How plants grow and move

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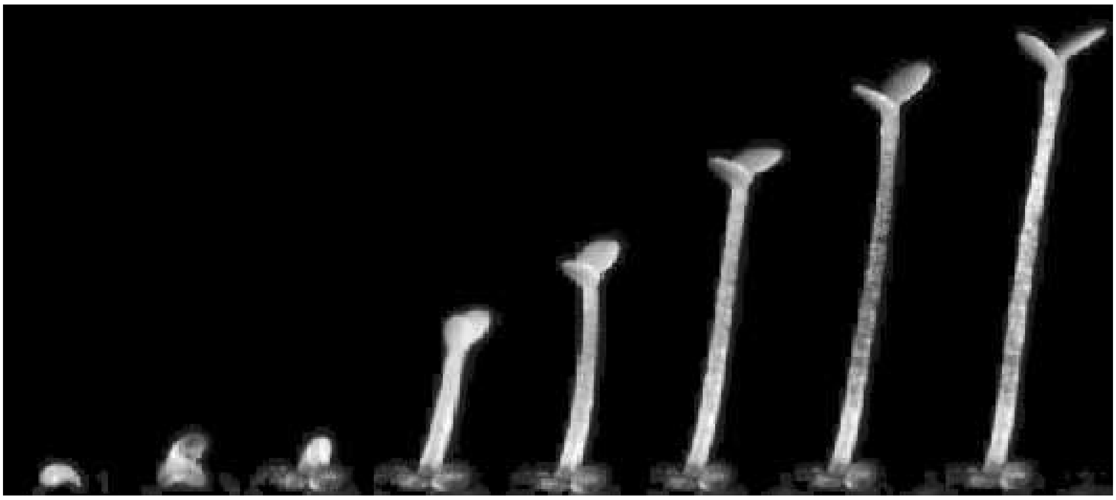
 My special thanks to Mareike Förster, Tübingen, who produced the indicated images using copies of originals. Thanks to Joachim Schuster, Winfried Hellrung and Andreas Neugebauer, who studied the growth of *Arabidopsis* and other plants for quite some time and with much patience. I had advices and help from the employies of the Botanical Garden Tübingen, especially Mr. Franz.



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

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Introduction

When I went to school, I was lucky enough to have teachers in biology, chemistry and physics who showed us pupils how to experiment. They tought us to observe and to ask nature for answers by doing experiments. Executing experiments brings us to an increasing understanding of nature.

In the meantime man has succeeded to grasp more and more of the processes in nature. We can inform ourselves in books and movies and other media. There are, however, also disadvantages in it. One disadvantage is, that we easily forget or never learn, how these informations were obtained and how cumbersome this usually is. An other disadvantage is, that we do not try anymore to ask questions and to find our own answers to them.

I have discribed in this book a few interesting stories concerned with growth and movement of plants.[1] I would like you to observe the described phenomena yourself. At various parts of the book I propose experiments, which answer questions and decide between hypotheses[2]. As is a matter of fact in life, the best way of learning is by doing, or, as the Romans said

> Verba docent
>
> exempla trahunt

in English: Words inform, examples bring us forward.

[1]In a book by Hensel (1981) numerous further examples can be found

[2]an (at the time beeing nonproven) assumption. It has to be proven first before becoming a firm inventory of our knowledge

Overview

I am sure that you have observed already a seed during germination. If not, it is high time to do so, since it is one of the most interesting events in the life of a plant. You can read in chapter 3, how to observe a bean seed during germination and what else one can see.

How does a seed come into being, what is its purpose, and how arises a plant out of it? The elongation of a seedling is more complicated than anticipated. The stalk does not just stretch. Instead it exhibits during elongation several different movements. We will learn different methods, which allow us to observe in more detail how this complicated growth takes place.

For a plant it is important to grow towards the light. If it grows in the shadow, it tries to find a way to get more light. The plant is afterward lopsided. This can be seen nicely at the edge of the woods where shrubs or even trees grow slanted.

If a plant has been thrown down by storm or rain or by both, it is often able to erect itself. The best way of observing this is a field of cereals. After a storm larger parts of the field can be found to lie at the ground.[3] If you come back to the field a few days later, most of the halmes have erected themselves again. You can see at the halmes, that the lower side of the nodes have grown more strongly than the upper side and that this has brought the halm back into the plumb line. We will perform an experiment, which shows, that a sunflower–seedling in trying to grow perpendicular, oscillates around the plumb line (section 1.11).

If you look at White Clover on a meadow (figure 0.1), you can recognize easily the

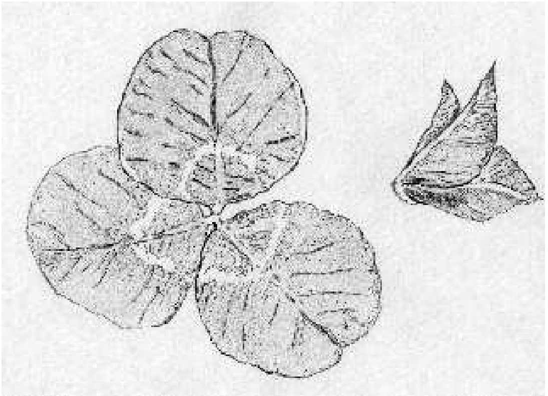

Figure 0.1: *White Clover in day– (left) and night position (right), from above*

three–parted leaves. If you check them in the evening, they are folded and wraped by the third leaf like a mantle. Each of the three leaflets has a joint. They sit at the end of the petioles and are responsible for the daily leaf movements. It can be demonstrated that they do react independantly of each other. If for example only one of the leaves is illuminated, it, and only it, changes its rhythm of movement. The other two don't.

Bean plants are also able to move their leaves. It is due to joints at the lower and upper end of the petioles. We should have a closer look at these joints, in order to understand, how the movement is brought

[3]This is especially the case in cereal varieties with long halmes. Today they are not much grown anymore, since farmer do not need as much strow as they used to in earlier times.

about. By doing so, we will also find out that in one and the same leaf of bean plant there are in addition to the daily movements also faster movements.

Much more plants do not only move during their growth. They are also able to lift and lower their leaves or even turn them. A master in this respect is the telegraph plant *Desmodium*. It possesses leaves, which are composed of leaflets (figure 0.2). The larger terminal leaflets begin to lower in the evening until they finally reach a vertical position. In the morning they rise again until reaching a horizontal position. These leaflets are thus a kind of hand of a day clock. There are furthermore one or two smaller lateral leaflets, which are a kind of hand of a minute clock. They too move up and down but need only a few minutes and do it during day and night.

Figure 0.2: *Telegraph plant* Desmodium gyrans *with larger terminal leaflets and* smaller lateral leaflets

1 A spiral journey

Many plants outlast unfavourable periods as a seed, while the mother plant dies. If the environmental conditions improve, the seeds germinate and the young seedlings elongate towards the light. This is often connected with revolving movements. Plants bend towards lateral light. Knocked over plants are able to erect themselves. At which part of the stalk all this happens can be found out by close up images using a video camera. Sunflower seedlings oscillate back and forth, if stimulated by the gravitational force.

A plant which has hibernated as a protected seed will germinate after the environmental conditions became favourable again. The root breaks through the seed case, the stalk elongates, the cotyledons unfold. That sounds simple, is, however, quite complicated, as you will notice after having read this chapter.

1.1 Seeds – embryos in a waiting position

If a frog has laid its eggs, an egg cell divides into two, four, eight and soon very many cells. They become the frog–embryo, which develops into the tadpole. It ecloses from the egg shell and metamorphoses finally into a frog.

In plants it is quite similar. In the seed lies an embryo, which is waiting to germinate and to grow into a plant, as soon as the conditions are favourable.

If we put a bean seed for 24 hours in water, it swells quite a bit. Put it on a piece of wet filter paper and observe, how it germinates (see figure 1.1). The seed case is broken by the root. Soon the two cotyledons break through the case. They are folded and directed downward, but erect soon and unfold, if the plant is in the light and elongates. Elongation will be looked at in more detail later.

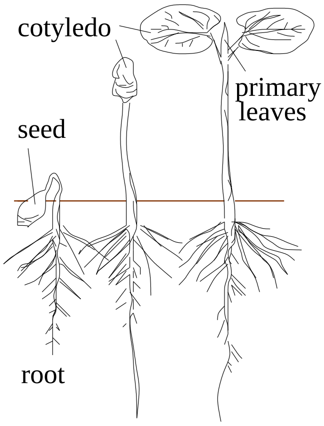

Figure 1.1: *After swelling of the seed (S) of a runner bean the root breaks through the seed case (left). Afterward the seedling with its cotyledons breaches the soil (center). The cotyledons serve the bean as a starch– and protein–storage. The primary leaves are also laid out already (right).*

1.1.1 How a seed originates

If we want to understand, what a plant seed is, we have first to see, how it arises. For this purpose we have a look at the flower of a plant (figure 1.2). It consists of a peduncle, a calyx, petals, stamen and in the center an ovary with a stigma. At the end of the stamen are pollen bags, containing numerous pollen grains.

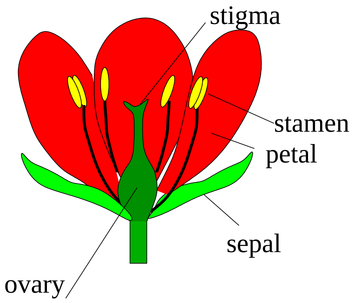

Figure 1.2: *Flower of a plant with peduncle, a calyx consisting of sepals, petals, stamen and in the center the ovary with a stigma. At the end of the stamen sit the pollen bags, containing numerous pollen grains.*

If pollen reaches the stigma, a pollen tube grows towards the ovary. There the nucleus of the pollen meets the egg cell and fuses with the nucleus of the egg. The female cell (in the ovary) is now a fertilized egg cell. It divides and, like the fertilized egg cell of the frog, becomes an embryo. You can recognize already the basic structure of the seedling (root, stalk, cotyledons). The story is, however, a bit more complicated, because around the embryo a nutrient tissue is formed which nurishes the seed.

In most cases the seed enters now a resting stage. It takes some time (for example the winter months), before it germinates to a new plant.

1.1.2 What a seed needs for germination

A dry seed can survive for quite some time. In contact with water it swells, the embryo breaks through the seed case and begins to elongate (see section 1.4). The cotyledons righten up and unfold. Later the seedling develops into a plant.

It is not only water, what the seed needs for germination. In quite a number of plants the seed germinates not before it has gone through a longer resting period. Often such seeds need a period of low temperature, before they are able to germinate at favourable temperatures. In other plants seeds possess an internal annual clock, which tells the time for germination. In still other plants the seeds need longdays (that is summer) for germination. Other seeds germinate only in light and are therefore called light germinators. Their seed must not be covered with soil. If that is the case, they have to wait until the soil is carried off by wind or rain. There are, however, also seeds, which germinate only in darkness. These dark germinators must wait, until they are washed into the soil or covered by soil which the wind carries along. There are finally seeds, in which the rain has to wash out of the case a substance, before they can germinate. All these tricks help the plants to germinate at a time, in which they can grow well, flower and set fruits.

1.2 Germination for light

For plants light is especially important. It supplies the energy necessary to produce all the substances needed for their growth. Therefore a plant tries to get as soon as possible light. If the seed lies in the soil and germinates, the young seedling elongates its stalk upward through the soil until it reaches the light. How this occurs is described in subsection 1.4 in an example.

To protect the cotyledons during elongation from becoming injured by the soil through which the plant has to grow, they are first bend downward (figure 1.3). Furthermore the seedlings are not yet green. Not before the plant has reached the light, the cotyledons erect and unfold into a horizontal position and become green. Elongation stops and the seedlings do not need to use the reserve substances in the seed for nourishment. They are able to produce with sun light and special provisions in the cells, the chloroplasts, sugar and other compounds which keep them alive and provide construction material for growth. The roots get the necessary water and the minerals.

Figure 1.3: *During germination of a salad–seed the plumula–hook between hypokotyl and cotyledons of the seedling is bend (left). It erects, as soon as the plant gets light (center). Afterward the cotyledons unfold (right).*

1.3 *Arabidopsis thaliana* – the fruitfly of the botanists

Botanists are people who study the life of plants. In current botany a plant is often used which grows at waysides and at waste disposal sites. Its name is Thale Cress or in Latin *Arabidopsis thaliana* (figure 1.4). Why have they choosen this particular plant? There are several reasons. The seeds germinate easily, the plants grow up fast and deliver seeds soon. Thus one does not need to wait long for the next generation. This is important, because the botanists use a special trick to understand the life of a plant: They search for mutants.

A mutant is distinguished from the wild type by certain properties: For example, a mutant could be longer than the wild type (figure 1.5). And why is this mutant longer? A gene of its genome has changed and thereby a certain step in its development differs. For example, a substance, which prevents the cells in the stem of the seedling to elongate, is not produced anymore. Therefore this mutant elongates further as the wild type does. With such mutants one can find out, what happens in the growth and development of plants.

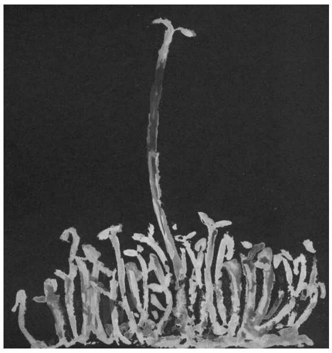

Figure 1.5: *A mutant of* Arabidopsis *with long hypocotyl. After a photography in Taiz and Zeiger (1998)*

Among zoologists (people, who study animals) a small fly has made furore. It is called fruitfly, in Latin *Drosophila*. The fly reproduces very fast, has many progenies, is easy to keep and many mutants have been produced. The same properties apply also to the Thale Cress. It is therefore called also jokingly *the Drosophila* of the botanists.

But we should come back to the seedling which has made its way out of the seed case and has elongated in order to reach the light above the soil. In this section I wanted to explain, why we have selected just this particular Thale Cress for studying growth.

Figure 1.4: *Arabidopsis as facultative longday plant. Left vegetative, right flowering*

1.4 A device for observing the growth of seedlings

To observe, how the Thale Cress is stretching to the light, we strew the very small seeds on a wet filter paper laying on a lid of a cuvette. To prevent it from drying out we put a stripe of filter paper underneath which reaches the water in the cuvette. After the seeds have germinated during the night, we distribute it evenly over the filter paper (figure 1.6).

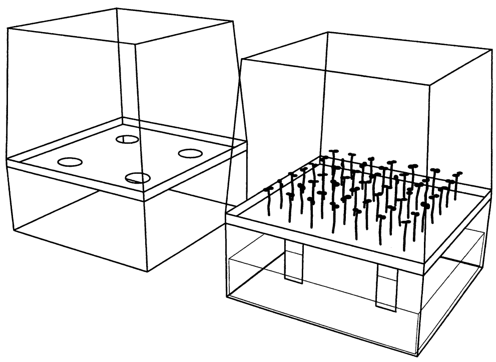

Figure 1.6: *Seeds of* Arabidopsis *on wet filter paper. It is positioned on the lid of a cuvette and connected with the water by a wick. Left: Cuvette with lid, four holes and cover on top. Right: With seedlings*

Unfortunately the seedling of *Arabidopsis* is very small. We have to observe it therefore with a lense. Andreas Neugebauer and Winfried Hellrung developed a device which allowed to take pictures of the elongation of the plants during day and night (figure 1.7). Some of the many pictures which were obtained will be shown and explained later. Andreas has written his PhD thesis on the elongation of seedlings in Tübingen (Neugebauer (2002), figure 1.8). Winfried studied control technique in Darmstadt and later some terms

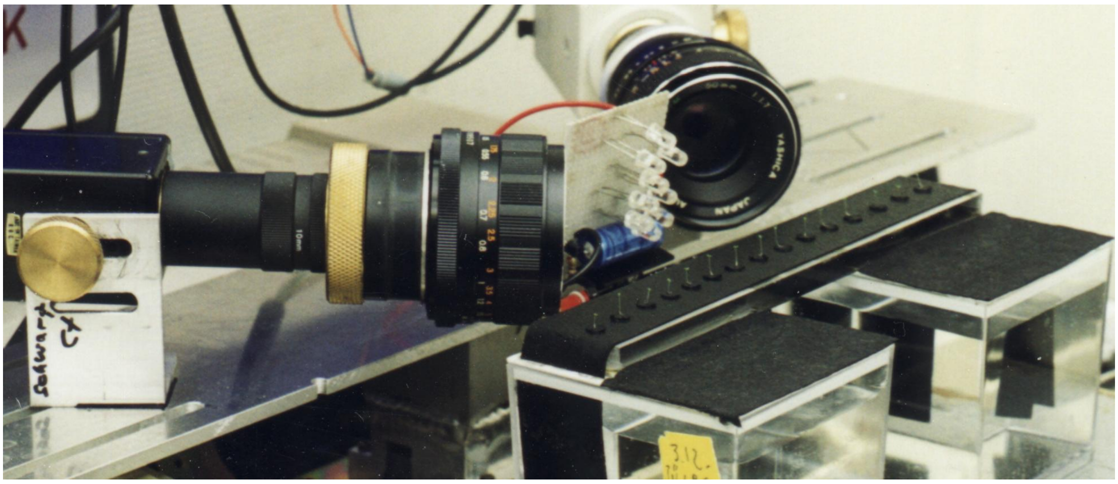

Figure 1.7: *A device allowing to take three dimensional pictures of* Arabidopsis *seedlings. Two cameras are mounted in a right angle to each other on a sled which can be moved horizontal, vertical and back and forth via a control unit. The location of the plants is adjusted and remembered by the control unit. It moves in front of a single plant, takes as many pictures as possible, averages the pictures and stores them. Afterward it moves in front of the next plant, does there do the same and so on, until after four minutes it starts again in front of the first plant. This and further pictures from* Neugebauer (2002)

biology in Tübingen. He is very familiar with computers and programming and developed methods to record the plants automatically and to evaluate the results, which is called image analysis.

First we will get to know the recording device. The seedlings are tiny. We need therefore a camera, which can take pictures of such small objects in such a way, that they can be seen enlarged on the picture. For this purpose a number of tubes are screwed between camera lense and camera case. We use a video camera and store the pictures in a computer (figure 1.9).

In order to take pictures of more than one plant we bought a machine, which is usually used to process metall pieces via computer–control. This machine allowed to move the camera in front of a bench on which up to twenty individual seedlings grew (figure 1.10). They are mounted on a black filter paper, which dips into a cuvette filled with water. In this way it stays wet all the time. The camera moves to a position, from which it can take a sharp picture of one of the seedlings. Afterward it moves to the next position, from where the following plant is photographed and so on, until it has reached the last plant. Now the sled moves back in the starting position, from where a picture of the first plant was taken and the sequence of pictures is repeated. This is done every four minutes.

But there is a problem. As we will see later, the seedlings do not grow straight upward, but turn during their growth. A seedling could for example in a certain moment bend towards the camera. It would therefore look shorter in the image. Therefore we have used two cameras and mounted them in such a way on the sled, that one of it sees the seedling from the left, the other from the right. The cameras are mounted rectangular to each other (see fig-

Figure 1.8: *Andreas Neugebauer (top) did his PhD thesis on the elongation of* Arabidopsis*–plants and Winfried Hellrung (bottom) has build the recording devices and wrote the programms*

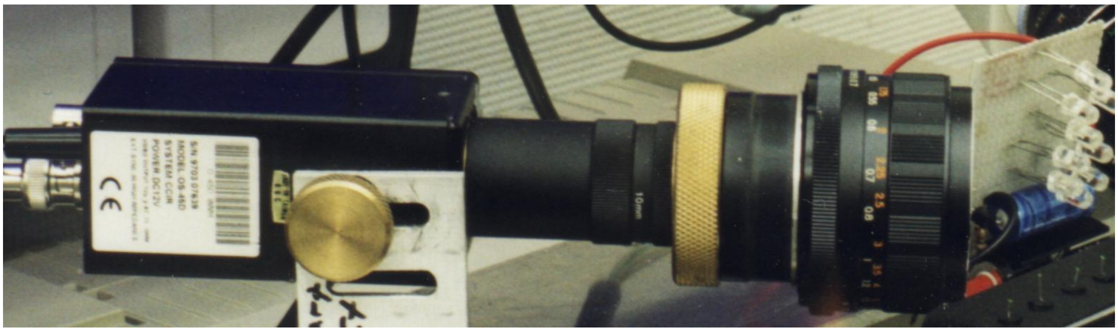

Figure 1.9: *Video–camera with tubes (yellow ring and smaller rings to the left of it) between lense (right) and camera case (left) for close up images of* Arabidopsis *seedlings. To the very right a set of infrared–light emitting diodes can be seen which are used to illuminate the seedlings*

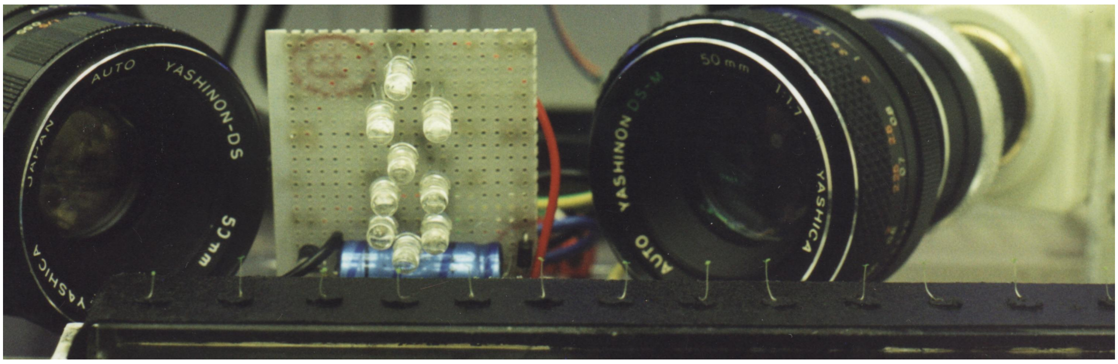

Figure 1.10: *Bench with* Arabidopsis*–seedlings, which are photographed sequentially by the two video cameras (next figure) in three dimensions. Infrared–light emitting diodes between the cameras for illuminating the seedlings*

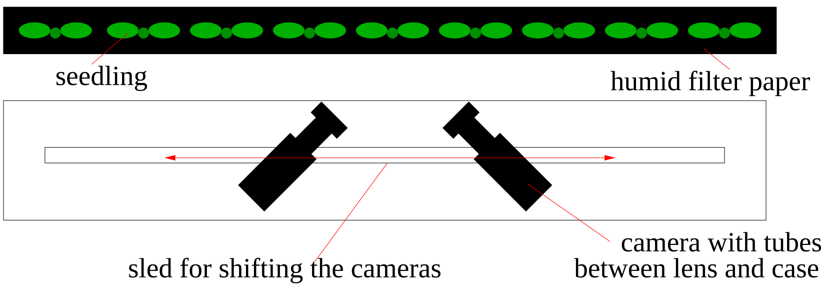

Figure 1.11: *Two video cameras are used for three dimensional images of* Arabidopsis *seedlings. They are moved on a sled from one plant to the next. At the end the sled moves back to the position from where it started and begins a new sequence of images of all the plants*. This is repeated *in four minute intervals*

ure 1.11). From the two images of the plant one can now calculate a three–dimensional plant.

We can even look at the plant three dimensionally (spacially), if one of the images is via software coloured green, the other red. If we use spectables with a red and a green glass, the eye with the green glass sees only the green image, while the red colour can not pass the green glass. The images made from the different angles are now composed in the brain to one spatial image. You can look at the movie in figure 1.12 with the red–green–spectacles enclosed in the CD case.

How the images are further processed, to understand what happens during growth, is shown in section 1.8.

1.5 Elongation and revolving: Slow and fast

When you watched the movie in figure 1.12 with the red–green–spectacle, you surely noticed, that the seedling did not just grew upward, but revolved around a vertical axis. These revolving movements are called circumnutations (circum – around, nutation – nodging). They were observed already quite early in other plants. The famous biologist Charles Darwin described them in his book 'The power of movement in plants'. Well studied are for example the pendulum movements of sunflower–seedlings grown in darkness. We will get to know them more precisely in section 1.11.

The stalk below the cotyledons is called hypocotyl. This is the structure were the circumnutation takes place. Joachim Schuster (figure 1.13) has described in his doctoral thesis circumnutations in seedlings of the Thale Cress, *Arabidopsis thaliana*. There are at the same hypocotyl different kinds of circumnutations. Some are faster, some more slowly (figure 1.14). If one looks from above at a growing seedling, one recognizes in a series of images circular, oval or pendulum movements. The revolving direction is different, but most of the fast circumnutations (SPN) occur clockwise, whereas the slower circumnutations (LPN) occur preferentially counterclockwise.

The fast circumnutations (SPN) take for one turn 15 to 60 minutes. They are observable mainly at the time the plant has germinated and elongates rapidly (see figure 1.15, red curve). Later during the elongation period the slow circumnutations (LPN, blue curve) prevail. One period takes more than an hour, partly even up to eight hours. Look at the figure minutely. It contains a lot of informations about what the tip of the seedling is doing. Besides the fast and slow circumnutations a daily rhythm occurs. It is recognizable by the steplike elongation of the hypocotyl. The elongation of a seedling is, as we have seen, much more complicated as anticipated. One has to look carefully at the process of growth to recognize the periodic revolving and the daily steps during elongation.

The time of one revolution is called period length or period for short. Figure 1.16 plots the number of oscillations at the various periods. Thus, a whole spectrum of circumnutations exist. How these oscillations come about is explained in more detail in section 1.8.

We saw already in figure 1.15, that the stalks do not elongate uniformly over time, but step–like. Elongation periods with fast oscillations are followed by periods, during which the hypocotyls hardly lengthen. This happens even if the plants are kept under continuous light at constant temper-

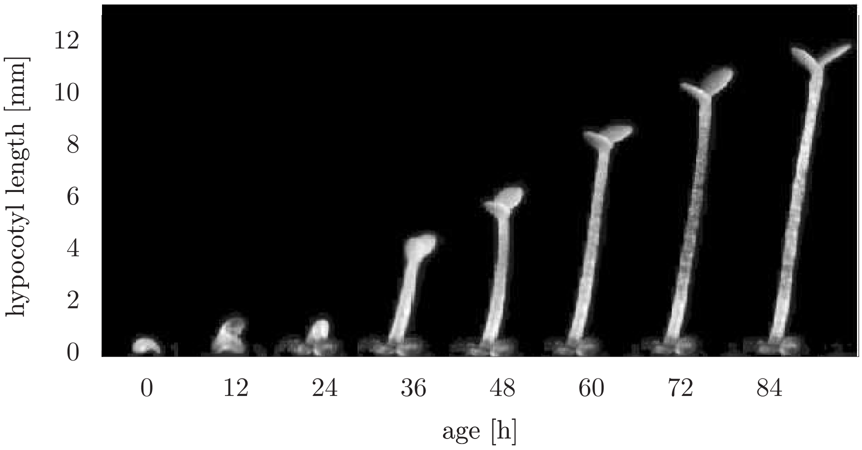

Figure 1.12: Arabidopsis–*plant during germination and elongation. For a demonstration see the time lapse movie at http://www.uni-tuebingen.de/plantphys/bioclox. With a red–green–spectacle you can follow germination and elongation three dimensionally. You will notice, that the hypocotyl is not just stretching, but additionally turning. This is called circumnutation*

Figure 1.13: *Joachim Schuster left with doctor hat, right in civil. After a doctoral thesis is finished, the graduate student is examined. Afterward the successfull examination is celebrated and colleagues and frinds decorate him with an adequate doctor hat, here with an oscillating sunflower seedling*

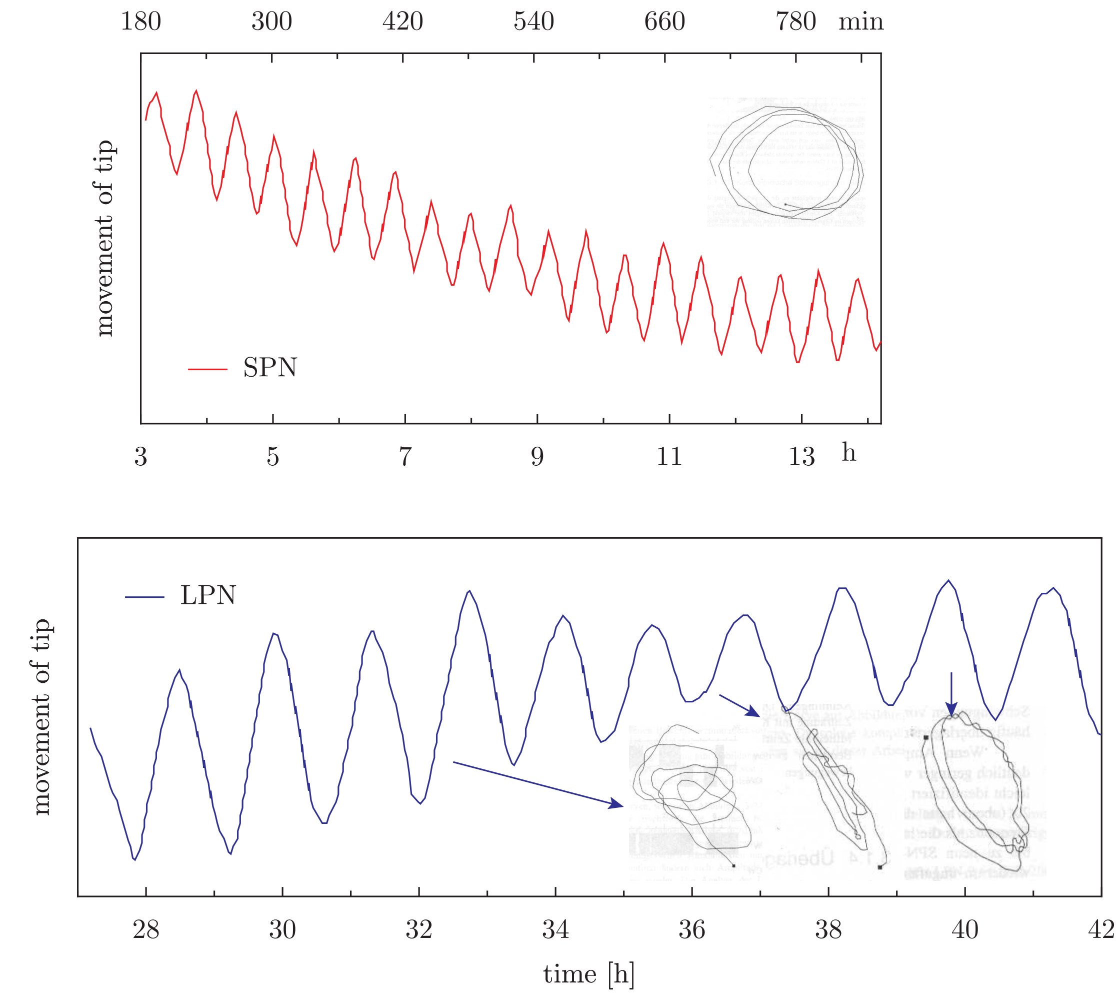

Figure 1.14: *During elongation of the hypocotyl the tip of the seedling* of Arabidopsis revolves. *Fast and* slow circumnutations *occur. Seen from the side the tip moves back and forth. The fast movement (SPN = short period nutation) is shown in the upper part of the figure (red, lower x–axis in hours, upper x–axis in minutes). The black curve shows the track of the movement of the tip while looking from above on the plant. It occured clockwise and ended at the point marker. In the lower part of the figure a slow movement (LPN = long period nutation) is shown (blue). In most cases they occur counterclockwise, as seen here (left blue arrow points to the view from above). From here onward the tip of the seedling began to move like a pendulum (arrow in the middle), and finally it revolved clockwise (right arrow).* From Schuster (1996), like the following figures

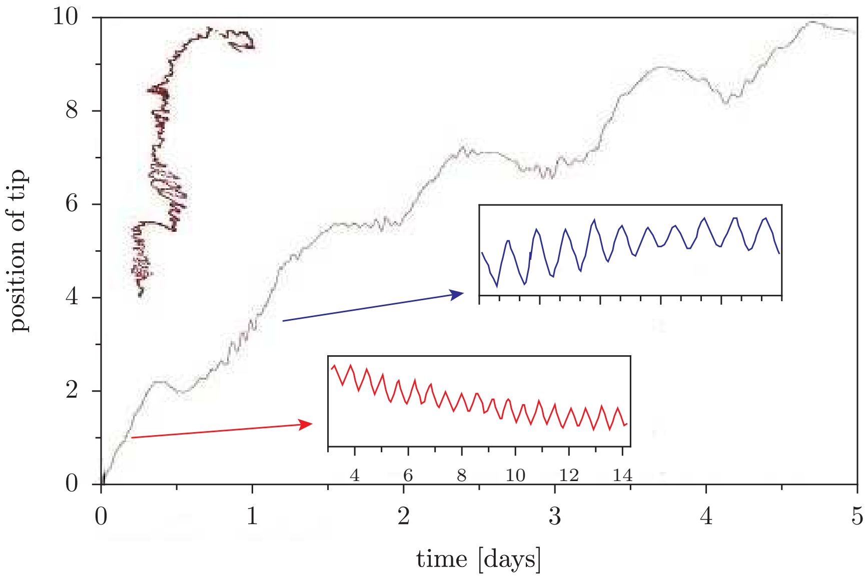

Figure 1.15: *The brown curve at the top left illustrates the growth and revolving of the seedling, if observed laterally. Only the track of the tip is shown. First the plant was small (lowermost position), than started to extend and simultaneously to revolve. Afterward the plant stopped extending for some time (the brown curve stays leveled or even drops a bit). Next the hypocotyl elongates again and revolves, but now much more slowly.*
Unfortunately this kind of plot does not allow to see at what time this all happens. It can be better seen if you look at the long stretched brawn curve in the figure. It has been plotted against time (days) at the horizontal axis. At the begin of recording it increases strongly: The seedling is stretching considerably and revolves at the same time. Since this is not well recognizable in the brown curve (the deviations from the plumb line are quite small), this part is shown magnified (red curve in the box). One revolution takes about 35 minutes. Than – during the night–time – growth stops for a while (the brown curve does not increase any more or even drops slightly). On the next day the hypocotyl continues to elongate. Again it is simultaneously revolving, but distincly slower (about one and a half hours, blue curve in the box). The elongation of the seedling slows down or stops and the tip does not turn anymore. This occurs each day until the end of the fourth day. This and further figures from Schuster (1996)

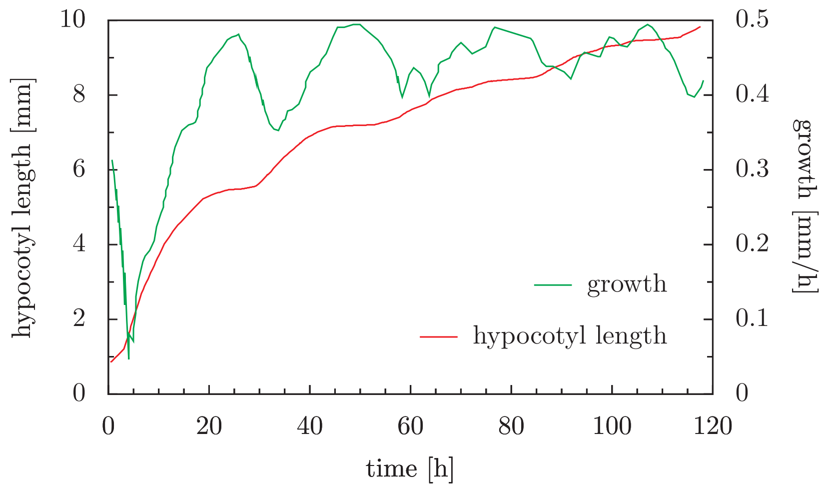

Figure 1.17: *The elongation of an* Arabidopsis *plant is controlled by a circadian clock. As a consequence the hypocotyl does not elongate uniformely, but step–like (red curve). During the steep part of the curve the seedling elongates strongly, during the flat part of the curve hardly. The horizontal axis shows time in hours, the vertical left axis the length of the seedling. The green curve shows the growth rate. This curve is obtained from the red curve by subtracting the values from their previous one.*

ature. If we mark on the time axis the times at which the seedlings stretch maximally and determine the length of time between these points, period lengths of 26.2 hours are found in this particular example. This is about the length of a day. The hypocotyl elongates thus in a daily rhythm at certain times strongly, at others hardly at all: Elongation is modulated by a circadian[1] rhythm. This can be seen even more clearly in figure 1.17. Here not only the length of the hypocotyl is plotted against time (red step–like curve), but also the growth rate (green curve). This curve is obtained by plotting the differences between successive measured values (mathematically it is the first derivative). The daily periods are well recognizable in this curve.

The circumnutations occur in daily patterns, and even if the plants are grown under continuous light at a constant temperature (figure 1.18). They occur only during strong growth. We will see later, that circumnutations are directly connected with the elongation of the stalk.

Further processes are controlled in a circadian fashion in *Arabidopsis* (figure 1.19). For example the cotyledons and the primary leaves move in a daily rhythm slightly up and down. Furthermore the stomata in the leaves open and close in a circadian way and parallel to it the water loss via the stomata (transpiration). Photosynthesis is also influenced in a circadian fashion by the day clock (Millar (1999)).

1.6 Bending to the light

Perhaps you have already noticed at a plant in a dark room how its stalk is bend to-

[1] circa = etwa, dies = day; also: etwa einen day dauernd

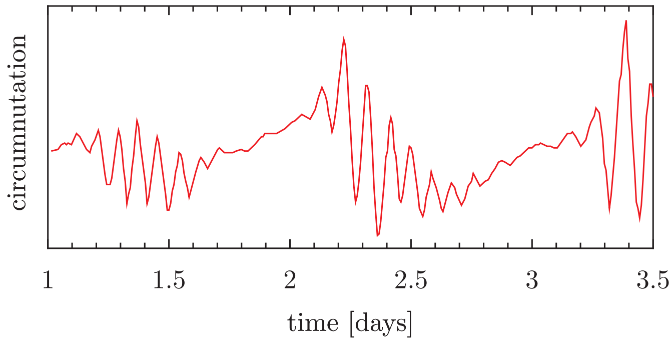

Figure 1.18: *Circumnutation of an* Arabidopsis *plant seen from the side. At the horizontal axis the time is plotted in days, at the vertical axis the particular position of the tip of the seedling*

wards a light window and how its leaves are positioned in such a ways that as much light is caught as possible. Light is of decisive importance for plants (figure 1.20).

If you illuminate the cuvette with seedlings (described at the begin of subsection 1.4) not from above, but from the side, you will soon notice, that all seedlings bend towards the light. This is called *phototropism*. This ability is important for plants, which germinated in the shadow of other plants and which must try to catch as much light as possible during their growth.

With our recording device we can also study the phototropic reaction more precisely. We can, for example find out, at which part of the hypocotyl bending towards the light occurs. We can determine the time it takes until the stalk starts to bend after the lateral light has been switched on. If the light is switched off again, the seedling grows straight again (although with circumnutations). We can study with this device also, which colour of light is especially effective.

1.7 Bending upward

The seedlings react not only to light, but also to gravity. This ability is called gravitropism. If we turn the lid of our cuvette with the *Arabidopsis* plants for some time upright, we will see how the stalks bend upward. We will describe some experiments in this respect later, but use larger plants for it (see section 1.11).

1.8 Understanding growth

In the last sections we got to know a number of reactions of plants which can all be recorded with our apparatus. But it does not help much to take pictures and to store them in the computer. We have to convert these images in such a way that we understand their meaning.

This is a difficult section, since it requires much mathematics and is based on programmes, which I do not catch either. But I do think that we will understand the principle. In any case we will see very nice colourful images which will help us to understand what is going on during the growth and revolution of the plants.

We have seen already before (figure 1.11)

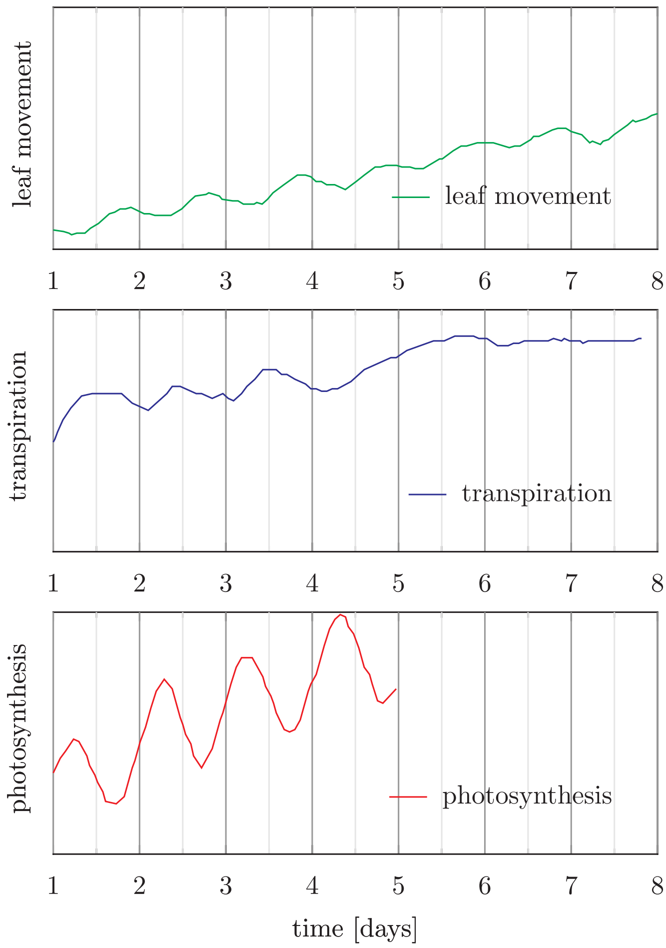

Figure 1.19: *Circadian control of events in* Arabidopsis*: Top: Movement of the primary leaf (green curve). Center: Transpiration (blue curve). Bottom: Photosynthesis (red curve). In this case the rhythmic regulation of an important gene of photosynthesis (LHC = light harvesting complex or antenna pigment) was recorded by using a genetical trick: Gene expression can be followed by bioluminescence (after Millar (1999))*

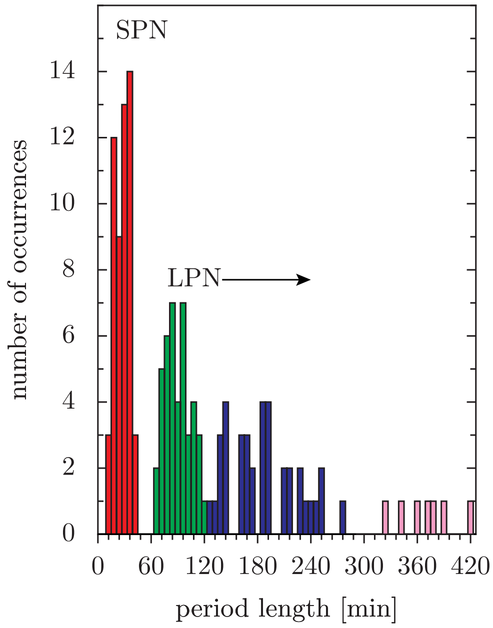

Figure 1.16: *On the vertical axis of the diagramm the number of occurrences of various circumnutations of* Arabidopsis*–seedlings is plotted. You see, that quite a number revolves in a 20 to 40–minute measure (SPN, red). Many are also found in a measure of up to 2 hours (LPN, green). Further to the right on the horizontal axis are oscillations with periods between 3 and 4 hours (LPN, blue). The first type (red) is called fast circumnutations, the second type (one hour or longer than an hour) slow circumnutations*

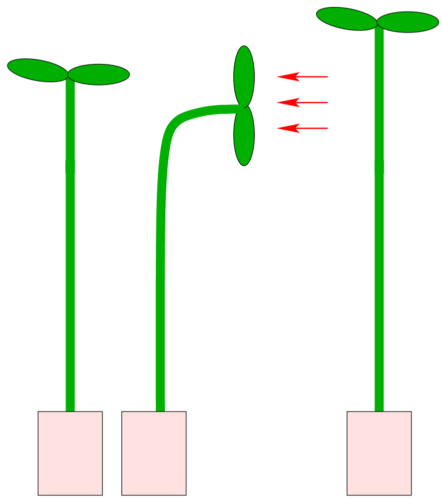

Figure 1.20: *An* Arabidopsis*–plant was reared under weak light (left) and subsequently illuminated from the side (center, red arrows). It bends towards the light source. If the light is switched off, the plant grows straight again (right)*

the two cameras used to take for each of the individual seedlings one picture from two different directions. From these two images a three-dimensional picture of the seedling can be constructed. Since we take every four minutes with both cameras one picture, we will have soon many such three–dimensional pictures (in one day after all 360, 2520 in a week).

To be able to represent the results clearly, Winfried Hellrung has used a nifty trick. First of all he has reduced the three dimensional image of the plant to one vertical line only. This is on the screen of the computer a single row of pixels (figure 1.21).[2] To avoid that we loose all the many informations which we need to un-

[2] A pixel (image point, image cell, or image element) is the smallest unit of a digital raster graphic and its depiction on a screen. Pixel is an artificial word consisting of picture and element

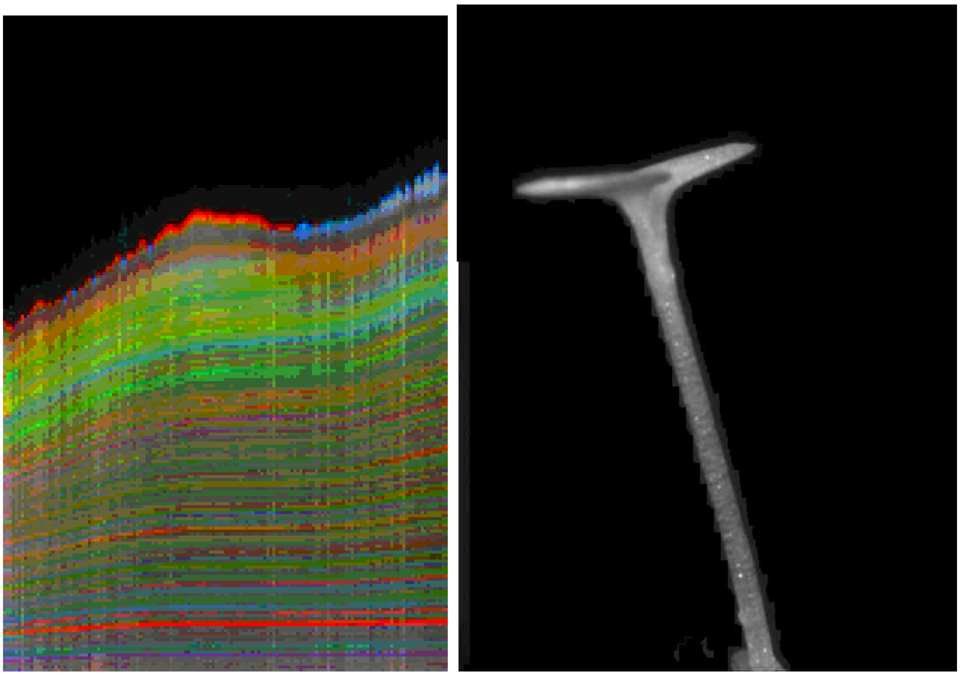

Figure 1.21: *Left picture: From the two pictures of the growing seedling a three dimensional plant was calculated and transferred into straight line. The bendings were colour coded for all locations of the hypocotyl using the colour circle in figure 1.22 (it represents the bending direction by the colour and the amount of bending by the colour saturation). Right picture: Plant at the end of recording. From Neugebauer (2002)*

derstand the behaviour of the plants, they were colour–coded.

For this purpose we assign a colour circle onto a cross section through the seedling (figure 1.22). Front is blue, back red, right

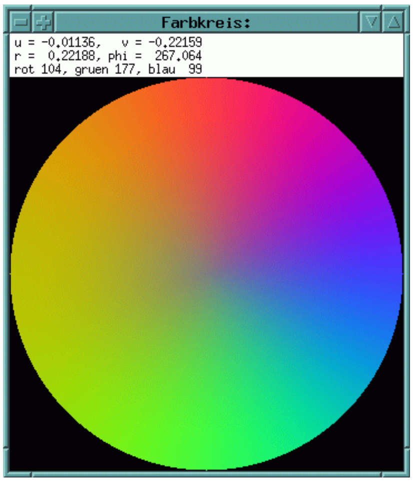

Figure 1.22: *Colour circle for coding the bending–direction and amount of bending in circumnutations. Yellow: Bending towards the camera, blue: away from the camera, red: to the left, green: to the right (Image by Hellrung)*

green and left yellow. In between are intermediate colours. Towards the periphery the colours are more intense, towards the centre less intense. The direction of bending of a particular spot of the hypocotyl is thus colour coded in our vertical pixel row. The strength of bending is represented by the colour intensity. We are now able to combine all our three dimensional images in a single coloured picture and still keep all the informations we need to understand the reactions of the stalk and its parts. The result is shown in figure 1.23 .

We will try to understand better the growth and revolving of the seedlings by using this method.

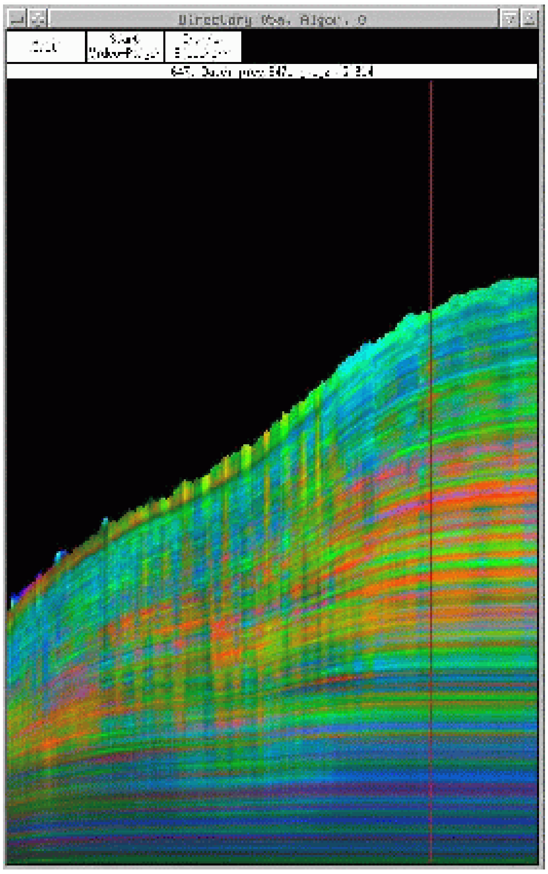

Figure 1.23: *Coloured picture of the time course of growth in* Arabidopsis*: The coloured lines produced according to figure 1.21 represent the bending direction and amount of bending of the hypocotyl in four minute–distances. The hight of the lines correlates with the length of the hypocotyl. The steps show, how much the seedling stretches: Steep parts correspond to strong elongation, flat ones to small elongation. The flat and step–like bands running to the right are produced by natural marks at or in the hypocotyl, for example by an uneven structure at the surface or cell borders of the epidermis cells. They show, which parts of the hypocotyl are still elongating (the middle and upper) and which do not elongate any more (the lower). From Neugebauer (2002)*

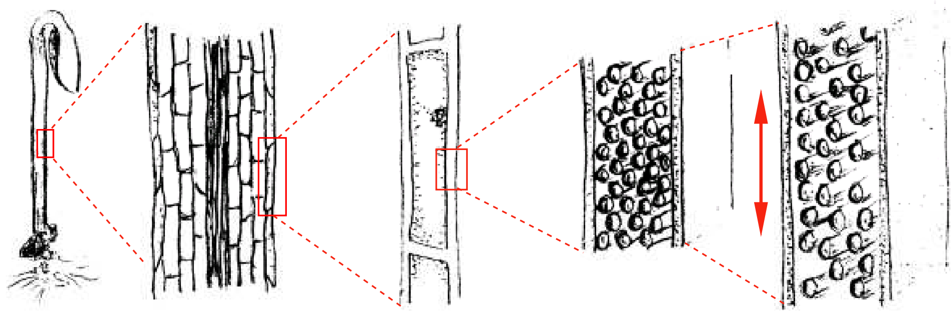

Figure 1.24: *Structure of the hypocotyl of* Arabidopsis*–seedlings: From left to right: A part of the hypocotyl of a seedling is shown enlarged. Of this, a single epidermis cell is depicted. A short piece of its wall is schematically shown in its original (penultimate picture) and during wall stretching (last picture). The cellulose–fibrills are pushed away by the turgor pressure of the vacuole and by other processes (see figure 1.26), before new cellulose–fibrills are produced and build into the cell membrane (red double arrow)*

1.9 How the hypocotyl elongates

Arabidopsis thaliana seedlings elongate vigorously if kept in darkness or in weak light. What are the mechanisms which underly elongation? To answer this question, we should first know how the hypocotyl of a seedling is constructed (figure 1.24).

Already in the embryo of the seed all cells are present which form the hypocotyl after germination. At the periphery the hypocotyl is surrounded by a mantle of thick–walled epidermis cells like a knight by his armour. In the interior are thin–walled parenchyme cells. No cell divisions occur during the entire time of elongation (Gendreau et al. (1997), figure 1.24, see also figure 1.12). How do the cells elongate? The wall of the epidermis cells is quite thick and solid. This stabilizes the seedling against wind. If the seedling is elongating, the walls of the epidermis cells have to be loosened first. They enlarge due to the pressure in the vacuole. The diameter of the cells does, however, not change during elongation. The reason is the structure of the cell walls. They are composed of microfibrills made of cellulose, which are constructed according to the belted tire principle in a circular arrangement (see figure 1.25). The cellulose–microfibrills depart from each other during the increase in volume of the cells (figure 1.25). In the space in between new cellulose–fibrills are added circularly without a change of the diameter of the cells.[3] In this way the increase in volumen leads to the elongation of the cells only. Consequently the hypocotyl growths rapidly upward in order to reach the light as soon as possible which is so important for plants.

The cell wall is structured in a complicated way. According to a modell of Carpita and Gibeaut (1993) (figure 1.25) it consists of three mutually interconnected nets with different structures. They confer strength to the wall, elasticity and the ability to enlarge (plasticity). If the cells stretch, contact points in the net of the cell wall have to disengage. For the plastic properties of the cell wall expansin

[3]Or the cell wall–material departs from each other, because the microfibrills change from a transversal into a longitudinal position (Roelofsen (1965)).

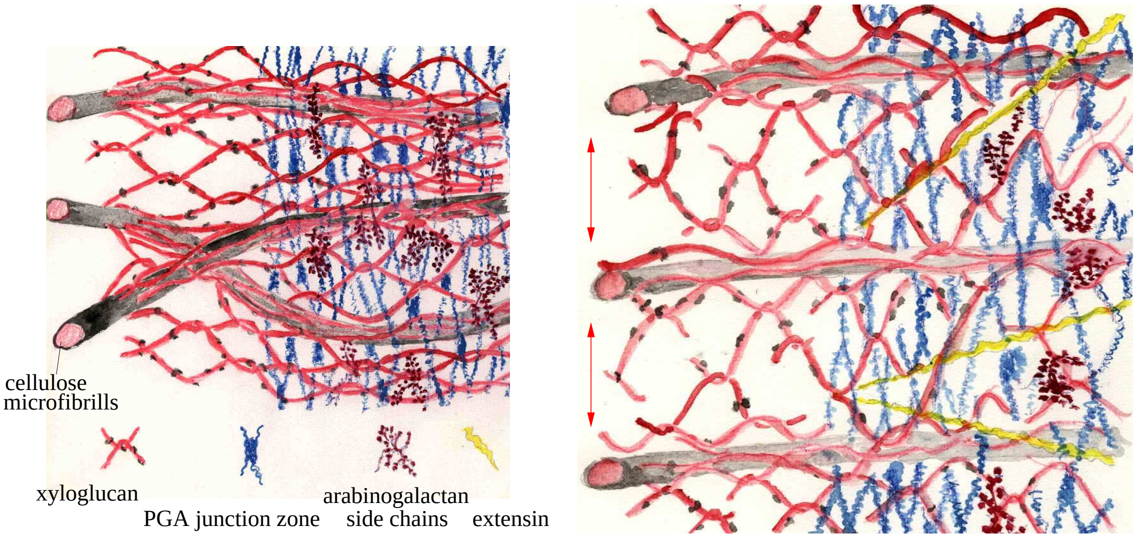

Figure 1.25: *Several nets constitute the cell wall of plants. The wall consists of three nets: one is formed by cellulose–microfibrills (30%, thick ropes) and hemicellulose (30%), one consists of a pectin–matrix (35%), and the last one is build from hydroxyprolin–rich glycoproteins (1–5%), which function as structure–proteins. Enzymes, among them expansin, play an important role during wall–elongation. Left picture: Close–mashed net of cellulose–microfibrills and hemicellulose. Right picture: Cross–linking is broken up and the turgor pressure in the interior of the cell is now able to push the nets apart. Later new wall constituents are added. Drawn by the author after an illustration in Carpita and Gibeaut (1993)*

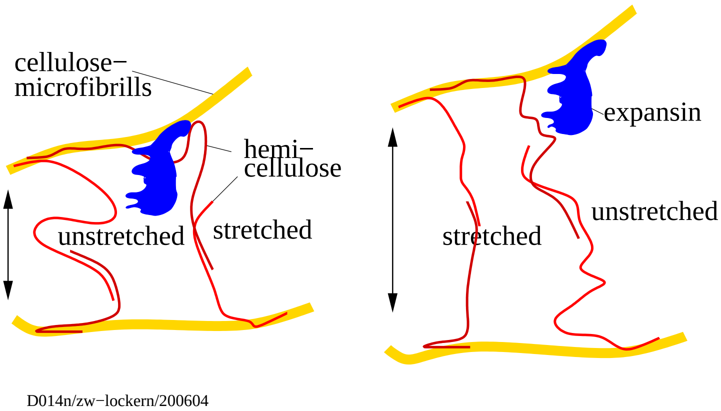

Figure 1.26: *The cell wall is loosened by expansin (blue) by disengaging the junctions between the cellulose–microfibrills (yellow) and the hemicellulose (blue, red, left). The microfibrills can now be pushed away from each other by the turgor pressure, the polymer creeps (right). New cellulose–microfibrills are formed and new hemicellulose is build in and connected (not shown). Other wall component are also stretched (not shown). After Cosgrove (2000)*

is responsible. It is a proteine, which loosens the walls. How this occurs is shown in figure 1.26. Depending on the activity or inactivity of receptors for expansin, the cells react to this proteine or not. During elongation the hormone auxin plays additionally a role. It promotes elongation.

1.10 How circumnutation is brought about

We have now an idea how the cells stretch and how the hypocotyl elongates. We have seen, that the hypocotyl cells do not stretch uniformely (figure 1.27). At the begin of germination the cells at the basis elongate (they are at that time reactive to expansin). Later the cells in the central and upper part of the hypocotyl lengthen. The lower cells are now insensitive to expansin.

But even in the same growth zone elongation does not proceed uniformly. Instead, one side of the hypocotyl stretches and the neighbor cells follow by and by with elongation (figure 1.28). A wave of stretching revolves around the hypocotyl region in question. Consequently the hypocotyl spirals. The elongation–growth occurs thus in time *and* in space in batches.

In section 1.5 we have seen already that the hypocotyl of *Arabidopsis thaliana* exhibits a whole spectrum of circumnutations. Faster and slower circumnutations differ also in their reactions towards light. It is therefore assumed, that different mechanisms are responsible which might additionally occur in different parts of the hypocotyl. The faster revolving movements occur mainly after germination, when the lower part of the hypocotyl begins to stretch (Schuster (1996)). The slower circumnutations could come about when later on the cells in the middle part of the hypocotyl stretch. But this is speculation and has to be checked first. One

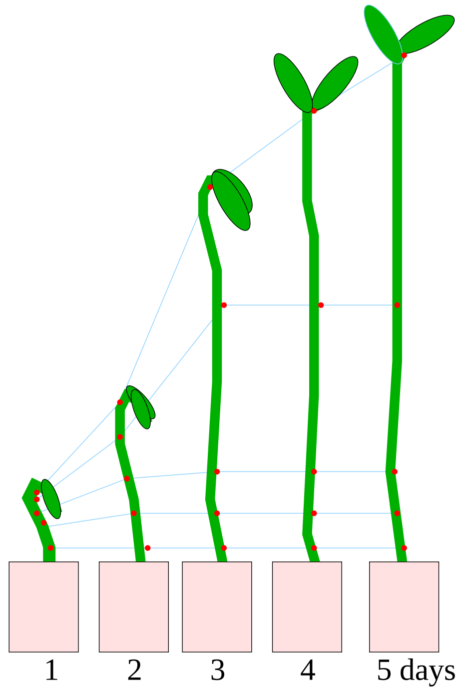

Figure 1.27: *At consecutive days* Arabidopsis–*seedlings were observed from the side. They were marked on the first day with small beads (shown red). The cells in the lower part have elongated already and stay therefore in the following days at the same position. At the second day the cells in the middle part of the hypocotyl lengthen, and at the third day the cells in the upper part. The blue curves connect the positions of the beats during the various days and demonstrate the elongation as a function of time. After Gendreau et al. (1997)*

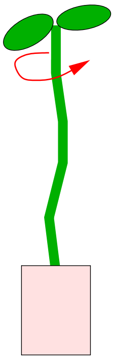

Figure 1.28: *How the circumnutation is brought about in an* Arabidopsis*–seedling: One side of the hypocotyl stretches and the neighbor cells follow by and by with elongation. A wave of stretching revolves around the hypocotyl region in question. Consequently the hypocotyl spirals. The elongation–growth occurs thus in time* and *in space in batches.*

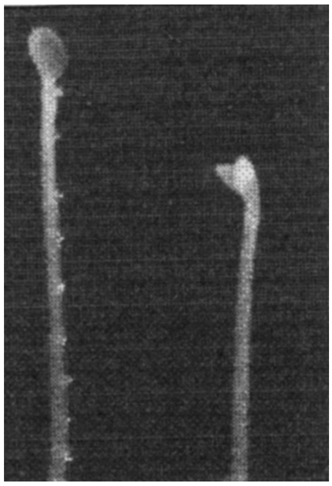

Figure 1.29: *Labelling of different parts of the hypocotyl with small plastic beads, to find out, at which parts circumnutations occur. From Neugebauer (2002)*

could for example label different parts of the hypocotyl and record with our recording unit the movements (figure 1.29).

We have also seen, that elongation of the hypocotyl of *Arabidopsis thaliana* is controlled in a circadian way. There are times with little and times with much elongation. Since circumnutations occur only during stretching, they are visible during this period. If elongation stops, they disappear. On the next day they occur again anew (red curve in figure 1.17).

1.11 Experimenting with the sunflower–pendulum

Unfortunately the seedlings of *Arabidopsis* are too small to observe them without lens or microscope easily. The back– and forth–movements of the hypocotyl can be observed, however, also on larger plants. Well suited are sunflower–seedlings (Engelmann (1999)).

Fill a small container such as a yoghurt–beaker with sand and put a sunflower–seedling on top. Strey some sand above it to cover the seed, and keep it wet. Keep it in a dark room and observe it with a flashlight which has been wrapped with a red or green foil. This kind of light does not affect the seedling, it is physiological darkness.

After a few days the seed germinates. Wait, until the hypocotyl reached a length of 5 to 6 cm. Now turn the vial with the seedling for 30 minutes sidewise and put it vertical again afterward (use always the safelight of the flashlight, never let white light hit the plant). You will see, that the plant bends. After having reached maximal bending, a counter–movement begins in the other direction. This pendulum movement continues for some time (figure 1.30). It is getting even stronger, if the hypocotyl has become longer in the dark. Finally the very

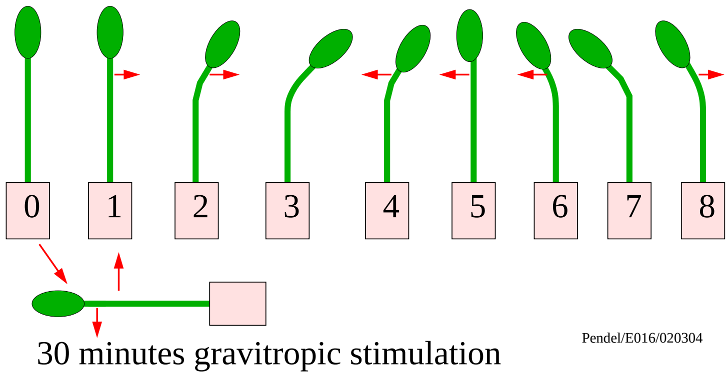

Figure 1.30: *A sunflower–seedling (0) is turned horizontally for 30 minutes (below) and afterward returned in the vertical position (1). The gravitational stimulus (short red arrow pointing downward) leads after a certain time to a reaction: The tip of the seedling bends (2) and bending increases (3). In the state of maximal bending, however, the hormonal unbalance of the two sides is lacking. A new gravitational stimulus induces again bending of the hypocotyl. It bends now to the counter side (4), overshoots the plumb line (5) and bends to the other side (6), until again a gravitational stimulus occurs (7) with counter–reaction (8). The arrows indicate the direction of bending. After Engelmann and Johnsson (1998)*

elongated stalk might even tip over (figure 1.31).

Plants do react very sensitively towards gravitation. Several hypotheses have been proposed to explain, how gravitation leads to a reaction (Salisbury and Ross (1991), Pickard and Ding (1993)). To explain the pendulum movement of sunflower seedlings, a feedback model was used (Johnsson (1977) and figure 1.32). According to this model the sunflower hypocotyl is stimulated by the gravitational force of the earth after turning the plant sidewise. As a reaction to it the hypocotyl elongates at the stimulated side and bends to the other side. This other side is now stimulated by gravitation, which leads again after some time to the bending of the hypocotyl, now in the other direction. In this way pendulum movements arise.

In the meantime, however, it was shown by experiments in a space ship, that in spite of weightlessness conditions bending reactions were still taking place (Brown (1993), figure 1.33). These results indicate, that the oscillations of sunflower seedlings are not exclusively induced by gravitation, but is based on circumnutations which we got to know already in *Arabidopsis*. A reanalysis of data from experiments in space show, that these movements are composed of oscillations with different period lengths, as is the case in *Arabidopsis* (Bardal et al. (2004)).

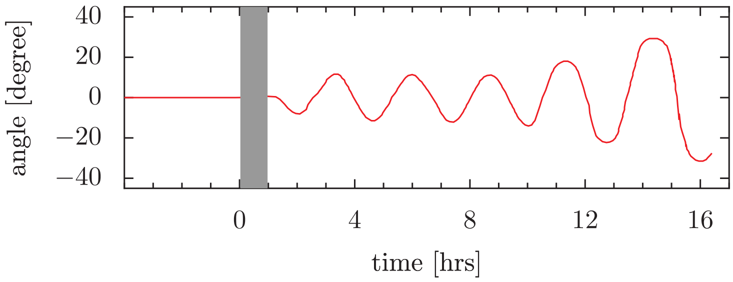

Figure 1.31: *Time course of the gravitropic pendulum of a sunflower seedling as shown in figure 1.30. At time 0 the seedling was exposed to a gravitational stimulus by turning the pot for one hour by* 90^0 *sidewise. After returning the pot into its old position, pendulum–like movements were observed*

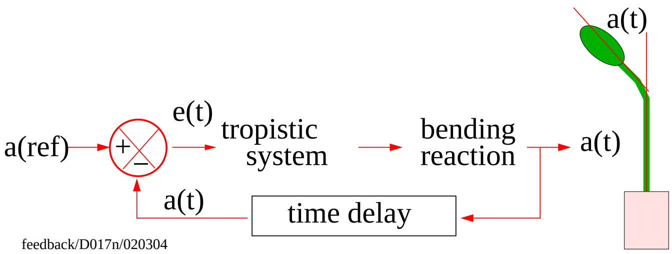

Figure 1.32: *Feedback model of the gravitropic pendulum. A reference value ('vertical growth'* $a(ref)$*) is compared with the actual value* $a(t)$*. If both values differ, an error signal* $e(t)$ *occurs. It is amplified by a tropistic system and weighted and compared with the reference value after a time delay. At the right the current angle* $a(t)$ *is explained as the deviation from the plumb line. After Johnsson (1977)*

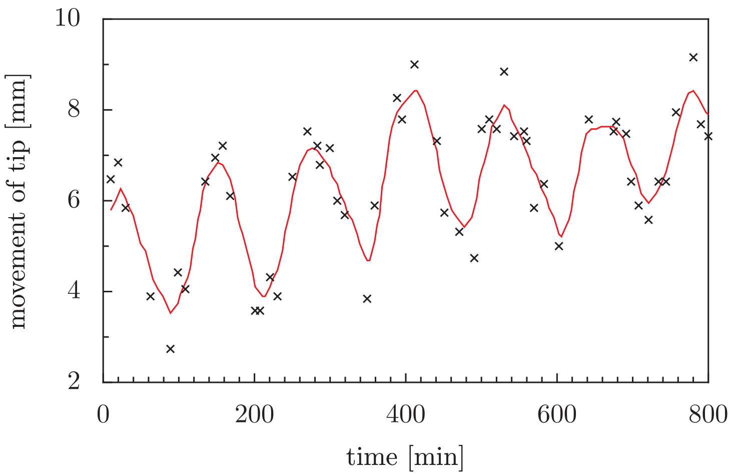

Figure 1.33: C*ircumnutations occur in sunflower–seedlings in a space–ship under weightlessness for some time if exposed to an artificial gravitational force produced by a centrifuge. Points are original values, the red curve is fitted to these values. Period length about 160 minutes. After* Bardal et al. (2004)

2 Sleep of clover leaves

The three leaflets of the Red Clover fold during the night and unfold during the day. Each leaflet has a joint, which is responsible for this movements. We will see how the joint functions and what these movements might be good for. There are other plants which are able to move their leaves.

In the previous chapter we got to know plants, the stalks of which moved by unequal growth in a pendulum–like or circular way. There are, however, also plants, which move the leaves. With this type of plants we will deal with in den next three chapters.

I am sure you know the Red Clover on meadows, in the garden or at the roadside. If you do not know it, it is time to get to know it. It does not only look pretty with its white flowers and the bright stripes on each of the three leaflets (Figure 2.1 left). Hiding behind it is also a detective story which has not been solved yet.

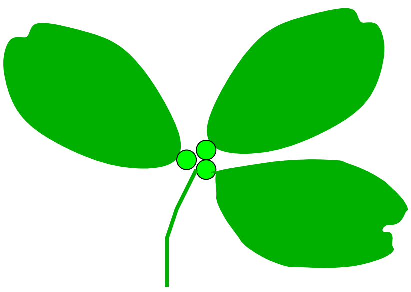

Figure 2.2: *Scheme of a Clover leaf. The leaf consists of three leaflets (dark green). Between them and the petiole are three joints (bright green), which are responsible for the movement of the leaflets. See also the photography in figure 2.1*

Looking at the day on a meadow for clover, we will notice the horizontally spread leaflets (figure 2.1 left). If many of them are growing there, it is like a green carpet. You should lay down on your belly and have a close look at the leaves. You will notice, that three small leaflets sit at the end of the petioles. And with sharp eyes or a magnifying glass you might even see at the upper end of the petiole three bulges (figure 2.2). The leaflets are attached to them. Detective, watch out! We need this for solving our detective story.

You should cut out with a knife a clover plant together with the roots and put it with soil in an old yoghurt beaker. Take it home and observe it in peace in the evening. Or you go back to the meadow in the evening and have another look at the clover.

Well, what happened? The green spot is not as green anymore as it was during the day. Everything looks a bit rough now. The lateral leaflets of the clover are folded together and the third leaflet has folded around the lateral leaflets (figure 2.1 right). Because the bottom side of the leaflets are haired, the whole spot looks a bit whitish.

That plants move is obvious: They grow and elongate, leaves form and increase in size, flowers are coming and produce seeds. But that mature plants such as our clover can move, each morning and each evening! The detective in us would like to know

- is this found only in clover?
- how are the leaflets able to move?

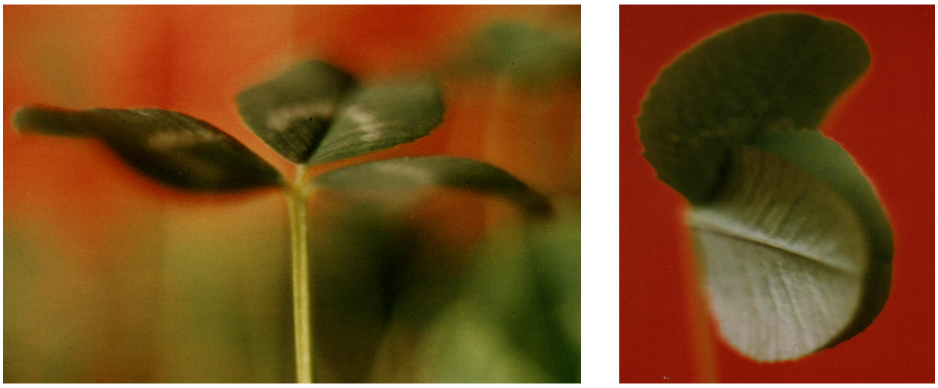

Figure 2.1: *White Clover in day- (left) and night position (right). The two front leaflets fold together during the night, the hind leaflet folds above the other two. From Mayer (1977)*

- why fold the leaflets in the evening and unfold in the morning?

2.1 Sleep movements are not only found in the clover

The Swedish botanists Carl von Linnè[1] has observed these leaf movements already 250 years ago (figure 2.3). He called them 'sleep movements´. Likewise the well known biologist Charles Darwin[2] studied these leaf movements and wrote a book about them (Darwin (1899), figure 2.4). Many biologists before and after him have been fascinated by these sleep movements of plants.

They are not only found in the clover. If you watch in a garden bean or pea plants during the day and during the night, you

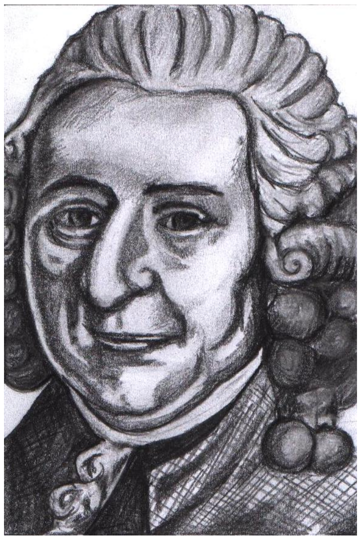

Figure 2.3: *The Swedish botanist Carl von Linnè. Drawn by Mareike Förster after a picture in Duden-Lexikon-Redaktion (1969)*

[1] according to him each organism gets a two parted name: In the same way as we have a family name and a surname the Red Clover for example is called *Trifolium repens*. *Trifolium* is the name of the genus (that would correspond to your family name), *repens* is the species name (that would correspond to your surname)

[2] he proposed, that all organisms have developed out of each other during the course of the history of the earth ('evolution theory´)

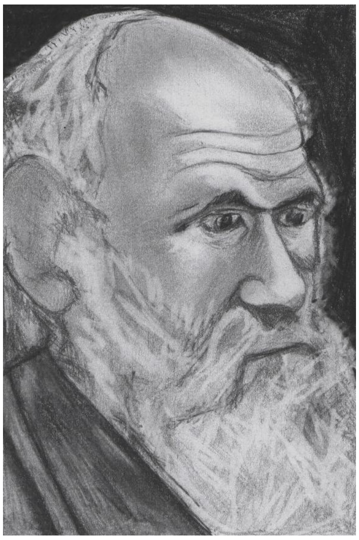

Figure 2.4: *Biologist Charles Darwin. Drawn by Mareike Förster after a picture in Duden-Lexikon-Redaktion (1969)*

Figure 2.5: *The Robinia belongs to the Fabaceae and grows often at railroad embankments. The flowers emit a strong fragrance. Photographed by Silyn–Roberts, Auckland (New Zealand)*

will also notice these sleep movements. In trees the leaves of Robinia show them (the spiny trees which one often finds at railroad embankments and which smell intensively in the spring, see figure 2.5).

Many flowers open and close at certain times of the day or night. The flowers of the american *Evening Primrose* (which is found in many gardens, see figure 2.6) open in the evening. You can watch the jerky opening. After opening they emit a strong fragrance which attracts moths. On the next day the flowers wilt. Since many flowers develop on the inflorescence, you can observe this spectacel each evening in the summer and fall. The Flaming Katy *Kalanchoe blossfeldiana* opens one and the same flower each morning and closes it each evening. The plants are available in the winter in flower shops (see figure 2.7 and also Engelmann (2003b)).

Figure 2.6: *Evening Primerose (Oenothera) open their flowers in the evening between 20 and 21 o'clock and emit a strong fragrance, by which moths are attracted*

How are leaves, leaflets or petals of plants able to move? Do you remember the three

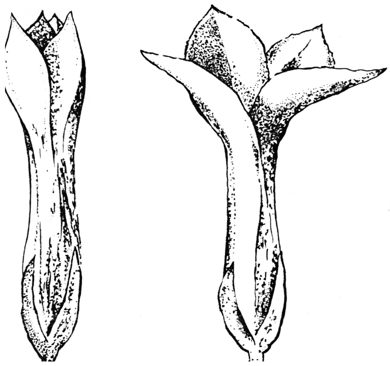

Figure 2.7: Kalanchoe *opens its flowers in the morning and closes it in the night*

bulges at the end of the petiole of the clover, at which the leaflets are inserted? These bulges are joints. In a similar way as our wrist moves the hand up and down, it moves the leaf. But since a plant does not have any muscle and bone and tendon, it has to work differently.

We have to look at such a leaf joint more closely. The joints of the clover are, however, quite small. It is easier to use the joints of a bean leaf. In the next chapter we will see, how we can cut the joint with a razor blade in thin sections and how to study the anatomy. We will also get to know a model, which explains the movements of the joint.

2.2 Why do plants possess sleep movements?

Why do sleep movements exist in plants? By opening the flowers during the night and emitting fragrancy (figure 2.8, left), moths are attracted. They pollinate the flowers. Seeds are produced and they germinate and grow into new plants. In other plants the flowers are fertilized by insects, which are active during the day (figure 2.8, right). These flowers open during the day.

But why do the leaves of some plants move? If a detective tries to clear up a crime, he will inform himself about everything he thinks might be important. Than he will think about who might be the criminal and about the way the crime might have happened. Finally he will test the assumptions.

In the same way proceeds a biologist, who wants to know, why some plants show sleep movements. He too will look at everything very careful. Than he will try to find out by what means and how the movement came about. Darwin thought, that the plants are protected from coldness by the vertical leaves during the night. One can indeed show, that vertical leaves are warmer in comparision to horizontal leaves (figure 2.9). But there are several other explanations. We do not know yet, which one is correct. Many experiments have to be made, to check, which one is the right one. Our detective story is still waiting for detectives who are able to solve the case 'sleep movement in the clover´.

Linnè called the movements of leaves 'sleep movements´. Are plants able to sleep? Surely not, since sleep is found only in animals. But in both, plants and animals, many processes are controlled by a day clock. In animals it determines, at what time they rest ('sleep´) and at what time they are active. This day clock works also in the cells of joints of some plants. They determine, at what time the leaves are lifted up and lowered (figure 2.11). If we use the term sleep a bit broadminded, plants are indeed able to sleep.

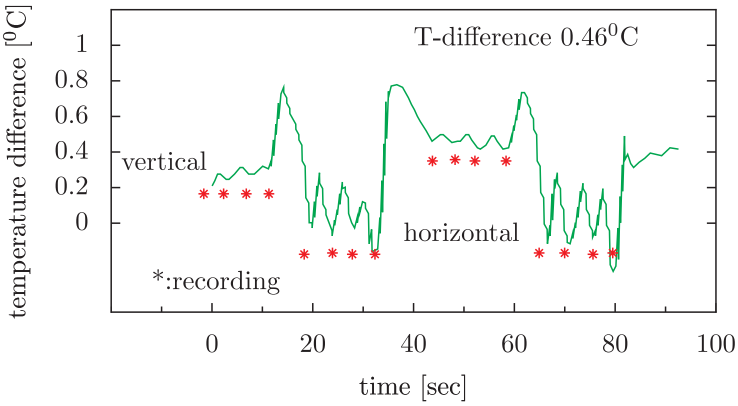

Figure 2.9: *According to Darwin leaf movements protect from coldness by reducing the emission of heat to the cooler night sky. This was tested by Enright in bean plants (*Phaseolus coccineus*). He recorded with a precise temperature–probe the leaf–temperature at leaves, which were vertically positioned during the night (4 recordings –red stars– at two different leaves) and at leaves, in which the movement towards a vertical position was prevented (leaf horizontal, 4 recordings at two different leaves). The vertical leaves were almost half a degree warmer. After Enright (1982)*

2.3 Observations and experiments

In the clover you can observe quite a number of interesting things. The movement of the leaflet can, for example, be influenced by light. If you transfer the plants in a dark room and illuminate them with a fluorescence tube during the night only, the leaflets will be extended during the night and vertical during the day (figure 2.10). Day and night have shifted for the plant. The plant gets apparently informations from the light–dark–cycle.

However, if you offer permanently light to the plants in a room without windows (or with blinds in front of the windows) and watch them frequently, you will notice that the leaves are still unfolding and folding (figure 2.11). The rhythmic movement works also without a light–dark–cycle. If you observe very precisely and frequently over the whole day (and also during the night), you will find out something astounding: The measure of folding and unfolding is not any more exactly 24–hours, but by about one to two hours longer. You must, however, write down precisely the times and values at which the leaflets were unfolded or folded. A centimeter–ruler is recommendable. It is even better to take pictures and to evaluate these. If you plot the values on millimeter paper, you will get a curve similar to the one in figure 2.11.

This observation shows, that the clover possesses an internal clock, which needs for a round about one day, but is somewhat slower than 24 hours. It is called a circadian clock.[3]

Where is this clock located? Researchers found out, that the joints even without petiole, plant and leaflets still move in the

[3] from (latin) circa = about, dies = day; thus: about a day

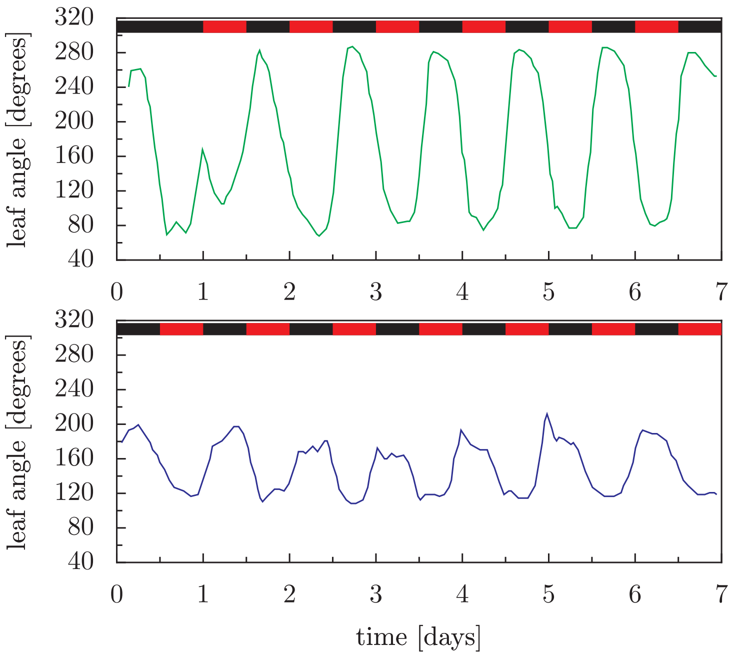

Figure 2.10: *Clover in a 12:12 hour light–dark–cycle (top, green curve) and in an inverted light–dark–cycle, which has light during the night and darkness during the day (bottom, blue curve). The leaves are vertical at large leaf angles and horizontal at small angles. The movements of the leaflets are reset by the new (inverted) light–dark–cycle in such a way, that the leaves are horizontal at the new light period (small leaf angles). After Robinson et al. (1979)*

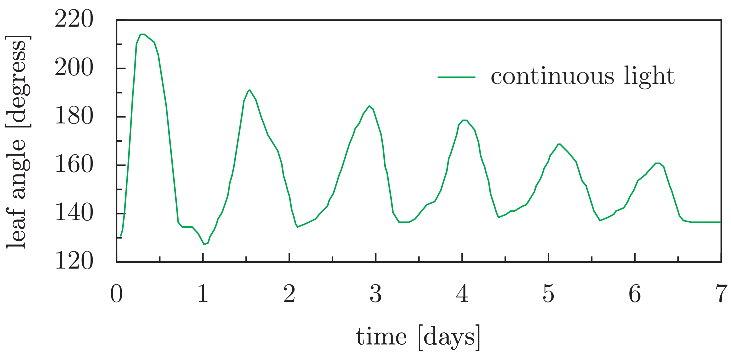

Figure 2.11: *Clover in continuous light continues to lift and drop its leaflets in a daily rhythm. This speaks in favour of an internal clock*

Figure 2.8: *Moth–attracting flowers (Soapwort* Saponaria, *top) and butterfly–attracting flowers (Thyme, bottom)*

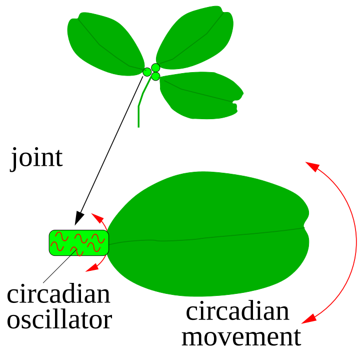

Figure 2.12: *In the clover the circadian clock for driving the movement of leaflets is located in the motor cells of the joint (bright green top and botton). The joints bend upward or downward (small red arrow) and by this move the leaflets up or down (larg red arrow)*

measure of this clock. You can verify it by cutting off all leaf tissue of the leaflet except a part of the midrib (the leaflet veine). The remaining two leaflets are also removed (see figure 2.13). However, you must take

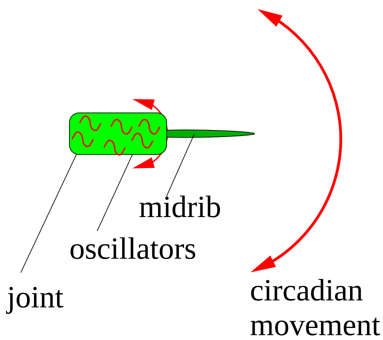

Figure 2.13: *In the clover the circadian clock driving the movement of the leaflets is located in the joint (top). They still move, even if cut off from the plant and with a piece of the midrib left as a hand*

care, that your preparation is kept moist. The best way is to lay it on a wet filter paper in a translucent cuvette. Otherwise it will dry out. The short piece of the midrib serves as a hand for the movement of the joint.

Even single motor cells are still able to move in a circadian way. The daily changes in the joints and motor cells can be reset by a shifted light–dark–cycle in the same way as we have seen in the intact plant. That is, the places where the plant recognizes the light–dark–cycle in the environment and synchronizes its internal day clock to exactly 24 hours (without this synchronization the clock would be slower) are also in the joints and motor cells (figure 2.12).

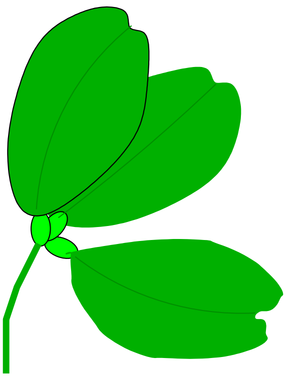

Figure 2.14: *Clover is kept under continuous light in a room in the cellar. If one of the three leaflets is illuminated at a time, at which the leaflets begin to fold, the illuminated leaflet unfolds and folds later the next day*

Do the individual leaflets influence each other? You could try to produce a fine light beam with you flashlight. For this purpose put a blind with a delicate hole in front of the flashlight and illuminate only one of the three leaflets. Offer the light at a time at which the leaflets are going to fold (the plants are kept in continuous light in a room in the cellar). If you observe on the next day the three leaves, you will notice that the illuminated leaflets unfolds and folds later than the other two (figure 2.14). The result shows, that the leaflets react independantly of each other to the light and do not influence each other.

3 Bean leaves during day and night

The Germans have an expression 'Interessiert mich nicht die Bohne'[1]*. We will see, that bean plants should have a much better reputation: They are interesting during germination, the leaves move in a day–night–rhythm, but in addition also in a faster rhythm, and they possess joints for these movements. The joint at the tip of the petiole moves in counter-measure to the joint at the base of the petiole. The joints consist of motor cells, which swell and shrink and as a consequence of it lift and lower the leaves.*

Interessiert mich nicht die Bohne! a German would say if he is not interested at all in what somebody is telling or showing him. As is the case with many such sayings, it is completely wrong. Beans are highly interesting plants (figure 3.1), the leaves of which are lifted and lowered in a day–night–rhythm (figure 3.2). Additionally shorter rhythmic movements are present during the day position (figure 3.3). In this chapter we will learn how to rear bean plants and how to observe the leaf movements. The leaves of bean plants move like the one of the clover with the aid of joints. We will have a close look at them under the microscope, to understand how they function.

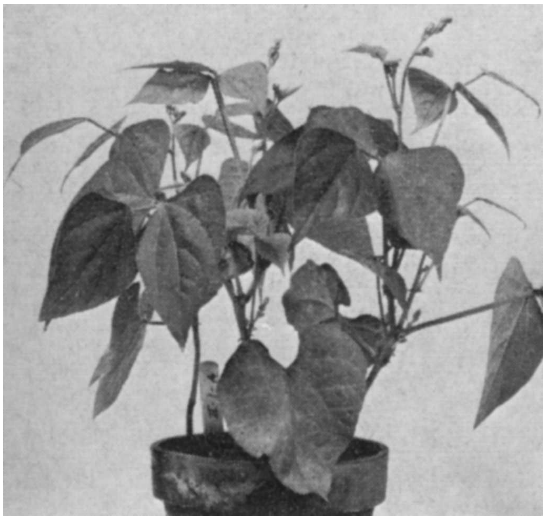

Figure 3.1: *The first leaves of the bean consist of a single leaf (directly above the rim of the pot). The younger leaves (above) consist of three parts*

Seeds of bean plants are available in flower shops and at gardeners. They are reared in pots with garden soil. The first leaves of the beans consist of a leaf blade and show leaf movements very clearly. Subsequent leaves consist of three leaflets (fig-

[1] *I am not at all interested in it*

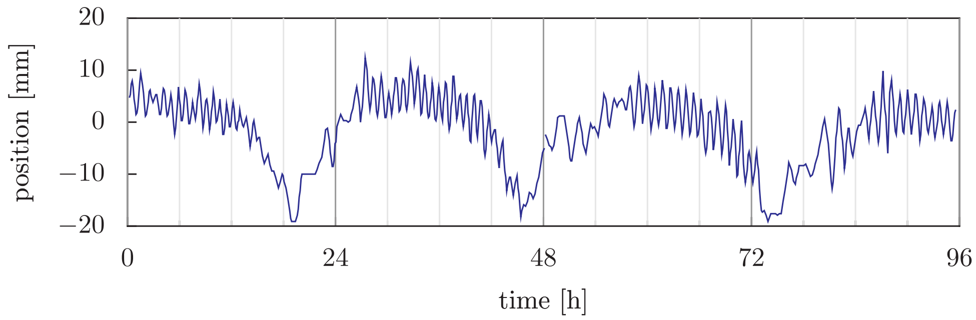

Figure 3.3: *The leaves of the bean* Phaseolus angularis *lift and dip in a day–night–rhythm, but show additionally during the day position (curve in upper position) shorter rhythmic movements. After Alford and Tibbitts (1971)*

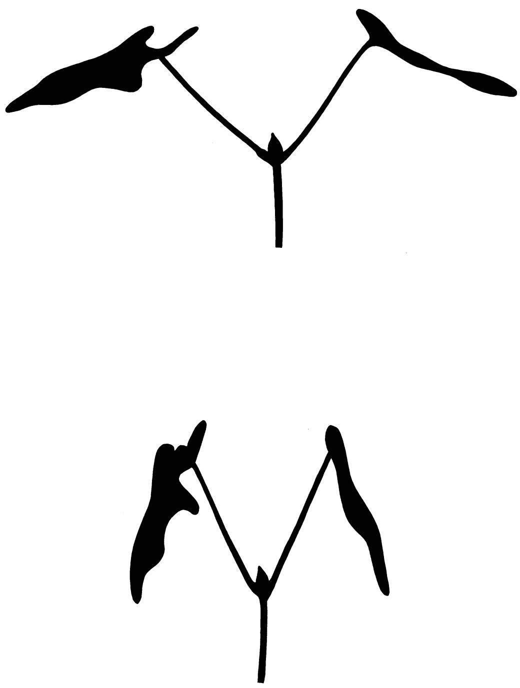

Figure 3.2: *The leaves of the runner bean during the day (top) and during the night (bottom). In the morning the petioles lower and the leaf bladen lift. In the night the petioles lift and the leaf bladen lower. From Bünning (1983)*

ure 3.1). They too move.

In contrast to the clover the bean leaves show additionaly short period movements during the day. The measure is about an hour. Here works thus besides the circadian clock additionally an hour clock. But its clockwork differs from that of the circadian clock: At higher temperatures the hour clock runs faster, whereas the day clock runs independant of temperature always in the same speed (it is said to be temperature–compensated, Table 3.1).

temperature in °C	period length in hours
15	28.3
20	28.0
25	28.0

Table 3.1: *The daily leaf movement of bean plants does not change its period at different environmental temperatures. The hour clock on the other hand runs faster at higher temperatures (no data given)*

The bean plants show another specialty: There are two joints per leaf (figure 3.4 and 3.5). One of it is situated between petiole and leaf (called the upper joint), the other one between stalk and petiole (lower joint). These two joints move out of measure: During the day the lower side of the upper joint swells, and as a consequence the leaf lifts. During the night the upper side of the lower joint swells. As a consequence the leaf lowers into the night position. The lower joint, however, lifts its petiole during the night and lowers it during the day (see figure 3.4).

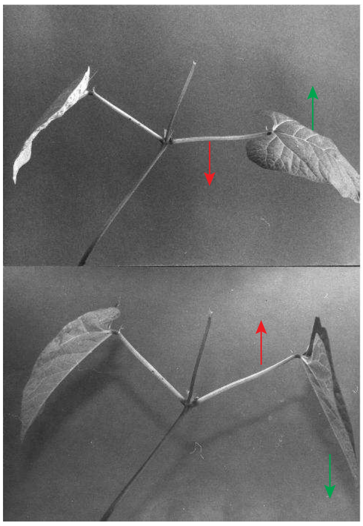

Figure 3.4: *Bean plants possess an upper and a lower joint at the petiole. The two joints move out of measure: During the day (upper figure) the lower side of the upper joint swells, and consequently the leaf lifts (green arrow). During the night the upper side of the upper joint swells (green arrow, lower figure). Consequently the leaf drops into its night position. The lower joint, however, lifts the petiole during the night (red arrow, lower figure) and lowers it during the day (red arrow, upper figure)*

You can indeed see, that the joint increases its upper side while lifting the leaf and shortens it at the lower side. Have a look at the silhouette of the joint with petiole and leaf insertion in figure 3.5!

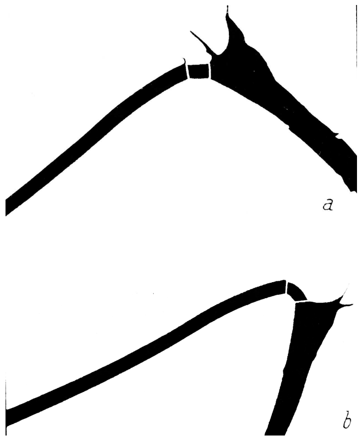

Figure 3.5: *The silhouette of the upper bean–joint (between petiole and leaf, white lines) show during the day (upper figure) about the same length of the upper and lower side. During the night (lower figure) the upper side of the upper joint is longer and the lower side shorter. Consequently the leaf drops to its night position. To the left is the petiole, to the right the leaf. After Bünning (1983)*

In the clover we were able to invert the leaf movement rhythm by applying light during the night and darkness during the day. This can be done also in the case of bean leaves.

If the plants are illuminated throughout day and night, the clock is slowed down, even more so as in the case of the clover (figure 3.6).

The bean plants possess large joints. They are more suited to recognize the structure of the joint. You can cut sections of the joint with a sharp razor blade, a calm hand and much exercise. As a support and help in cutting embed the joint in a piece of Elder pith.[2] Put the sections in a wa-

[2] cut out the inner pith of an Elder branch and drill a hole into it big enough to fit the joint.

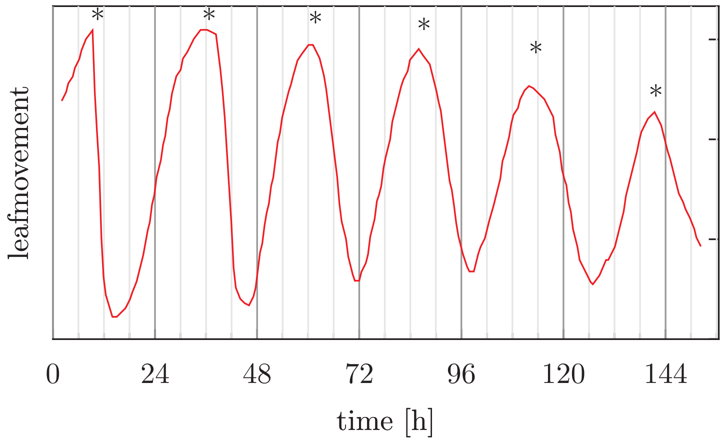

Figure 3.6: *Illuminating bean–plants during day and night slows the day clock. Its measure amounts to 26 hours. The vertical marks in the curve indicate 24–hour–distances. The maxima of the curve (marked with *) occur each day about 2 hours later. 24+2 gives a period of 26 hours. Lifting of the leaf plotted upward, lowering downward. After Bünning (1983)*

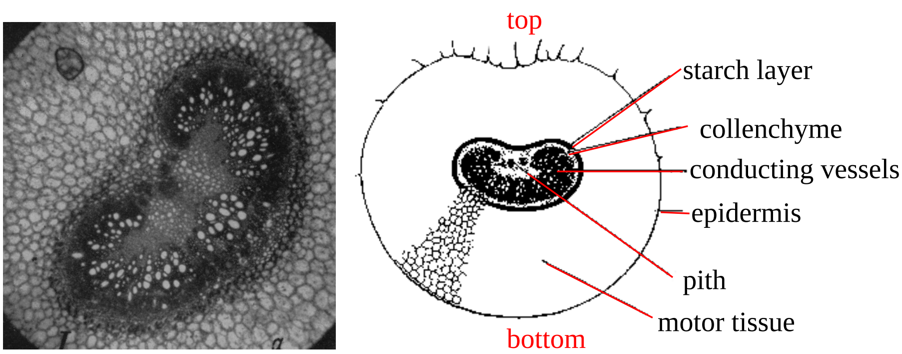

Figure 3.7: *Left: Cross section of a joint of a bean leaf. In the darker central part the tube–like water vessels and sap vessels to and from the leaf are sectioned. Since most of them are tiny, only a few can be recognized as circles. This inner part is surrounded by motor cells. The upper part is the 'flexor´ or bender. If these motor cells swell, the leaf lowers. The lower right part is the 'extensor´ or stretcher. If its motor cells swell, the leaf lifts. Right: Scheme of the cross section of a joint and its lettering*

ter drop and look at it with a strong magnifying glass or better with a microscope. What you see is shown in figure 3.7.

The motor cells are like balloons. If you blow air into a balloon, they become plump. If you release the air, they collapse. Of course plant cells do not contain air. In spite of it they are able to extend by pumping salts into the interior. Salt attracts water (if you leave salt on a table, it becomes wet with time). As a result the cell expands and becomes plump. If all the motor cells at the lower side become plump, the leaf lifts. If all the motor cells of the upper side become plump, the leaf lowers.

In the next chapter the joint of the telegraph plant is described in detail. The bean has a very similar joint. It functions also in the same way as the joint at the terminal leaflets of *Desmodium*. However, the leaf movement is influenced additionally by an hour clock. *Desmodium* possesses instead in the small leaflets a minute clock. This will be presented in the next chapter.

The area is now bigger and it is easier to cut thin sections

4 *Desmodium*: Why does a plant need a fan?

In the Indian telegraph plant Desmodium gyrans *the small lateral leaflets show circular or pendulum–like up– and down movements. One revolution takes some minutes and is faster at higher temperatures. The larger terminal leaflets move also up and down, but the time for one revolution takes about a day and temperature of the environment does not influence the period of the oscillation. The movements of the small and large leaflets are due to joints between the leaflets and the stalk. The joints consist of numerous motor cells which shrink and swell thus lifting or dropping the leaflets. It is caused by pump mechanisms in the cell membranes, which pump mineral salts into the cells or out of the cells. Why the lateral leaflets move is unknown. We will speculate about it. Some experiments are proposed.*

4.1 The automobil plant

Desmodium gyrans belongs to the *Fabaceae* (figure 0.2). The plant possesses leaves consisting of smaller leaflets (figure 4.1). Each leaf has a larger terminal leaflet and one or two smaller lateral leaflets. Sometimes the lateral leaflets are missing. In figure 4.3 the plant is shown during the day (left) and during the night (right). The larger terminal leaflets lower during the evening and are finally in a dropped position. In the morning they move again upward until they have reached a horizontal position. These leaflets are thus a kind of day clock. The one or two smaller lateral leaflets are

Figure 4.1: *The telegraph plant* Desmodium gyrans *possesses leaves consisting of a larger terminal leaflet and smaller lateral leaflets*

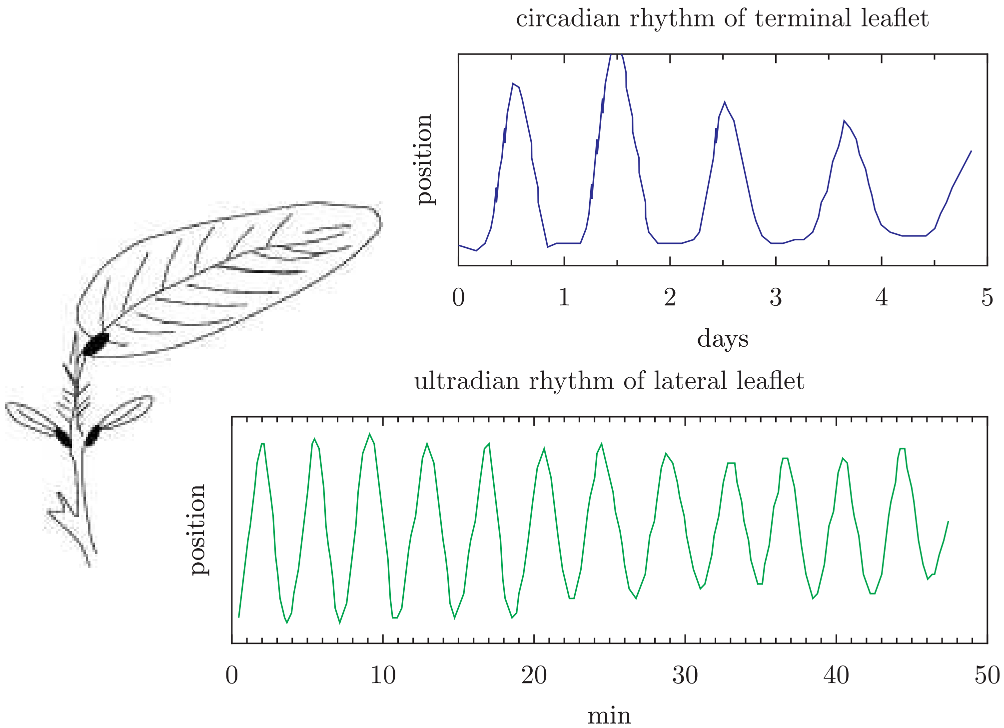

Figure 4.2: *Terminal leaflets (top) and lateral leaflets (bottom) of* Desmodium gyrans *move rhythmically up and down. The upper curve represents the circadian movement of the terminal leaflet (horizontal axis: Days). The lower curve represents the ultradian movement of a lateral leaflet (horizontal axis: Minutes)*

a kind of minute hand. They too move up and down, need however only a few minutes and the movement continues during day and night (figure 4.2).

Because of the revolving movements of the lateral leaflets the plant was called 'Indian telegraph plant'. It was assumed that they would send messages to each other. In its home country India it is called Bon Charal which means 'dancing to the clapping of the hands'. In France the plant is called 'automobile'. This is less poetic, but to the point.

4.2 Lateral leaflets as a minute hand

In figure 4.1 the lateral leaflets of *Desmodium* are recognizable. The circular or oval movements are very regular (figure 4.8 and 4.2). How do these movements arise? With a magnifying glass we can see, that the location between the stalk and the lower end of the leaflet is thickened. This is a joint. It can bend up– and downward and is responsible for the movements. In the left part of figure 4.4 a cross section through a joint is shown, at the right a longitudinal section, and below a three–dimensional image. In a central cord we find the conducting vessels for the water transport from the root to the leaflets and the conducting vessels for the transport of substances which are produced in the leaves. They are surrounded by supporting elements. If these conducting– and supporting elements would lie more peripheric as they usually do in stalks and petioles, the tissue could not bend. Around the central cord lie motor cells. The outermost layer consists of epidermis cells, which protect the joint.

The joints can bend up and down, because the motor cells shrink and swell. But

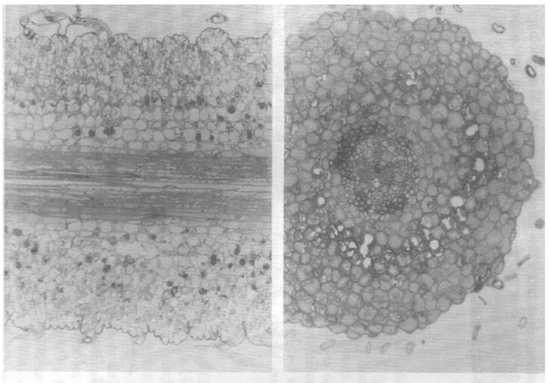

Figure 4.4: *Cross section (right) and longitudinal section (left) through the pulvinus of a lateral leaflet of* Desmodium gyrans. *From the outside to the center: Epidermis, motor tissue, central cylinder. In figure 4.7 a three dimensional view is shown. After Engelmann and Antkowiak (1998))*

this occurs only in the longitudinal direction of the motor cells. They can not shrink and swell perpendicular to it, because cellulose–microfibrills are arranged in the walls in rings and surround the cell like ribbons. These ribbons can, however be driven apart by the turgor pressure of the cells, whereby the motor cells lengthen or shorten (left picture in figure 4.5). The changes in the length of the motor cells are brought about in the following way (right picture in figure 4.5):

Proton pumps in the outer cell membrane (plasmalemma) pump protons (hydrogen–ions, H^+) out. Since protons are positively charged, a negative charge will build up in the cytoplasm of the cell. Negative charges attract, however, positively charged particles. In this way potassium–ions (K^+) enter the cell. There is furthermore an outlet in the cell membrane for chloride–ions (Cl^-). Although they are charged negatively, and therefore the interior of the cell would repell them,

Figure 4.3: Desmodium–*plant in day– (left) and night position (right). During the day the terminal leaflets are spread horizontally, during the night they are completely dropped. Water colour by the author*

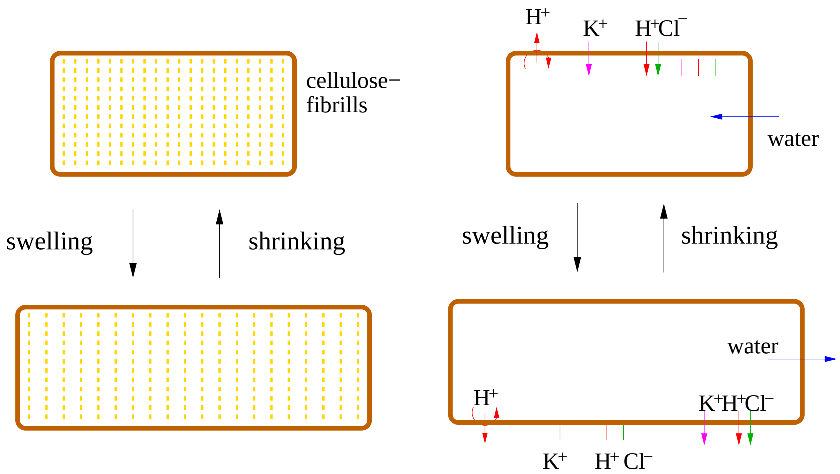

Figure 4.5: *How motor cells of the joint of* Desmodium *swell and shrink. Left: Cellulose–microfibrills run in rings around the longitudinal wall. They prevent the increase of the cell diameter in this direction, but allow lengthening (swelling: bottom) or shortening (shrinking: top). The longitudinal axis of the cell corresponds to the longitudinal axis of the joint.*
Right: The motor cell swells (upper figure), because they take up K^+ *and* Cl^- *and consequently water is taken up. Driving forces are proton pumps, which expell protons (*H^+*) . As a result the interior of the cell becomes negatively charged and positive ions such as* K^+ *are attracted and enter the cell. The motor cell shrinks (lower figure), because the membrane potential collapses and as a result* K^+ *and* Cl^- *move out of the cell. As a consequence water leaves (lower figure right). The cell shrinks (upper figure). The coloured arrows show kind and direction of the ion flows. Coloured lines without arrowheads represent ions, which can in the particular state not pass the cell membrane. After Engelmann and Antkowiak (1998)*

they are able to enter the interior, because they are transported together with positive protons via so called symport–channels. K^+ as well as Cl^- act osmotically: They attract water in the same way as salt ($NaCl$) strewn on icy roads: The cell swells and the mechanical tension of the cell wall increases. From a certain point onward Ca^{2+} channels are activated which induces the outflow of Cl^- and $K^+$from the cell via ion–channels. Together with the ions water leaves the cell and it shrinks. This event repeats: Proton pumps charge the cell electrically negative and ions are able to enter the cell together with water. The cell swells. In this way the joints swell and shrink periodically and the leaves lift and lower. At higher temperature the period of the oscillations shortens.

4.3 Why all this?

For a long time the movement *of the Desmodium*–lateral leaflets has fascinated lovers of nature. Thus the poet, novelist and observer of nature Goethe has described this movement. Ever since one has asked for the purpose of these leaf–movements. Since *Desmodium* is a plant which grows in warm and humid areas, one could for example image, that due to the movement water is removed from the leaflets, thus preventing fungi from growing on the leaves. The terminal leaflets are often kicked by the lateral leaflet during their movement and get a jerk which removes accumulated water drops.

It might, however, also be, that the movements are only for the observer so conspicuous, but for the plants only a side effect. Perhaps the joints are only a kind of pump for minerals. If an alian would see the piston of an oil pump, the movements of the conrod would probably also distract him from its actual function, namely to pump oil. It has indeed been found that in plant cells a pump state (minerals are taken up as ions) alternates with a state in which ions leave the cells (Gradmann and Buschmann (1996a), figure 4.6). These periodic alternations have about the same period length in the minute range as the lateral leaflet movements of *Desmodium*. This type of rhythm seems to be more widespread in plants as known so far (Engelmann (1996), figure 4.6).

4.4 Terminal leaflets as a hand of the daily clock

The terminal leaflets of *Desmodium* move up and down in a circadian manner. During the day they are spread out horizontally and are able to absorb sunlight optimally. In the evening the terminal leaflets drop in a short time and hang down (figure 4.3).

As in the lateral leaflet, here too joints between the leaflets and the petioles are responsible for the movement. The joints are larger than the one of the lateral leaflets. But they are structured in a very similar way. Here too the conducting vessels and supporting tissue is displaced into the centre of the joints. Otherwise the joints could not move. Between these central cords and the outer cell layer (epidermis) lie the motor cells, which function in the same way as the motor cells of the joints of the lateral leaflets (figure 4.7).

There are, however, some differences: Whereas the lateral leaflets move faster at higher temperature, the terminal leaflets have a period, which is, independant of the environmental temperature, always about 25 to 26 hours. To observe this 'freerun', the plants have to be kept under continuous

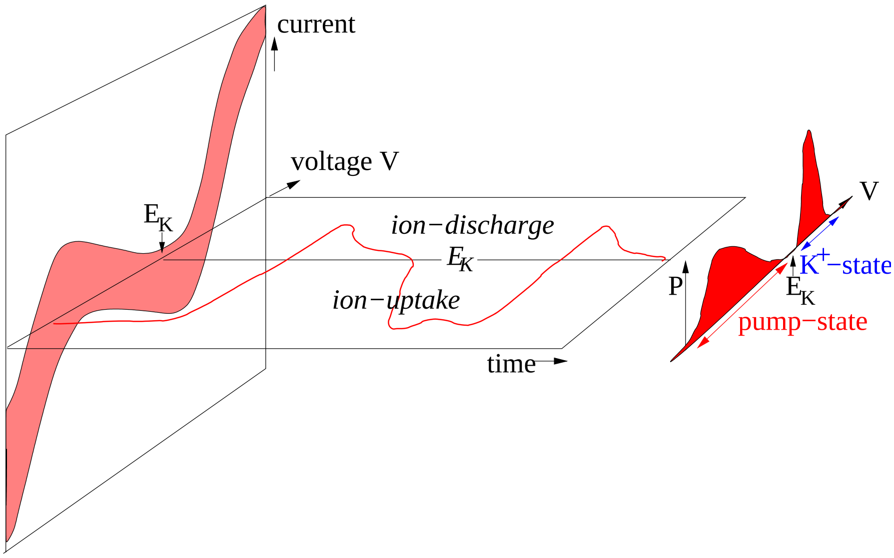

Figure 4.6: *The voltage in some plant cells alternates periodically between a low negative or even positive value (axis voltage V left backward) and higher negative values (axis voltage V left forward). With voltage the current changes (vertical axis of left vertical area) in a characteristic way, as shown by the red band at the left. As a result minerals are taken up as ions at higher negative values (*ion–uptake *shown on the flat plane, front). This pump–state of the plant cells alternates with a state, in which ions leave the cells (*ion–*uptake at the flat plane, back), if the voltage (V) in the cells becomes less negative or positive (in direction of the arrow of the voltage backward). These two states, pumping and ion discharge, alternate with each other (shown in the curve on the flat plane to the right plotted against time). The states take turns, because the probability P,* to stay between the *pump state and the ion–discharge–state, is low (the probability is plotted against voltage (V), see curve on top of the red vertical area at the right). At E_k the probability P has its minimum. Therefore this state occurs only very briefly and the cell is normally – depending on the voltage – either in the pump state or in the state of ion discharge. After Gradmann and Buschmann (1996b)*

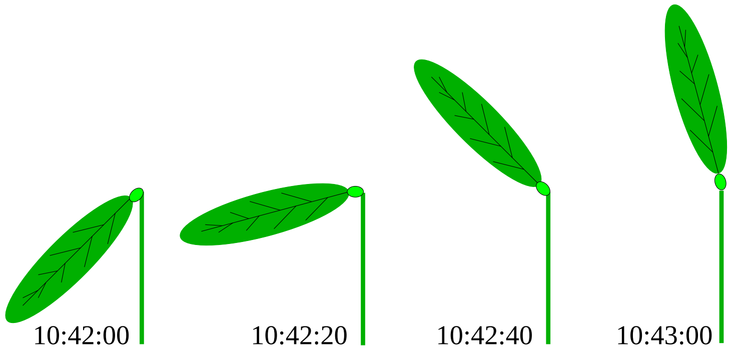

Figure 4.8: *All 20 seconds the position of a lateral leaflet of* Desmodium *was determined and plotted. At 10:42 it was dropped, one minute later lifted. An oscillation took therefore in this case* $2 * 30 = 60$ *seconds. The period length depends strongly on the temperature (figure 4.10). The leaflets can be in a revolving state or oscillate like a pendulum or alternate from one state to the other. They are thus more flexible in their movements as compared to the terminal leaflets, which move only up and down*

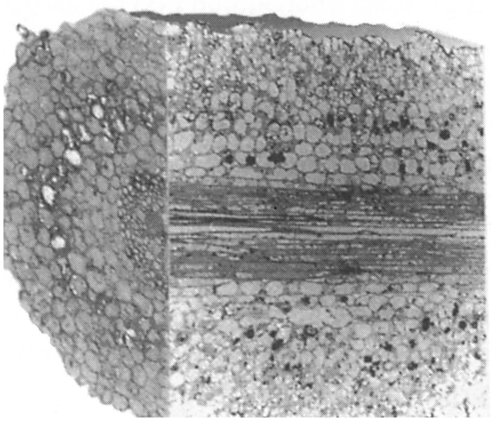

Figure 4.7: *How the* Desmodium*–joint is build: Conducting vessels and supporting tissue are displaced into the centre of the joint. Otherwise the joints could not move. Between these central cords and the outer cell layer (epidermis) are the motor cells. They swell and shrink, which results in the up– and down–movement of the joint. After Engelmann (1996)*

light and the temperature should not alter. In the light–dark–cycle of the natural day or in a temperature cycle between day and night the period length would amount to exactly 24 hours. The reason is, that these day/night–differences synchronize the circadian clock. This raises the second difference to the lateral leaflets: Light influences this rhythm, whereas the movement of the lateral leaflets is not influenced by light and darkness. A third difference: The movement of the terminal leaflets is fixed: They lift and lower. The lateral leaflets, however, are able to turn or to change the movements from a pendulum–like to circular or oval revolution. They are thus more flexible in their movements (figure 4.8).

4.5 Own observations and experiments

Movements are widespread in plants. In the Internet you will find several very impressive examples as short movies (see www.amillar.org with links to other sites)[1]. They might stimulate you to observe these plants or others and to experiment with them.

In *Desmodium gyrans* it can be observed easily, how the period of the rhythmic movement of the lateral leaflets shortens with increasing temperature, whereas the period of the movement of the terminal leaflets is not influenced by the environmental temperature.

It takes, however, quite some time until the plants are reared from seed. It is furthermore not so easy to obtain the seeds. One source is A. Schenkel company, Blankeneser Hauptstr. 53a, D22587 Hamburg. You might ask in a botanical garden for a few seeds. Put a seed in a flower pot filled with garden soil. You need much patience until they have germinated. The plants like humid warmth. The best way to obtain these conditions is to build a small green house out of a frame covered with translucent plastic foil (figure 4.9). If you wait long enough and nurse the plants well, you are rewarded with a living clock.

The movement of the lateral leaflets can be observed also in a cut leaf. Put it in a small vial filled with water and stop the time with a stop watch how long it takes until the lateral leaflet has moved from the upper position of the leaflet via the lowest position back to the highest again. Repeat this experiment several times and calculate the mean value. Afterward you can perform the experiment in a room with higher or lower temperatures. If you plot the period length against temperature, you should obtain a curve like the one shown in figure 4.10.

More can be found in a book (*Rhythms of life, an introduction using selected topics and examples,* Engelmann (2002)). It is available in the Internet (http://www.uni-tuebingen.de/plantphys/bioclox).

If you own a microscope or can use one of a friend or at school, it is worth to look at a joint in a cross section and in a longitudinal section (figure 4.4). Use for cutting a piece of Elder pith as described in the book *Flower clocks, time remembering and time forgetting* in the chapter on the Kalanchoe–clock (Engelmann (2003b)). Insert the joint into a slit in the pith and cut it with a new (that is sharp) razor blade in sections which are as thin as possible. You have to practice it. The sections are put in a drop of water which you have added to the surface of a microscope slide. A thin glass (*cover slip*) is used to cover the sections and prevent them from drying out, to flatten the sections, and to protect the objective of the microscope from the water.

[1] sunflower.bio.indiana.edu/~rhangart/-plantmovies/movements/nastic/nastic.html
deepgreen.stanford.edu
the internet movie database

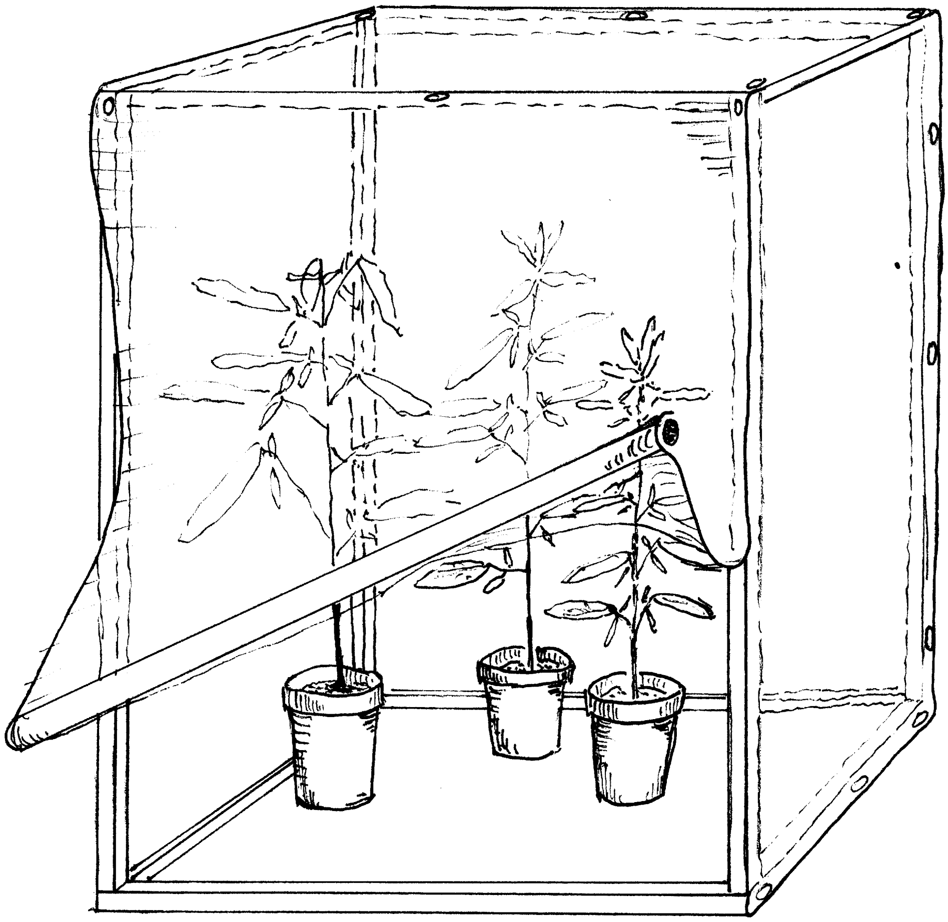

Figure 4.9: *Small green house for* Desmodium *made out of a wooden frame covered with a translucent foil. The foil is fastened with tacks at the wooden frame. At the front the foil is weight with a roller which can be lifted up. Illumination with fluorescence tubes from above. The plants like humid warmth. Sketch of the author*

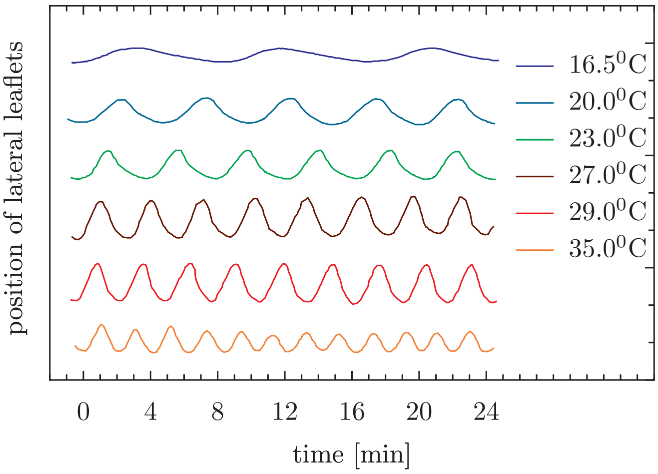

Figure 4.10: *Movement of lateral leaflets of* Desmodium *at the indicated temperatures*

5 Further books

I have written some more books or am in the process of writing. They are also concerned with topics which have to do with rhythmic processes in organisms – my special field as a scientist (*Biocalendar: The year in the life of plants and animals:* Engelmann (2003a), *Flower clocks, time memory and time forgettingEngelmann (2003b), Flying clocks:* Engelmann (2003d), *Clocks which run according to the moon Engelmann (2003e), Depressions: Experiments in Spitsbergen:* Engelmann (2003c), *Rhythms in structures of organisms:* Engelmann and Hellrung (2003), *How to stop a biological clock: Point of singularity:* Engelmann (2004)). They are available under http://www.uni-tuebingen.de/plantphys/bioclox as pdf-files.

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