

Bio-Calendar

The year in the life of plants and animals

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Dedicated to Erwin Bünning and Georg Melchers

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As long as the earth endures,
seedtime and harvest,
cold and heat,
summer and winter,
day and night
will never cease

1 Mose 8, 22



Introduction

I lie in a bed of the sleep laboratory of the University of Basel, hooked up with cables for all kinds of measurements at the head, body, extremities. In a study of the division of Chronobiology it is planned to find out how the sleep of elder and younger participants differs. I use the time between the different tests and the regular simple food uptake to write this book.

For many years I have studied rhythmic processes in different organisms. Already my doctoral thesis was concerned with a topic from this field. In addition to my scientific work in this area I offered each term lectures, seminars and courses to students. Laymen, for whom I lectured about my interests, found much interest in this field and its questions and problems. I began therefore to write some books on different parts of this field.

This book is one of it. It is written for those people who like to think about nature and its many riddles and who want to ask questions to nature. Scientists do that by observing accurately, putting there questions in words and thinking of experiments which pry secrets from nature. At the end of the book (chapter 8) I propose experiments which on the one hand demonstrate the methods used and which on the other hand point out the many unanswered questions which can be clarified by observing, contemplating and experimenting.

The government and thus the citizen spend a lot of money for research. The public has therefore the right to hear about the results of these studies. And the scientists should feel obliged to tell about their work

in such a way that the interested layman understands, how research is done and how it proceeds. I hope that I will be successful in pursuing this goal.

Introduction

1 How organisms find their way in the course of the year

Most people of the Northern and Southern hemisphere are very familiar with the seasons of the year. To know, whether it is summer, winter, fall or spring, we do not need to look at a calendar. Nature changes so profoundly during the course of a year, that already as children we know the typical appearance of the season: Coldness and bare trees in the winter, heat and green woods in the summer, colored leaves in the fall and orchards in full bloom during springtime (see figure at begin of book).

It is more difficult for people living in tropical areas close to the equator. There the seasons do barely differ. It is always warm, wet and green, the trees carry flowers and fruits throughout the year. If one wants to know the time of the year there, a calendar is needed. It divides the year in twelve months with 30 respectively 31 days (exception: February, which is shorter). On the Northern Hemisphere the summer begins on the 21st of June and lasts until the 21st of August, the winter begins on the 21st of October and lasts until the 21st of March, and spring and fall lie in between. On the Southern Hemisphere the summer begins on the 21st of October and lasts until the 21st of March, that is the time during which winter prevails on the Northern Hemisphere. The winter of the Southern Hemisphere begins on the 21st of June and lasts until August 21, the summer time of the Northern Hemisphere.

In the moderate and higher latitudes not only the average temperature changes dur-

ing the year, but also the length of the daily light period (*daylength*). In fact, they are much more reliable as compared to the temperature. Figure 1.1 shows, how the daylength changes during the course of the year at a particular location (here Tübingen near Stuttgart, Germany). If we want to know the time of year at a particular day, we need only to determine the length of the light period, find it on the y-axis on our diagram and go down to the time axis to find the day¹.

Many organisms are able to recognize the time of the year. They do so by either using an internal annual clock or by measuring daylength, which changes during the course of the year. How this might function will be discussed in this chapter.

Many organisms are able to measure daylength. They possess an annual calendar. In this book it will be shown in some examples, how widespread this calendar is. In the next chapter a dinoflagellate is presented, which in addition to being able to measure daylength possesses an annual clock. Annual clocks exist also in plants (chapter 3) and animals (chapter 6 and 7). In many plants specific daylengths are necessary for inducing flowering. *Kalanchoe blossfeldiana* serves as an example (chapter 4). Subterranean tubers are often formed in shortdays, onions under

¹This daylength occurs twice a year, for instance in the spring and also in the fall. However, in the spring the days will become longer, and in the fall shorter.

1 How organisms find their way in the course of the year

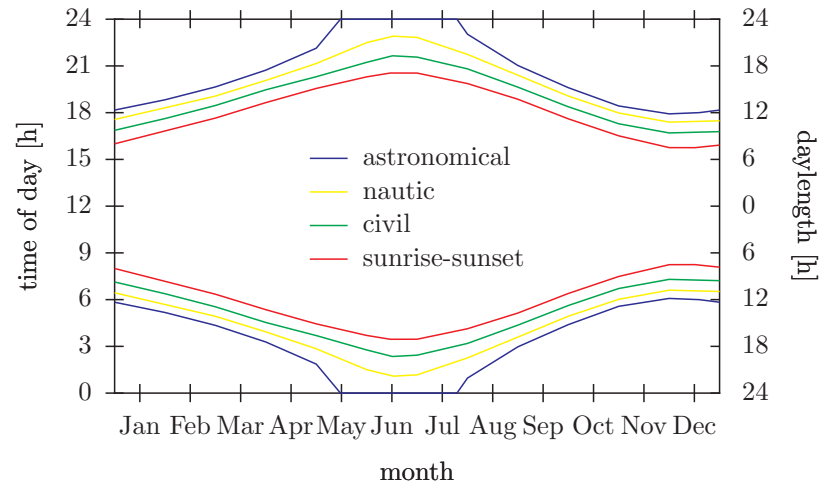


Figure 1.1: How the daylength changes during the course of the year. Here as an example for Tübingen, 42° northern latitude, 35 km south of Stuttgart. In the winter the civil twilight begins in the morning shortly after 7 o'clock and ends in the evening around 17 o'clock. In the summer, however, civil twilight begins already at 3 o'clock in the morning and ends at 21 o'clock in the evening. The longest day is thus almost 18, the shortest 9 hours. Figure from data in www.GeoAstro.de

longday conditions (chapter 5).

2 The smallest calendar

The *Dinoflagellate* *Alexandrium tamarense*¹ lives at the surface of oceans². In the fall cysts are formed, which sink to the bottom of the sea where they outlast the winter. In the spring the algae hatch from the cysts and ascend with the help of flagella to the surface of the sea. There they grow and propagate³

The algae use a built-in annual clock as a calendar. This clock signals the time of the year at which the algae eclose from the cysts and return to the surface of the ocean.

Cysts are formed also in another species, *Lingulodinium polyedra*. However, here the right time is determined by the daylength. If the days shorten in the fall, the algae sink to the bottom and form cysts. An internal daily clock measures the length of the light period. This clock controls also other processes in the algae such as the bioluminescence during the night, the division of the cells, and photosynthesis. Melatonin and 5-methoxytryptamine are the messengers of the dark period.

¹old name is *Gonyaulax tamarensis*

²Japan, Europe, northwestern America and other oceans.

³asexual propagation by division. Sexual cycle by anisogame mating types. The gametes adjoin each other and fuse to planozygotes which form cysts.

2.1 Luminous algae

Perhaps you have bathed already by night in the Mediterranean sea or in other warm seas. At this occasion you might have experienced how thousands of small luminous spots fuse to a luminous cloud while jumping into the water. The reason for this spectacular firework are small dinoflagellates such as *Noctiluca* in the Mediterranean sea, *Alexandrium tamarense* in the Gulf of Main and *Lingulodinium polyedra* (figure 2.1) in the Pacific.

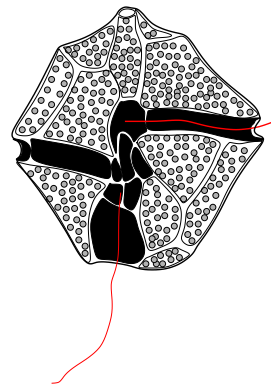


Figure 2.1: *Lingulodinium polyedra* is a *Dinoflagellate* with an armour consisting of cellulose plates. In both, a transverse and a longitudinal groove a flagella is found which serve to propel and turn the alga. Seen from below. Diameter 40 μm . Drawn from the author after an illustration of [Schussnig \(1954\)](#) and an electron microscopic image of [Hastings \(2006\)](#)

If a culture of *Lingulodinium polyedra* is kept in a flask with sea water, a faint mysterious blueish glow can be seen during the

2 The smallest calendar

night (figure 2.2). Its intensity depends, however, from the time of the night. If the culture is kept in the dark also during the day, the glow is absent.

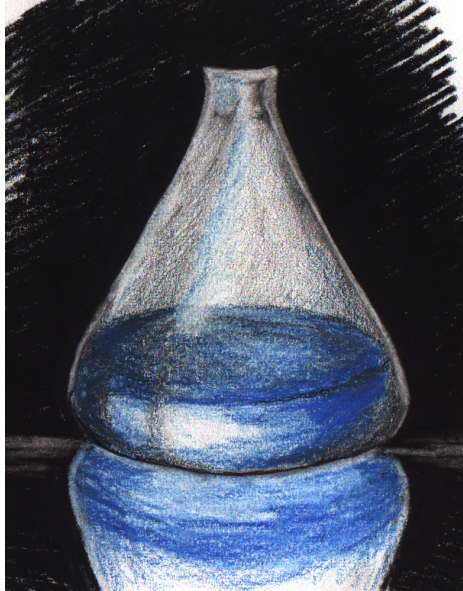


Figure 2.2: *The bioluminescence of Gonyaulax polyedra-algae in a flask was photographed after shaking the culture. Painted from the author after a photography of Taylor in Hastings (1994).*

The reason for it might be, that our eyes are during the day less sensitive to weak light as they are during the night. We see during the day with cones, small light sensitive cells in the retina, the light sensitive layer of our eyes (see <http://webvision.med.utah.edu/>). There are three different types of cones, which are responsible for blue, red respectively green light. During the night the rods are used for the weak light of the moon and stars. They can not detect colors, but are much more sensitive.

How can we find out, whether the algae do not emit light during the daytime or whether our eyes are less sensitive to light?

We could, for instance, adapt our eyes for a long time to darkness. If we are still not able to see luminescence of the algae, they are not likely to emit light during daytime. If you are still sceptical, because your eyes might not have adapted well enough to darkness, you could use a cardboard with a small hole (made with a needle) in front of a flashlight. Cover the hole with several sheets of paper, until the light passing the hole is as weak as the bioluminescence of the algae during the night. If you use this setup while looking in the dark at the culture during its daytime and you can see the weak flashlight, your eyes are well adapted and the algae are not glowing.

Still better is to use a sensitive instrument to measure the emitted light. With it you can directly measure the light emitted by the algae at their day- and their night-phase. Such devices are, however, expensive. An automatic recording device for the bioluminescence of *Lingulodinium* is shown in figure 2.3.

There is, however, another method to answer our question. If the algae do not emit light during their day phase, even if kept in constant darkness, one could try to reverse the bioluminescence rhythm. For this purpose we would illuminate the cultures during their night phase and keep them in darkness during their day phase (figure 2.4). After a few days the rhythm is shifted: The night phase of the algae is now at our day, and their day phase at our night. We are thus able to observe the algae during their day phase at our night phase, when our eyes are most sensitive to light. We should see their glow, if they do emit light during their day phase. But that is not the case. Thus the algae do not glow during their day phase, and it is not due to our eyes being insensitive.

Apparently the algae possess a daily

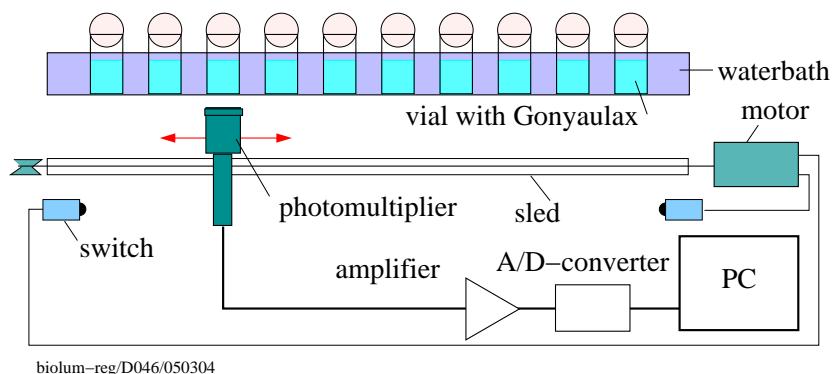


Figure 2.3: Device for recording the bioluminescence of *Lingulodinium* in different vials with a photo-multiplier. The sensor is moved automatically with a sled from one vial to the next and measures the intensity of the bioluminescence. After having reached the end, a switch reverses the direction and the photo-multiplier is driven to the first vial. After one hour a new measuring cycle begins. The analog data are digitized and stored in a computer. The vials are kept in a water bath with constant temperature.

rhythm with strong glow during the night and without glow during the day. Furthermore this rhythm occurs also, if the light-dark cycle is absent and the algae are all the time in darkness. It seems as if in these tiny marine algae an internal clock is working, which is responsible for the periodic glowing (overview Roenneberg and Rehman (1998)).

However, it is even more magic: If we shake the flask with the culture of algae in the dark, a quite bright light consisting of thousands of light flashes is emitted. This is, what we see, if we jump in the warm sea containing luminescent dinoflagellates. As with the glowing this flashing is rhythmic too: Shaking the culture at different times during the day- or night-phase shows, that the algae emit light mainly during the night phase. The maximum of light emission occurs, however, at a slightly different time as compared to the maximum of the glow rhythm. Furthermore, the period length of the two rhythms differs (figure 2.5).

These algae possess internal circadian

clocks, which control flash and glow rhythm. They furthermore control other processes in a circadian way, such as photosynthesis and cell division (figure 2.6). Furthermore the algae accumulate during the night phase in clouds, whereas they are uniformly distributed in the water during the day phase.

But what has all this to do with our bio-calendar? We will come back to it in section 2.3. Before we will get to know another phenomenon which can be observed in these algae.

2.2 How luminescent algae hibernate

If *Lingulodinium*-algae are kept in a 10:14 hour light-dark cycle instead of a 12:12 hour change (a daylength occurring during the fall, when the daylength shortens), the algae shed their flagella and sink to the bottom of the vials (figure 2.7). The cells hatch from their armored shell and form a

2 The smallest calendar

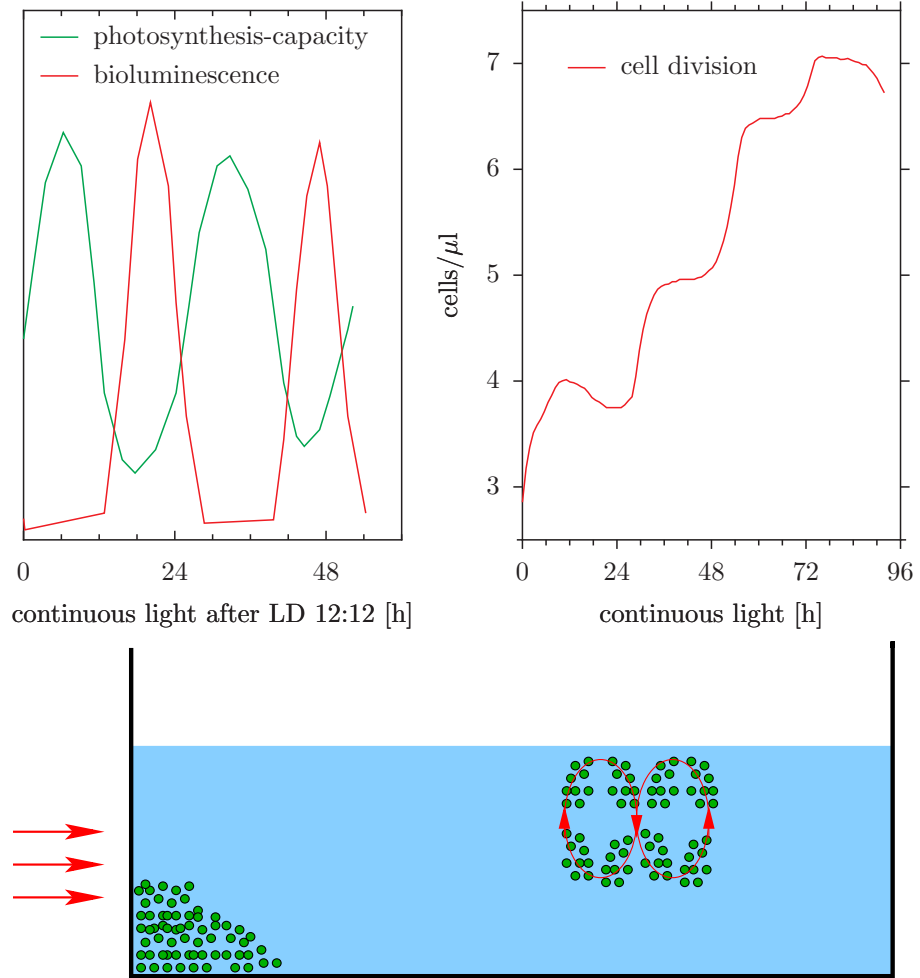


Figure 2.6: *Photosynthesis-rhythm (top left, green curve), bioluminescence (top left, red curve), cell division rhythm (top right, number of cells increases in daily steps) and cell accumulation (bottom, cells in day phase at water surface with up- and down movements - red rings and arrows, during the night at the bottom and side from where the light comes)*

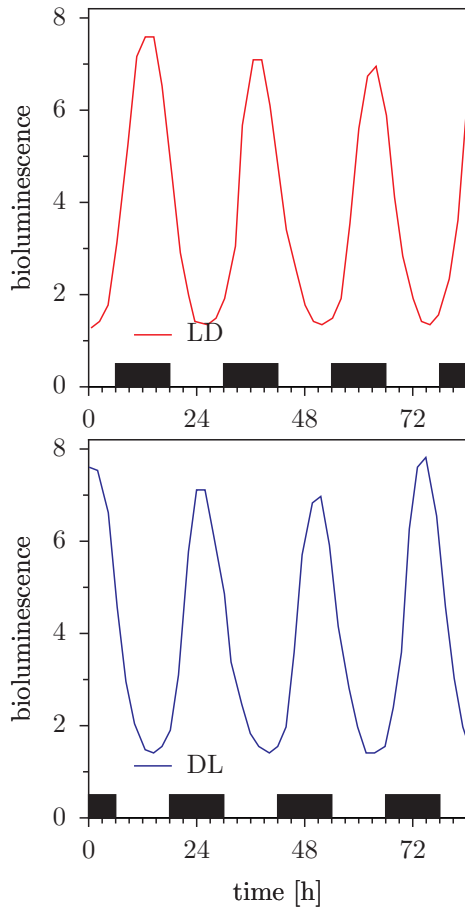


Figure 2.4: *Lingulodinium*-culture in a normal (top, blue) and in a reversed light-dark cycle (bottom, red). Here the *Lingulodinium*-culture was illuminated during the night and kept in the dark during daytime. After a few days the rhythm of the algae has been shifted. Their night is now during our day, their day during our night

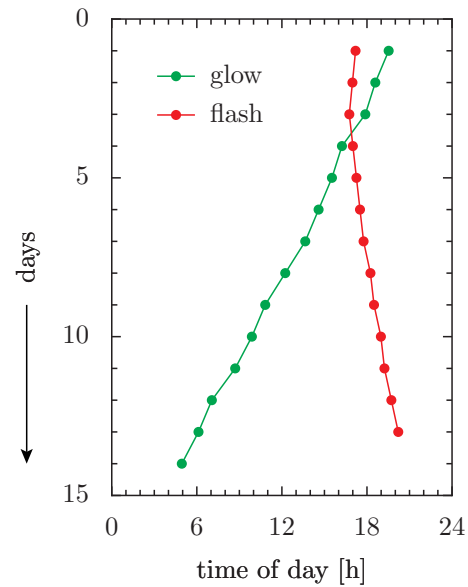


Figure 2.5: *Shaking a Lingulodinium*-culture in regular intervals during fourteen days and plotting the time of the daily maxima of the flash light intensity (red triangles) shows a free-run period of slightly longer than 24 hours. At the same time the glow rhythm of another sample of the same culture was automatically recorded and the time of the maxima of the light intensity plotted as green triangles. Its free-run period is shorter than 24 hours. Glow and flash rhythms are thus driven by two different daily clocks.

2 The smallest calendar

cyst. This is also found in nature. The algae sink to the bottom of the sea and hibernate there. In the spring the algae eclose from the cyst, build the armored shell, form two flagella and reach the surface of the sea.

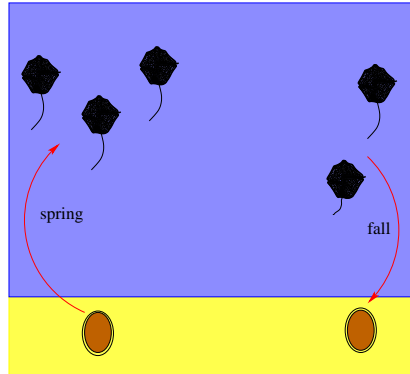


Figure 2.7: In the fall *Alexandrium tamarense* drop their flagella, sink to the bottom of the sea (yellow) and hedge from the armored shell (brown). A cyst shell is formed in which the algae hibernate. In the spring the cells eclose from the cyst, build an armored shell and two flagella

How do the *Lingulodinium*-cells notice, that fall has started and what makes them form cysts?

2.3 How an algal calendar works

The cyst formation of *Lingulodinium polyedra* is controlled by the daylength (figure 2.8, Balzer and Hardeland (1991) and Balzer and Hardeland (1992)). In longday with 11 or more hours light per day the algae reside at the surface of the sea. In shortdays with 10.5 or less hours of light all cells are in the cyst stage at the bottom of the sea. If the daylength is measured by organisms and used to control certain processes or behavior, it is called photoperiodism. De-

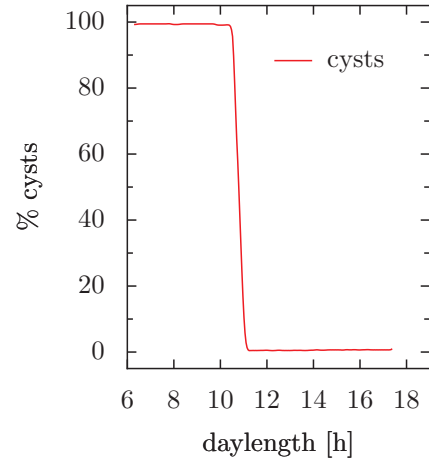


Figure 2.8: In short day the dinoflagellate *Lingulodinium polyedra* forms cysts (left part of diagram). If the light period is 11 hours or longer, no cysts are formed (right part of diagram). The percentage of algae is shown on the vertical axis as a function of the daylength (horizontal axis). After Balzer and Hardeland (1992)

pending on the organism a shortday or a longday evokes the photoperiodic reaction. In *Lingulodinium* it is the short day which induces cyst formation. The algae will sink to the bottom of the sea. During long days, however, the algae are at the surface of the sea, where they grow and divide.

A feature of photoperiodic reactions is, that a short day effect can be canceled if in the middle of the dark periods light is given. It is as if long day was prevailing. This was found also in *Lingulodinium*: No cysts are formed, if the culture is illuminated for two hours in the middle of the dark period. We are thus dealing with a true photoperiodic reaction and not a reaction, which was induced by the amount of light.

For the photoperiodic reaction -that is cyst formation- it is necessary that the temperatures are 16°C or lower. If the sea water is warmer than 16°C , no cysts are formed, in spite of short day. Cysts are, however, formed even in long days,

2.3 How an algal calendar works

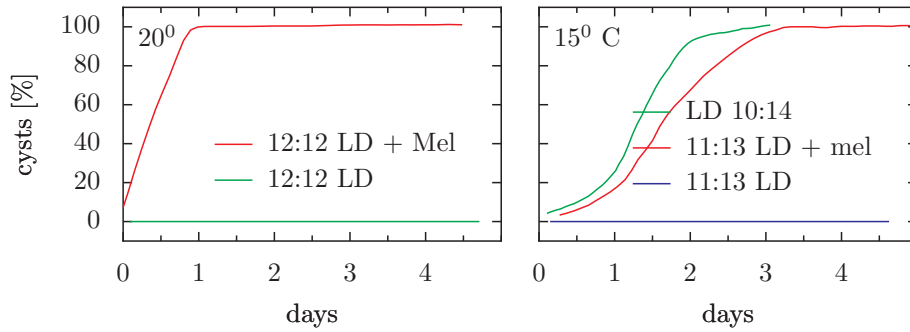


Figure 2.9: *Cyst formation in Lingulodinium with melatonin. Right: In short days (10 hours light, 14 hours darkness) cysts are formed after a few days (green curve), whereas in long days (11 hours light, 13 hours darkness) no cysts are found (blue curve). However, if melatonin is added to the medium in long days (right part, red curve), cysts are formed in the following days. Left: Melatonin allows cyst formation even at 20°C (red curve). At this temperature normally no cysts are formed (green curve). After Balzer and Hardeland (1992)*

if melatonin is added to the medium (figure 2.9). Even more effective is 5-methoxytryptamine. Adding it to the water cysts are formed under continuous light and at 20°C.

In vertebrates daylength is also signaled to the organism by melatonin (see chapter 6). As in vertebrates, the concentration of melatonin fluctuates also in *Lingulodinium* rhythmically during the day (figure 2.10). In the algae as well as in the vertebrates the melatonin-production is maximal shortly after onset of darkness. Melatonin serves the organism as a messenger of darkness. This seems to be true for the unicellular *Lingulodinium* as well as for mammals. Perhaps melatonin has been retained as a dark-hormone during the evolution of organisms.

A number of question remain unanswered. How is, for instance, the daylength measured by the algae?

It turned out, that in vertebrates (see chapter 6) as well as in *Lingulodinium* an internal daily clock is used. And in both

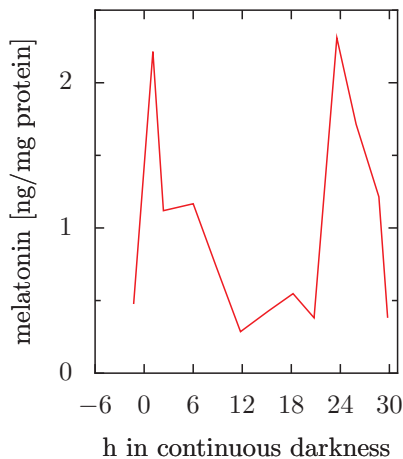


Figure 2.10: *Circadian rhythm of melatonin formation (ng/mg protein) in Lingulodinium polyedra after transfer of the culture from a 12:12 hour light-dark cycle into continuous darkness (at the time 0, abscissa). After Balzer et al. (1993)*

cases the length of the dark period and not the length of the light period is measured. If the night is long enough, melatonin is synthesized and cysts formed. The circadian clock of *Lingulodinium* we got to know already in section 2.1. It controls luminescence, division and accumulation of the cells. Unfortunately it is not yet known how in *Lingulodinium* the night length is measured by this clock. Likewise, it is not known which steps lead finally to cyst formation. It is only known, that melatonin and related substances are able to induce cyst formation.

A final question is, how the algae recognize the end of the winter. This shall be discussed in the next section.

2.4 Dinoflagellates with an annual clock

How do the algae in the cyst stage detect, that winter is over and time to eclose out of the cyst, form an armored shell and flagella and to arrive at the surface of the sea? This has not yet studied in *Lingulodinium polyedra*. There is, however, a related species, *Alexandria tamarensis*, which inhabits the Gulf of Main at the East coast of the United States. How an annual clock causes eclosion from the cyst in the spring was studied in detail by Anderson and Keafer (1987):

Under certain conditions these algae appear in huge amounts (*Algenblüte*) and the population is visible even during the day by the red fluorescence of the chlorophyll ('red tide', figure 2.11). These algae produce a toxic substance. If fishes eat those algae and the fish is consumed by humans, poisoning might occur. To prevent this, fishing has to be stopped during red tides in affected areas. In the Gulf of Main such episodes occur between April and Novem-

ber. During this time the cells are vegetative and move around.

As in *Lingulodinium polyedra* the algae sink to the bottom of the sea in the fall, cast off their armored shell and the flagella and form cysts. They hibernate in the cysts for 2 to 6 months. In the spring the algae eclose from the cysts, form a new armored shell and arrive at the surface of the sea.

Since the algae hibernate in a depth of 100 to 200 meters, they do not get any information concerning the time of the year. Daylight does not penetrate to this deepness and the water temperature does not fluctuate more than one degree. Anderson and Keafer (1987) found, that eclosion from the cyst is controlled by an annual rhythm: If at different time of the year samples from the sediment at the bottom of the sea are brought to the laboratory, the algae emerge from the cysts in an annual rhythm (figure 2.12, top part). This rhythm is also found, if a larger sample collected in August is kept in a refrigerator with 2⁰C and individual samples transferred to 15⁰C at different times of the year (figure 2.12, lower part). A rhythmic eclosion was observed for more than two years. We are thus dealing with a true *endogenous* annual rhythm.

Algal populations in shallow coastal water does not show this endogenous annual rhythm. They might receive periodic annual informations and do therefore not need an endogenous annual rhythm.

Annual rhythms are known also from other dinoflagellates.

In the natural science faculties of universities practical courses are offered in which students learn how to observe, and how to execute and evaluate experiments. In such a course we made also experiments with the bioluminescence-rhythm of *Lingulodinium polyedra*. Parallel to the course a seminar was offered. In the seminar publications of



Figure 2.11: *Aerial photograph of a ‘red tide’ at day time: The density of the dinoflagellates is so huge, that the population is visible by the red fluorescence of the chlorophyll. Painted from the author after a photography of Taylor in Hastings (1994)*

researchers in scientific journals were read, which had to do with the topics treated in the course. To practice how to summarize it in such a way that an interested layman will understand it, we have read together the already mentioned paper of [Anderson and Keafer \(1987\)](#). Afterward each student was asked to write an article for a newspaper on the annual rhythm of *Alexandria tamarensis*. You can read one of the articles in the following. The heading ‘The smallest calendar’ is also from the author.

***The smallest calendar. -
Unicellular with build-in annual
rhythm discovered***

The marine dinoflagellate Alexandria tamarensis, a unicellular with a cellulose carapace which modern biologists put between animals and plants, is often a headliner: It belongs to the poisonous algae participating in the dreaded annual algal pest. An American team at the oceanographic Institute in Woods Hole, Massachusetts, noticed that the encapsulated resting stage of this species at the bottom of the sea hatched only from late winter to early spring, in due time for the algal pest. This was surprising, since even algae from a depth of 30 m and more knew the time of the year. And this in spite of the constant conditions prevailing there. To get to the (sea) bottom of things a larger sample of soil was taken, for two years kept under constant laboratory conditions and each month a germina-

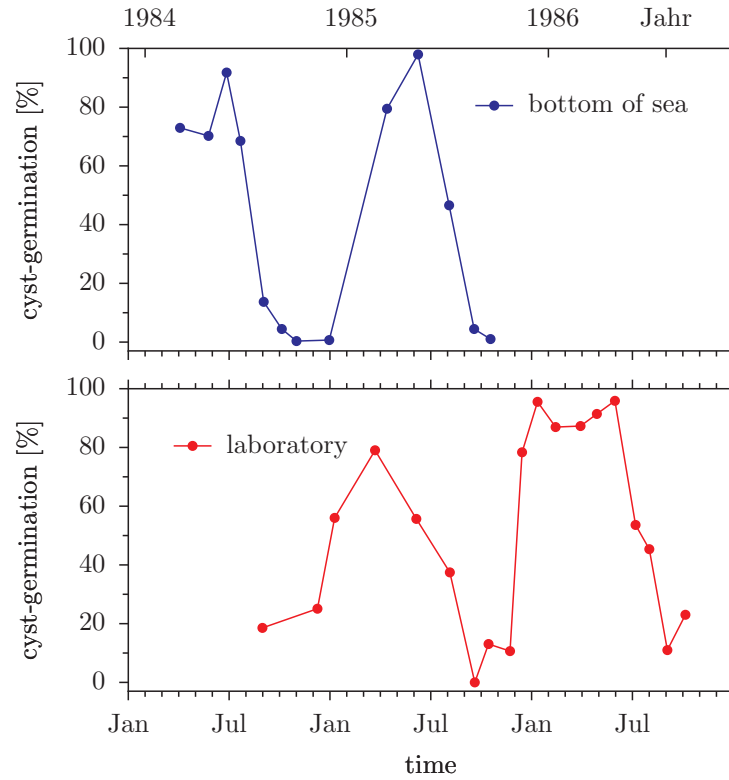


Figure 2.12: *Alexandria tamarense* from the Gulf of Main eclose from the cysts in an annual rhythm. Top: Each blue point shows for a sample from the bottom of the sea the percentage of hatched algae with high values in the summer and low values in the winter of 1984 and 1985. Bottom: A larger sample from the bottom of the sea was kept in a refrigerator at 4°C . Samples were taken at different times of 1985 and 1986 and transferred to 18°C . Again the percentage of hatched algae is shown as a function of the time of the year. The red curve shows, that again an annual rhythm is found. After [Anderson and Keafer \(1987\)](#)

tion test performed. The algae with only a thousands of a millimeter had even under these conditions not forgotten their calendar. Interestingly members of this species from shallow waters germinated without an annual clock by using environmental cues. Alexandria tamarensis has thus to be divided into two subspecies. One of it controls its life cycle via an endogenous annual clock.

Schmitt

2 *The smallest calendar*

3 Annual clocks in seeds

Higher plants proliferate and propagate by seeds. In this way they outlast unfavorable conditions. Some seeds germinate only during certain times of the year. An internal annual clock is responsible for it.

In this chapter we will deal with a secret of nature, which has not yet clarified, namely seeds of plants, which germinate only at certain times of the year. We will first look at a few examples. Afterward the significance of seeds for plants is explained. Finally we will speculate on how seeds are able to germinate at the right time of the year.

3.1 Seeds of plants germinating at certain times of the year

In many regions of the earth the living conditions for organisms change dramatically during the course of a year. There are times with favorable temperatures allowing plants and animals to develop and propagate. During the winter, however, many plants die or stop growing. In other regions it is now the low temperature, but aridity which is problematic for the organisms.

Higher plants have developed a large number of different strategies to outlast such unfavorable seasons. The most successful one is to form seeds. Seed formation is characteristic for gymnosperms and angiosperms.

Many plants which we grow in the garden germinate as soon as water is available and the temperature is favorable. It does

not matter, whether the seeds are sown in the spring, summer or early fall. They are, however, often special varieties. The seeds of the wild types germinate only at special times of the year and other times at which they enter a resting period.

The development of plants in the temperate and higher latitudes of the earth has to be synchronized with the season. For many annual plants it would be deadly to germinate and grow in the fall. The first frost would kill them. Therefore higher plants develop seeds in good time before the winter begins. They germinate in the following spring or summer. Rest and germination of seeds are adapted to the seasons. For more information regarding seed germination see [Hegarty \(1978\)](#), [Leubner \(2000\)](#) and [Finch-Savage and Leubner-Metzger \(2006\)](#).

What causes seeds to germinate at certain times of the year? In quite a number of plants photoperiodic signals are needed for germination of their seeds.

There are, for instance, seeds which germinate only under long days (summer), such as the wild lettuce *Lactuca sativa* or the brown birch *Betula pubescens* (figure 3.1) and the silver birch *Betula pendula* ([Vanhatalo et al. \(1996\)](#)). The rest of the seeds can also be controlled photoperiodically such as in *Desmodium barbatum*. If seeds of this species ripen under short days (for instance a light period of 8 hours), more of them germinate as compared to those which ripen under long days (for instance a light period of 18 hours, [Siqueira and Valio \(1992\)](#)). Other seeds germinate only under short day (spring or fall).

3 Annual clocks in seeds

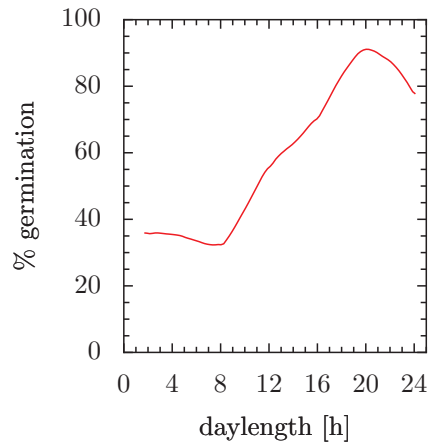


Figure 3.1: *Seeds of the brown birch* *Betula pubescens* show under short days (2 to 8 hours light per day) a low germination rate, whereas under long days (20:4 hour light-dark-cycle) 90 % germinate (at 15°C). After [Black and Wareing \(1955\)](#).

In other plants low temperatures for several days or even weeks are needed to terminate the resting period of the seeds. Scientists call this vernalisation. The seeds would germinate only if frost was present for some time in the winter. If the soil temperature becomes favorable in the spring, the seeds germinate and the plants begin to grow.

3.2 What it a seed?

The development of an angiosperm plant is shown schematically in figure 3.2. After fertilization a seed with an embryo and endosperm develops from the seed anlage. The embryo in the seed contains already all the important structures of the young plant: Root-anlage, stalk, and cotyledons. Before developing, the metabolism is reduced and the seed enters a resting stage. In this ‘dormancy’ the seeds are protected from the rigors of the winter and outlast

the low temperatures without damage (see [Taylorson and Hendricks \(1977\)](#)).

3.3 Seeds with an annual clock

There are a number of plants in which seed germination is neither induced photoperiodically nor by vernalisation. Instead an internal annual clock controls germination. The ability to germinate was studied in 335 species by Bünning and his coworkers in the years between 1940 and 1960. The seeds were kept at different temperatures (2, 20 and 35°C) either in continuous darkness or in continuous light. It was tested, how they germinate at different times of the year ([Bünning \(1951\)](#)). From the checked plants 10 showed a pronounced annual rhythm: They germinated always at a characteristic time of the year, depending on the species. Examples of those plants are St. Johns wort *Hypericum*, the foxglove *Digitalis lutea* (figure 3.3), the cinquefoil *Potentilla molissima*, hedge hyssop *Gratiola officinalis*, *Chrysanthemum corymbosum*, the mistletoe *Viscum album* and the wild strawberry *Fragaria vesca*.

The storage temperature does not influence the annual rhythm of germination.

Since the conditions, under which the seeds were kept, were constant, an internal annual clock must have induced germination. This clock somehow lead the embryo in the seed to terminate dormancy and to start development.

That indeed an internal annual clock exists is shown by dry seeds. A dormant seed has a very reduced metabolism, but some respiration is still present. If measured by a suitable device, an internal annual rhythm shows up. Parallel to this rhythm another annual rhythm exists for water penetrating the seed shell and causing the seed to swell (figure 3.4). The physiological basis of this

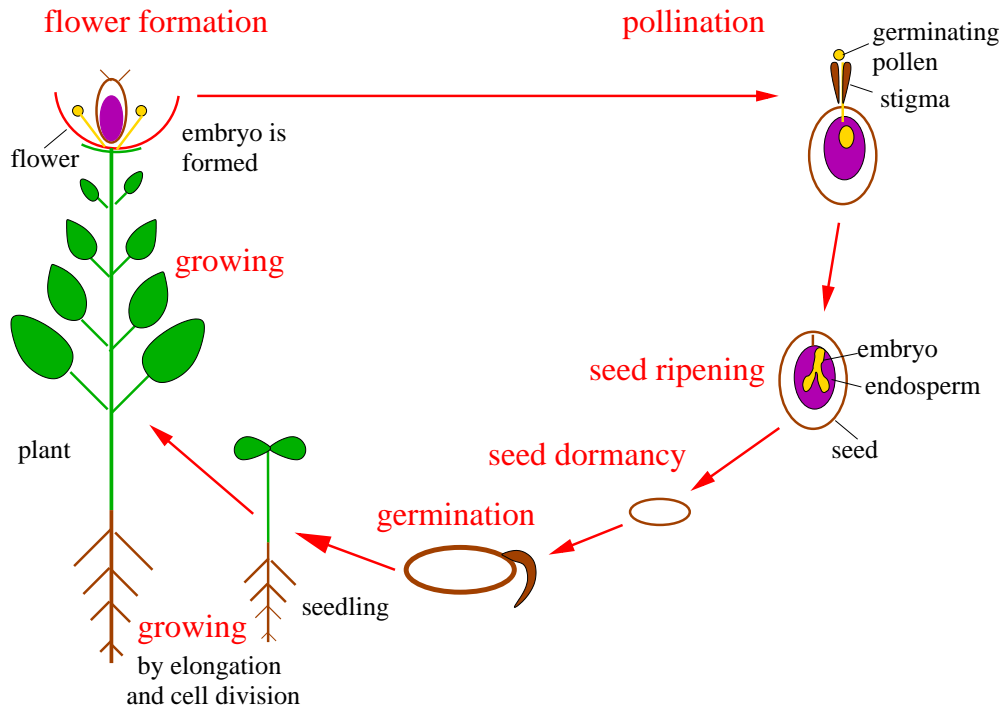


Figure 3.2: *Development of an angiosperm plant: In the flower the seed anlage with an embryo is formed in the ovary. The seed ripens, drops and enters a dormant stage. In this stage it can outlast unfavorable conditions such as frost and dryness. If the environmental conditions become favorable again, the seed germinates and the seedling develops into a new plant. This plant forms flowers with stamen and carpel in which the ovary is pollinated and together with the endosperm forms a seed*

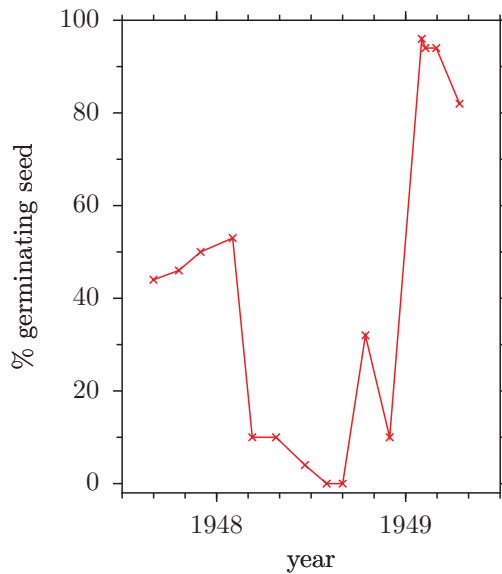


Figure 3.3: Annual rhythm of germination of the foxglove *Digitalis lutea*. The seeds were kept at 3°C and samples transferred about every 45 days to wet filter paper at 23°C in continuous darkness. The percentage of germinated seeds is plotted against the time of the year. After *Bünning (1949)*

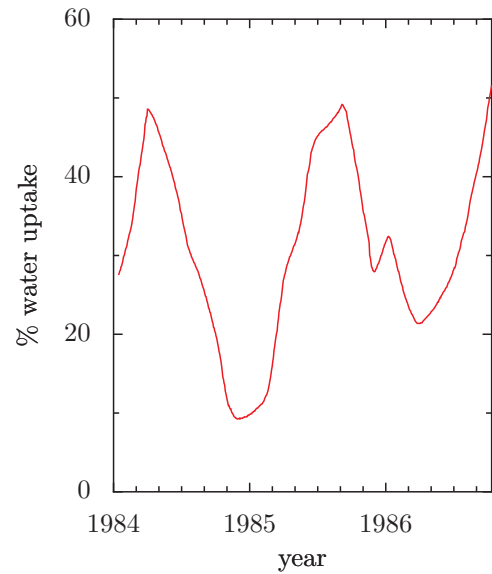


Figure 3.4: Annual changes in water uptake (in percent of dry weight) of dry bean seeds (*Phaseolus vulgaris*) during four hours at 25°C in the dark from June 1984 to July 1986. After *Spruyt and De Greef (1987)*

annual rhythm is unknown.

In the strawberry *Fragaria vesca* the mother plant influences the readiness to germinate: Seeds ripened at different times of the year was harvested and the readiness to germinate measured in the following months. It was in all samples independent of the time of harvesting and highest in October (figure 3.5). How the annual rhythm of seed germination is synchronized is not known.

3.4 Examples for annual rhythms in plants

Annual rhythms are found not only in the germination of some seeds. They exist also in the rooting of willow-cuttings, in the growth of duckweeds *Lemna* and oat *Avena*, in the dropping of leaves in the fall,

3.4 Examples for annual rhythms in plants

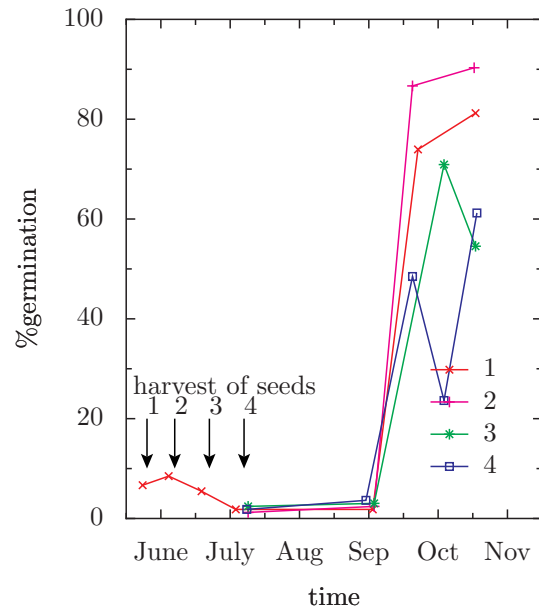


Figure 3.5: *Readiness to germinate in seeds of *Fragaria vesca*, which ripened at different times of the year, is independent of the time of harvesting (arrows) in all samples (colored and numbered curves) and maximal in October (Bünning (1949))*

in the adding of new wood in stems of trees as shown by the annual rings (see figure 3.6), in frost hardiness and bud dormancy. It is not yet known how these annual clocks work.

Examples for annual clocks exist also in animals. We will hear about it later in the Dsungarian hamster section 6 and in the bird migration section 7. In the next chapter we will see that organisms can use the daylength as an external calendar, which determines certain developmental steps such as flowering in plants.

3 Annual clocks in seeds

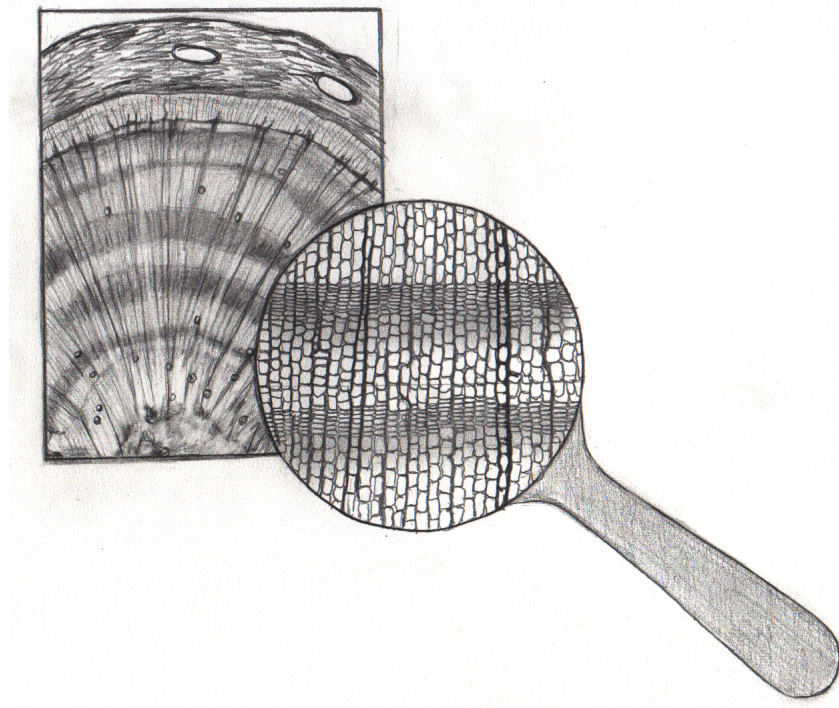


Figure 3.6: *Cross section through the stem of a pine (radius about 25 cm). Periphery (top) bast and outermost part cork. The wood (inner part) shows annual rings consisting of larger cells in the spring and summer and smaller cells later in the year. With a magnifying lens the early wood (large cells with thin cell walls) and the late wood (small cells with thick walls) can be seen better. The sharp boarder is the end of an annual ring and reflects the absence of growth during the winter. By Mareike Förster*

4 How to make *Kalanchoe* thick and induce flowering

Many plants use the daylength as an external calendar. A short day plant such as *Kalanchoe blossfeldiana* flowers in short days, long day plants in long days. In *Kalanchoe* the form of the leaves is also photoperiodically determined. Under long days the leaves are large and relatively thin, Under short days small and thick.

How the photoperiodic induction of flowering works and how in this connection the daylength is measured, is described in a section.

Another section deals with the 'eyes' of plants, with which they see the daylength.

If plants are photoperiodically induced to flower, a flower impulse is induced in the leaves and a signal sent to the location where flowers are formed.



Figure 4.1: *Kalanchoe blossfeldiana* in full bloom. The flowers are red colored with four petals forming a tube, a green calyx and a flower stalk. The leaves are fleshy

4.1 Thick leaves under short days

Kalanchoe blossfeldiana is found in arid locations on the island of Madagascar east of Africa. It belongs to the *Crassulaceae*. To protect the plants from drying out during the hot season they possess thick fleshy leaves in which water can be stored. They begin to flower at the end of the winter in Madagascar. Up to 300 flowers are found on a plant. They are deep red and pretty. Therefore and because they flower during the winter, *Kalanchoe* is an ornamental plant (figure 4.1).

As in many *Crassulaceae* the succulence of the leaves is controlled photoperiodically. Under short days small, brittle succulent leaves develop. Under long days, however, the leaves are thin, flexible and large (figure 4.2).

The leaves become succulent by taking up water and increasing the size of the cells in transverse direction. For this purpose a substance is formed under short days which induces succulence. This substance can be transferred by grafting a leaf from a plant kept under short days on a plant kept under long days. The leaves of the host plant

4 How to make Kalanchoe thick and induce flowering

become succulent, although they are still under long day conditions.

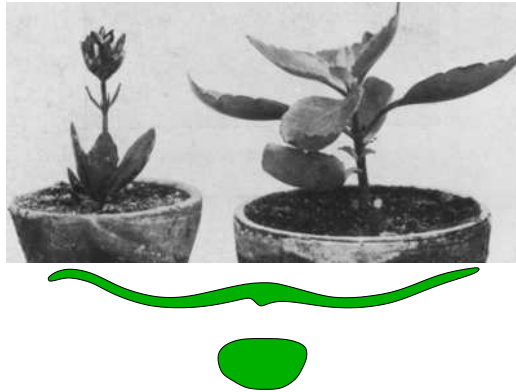


Figure 4.2: *Leaves of Kalanchoe blossfeldiana kept in long days (left) and in short day (right). Below: Schematic cross section through a long day leaf (top) and a short day leaf (bottom). After Harder and Witsch (1941)*

4.2 Flower induction in short day

Short days not only thicken the leaves of *Kalanchoe blossfeldiana*, but the plants are also induced to flower. This is the reason, why the plants are usually sold before Christmas in flower shops and market gardens. Plants kept under long days do not flower, and the leaves are thin and expanded. Figure 4.3 shows the mean number of flowers as a function of the length of the light period. The figure illustrates, that short day plants flower, if a critical dark period is exceeded.

In the latitudes of Europe, Asia and North America many plants flower under long days, that is in the summer. They are therefore called long day plants. In their case the light period has to fall *below* a critical dark period in order to induce flower-

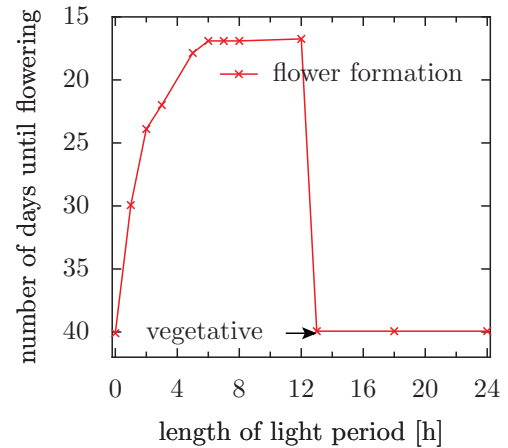


Figure 4.3: *How the flower induction of Kalanchoe blossfeldiana depends on the length of the light period: In days with short light periods (long nights in the winter) flowers are induced, but they stay vegetative in days with long light periods (short nights such as in the summer). In very short light periods (up to 4 hours), which never occur in the habitat Madagascar, a few flowers are found (decline of the left part of the curve). The dark period, at which just half of the maximal number of flowers are induced, is called critical dark period. It is 11.5 hours (corresponding to 12.5 hours of light; note, that the light period is plotted on the x-axis)*

4.2 Flower induction in short day

ing (figure 4.4). Long day plants are for instance the rye grass *Lolium perenne* and the thale cress *Arabidopsis thaliana* (figure 4.14). There are, however, also plants which flower only after having been for some time in short days and afterward in long days (so called short-long day plants). In long-short day plants it is the other way round. Day-neutral plants flower independent of the daylength in short- and long days. But they too need a certain age before being able to flower. Instead of using this autonomous path for flowering many plants react to environmental factors such as the day length.

There are short day plants, which are induced to flower by a single short day (for instance *Pharbitis nil*). The same is true for long day plants (for instance rye grass *Lolium perenne*). These plants are particularly well suited for experiments, since flower induction occurs under just one effective photoperiod. If in addition the first signs of flower formation are visible soon after the induction, one can get results of the treatment already a short time after the experiment. Most plants need, however, several days with the correct photoperiod for flower induction (they possess a *photoperiodic counter*). This is the case for *Kalanchoe*. This plant needs at least seven 'inductive' short days, in order to induce at least some flowers. With an increasing number of short days more flowers are formed.

Photoperiodic flower induction is very important for agriculture and horticulture (Overview: [Salisbury \(1985\)](#)).

How the daylength controls flower formation will be treated in the next sections. We have to deal with different processes in more detail, in order to understand flower induction:

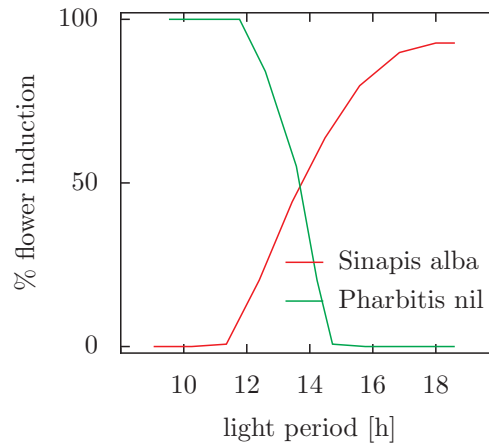


Figure 4.4: *Short day plants flower, if a critical dark period is exceeded (green curve, Pharbitis nil, strain Violet), long day plants, if a critical dark period falls below a critical dark period (red curve, senap Sinapis alba). Note, that the light period has been plotted on the x-axis*

1. In the leaf the photoperiodically effective light is perceived by photoreceptors and its length determined by a circadian clock.
2. At the proper day length and a sufficient number of inductive cycles a flower-inducing substance is formed in the leaf.
3. This 'Florigen'¹ is transported to the apex.
4. Florigen re-tunes the apex. It does not grow vegetatively anymore, but forms finally flowers (flower induction).
5. In the re-tuned apex gene activities are

¹the expression was created by [Chailakhyan \(1936\)](#). According to other hypotheses several hormones are involved which act together ([Bernier et al. \(1993\)](#)) or an inhibitor of flowering is removed

changed which leads to flower formation.

4.3 How the daylength is recognized in the leaf

First of all the plant has to recognize the daylength in order to induce flowering. A short day plant such as *Kalanchoe* has somehow to find out whether the critical dark period has been reached or exceeded. Where in the plant does it occur? It was found that it does not happen at the tips of the stalk, where later the flowers are formed, but in the leaves². In the tissue of leaves are provisions to perceive daylight, so called photoreceptors. In the same way as pigments in the photoreceptors of the retina of our eyes absorb light and convert it into signals for the brain, receptors for the photoperiodically effective light are in the leaves of plants. These receptors are different from chlorophyll, which plants use to convert sugar into starch by using energy from the sun light. Phytochrome is the most important light receptor in flower induction (Weller et al. (1997)). It is probably also used in the flower induction of *Kalanchoe*. Phytochrome is most sensitive for red light.³

²probably in the mesophyll and in the epidermis, as shown in *Kalanchoe blossfeldiana* (Schwabe (1968)), in *Solanum* with periclinal chimera and in further plants where the leaves were photoperiodically illuminated either from the upper or the lower epidermis (Bünning and Moser (1966), Schwabe (1968), Mayer (1973)). The epidermis of different plants can show special anatomical properties which are able to absorb sufficient light even during twilight (Haberlandt (1905)).

³There are other photoreceptors for photoperiodic reactions. In Brassicaceae such as the thale cress *Arabidopsis* blue light receptors (cryptochromes) are responsible for it (Guo et al.

A timing system and a photoperiodic counter play an important role (figure 4.5). The time measurement is made by a circadian system (see section 4.4). In long day plants apparently a critical daylength (light period) is measured, whereas in short day plants a critical dark period is measured. If a critical dark period is exceeded (short day plants) or falls short of (long day plants), the cells in the leaf produce florigen.

4.4 Models for photoperiodic flower induction

In the photoperiodic induction of flowering a short day plant will flower in short days, but not in long days. What are the underlying processes in the plant. Many researchers work on this question.

If a scientist has a problem which he can not solve, he uses the same method a detective would use who has to solve a case: He puts forward different *hypotheses* about how the case could have occurred. Hypotheses are models of reality. These models are tested by comparing them with reality. Natural scientists do this by experimenting. The models have to be improved if the experimental results differ from expectation.

Many decades ago it was already known that in photoperiodic reactions the daylength (or the night length) is measured by an internal daily clock of the organisms. Actually one would have expected a simple daylength measurement in the way the sprint time is recorded with a stop watch in a foot race. One could imagine, that with the onset of the dark period a substance is produced which after a certain duration of the night accumulates to such an amount, that flowering is induced.

(1998)).

4.4 Models for photoperiodic flower induction

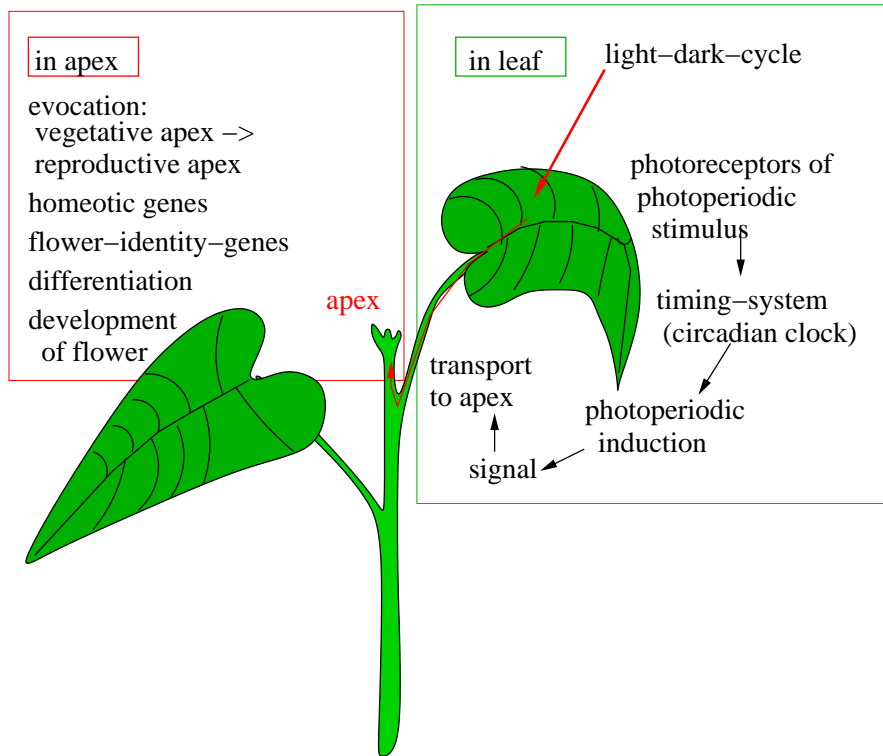


Figure 4.5: *In the leaf the photoperiodic signal of the environment (the light-dark cycle) is perceived via photoreceptors (red arrow top right). The daylength (night length) is detected in a timing system (circadian clock). With the proper length (for instance short day in short day plants) photoperiodic induction takes place in the leaf. A signal is produced and (red line with arrow) transported to the apex. It switches the apex from growing vegetatively to forming flowers ('evocation'). The apex differentiates to a flower or an inflorescence and the flowers develop. After Bernier (1971)*

4 How to make *Kalanchoe* thick and induce flowering

It turned out, however, that the idea of [Bünning \(1936\)](#) is valid for most photoperiodic reactions: Accordingly the photoperiodic time measurement is done with the daily clock which runs periodically (every 24 hours the clock is in the same condition). This Bünning-hypothesis has been tested meanwhile frequently with critical experiments and was partly varied, but the basic idea is today accepted by most researchers.

How the flower induction occurs according to this vision is explained in figure 4.6. Light has two functions: It synchronizes the circadian clock and, depending on the photoperiodic constellation of the season (long days or short days) and the photoperiodic situation of the organism (for instance long day plant or short day plant) induces the photoperiodic reaction or not.

The principal of this model is thus, that an internal clock interacts in different ways with the external rhythm of the light-dark-cycle.

How the external light-dark-cycle synchronizes the internal clock, depends on whether the onset or end of the light period influences the phase more strongly. In the example of figure 4.6 it is the onset of the light period, which sets phase in long day and in short day. It is, however, more realistic to assume that both light-on and light-off set phase. More to it later.

Bünning's hypothesis was modified in such a way that only a short section of the oscillation is light-sensitive, called light-inducible phase Φ_i . The external light-dark-cycle has to coincide with Φ_i in the right way (see [Pittendrigh \(1964\)](#) and figure 4.7). This model was therefore called *external coincidence model*. There is, however, not a fundamental difference between the two models: The external light-dark-cycle might well evoke an internal light-dark-rhythm in the organism which inter-

acts with the critical phase of the circadian clock.

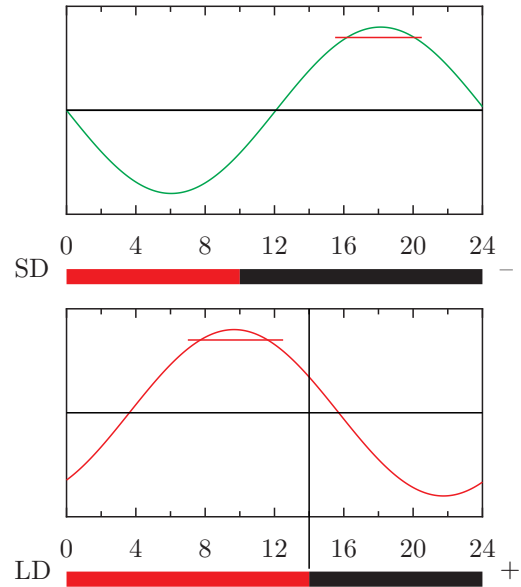


Figure 4.7: *External coincidence model for photoperiodic reactions. Light synchronizes the circadian clock to the external light-dark-cycle. The phase of the oscillation is in this case not fixed to the onset of light. Upper curve (red): In a 14:10 hour long-day. Lower curve (blue): In a 3:21 hour short day. A light-sensitive phase θ_i of the oscillator has to coincide with light, if a photoperiodic reaction should occur. After [Pittendrigh \(1964\)](#)*

This leads us to another proposal, the internal coincidence model. I experimented with *Kalanchoe* plants in bloom ([Engelmann \(1960\)](#)) which were kept for several days in continuous light. The flowers had stopped opening and closing their petals and were almost completely closed. If I transferred the flowers afterward for some days into darkness, they began to move again in a daily rhythm. I called this the 'light-off rhythm', because it began after the light was switched off (figure 4.8). If the

4.4 Models for photoperiodic flower induction

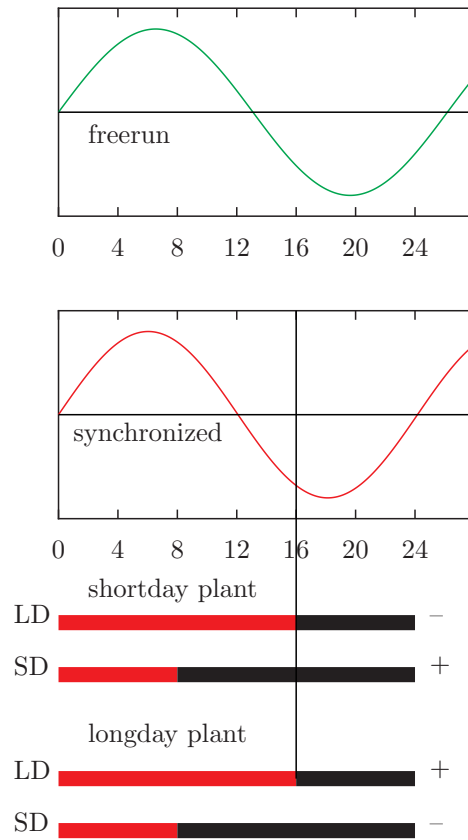


Figure 4.6: *Bünning-model of photoperiodic induction of flowering (or other processes). Light has two tasks:*

First, it synchronizes the circadian clock with the light-dark cycle. The upper curve shows a free-running oscillation of the circadian clock, that is, under constant conditions without Zeitgeber. The two lower curves are synchronized by light-dark cycles (long day, middle, short day, bottom) to the 24 hour day.

Second, light influences the photoperiodic system differently, depending on whether short day or long day prevails. In long days the long light period (white area above the x-axis) coincides not only with the so called 'photophilic phase' ('light-loving', red part of curve), but partly also with the skotophilic phase ('dark-loving', gray part of curve). In this case flowering would be induced in a long day plant, but inhibited in a short day plant. Under short day the skotophilic phase is not illuminated and a long day plant will not be induced to flower. Short day plants, however, would be induced. After [Bünning \(1983\)](#)

flowering plants were for more than 14 days in continuous darkness⁴, the petals have stopped moving and are maximal opened. If I transferred the flowers into continuous light, a circadian petal-movement was started which I called ‘*light on rhythm*’.

The figure shows, that the first maximum of the light-on-rhythm occurs 5 hours after the transfer into light and that the first maximum of the light-off rhythm 15 hours after transfer into darkness. If the flowers receive a period of 10 hours, the two rhythms superimpose in such a way, that the first light-off maximum and the first light-on-maximum coincide. That corresponds surprisingly the daylength, at which the photoperiodic flower induction of *Kalanchoe* begins. I proposed therefore, that the superposition of a ‘*light-on-rhythm*’ and a ‘*light-off-rhythm*’ are responsible for the photoperiodic reaction (here: flower induction). In this model the photoperiodic time measurement of an organism is induced by an internal rhythm started by the onset of light and another internal rhythm by the onset of darkness (Engelmann (1966), Engelmann (1967)). Both rhythms superimpose each other. Depending on the phase relationship of the two rhythms the amplitude of the curve representing the sum of the two oscillations are increasing or decreasing. Oscillations with a high amplitude lead to a photoperiodic reaction.

To explain this internal coincidence model, the flower induction of *Kalanchoe* in the following and the petal movement serves as a hand of the two different oscillators.

⁴This is possible in Crassulaceae; they possess sufficient reserve substances to overcome the long starvation time; green light can be used as physiological darkness and facilitates watering and handling of the plants

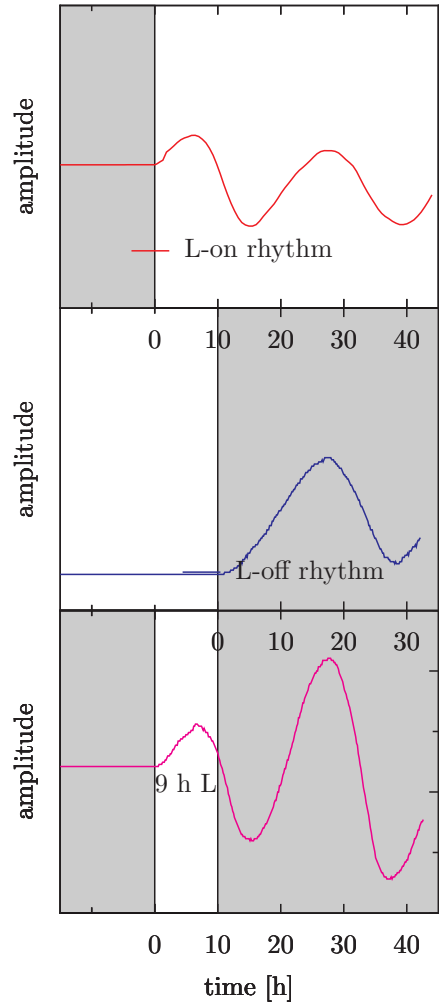


Figure 4.8: *Internal coincidence model of the photoperiodic induction of flowering.* Light-on rhythm (red) of *Kalanchoe*-flowers (chapter 4) after transfer from DD (gray) into LL (bright). Flowers continue to open, maxima 6 and 27 hours after onset of light. Light-off rhythm (blue, flowers maximally closed) after transfer from LL into DD. Next maximum 18 hours after onset of DD. Magenta: Flowers for some days in DD, afterward 9 hours in light and back into DD. Both rhythms superimpose, amplitude is increased. Longer as well as shorter light periods would lead to smaller amplitudes (not shown). *Kalanchoe* is induced by this 9:15 hour LD to flower maximally. The superposition of the light-on- and the light-off rhythm reflects the strength of the photoperiodic induction in different LD-cycles

The most important point of Bünning's idea was, that a circadian clock is used for photoperiodic reactions: An internal oscillator in an organism (1) synchronized by the external light-dark-cycle. (2) Depending on whether the dark-loving phase falls into darkness or is partly illuminated, a chain of events is induced which leads to a photoperiodic reaction or not. There are, however, a number of points which are unclear.

One of these points is, how the oscillator is synchronized by the light-dark-cycle. In the case of the short day plants, as shown in figure 4.6, Bünning assumed, that the transition from darkness to light sets the phase of the clock (the photophilic phase, red in the figure, falls together with the light-on of the longday *and* the shortday). That must not be so necessarily. Nor must the light-off set the phase. Rather, it is more likely that synchronization is more complicated. We will come back to this (see figure 4.9).

A second vague point is, which phase of the cycle of the internal oscillator interacts in which way with the light-dark-cycle. That has been mentioned already in the external coincidence model, but needs more thoughts.

To answer this question, we (Bollig et al. (1976)) used a feedback model which was originally developed to describe ultradian rhythms and extended later to circadian rhythms (Johnsson and Karlsson (1972), Karlsson and Johnsson (1972)). The model-oscillator is driven by the light-dark-cycle (simulated with an analog-computer, in which the different light-dark-cycles were fed into the program) and the resulting data plotted as curves (see figure 4.9). A number of experiments on the flower induction of the red-goose foot *Chenopodium rubrum* were done parallel to the simulations using the same combinations of light-

dark-cycles. We tried to find from the simulations an indicator of the photoperiodic induction. The distance between *light-on* and the next minimum of the oscillation (regardless of the sign) was called ψ and the mean value of all ψ 's turned out to be a fairly good indicator of the photoperiodic induction (see figure 4.10 and figure 4.11).

Instead of assuming, that the transition from darkness to light (light-on) or the light-off sets the phase of the clock, it is much more likely, that the synchronization by the light-dark cycle is more complicated and corresponds to the situation just described. Which part of the cycle of the internal oscillator interacts with the light-dark-cycle was already indicated.

4.5 Flower hormone florigen and its transport

Now back to the biochemical basis of flower induction. Is there an universal flower hormone? The following speaks in favor of it:

- If a photoperiodically reacting plant is kept under non-inductive conditions and only one or a few leaves are photoperiodically treated, the plant will flower (Zeevaart (1984)).
- If a shoot or leaf is photoperiodically induced and grafted on a non-induced plant under non-inductive conditions, the plant will flower (figure 4.12).
- If a photoperiodically induced leaf is grafted on a plant which is photoperiodically insensitive, it will flower earlier than usual (Lang and Melchers (1948)).

This flower hormone was assumed to be an organ-forming substance (an *inducer*, Sachs (1880)) or substances which

4 How to make Kalanchoe thick and induce flowering

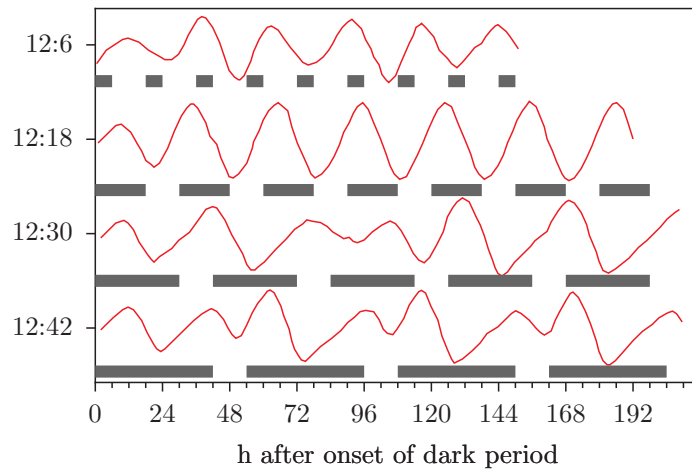


Figure 4.9: *Simulation of oscillations with a feedback model. Different light-dark-cycles were used, as indicated on the y-axis (LD 12:6, 12:18, 12:30, 12:42 hours). The model-oscillator is driven by the light-dark-cycle (simulated with an analog-computer, in which the different light-dark-cycles were fed into the program) and the resulting data plotted as curves. The distance between light-on (changes between gray and white areas) and the next minimum turned out to be an indicator of the photoperiodic induction (see figure 4.11 and 4.10). After Bollig et al. (1976)*

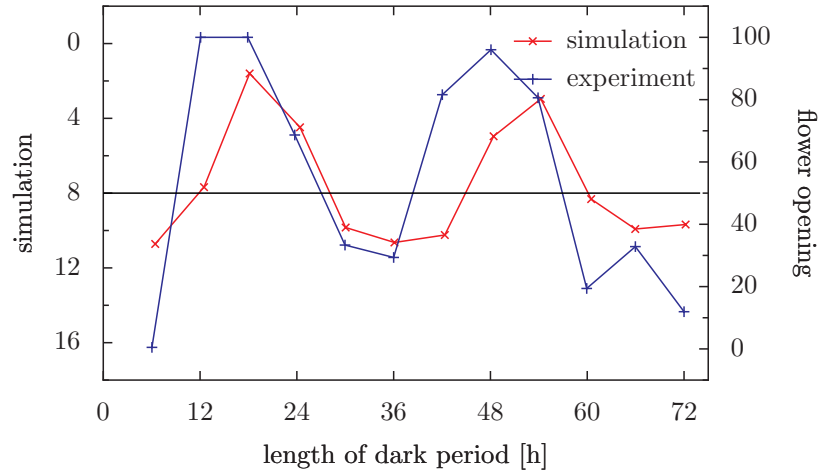


Figure 4.11: *Simulation (red curve) and experimental values (green curve) of flower induction in *Chenopodium rubrum* plants Ecotype 374 under LD-cycles consisting of a 6 hour light period and varying dark periods (abscissa). The flower induction (right y-axis) and the ψ values (left y-axis) show a similar pattern. After Bollig et al. (1976)*

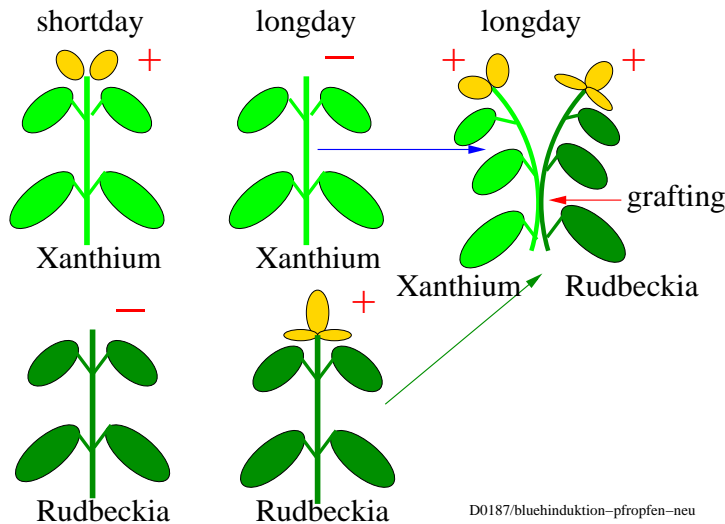


Figure 4.12: Grafting a photoperiodically induced short day-scion of the short day plant *Xanthium strumarium* (flowers April to May) on a non-induced long day-host plant *Rudbeckia hirta* (flowers July to September) induces flowering. After *Okuda (1953)*

turn on flower genes (*Zeevaart (1984)*). It was also presumed that the flower-controlling signals might consist of promoting **and** inhibiting components. By using mutants of *Arabidopsis* it was recently successfully tried to understand the processes better. Modern genetic methods (forward- and backward-genetics), genome-technology (*Wellmer and Riechmann (2005)*) and microarray-technique (*Yamada et al. (2003)*) were used (see section 4.8).

4.6 Transport to the apex and flower formation

In any case the flower-inducing substances have to be transported to the apex. It is not known, whether this is alike in short day plants and long day plants. Once the flower-inducing substances have arrived in the tissue of the apex, it is reprogrammed in such a way that now instead of vege-

tative shoots flowers are formed. Certain genes play a role which change the meristem in the apex correspondingly. Furthermore genes are activated which are responsible for the time of flowering. At the same time the metabolism in the meristem of the apex changes. A few hours after the arrival of the flower-inducing substances the differentiation pattern at the apex has changed already (the morphologically visible differences are shown in figure 4.13). The apex is now determined to develop into flowers. These processes are called *evocation*.

4.7 Examples for long day plants

Examples of long day plants are among others the thale cress *Arabidopsis thaliana*, spring varieties of oat *Avena sativa*, senap *Sinapis alba* and red clover *Trifolium pratense*. A single long day induces scarlet pimperl *Anagallis arvensis*, senap

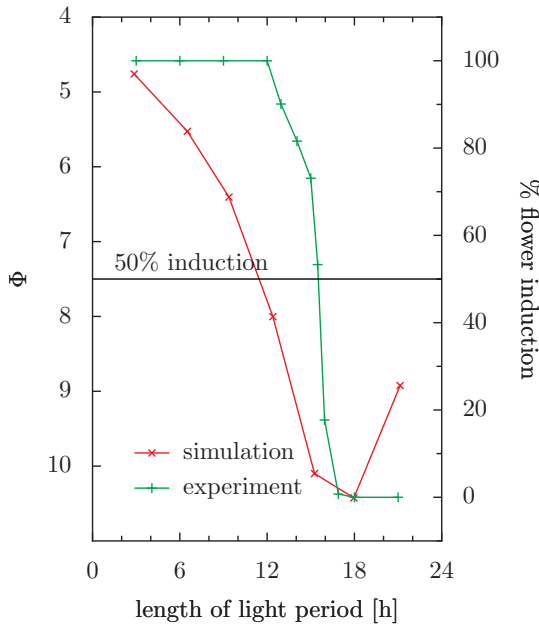


Figure 4.10: Simulation of oscillations and results of photoperiodic experiments with different light periods (x-axis) and corresponding dark periods in 24 hours-cycles. Plants of ecotype 374 of the red-goose foot *Chenopodium rubrum* were reared in continuous light and transferred to three cycles consisting of different LD periods, The percentage of flowering plants (blue curve) is shown together with the ψ value of the simulations (red curve). Strong flower induction up to 12 hours, no induction beyond 16 hours. The time course of the (red) ψ values resembles the curves of the experimentally found one (blue). Details in *Bolig et al. (1976)*

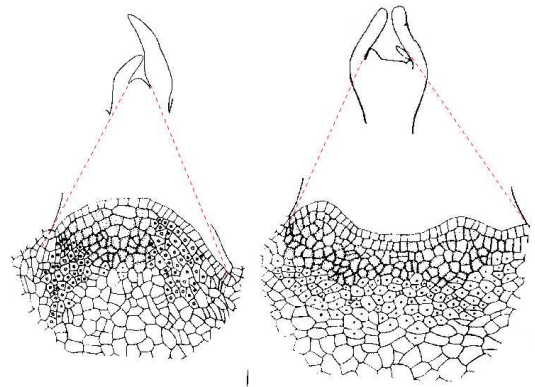


Figure 4.13: Development of the apex of *Pharbitis nil* under non-inductive photoperiod (long day, left) and after photoperiodic induction by short day (right). The differences are made clear by macroscopic (top) and microscopic (bottom) images of the apex. After *Imamura and Marushige (1967)*.

Sinapis alba, and Darnell *Lolium temulentum* (a grass) to flower. Two to three days needs the henbane *Hyoscyamus niger*, four days the thale cress *Arabidopsis thaliana* and six days campion *Silene armeria*.

Particularly well studied is the photoperiodic induction of flowering in *Arabidopsis thaliana* (figure 4.14). This plant has a number of advantages, such as a short generation time of three weeks only. In this way seeds are rather soon available. *Arabidopsis thaliana* is small and undemanding. It can thus be grown easily in the laboratory. The plant has a small genome, that is, relatively few genes. Being a self-pollinator the plant does not need wind or insects for pollination. Long day accelerates flowering, short day retards it, but can not hamstring flowering. Different varieties exist the photoperiodic reaction of which differs. The earliest variety flowers already eleven days after germination, the

latest 112 days later. For the induction of flowering four days of continuous light are sufficient.

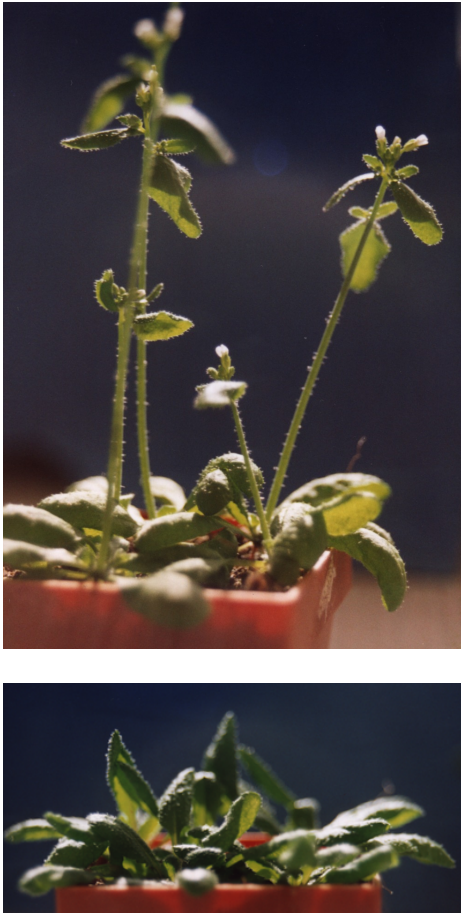


Figure 4.14: *Cress thale Arabidopsis* as a facultative long day plant, on top blooming, below vegetative under short day with rosetta-like leaves

Numerous mutants of *Arabidopsis thaliana* are available in which the photoperiodic reaction is changed. More than 80 genes influence the time of flowering (they are called *flowering time genes*). Other mutants affect the photoreceptors of the photoperiodic stimulus. Furthermore mutants exist in which the transfer of the signals is affected which transfer the

photoperiodic stimulus after its perception.

4.8 Biochemistry of flower induction

systems in order to flower and set seeds at the right time of the year (see right part of figure 4.15 and the legend there). One of these controls is the autonomous path (A.P.). The plant has to go through a juvenile phase first and reach a certain developmental age before being able to flower. Other control systems use environmental signals such as the daylength (P.P), temperature (V.P.) and humidity of the soil. If the right conditions are signaled, flowering is induced. Which of the various paths are dominating depends on the species and varies a lot (Aukerman and Amasino (1996)). Figure 4.15 shows a scheme of the photoperiodic flower induction of the long day plant *Arabidopsis thaliana*. After perception of the light by photoreceptors in the leaves a circadian clock located there measures daylength. If the light period is long enough, flowering is induced. Thereby the protein CO (CONSTANS) plays a central role: It mediates between environment, the circadian clock and the flower induction (Hayama et al. (2003)). Since CO in long day plants such as *Arabidopsis thaliana* is stable only in the light and is degraded by proteosomes in the dark, it accumulates only in long days, but not in short days. It induces the synthesis of FT by the *ft*-gene (Valverde et al. (2004)). The mRNA of FT is transported from the leaves to the apex⁵ and combines there with the already existing FD. Without FT the FD is inactive. The FT-FD-complex triggers the flower formation in the meristem of the

⁵perhaps also FT, since it is a small protein (Huang et al. (2005))

4 How to make Kalanchoe thick and induce flowering

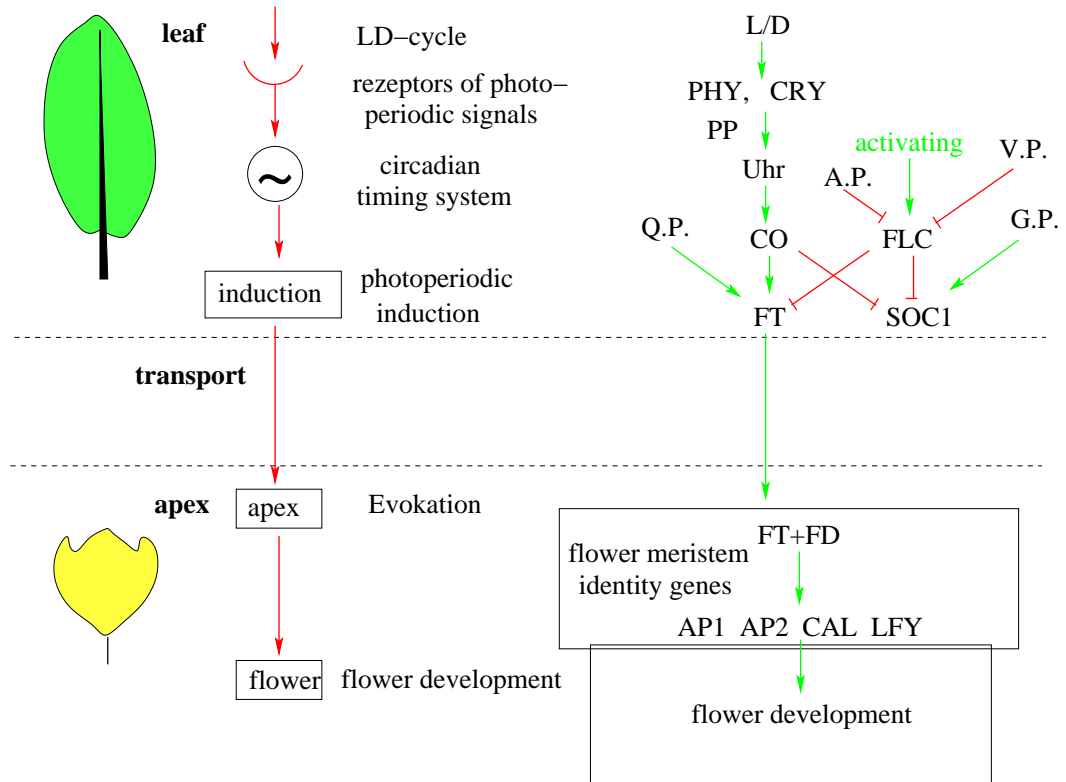


Figure 4.15: Basis of photoperiodic flower induction in Arabidopsis. Left: In the leaf the kind of light-dark (LD) cycle (long- or short day) is recognized by photoreceptors and the daylength determined by circadian timing system. At the right daylength photoperiodic induction occurs. A signal is transported to the tip of the plant (apex, plural apices) and it induces there flowering. Right: Biochemical basis of flower induction (green arrows: promoting, red swards: inhibiting processes). In the photoperiodic path (PP) light is absorbed by phytochromes and cryptochromes, the daylength measured by a circadian clock and transformed into the signal FT via the protein CONSTANS. FT combines in the apex with FD. In this way flower meristem identity genes are induced, which lead to flowering. There are furthermore an autonomous path (A.P.), a vernalisation path V.P., a gibberellin acid path (G.P.) and a QP (Q.P), each of which can also lead to flowering

apex (*evocation*, Abe et al. (2005), Wigge et al. (2005)). During this process flower meristem-identity -genes such as *ap1*, *ap2*, *cal* and *lfy* are activated and the flower formation begins, as described by the ABC (DE) -model (Parcy (2005)).

The interaction of CO and FT seems to be widespread among plants (Griffiths et al. (2003), Izawa et al. (2003)). The photoperiodic reactions use the same genetic paths in the long day plant *Arabidopsis thaliana* and in the rice *Oryza sativa*. However, the functions differ (Hayama and Coupland (2003)): CO inhibits in short day plants FT and in this way also flower induction. Long dark periods would thus promote the expression of FT, because the CO activity is low. How the switch functions on the biochemical level which takes care that long day plants flower under long days and short day plants under short days is not yet known (Cremer and Coupland (2003)).

4 *How to make Kalanchoe thick and induce flowering*

5 Potato tubers

Potato tubers are formed on subterranean shoots by thickening of the tips and by depositing starch into the cells. The tubers outlast unfavourable conditions and sprout afterward to form new plants. The tubers are produced in the fall and induced by the shorter days. The daylength is perceived by the leaves. They form inhibiting and promoting signals which reach the subterranean shoots where they induce the tubers. Onions are induced by long days, that is, in the summer.

Potatoes are next to rice, wheat and rye the fourth important basic foodstuff of mankind. They originate from South America and were cultivated there by the Indians.

5.1 Potatoes are tubers on subterranean shoots

Perhaps it is new for you (and many other persons), That potato tubers are not swellings of roots, but are formed on subterranean *shoots*. These shoots growing in the soil are called *stolons*. Potato tubers are formed by swelling of the stolons at their tips (figure 5.1). The cells extend perpendicular to the longitudinal axis and incorporate starch.

That potato tubers arise indeed at - normally overground growing- shoots can be recognized in the small scale leaflets. They surround the 'eyes' of the potato tuber, drop however early. The leaf scars can still be seen. In the leaf scars the buds can be seen. They develop later to lateral

shoots after the farmer has layed the potato tubers into the soil.

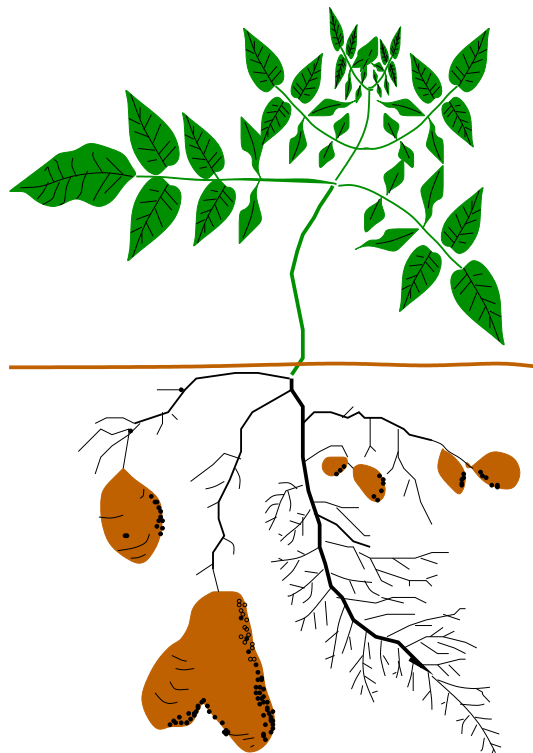


Figure 5.1: *In short days potato plants form tubers on subterranean stolons which swell perpendicular to the longitudinal axis. The horizontal line indicates the surface of the soil. Root system below the stolons.*

5.2 Potatoes are formed in short days

The tuber formation of potatoes is influenced by a number of factors such as environmental temperature, nitrogen content

of the soil, the physiological age of the plant and above all by the daylength. In the original varieties from South America the tubers are induced under short days. Long days inhibit tuber formation (Ewing and Struik (1998)). Even the South American cultured species and varieties such as *Solanum demissum* and *Solanum tuberosum* ssp. *andigena* develop tubers in short days only. Decisive is the length of the dark period, since with a light pulse interrupting the night no tubers are formed. Red is the most effective light. Phytochrome is used by the plant as the photoreceptor. Most European and North American cultured varieties show only a weak photoperiodic reaction or develop tubers also on long days (early potatoes!).

In the same way as in flower induction the photoperiodically effective light for tuber formation is perceived in the leaves. And here too the length of the dark period is measured by a circadian clock. If the dark period is longer than a critical value, a signal is formed¹ and transported to the subterranean shoots (Stolons). This signal consists probably of a substance which accumulates under inductive conditions and an inhibiting substance which wanes under inductive conditions. Gibberellines might serve as inhibiting substances (Tizio (1971)). Jasmonic acid and tuberonic acid are perhaps promoting substances (Koda et al. (1988), figure 5.2).

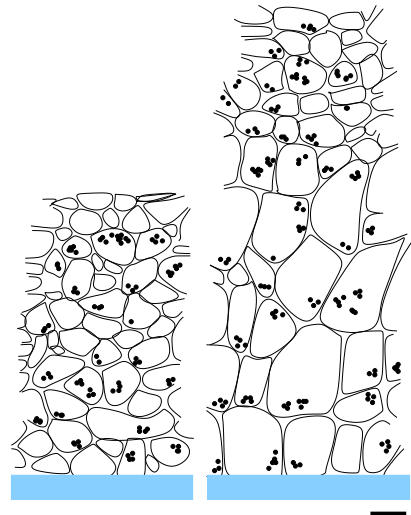


Figure 5.2: If potato slices are placed on agar (blue) containing $10^{-4}M$ jasmonic acid (right part of figure), the cells increase in size. Control without jasmonate left. Scale $100\ \mu m$. After a photography of Takahashi et al. (1994)

5.3 Onions are formed under long days

Onions are formed -in contrast to tubers of potatoes- in most cases under long day conditions. The common onion *Allium cepa* and garlic *Allium proliferum* are examples (figure 5.3). The yellow Zittau onion needs long day of at least 14 hours light. In southern latitudes varieties are common such as the sweet Spanish onion, which forms bulbs also in shorter daylengths (12-13 hours). Its critical daylength is thus shorter. In order to form onions, *Allium ascalonicum* needs 7 to 28 long days (Esashi (1961)).

¹This signal can be established by grafting: Short-day treated leaves of potatoes induce tuber formation in plants kept in long days, if grafted successfully. Short day treated leaves of tobacco are able to induce tuber formation in potatoes in long days after grafting. Tobacco and potatoes belong to the same family (Chailakhyan et al. (1981), Martin et al. (1982)).

5.3 Onions are formed under long days

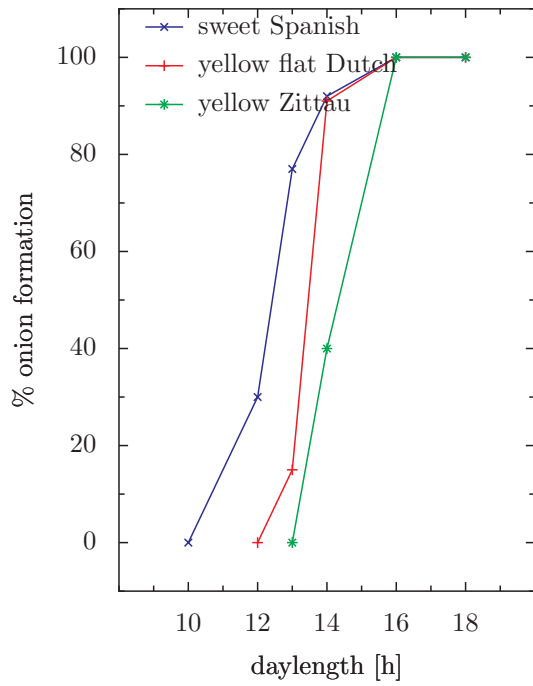


Figure 5.3: *Dependency of onion formation (percentage) from daylength (hours) in three different Allium cepa varieties. After Magruder and Allard (1937)*

5 *Potato tubers*

6 Annual clock of the Dsungarian dwarf hamster

The Dsungarian dwarf hamster possesses an annual clock. It controls the weight of the animals, fur color, size of the male gonads, time of reproduction and the behavior during the course of the year.

Since this internal annual clock is not precise enough, it has to be set continuously. For this purpose the daylength is used, which changes during the course of the year in a characteristic way and can be used as a precise calendar.

To proof that an annual clock is indeed running, experiments have to be performed in air conditioned chambers in which temperature is kept constant and the daylength does not vary.

Where in the brain of mammals the annual clock is located is not yet known.

An annual clock has advantages for animals.

The Dsungarian dwarf hamster (*Phodopus sungorus*, figure 6.1) lives in subterranean holes in the steppe of Dzungaria in the Northwest of China. They feed on grass- and herb seeds, green plant parts and insects. In this regions the environment changes during the course of a year heavily. This is true especially for the temperature. It might rise up to 45⁰C during the summer and drop in the winter to -64⁰C. The animals are therefore adapted to these extreme conditions in respect to their metabolism, physique and behavior.

Remember: We got to know in the preceding chapters organisms which recognize the time of the year photoperiodically by

measuring daylength with a daily clock. But we dealt with cases in which an internal annual clock allowed to know the time of the year. This annual clock is in most cases photoperiodically set, since it is not precise enough to allow an organism to go along without synchronization with the environment.

That is also the situation in the Dsungarian dwarf hamster. The animals reproduce only at seasons where the offspring has the best chance to survive. This is controlled in these animals not only photoperiodically, but underlies also an annual rhythm (see annual changes of the gonad weight in figure 6.2 and further annual rhythms in this figure, Hoffmann (1978)). In the same way reproduction of other rodents such as the Syrian hamster is not only influenced by daylength, but in addition by an annual clock. However, in the Dsungarian dwarf hamster the influence of the annual clock is much more pronounced. In spite of this dominance the internal annual clock by itself would not suffice to restrict the necessary changes in the body to a narrow period. The annual clock's rhythm deviates in its period slightly from the length of a year. Therefore the annual clock has to be additionally synchronized by the daylength (photoperiod) with the annual rhythm of the environment. In this way it is for example accomplished that in the Dsungarian dwarf hamster all males produce sperms at the same time and that the females will have their estrus shortly afterward. This

6.2 Where is the annual clock located and how does it function?

provides security for the propagation of the animals.

During the synchronization by daylength the hormone of the pineal, melatonin, plays an important role, as is the case also in other vertebrates. It is produced only during the night and needs a certain length of the dark period, in order to be made. It tells the animals whether the night is long or short. In this way the animals can recognize the season.

Annual rhythms are also known in other mammals such as ground squirrels (hibernation in golden mantled ground squirrel *Spermophilus lateralis*), squirrels (body weight of *Tamias striatus*), bats and sheep.

6.1 The distinctiveness of annual clocks

When are we allowed to speak of an annual clock? It could well be, that certain changes in the environment during the course of a year are perceived by the animals. In this case they do not need an annual clock. If we are indeed dealing with an internal annual clock, we have to show, that it is running also under constant conditions. We must make sure that the animals are not exposed to the natural light-dark cycle, which changes during the course of the year. Instead the animals must be kept all the time under the same daily conditions consisting of 12 hours light and 12 hours darkness. Likewise the environmental temperature has to be constant. Furthermore, the process, which is controlled by the annual clock, has to be observed for not just one year, but for at least two years, possibly longer. In the case of a true annual clock it will be seen, that the observed annual rhythm of the animal does not last exactly 12 months, but is somewhat shorter

or longer. A genuine annual rhythm should furthermore be robust and its period should mostly be independent of the temperature in which the animals live (for instance in one experiment 15⁰, in another experiment 25⁰C).

Since the beat of the internal annual clock is not exactly 12 months, but somewhat longer or shorter, an annual Zeitgeber must synchronize the clock to exactly 12 months. In nature the daylength is used as a rule, as mentioned before. The light receptors for the photoperiodic Zeitgeber of the annual clock are unknown. But other Zeitgeber are able to synchronize the annual clock such as for example temperature changes, lack of food, monsoon-rainfall, social interactions.

6.2 Where is the annual clock located and how does it function?

Where in the mammal is the annual clock located? After [Stetson \(1971\)](#) and [Kuenzel \(1972\)](#) the hypothalamus in the brain seems to be involved. The signals of this clock are passed via the neuroendocrine axis eye, hypothalamus and gonads. Details are not yet known. It is, for example, completely unknown how the time measurement functions. It has furthermore not yet been studied which neurotransmitters are involved in the annual clock or are used to pass informations from the clock to the target organs. Whether hormones play a role in annual rhythms could be studied by eliminating or adding them.

The gonads of mammals do not seem to be needed for the function of the annual clock, because castrated animals do still possess an annual rhythm. Although the annual clock determines

via the hypophysis-hypothalamus-system, when the gonadotrophin-secretion begins and ends. The annual rhythm functions, however, independently of the secretion of the gonadal hormones. Likewise, the pineal does hardly influence the annual rhythm, as shown by removing this organ. The pineal is, however, necessary for the photoperiodic effect.

Annual rhythms have a genetic basis. Thus, cubs of the golden mantled ground squirrel (*Citellus lateralis*) possess an annual rhythm even if born under constant conditions.

Annual rhythms are wide spread among organisms. We find them not only among mammals, but also among vertebrates, invertebrates and in plants and unicellulars (see chapter 2).

What is the significance of annual rhythms? Using an annual clock the time of the year can be predicted by the seed of a plant and germination can begin at the right time (see chapter 3). If annual Zeitgeber of the environment are missing such as at the bottom of the sea (see chapter 2), an annual clock is certainly of advantage. A migrating bird is reminded by an annual clock even in the tropics, that it is now time to fly to higher latitudes. Furthermore organisms with an annual clock can safeguard them self against unreliable influences of the environment such as temperature and humidity. More in the next chapter.

7 Bird migration

Annual rhythms have been studied especially in birds such as migration to the winter quarters and the summer quarters and the behavior connected with reproduction. Examples are gonadal growth and the preparation to migrate, moult of feathers, changes in body weight and food preference at different times of the year.

Each year about 600 million birds migrate to their breeding areas or winter quarters (see figure 7.1). Migration might take many months (up to nine), whereas breeding can be brief (one month). Therefore a precise timetable is necessary. This timetable is an endogenous timing program. It is genetically fixed.

Short distance migrants are more flexible. Therefore the time of leaving and arriving varies to a larger amount. They migrate for nine months and breed for one month.

How precise these annual rhythms work can be seen in so called ‘calendar birds’. They arrive during just a few specific days of the year from their winter quarters in our latitudes. The spotted Redshank *Tringa erythropus* for example arrive in the area around Helsinki between the first and eighth of May (4.5 ± 2.06 days), the Northern cliff swallow *Pterochelidon albifrons albifrons* arrives in San Juan Capistrano in California around the 19th of March (figure 7.2).

7.1 The benefit of an annual clock

Annual rhythms are of advantage to tropical birds and migrators near the equator.

The differences in daylength are namely in these areas too small to be useful for photoperiodic signaling of the migration time. The animals live practically under constant conditions. They must, however, initiate at certain times of the year distinct processes or behaviors. For example migrating birds which hibernate in tropical regions must begin migration to the breeding quarters at the right time. Without an annual clock they would be cue ball of environmental conditions and a temporal irregularity could have disastrous consequences.

The synchronization with the environment consists of an interplay between internal annual clock and Zeitgeber of the environment such as photoperiodic signals. There are, however, also additional fine tuners. By and large the adaptation to the environment becomes on the one hand quite flexible, but on the other hand also quite reliable.

An internal annual calendar allows the animals to prepare for changes of the environment internally. In this way they are not taken by surprise. This is perhaps the reason why most endogenous annual rhythms are shorter than 12 months. The internal annual clock ‘rings’ already before the expected event occurs and the animals can prepare for it in time. In this way for instance reproduction and hibernation can occur at the right time of year, the sexes can be synchronized at the begin of the reproductive time and specific timing programs can proceed as action chains.

The annual clock controls also the du-

7 Bird migration



Figure 7.1: *Barnacle geese (Branta leucopsis) on migration from Greenland passing Iceland (this image) to Scotland. Painted from the author after a photography in ?*

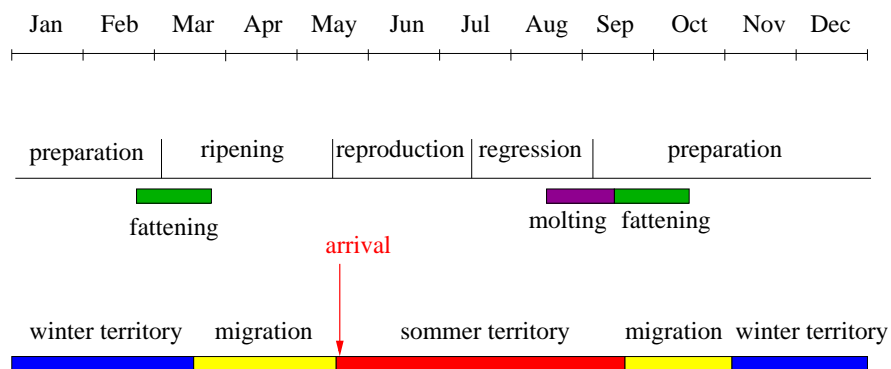


Figure 7.2: *Typical annual breeding- and migration-cycle of birds of temperate latitudes. Very top: Time of year (months). Middle: Sequence of events in a bird with preparation of the body for migrating to the summer quarters (feeding a lot for reserves, ripening of gonads), reproduction, regression of gonads and preparation in the fall for migrating to the south (moult and carving). Bottom part shows the times spent in the winter quarters and summer quarters and the time of migration. In calendar birds the arrival time is restricted to a few days of a certain month. After Beck (1963)*

7.1 The benefit of an annual clock

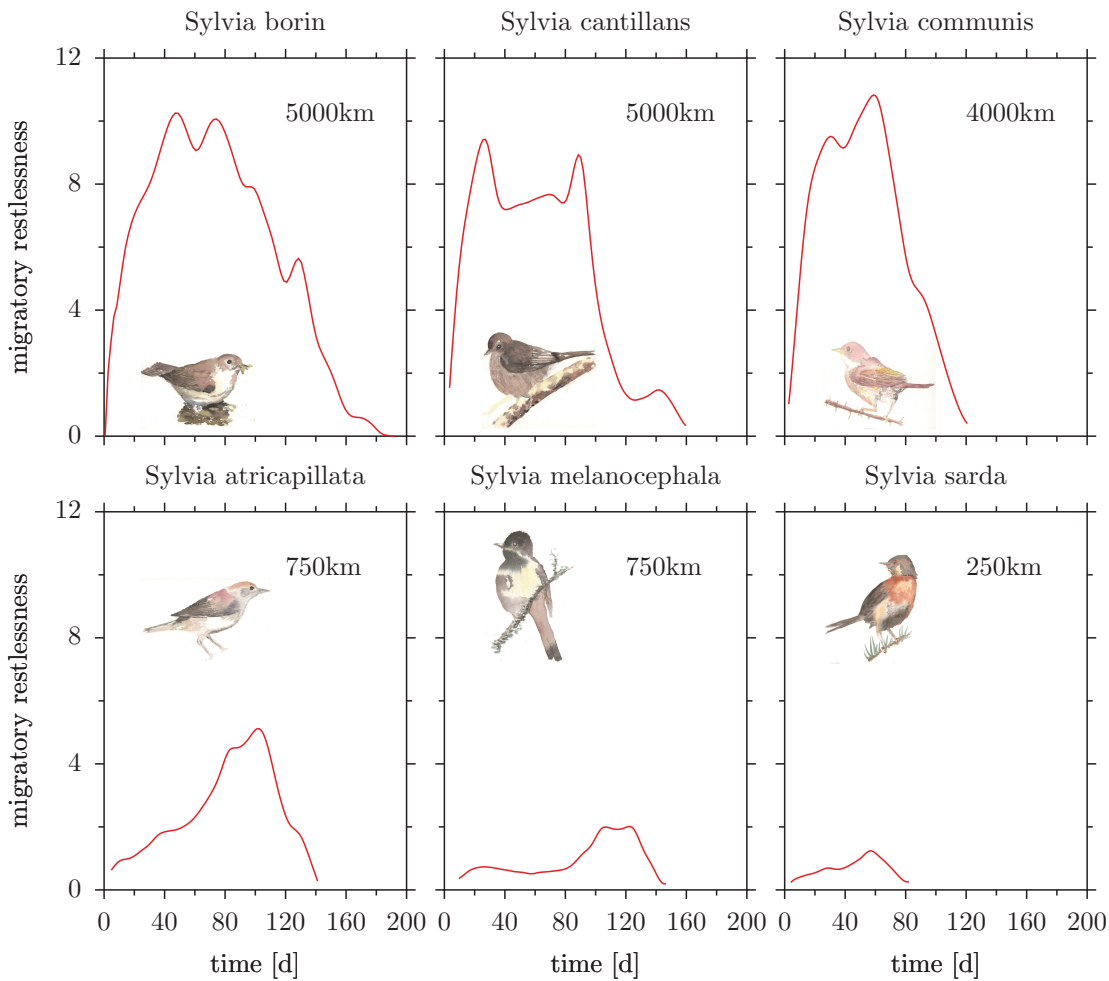


Figure 7.3: An annual clock controls duration and amount (amplitude) of the nocturnal activity. In the case of warblers (lower images) Garden warbler *Sylvia borin*, black cap warbler *cantillans*, whitethroat warbler *communis*, atricapillata, Sardinian warbler *melanocephala* and Marmora's warbler *sarda*) duration, amount and pattern of migratory restlessness are preprogrammed. The nocturnal activities (curves in upper part, number of nocturnal 0.5 hour-intervals with activity) are highest and longest in the long-distance migrants. After [Berthold \(1973\)](#) (curves). Images painted by the author after photographs in [Delin and Svensson \(1989\)](#)

7 Bird migration

ration and vigor of events. That means, the amount of fat deposited for the different phases of hibernation are preprogrammed. In different warbler species (here shown in figure 7.3: Garden warbler *Sylvia borin*, black cap warbler *cantillans*, whitethroat warbler *communis*, *atricapillata*, Sardinian warbler *melanocephala* and Marmora's warbler *sarda*) the duration, amount and the pattern of migratory restlessness are preprogrammed. By using vector-navigation even inexperienced migrating birds find automatically their destination.

7.2 Migration, migratory restlessness and moult

The drive to migrate in the search for food is present in many animals. They pull away in the winter from the mountains into the valleys or in the hot season from the steppe in more humid areas. It is even discussed that dinosaurs migrated (see [Engelmann and Hellrung \(2003\)](#)).

In birds the migrations are much more pronounced. Often bird migration begins already when plenty of food is still available, as for instance in the golden oriole and the swift. They become restless before migration (Zugunruhe). Some bird migrants cover long distances between the winter- and summer quarters. The arctic stern *Sterna macrura* migrates twice a year a distance of about 10 000 km. The swallow *Hirundo rustica* migrates in the fall to South Africa and comes back in the spring. Even small birds such as the ruby-throated hummingbird *Archilochus colubris* cover long distances. This bird crosses the Gulf of Mexico. It weights normally only 2g and adds another 2g to its weight before migration. During its flight many birds use

the sun or, if they migrate during the night, the stars for navigation.

Observations in the willow warbler (*Phylloscopus trochilus*, [Gwinner \(1968\)](#)) gave the first hints that bird migration is controlled by an annual clock. This song-bird resides for a long period in equatorial regions. In March it begins to migrate in higher northern latitudes, in late July and August it returns to the equatorial winter quarters. Like many other small migrating birds it migrates during the night, even though it is normally a day-active bird. If kept in cages, it develops migratory restlessness around these times of the year. This activity can be measured in Emlen-cages (figure 7.4).

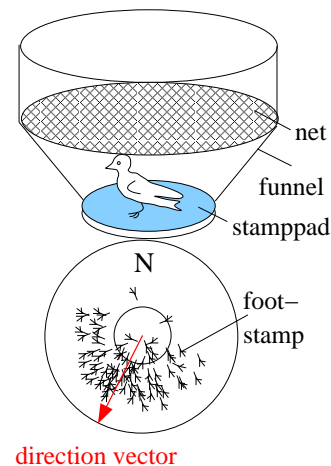


Figure 7.4: In an Emlen-cage migratory restlessness can be recorded. A stamp pad (blue) in the center stains the feet of the bird. Under migratory restlessness the bird tries to fly in the direction of its migratory flight. It jumps at the paper in the funnel staining it with the stain of the stamp pad. From the author after [Emlen and Emlen \(1966\)](#)

The birds prepare them self for migration: The feathers are changed (moult).

7.2 Migration, migratory restlessness and moult

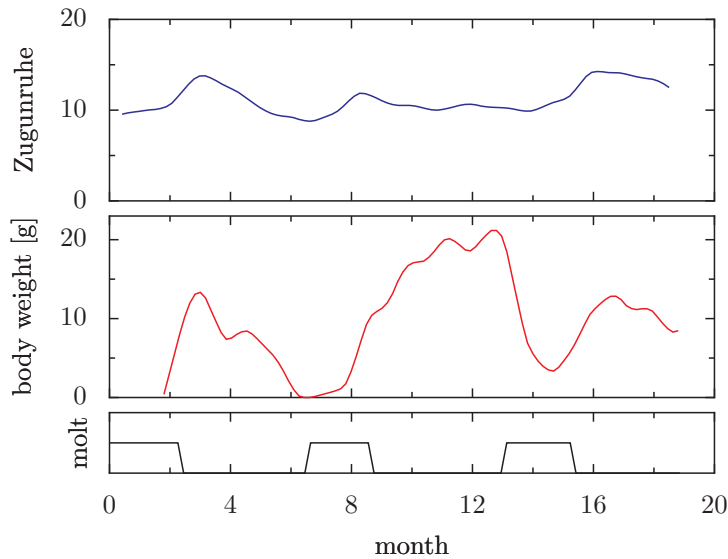


Figure 7.5: Circannual rhythm of body weight (blue curve), nocturnal activity (red curve) and moult (black bars) of a willow warbler (*Phylloscopus trochilus*), which was kept for 18 months under constant temperature and in a 12:12 hour light-dark cycle. Number of nocturnal ten-minute intervals with activity plotted against the time of the year (months). After Gwinner (1967)

Furthermore fat is deposited in the body. As a consequence body weight increases considerably. It was first assumed that these events are triggered by the daylength. The differences in daylength are, however, in equatorial regions very small. It was therefore not astonishing when it turned out that the changes in physiology and behavior occurred also if the birds were kept for a longer time in the laboratory under a constant daylengths (12:12 hour light-dark cycle). After 28 months of recording the nocturnal activity the curve shown in figure 7.5 was obtained. A circannual rhythm of 10 months (Gwinner (1967)) can be recognized. Thus, even without external Zeitgeber an endogenous annual program in the bird is executed which steers the preparations for migration and the migration. Since the 'freerun period' differs clearly from the length of a year (10 in-

stead of 12 months), we are dealing with an endogenous rhythm.

Similar studies were performed on other warblers. Figure 7.6 shows, how the size of gonads of garden warblers (*Sylvia borin*) changes during 33 months at a constant temperature of 20°C and in a light-dark cycle of 12:12 hours as compared to a group kept in natural days (Berthold et al. (1972)). Blackcaps (*Sylvia atricapilla*) were even kept for more than 8 years in a 10:14-hour light-dark cycle (Berthold (1978)). They too showed an endogenous annual rhythm, namely in the moult. The period length was 10 months; thus in the eight years nine endogenous years elapsed.

Annual rhythms of size of gonads were studied intensively in starlings (*Sturnus vulgaris*) (Gwinner (1981)). Figure 7.7 shows the results of an experiment in which the birds were kept for 43 months in a 12:12

7 Bird migration

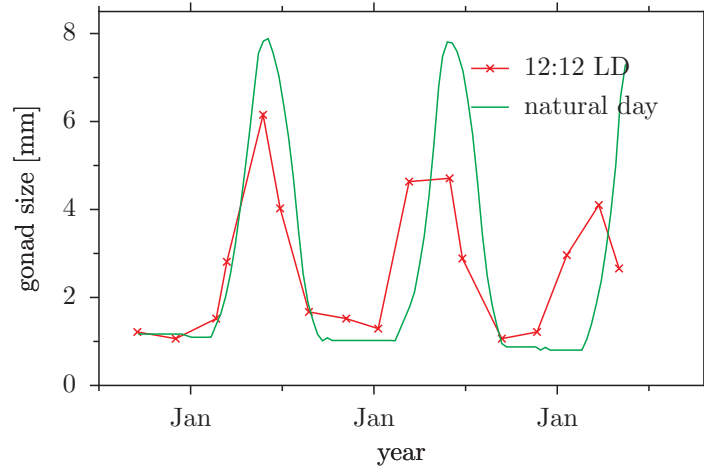


Figure 7.6: Annual rhythm of the body weight, migratory restlessness (thin bars) and moult (thick bars) of garden warblers (*Sylvia borin*) during 33 months at constant temperature of 20°C . One group (green curve) was kept under natural days, another group (red curve) under 12:12 hour light-dark cycles. Each January is numbered. After [Berthold et al. \(1972\)](#)

hour light-dark cycle. The curves represent the annual rhythm of the size of gonads and of moult.

7.2 Migration, migratory restlessness and moult

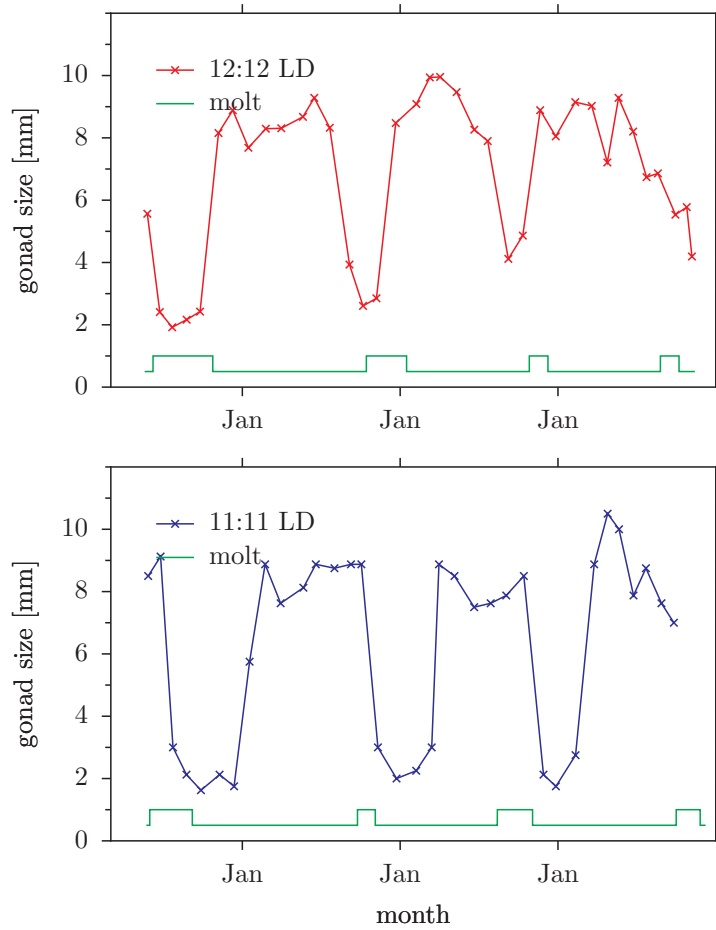


Figure 7.7: Circannual rhythm of size of gonads (curve) and of moult (bars) in starlings (*Sturnus vulgaris*). Individual birds were kept for 43 months in a 12:12 hour light-dark-cycle (upper curve, red) or in a 11:11 hour light-dark-cycle (lower curve, black). After [Gwinner \(1981\)](#)

7 *Bird migration*

8 Experiments

Some experiments are described which can be performed without much effort: Formation of aerial bulbs under short day in begonia, whether the sprouting of potato tubers occurs in an annual rhythm, annual rhythm of germination of several plants, photoperiodic flower induction of morning glories and whether this is temperature dependent.

8.1 Induction of aerial bulbs in *Begonia evansiana*

*Begonia evansiana*¹ belongs to the tropical and subtropical (Begoniaceae). This family consists of five genera and 820 species, herbs and semi-herbs mainly at humid habitats. They are often found in the woods, but also in dryer and cooler places. They are frequently kept in botanical gardens.

The genus *Begonia* consists of about 800 species. *Begonia rex* (rex-begonia or leaf-begonia) and numerous breedings as well the bulbous begonia with their large flowers and the lush Flor belong to it. Bulbs store water and help to outlast unfavorable times. One representative is *Begonia evansiana*. Try to get a plant or a bulb from a flower shop or a botanical garden (tell the gardener what you are planning to do with it)².

¹synonym with *Begonia grandis*. Flowers from July to August.

²to rear plants from seeds takes a long time, up to a year. The aerial bulbs form in the axles of the leaves and can be planted after keeping

In *Begonia evansiana* one to two short days are already sufficient to induce the formation of aerial bulbs. The best season for this experiment is the summer, because under long days no tubers are produced. If you put the plants for two nights in a dark room or cabinet and make sure they receive 16 hours darkness per day (short days), aerial bulbs will develop.

If you have several plants available, you could vary the length of the dark period. At which night length are still tubers produced? Try out dark periods of 16, 14, 12, 10 and 8 hours.

What happens if you illuminate the plant in the middle of the long dark period for one hour?

8.2 Sprouting of potatoes at different times of the year

If the potatoes have been stored in the cellar for the winter, you could every month put some of them in flower pots with humid garden soil and observe, whether they sprout and how long this takes. Do not forget to label the pots with the time of planting. Put the pot in a dish with water, which keeps the soil humid.

Are there times of the year at which the potatoes sprout faster?

them in a cool place -but without frost- in the spring. Use well aerated, neutral to slightly acid (pH 6-7) soil and keep the plants wet. Moderate shadow is favorable for rearing.

8.3 Germination and annual rhythm

In chapter 3 it was mentioned that the seeds of the following plants germinate in an annual rhythm: Perforate Saint Johns wort *Hypericum perforatum*, foxglove *Digitalis lutea* (figure 3.3), cinquefoil *Potentilla molissima*, hedge hyssop *Gratiola officinalis*, *Chrysanthemum corymbosum*, the mistletoe *Viscum album* and wild strawberry *Fragaria vesca*. Collect seeds of one or several of these plants and keep them in well closed receptacles containing a bag of silica-gel (which keeps the vessel dry)³. Take a sample of the seeds every 14 days and sow them on soil in flower pots. As you have done with potatoes you can put a dish with water underneath the pot to keep the soil wet.

Are there times of the year at which the seeds germinate faster?

8.4 Experiments with the shortday plant *Pharbitis*

The cover of this CD contains seeds of the morning glory *Pharbitis nil*⁴ (figure 8.1). The variety 'violett' can be induced to flower by a single short day photoperiod (figure 8.2). The plants can be induced already at a stage where the cotyledons

³the berries of the mistletoe should not dry out. Keep them in a receptacle with a net on top of water. Furthermore the berries have to be illuminated with small fluorescent tubes (or white photo diodes), because they do not germinate any more if kept more than three days in the dark. The cable for the light source can be laid through the sealing of the door of the refrigerator. The chocking coil should be kept outside the fridge because of the heat it produces.

⁴Seeds can be purchased from the Maruthane Trading Company in Tokyo (Japan). They can be stored a few years in a refrigerator.



Figure 8.1: *Morning glory* *Pharbitis nil*. Left: vegetative plant kept in long day. Right: flowering plant kept in short day

have just unfolded and no other leaves have yet been produced. The early indications of flower induction can be seen under the binocular already a few days after the photoperiodic induction. This allows to perform experiments in a short time. More details for rearing in Engelmann and Klemke (1983). The seeds are soaked over night in tap water and planted 15 mm deep in pots with garden soil. They germinate under white fluorescent tubes.

8.4.1 Determination of the critical dark period

In seedlings of *Pharbitis nil* strain violet a single short day with a 16 hour dark period induces flowering. Shorter dark periods induce also, up to the critical dark period of 9-10 h. You can test this in an experiment:

If the cotyledons are fully extended, the plants are photoperiodically induced to flower by a single dark period. Bring the plants in groups of 5 in a dark room (ex-

8.4 Experiments with the shortday plant *Pharbitis*

cept five plants which stay as controls in the light). After 6, 8, 10, 12, 14, 16, 18 and 20 hours put the various groups back under the white fluorescent tubes⁵ (see figure 8.3).

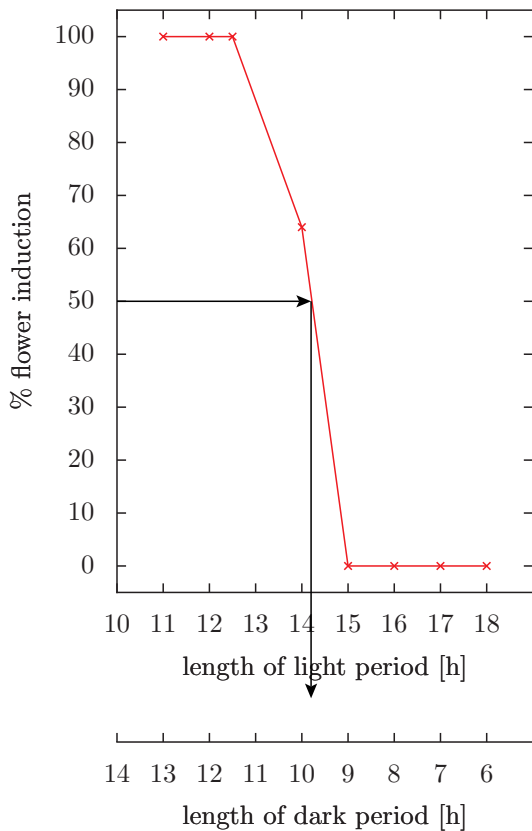


Figure 8.2: Effect of varying light periods on the induction of flowering in the morning glory *Pharbitis nil*. After *Takimoto and Hamner (1964)*

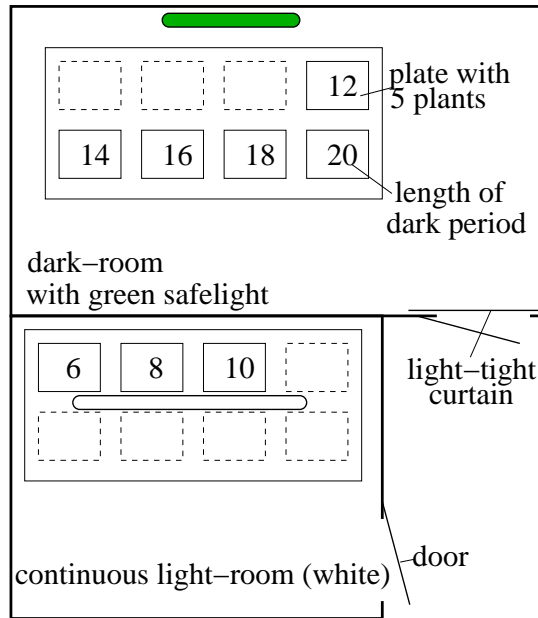


Figure 8.3: In two cellar rooms the critical daylength can be determined. First the morning glories are reared in eight groups of five plants each under continuous light produced by a white fluorescent tube. When the cotyledons have unfolded, all eight groups are transferred to the dark room. A green fluorescent tube serves as safelight. After 6 hours one of the groups is returned to the room with continuous light. After 8 hours the next, after 10 hours the third group is transferred. After 20 hours all groups are back in continuous light. A week later you can check under the binocular which plants flower

Already one week after the dark period

⁵no light should fall on the other groups in darkness. You could use a green foil in front of a flashlight as safelight

8 Experiments

treatment you can check under the binocular or with a strong magnifying glass the buds. Flower buds are characterized by two long bracts and a broad apex, whereas the vegetative buds have short bracts of unequal size and a pointed apex (figure 8.4).

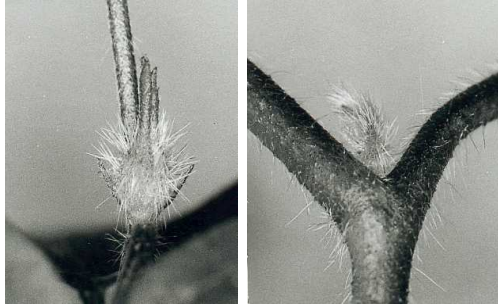


Figure 8.4: *Flower buds and vegetative buds of Pharbitis nil: left flower bud, right vegetative bud*

Determine the mean number of flowers per plant for each group. Plot the results against the length of the dark period. The critical dark period lies at 50% of the maximally possible induction.

8.4.2 Does the critical dark period depend on temperature?

You can do the experiments also at another environmental temperature (for instance in the winter in the cellar at 15°). This allows you to check whether the critical dark period of flower induction is influenced by temperature. Circadian rhythms are, as you might recall, temperature-compensated in their period length.

Photoperiodic experiments in *Pharbitis nil* are described in a book ([Engelmann \(1999\)](#)).

9 Further books

I have written further books or am in the process of writing. They are also concerned with topics, which have to do with rhythmic events in organisms - my specialty as a scientist (*Engelmann (2007)*, *Engelmann (2004c)*, *Engelmann (2009b)*, *Engelmann (2009a)*, *Engelmann (2009b)*, *Engelmann (2009c)*, *Engelmann (2008)*, *Engelmann (2004a)*, *Engelmann (2004d)*, *Engelmann (2004b)*).

9 *Further books*

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