examples house flies, fruit-flies (*Drosophila*), and Syrian hamsters will be described here and recording methods presented.*

The eclosion rhythm of fruit-flies out of the pupal case serves as an example for a rhythm observable only in a population of animals.

Finally we will get to know some circadian rhythms of man and their significance for our life.

8.1 Locomotor activity rhythms of animals and their analysis

The behavior of most animals is controlled in a diurnal way. Thus, flies are active during the day and rest at night, whereas it is the other way round in Syrian hamsters. *Drosophila pseudoobscura* flies eclose mainly in the early morning hours out of their pupal case. In the late morning only few emerge and in the evening and night none. Those rhythms are not only widely found, but also easily recorded. We will use again an imaging system for recording (see page 41).

First we will mention briefly the background of circadian activity rhythms. Afterward we describe how to get or rear the animals needed and how to maintain them.

Secondly we describe, how the different activities are recorded and analyzed. We will use the methods described already in the first part of the book (imaging system and light beam method).

On page 124 the construction of an air condition box is described. It can be used to keep temperature and illumination constant.

Finally different experiments are proposed. We refer to a handbook in which the recording methods are described in detail and for which programs and data are available which help to understand the procedures better (Engelmann (28)). With this book comes also a collection of activity data which can help to learn the analysis and the understanding of actograms.

8.1.1 Background

Locomotor activity of many animals is adapted to the light dark cycle. Night active animals rest during the day, day active animals during the night. Examples for the former are cockroaches, owls and mice, examples for the latter flies, many birds, lizards (figure 8.1).

This daily change in activity and rest is continued in a room with continuous light and constant temperature and food and water available ad libidum. It looks like the animals are still able to notice the change between day and night. This could be explained in two ways. Either one or more environmental factors are still synchronizing the animals which we haven't been able to eliminate, or the animals use an internal clock which tells when to be active or when to rest. It is easy to decide between

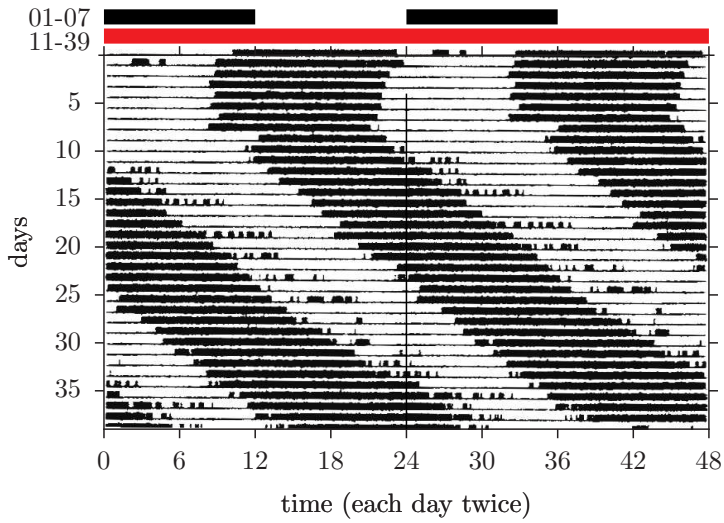


Figure 8.2: *Example of an actogram (kind of black box) of a housefly, the locomotor activity of which was recorded in the first 7 days in a 12:12 hour light-dark cycle and then 30 days under constant conditions at weak red light in* 22 *°C. Activities of subsequent days plotted below each other, time of day at x axis (scale from 0 to 24 o'clock); each day plotted twice. Data were obtained with the described imaging method and displayed with a plot program. A straight line connecting the onsets of activity can be used to determine the period length*

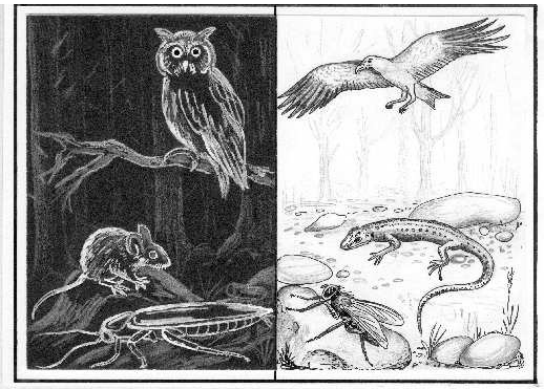


Figure 8.1: *Examples for day- and night active animals (left: cockroach, owl, mouse) and day active animals (right: fly, bird, lizard). Drawn by C. Förster*

both possibilities. In the case of synchronization by an external (unknown) factor the rhythm of the animal should continue to be exactly 24 hours, whereas an internal clock is unlikely to run with exactly 24 hours. The observation of our animals shows: whereas in a 24 hour day with light dark cycles the animals became active or tired about the same time of the day, they started to be active under conditions without time cues each day somewhat earlier or later, depending on the speed of their internal clock. From this we conclude, that the internal day of an animal is *circa* 24 hours (*'circadian'* from Latin *circa*, about, and *dies*, day), although very precise for each individual.

In figure 8.2 the locomotor activity of a housefly is shown. The animal was for 7 days in a light dark cycle and for another 30 days under constant conditions of weak red light and an environmental temperature of 22^0 throughout. This figure 8.2 is a so called actogram, a kind of black box of the fly (as used in trucks and air planes to trace the 'activity' of the car or plain, respectively): Activity is represented by vertical dashes, rest by a horizontal line. Time of day is shown on the vertical axis (from midnight to midnight; however, for better visibility the actogram is shown twice next to the midnight-midnight plot: a so called double plot), sequential days below. During the time of light dark cycles the animal became active with onset of light and stopped activity with the begin of darkness. When the change of light and darkness was discontinued, periods of high activity were still alternating with periods of rest. However, activity (and rest) began each day about 35 minutes later and stopped 35 minutes later. The day of this particular fly was therefore 24 hours and 35 minutes.

8.1.2 Animals, rearing, keeping

Musca domestica can easily be reared. However, since we need only a few flies and since they are usually found throughout the year in buildings, we will give a reference only for rearing (West (114)). The identification of the species should not be difficult (figure 8.3).

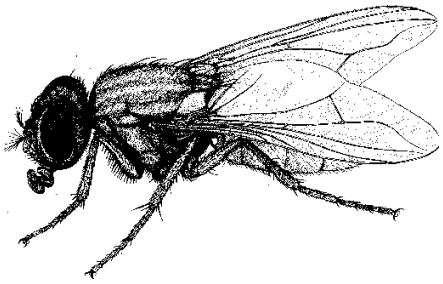


Figure 8.3: *Housefly* Musca domestica. *Drawn by C. Helfrich-Förster*

Drosophila melanogaster can be obtained from genetics departments or purchased. During the summer and fall months they can easily be caught on fermenting fruits. Cultures can be reared on food which is

prepared in the following way:
Measure

- 850 ml tap water
- 100 g sirup without sulfur
- 15 g Isabgol [1] (or 10 g agar)
- 100 g maize- or wheat-Simula
- 8 g yeast flakes

in the given order and stir when pouring the items into the warm water to prevent clumping. Boil for 5 minutes under constant stirring and add

- 5 ml Nipagin (=4-hydroxi-benzoic acid-methylester, 2.5 g in 50 ml 96% ethanol)
- 1.5 ml propionic acid

poor while still hot in glass or plastic vials, close with foam or cellulose stoppers and wait until cool. This food can be kept in the refrigerator for some weeks. A little bit of dry yeast should be added before flies are added (West (114)). Adult animals are transferred via a vial with the same diameter of the opening. If the walls of the vial with food are wet, they should be dried with filter paper and/or a piece of filter paper should be put on the surface of the food before introducing the animals[2]. Mark the vials (species, mutation, date) and rear the next generation in a 12:12 h light dark cycle at about 25^0C. Larvae eclose from the eggs and feed on yeast growing in the food. Pupation occurs about 14 days later on the wall of the vials or on dry parts of the food.

[1] a substitute of agar from Indian plantain-seed pots

[2] to protect the flies from getting stuck to the water

Syrian hamsters can be purchased in zoo shops. There one gets also hamster cages which are already equipped with running wheels. Special hamster food can be bought, but corn or kitchen garbage are also sufficient.

8.1.3 Recording of locomotor activity of animals

Locomotor activity of animals can be recorded by different methods. Running wheels, tilting cages and photo-electric methods, in which the animal lives in a small container and interrupts a light beam when walking around, are examples. Here we use an imaging procedure for the recording of the locomotor activity of houseflies. This system has been mentioned already in the first part of this book (section 2.1). The program determines the position of the animal in the cage. If it has not changed after a certain interval it is regarded to be inactive during this time. This is repeated for instance every 4 minutes and continued for several days. The results can be displayed on screen as an 'actogram' and observed during recording. Additionally the data are stored in a file for later analysis.

For the recording of the locomotor activity of *Drosophila*-flies we use the system described in the first part of the book (page 42).

8.1.3.1 Set up and start of the recording system

With the locomotor activity of houseflies as an example the setup of the recording system will be demonstrated (figure 8.4).

A hole (5mm diameter) is drilled in the bottom of a Petri dish of 10 cm diameter, through which a fly, head first, can be transferred into the dish. A wick made of a

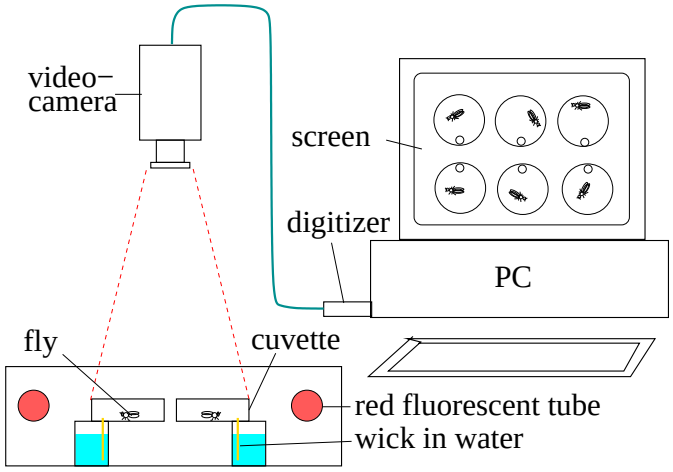


Figure 8.4: *Recording system for locomotor activity of insects using video camera, frame grabber (digitizer), and a computer. Petri dishes with flies are placed in an air conditioned box. Illumination with red light from the side*

strip of a kitchen cloth is inserted into the hole in such a way that it sucks water from a container and allows the fly to drink. A lump of sugar is fixed with plasticine to the Petri dish and serves as food. Either several such cages are placed on a diffusing glass plate on top of red homogeneous light[3] or, alternatively, the red light comes from the side as shown in figure 8.5. The whole system is placed in the air conditioned box described already (page 124). The video camera is mounted on top of the air conditioned box and looks upon the dishes with flies. A cable connects the camera with the frame-grabber, which is inserted into a slot of the computer. For details see (Engelmann (28), Engelmann (29)).

For the time span between the single pictures (sampling rate) we could choose for instance 4 minutes, for the recording time 7 days.

[3] red fluorescence tube with red Cinemoid foil no. 6, primary red

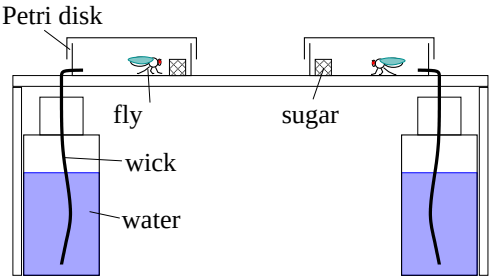


Figure 8.5: *Petri dishes for housing house flies. Wick for water supply and sugar as food*

8.1.3.2 Analysis

With an analysis program described in part 1 the activity data obtained with the setup are displayed as an actogram (figure 8.2) and the period length can be determined (page 49).

8.1.4 Examples and proposals for experiments

Experiments to study locomotor activity rhythms of house flies, *Drosophila* and Syr-

ian hamsters are proposed in the following.

8.1.4.1 Activity rhythm of house flies under constant conditions

Houseflies are individually kept under 12:12 h light-dark-cycles for 7 to 10 days in the containers described before. They are then recorded for 7 to 10 days in red safe light. A change between activity and rest is still found under these constant conditions. However, the period length is not 24 hours any more as was the case in the 12:12 hour light-dark-cycles, but either somewhat shorter or longer (figure 8.2, Helfrich et al. (51)).

A further experiment would be the demonstration of the temperature compensation of this free run rhythm. Independent of the (constant) environmental temperature (use between 18 and 35^0C) the period length is almost the same.

8.1.4.2 Locomotor activity rhythm of mutants of *Drosophila melanogaster*

A number of mutants of *Drosophila melanogaster* are available with different period lengths. The best studied one are the *per*-mutants. This mutation affects eclosion rhythm and locomotor activity rhythm. per^s has a shorter, per^l a longer, and per^0 no rhythm. As an experiment it is proposed to compare the period lengths of these mutants with the one of the wild type. Since the *Drosophila*-flies are smaller than houseflies, the imaging system might pose problems. More suited is a light beam recording system which was especially developed for it. It was already described in the first part of the book. The recording cages are also different. We use plastic cuvettes as used for spectral photometers. Figure 8.6 shows the construction of a recording unit for flies. Individual flies are transferred into cuvettes containing a small piece of sugar and a wick for water supply. They are first recorded for a few days in a 12:12 hour light-dark cycle at constant temperature (for instance 22^0C). Afterward the recording of activity is continued under weak red light. The activity pattern and the period length of the individual animals is determined and the mean value calculated.

8.1.4.3 Locomotor activity of Syrian hamsters

Male Syrian hamster are kept individually in cages[4]. Water is supplied from bottles with a drink nipple, hamster pellets[5] serve as food.

For recording, the imaging system can be used again. If the hamster is recognized while in the vicinity of the drink nipple, the food container and in the running wheel, drinking, feeding and running wheel turning can be detected separately (see Engelmann (29)).

As an experiment the dependency of the circadian rhythm from the intensity of the (weak!) continuous light can be demonstrated. The period length and the expression of the rhythm are determined for the different light intensities (for example 0.1, 1, 10 lux).

8.2 Population rhythms

We record the eclosion rhythm of *Drosophila pseudoobscura* flies with an imaging system. The rhythms are

[4] In females the circadian rhythm is less well expressed because it is superimposed by the sexual cycle

[5] for instance from Altromin company

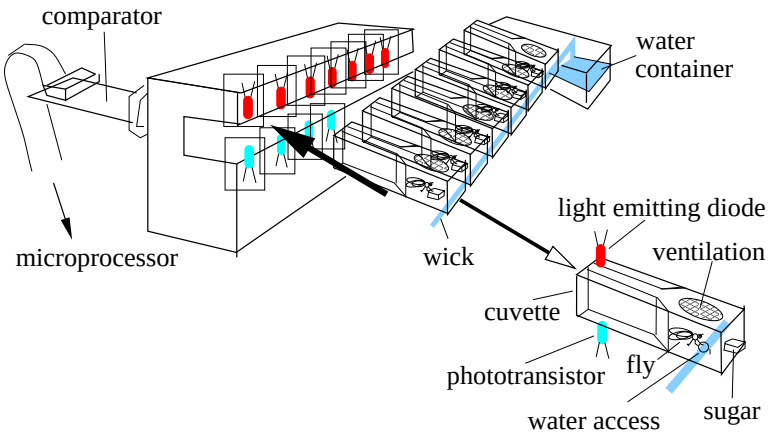


Figure 8.6: *Recording of the activity of* Drosophila *in cuvettes with infrared-light beam and computer system (After Förster)*

analyzed by digital filtering. Proposals for experiments with *Drosophila* and mutants are made.

8.2.1 Introduction

With the imaging system we can not only record the activity of flies, but also the eclosion rhythm of *Drosophila* flies. This is a population rhythm, which can be observed only in a large number of animals (Winfree (120)): The eclosion of a fly out of the puparium (pupal case) happens for the individual fly only once. That this event is also controlled by a circadian clock is shown by the behavior of the population only: Eclosion is not evenly distributed over the day, but occurs during a special time window during the day. In a 12:12 hour light-dark cycle *Drosophila pseudoobscura* flies eclose a few hours after onset of the light period. In a subsequent continuous dark period (or continuous red light as physiological safe light) this eclosion rhythm continues.

8.2.2 Animals, rearing, maintaining

Drosophila pseudoobscura can be obtained from genetics departments or from *Drosophila* stock centers. This species lives in dry areas in the southern part of the United States. *Drosophila pseudoobscura* is reared in the same way as *Drosophila melanogaster* (page 86). The development takes somewhat longer (at 20^0C 21 days).

8.2.3 Recording of eclosion rhythm

The pupae are inserted individually in holes with 3 mm diameter in a rectangular metal plate using a special pair of tweezers (figure 8.7). A delicate white nylon net on the lower side of the plate prevents the pupae from falling through. A sooted slide (50∗50 mm) is put on top of the metal plate[6]. Allow toxic substances to disappear from the sooted glass plate by waiting for a night.

Underneath the metal plates is a red safelight[7]. As soon as the flies eclose from the

[6] soot with a candle. Cheap candles make more soot and are in this case to be preferred.

[7] Philips TL20W/15 with three layers of red Cinemoid foil no. 6 and one layer of yellow foil no.

puparium they try to free them self. In doing so they scrape off the soot on top of the hole. They die quickly and dessicate. Red light can now pass through the hole (figure 8.9). A video-camera is mounted on top of an air conditioned box. The lens is inserted in a hole and sees the metal plates with the pupae in the box on top of the red light (figure 8.8). The increase of the bright spots corresponds to the course of eclosion. The number of illuminated holes (eclosed flies) is recorded hourly with the imaging system described before. The data are stored on the computer (figure 8.10). If we record $3 * 3 = 9$ metal plates (with $10 * 10 = 100$ holes containing pupae), we obtain for 9 groups of 100 flies each the course of eclosion in step-like curves. They can be shown as diagrams during recording on the screen.

The setup of the system is described in the first part of this book and in a handbook (Engelmann and Schuster (35)).

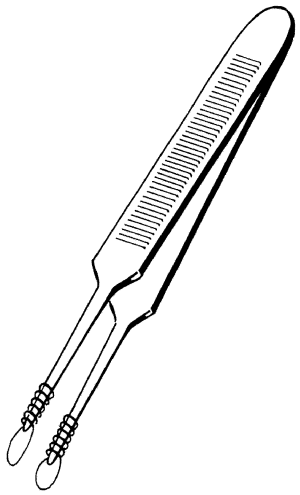


Figure 8.7: Tweezers, around the ends of which two wire loops are fastened. They facilitate the handling of *Drosophila* pupae.

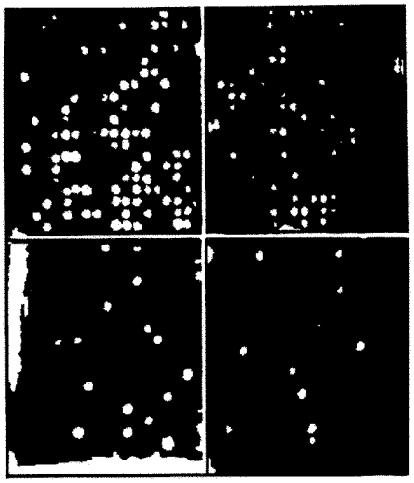


Figure 8.9: *Soot recording method for eclosion rhythm of* Drosophila pseudoobscura*-flies out of the puparium. Pupae are individually placed into the 100 holes of the metal plates, as described in the text, and a sooted glass plate put on top. During eclosion the flies scrape off the soot and light falls now through these spots and is recorded by the video camera*

8.3 Circadian rhythms in humans

Here are instructions to measure the diurnal rhythm of activity and body temperature of man and to find a possible correlation with the chronobiological type (morning-, indifference-, evening type).

8.3.1 Introduction

On an average we sleep for about 8 h per day and stay awake for 16 h. This alternation of activity and rest is caused by an oscillator which functions even in the absence of external Zeitgeber such as light-dark cycles or social contacts (Aschoff (7), Aschoff (5), Schulz and Lund (101)).

5a.

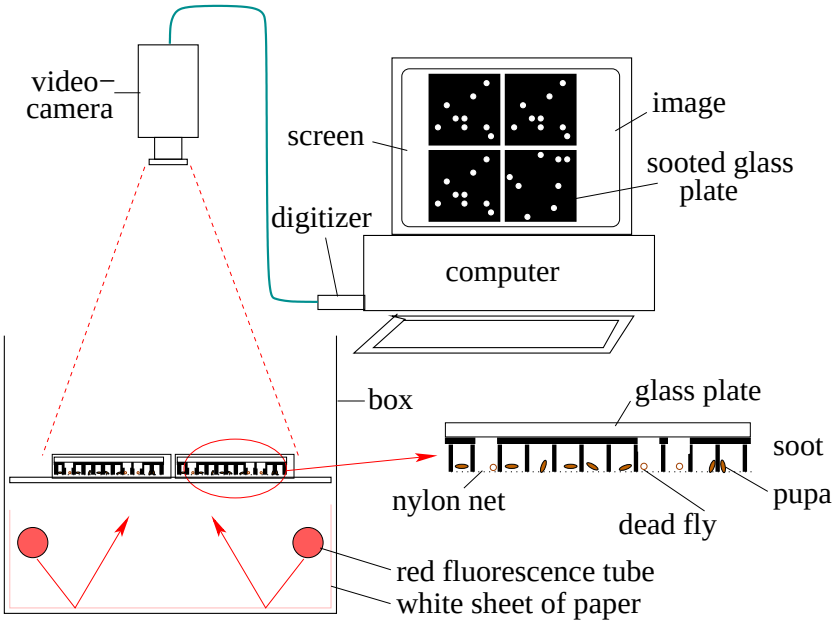


Figure 8.8: *System for recording eclosion of* Drosophila *flies out of the puparium with video camera, frame-grabber and computer using a soot method. Light of two red fluorescence tubes is reflected from white sheet of paper to stacks of holders for pupae shown in more detail at right lower part of figure: A metal plate with 10*10 holes, each holding a pupa. A white fine-mashed nylon net keeps pupae on place. Eclosing flies wipe of the soot underneath the glass plate on top of the stack. Red light can pass these holes and is seen by the camera (see screen of monitor and figure 8.9). Data are stored and plotted as a function of time (see figure 8.10)*

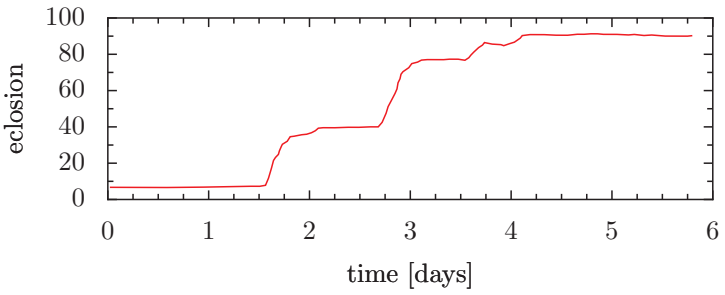


Figure 8.10: *Example for a curve of eclosion rhythm of* Drosophila *pseudoobscura. The data were obtained with the soot recording and an imaging system*

Humans staying in well-insulated isolation units or bunkers (Wever (115), Moore-Ede et al. (84)) or inside caves (Siffre (104)) still continue to go to bed and awake on a near-daily basis thus experiencing subjective night and subjective day. Parallel to these sleep-wake cycles rhythms in the profiles of body temperature, urine output, concentration of ions in urine, bodily and psychological alertness are found (Aschoff (5), Aschoff (7)). However, the period length of these rhythms, as for instance between successive maxima is not exactly 24 h any more as under normal day-night conditions. It is now typically 25 h or slightly more. This 25 h 'free running rhythm' expresses itself in newborn human babies under natural day-night conditions, even though it is often punctuated by short term sleep-wakefulness cycles (figure 8.11). It is only after the lapse of the first few weeks that human babies entrain to the 24 h day-night duration of the outer world (Kleitman (66)). In the course of subsequent years in life humans develop tendencies to belong to 'early morning', 'late evening' or indifferent types (Östberg (88), Kerkhof (65)).

We propose to conduct a test which will show to which phase type you belong. Besides you can record your own body temperature automatically and determine temperature maxima and minima in each cycle. We could expect that the 'morning' type of persons experience temperature minima to occur earlier in the night than do 'evening' type persons. The locomotor activity could also be measured using special recording devices.

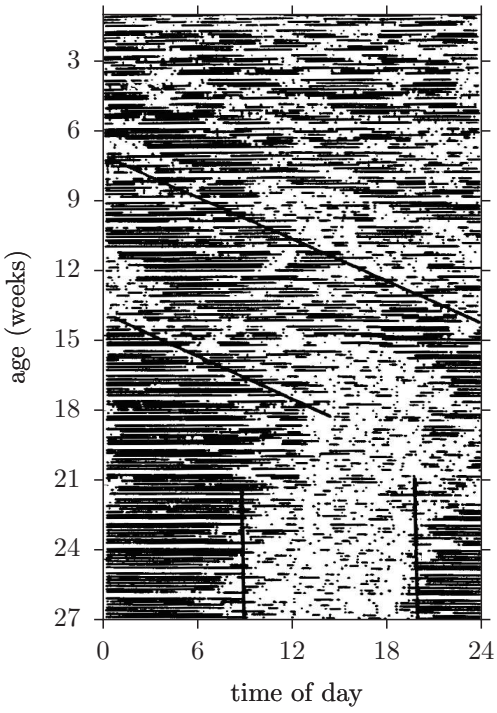


Figure 8.11: *Sleep-wake periods of a baby in the first 26 weeks of life (after Kleitman (66), changed). In the first 16 weeks the periods of sleep (—) are characterized by frequent awakening. A 'free run' of about 24.5 hours can be seen (indicated by the straight line in the upper part of the figure). From the 16th week onward the child is synchronized to the 24 hour day. Activity is now mainly restricted to the day, sleep to the night, the parents relax*

8.3.2 Material and Methods

The test is conducted in the form of a questionnaire (see page 93). For recording

body temperature we use a portable instrument with a thermo-probe which is inserted into the rectum. The rectal temperature is recorded at certain time intervals and the data stored. The wrist movements can also be recorded using the same apparatus.

The filling up of the questionnaire should pose no problems. Follow the instructions. Table 8.1 shows the evaluation of the chronobiological phase type.

A few methodological hints may be in order at this juncture. A protective covering is pulled over the thermo-probe (such as the disposable protective covering for fever thermometers 'steritemp'). The thermo-probe consists of polyvinyl chloride with a polyvinyl cable. Therefore no organic solvents such as alcohol, acetone and others shall be used. Medical desinfectant should not act on it too long. The probe with cable should be slowly and gently inserted into the rectum about 6 cm almost until the end of the protective covering (moisten or use some cream). The best way to do this is by lying on the back and spreading the legs. The cable from the probe to the instrument should not be tight, in order to prevent the cable from braking. Relieve the cable at the plug end by using a loop. The best way to carry the instrument is on the belt of the trousers.

The function and application of the recording device are described in the instructions of the maker. The apparatus has to be equipped with a new set of batteries. The data are transferred via an interface to a PC and displayed as a function of time on screen using special software supplied by the maker.

A log note-book should be available to enter time of rising, meal times, nature and time of activities during the day, special events, time of going to bed, time of falling asleep and wakeup.

8.3.3 Proposal for experiment

Try to find out whether there is a correlation between the time of temperature minima and the classification of the 'morning-evening' types (which is obtained from the questionnaire). This would of course mean that your own experiment and data are only one brick in many, and that a series of experiments have to be performed on numerous persons, until a clear-cut answer can be found. For this very reason it is important to maintain a detailed description of procedures and entries which is intelligible also to others and to add your results to those collected by earlier workers. This explains why the experimental strategy has been explained at greater length than those for other experiments.

8.3.4 Planning and executing the experiment

8.3.4.1 Answering the questionnaire

After answering all questions you should add up the sum of the scores of the individual question items. The chronobiological phase type can be obtained from table 8.1. Add important data such as age, sex, profession, working time, structure of day.

8.3.4.2 Recording of rectal temperature and activity

Use the instructions coming with the recording devices and start recording.

Record rectal temperature and activity as described for at least a week continuously. It is important to note down the exact time of wakefulness, rising from bed, meals, physical activities, drinks and their nature (especially alcohol, coffee, tea, cocoa), sitting, standing, movements, lying down, going to bed etc. It is recommended

Table 8.1: *Evaluation of the chronobiological phase type from the results of the Netherlands questionair*

chronobiological phase type	score
extreme evening type	7-10
weakly expressed evening type	11-14
indifference type	15-21
weakly expressed morning type	22-25
extreme morning type	26-31

to make notes elaborately, which might be needed later to interpret the recorded data and their time course.

8.3.4.3 Analyses of data

The results can be graphically presented after the data have been entered into the computer. With a special program body temperature and activity can be displayed on the monitor screen and printed on a printer. The data can be analyzed with time series analysis programs.

An example for such a temperature- and activity curve is shown in figure 8.12. A rough estimation of the period length can be made from the graph. More precise is, however, the evaluation with a time series analysis procedure. They are described on page 48. In order to transfer the data in this or in other programs, they have to be transformed into a suitable form.

With a signal average method an 'average day' can be obtained (figure 8.13), from which the temperature minimum can be obtained. These values and the activity minimum are entered into table 8.2 and into the graphic display (figure 8.14).

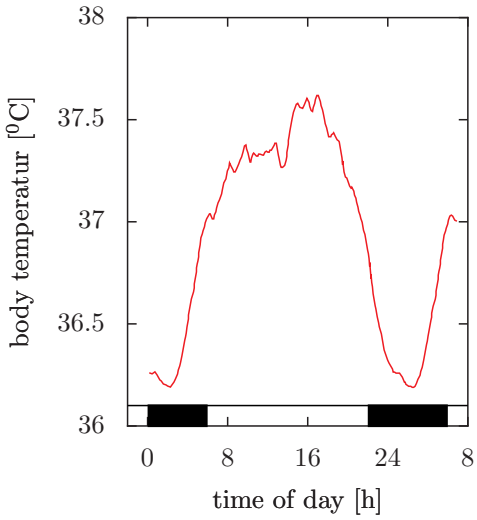


Figure 8.13: *'Average day' of rectal temperature of a person. The dark bands above the time axis represent sleep time, the time in between is wake time*

8.3.4.4 Statistics

When sufficient data become available (from other persons) a correlation analysis should be undertaken. For this purpose

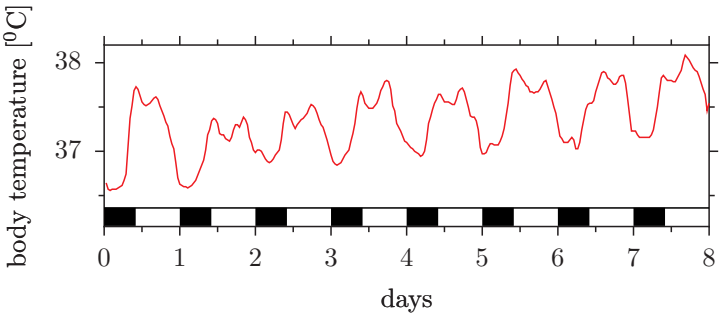


Figure 8.12: *Course of rectal temperature and sleep-wake periods of a person. Sleep: dark, wake: bright stripes*

Table 8.2: *Chronobiological phase type of different persons*

full name	pre-name	M/F	age	type	temp.min.

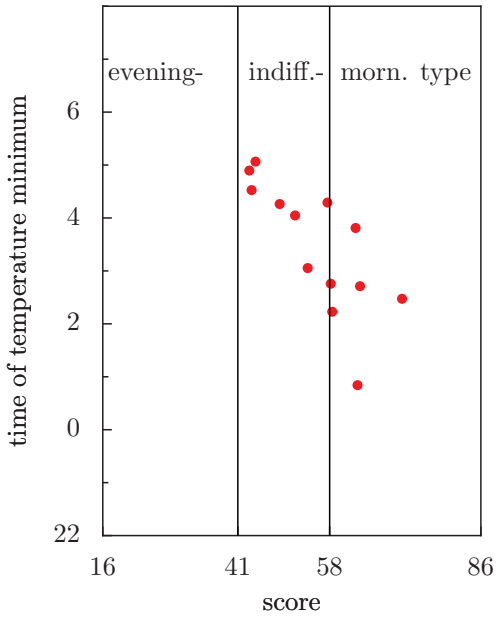


Figure 8.14: *Dependency of the time of nocturnal body temperature minimum (ordinate) from chronobiological phase type (evening-, indifference-, morningtype). Score 16 represents extreme evening type, score 86 an extreme morning type*

enter the phase type score and the clock time at which the temperature minimum is found. The correlation coefficient r^2 is calculated. Similarly r^2 is calculated for a possible correlation between activity minima and the phase-type number. In case the data points do not depent linearly on the phase-type other kinds of data fitting should be considered.

8.3.4.5 Interpretation

When data of at least 15 person are available the results should be interpreted. It is possible that there is no noticeable correlation between the chronobiological phase-types and temperature- respectively activity minima. It might be that inconsistencies occur in the data for only some persons. It would be of interest to look for personality traits and other factors that account for these deviations. In such instances it would be better to correlate the clear cases only and try to find simple criteria which would characterize the other 'type'.

8.3.4.6 Report

Report on the results and use also the results of other persons in order to answer the initial question ('Can chronobiological phase-types be correlated with the temperature and/or activity minima?' (page 94, Kerkhof (65)).

8.3.5 Significance, prospects and practical applications

The experiment performed was supposed to answer the question whether the chronobiological phase type, as indicated in the questionaire, was indeed related to the temperature and/or activity minima. In the case of evening phase types one could expect minima later in the subjective night

and in the morning phase types earlier minima. These results are important in the context of shift work since it turns out that markedly morning phase type persons are unsuited for night shift work (Akerstedt (1), Döhre (26), Knauth and Rutenfranz (68)).

Other practical applications to these findings might emerge. There are indications that the chronobiological phase types may in some cases arise as a function of age and that fluctuations are found during the age children go to school. Unfortunately our society does hardly take any notice of these phenomena. In fact many traffic accidents involving school children might be avoided by adjusting school timing to suit the chronobiological behavior traits of children. Obviously the alertness and performance skills of children are also very strongly correlated to the chronobiological phase types. This can be demonstrated easily by experiments. A vast array of experimental possibilities lies in this area of chronobiology. Someone remarked that more marriages might break up because of chronbiological phase incompatibilities of the partners than of other reasons. Watch out!

8.3.6 Suggestions for independent experiments

Besides body temperature and bodily activity several other physiological processes display a distinct circadian rhythmicity in humans. Those who wish to investigate a few of these are referred to Koukkari et al. (71). Even body temperature and activity can be used to answer a series of other questions, as for example:

- Do drugs and stimulants (smoking, alcohol, methylxanthines like coffein and theophyllin) influence circadian rhythms?
- Are there changes in the circadian profile of the temperature curve during the menstruation cycle?
- Is the circadian rhythm influenced by lighting conditions?
- Does the activity rhythm show changes related to age, e.g. in school children? Is for instance school timing appropriate to the physiological state of the children?

8.3.7 Questionaire for the chronobiological phase type

This list consists of questions which relate to your activity and feeling of being awake in the morning and evening. In answering questions 1 to 4 you should assume that you have to work during the day for 8 hours at a self-selected time. Answer all questions honestly. Mark only one item for each question.

How difficult would it be for you if you had to go to bed each day at 01:00 o'clock?

- **4** Very difficult, I would be terribly tired for a long time.
- **3** Rather difficult, I would feel tired for some time.
- **2** Not difficult, I would feel a little bit tired.
- **1** No difficulty, no problem.

How difficult would it be for you if you had to rise up in the morning each day at 06:00 o'clock?

- **1** Very difficult, I would be terribly tired.
- **2** Rather difficult, I would feel tired
- **3** Not difficult, somewhat unpleasant, no big problem.
- **4** No difficulty, no problem

Imagine you decided to participate in a fitness training. Your friend proposes an hours training twice a week. For her/him the best time in the morning would be from 7 to 8 o'clock. How would this be for you?

- **4** This time would be optimal.
- **3** Would be all right.
- **2** I would have difficulties, I would prefer a later time.
- **1** It would be to hard for me.

Imagine you decided to participate in a fitness training. Your friend proposes an hours training twice a week. For her/him the best time in the evening would be from 23 to 24 o'clock. How would this be for you?

- **4** This time would be optimal.
- **3** Would be all right.
- **2** I would have difficulties, I would prefer an earlier time.
- **1** It would be to hard for me.

Underline at which time you *normally* go to bed

20:00	21:00	22:00	23:00	24:00	01:00	02:00	03:00

Example:

20:00	21:00	22:00	<u>23:00</u>	24:00	01:00	02:00	03:00

Underline at which time you *normally* rise up from bed

05:00	06:00	07:00	08:00	09:00	10:00	11:00	12:00

Are you a morning- or evening active person?

- **5** extremely morning active.
- **4** rather morning active.
- **3** neither.
- **2** rather evening active.
- **1** extremely evening active.

The evaluation of the questionaire is on page 93

9 Significance of circadian rhythms: Photoperiodism

Overview:
Quite a number of organisms use daylength for photoperiodic reactions. In this way they are able to adapt them self to changes in environmental conditions during the course of a year. Experiments for photoperiodic reactions in Drosophila *flies and for flower induction in short day plants are described.*

9.1 Introduction

Organisms living in temperate or higher latitudes of the earth had to adapt them self to the seasonal changes of the environment. They mostly use the daylength as the most reliable indicator of the time of year. The shorter days in the fall signal that winter is coming and with it unfavorable life conditions. At that time of the year the organisms have still enough time to prepare for it by behavioral (e. g. larvae crawl into the soil) or metabolic changes (insects for instance protect the body against freezing).

There are numerous examples for photoperiodic reactions. In plants the daylength often determines whether flowers are formed. In temperate or higher latitudes this occurs usually uner long day (such as shooting of salad). But there are also short day plants, in which flowering is induced by short day. One of them is the Crassulacea *Kalanchoe blossfeldiana* from arid areas of Madagaskar.

Many insects survive unfavorable conditions in the state of diapause. Diapause is in most cases photoperiodically induced.

In mammals the color of the fur can be controlled photoperiodically. The Siberian hamster is white in the winter and brown in the summer. Another example: The rutting season is induced by certain daylengths. For this and further examples see Aschoff (6).

Photoperiodic reactions are of practical significance in agriculture (chicken for instance lay eggs only under long day. By using additional light in the night egg laying is maintained also in the winter). Furthermore, in horticulture photoperiodic treatment is used to induce flower induction at certain times of the year when those plants would usually not flower.

9.2 Experiments in flower formation of *Pharbitis*

Quite a number of plants can be induced by short days to flower. The morning glory *Pharbitis nil* is one example. In the strain 'violett' a single short day is sufficient to induce the photoperiodic reaction. Furthermore this plant can be induced already in the seedling stage. It takes only a few days until the photoperiodic reaction is visible under the binocular. This allows to conduct experiments in a short time.

9.2.1 Rearing the plants

Seeds can be purchased from the Maruthane Trading Company in Tokyo (Japan). They can be kept in the freezer for a few years. The seeds are treated with concentrated sulfuric acid (45 minutes), the acid poured off (in much water, attention, never poor water in the concentrated acid, danger of splashing!) and much tap water added to the seeds. They are heated for a short time which synchronizes germination. After 12 hours of watering in running tap water (with a net on top of the seeds to prevent the seeds from floating away) plant the seeds 15 mm deep in garden soil in pots and allow to germinate under continuous light.

Figure 9.1: *Left: A vegetative bud. Right: A flower bud of* Pharbitis nil

9.2.2 Determination of the critical dark period

After the cotyledons have unfolded them self the plants can be induced photoperiodically. A single dark period is sufficient in the strain 'violett'. Afterward the plants are transferred again in continuous light. Vary the length of the dark period for groups of 10 plants between 6 and 20 hours.

Already one week after the dark treatment the condition of the buds can be determined under the binocular. Flower buds have two long bracts and a broad apex, whereas vegetative buds possess a pointed apex (figure 9.1). Determine the mean number of flowers per plant in each group. Plot the results against the length of the dark period. The critical dark period is the length where the plants show 50% of the maximally inducible flowers (figure 9.2).

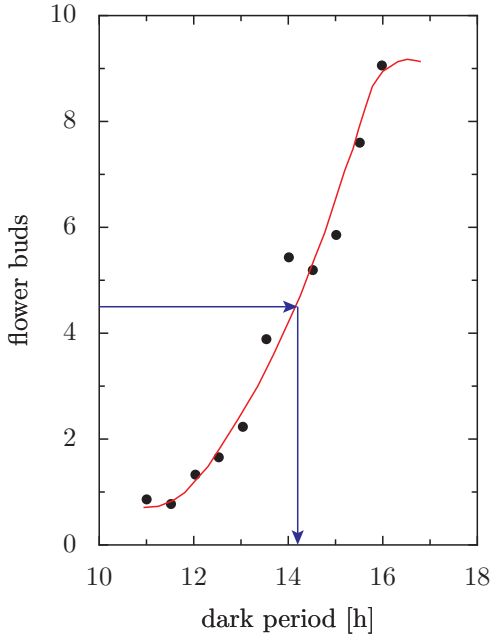


Figure 9.2: *Flower induction as a function of the length of the dark period in* Pharbitis nil. *Ordinate: Average number of flowers per plant (10 plants per value). Abscissa: Length of dark period in hours*

9.2.3 Does the critical dark period depend on the temperature?

The experiments can be performed at another environmental temperature in order to test, whether the critical dark period is influenced by it. Remember that circadian rhythms are temperature compensated in their period length. According to Bünning (17) the day-length measurement in photoperiodic reactions occurs with the help of the circadian clock. Accordingly the critical day-length should be independent of the environmental temperature.

9.3 Photoperiodism in *Drosophila littoralis*

In numerous insects developmental steps are photoperiodically controlled. Take the development of ovaries of female *Drosophila littoralis* as an example. It is blocked in short day. Cultures are kept at the department of genetics, SF 90570 Oula (Finland). There are various strains, the critical day-lengths of which differ. Strain 1008 from the Tessin has no diapause ('Ticino'), strain 1036 has a critical day-length of 20 hours ('Oulu 1A'), im-3 ('Kutaissi'), and 1052 ('Batumi') of 12 hours. The further south the species is found, the shorter is the day-length at which ovary development is stopped.

9.3.1 Rearing

Rear in 250 ml bottles. For oviposition to occur flies must be at least 2 weeks old. Wash the larvae out with water, bring them in small quantities in vials which have to be kept humid and supplied with yeast. Use cotton- or cellulose plugs. Food recipe see Lumme (74). Nipagin prevents mold growth. At 19 to 20^{0}C the development from oviposition to eclosion takes 3 weeks. Eclosion is spread over a week. Change food every fourth day and use about 100 flies per vial. Transfer flies with an exhaustor (figure 9.3).

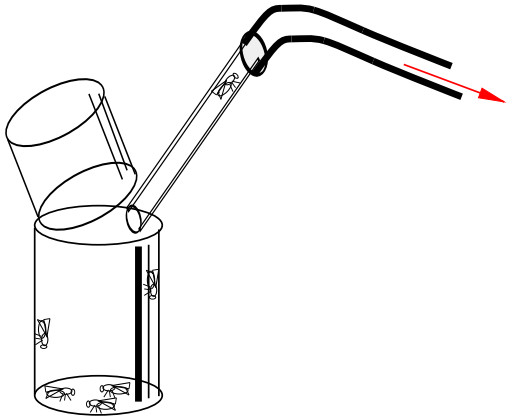


Figure 9.3: *Exhaustor for transfer of* Drosophila-*flies*

The adults are photoperiodically sensitive (Lumme (74), Saunders (99)). After eclosion every second day flies are collected and transferred into different photoperiods at 16^{0}C. For instance 6, 9, 12, 13.5, 15, 16.5, 18, 21 h light per day. The best method is to use light tied boxes in continuous light. They are closed with lids during the dark periods. The opening and closing of the lids can also be automatically controlled by a motor via an electric timer.

After (2-) 3 weeks the females are checked for the development of the eggs (the males are easily recognizable by their red testes). Pull with two pointed forceps the abdomen apart and check at a magnification of 25* the egg dotter under a drop of water. The developed ovaries are much larger. It takes about 15 minutes to check 100 flies.

For each record about 200 flies from each culture are needed. Enter the results in a table and plot the values graphically (% di-

apause as a function of length of light period).

Part III

Teaching about rhythms

Introduction

This part of the book is somewhat preliminary and will be worked at in the future. I consider it important, since the development of the field of biological rhythms depends on the young people coming in contact with it, and on the interest of the public.

In the first chapter of this part, books are listed and commented on which introduce the interested layman into the field. Proposals are made for projects in adult education courses, and teaching material is mentioned.

In the next chapter projects for schools are described on two levels. The lower level is aiming at pupils and students who want to be introduced into the field and to back it up with observations, simple experiments and modeling. At the advanced level more elaborate projects are introduced and weight is put on own planning and execution of experiments. In this chapter reference is frequently made to methods described in part I and examples given in part II.

A third chapter is concerned with teaching this field at the university level, where I have much own experience. Lecture topics, seminar proposals and -styles, are briefly mentioned.

In the last chapter didactic considerations will be discussed.

Furthermore, instruments, recording systems, laboratory material, sources for material and organisms, handling and rearing of unicells, plants and animals which have been proposed to be used in this book are described.

10 Chronobiology for the interested layman

Overview:
There are a number of books which introduce into the area of chronobiology. Some of them are listed in the following section and their content is summarized. In a further section topics for small projects are proposed which interested laymen could tackle for instance in the frame of an adult education course. The chapter closes with hints for audio- and visual material.

The following books are useful as an introduction to chronobiology and *their content is summarized briefly*:

- Beck, S.D.: Animal Photoperiodism. Holt, Rinehart and Winston Inc. New York 1963. *Photoperiodism in mammals, man, birds, insects. Significance and relation to circadian rhythms*

- Brady, J.: Biological clocks. Studies in Biology No. 104. Arnold Ltd., London 1979. *Daily, tidal, lunar and annual rhythms. Examples for circadian rhythms. Time sense. Sun compass orientation. Photoperiodism. Mechanisms of biological clocks.*

- Brady, J.: Biological time-keeping. Soc. Exp. Biol. Seminar series 14, Cambridge Univ. Press 1982. *Contributions of S. Daan (circadian rhythms in animals and plants), E. Naylor (tidal and lunar rhythms), K. Hoffmann (sun compass orientation), D. Saunders, B.K. Follet und D. Vince-Prue (photoperiodism), J. Brady, J. Aschoff, M. Kluge and J.W. Jacklett (physiology and mechanisms of circadian rhythms in plants, animals and man).*

- Brown, F.A.; Hastings, J.W.; Palmer, J.D.: The Biological clock. Two views. Academic Press New York, London, 1970. *Introduction into biological rhythms and contrasting a hypothesis of F.A. Brown (circadian rhythms are caused by exogenous rhythms) with another hypothesis presented by J.W. Hastings that circadian rhythms are endogenous.*

- Bünning, E.: The physiological clock. Circadian rhythmicity and biochronometry. 2. edition, Springer Berlin, Heidelberg, New York 1977. *Classical work with overview on time measurement by organisms based on circadian rhythms, effects of external factors on circadian rhythms, physiological bases and models, significance of rhythms for orientation and photoperiodism, rhythm disturbances and their consequences.*

- Conroy, R.T.W.L., Mills, J.N.: Human circadian rhythms. J. and A. Churchill, London 1970. *Different rhythms, sleep-wake, time sense, daily rhythms, applied and clinical aspects.*

- Engelmann, W., Klemke, W.: Biorhythmen. Biol. Arbeitsbücher 34. Quelle und Meyer 1983. *Instructions for experimenting with rhythms*

in unicellulars, fungi, insects and vertebrates with references to methods.

- Gwinner, E.: Circannual rhythms. Springer Berlin, Heidelberg, New York, London, Paris, Tokyo 1986. *Examples for annual rhythms of organisms, their properties and influences by external factors. Mechanisms and significance.*

- Haupt, W.: Bewegungsphysiologie der Pflanzen. Thieme Verlag Stuttgart 1977. *In the 14th chapter circumnutations and circadian movements are described.*

- Held, M., Geissler, K.A.: Ökologie der Zeit. S. Hirzel Wissenschaftliche Verlagsgesellschaft Stuttgart 1993. *Interesting contributions by Roenneberg (Time as 'Lebensraum'), Zulley (Sleep and wakefulness), Lemmer (Circadian rhythms and medicine).*

- Hildebrandt, G.: Biologische Rhythmen und Arbeit. Bausteine zur Chronobiologie und Chronohygiene der Arbeitsgestaltung. Springer Verlag, Wien, New York 1975. *Significance of chronobiology for humans at work, recovery and during sleep, in shiftwork and flights through time zones. Morning- and evening types in humans.*

- Hildebrandt, G., Moser, M., Lehofer, M.: Chronobiologie und Chronomedizin. Hippokrates Verlag Stuttgart 1998. *After an introduction into the field of chronobiology the connection between biological rhythms and medicin is discussed, methods described and results of studies in man presented.*

- Hobson, J.A.: Gehirnaktivität im Ruhezustand. Spektrum der Wissenschaft Verlagsgesellschaft Heidelberg 1990. *Sleep and sleep physiology.*

- Lofts, B.: Animal Photoperiodism. Arnold Publ. London 1970. *Short overview of photoperiodism in mammals, birds, lower vertebrates and invertebrates. Mechanisms.*

- Moore-Ede et al.: The clocks that time us. Harvard Univ. Press 1971. *Characteristics of circadian rhythms, organization of the circadian system and its neuronal basis. Circadian control of physiological systems (Sleep-wake-cycle, eat, drink, temperature regulation, endocrine system, kidney activity, reproduction). Structure of the circadian system of man, medical aspects of circadian rhythms.*

- Palmer, J.D.: An introduction to biological rhythms. Acad. Press New York, San Francisco, London 1976. *Introduction into biological rhythms with examples from plants, animals and man, tidal rhythms, orientation and photoperiodism.*

- Palmer, J.D.: Biological clocks in marine organisms: The control of physiological and behavioral tidal rhythms. J. Wiley and sons. New York, London, Sydney, Toronto, 1974. *Activity rhythms, vertical migration rhythms, color change, rhythms in oxygen consumption, rhythms depending on the moon.*

- Palmer, J.D.: The biological rhythms and clocks of intertidal animals. Oxford University Press 1995. ISBN 0-19-509435-2

- Reinberg, A., Smolenski, M.H.: Biological rhythms and medicine. Cellular, metabolic, physiopathologic, and pharmacologic aspects. Springer Berlin 1983. *Articles of different authors on e.g. chronopathology, chronopharmacology, chronobiology and food intake.*

- Rensing, L.: Biologische Rhythmen und Regulation. Gustav Fischer Verlag Stuttgart 1973. *Rhythms of movements and membrane processes in cells, excitable systems, enzyme systems and gene activities. Spectrum of the different rhythms. Mechanisms. Significance. Results and goals.*

- Saunders, D.S.: An introduction to biological rhythms. Blackie, Glasgow, London 1977. *Rhythms adapted to environment, endogenous character, synchronization, photoreceptors, time sense and celestial orientation, photoperiodism, localization of oscillators, mechanism.*

- Saunders, D.S.: Insect clocks. 3rd edition, Elsevier Boston, London, New York, Oxford, Paris, San Diego, San Francisco, Singapore, Sydney, Tokyo 2002. *Circadian rhythms of activity and other properties of insects, their synchronization, physiology and anatomical localization, population rhythms, photoperiodism, models.*

- Schmidt-König, K.: Migration and homing in animals. Springer Berlin, Heidelberg, New York 1975. *Compass orientation in animals.*

- Sweeney, B.M.: Rhythmic phenomena in plants. Academic Press, London, New York, 1969. *Observations, terms, adaptation to environmental rhythms, other rhythms, rhythms in cell division, mechanisms.*

- Ward, R.R.: The living clocks. Alfred A. Knopf Inc., New York 1971. ISBN 0-394-41695-3 *Introduction into the field of chronobiology: reports on the prominent workers in the field and their specialties, up to 1970.*

- Winfree, A.T.: The timing of biological clocks. Scientific American Library, Scientific American Books, Inc., New York 1986. *Interesting exposition of different problems concerning biological clocks, topological description of oscillations and the possibility to bring rhythms into a singular state, where they do not oscillate anymore. Significance of these rhythms. Special color pictures.*

10.1 Topics and project proposals for the interested layman and for adult education courses

The following topics are useful for smaller projects:

Movements in leaves and flowers: Quite a number of plants show leaf movements, which are adapted to the daily rhythm. *Leguminosae*, *Oxalidaceae* and *Maranthaceae* possess special joints which are responsible for the movement (Pfeffer (90), Satter et al. (98)). There are, however, also leaf movements in plants without special joints. Here daily periodic differences in growth between the upper and the lower side lead to movements.

Flowers might open and close in a diurnal manner. The movement can be brought about by turgor changes in the cells of the petals. Examples are the *Kalanchoe* petal movements we have heard about already (page 74). The petals of water lillies do also move in a circadian way. Even cut stripes of the petals show this movement (Bünning (18)).

Some flowers open at certain times of the day. This is especially impressive in *Oenothera*. The process of opening occurs in a few minutes. An interesting project is to determine the time of opening as a function of time of the year (and consequently as a function of day-length). The first flowers open in the begin of July, the last one in October or even November. Is the time of day at which they open influenced by temperature, clouds, wind? The flowers of morning glory *Pharbitis* open also at certain times.

The opening of flowers is often connected with fragrance production. Other flowers such as in the Persian Violet *Exacum* are open all the time, but the fragrance occurs rhythmically. One should also test whether perhaps the sensitivity of the human nose and not the fragrance of the flowers changes rhythmically. For this purpose fragrant substances can be smelled in different concentrations at different times of the day. The threshold value (lowest still recognizable concentration) should be constant, if the sensitivity of the nose does not change. Many petal movements or openings of flowers or fragrance productions of plants are connected with the attraction of insects, to ensure fertilization. This offers also the opportunity to identify the fertilizing insects.

Rhythms in algae: Use samples of water with algae from lakes, ponds, puddles and pools. To isolate species, the samples are diluted considerably, poured in test tubes and closed with a cotton plug. In this way one can succeed in having only individual cells in the diluted sample. After several division steps one ends up with a dense population. To determine the algae a microscope is needed. Use Ettl et al. (38) to find out the species. It is, however, easier to buy algae from a place were collected algae are kept, such as the Pringsheim collection of algae in Göttingen (Schlösser (100)).

The algal suspension in the test tube is observed every 3-4 hours and the density recorded. In many algae a substantial part of the population settles during the night at the bottom and during the day they are more or less uniformly distributed in the medium. The cause for it is in the case of *Euglena* a difference in beat activity of the flagellae respectively a loss of flagellae during the night. There are, however, also indications of diurnal vertical migrations based on a different density of the individual cell (Ettl et al. (38)). These events can be documented by photography or video pictures. In this way it is easier to compare the density differences in the various parts of the test tube.

Especially interesting would be studies on blue-green algae (*Cyanobacteria*). It was only in the last years that diurnal and circadian rhythms were described in these prokaryotes

(Huang et al. (56)). These rhythms show up in photosynthesis, nitrogen fixation and carbohydrate. Other movements in *Cyanobacteria* are perhaps also controlled in a circadian manner. *Cyanobacteria* show vertical movements due to density differences. To determine *Cyanobacteria* see Bittner (11).

Fairy rings in fungi: In the woods or on meadows one can often find fungal fructification bodies in a ring-like structure, so called fairy rings. They are formed because the mycelium which grew radially from a single spore fructified simultaneously. Here we will study another kind of fairy ring which is often found in molds. We use an agar-fungal medium in a Petri dish of 10 cm diameter and keep the dish open for some time. They are then closed and controlled daily. Germinating spores of different species of molds form mycelia which grow radially. They are recognizable by their circular pattern. Some show a daily rhythm (testable by daily marks at the growth front of the mycelium), which might even be temperature independent (Lysek (75)). The method is useful also to check for a periodic spore flight during the course of a day. To determine molds, use Müller and Löffler (85) or Reiss (96).

Rhythmic events in insects: In grasshoppers, crickets and cockroaches diurnal depositions of the chitin coat can be observed. An animal is killed with chloroform and the tibia of a leg cut off. It is embedded in a piece of elder pith or polyurethan and cross-sectioned with a new razor blade. A pattern of layers can be seen under the microscope at about a 500 fold magnification (figure 10.1). It is especially well recognizable under polarized light. Use a Polaroid foil (see sources of supplier on page 121) in front of the microscope lamp. This pattern is brought about by chitin fibers arranged in preferential directions. Processes which are connected with the development of insects are often controlled in a circadian way. Thus in some species egg deposition, larval shedding, pupation or eclosion of the adults from the pupal cast occur only at certain times of the day. This happens of course in the individual animal only once. The rhythmic control can therefore be studied only in a population of animals (see page 88). On the other hand rhythmic events are also observable in individual animals: running and flight activity, attraction of males by pheromones (e.g. in butterflies) or by light signals (e.g. in fireflies) are examples. In the eye of several arthropods one can observe pigment migration in a diurnal or circadian way. This protects the eye from too heavy light exposure (Fleissner and Fleissner (41)). A time pitfall: The daily activity of *Drosophila* flies can be demonstrated in field experiments using a time trap. Deposit a glass vial with fermentizing banana in an area where *Drosophila* is found. Change every hour for a new one, close the old one and determine the species (Markow and O'Grady (78)) and number of flies using a binocular microscope. The number of flies caught depends in a characteristic way on the time of day for the different species.

Daily rhythms in man: The efficiency of our sense organs are modulated in a diurnal way. This can be shown for vision, hearing, smell and taste (Moore-Ede et al. (84)). Performance is also fluctuating during the day, as can be shown. On page 90 it was already referred to the diurnal rhythmicity of locomotion and of body temperature of man. The phase of these rhythms depends on the chronobiological phase type, i.e. whether somebody is more a morning or an evening type. Use the questionair on page 93, to determine your phase type.

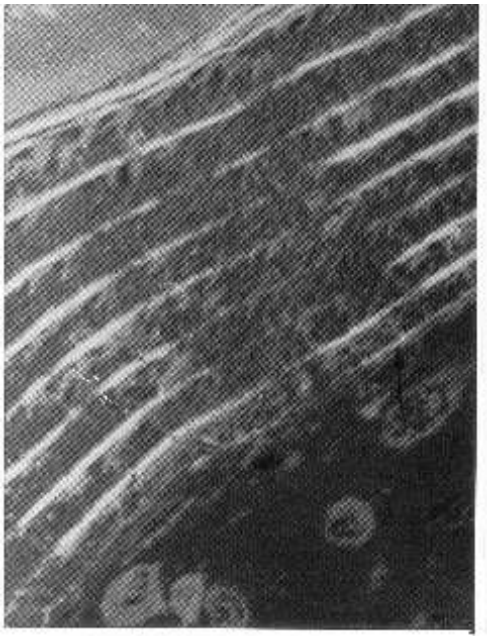


Figure 10.1: *Rhythmic deposition of chitin layers in the tibia of a cockroach*

Chronobiological topics are also interesting for adult education courses and are used there. Scientist working in this field are usually willing to report about their work. Especially motivating are topics concerned with rhythms of man and their significance, such as shift work or flights to the east or west ('jet lag). Proposals for own observations and projects can be offered such as the one on page 111. Movies and references to literature enrich the program.

Addresses of groups working in the field of chronobiology in Europe can be found in the internet under the website of Euclock (http://www.euclock.org/). Further informations under Euclis (Euclock-information system, http://www.bioinfo.mpg.de/euclis/).

11 Teaching chronobiology in advanced courses in schools

Overview:
Some books are mentioned which in addition to the one of the last chapter introduce into the area of chronobiology. Ten chronobiology topics for an advanced course at schools are proposed.

11.1 Introductory literature

As an introduction the following books and articles are suited:

- Brady, J.: Biological clocks. Studies in Biology No. 104. Arnold Ltd., London 1979
- Brown, F.A.; Hastings, J.W.; Palmer, J.D.: The Biological clock. Two views. Academic Press New York, London, 1970.
- Bünning, E.: The physiological clock. Circadian rhythmicity and biochronometry. 3. edition, Springer Berlin, Heidelberg, New York 1977.
- Palmer, J.D.: An introduction to biological rhythms. Acad. Press New York, San Francisco, London 1976
- Saunders, D.S.: An introduction to biological rhythms. Blackie,Glasgow, London 1977.
- Winfree, A.T.: The timing of biological clocks. Scientific American Library, Scientific American Books, Inc., New York 1986.

A multimedia show will be composed which aims at motivating the student for this field. It will offer an overview of the different rhythms at different organisms from unicellulars to man.

11.2 Chronobiology topics for schools

Topics from the field of chronobiology are well suited for an advanced course at schools. In the following, ten units are described with content, time necessary to do them, and the kind of instruments needed.

1st unit: Introduction: A short introduction into the field offered by the teacher or by proposing literature. Observation of the lateral leaflet movement of *Desmodium motorium*. Determination of mean period length of an oscillation at different environmental temperatures with stop watch or watch with hand for seconds. Grafic representation of the results: Strong temperature dependence of the rhythms. Comparison of the results with those from the literature. Q_{10} of 2 (see Glossary). Mentioning of circadian movement of terminal leaflets. Aim: to demonstrate a short term oscillation and dependency of the period on temperature. Discussion: what is the physiological basis of these oscillations? Chemical pro-

cesses? Can chemical processes exhibit oscillations? Gears: Stopwatch or clock with hand for seconds. Time needed: one hour.

2nd unit: Chemical oscillations: Observation of Belousov-Zhabotinsky-reaction (how to make it, see 61 or movie (page 123). Differences to water waves. Demonstration of the Belousov-Zhabotinsky-reaction in time. Temperature dependency. Aim: To show that even chemical processes can oscillate. Discussion: The sequences of reaction is quite well known. Feedback and amplification as prerequisites for oscillations (see also unit 10).

3rd unit: Sleep movements of bean plants: In the following unit bean leaves are recorded and analyzed using the kymograph method or the described video system. The course of the leaf movement is studied in a natural day, in an artificial light-dark-cycle with 12 hours light and 12 hours dark, normal and inverted (i.e. dark during the day, light during the night) and under constant conditions. Aim: Circadian processes show free-run under constant conditions, but are synchronizable to 24 hours by a light-dark-cycle. Analysis of the experiment after a week. Instruments: Kymograph, video recording system with imaging (page 41), fluorescence light, timer, air conditioned box. Time needed: One hour each to start the experiment and analyze the results, one week of recording.

4th unit: banding in bread mold: The banding of *Neurospora* is easy to demonstrate or to perform because of the simple (self-recording) method (Engelmann and Klemke (34)). A week later analysis of the experiments by the students. Determination of period length with a ruler. Aim: Demonstration of timing of reproductive and vegetative processes. Discussion: Hints to other similar events (see unit 6, control of semilunar rhythm). frq-mutations of *Neurospora*. Instruments: growth tubes made from 10 mm diameter glass tubes the ends of which are bended upward. Inoculation wire, explained in (Engelmann and Klemke (34)). Time needed: One hour for medium (prepare in advance), half an hour for inoculation and marking.

5th unit: Locomotor activity of cockroaches: Locomotor activity of cockroaches in light-dark-cycle and in weak continuous red light. Evaluation after a week by the students. Aim: To demonstrate circadian rhythms in insects. Discussion: Insects as suitable objects for the localization of the control centers of rhythms. Instruments: Video-computer recording system with imaging program. Program to analyze the data. Time required: Setup of the recording system and putting animals into the recording dishes about one hour. One week recording. One hour for evaluation of the data.

6th unit: Lunar rhythms: Show two movies on moon dependent tidal rhythms: *Clunio* and grunion-story (Neumann (86)Walker (112)). Aim: Lunar- and tidal rhythms. Discussion: Significance of these rhythms for the biotope sea-coast. Increases chance

for fertilization. Palolo worm as an example. Instruments: Movie projector. Movies from IWF in Göttingen. Time required: One hour.

7th unit: Photoperiodism: Measurement of day-length as a means to orientate during the course of the year. Short day plant *Pharbitis nil*, long day plant *Lolium perenne*. Evaluation of photoperiodic induction of flowers under the binocular by the students. Aim: Photoperiodism as the most precise method for an organism to determine the time of year. Discussion: How is the day-length measured? Instruments: Binocular, pair of tweezers, preparation needle. Seeds (page 102), concentrated sulfuric acid, Erlenmeyer flask, garden soil, flower pots. Time required: One hour for preparation and sowing, after one week photoperiodic induction in darkroom or box (timer!), after another week one hour for evaluation.

8th unit: Rhythm of man: Temperature rhythm and activity rhythm in man, chronobiological phase type. Fill out questionaire. Distribution of phase-type classes in the school class. Demonstrate daily rhythm of body temperature with extreme morning and evening types. Aim: Demonstration of diurnal rhythms in man. Other diurnal rhythms in man. Development of circadian rhythms in babies. Instruments: None. Questionair. Time required: One hour to fill out and evaluate the questionair.

9th unit: Gravitropic pendulum: Demonstration of the phenomenon during an one hour lesson. Demonstration and interpretation of curves. Aim: Exogenously induced oscillation, feed back principle. Discussion: How the gravitropic pendulum functions. Experiments in space. Instruments: Video-computer recording system with imaging program. Program to evaluate the data. Time required: One hour.

10th unit: Models: Simulation of oscillations with program 'Modus'. Explain program and demonstrate the predator-prey model. Students in groups of two change parameters. Damping, increase in oscillation. Aim: Models as tools for scientific work. Discussion: Predator-prey oscillations exemplified with the furs of snow hares and lynx sold to the Hudson Bay Company. Effect of other factors. Instruments: PC computer with mouse and color monitor. Modus program and handbook. Time required: at least five hours.

Slides, movies, videos and suitable computer programs are mentioned in the last chapter of this part ('teaching aids').

12 Teaching at the universities, research projects

Overview:
Lectures, seminars, courses as introductions into chronobiology at universities, research projects, research groups.

The field of chronobiology can be offered in courses, seminars, colloquia and lectures. Details are found in the catalogue of lectures of the universities. See also page 114.

I have experience of many years in teaching chronobiological topics. In a lecture cycle 'adaptation of organisms to the time structure of the environment' I have covered in five terms the following topics:

- Photoperiodism and annual rhythms
- Rhythms in unicellulars
- Insect clocks
- Rhythms in higher plants
- Rhythms in man

The content of these lectures will be presented on the Internet (see page 10.1). Here are some short overviews of the content:

Photoperiodism and annual rhythms: Introduction, historical background, examples from the plant- and animal kingdom, significance, photoperiodic time measurement and circadian system, flower induction in short- and long day plants, models for photoperiodic reactions (external, internal coincidence, amplitude model), diapause, photoperiodic counter, photoperiodism and annual rhythms, mechanism of photoperiodic reactions, localization, annual rhythms in birds, mammals, plants and unicellulars, timing of annual rhythms.

Rhythms in unicellulars: Introduction, evolution and adaptation of circadian rhythms, circadian rhythms in prokaryotes, fungi, amoebae (*Thalassomyxa*), algae (*Euglena*, *Chlorella*, *Chlamydomonas*, *Acetabularia*, *Gonyaulax*), ciliatae (*Paramecium*), minimal systems (erythrocyts, seeds, *Cyanobacteria*), rhythmic cell division, recording and analysis, mechanisms and models, genetical aspects.

Insect clocks:Introduction, examples (cockroaches, crickets, butterflies, flies, mosquitoes, beetles), population rhythms (predator-prey interaction, eclosion rhythm), annual rhythms, photoperiodism and diapause, tidal- and lunar rhythms, sun compass orientation and time memory, circadian system, localization of controlling centers, mechanism of circadian rhythms, genetical and molecular biological studies, models.

Rhythms in higher plants: Introduction, gravitropic pendulum, heliotropic movement, growth rhythms, movement of lateral leaflets of *Desmodium*, sleep movements of plants, pulvinus

as a motor organ, synchronization, flower clock *Kalanchoe*, transpiration rhythm, layer formation, deposition rhythms, CAM metabolism, photosynthesis rhythm, molecular biological methods.

Rhythms of man: Introduction, spectrum of rhythms, examples of circadian rhythms (temperature-, locomotor activity-, sleep-wake-rhythms), localization of the circadian oscillators, influencing and perturbing the circadian system (diseases, shift work, jet lag), ontogeny.

One of the main obstacles in preparing these lectures was the lack of more recent books and review articles. Therefore one had to use quite often original papers, to select the most important findings and to try to cover the newest results of this rapidly expanding field.

Seminars are useful to introduce into the field of chronobiology. I have used different strategies and found the Epstein-method (Epstein (37)) useful. All the participants read the same publication at home and it is discussed in the seminar. The basic idea is, that an original paper reflects quite accurately the way of scientific working of a scientist. This strategy stresses mainly the methods used and the way of presenting the results, whereas the subject content of the publication is less important. In this way the learned is transferable to other fields in biology and natural sciences.

I have also offered seminars in which teaching aids were produced by the participants, and some of them are referred to in this part. This kind of seminar is especially interesting for students which want to become teachers.

Courses are the most effective teaching arrangements to get to know the field of chronobiology. At the university of Tübingen we propose projects which can be worked at in small groups. The students have 2 to 3 weeks time for the practical studies. They are, however, already familiar with the field and the methods by an introductory seminar. It is of much value to work on projects which have not been studied so far. The knowledge to work at the frontier of science balances the danger, that such a study might also lead to an unun-exciting result. It is, however, necessary to have the recording methods and other aids available. Otherwise too much time has to be invested which is then lacking for the studies them self. One reason to write this book was to offer the necessary prerequisites for this.

Often research studies in the field of chronobiology are started as a consequence of working with such a project in a course. A number of students become so motivated that they decide to continue the project in form of a diploma-, teachers examination- or even doctoral thesis.

How to find a topic or project has been described already before (page 111). A list of groups working in the field of chronobiology is found on the Internet (page 114). Lecture catalogues or lists of research activities at universities are of help as is the Internet. Using the publications of the research groups allow to get an overview of the work done in these groups.

13 Didactic considerations and concepts

Overview:
Didactic considerations, teaching aims for chronobiology such as programs, movies, slides, instruments, laboratory material, supply sources and rearing of experimental organisms.

Didactic aspects are important in schools, adult education courses and universities. This applies for the field of chronobiology to the same extent as for other fields of natural sciences. I will mention here just briefly some high level teaching aims, which are essential, and refer to literature (Schwab (102)Siedentop (103)).

Important teaching aims:

- encourage curiosity behavior
- get to know scientific working
- search for problems and find solutions
- carry out projects for yourself or in groups
- self-controlled learning, learning by teaching, discussions
- democratization of knowledge

To reach these goals, a number of **aids** have to be offered such as

- introductions into the subject
- written overviews
- compendia
- sequence of operation, organization, organizer
- counseling, consulting hour of experts, inquiry hour
- display literature (books, articles, reports)
- lectures and counseling by specialists (e.g. guest lectures)
- offer suitable learning- and working places
- provide resources
- arrange excursions
- test success of learning

To offer learning- and working places and to provide resources is, especially for research work, the most elaborate task.

13.1 Teaching aids

As teaching aids the following should be available:

- Information by literature, audio-visual aids such as slides, movies, video tapes, computer programs.
- Instructions for instruments, programs, procedures. Training.
- Experimental organisms (rearing, maintaining, recording)
- Laboratory material
- Analysis procedures

The aids described in the book and the supply sources are compiled in the following:

13.1.1 Programs and their description

- Recording systems: Recording of movements in plants and animals, recording of temperature and activity in man.

- Graphics- and analysis programs: Quite a number of plot programs for graphic display and description of recorded data are available (Matlab, Techplot, ...). Data can be analyzed by different time series analysis methods (see page 45). Recording programs for the Atari computer in connection with a digitizer were developed by J. Schuster (Tübingen) (OXALIS, OXALAKTO and OXALIMAG). Programs for data collection of locomotor activities of animals (e.g. insects such as *Drosophila*) using PC's under the Linux operational system were written by W. Hellrung (Tübingen). Programs for recording temperature and activity in man have been supplied by W. Himer (Tübingen) and by companies selling corresponding recorders. A time series analysis program is 'Timesdia' by W. Martin (Bonn), programs of De Prince (Bruxelles), the program 'Circadian' (Harvard University), the program 'Chrono' (T. Roenneberg, München), the program 'Chronobio', (Diez-Noguera, Barcelona), the programm 'SCK' (Stanford), the program 'Tau' (Oregon), and the programs 'Oxaldifi' and 'Oxalakto' (Schuster, Tübingen). Trend removal, runtest, autocorrelation, Fourier analysis, spectral analysis, periodogram analysis, digital filtering (in the program OXALDIFI), complex demodulation, maximum entropy spectral analysis, frequency folding and others are used. For digitizing diagrams the program 'Scandata´ can be used in order to obtain data from curves in figures from publications. The diagrams can be plotted by plot programs. I used mainly 'Techplot´. Both programs, Scandata and Techplot, are from Dittrich, Braunschweig.

- Models and simulations: The program package 'Matlab' contains a simulation part. Other program packages are available. We used the program 'Modus', 'CoMet Verlag für Unterrichtssoftware, Duisburg', the 'DSP-programs' (Copyright 1993 by MAXON Computer GmbH Eschborn, 'Simbion' (Witte, Wiesbaden) and the program 'Chronobio' (Diez-Noguera, Barcelona). Most of these programs contain examples for oscillating systems such as the predator-prey model, feedback models, and others.

- Examples for data, data banks, time series analysis programs are found in the literature on biological rhythms (see also the Internet, Current Contents, Biological Abstracts, Medline, literature service of the Center for Biological Timing in Charlottesville (USA), Sheffield Service 'Biological Rhythms' of the university of Sheffield (Great Britain).

13.1.2 Movies, video films, slides

Movies from the catalogue for scientific movies of the 'Institut für den wissenschaftlichen Film' (IWF) (Göttingen) (partly available in English versions also).

Chemical oscillation:

- Hock, B., Bolze, A. 1980. Die Zhabotinsky-Reaktion als Modell einer Musterbildung. (The Zhabotinsky-reaction as a model for pattern formation). C1473 IWF
- Hock, B., Bolze, A. 1980. Die Briggs-Rauscher-Reaktion als Modell einer chemischen Uhr (The Briggs-Rauscher-reaction as a model of a chemical clock) *Chemical oscillator of a iodine-starch-complex).* E1495 IWF
- Gross, W.O. 1977-1980. Fibroblast - caused cardio-myogenesis in vitro. Synchronization of muscle cell pulsations of the heart. E2673 IWF

Transpiration:

- Trolldenier, G. 1968. Movements of stomata. *Turgor changes of guard cells in stomata. Type of guard cells.* C973 IWF

Circumnutation:

- Gradmann, H. 1925, 1926. Bewegungen der Rankenpflanze *Sicyos angulatus* (Cucurbitaceae). (Movement of the tendril plant *Sicyos angulatus* (Cucurbitaceae)). B1419 IWF
- Gradmann, H. 1925, 1926. Zusammenwirken von Geotropismus und Phototropismus bei der Haferkoleoptile. (Interaction of geotropism and phototropism on the oat coleoptile) B1420 IWF
- Rawitscher, F. 1926. Gradmann, H. 1925, 1926. Kreisen und Winden bei *Calystegia sepium.* (Orbiting and winding in *Calystegia sepium*) *Circumnutational movements of* Calystegia sepium) W918 IWF
- Baillaud, L. 1966. Mouvements des tiges volubiles du haricot. *Tendril movements of bean plants.* C918 IWF
- Denffer, D.v. 1953. The movements of tendrils. - I. Basic phenomena. C 677 IWF
- Denffer, D.v. 1953/54. The movements of tendrils. - II. Region and age of stimulation, types of reaction. C678 IWF
- Url, W. 1981. *Desmodium gyrans* (Fabaceae) - Gyration. (Gyration) *Lateral leaflet movement of the Indian telegraph plant.* E2619 IWF

Nyctinastic movements:

- Calabek, J. 1959. The autonomous movements of plants. W531 IWF *Nutational movements*
- Url, W. 1972. Plasmolysis and Cytorrhysis. C1144 IWF. *Demonstration and experiments regarding plasmolysis.*
- Nultsch, W., Schuchart, H. 1982. Traube cell. C1454 IWF. Water uptake by osmosis

Rhythms in unicellulars and algae:

- Hemleben, C., Spindler, M. 1983. Life cycle of the planctonic foraminifere *Hastigerina pelagica.* C1516 IWF. *Lunar periodic reproduction cycle*
- Grell, K.G. The change of phases of *Thalassomyxa australis* (Promycetozoida). C1631 IWF.

Insect rhythms:

- Schimanski, B. 1979. Imaginal hatching. Housefly. E2489 *IWF Metamorphosis and eclosion of Musca domestica (Muscidae)* E2489 IWF
- Ede, D.A., Counce, S.J. 1958. The development of *Drosophila melanogaster* - I. Normal development. *Eclosion of fruit flies out of the puparium.* W361 IWF

Insects and pollination:

- Baumann, H. 1998?: Der Herr der Blüten (The lord of the flowers) Video tape

Mammals, man:

- Borbely, A., Tobler, I., Achermann, P., Geering, B. 1999. Bits of sleep. Explore the facts behind the mystery. University of Zürich. http://www.unizh.ch/phar/sleep

Tidal and lunar rhythms:

- Neumann, D. 1970/71. Semilunar reproduction of *Clunio marinus* - Biological timing in the intertidal zone. C1091 IWF *semilunar rhythm of pupation and circadian rhythm of eclosion in a marine chironomid*
- Hemleben, C., Spindler, M. 1983. Life cycle of the planctonic foraminifere *Hastigerina pelagica.* C1516 IWF. *Lunar periodic reproduction cycle*
- Walker, B.W. Fish, moon and tides - The Grunion story. W791 IWF. *The grunion fish deposits its eggs at the California beach at certain phases of the moon and the tides. Nine days later the baby fishes hedge.*

Sun compass orientation:

- Frisch, C. v., Lindauer, M. 1979. Indication of distance and direction in the honey-bee round- and wiggledance. C1335 IWF (Entfernungs- und Richtungsweisung durch Rundtanz und Schwänzeltänze).

Models:

- Meinhardt, H., Gierer, A. 1984. Activator-inhibitor - A model of biological pattern formation. D1571 IWF. *Oscillating patterns, periodic patterns*

13.1.3 Instruments, -instructions, laboratory material, supply sources

Construction of a temperature box: To allow studies under constant temperature and in controlled light conditions, we propose the construction of an air conditioned box. This small room for constant conditions consists of a solid basis (plywood or chipboard), a 10 cm thick wall of polystyrene plates for thermal isolation and a plywood bench which is inserted into the box. On the top of this bench is a window covered with plexiglass to allow light from three fluorescence tubes to enter. The fluorescence tubes are switched on and off by an electric timer. The fittings and ballasts of the lamps are also mounted on top. At the left side of the bench a tangential ventilator maintains permanent circulation of the air. Below the ventilator are two

heat foils mounted (80 Watt efficiency each). The heating foils are controlled by a thermostate. The number of revolutions has to be reduced by adding a resistance (e.g. a 25 Watt incandescence lamp). Otherwise the air stream is too strong. Depending on the required temperature and precision a counter cooling has to be added. For this purpose a coiled copper tube (8mm diameter) can be mounted at the right side of the bench. Cooling water constantly flowes through the tube ($12 - 14^0$C). The front wall contains a door, which allows handling in the box. Holes for air exchange as well as openings for e.g. a recording camera can be inserted with a knife. For required temperatures below the room temperature (lowest limit at about 15^0C) the cooling copper tube has to be dimensioned larger or the flow of water has to be increased.

Illumination: Safety ligth with red or green fluorescence tubes. Filter and foils (Cinemoid or Rosco, Dedo Weigert Film GmbH, Karl Weinmair Str. 10, 80807 München). Slideprojectors as light sources. White fluorescence tubes. Electric timer to obtain light dark cycles ('photoperiods'). Measuring of light intensity with light measuring instrument, setting the intensity with grey foils. Polarized light using polarisation foil.

Computer: Description of advisable features see Engelmann (30). Framegrabber as an A/D-converter (digitizer) to connect a video-camera to the computer. Programs for recording see Engelmann (30).

Recording of voltages, currents, resistances with the computer using special printed cards (analogue/digital converters)

Temperature recorder: Powerline independent apparatus for recording body temperature of man.

Activity recorder for counting rates (e.g. arm movement as a measure of locomotor activity).

Video equipment with time laps see Engelmann (30)

Recording system of W. Hellrung for locomotor activities of animals with infrared lightbeam, multiplexer, interface and peripheric processor unit, radio controlled clock as time reference.

Recording of eclosion rhythm of *Drosophila*-flies with metal plate containing holes, soot method.

Transpiration measurement with humidity sensor of Driesen und Kern company, Wiesenweg 2, PF 1126, 2000 TANGSTEDT Tel. 04109 6633 Fax 0419 1359

Aquarium pump in aquarium supply shops

flow meter laboratory supply shops

Cages Fly cage, tilting cage, hamster cages, food pellets, drink nippel, running wheels.

Hand microtome for cutting plant material or legs of insects.

Cuvettes for *Kalanchoe* flowers with polyurethan plates, for *Oxalis*- und clover leaves, spectral photometer cuvettes to record the locomotor activity of *Drosophila*.

Koukkari-recording method for leaf movements.

Microscope for anatomical work (pulvinus sections, petal structure of *Kalachoe* flowers)

Temperatur recorder, electronic. For recoding the temperature in chambers and boxes.

13.1.4 Rearing of experimental organisms, supply sources

- *Amphiprora*, marine alga as food for *Thalassomyxa*. Department of Zoology, University of Tübingen, Auf der Morgenstelle, Algensammlung Pringsheim, Göttingen
- *Avena sativa*, oat, Gramineae, seed from seed shops, sowing in garden soil or vermiculite, use plants for transpiration measurements 7 days after germination (primary leaf fully expanded).
- *Phaseolus coccineus*, bean, Fabaceae, seeds from seed shops, soak in water over night, plant in garden soil in flower pots. Circadian leaf movements can be observed as soon as the first leaves are unfolded.
- *Cestrum nocturnum*, Solanaceae, fragrance rhythm with maximum during the night. Botanical gardens, gardeners.
- *Chlorella*, marine alga as food for *Thalassomyxa*. 'Algensammlung Pringsheim', Göttingen
- *Clunio marinus*, marine Chironomid (midge) of the atlantic coast from southern Spain to northern France, north sea coast up to Norway. Prof. Neumann, Zoology department, University of Köln, Köln
- *Desmodium motorium*, 'telegraph plant', 'automobile', Fabaceae, seeds from A. Schenkel company, Blankeneser Hauptstr. 53a, D22587 Hamburg.
- *Drosophila melanogaster*, fruitfly, Diptera, Phywe Göttingen, Postfach 665, 3400 Göttingen, or genetics departments. per-Mutanten per^s, per^l und per^0 Prof. Rensing, biology, University of Bremen, Bremen. Food: see page 86
- *Drosophila litoralis*, Diptera, Dr. Lankinen, Genetics Department, University of Oulu, Finland.
- *Dunaliella*, marine alga as food for *Thalassomyxa*. Department of Zoology, University of Tübingen, Tübingen, 'Algensammlung Pringsheim', Göttingen
- *Exacum affine*, the Persian Violet, Gentianaceae, fragrance rhythm with maximum at noon. In flower shops or on flower markets.
- Hamster, sibirian, *Phodopus sungorus*, food pellets of Altromin company.
- *Helianthus annuus*, sunflower, Compositae, seeds from flower shops, imbibe in water over night, put seeds in garden soil in 2-3 cm diameter plastic flower pods. Keep at room temperature in the dark or red safeligth until hypocotyl is about 6 cm high. Use for geotropic pendulum experiment in red light.

- *Kalanchoe blossfeldiana*, panda plant, succulent plant *Crassulaceae*. Seeds from Engelmann, Biologie I, Tübingen, Auf der Morgenstelle. Verry small seeds, needs light for germination, sandy garden soil. Keep from germination onward in long day (13 h light, 11 h darkness per day). Flowers are induced by short day treatment (11 h light, 13 h darkness). About 3 month in long day, 1 month in short day until flower formation.

- *Leucophaea maderae*, cockroach, rearing in glass containers with dog food or kitchen garbage. From Engelmann, Biologie I, Tübingen, Auf der Morgenstelle 1

- *Mesocricetus auratus*, Syrian hamster, in zoo shops. For studies of locomotor aktivity. Hamster cages in special shops (Zoohandlung, Fa. Becker und Co. GmbH, Postfach 546, 4620 Castrop-Rauxel, Fa. Wagner und Keller GmbH und Co., Uhlandstr. 13-21, Postfach 1125, 7140 Ludwigsburg), food Altromin Company.

- *Musca domestica*, housefly, Diptera. In buildings and staples. Reared in some departments, e.g. Max Planck Institut für biologische Kybernetik, Tübingen. Culture on cheese or meat, for recording of the locomotor activity water and a lump of sugar in a Petri dish are sufficient.

- *Neurospora crassa*, red bread mold, Ascomycetes.

- *Nymphaea* water lilly flowers.

- *Oenothera* evening prime rose often in gardens.

- *Oxalis regnellii*, wood sorrel, Oxalidaceae, botanical gardens. For propagation put bulbs in garden soil or pith soil.

- *Paramecium*, Hymenostomatida. Found in large amounts in hay covered by water for a few days. Departments of Zoology.

- *Pharbitis nil*, morning glory. Seeds from the Maruthane Trading Company, Tokyo.

- *Thalassomyxa australis*, marine naked amoeba, Institut für Zoologie, Universität Tübingen, culture in seawater with marine algae (see *Amphiprora*, *Dunaliella*, *Chlorella*) in glass bowels. Light-dark-cycle 12:12, 15 to 22^0C.

- *Trifolium repens*, white clover, Leguminosae, from meadows, lawns, road sides.

Index

Glossary

Remark: in *italics* latin names and references to other entrances in the glossary

agar or agar-agar, gelatine like product (polysaccharide) of red seaweeds. Used as solidifying component for culture medium. Absorbs as much as 20 times its weight water.

actogram graphic presentation of locomotor activity of animals

Amphiprora diatome

amyloplast Organell (leukoplast) of plant cell, in which sugar is converted into starch

apex tip of shoot of higher plants from which all the tissue of the stem arises

Arrhenius-equation desribes effect of temperature on velocity of a chemical reaction. Basis for calculating reaction rate constants

arthropod member of the largest phylum of animal kingdom. Largest group of this phylum are insects. Furthermore *Chelicerata* (spiders, scorpions, ticks, mites), crustaceae (shrimps, crabs, lobsters, crayfish, sand fleas) and *Trilobita*. Bilaterally symmetrical, segmentated body with exoskeleton

autocorrelation measure of how strong a momentarious value correlates with a later one

***Avena sativa*,** oat, cereal with edible, starchy grains, widely cultivated in temperated regions of the earth. Family *Poaceae*

background noice statistical deviations of recorded data

bandpass filter filter which passes frequencies between two border frequencies only

Belousov-Zhabotinsky-reaction an oscillating chemical reaction described by B. Belousov in 1958 and studied especially by A.M. Zhabotinskii

binary numerals used in the binary system are two distinct symbols only, 0 und 1. Used in computer devices

bract(s) modified leaves intermediate between the calyx (the outermost of the floral envelopes) and the normal leaves

caffein nitrogenous organic compound of the alcaloide group. Purin derivate trimethylxanthin. White powder. In tea, coffee, cacao and other plants

CAM see *crassulacean acid metabolism*

carbohydrate member of organic substances that include sugars, starch and cellulose. General formula $C_x(H_2O)_x$

Cestrum night jasmin, nightshade family *Solanaceae, Cestroideae.* Shrub with few seeded berries

chitin white horny substance which forms the outer skeleton of insects, crustaceans, and the cell wall of fungi. Formula $(C_8H_{13}NO_5)_n$, a complex carbohydrate with molecular weight of 400 000 that is derived from N-acetyl-D-glucosamine. Similar to the cellulose molecule.

Chlorella genus *Chlorococcales* of green algae, in fresh or salty water or soil. Spherical cubshaped *chloroplast.*

chloroplast cell organell for *photosynthesis*

chronobiology describes and studies the time structure of organisms

circadian cycles of about 24 hours in organisms

circumnutation climbing, circular or pendulum like movements of plants or plant organs. Based on unequal growth of different flanks

Clunio marine midge (chironomid), insect

cockroach or roach. Primitive, often large sized winged insect of the order *Blattaria. Blattoidea.* Usually found in tropical or other mild climates.

coleoptile protective sheet which covers embryonic leaves of grasses during germination

colloquium scientific talk, meeting of scientists and students

complex demodulation *time series analysis* method to determine the *period* length and phase position of data. Also usable for data sets with non-stationary periods.

correlation measure of association between two or more *variables* and its mathematical description. Correlation coefficient between -1 and +1 (0: no correlation)

cotyledon first leaves to appear after germination

Crassulaceae stonecrop or orpine family of perennial herbs or low shrubs. Native to warm and dry regions of the earth. Thick leaves. Order *Rosales*

Crassulacean acid metabolism or diurnal acid metabolism. Special mechanism in many succulent plants to fixate carbon dioxide ('CAM'-plants)

cuticula membrane lamella covering the outer walls of *epidermis cells*

cuvette small container out of glass or plastic material

cyanobacteria bacteria with bluegreen pigment which *photosynthesize*

daylength ,critical length of the light period in a 24 hour day at which 50% of a *photoperiodic reaction* has occurred. Example: at a critical daylength of 11.5 hours of a particular *shortday plant* half of the experimental plants would flower, at shorter light periods more, at longer less. At the critical daylength of a particular *longday plant* half of the experimental plants would flower,

at shorter light periods less, at longer more.

daily periodicity Periodic process with period length of 24 hours. See also *circadian*.

daily rhythm rhythm found in organisms the period length of which is synchronized to 24 hours by 24 hour time cues (*Zeitgeber*)

Desmodium tick trefoil, *Fabaceae,* Indian telegraph plant

diapause spontaneous interruption of development of certain animals (especially insects) for a certain time span. Marked by reduction of metabolic activity. Serves to survive unfavorable conditions of the environment. May occur during any life stage

dielectricum Non-conductor with high specific resistance. Isolator in condensors

dielectricity constant indicates how much the capacity of a condensor is increased if a material with dielectric properties is brought betwee the condensor plates

digitizer see frame grabber

digitizing transformation of analog data in binary information needed as input for the computer

Discette, disk magnetic data storage for disk drive of computer

Drosophila vinegar fly or fruitfly, genus *Drosophilidae*, *Diptera*, Insect

Dunaliella *Dunaliellaceae*, *Volvocales*, unicellular flagellated green alga

endogenous caused by internal reasons

endoplasmatic reticulum 'ER', intracellular, heavily branched membrane system of all eukaryotes

enthalpy sum of the internal energy and product of pressure and volume of a thermodynamic system. There is free and bound enthalpy

epidermis outermost layer of cells covering the different plant parts. With its waxy cuticle it provides a protective barrier against mechanical injury, water loss and infection.

erythrocyte red blood cell, component of blood which give it the characteristic colour. Circulates in the blood and its hemoglobin transports oxygen from lung to tissue. Without nucleus in humans

Euglena alga, single-cell protozoa, one or two flagella, spindle shaped, usually green, commonly found in stagnant water

eukaryote cell or organisms that possesses a clearly defined nucleus. Eukaryotes have nuclear membrane, well defined chromosomes and other organelles. All other organisms belong to *prokaryotes*

evolution, theory of, postulates that the various types of organisms have their origin in other preexisting types and that the differences are due to modifications in successive generations

Exacum affine the Persian Violet, *Gentianaceae*

exhaustor gadget used here to suck up *Drosophila* flies made of a glass tube, net and rubber tube. Facilitates transfer of single flies

fairy ring circular appearance of fruiting bodies of fungi, caused by radial growth of the *mycelium*

feedback in biology: a response within a system that influences the continued activity or productivity of that system. Control of a biological reaction by the end product of that reaction

ferroin 1,10 phenanthrolin-ferrisulfate-complex, redox indicator

filter, digital mathematical procedure to filter *time series*

flow meter for recording and controlling the flow of fluids and gasses

forcept elastic plier to grabb small objects

Fourrier analysis determination of the harmonic components of a *time series*

frame grabber printed circuit of a computer for *digitizing* analog recorded values

freerun course of biological rhythms without synchronising *Zeitgeber*.

frequency folding partitioning of a *time series* in parts which correspond to the *period* length of the recorded event. In this way sequential cycles are positioned underneath each other. Simple and sensitive procedure for determining the period length

frq-mutants *mutants* of *Neurospora* with changed *circadian* period length

fructification fruit formation. Here: formation of fruiting bodies in club fungi. See also *fairy ring*

fruitfly see *Drosophila*

gravitropic pendulum (same as geotropic pendulum), unequal growth of flanks of plant tips and tendrills which is induced by gravity and leads to pendulum like movements

grunion *Leuresthes tenuis*, Atherinidae, fish. Eggs are fertilized and deposited on the beach at certain times of the lunar and tidal phases

guard cell special, often beanlike epidermis cell. Two guard cells surround a *stoma*

hamster, Sibirian *Podopus sungorosus,* dsungarian hamster. Order *Rodentia* of Family *Critecidae.* Short tailed with cheek pouches for carrying food.

hamster, Syrian *Mesocricetus auratus,* golden hamster. Order *Rodentia* of Family *Critecidae.* Short tailed with cheek pouches for carrying food.

hamster pellet hamster food pressed into small pieces

Hellrung-system *infrared-lightbeam* system for recording the locomotoric behaviour of animals

humidity sensor electric sensor to record humidity

hyperpolarisation increase of the negative membrane potential of cells

hypocotyl part between *cotyledons* and root of plants

hypothesis statement without contradiction, which could be true

indolyl acetic acid (IAA), plant hormone for e.g. elongation

infrared-lightbeam infrared emitter (infrared light emitting diode) and -receiver (phototransistor). An interruption by an animal leads to an electrical signal

inhibitor substance which inhibits a chemical reaction or a physiological process

interface printed circuit used to convert signals

inverted light-dark-cycle in an inverted 12:12 hour light-dark-cycle the normal light period is replaced by darkness, the dark period replaced by light

isabgol cheap agar substitue from the seedpods of an indian plantain

jetlag disturbance of the circadian system after jet plane travelling through time zones (to the west or east). It takes several days until the human circadian system is adapted to the new conditions

Kalanchoe panda plant, genus of succulent plants of the stone crop family *Crassulaceae*

larva juvenile stage of certain animals that undergoes changes in form and size to mature into the adult

larval molding shedding of the old *cuticula* when changing from one larval stage to the next

latency time time from the stimulus until the reaction is first seen

lateral leaflet lateral leaflets of pinnate leaves

legumes see *Leguminosae*

Leguminosae plant family *Fabales*, subfamily *Fabaceae* (*Papilionaceae*), largest group of legumes

lens piece of glass or other transparent substance to form an image of an object by focusing on it. Compound lenses are used in cameras, microscopes, telescopes. Lense system of an optical apparatus facing the object

light beam see *infrared-lightbeam*

light, polarising the waves of this light vibrate in a specific direction rather than randomly in all directions as in ordinary light

longday day with long light period and short dark period (e. g. 13 hours light, 11 hours darkness)

longday plant flowers in *longdays* only. See also *daylength, critical*

Lotka-Volterra model mathematical desciption of a *predator-prey* system by Lotka (1925) and Volterra (1926)

lunar rhythm Rhythms with periods in the range of a lunar cycle (28 days). See *semilunar rhythm*

mammals member of the *Mammalia*, class of vertebrates. Young are nourished with milk of the mother. Hairy, warm blooded, four limbed.

manuscript document submitted for publication

Marantaceae prayer plant, family of monocotyledonous plants of the order of ginger (Zingiberales) native to moist or swampy tropical forrests particularly in the Americas

matrix rectangular scheme of elements, here: division in horizontal and vertical fields

maximum entropy spectral analysis *time series analysis* method for determining the period length of data sets which can be rather short

mean value designation of a value *overlinex*, to which n given values are appointed to according to certain rules. It lies between the largest and the smalles value. Arithmetic, geometric, harmonic and quadratic mean value

mean value, gliding formation of average values of a series of values which are shifted by one value after each averaging

menstruation periodic discharge from vagine of blood, secretion, and disintegrated tissue that had lined the uterus of women

mesophyll parenchymatous tissue of leaves (pallisade- and spongeous parenchyme)

Mimosa sensitive plant, member of a genus in the *Mimosaceae* family, native to tropical and subtropical areas at the northern and southern hemisphere.

Modus-program special program for model simulation

mold mass of *mycelium* (masses of vegetative filaments) produced by various fungi

molecular genetics Subarea of genetics, in which the structure and fuction of genetic information is studied on the level of molecules (nucleic acids, proteins).

monograph a written account to a single subject

morning glory *Pharbitis nil*, twining plant in genus *Ipomoea*, *Convolvulaceae*

motor cells cells of the *motor tissue* of the *pulvinus*

motor tissue conglomerate of special *motor cells* which allow the *pulvinus* of plant leaves and -stalks to move

multimedia presentation Presentation which uses different technical media

multiplexer way of signal transduction in which each channel is used several times (there are time- and frequency-multiplexer)

Musca domestica common housefly (*Diptera*, *Muscidae* family)

mutant an individual strain or trait resulting from *mutation* of the wild type

mutation a relatively permanent change in hereditary material

mycelium mass of branched, tubular filaments (hyphae) of fungi

Neurospora red bread mold. Fungus of the *Ascomycetes* class, *Xylariales* order. Often found on wet bread

nipagin 4-hydoxibenzoic acid-methylester, fungicid (kills fungi)

nitrogen fixation process of binding nitrogen of the athmosphere and converting it into protein

OCR optical character recognition: Program for the recognition of characters and its conversion into computer readable signs

Oenothera evening prime rose, *Onagraceae* family, *Myrtales* order

organ a group of tissues in an organism wich performs a specific function. Consists of certain kinds of tissue and is arranged in a certain way

ovary germ gland of females. Harbours, nurtures, and guides the development of the egg. Furthermore important endocrine functions.

Oxalis wood sorrel, *Oxalidaceae*

palolo worm *Eunice viridis*, annelide in corall reefs of polynesia

paramecium Pantoffeltierchen, free living protozoon of *Holotricha* (order) of *Hymenostomatida*. Covered with fine hairlike filaments (cilia) that beat rhythmically to propell them

parameter a *variable* for which the range of possible values identifies a collection of distinct cases in a problem

per-mutants of *Drosophila melanogaster* without circadian rhythm of locomotor activity or eclosion (per^0) or with changed *period length* per^l (shorter), per^s (longer)

period period length, time after which a certain *phase* of an oscillation occurs again

periodicity in regular distances reoccuring events

periodogram analysis mathematical procedure to determine the *period length* of an oscillation

Petri dish dish with nutrient medium for cultures of microorganisms after R. J. Pétri (1852-1921), bacteriologist

Pharbitis morning glory, twining plant in genus *Ipomoea*, *Convolvulaceae*

phase see phase position

phase diagram or phase plot: graphic display of two *variables* plotted on x- and y- axis respectively

phase position particular state in a cycle of changes. See also *Period*

pheromone substance for the chemical communication between organisms of a species. It is effective in extremely low concentrations

phosphoinositol cycle special cycle in cells for calcium release

photoelectric method recording method with electric light beam. See *Infrared-lightbeam*

photoperiod (1) length of the light period of a day (2) ratio between the

duration of the light and the dark period of a day

photoperiodic induction induction of a physiological reaction by the day length

photoperiodic reaction physiological answer of an organism to a *photoperiodic* treatment

photoperiodism Behaviour of an organism in respect to daylength. See *shortday*, *longday*

photosynthesis synthesis of organic compounds with the aid of light, especially formation of carbohydrates from CO_2 and H sources (as water) under the catalysis of chlorophyll in *chloroplast* containing tissue of plants

pH value potentia hydrogenium (latin), hydrogen ion content of a solution, characterizes the acid, neutral oder basic character. pH 7 means 10^{-7}g H-Ions in a solution

physiological darkness light which has in the particular physiological process no effect. In this way the process can be observed without influencing it. see also *safety light*

pigment colouring matter in organisms

pipette calibrated thin glass tube for measuring volums

pixel any of the small discrete elements that together constitute an image (as on a monitor screen)

plasticin moulding made out of caolin, zinc, chalk, pigments, waxesand oils

point of inflection point of a curve in which the bending changes its sign

polarisation of light see *light, polarizing*

polynome curve mathematical expression in which the single memers are connected with each other by + or − only

polyurethan light polymeric material consisting of alcohols and isocyanates

population sum of individuals of a species in a certain area, They are genetically connected with each other over several generations

potential measure for the energy at a certain point in a field (e. g. an electric field)

practical course teaching unit for providing practical skills

predator-prey-modell describes the mutual interaction between the *populations* of predators and prey

primary leaf the first leaf following the *cotyledons*

procaryote all organisms (bacteria, bluegreen algae) with nucleus equivalent or nucleoide instead of a true nucleus as found in *eukaryotes*

processor the part of a computer that operates on data (central processing unit)

propionic acid stinging fluid with antimicrobial effect

protocol detailed description of a scientific experiment, treatment or procedure

pulvinus cushion, a mass of large thin walled cells surrounding a vascular strand at the base of a petiole or petiolule and fuctioning in turgor movements of leaves or leaflets. Found especially in *Fabaceae, Oxalidaceae, Maranthaceae.*

puparium a rigid outer shell formed during metamorphosis of insects from the larval skin that covers and protects a pupa

pupation to become a pupa. In the pupal stage the larva metamorphoses (changes) into the adult insect

Q_{10}**-value** measure of the temperature dependence of a process. Calculated from

$$Q_{10} = (\tau_1/\tau_2)^{10/(t_2-t_1)}$$

where τ_1 period length at temperature t_1 and τ_2 period length at temperature t_2

reaction, radical reaction involving radicals i.e. group of atoms bonded together to an entity

recording continuous measurement of physical entities

rectal temperature temperatur in the anus

repolarisation to restore the *hyperpolarised* condition

rhizome bulb more or less thickened rhizome, which are clearly different from roots

rhythm a regularly recurrent quantitative change in a *variable* biological process. See also *oscillation*

***Robinia pseudacacia*,** locusts. *Fabales*, *Leguminosae* family

ROM-port input into a computer for read only memory ('ROM') storage containing special purpose programs which can not be altered.

run-test mathematical procedure to test whether the values of a *time series* are randomly distributed or not

safety light *physiological darkness*, light without effect in a special physiological process. It allows to observe a process without interfering with it

sample a representative part of a larger whole or *population* especially when presented for inspection

scanner device to scan point- or linewise objects such as images or text and store it in binary form. These informations can be transferred and worked at with a computer

seed propagative plant structure: fertilized ripened ovule of a flowering plant containing embryo, seed shell and usually also nurishing tissue. Capable of germinating to produce a new plant

semilunar rhythm rhythm with periods of 14 days, corresponding to half the lunar cycle. *Lunar rhythm*

seminar Teaching method at universities. Introduces in autonomous (independent) scientific work

shift work working hours are divided in two or three shifts (early, late and night shift)

shortday day with short light period and long dark period (e. g. 11 hours light, 13 hours darkness). See also *photoperiodism*

shortday plant plant which flowers under short day conditions only. See also *daylength, critical*

signal-average method for *time series analysis*, see in chapter *'display and analysis of time series', 'display of actograms'.*

simulation technique that reproduces systems, actual events and processes by using models, often involving highly complex mathematical procedures.

singlet certain ground state of a molecule

sleep movement periodic vertical movement of leaves. See also *pulvinus*

smoothing mathematical procedure to reduce the deviations of recorded data. The smoothing window determines the kind of smoothing. See *gliding average*

snowhare *Lepus timidus*, mammal in forests of the northern hemisphere and the alps

Solanaceae night shade or potato family. Order *Solanales*, 95 genera with at least 2400 species, many of considerable economic impact such as the tomato, potato, tobacco

spacelab ESA (European Space Agency) build space station providing room and facilities for research in space.

spectral analysis method to measure the spectrum of a substance with *spectral photometer*

spectral photometer see *spectral analysis*

spore asexual germination- and propagation cell, often of considerable resistance against unfavourable conditions

standard deviation statistical measure of variability (dispersion or spread) of any set of numerical values about their arithmetic mean

standard error s*tandard deviation* devided by the the root of n of the single cases

stoma plural stomata, microscopic openings or pores in the *epidermis* of plant leaves and young stem. They provide for the exchange of gases between the outside air and the branched system of interconnecting air canals within the leaves. Surrounded by two *guard cells*

structur diagram Presentation of the structure of a dynamical process in form of a modell

subsidiary cell cell neighbouring the *guard cell* of *stomata*, which are different from the normal *epidermal cells*

suction force positiv value of the negative *water potential* ($S = -\Psi$).

sulfuric acid H_2SO_4, strongly hygroscopic and agressive fluid

suncompass orientation ability of organisms to navigate by using the

sun direction (directly or by the *polarization* pattern of the sky). The daily and annual change of the sun is thereby taken into account.

synchronisation condition of two or more rhythms which have the same period length due to interactions

synchronous culture cell culture which divides at the same time

Syrian hamster see *hamster Syrian, Mesocricetus auratus*

system-dynamics complex network with one or more feedback loops in which the effects of a process return to cause changes in the source of the process

Tamarindus Tamarind, *Caesalpiniaceae*, tropical tree in Asia

telegraph plant see *Desmodium gyrans*

temperature compensation the period length of *circadian* rhythms is not or only slightly dependent on the temperature of the environment

testis or testicle. Male gonads. Contain germ cells that differentiate into mature spermatozoa, supporting cells (Sertoli cells) and testosterone producing cells (Leydig cells)

tetraethylammoniumchloride $[(C_2H_5)_4N]^+Cl^-$, inhibitor of potassium channels

Thalassomyxa marine naked amoeba

theophyllin purin-alcaloidal (methylxanthine) from leaves of tea plant and other plants. Chemically related to *caffeine* and theobromine

thermodynamics fundamental science of energy and its transfer from one place to another

tibia part of insect leg. This consists of *coxa* (proximal to body), the small *trochanter*, *femur*, *tibia* and *tarsus* (with several segments and claws)

tidal rhythm periodic biological fluctuation in an organism that corresponds to and is in response to tidal environmental changes (regular ebb and flow of oceans). Two high tides and two low tides occur each day 24.8 hours apart. Thus the period of the tidal rhythm is around 12.4 hours.

time-diagram graphic display of a *variable* (y-axis) as a function of time (x-axis)

Timesdia program for the analysis of timeseries, written by W. Martin

time series serie of data (usually equidistant) of a *variable* during a certain time span

time series-analysis statistical analysis of *time series*, in order to determine for instance trend, influences of random events and periodicities

transpiration loss of water mainly through the *stomates* of plant leaves

trend here: tendency of a *time series* in a certain section

trend removal mathematical removal of a *trend*. In this way a *periodicity* can be better recognized if originally superimposed by a trend

***Trifolium repens*,** clover, *Fabaceae*

turgor hydrostatic pressure caused by water in the *vacuole* of plant cells. Turgor is the cause of rigitity in living plant tissue

ultradian rhythm oscillation with period shorter than *circadian* oscillations, i.e. in the range of minutes to about 8 hours

vacuole cytoplasmic organelle performing functions such as storage, ingestion, digestion, excretion and expulsion of excess water. In plant cells large central space that is empty of cytoplasm, lined with membrane and filled with fluid

variability fluctuation, deviation from the norm

variable factor which can take different values during the course of observation. The *independent variable* is plotted on the x-axis, the *dependent variable* on the y-axis

vegetative asexual reproduction. No union of sperm and egg occurs

vertical migration up and down movement of small organisms in rivers, lakes and seas

Vicia faba broad bean, *Fabales*, *Fabaceae*

voltage recorder device to continuously record voltages or other values which can be converted into a voltage

World Wide Web part of the internet, an electronic information system

tilting cage cage which is balanced in such a way as to change its position if the animal is moveing. Contacts at the floor of the cage sens the movements

time-lapse recording with movie- or videocameras in a lower frequency as normal. The film runs therefore faster as in reality

Zeitgeber (german) time giver, time cue. It *synchronizes* a biological rhythm

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