Flower clocks, time memory and time forgetting

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Karlheinz Baumann dedicated in admiration of his nature movies

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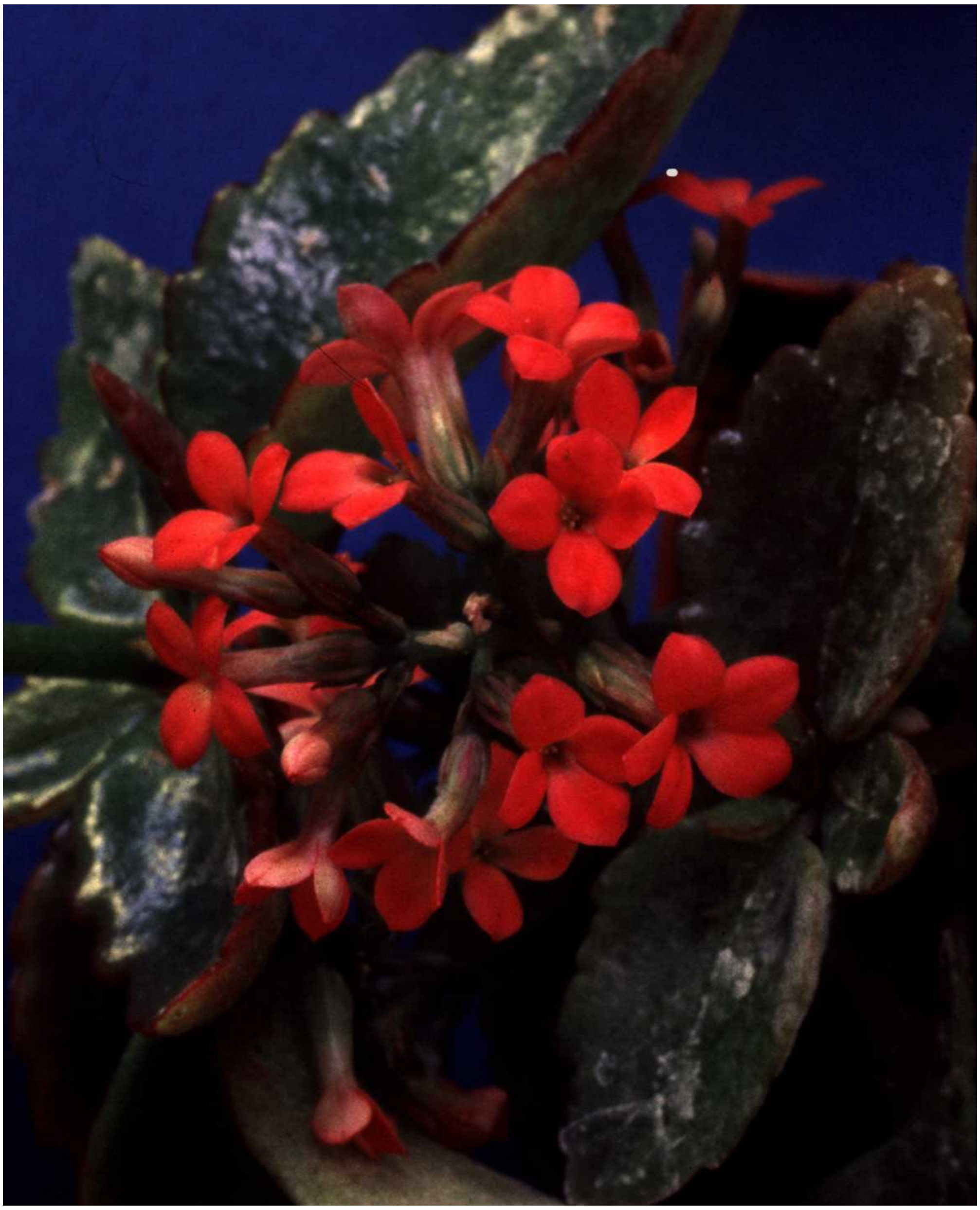

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Once upon a time ...

It was long ago. I was a child and asked, asked, asked, as is usual in this age: Daddy, why ... At my ninth birthday I received as a gift a book about the harbor of Hamburg, with many illustrations of ships, their unloading and loading, the docker and sailor, the sheds and dockyards. It was my favorite book, and when briefly afterward our apartment was destroyed by an aircraft bomb, I sorrowed after it more than anything else. later I received a book, which cast a spell upon me. It dealt with two neighbour children Traute and Dieter, who were allowed to accompany doctor Kleinermacher [1] on his exciting journeys. He had found a panacea which after swallowing it let the person shrink. In this way the children could enter with him plants, an anthill, a pond, a drop of water. The children asked, asked, asked, as is usual in this age: Doctor Kleinermacher, why .. And he showed them the creatures in a water drop, took them along on a journey in a mini-submarine through a pond, explorated together with them an ant hill and crept with them in a plant, in order to get to know this miracle of nature. I have read this book often, but more so I had long close looks at the illustrations, which allowed to interpret much into it and to use ones imagination.

It was years ago. My children asked, asked, asked, as is usual in this age: Daddy, why ... And one of their questions was, what I am doing in the department of biology at the university. And I told them about the ideas and plans and showed them our experiments. They were thrilled by it and I thought, I should write a book about it for children. But nothing came out of it.

Later, when the children of a graduate student of mine, Charlotte Helfrich-Förster, grew up and asked, asked, asked, as is usual in this age: Wolfgang, why ..., I proposed to write together a book for children. We planed even and Charlotte painted some illustrations. But nothing came out of it, because there was not enough time.

In the meantime my chidren have children, and these grandchildren grow up and ask, ask, ask, as is usual in this age: Grandfather, why ... And this time I am going to realize my plans, since I am retired and can take the time for more important things such as showing children how interesting nature is.

[1] Paatz (1938), Paatz (1947). Herbert Paatz, pseudonym for Herbert Fiebrandt, born in 1899, reported missing in the second world war

1 Flower clocks

Here we will talk about flowers, the petals of which move rhythmically. In the case of the Forsythia this occurs in a 90-minute measure. The flowers of the flaming Katy open during the day and close during the night for about 14 days. Other plants open their flowers at certain times of the day or night, but they stay open.

Bees remember at what time flowers provide nectar and pollen and collect food for themself and the hive. They orient themself by using an internal day-clock and a sun compass. The collectors are able to tell co-collectors in the hive by a special dance, where to find food.

Leafcutter bees are reared and sold to farmers which grow Alfalfa on their fields. These bees fertilize the flowers. In this way the yield of seeds is increased by a factor of ten.

1.1 Surprise in *Forsythia* flowers

1.1.1 A letter from Minnesota

Some time ago I got an EMail from a city in Minnesota in the USA.

Petal movements in plants.

From: "Van D. Gooch" <goochv@mrs.umn.edu>
To: Wolfgang.Engelmann@uni-tuebingen.de
Date: Tue, 22 Oct 2002 10:49:56 -0500
Dear Wolfgang:
As you may know, I have played around with time-lapse video and circadian rhythms for several years. By accident we have observed an interesting high frequency rhythm in petal movement in Forsythia. The oscillation occurs when the flower blooms and petal bends in the middle with a damped oscillatory frequency of about 90 minutes. We have searched the literature for evidence that his type of phenomenon has been observed before. Although we have found a few reports on the movement of flower petals in the middle, we have been unable to find any reports on rhythms or the mechanism of such movement (of course we are aware of the literature on the movements that emanate from the base of the petal.)
Having probably done more research on plant movements than any other researcher, are you aware of any research reports done on the rhythmic movements of plant petals where the bending occurs in the middle of the petal?
Do you know of any reports on high frequency rhythms in Forsythia? It is hard to believe this has not been observed before.
Are there any other current researchers that we should contact?
Your help and insight would be greatly appreciated.
Van D. Gooch
University of Minnesota-Morris,
Division of Science & Mathematics,
Morris, MN 56267
320-589-6327,
(lv message at 320-589-6300)
(Home:320-589-3075)
goochv@MRS.UMN.EDU
www.mrs.umn.edu/academic/biology/gooch.html

What is the subject matter of it? The petals of the *Forsythia* (figure 1.1) move rhythmically by bending the upper half of the petal outward and back again. They thus move like a swing, but much more slowly, namely about 90 minutes, until they are in the same position as at the time we started to observe them. We will, however, see, that it might take much more time for oscillations found in other organisms.

That is indeed a crazy story. There are plenty of *Forsythia* allover the cities and villages, because they cast a spell over gardens and parks with their beautiful yellow flowers. And nobody had so far noticed,

Figure 1.1: Forsythia *flowers in spring. The flowers occur before the leaves. The* Forsythia *belongs to the olive family (*Oleaceae*). Further members of the family are besides the olive tree the ash, lilac, privet and jasmin. The european Forsythia (*Forsythia europaea*) originates from Albania. Other* Forsythia*-species come originally from East-asia. Common are bastards between* F. suspensa *and* F. viridissima*. The nicest variety is* F. spectabilis*. The twigs can be budded in the winter. They must, however, have undergone a period of low temperature (if no frost had occurred yet, this can be done in a refrigerator). Pictures by the author*

that the petals move.

Perhaps you have a look at the next spring on one of the *Forsythia* flowers. With a camera you can take a picture every 10 minutes. After two hours (12 shots) you will have a complete serie of the rhythmic movement. Measure the distance between the tips of the petals with a ruler and use the data for producing a curve. You will see the oscillation and you can determine its period length (90 minutes?) precisely. How to do that is described in the next subsection.

1.1.2 We observe and record the movement of a *Forsythia* flower

Cut a twig from a *Forsythia*, take it home and put it in a glass or in a vase. The flowers can be seen better in front of a dark background. If there is no dark background in the room, use a sheet of black paper or cardboard behind the twig. Mount a camera in front of the twig in such a way, that the single flower is photographed as large as possible. Two petals should stay opposite to each other. The camera must have a solid stand and should not move if you press the shutter (a camera-tripod would be ideal). The photos are developed. You can now measure the distance between the facing tips of the petals with a ruler. It is easier, if a digital camera is available. You transfer the images to a computer and measure the distance between the tips of the flowers on the screen. The values are noted (see table 1.1).

time of day	distance [mm]
9:00	
9:10	
9:20	
9:30	
9:40	
9:50	
10:00	
10:10	
10:20	
10:30	
10:40	
11:00	

Table 1.1: *Table for entering the distance between the tips of two fronting petals of a* Forsythia

Next you plot the data of the table on millimeter paper. Or you use a spreadsheet programm for the computer which allows you to plot the values as a curve in a diagram.

Use the curve to determine the length of the oscillation. It is the distance between one maximum to the next. It might amount, for instance to 85 minutes. The period length of the oscillation is thus 85 minutes.

1.1.3 Why do the petals of the *Forsythia* move?

Why do the petals of the *Forsythia* move? Why-questions are often not easy to answer. In the case of the Forsythia we do not know it yet. This is often the situation with observations we make in the nature.

If we want to understand something we have observed in nature, we are in the situation of a detective who tries to find out. Something was stolen and the detective is ordered to find the thief. He will first visit the site and look around carefully. He will try to find traces and secure them. He is, with other words, concerned with the question: **How** did the thievery occur? He will interrogate people who have been at or around the site before, during and after the thievery. He will afterward try to think of persons who might be worth considering. These persons he has to examine carefully.

Likewise with our question 'Why do the petals of the *Forsythia* move?'. It is important to observe carefully. Before the **why** comes the question **how**. How do the petals move? For this purpose we have to look at the petals carefully. We could use a binocular and try to find out which part of the petal moves. By preparing thin sections through the petal we can observe under a microscope the arrangement of the cells and how they change during the opening and closing of the petals. How this is done will be demonstrated in the next section by another example, the petal movement of the flaming Katy. And afterward comes the even more difficult question 'why´.

1.2 Flower clock *Kalanchoe*

Many years ago, when I planned to finished my biology studies with a thesis, my 'doctor-father´ Erwin Bünning (figure 1.2) proposed to work with the flaming Katy. Its latin name is *Kalanchoe blossfeldiana*.[1] An illustration of the plant with open flowers is shown at the begin of the book and with closing flowers in figure 1.3. It is native to the island of Madagascar east of Africa. There it grows on dry places. To prevent drying out during the hot season it

Figure 1.2: *Erwin Bünning was born on January 23rd, 1906 in Hamburg, studied in Berlin and Göttingen, got his PhD at an age of 22 years and worked at the universities of Frankfurt (Main), Utrecht (Netherlands), Jena, Königsberg, Strassburg, Köln and Tübingen. Photography by Walter-Erich Mayer, Tübingen*

[1] animals and plants are named in such a way that the first part (here: *Kalanchoe*) stays for the genus (the genus human would be *Homo*) and starts with a capital letter. The second part of the name stays for the species (here: *blossfeldiana* after a seed trader Blossfeld; the species of the present humans would be sapiens; the complet names are therefore *Kalanchoe blossfeldiana* and *Homo sapiens*). The species name has no capitals. Other species of the same genus are *Kalanchoe daigremontianum* (devils backbone) for our plant example and *Homo heidelbergensis* for an extinct human species

possesses thick fleshy leaves, in which water can be stored. After the winter in Madagascar it begins to flower. Up to 300 flowers can develop on one plant. They are deep red and look pretty. Therefore and because they flower during winter time, *Kalanchoe* is gladly used as an ornamental plant.

Figure 1.3: *The flaming Katy* Kalanchoe blossfeldiana *in full bloom. The flowers consist of a flower tube with four petals which are colored red. At the basis of the flowers is a green calyx and a peduncle. The leaves of the plant are fleshy*

1.2.1 How *Kalanchoe*-flowers move

But *Kalanchoe* does not only look pretty. It is also able to move its petals: In the morning the flowers open, in the evening they close, and during the night they are completely closed (see figure 1.4). Thus the flowers can be used as a clock. Even more, you don't need the whole plant: We brake off individual flowers with a tweezer and mount them with their peduncles in holes

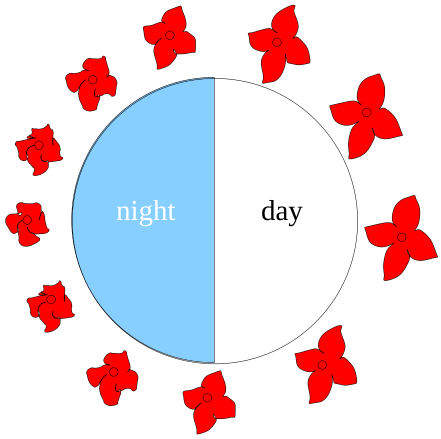

Figure 1.4: *How the petals of a* Kalanchoe-*flower open (right) during the day and close during the night (left), is shown here in intervals of two hours (later an animation is planned here)*

of a thin plastic disc. The disc floats on a cuvette filled with water (figure 1.5). If we watch the flowers, we will notice that they continue to open in the morning and close during the night (figure 1.6).

Now the detective in us lives up again and asks: How are the petals able to move? We have to look closer at the flowers. They consist of a green calyx on top of the peduncle, a red flower tube, four red petals and the ovary with the stigma and the stamen (figure 1.6). The petals are the one which move. They bend during the day outward: The flowers open. During the night the petals move upward, resulting in closed flowers. In order to understand, how the movement comes about, we have to look at these petals.

For this purpose we cut the petals with a razor blade in delicate sections and put them on a microscope slide[2] and observe

[2] a piece of glass, 76 times 26 millimeter sized. Available in shops for laboratory supply

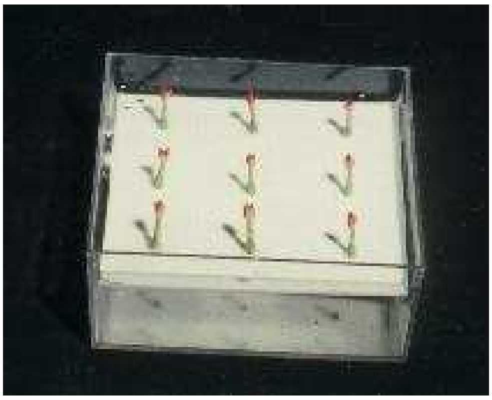

Figure 1.5: Kalanchoe-*flowers are mounted in holes of a plastic disk which float in a water-filled cuvette. They can be photographed for instance every three hours from above. The flowers open during the day and close during the night in the same way as in intact plants. Here the flowers are closed*

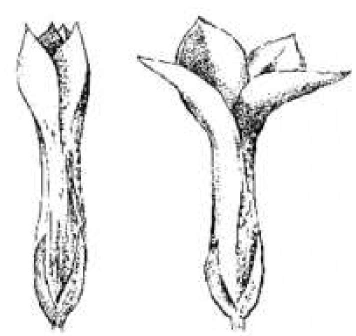

Figure 1.6: Kalanchoe flowers *during the night (left) and during the day (right). In the night the petals are bend upward and the flowers therefore closed. During the day the petals bend outward: The flowers open. Details and how it functions is explained in figure 1.8, 1.9 and 1.10*

them under the microscope[3]. How the cuts are made is illustrated and described in figure 1.7. Under the microscope one can

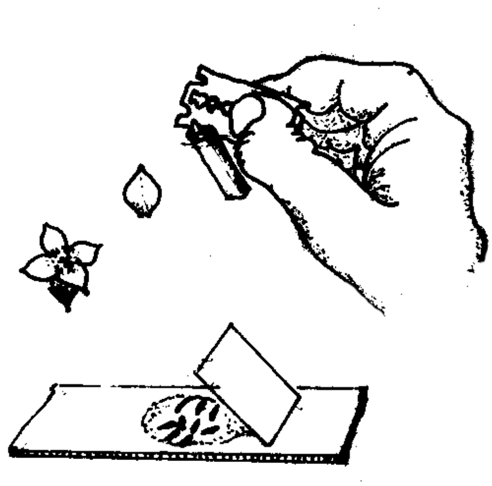

Figure 1.7: *The pith of a elder twig is cut out with a knife. It is wettened with water and notched with a new rasor blade at the top center (be careful, very sharp). The tip of a petal is clamped in the slit of the elder pith in such a way that the tip is a bit above the pith. With the razor blade very thin cross sections of the petal are made. The sections are transferred with the tip of a wet brush and placed in a drop of water on a microscope slide. The cut elder pith sections are thrown away. Make a lot of sections, because you need some praxis in order to obtain real thin sections. With a pair of forceps a cover slip is put with its edge close to the drop of water and slowly laid on the sections in the drop. Under the microscope you can look for the best section*

recognize the cells in the various tissues of the petal. There is an upper cell layer

[3] I propose to ask at school, whether you might use there a microscope. Somebody should show you how to work with it.

(*epidermis*) with papillae like cells (kind of half a balloon, see figure 1.8). They are coloured red. Below are about 15 layers of so called *parenchyma cells*. They are loosely arranged cell accumulations with a lot of air betwen the cells. The lowest cell layer is a *cobble epithelium*: The cells *are interlocked with each other* (bottom of the section in the figure).

Figure 1.8: *A section through a petal show an upper layer of so called* epidermis cells, *which are structured like papillae. They are colored red. Underneath are several so called* parenchyma cells. *The lowest cell layer is a cobble* epithelium*: The cells are interlocked with each other (here not recognizable. See* Engelmann (2004b)*)*

The opening and closing of the petals is brought about by the parenchyma cells. They are therefore also called motor cells. How does it work? To understand it, we have to look at a cell in more detail (figure 1.9). The motor cells shrink and swell, depending on their salt content of their vacuoles. The salt content[4] can be changed by ion-pumps[5] in the membrane between the cell sap and the vacuole. The lower epidermis serves probably as a bendable counterfort for the extending or shrinking motor cells in the parenchyma. The upper epidermis with its papillae cells are able to enlarge, if the motor cells in the interior of the petal change their length. How this might happen is shown schematically in figure 1.10.

Similarly the papilla cells can shrink and swell.

1.2.2 An internal clock opens and closes *the Kalanchoe*-flowers even if the day-night cycle is excluded

Now the detective in us awakes and asks: Are the flowers able to open and close also if they are not placed under a day-night-cycle? We could observe them in a cellar, in which all the time a weak green[6] light is on. Surprize: Even without the light-dark change the petals still move in a daily fashion. In most cases the temperature in the cellar is quite constant during day and night. It is therefore unlikely, that the flowers move rhythmically because of tempera-

[4] salt is here not table salt (sodium chloride), but salts in a chemical sense such as potassium chloride

[5] if table salt is solved in water, ions are formed: Sodium ions are positive, chloride negative charged particles

[6] at white light the flowers do not move any more. Green light is for the flowers like darkness, but it allows us to observe what happens. We should furthermore add 68 gramms of sugar to 1 liter of water or 6.8 gramms of sugar to 100 milliliters of water. The flowers move now much longer as compared to pure water

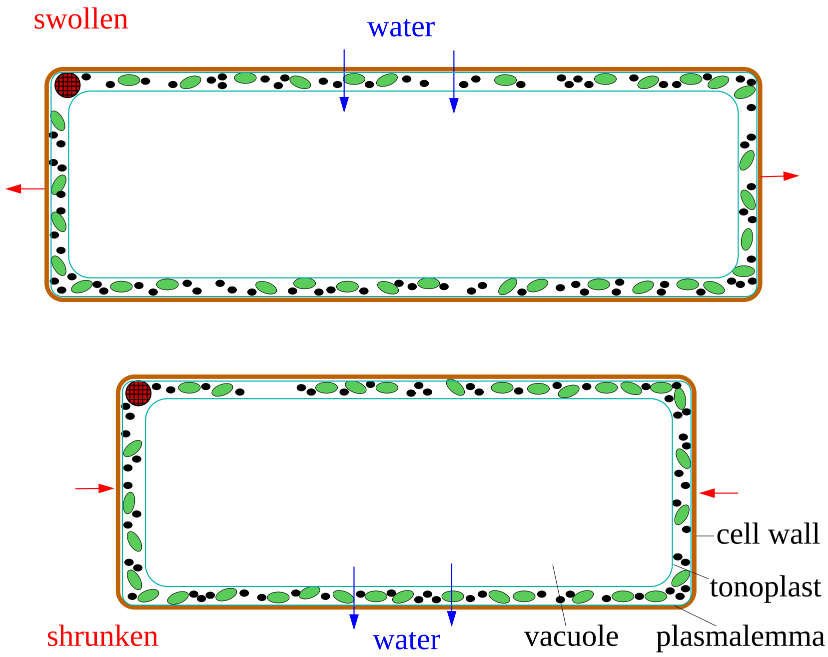

Figure 1.9: *A motor cell of* Kalanchoe *consists of a wall (thick), the* cytoplasm *and a central bubble (*vacuole*). Between cytoplasm and cell wall ist a membrane called* plasmalemma*. Between cytoplasm and vacuole is a membrane called* tonoplast*. The cytoplasm contains a nucleus (red blob top left) and smaller items called organelles with diverse tasks. Mitochondria (small items, drawn black) support the cell with energy, in the chloroplasts (oval green) carbon dioxid is converted to sugar with the aid of light. The vacuole is a bag filled with water and salts solved in it. If there are plenty of salts in the vacuole, it sucks in water (upper image, blue arrows). As a consequence the cell swells (red arrows) and becomes turgescent. If the vacuole contains few salt, the turgescence of the vacuole is low, the cell looses water (lower image, blue arrows) and shrinks (red arrows). The constituents of the salts (*ions*) are transported inward or outward through channels by pumps in the membrane.*

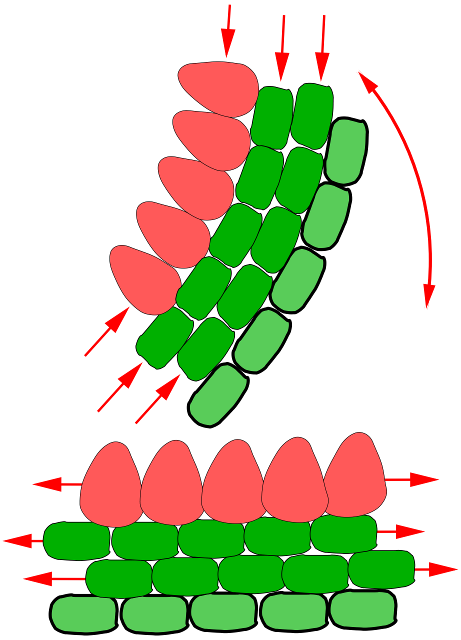

Figure 1.10: *The motor cells shrink and swell depending on the salt content of their vacuoles (see figure 1.11). If they swell, the parenchyma cells lengthen (lower part of the figure, red arrows. Here in the scheme only two cell layers are shown instead of 15). The lower epidermis serves as a bendable counterfort. It is not able to lengthen or shorten, but can bend down, if the parenchyma cells swell, or bend upward, if the parenchyma cells shrink (upper part of figure, red arrows). Similarly the papilla cells of the upper epidermis can shrink and swell and in this way adjust to the length of the motor cells in the interior of the petals (red arrows at the red papilla cells)*

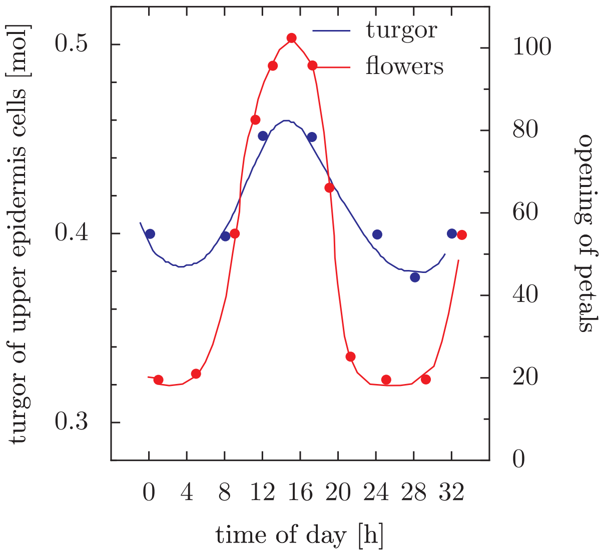

Figure 1.11: *The width of opening respectively closing flowers were measured (red points with curve) and the turgor determined at the given times (blue points and curve). For this purpose the upper epidermis, that is the papilla cell layer, of a flower was removed with a pair of forceps at the indicated time and its turgor determined (how this is done is described in Engelmann and Klemke (1983)). With increasing turgor (blue curve turns upward) the flowers open, with decreasing turgor they close*

ture cycling.[7] If we determine now the period length (that is the time between fully opened flowers until the next point in time of fully opened flowers) of this petal movement under continuous green light (and at constant temperature), it is not anymore exactly 24 hours, but only 22 hours. Thus the flowers open each day two hours earlier as they would under a normal day with light-dark cycling. Apparently the flowers possess an internal clock which controls the opening and closing. It is by two hours shorter as compared to the length of a natural day with its 24 hours. This clock is therefore called circadian clock (from latin circa = about and dies = the day).

1.2.3 The circadian clock runs independent of the temperature with the same speed

A clock is only reliable, if it runs at various temperatures with the same speed. Therefore man has invented and built in special mechanisms for their clocks which make sure that they run with the same speed independent of the temperature. Circadian clocks are also independent of the temperature (figure 1.12). If *Kalanchoe*-flowers are observed under weak green continuous light at 15° C and at 20° C, the period length is about the same. That is not self-evident, because most processes in organisms proceed at higher temperatures faster. The circadian clock has therefore a special mechanism, which provides security for variations in the environment. The circadian clock is said to be temperature-compensated.

[7] Even better: If the flowers are observed in a room with an air conditioner which keeps the temperature constant, the petals continue to move in a daily fashion

1.3 A flower clock in the school garden

We have just seen that the flaming Katy *Kalanchoe* opens and closes its flowers each day.[8] The movement terminates after the flowers are pollinated and the seeds have formed; the petals wilt.

Other plants open their flowers at certain times of the day. This can be observed for example in the morning glory *Pharbitis*: It opens its flowers in the morning (therefore its name) and wilt in the evening (figure 1.13, Winfree (1976)).

Some plants have even been named according to the time of day at which the flowers open. The english name (and also the Indonesian name) of *Mirabilis jalapa* is four o'clock flower, since they open their petals around 4 o´clock in the afternoon.[9] Linné (figure 1.14) constructed in 1751 a flower clock: Various plants are planted as a kind of clock-circle in a round garden bed in such a way, that their flowers open or close at the corresponding day- and night-times (figure 1.15).

Many butterflies visit flowers at specific times of the day. The reason is not only, that butterflies are active at certain times of the day, but also, that the flowers of various plants do not produce the nectar

[8] Unfortunately the gardeners have selected *Kalanchoe* plants in which the flowers do not move any more. They thought the plants would look more attractive if the flowers do *not* close. But it is so much more interesting, if one can observe these movements. You should buy the plants in the evening and check, whether the flowers have closed

[9] This is, however, not the case for plants observed in the Netherlands, as Rob Soekarjo (r.soekarjo@phys.uu.nl) told me. They flower in the late afternoon or even during the night. Furthermore the time of flowering seems to depend on the colour of the flowers (EMail of February 4th, 2002)

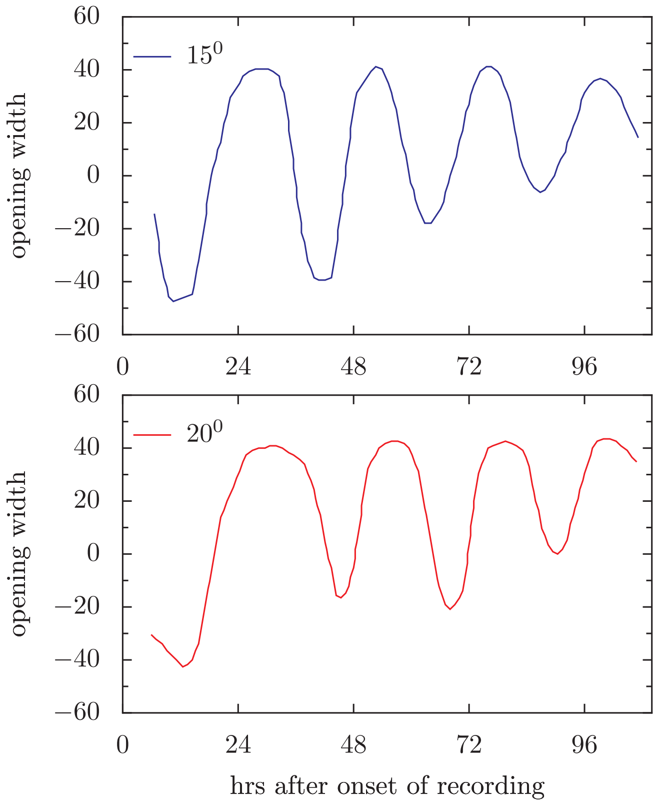

Figure 1.12: The opening width of Kalanchoe-*flowers was recorded at a temperature of 15°C and of 20°C at different times. The obtained values were plotted (blue curve, 15°C, red curve, 20°C). At both temperatures the flowers open (increasing values) and close (decreasing values) rhythmically. Maximal opening is somewhat earlier at 20°C as compared to 15°C, but that is true also for the maximal opening in the following three days. The intervals (called period lengths) between the times of maximal opening are, however, almost identical. Thus, the clock in the motor cells runs with the same speed*

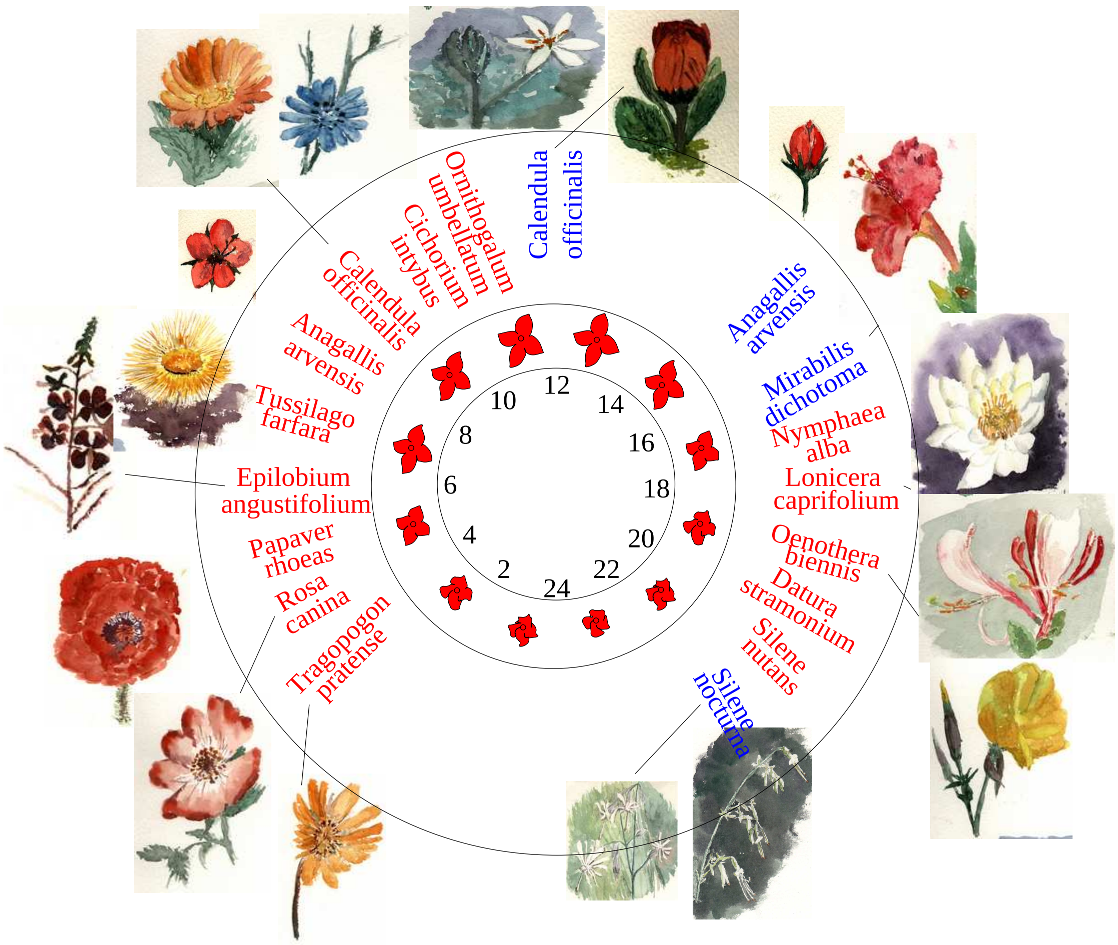

Figure 1.15: *A flower clock: Plants are shown from 12 o´clock noon (top) and midnight (24 o´clock, bottom) to 12 o´clock, the flowers of which open (names red) or close (names blue) at the indicated times. Illustrations of these plants at the margin. In the center* Kalanchoe*-flowers are shown at various times of the day (see also figure 1.4). Here are the German and Latin names with opening- (o) and closing times (z). Top row from left to right: o 8 Scarlet Pimpernel* Anagallis arvensis*, o 9 Common Marygold* Calendula arvensis*, o 6 Chicory* Cichorium intybus*, o 10-11 tar of Bethlehem* Ornithogalum umbellatum*, z 12 Common Marygold* Calendula arvensis*, z 15-16 Scarlet Pimpernel* Anagallis arvensis*. Rechte vertical Reihe: z 16-17 zweiteilige Wunderblume* Mirabilis dichotoma*, z 17 White Sea Lilly* Nymphaea alba*, o 18 Perfoliate honeysuckle* Lonicera caprifolium*. Bottom row from right to left: o 19-20 Thorn Apple* Datura stramonium*, o 20-21 Nodding Catchfly* Silene nutans*, z 21-22 Night-flowering Catchfly* Silene nocturna*, o 3-5 Goats-beard Tragopogon pratense, o 4-5 Wild Rose* Rosa canina*. Left row from bottom to top: 5 Common Red Puppy* Papaver rhoeas*, o 6-7 Rose-Bay* Epilobium angustifolium*, 7 Coltsfoot* Tussilago farfara*.*
After Hess (1990), Böer (1948) and, with hand written corrections of the opening and closing times by Bünning, after Jores (1937). At page 92 these and further plants are specified. Water coloured by the author

Figure 1.17: *The flowers of the Evening Primrose* Oenothera biennis *open in the evening so rapidly, that one can watch it. This series of pictures was obtained between 20:00 (top left) and 21:15 (bottom right). The first three images were taken in 20 minute intervals, the last five in a few minutes. Photographs by the author*

throughout the day, but at distinct times of the day. Therefore one can also establish a butterfly clock (see figure **??**). The Evening Primrose *Oenothera biennis* (family of *Onagraceae*) originates from North America. It has spread in our gardens because of their nice large yellow flowers (figure 1.16). It flowers from June until end of October. Each evening between 20 o´clock and 22 o´clock one or several flowers open so rapidly, that one can watch it (figure 1.17). You should absolutely have a look at that pageant. The petals unfold partly quite jerkely and after a few minutes the flower is completely unfolded. At the same time the flowers emitt a sweet fragrance which attracts moths. Since new flowers develop daily, one can observe this fascinating event for several months each evening. The opening of the flowers occurs also under continuous light. It is thus controlled by a circadianen clock (Arnold (1959)).

Figure 1.13: *The flowers of the* morning glory Pharbitis *come into bloom in the morning by unfolding the petals which are folded in the flower bud, to start with. In the late morning they have opened. In the afternoon they close and in the evening they wither (at higher air temperature they wither earlier as compared to cooler weather). On the next day new flowers will open. After Winfree (1976)*

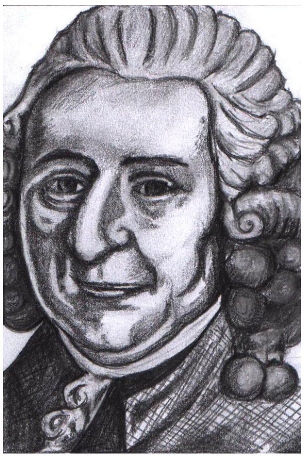

Figure 1.14: *The Swedish botanist Carl von Linnè. Drawn by Mareike Förster after an image in Duden-Lexikonredaktion (1969)*

Figure 1.16: *Evening Primrose* Oenothera biennis *flowers from June until end of October. Each evening between 20 o´clock and 22 o´clock one or several flowers open (see the series of images in figure 1.17). At the same time the flowers emitt a sweet fragrance which attracts moths*

2 Flowers and insects

Many flowering plants are fertilized by insects. Cross polination is of advantage for the plants. The insects are attracted by fragrance, colour and special shapes of the flowers. They are rewarded with nectar and pollen. Bees remember the time at which flowers offer nectar and pollen and collect it as food for themself and the hive. They use an internal day clock and a sun compass.

Why are there so many plants the flowers of which open and close each day or once at a certain time of the day? It has to do with insects which collect the pollen and fertilize the flowers of plants of the same species. This prevents, that flowers are fertilized with their own pollen. For organisms *cross pollination is more advantageous than self polination*, since the genom differs more. For this reason many mechanisms have evolved to prevent self-pollination. For humans it is usually a rule that close relatives do not marry. And if they do, like for instance the pharaos, problems arise easily: Often the children suffer under hereditary disease.

Insects are attracted mainly by the pollen and the nectar of the flowers as a source of food. Partly the flowers might also offer shelter and warmth.

The flowers adapted during the course of evolution of life on earth to certain insect groups and are fertilized by them. There are plants, which are pollinated by butterflies (butterfly flowers, figure 2.1), and others, in which bees take care of pollination (bee flowers, figure 2.2). Parallel to this adaptation the insects adapted also to the flowers. For the insects it is important to find the plants, to remember good nectar- and pollen donators and the time at which food is offered by the plants. For further and more detailed information in the context of flowers and insects see the book by Hess (1990).

Figure 2.1: *Phlox as an example of a butterfly flower. Moths fetch only nectar and use a long proboscis which they roll out and insert in the flower tube in order to suck out nectar. Therefore butterfly flowers possess a long flower tube and offer plenty of nectar. Furthermore they are very fragrant allowing them to attract the moths over long distances*

Flowers open and close often rhythmically or at certain times. We have heart about it already in the first chapter. Other rhythms are found in flowers: Pollen- and nectar offering, fragrance, heat production.

Figure 2.2: *Left: Bee wipes off pollen from a willow catkin and forms pollen panties at the hind legs. Center: Bee licks nectar on a* borrage flower *(*borrage Borrago officinalis*, flowers from June to the fall). Right: Bee visitor at thyme. Bee flowers are often blue, yellow or white coloured (often including UV-marking which we can not see in contrast to the bees). They offer besides nectar plenty of pollen. Fragrance is of intermediate strength and often honey-like. Bee flowers are open during the day. Bees must be able to land on the flowers*

It is furthermore advantagous for insects, to remember the time of opening of flowers. It saves energy and the insects can start to collect at the most favorable time. It is also of advantage to visit flowers of the same kind. This allows to get to know the flowers better and find pollen and honey faster and effektivermost effectively. This behaviour is called flower constancy. It is especially well developed in bees. For the plants flower constancy means, that the bees transport pollen of the same species and warantees safe pollination of the flowers of this species.

2.1 Time sense of bees

Bees can be trained easily to find food. This was observed by Forel (1910) while having his breakfast on the veranda of his vacation home. The bees helped themself with the jam on the table, but arrived on the following days already *before* the jam was served. Once, when the family stayed inside the house because of bad weather, the bees still arrived and searched for food. Forell concluded, that the bees must have a kind of time sense which helped them to look for food. Some kind of internal clock reminded the bees that soon jam will beserved at the house of the Forel's.

Later von Frisch and his students did numerous training experiments with bees (Frisch (1965)). They offered the bees concentrated sugar water and at the same time specific signals such as colour, fragrance, flower shapes. Once the bees had learned to find sugar water in a small bowl for example on a blue cardboard disk, they flew after successful training to the blue disk even if the bowl contained only water without sugar (figure 2.3). The scientists found out that the following signals are important for finding food: Fragrance, color, time and shape of the flower. Fragrances are especially important. The bees learned already after a single trial. For colors they needed three to four trials and for recognizing the form of the flowers 30 to 40 trials. Why fragrances are so effective is obvious: Mixtures of fragrances are characteristic for flowers and they can be distributed in the hive to the fellow forager bees. Memorization of the fragrances disappears after some time, but reappears after 24 hours. Because pollen and nectar and the connected signals such as fragrance are offered by the flowers often only at certain times of the day, bees use special collecting hours.

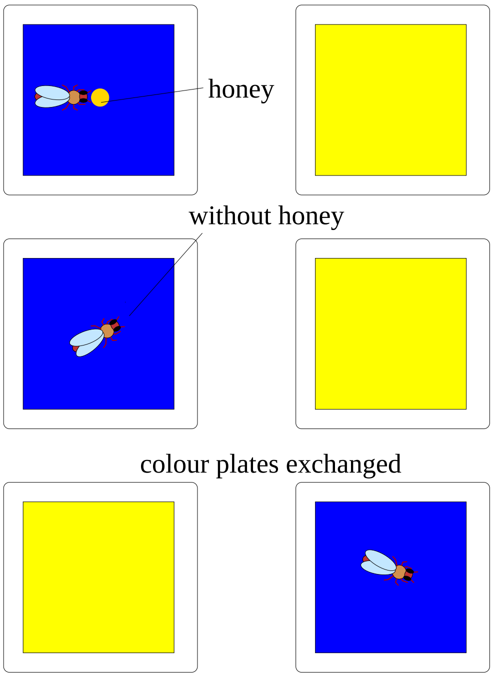

Figure 2.3: B*ees are trained for certain colors, here blue, by offering some honey on a glass plate. A colored cardboard was placed underneath the glass. The glass plate on top of the yellow color was left empty. After the training period a glass plate without honey is placed on the coloured cardboard. Bees trained to blue still fly to the blue color expecting to find honey there. If the blue and yellow plates are exchanged, the blue one is still selected. That shows, that the bees did not just memorized the location where honey was offered*

The proficiency of bees in searching for pollen and nectar is even more admirable. Once a forager bees has found a new source of food, it is able to tell their co-workers in the hive, in which direction and how far they have to fly to find the flowering plant. In this way the bees find food faster and more economic and save a lot of energy. How this is done is explained in figure 2.4.

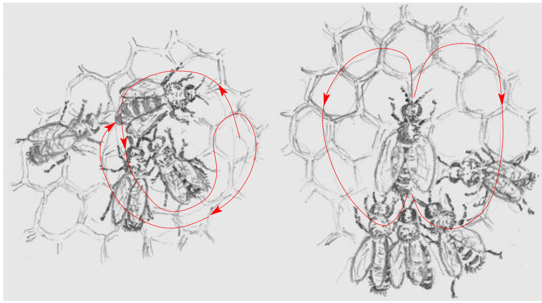

Figure 2.4: *Forager bees are able to communicate to their coworker bees in the hive, in which direction a source of food can be found and how far away it is. Furthermore fragrance and quality of the food is signalled. Left: Round dance. The forager bee dances at the vertically hanging comb in the hive in circles. The direction is frequently changed (red track). Other foragers follow the dancer. This kind of dance tells the co-foragers, that food is available in the vicinity of the hive. Furthermore fragrance plays an important role. If the food is further away from the hive, the round dance changes to a sickle dance (not shown). Right: Wag dance. If food is 250 meter or further away, the foragers in the hive are informed about the food by a wag dance. It looks like a flat 8 and informs about direction and distance of the food. The path is shown as a red track in figure 2.5.*

A bee flew at the time of blooming of the orchard trees in the spring time in search for nectar and pollen in the direction of the sun and found a renumerating cherry tree. It collected plenty of pollen from the flowers and striped it as panties at its hind legs. Furthermore it sucked nectar from the flowers and stored it in its honey stomach. Bang full it flew back to the hive. There it landed at the entrance board and ran in the dark hive to the combs where breed was reared.

At the comb it performed a dance, which is shown schematically in figure 2.5 in the interior of the hive: It runs upward (long red arrow) and wags with its abdomen. Than it turns, runs downward (short red arrow) and this wag dance (long red arrow) is repeated several times. During the dance other forager bees follow. They obtain a lot of informations: whether the forager found either pollen or nectar or both, which fragrance and quality the food had, and how much of it is available.

Furthermore the direction of the food source is reported in the following way. The direction of the sun is transposed in the dark hive upward. Since our bee found food in direction of the sun, its wag dance will point upward (long red arrow). An other bee, which found an opulent flowering pear tree 30° left of the sun does not run upward, but 30° inclined to the left (lower example in figure 2.5). To transmit the direction, the angle between the food position and the sun is coded in the wag dance. This *sun compass orientation* works also under overcast sky. Small patches of blue sky still allow the bees to orient by using the polarization pattern of the sky to find out the suns direction (see figure 2.6).

You might like to observe this typical polarization of the sky and how it turns during the day with the suns orbit by looking through a polarization star-foil (see figure 2.7).

If individual bees are caught on the way from the food source to the hive and kept for some time in darkness, they are still

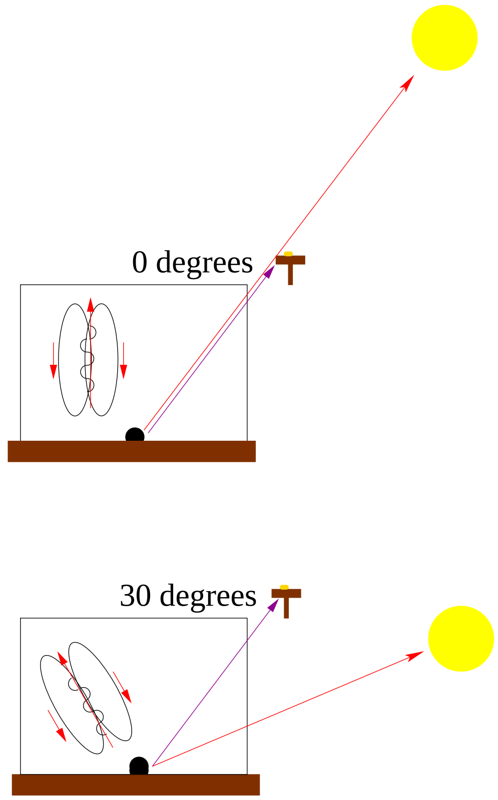

Figure 2.5: *Sun compass orientation of the honey bee and wag dance. In the upper sketch food (F) is in the direction of the sun. The forager projects this direction in the vertically hanging comb of the dark hive downward. It informs other foragers about the direction by performing a wag dance as described before. At the roller coaster the bee runs down at the side and than in the center up while wagging (how often it wags, signals the distance of the food), and afterward down again at the side. In the lower sketch the food place T* 30^0 *is left of the sun. The wag dance is conseqently inclined by* 30^0 *to the left. After Frisch (1965)*

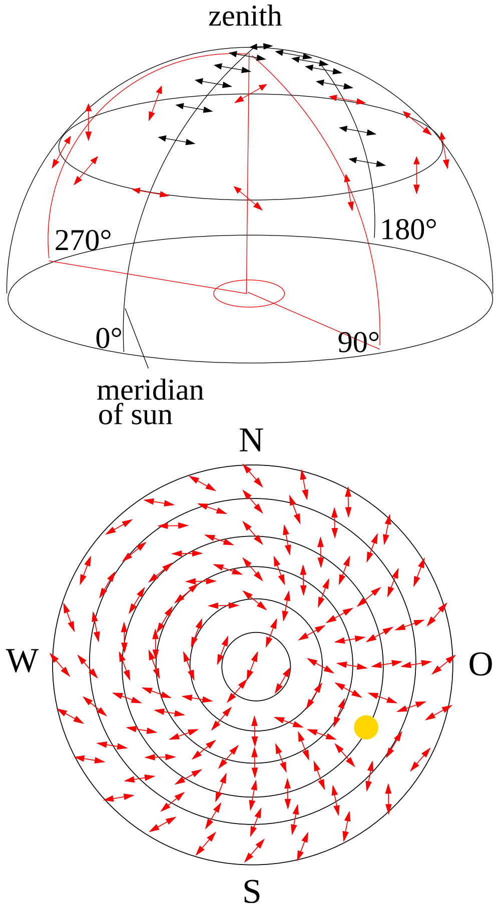

Figure 2.6: *The polarization pattern of the sky changes in a characteristic way during the day. It can be recognized by the bees and with it the time of day, provided a small piece of blue sky can be seen. Top: Hemisphere of the sky showing the direction of the polarization of light, indicated by arrows for the various locations in the sky. Below: View from the center of the upper figure (red arrows on the dark ring) into the sky. Sun in south-east-east (yellow circle). After Nitschmann and Hüsing (1987)*

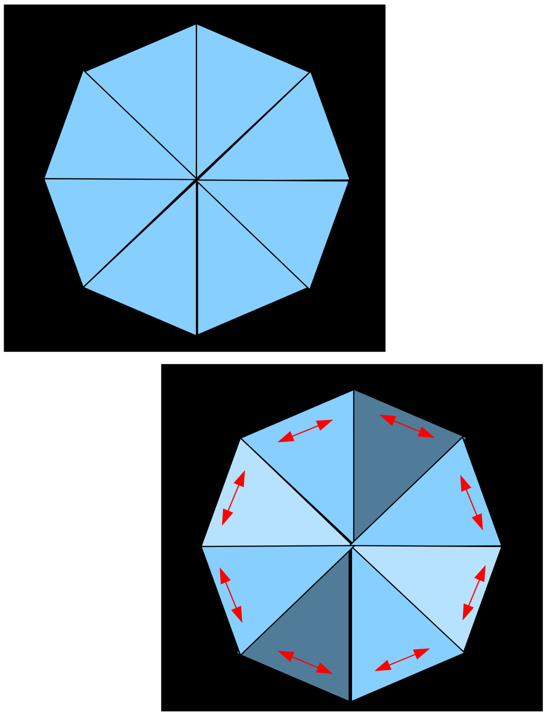

Figure 2.7: *Cut 8 triangles out of a polarization foil in such a way, that the direction of polarization (red doubble arrow) runs parallel to the outer margin of each triangle. Insert them between two glass plates and put an adhesive at the edges to prevent the foils from getting out of place. Watch the light of an incandescent bulb (unpolarized light, upper image) and the sky at different times of the day and find out, where it is brighter or darker (lower illustration). The polarization pattern of the sky is the result of scattering of the sunlight in the atmosphere, whereby it is polarized. Bees possess at the inner edges of their upper eyes UV-sensitive ommatidia with pigment molecules which are oriented differently. Each ommatidium has thus a slightly different direction of its polarization. With these ommatidia the bee sees the polarization pattern of the sky differently depending on the time of the day (see figure 2.6) and in a way as you see it with your star foil. After Nitschmann and Hüsing (1987)*

able to transmit the direction of the food correctly to other foragers, if brought back into the hive (figure 2.8). Since they were unable to observe the way of the sun during their confinement, they must possess an internal clock with the 24-hour-rhythm of the orbit of the sun. This clock allowed the bees to remember the time interval before beeing transferred into the hive. They have therefore taken into account the course of the sun (15° per hour) and made provisions for it when displaying the wig dance.

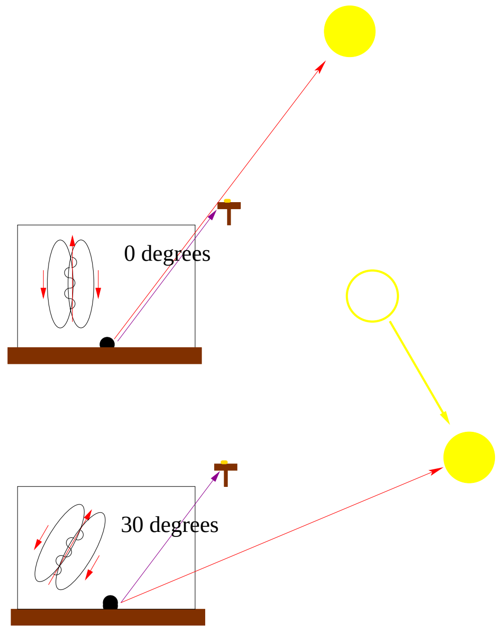

Figure 2.8: *Upper image: A bee displaying a wig dance in the hive: It found food in direction of the sun and transmitted this direction to other foragers by running upward on a comb. Lower image: Bee displaying a wig dance after having been confined for two hours in a dark box on its way from the food source to the hive. It was released directly in the hive and could therefore not see the current position of the sun. Since the sun is after two hours 30° more westward, the transmitted angle should be 30° right of the plumb line. This is indeed the case. Since the bee could not see the movement of the sun during its confinement, it must possess an internal clock. This clock told the bee, how long the time was during the confinement, before being returned to the hive. The bee took the path of the sun (15° per hour) into account and indicated the correct direction during its wig dance*

Other events in the life of bees are also controlled by this clock. They can, for instance, remember fragrances and food 24 hours after experiencing them. This is very practical, since many plants offer nectar and pollen in a 24 hour rhythm.

The distance from the hive to the food is also signalled. If the food source is far away, they wig less frequently with their abdomen as compared to food closer by (figure 2.9).

Since the bees recognize the direction as well as the distance of the food source, one speaks of vector-navigation (vector: distance and direction). Thus the sun compass orientation serves the bees to orient in space, to collect food and to communicate.

After having informed the foragers in the described way, the nectar is transferred into a cell of a *honey comb* . The pollen is transferred to a *comb cell*, which was prepared for the queen to lay an egg into it (figure *2.10*). Out of the egg ecloses later a larva which feeds on the forhandedly deposited pollen. Finally a young bee would emerge and take over the necessary work in the hive allowing the population to survive.

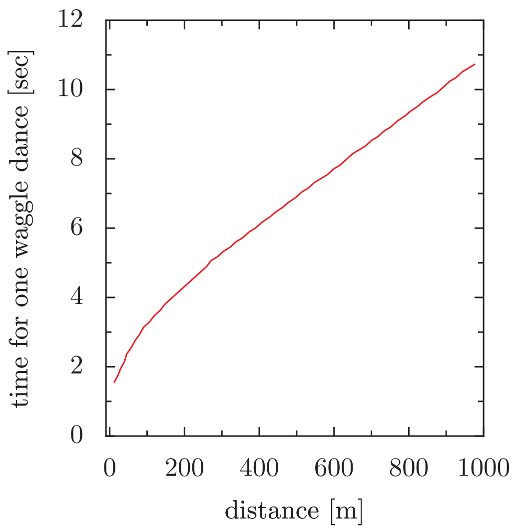

Figure 2.9: *During a wag dance the distance of food from the hive is signalled to other forager bees by wagging the abdomen more frequently at shorter distances and less frequently at longer distances. The curve shows, how many seconds the forager needed for one completed wag turn at different distances of the food. For food further away it needs more time. After Frisch (1965)*

Figure 2.10: *View at cells with pollen (bottom left, yellow), and uncovered (center and bottom right, dark shiny) and covered honey cells (top and right part, grey lids) in the comb of a bee hive.*

2.2 Other tricks of plants to become pollinated by insects

In bogs and wet, agriculturally not much used swampy meadows, but also at wet parts of dryer meadows you might find Grass of Parnassus *(Parnassia palustris)* (figure 2.11).

The flowers contain five filamented stamen arranged in a circle around the ovary (figure 2.12). One of the five stamen is shifted over the top of the ovary by extension of the filament. The anther opens face up and the pollen can be spread by flies landing on the flowers and getting brushed with pollen at their belly. On the next day the filament bends outward and the anther is shed at the outer edge. A new anther is shifted over the top and opens. This is continued for five days lang, until all anthers are shed. Now the stigma at the tip of the ovary opens. If a fly covered with pollen at its belly from another Grass of Parnassus lands, the stigma is pollinated. This mechanism makes sure that the flowers are cross pollinated from plants of the same species and that self pollination is prevented.

Pollen is offered by some plants to the pollinators at certain times of the day only. It makes sense and saves energy if the insects visit the flowers only at these times.

In other plants the nectar is produced at certain times of the day only. In the jungles of Trinidad *Anguria*, a pumpkin plant, is pollinated by the butterfly *Heliconius*. There are two different species of *Anguria*. Nectar secretion of *A. umbrosa* occurs between 7 and 12 o'clock. *Anguria triphylla* offers its nectar from 12 to 19 o'clock (figure 2.13). The butterfly has adapted to it: There are two different species*, one of which pollinates the species Anguria umbrosa* and the other species *Anguria triphylla*. This shows that nectar secretion, if restricted to a certain time of the day, can make insects to adapt to these times and to form a new species in the course of time.

Figure 2.11: *Grass of Parnassus* Parnassia palustris *is found bogs and in wet, agriculturally not much used swampy meadows, but also at wet parts of dryer meadows. Blooms from July until September. Its flower is shown enlarged in figure 2.12. Photography of the authors*

Fragrance is often secreted at certain times of the day. In turn, the pollinators of those plants, insects, birds and bats use their own clocks and possess often special mechanisms for orienting, in order to adapt to timing of the flowers. In the next section we will have a closer look at this.

2.3 Fragrance of flowers and fragrance rhythms

Surely you know, that many plants smell (or also stink). Roses, spices such as lavender and thyme, or the Evening Primrose (we talked about it on page 15) are ex-

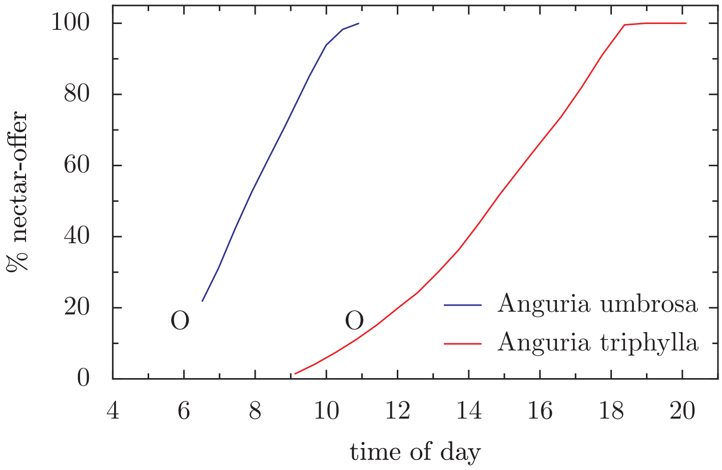

Figure 2.13: *Nectar secretion of* Anguria umbrosa*, a pumkin plant from the djungel of Trinidad, occurs between 7 and 12 o'clock (green), pollen a bit earlier (bottom left). In* Anguria triphylla *the nectar is secreted between 12 and 19 o'clock (red), the pollen (bottom center) between 10 and 12 o'clock. After Hess (1990)*

amples. At least 30% of all higher plants produce substances, which vapourize easily (are volatile) and smell therefore. In higher concentrations they are dangerous for the plants. They are therefore stored as etheric oils in special cells, fragrance fields or glands of the flowers.

Fragrance cells possess thin walls, which allows easy transpiring. This property allows to find out whether flowers possess fragrance cells or fragrance fields. The flowers are cut and the stalk put into water. A small amount of neutral red was added before to the water. Neutral red is a colour which solves easily in water and does not harm the plants. At spots where fragrant substances are given off the neutral red accumulates, because water is also given off in larger amounts leaving the neutral red back in the cells.

This can nicely be seen in daffodil which possess a crown between the petals and the stamen and ovaries. It serves as a fragrance and colour structure and possesses furthermore special markings which can easily be seen under ultraviolet light[1]: If daffodil flowers are stained with neutral red, the yellow crown turns red (figure *2.14*). The crown attracts the pollinators. Coloured markings attract additionally. They indicate the places where food is offered to the insects. For the plants these places are important for pollination. Insect flowers use other colours and fragrances than bird- and bat flowers (figure 2.15 and 2.16). Bird flowers are usually red, but also blue and green-yellow and the fragrance is faint. They offer throughout the year plenty of nectar. Bird flowers are found in the tropics and subtropics. More than hundred plant species possess bird flowers. Plants with bat flowers are also found in the trop-

[1] we are not able to see ultraviolet light with our eyes, but insects and especially bees can. Often these flowers show under ultraviolet light patterns which point towards the internal structures of the flowers, thus leading the way for the insects to the places where something is offered to them (and where the plant is pollinated)

Figure 2.16: *Letf: As an example of a bat flower* Campsis radicans *is shown. Bat flowers occur in the tropics and subtropics, possess tough structured flowers with a lot of pollen and a slimy nectar. The flowers sit on long stalks elevated over the leaves. Photography of the author. Right: Bat flower in front of a flower (sketch of the author after a figure in Hess (1990)).*

ics and subtropics, the flowers are tough with plenty of pollen and a slimy nectar. They smell slightly acidulous like fruit or butyric acid, like bats do. The flowers are positioned on long stalks elevated over the leaves or directly at the stem.

Fragrances and fragrance markings of plants, which flower during the day, attract the visitors at short distances. Orienting in the interior of the flowers is facilitated in this way. In contrast, night-blooming species possess a more intensive smell which works over long distances. The fragrance increases strongly with progressing darkness. This attracts night active moths such as the Sphingids. Furthermore the flowers of night flowering plants are white coloured, allowing to be recognized more easily by flying insects in the weak light of the twilight and the night (figure 2.17).

The fragrances of flowers are usually composed of many scents. They are also used by the parfume industry by extracting them from the flowers with special solvers or with water vapour. However, many scents are nowadays also artificially produced ('synthetized'), because the chemical composition is known.

In the Gentianaceae *Exacum affine* (figure 2.18), the Persian Violet, flowers are opened continuously, but the scent intensity varies rhythmically. It is strongest at noon and weak in the evening, night and morning. The plants can be obtained in flower shops and at gardeners. It is worth to observe them at home and to take a smell at them. If it smells strongly, we enter in table 2.1 under the observation time a 3, if the fragrance is weaker, a 2, and at very weak fragrance a 1. If it is not smelling at all, we enter a 0. The values can be plotted

Figure 2.12: *The flowers of the Grass of Parnassus contain five filamented stamen arranged in a circle around the ovary. One of the five stamen is shifted over the top of the ovary by extension of the filament. The anther opens face up and the pollen can be spread by flies landing on the flowers and getting brushed with pollen at their belly. On the next day the filament bends outward and the anther is shed. A new anther is shifted over the top and opens. This is continued for five days, until all anthers are shed. Only the stubs are left. Now the stigma at the tip of the ovary opens. If a fly covered with pollen at its belly from another Grass of Parnassia lands, the stigma is pollinated. This mechanism makes sure that the flowers are cross pollinated from plants of the same species and that self pollination is prevented. Meanly the flies do not get any nectar from these flowers. They are beceived by small yellow-green knobs which shine like honey. They are stamen without anthers (recognizable as small structures on the petals). Unexperienced flies are indeed bluffed. Photography of the author. See also Hess (1990)*

Figure 2.14: *Left: Daffodil in bloom. Center and right: flowers of daffodil with crown stained by neutral red. Center: flowers in water. Right: flowers in water with neutral red. Nach Hess (1990)*

Figure 2.15: *As an example of a bird flower a mallow is shown which a rubinthroated hummingbird (*Archilochus colubris*) is visiting and pollinating. Hummingbird flowers hang freely, since hummingbirds get the nectar by whirring in front of the flowers. The flowers offer much nectar throughout the year. After Mohr (1979)*

Figure 2.17: *The Night blooming Jasmine* Cestrum nocturnum *flowers at the onset of the night with white flowers which possess a strong fragrance. The plant belongs like tomatoe and potatoe to the nightshade family (*Solanaceae*).*

in a diagram in the same way we did by observing the movement of Forsythia flowers (see the red curve in figure 2.19).

Figure 2.18: *The* Persian Violet Exacum affine *belongs to the* Gentianaceae*. Its blue flowers emit a strong fragrance, especially at noon*

But perhaps the flowers are smelling alike all the time and it is our nose which reacts differently at the various times of the day. To test this, we could for example pour some spiritus in a small flask (number 1) and dilute it. Spiritus is denaturated alcohol and smells of pyridine. If we poor half of the content of flask number 1 in flask number 2 and add the same amount of water, we have only half the concentration of flask number 1. Now we take half of the solution of flask number 2, pour it in flask number 3 and add again the same amount of water. This flask has now only 1/4 of the concentration of flask number 1. In this way we can produce a series of dilutions. The weakest concentration should be diluted in such a way that one cannot smell it anymore. Now we can smell these control series at different times of the day before testing the flowers. At which flask are we not able any more to recognize the pyridine smell? This value is put in the last column of table 2.1. The threshold value (lowest recognizable concentration, for example at flask number 8) should be constant, if our nose has always the same sensitivity (see the blue curve in figure 2.19).

time of day	fragrance intensity	control
7:00		
9:00		
11:00		
13:00		
15:00		
17:00		
19:00		
21:00		
23:00		

Table 2.1: *Fragrance intensity of the Persian Violet at different times of the day. No fragrance: 0, very weak fragrance: 1, notedly fragrant: 2, strong fragrance: 3*

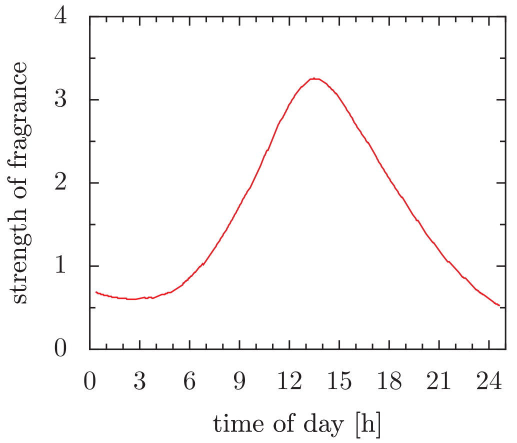

Figure 2.19: *Diagram of the fragrance rhythm of the Persian Violet* Exacum affine. *Clock time at x-axis, fragrance strength at y-axis. The red curve shows the fragrance strength over the day (maximum at early afternoon)*

Our experiment has shown according to the results in figure 2.19, that the intensity of the fragrance *of* the Persian Violet *Exacum affine* has changed in a daily pattern.

In other plants it has also been shown that fragrance secretion is controlled by a circadian clock. But in most cases the strength of fragrance is highest in the evening or during the night and serves to attract insects which are active during the night.

This is the case in the flowers of the Night blooming Jasmine *Cestrum nocturnum* (Overland (1960)) and the Soapwort, *Saponaria officinalis* (plant: figure 2.20, curve of fragrance of *Saponaria*: figure 2.21). In the same way the intensity of fragrance changes in the Wax Plant *Hoya carnosa* (Altenburger and Matile (1990)). In this and most other plants fragrancy consists of several components. In *Hoya carnosa* the various components reach their strongest fragrance at the same time.

In other plants the times of strongest fragrance can differ. In *Stephanotis floribunda* the maxima of 1-nitro-2-phenylethane and methylbenzoate are displaced by 12 hours from each other (Matile and Altenburger (1988) and figure 2.22). Thus the fragrance of flowers of this plant differs at the different times of the day. The up to now studied flowers which scent during the day such as the Bitter Seville Orange *Citrus aurantium* or *Odontoglossum constrictum* do not display an endogenous rhythm of fragrancy (figure 2.23).

Some plants are even able to ask for help by using scents when caterpillars or aphids feed on them. The scents attract natural enemies of the varmint insects as for example Ichneumon wasps. The Ichneumon wasp females pinch the animals with their ovipositor and lay their eggs in their boddy. From the eggs eclose larvae which begin to eat the caterpillars or aphids from within. Ichneumon wasps are

Figure 2.20: *Left* soapwort Saponaria officinalis. *Strongest smell like that of the wax plant* Hoya carnosa *(center) at the onset of night. Right* Stephanotis floribunda

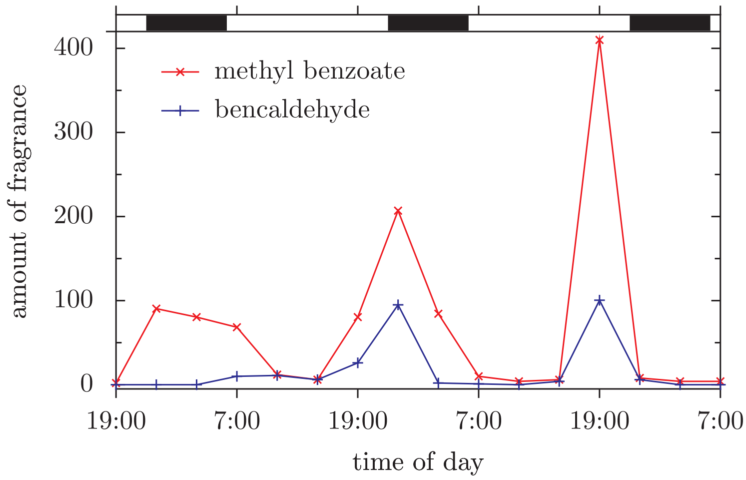

Figure 2.21: *Fragrance production of the soapwort* Saponaria *was measured. The two compounds methyl benzoate (red curve with red values) and benzaldehyde (blue curve with blue values, actual values multiplied by ten, since the curve would have been too small) are both secreted in the evening, but at different amounts. The black bars on top indicate night, the bright once day. After* Neugebauer (1997)

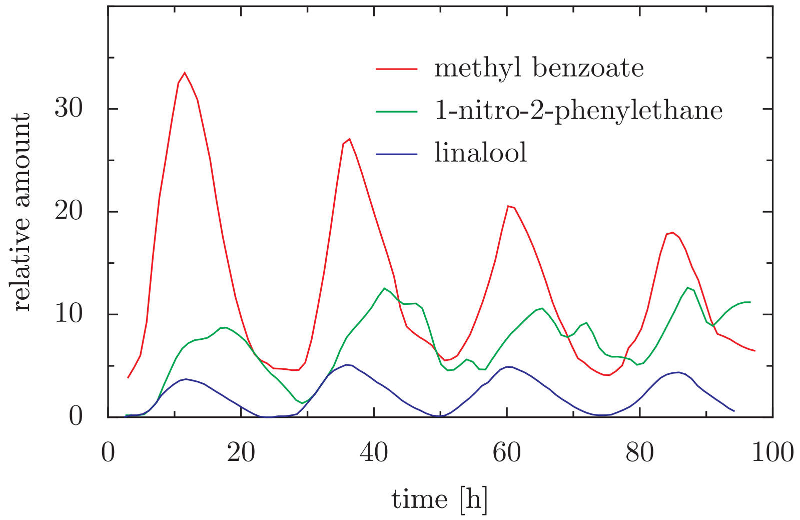

Figure 2.22: *The fragrance production in* Stephanotis floribunda *was recorded with a gas chromatograph. The two fragrant substances methylbenzoate (red curve) and linalool (blue curve) are emitted at the same time of day, but in different amounts. The fragrant 1-nitro-2-phenylethane (green) has, however, its maximum 12 hours later as the other two fragrants. After Matile and Altenburger (1988)*

Figure 2.23: *Day fragrant plants such as the Bitter Seville orange* Citrus aurantium, *an ornamental plant, or the orchid* Odontoglossum constrictum *(Constricted Odontoglossum) does not possess an endogenous rhythm of fragrance. Water colour of the author.*

Figure 2.24: *Fragrances of plants attract enemies of varmint insects as for example ichneumon wasps. They oviposit the eggs in the* caterpillar*. The larvae of the Ichneumon wasps eat the caterpillars from inside. After Tumlinson et al. (1993)*

also attracted by scents of the excrements caterpillars (Tumlinson et al. (1993), figure 2.24).

2.4 How to earn money with leafcutter bees

Many flowers are pollinated by insects. Without insects we had no fruits. Many other cultivated plants depend on insects. They are thus of high economic importance for humans.

Alfalfa (*Medicago varia*) is an interesting example (figure 2.25, Dorn and Weber (1988)). This clover is especially in the United States, but also in Europe the most important food for domestic animals, because it contains much protein, is perennial and frost resistant: Furthermore the Rhizobium bacteria in the roots accumulate nitrogen compounds in the soil. To obtain seeds, the plants have to be pollinated in the partly very huge Alfalfa fields. Honey bees are not well suited for this task, because the flowers are equipped with a special trigger mechanism which discharges the pollen to the insects (figure 2.27). A bee inserting its proboscis into the flower for obtaining nectar sets in motion a flap mechanism which ruptures the suture of the shuttle and this opens as a result. Stamen and stigma which where held by the shuttle flip upward.

In contrast to the honey bee the leafcutter bee *Megachile rotundata*[2], which belongs like other bees to the Hymenoptera (figure 2.26), is not bothered by the 'hook to the chin' of the stamens of the alfalfa. Therefore it polinates successfully this food clover.

Figure 2.26: *Leafcutter bee* Megachile rotundata, *an important pollinator of alfalfa-flowers. After* Dorn and Weber *(1988)*

The leafcutter bees do not appear before end of May and the flights end in August. This is also the time at which alfalfa is in bloom. The females search for a place in hollow plant stalks, boreholes in wood, empty snail shells and other hideouts. They are first inspected, than upholstered with small oval pieces which they cut out of leaves from alfalfa- or other plants with their mouth tools (figure 2.28 and 2.29). The bee roles the leaf cut to a bag and flies with it to the borehole (figure 2.29 bottom). There the leaf is pushed in the borehole and pressed against the wall. The edges of the piece of leaf are chewed. Juice of the leaf and saliva serve as an adhesive to seal up the breading cell. The walls consist of several leaf layers, the bottom of circular pieces of leaf, the edges of which are

[2] Leafcutter bees are distributed allover the earth. Among them is the largest bee species *Eumegachile pluto*. It is 4 cm large and native to Indonesia. The smallest leafcutter bee is *Megachile minutissima*. The females are only 6 mm in length, the males with 3.5 mm even smaller. They are found in Egypt. The leafcutter bee *Megachile rotundata* originated from Eastern Europe and West Asia and does not live like the honey bee in hives, but solitary.

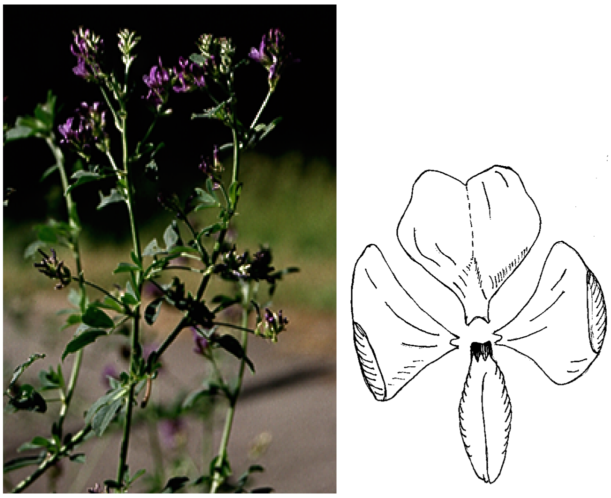

Figure 2.25: *Alfalfa (*Medicago varia*) is the most important food plant on earth because it contains much protein. It belongs to the Papilionaceae and the flowers (right) consist of a standard on top, two wings at the left and right and a keel at the bottom*

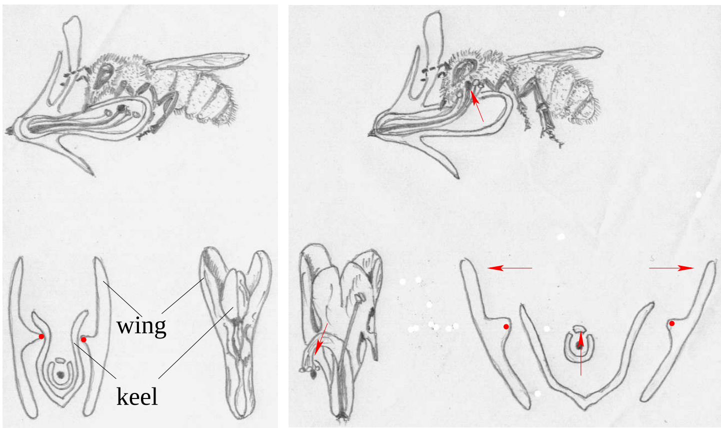

Figure 2.27: *Pollination mechanism of alfalfa: A leafcutter bee tries to suck nectar from the flower of an alfalfa (top left). By doing so its head hits the flap mechanism which flaps the stamens of the flowers on its chin (top right). Stamens and pistil are kept down by the inner lip of the keel (marked by red points bottom left), to begin with. If a bee lands on a flower (top right), the outer petals (wings) are pushed to the sides (bottom right, horizontal arrows). They release the inner lips of the keel which used to be buckled by the wings (red vertical arrows bottom right). They flip upwards and hit the chin of the bee (top right, red arrow). The pollen is discharged on the bee and pollinates other flowers at the bees visit to them. Drawn by the author after illustrations in Hess (1990)*

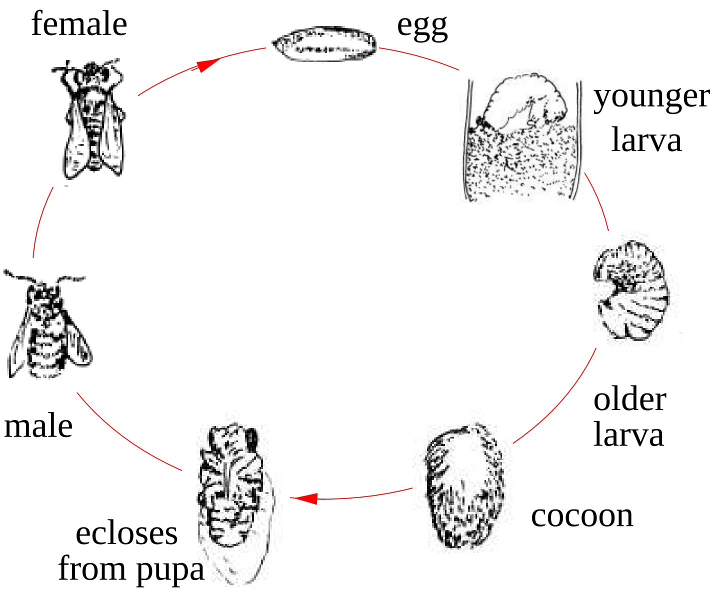

Figure 2.31: *Life cycle of an insect with metamorphosis shown in the example of the leafcutter bee: From the egg ecloses a larva which lives in a breeding cell with food. It grows until the larval skin can not extend anymore. A new skin is formed underneath the old one, the old skin rupures and the new larva ecloses out of the old skin. Two more larval stages follow until finally a prepupa is formed. This prepupae spins a cocoon, in which it hibernates or, in the summer, it directly metamorphoses into an adult bee. Instead of the bag-like body of the larva the adult body is composed of a head with antennae, large eyes and completely different mouth tools, a thorax with four wings and six legs and an abdomen, in which in the case of females the eggs and in case of the males the sperms are produced. The males fertilize the females and the fertilized eggs are again oviposited in breeding chambers filled with honey and pollen. A new generation of leafcutter bees starts. Drawn by the author after Dorn and Weber (1988)*

Figure 2.28: *If you find such punched leaves, you have found the work of a leafcutter bee. Water colour of the author after Müller et al. (1997)*

Figure 2.29: *Top: A leafcutter bee cuts a piece out of a leaf and uses it to coat the tube for its breed. Bottom: Briefly before 'takeoff'. After the last connection to the edge of the leaf is gnawed off, the leafcutter bee flies with the leaf bag underneath its abdomen to the breeding hole and covers the walls. Water colour of the author after a photography in Müller et al. (1997) and Dorn and Weber (1988)*

turned inward. The whole structure looks like a thimble. Now the bee collects pollen and nectar and mixes both to a delicate dough and fills the thimble to about two third with it. Finally pure nectar is added as a thin layer on top of the daugh. In less than a minute an egg is layed onto the honey pulp and closed off with several round leaf pieces (figure 2.30). The first breeding chamber has now been finished. The second one will be build on top of the first in the same way as the first one and again filled with pollen and a final nectar layer. Further breeding cells are added, until finally the last cradle in the tube (e.g. a stalk) is finished. With a thick plug the whole artwork is sealed. After two to four days larvae eclose from the egg (figure 2.30). They live in cockaigne and need only to feed on the mush of pollen and nectar (figure 2.31). Insects possess a skin which is able to extend for a while in the growing larvae. However, at some time the skin is streched so much, that it would burst, if the larva would continue to feed. It stops feeding and the skin does indeed burst. However, before it a new larger and softer skin had formed. Now the larva

Figure 2.30: *The leafcutter bees use tube-like structures as for example stalks as breeding chambers. With their mouth tools they cut pieces out of leaves (see figure 2.29) and cover the inner walls with it. Pollen is collected and deposited, and the final layer covered with nectar. An egg is deposited on it (left) and the chamber closed with several pieces of leaves. After oviposition (center) a larva ecloses (right), which feeds on the supply in the breeding chamber, grows and moults several times*

can continue to feed, until it has to moult again. That occurs four times and takes altogether ten days. The gut is closed during the larval stages thus preventing the food from becoming spoiled by the faeces. After about three weeks the food is used up. At the last moult the gut is emptied and its content deposited in the interior of the breeding cell. This time no larva ecloses. Instead, a dense, silky shining cocoon is spun. The grown up larva becomes a resting larva. In this life stage, called prepupa, the animals hibernate until the cold winter has gone and the alfalfa is growing again in the fields. Many insects interrupt their development, before the winter comes. They undergo a diapause.

In the spring the prepupae changes into a pupa. It is first whitish, than the eyes turn pink to black, and finally the whole pupa gets greyish black. The pupal skin is striped off, the wings unfold and the soft surface of the body hardens. The larva has turned into a bee. I chews a hole in the cocoon and pushes the lid of the breeding cell off in order to escape into the nature.

The adult bee looks completely different from the larva. It is composed of a head with antennae and large eyes, completely different mouth tools, a thorax with four wings and six legs and an abdomen, in which in the case of females the eggs and in case of the males the sperms are produced. The males fertilize the females and the fertilized eggs are again oviposited in breeding chambers filled with honey and pollen. A new generation of leafcutter bees starts.

To pollinate one hectar of an alfalfa field[3] 5000 females are needed although each individual provides for 418 000 seeds. In the USA, to which the leafcutter bees were introduced in 1930 by man, a whole industry has developed in the meantime which reares and sells *Megachile*. Tobacco plants are used, and the bees utilize the leaves to cut out the pieces for their breeding tubes. These tubes together with the larvae are sold to the farmers growing alfalfa for seed production. Since this is done briefly before eclosion of the adults; the bees eclose soon and fly in the fields to search for food. They fertilize the flowers. In large fields huts are build in which the breeding tubes are placed (figure 2.32). It is worthwhile for the farmers to buy the leafcutter bees, since they get instead of one to three and a half dt twentytwo decitons of alfalfa-seed per hectar.

But how do the insects know, that winter is approaching and that it is time to enter diapause? To wait until the first frost comes is too risky. The body can not

[3] a hectar is an area of 100 times 100 meter, that is 10000 square meters

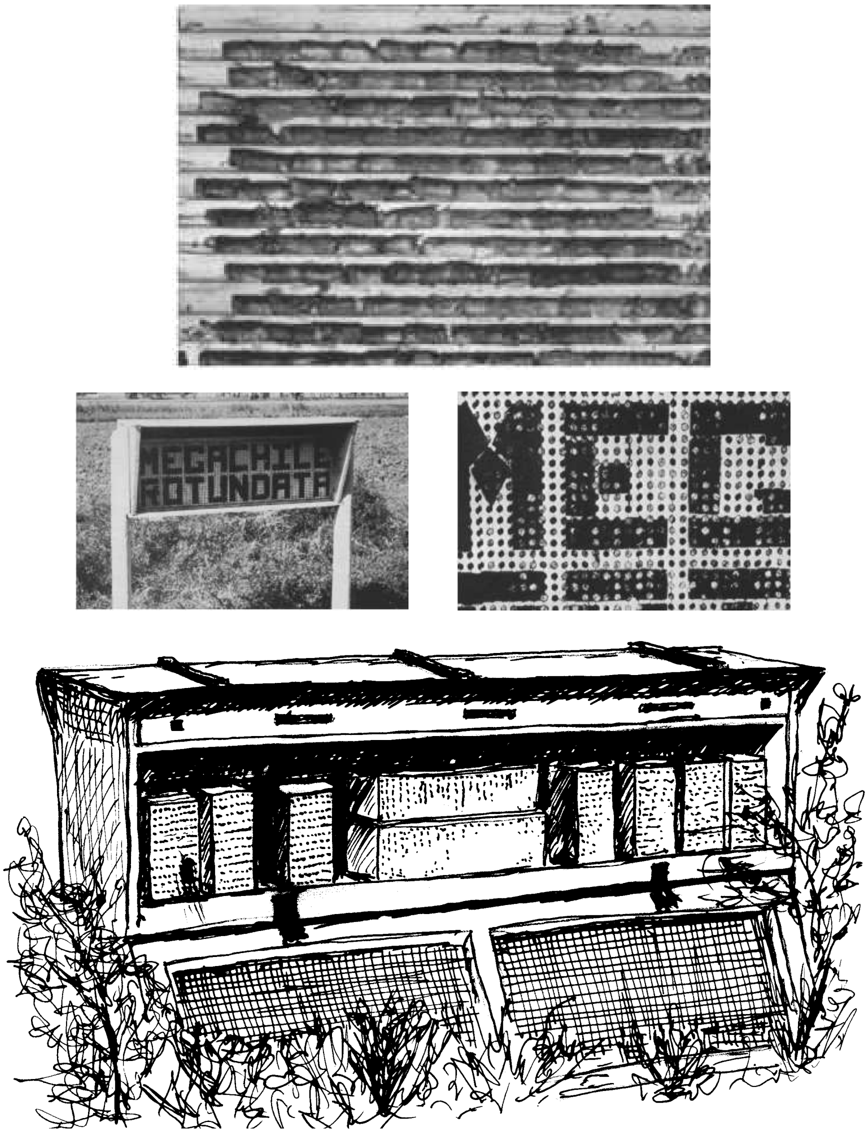

Figure 2.32: *Breeding help made of wood with long holes (top, opened and showing the numerous breeding cells). Many of these breeding helps are hosted in a shelter on the experimental field (center, the entrance has been brushed with 'MEGACHILE ROTUNDATA'). Huts (bottom) for hundreds of rearing tubes of the leafcutter bees are placed on large alfalfa fields at which seed is harvested for the farmers who use the plant as food for animals. The bees eclose from the breeding cells and polinate the alfalfa flowers. In this way the farmers obtain more than ten times as many seeds as they wood without the bees. From* Free *(*1970*) (top and center) and after* Dorn and Weber *(*1988*) (bottom)*

adapt quickly enough to the unfavorable conditions and the animals would freeze to death. They must therefore use other signs of the environment which indicate that winter is coming. Most insects use for this purpose the daylength: In the fall the days become shorter and in the winter they are much shorter as they are in the summer. If a certain daylength is reached, as for instance 12 hours at the 21st of September, development is ceased in a certain stage until the winter has passed and the environmental conditions are favourable again. More about it in chapter 3.

In the case of the leafcutter bee it is, however, not the daylength which is used for triggering diapause. It would anyway be worthless, since the larvae are inside the breeding tubes and surrounded by several layers of leaves which are additionaly in a stalk, an empty snail shell or another shelter. Although the larvae live in cockaigne, they are in the dark. They are therefore not able to recognize daylength.

But they know what to do. If in the fall the nights become longer, it is also for a longer period of the day colder. If the animals would instead of the length of the dark period measure the length of the cold period of the day, they would have a kind of annual calendar at their disposal and would realize, that is is fall and the winter approaching. This ability to recognize the season with the help of the length of the daily temperature has been called *thermoperiodism* (figure 2.33). Other insects, which use the daylength (or length of the night) as a calendar, show *photoperiodism*.

If the breeding chambers are kept in the refrigerator at 7^0C, diapause can last up to two years. Only after several days of temperature beyond 17^0C suffices to brake diapause. The prepupae transmute in a bee and leave the winter quarter trying to find

Figure 2.33: *Thermoperiodism is used by the leafcutter bees as an annual calendar for winter rest. If the length of low temperature (usually the night) exceeds a certain number of hours per day, the animals in the third larval stage develop only to the prepupa. In this stage they hibernate (they enter* diapause*). These prepupae can be seen in the two opened breeding cells. Not before spring, when the temperatures exceed 17°C for several days, this resting stage is terminated. The animals pupate and metamorphose into adult bees*

a partner, to mate and to start a new generation of leafcutter bees.

3 Diapause: How insects hibernate

Surely you have been concerned about the fate of insects during the winter. Butterflies, flies, beetles, grasshoppers... It is a difficult time in our latitudes, with coldness and lack of food. However, since in the spring of each year these insects show up again, they must have survived somehow the winter. How these animals do it and what happens in detail shall be demonstrated in a few examples in this chater.

Wouldn't it be stunning if you could retreat somewhere in order to bridge bad times or bad mood or just to sleep for some time? And after some days, weeks or months *of 'time forgetting'* you would be fully back again? Many insects are able to do this and they use this ability to survive the winter or other unfavourable times.

3.1 How a midge fools the tricky pitcher plant

After having written my doctoral thesis in Tübingen I went for two years to Ann Arbor and worked there at the University of Michigan with Professor Shappirio (figure 3.1). He is zoologist and concerned with the development of insects. He introduced me to the pitcherplant midge *Metriocnemus knabi* and its strange biotope.

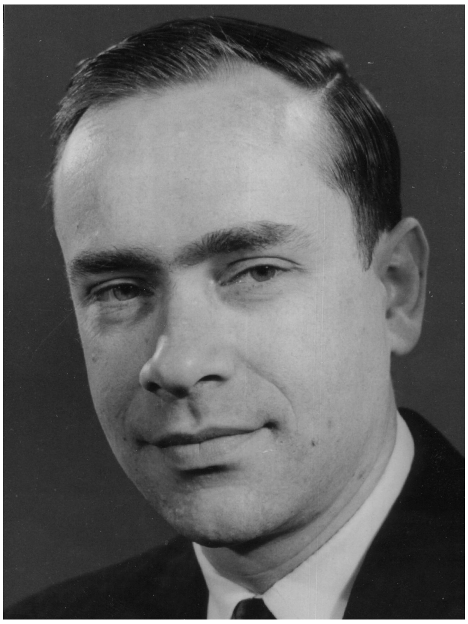

Figure 3.1: *David Shappirio, insect-physiologist at the University of Michigan in Ann Arbor (USA)*

Tramping through the bogs and swamps of Michigan (figure 3.2) one finds often plants with very peculiar leaves. They look like little pots and are called pitcher plants. The pitcher leaves are open at the top, but a kind of collar surrounds the opening. Looking into a pitcher leaf you will

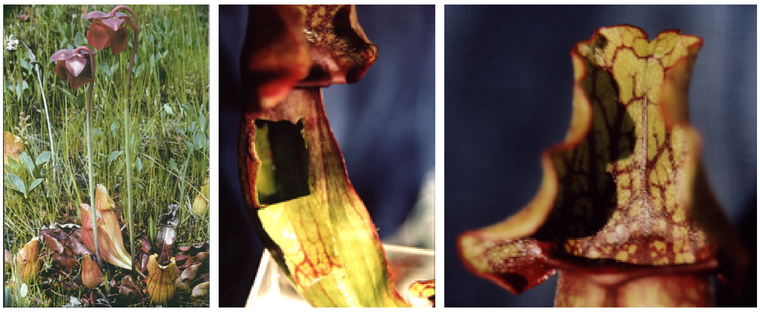

Figure 3.3: *Flowering pitcher plant* Sarracenia purpurea *with pitcher leaves (left). Center: Pitcher leaf in closeup, cut open laterally. Right: Opening of the pitcher leaf. Note the hairs directed downward in the interior of the entrance. Left photography from the home page of Dr. Bradshaw and Dr. Holzapfel in the Internet, with permission. reamining photographies made in the botanical garden of Tübingen*

Figure 3.2: *Bug close to Ann Arbor in Michigan, USA, habitat of the pitcher plant* Saracenia purpurea. *They and its pitcher plant-midges are studied by Dr. Holzapfel (left) and Dr. Bradshaw (right) for a long time. Both are at the University of Oregon. From the homepage in the Internet, with permission*

notice that it is partly filled with water (figure 3.3).

If the water of such a leaf is poured into a flat dish (figure 3.4), one understands the strange name of this plant: In the fluid are hundreds of drowned insects or parts of them. These insects landed on the lid of

Figure 3.4: *The content of a* Sarracenia-*leaf was poured into a dish. Numerous drowned or partly disintegrated animals can be seen. The larvae of the pitcher plant (*Metriocnemus knabi*) midge feed on it. - Image missing*

the pitcher plant leaf and, perhaps allured by the water in the leaf, had fallen into the pitcher. This is usually not fatal for most insects. They paddle to the frindge of the water and crawl out. However, this is not possible in the case of the pitcher plants. A tricky surface at the interior of the leaf makes the insects slip off. Each trial to escape the water in the pitcher ends with a splash in the mini-lake. At the end the insects have no power left and drown in the water.

If we cut a window in such a leaf and

look carefully at the inner surface, we understand, why the insects have no chance to escape this deadly pitcher: The upper layer (*epidermis*) consists of cells, the surface (*cuticula*) of which is arranged like tiles on a roof. Furthermore the cuticula is slippery. It is an ideal mechanism to prevent the insects and other small animals from escaping the pitcher after having fallen into them (figure 3.5).

Figure 3.5: Saracenia-*leaf with cut out window showing the fluid in the interior and the slippery walls. Insects slip off and have no chance to escape from the water in the leaf. Only the larvae of the pitcher plant-midge* Metriocnemus knabi *manage to live in this deadly pond and to feed on the dead bodies of insects*

The detective in us would like to know, why during the evolution such pitcher plants developed. Minerals, particularly nitrogen, are scarce in ponds and bogs. The reason is, that many mosses grow in this biotop, especially peat moss (*Sphagnum*). They grow on top of the older peat, which die because they do not get enough light. They are finally turning into peat. The upper mosses have no contact to the soil and the minerals and have to obtain these substances from the dead plants. A plant which is able to retrieve minerals and nitrogen from the dead insects has thus a huge advantage against other plants.

There is, however, an insect, which fools the pitcher plant. It is a Chironomid, a non-biting midge (*Chironomidae).* Their larvae live in the water of the pitcher leaves and feed on the drowned and dissolved animals. It is called pitcher plant midge *(Metriocnemus knabi*). Ils life cycle is descibed in figure 3.6. After about 4 weeks (at 23^0C) the fourth larval stage is terminated. The larva becomes a prepupa, crawls out of the water and produces with its salivary glands a jelly like cocoon, which adheres closely above the surface of the water to the wall of the leaf. In this jelly the prepupa lies like in a cradle. It transmutes into a pupa and after 2-3 days (at 23^0C) the midges eclose. The males and females are winged and thus able to fly out of the pitcher leaves and to mate. The females look for a new pitcher plant and oviposit a package consisting of many eggs on the surface of the water.

Like the leafcutter bee (see section 2.4) the picher plant midges have to enter a *diapause*-stage for hibernation (figure 3.7). From September onwards no prepupae are found anymore in Michigan (35^0 northern latitude). Instead, the animals stay in the last (fourth) larval stage in the water of the

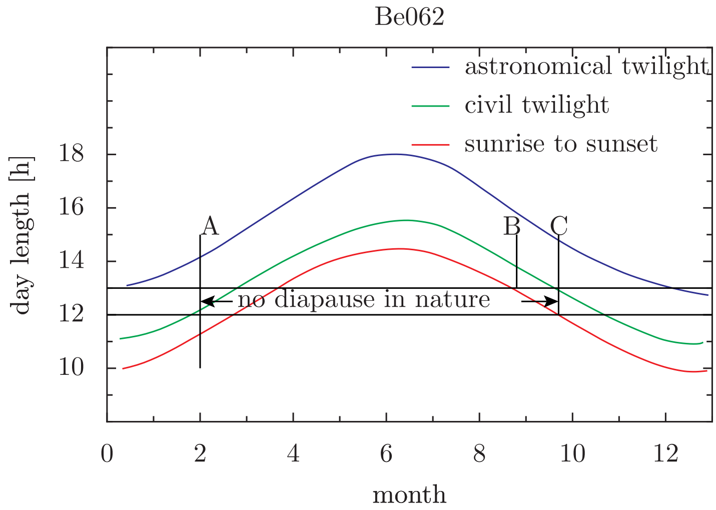

Figure 3.7: From *end of February/begin of March the first pupae of the pitcher plant midge* Metriocnemus knabi *can be found in nature (A with vertical marking). That is the time at which diapause is terminated. Pupae are found until middle of September (B with vertical marking). At the end of September (C with vertical marking) no pupae are found anymore. Between B and C lies thus the date at which diapause is induced. After Paris and Jenner (1959)*

pitcher leaves. The metabolism is reduced and the larvae become insensitive against frost. In the same way as we protect the water in the radiator of our cars against frost the insects accumulate glycerol in the body fluid. Now the water in the pitcher plant leaf can freeze completely without killing the larvae. In February/March diapause is terminated. The larvae crawl out of the water and pupate at the interior walls of the pitcher leaf as we know already from the animals during the summer. They do, however, not undergo diapause: The larvae pupate, the midges eclose and propagate without interruption of development as is the case in the fall.

In contrast to the leafcutter bees in these insects diapause is not induced by thermoperiodism (that is by the length of the cooler time of the day), but photoperiodically. The daylength is measured. As is known, daylength varies systematically in the temperate and higher latitudes during the course of the year. To understand this, we have to take a little side trip and realize, how day and night come about and why the days in the winter are shorter then during the summer.

During the course of a year the earth orbits the sun. Additionally the earth turns each day once around its axis. Since the axis of the earth is not perpendicular to the earth-sun-line, but inclined by 23°, the northern hemisphere receives on summer days longer light as on winter days. This is shown in figure 3.8. To realize this context, you should look at a globe in a dark room with one window only. If the axis of the globe is inclined towards the window (=sun; it is summer time at the northern hemisphere) and if you turn the globe slowly and uniformly around the axis, a city

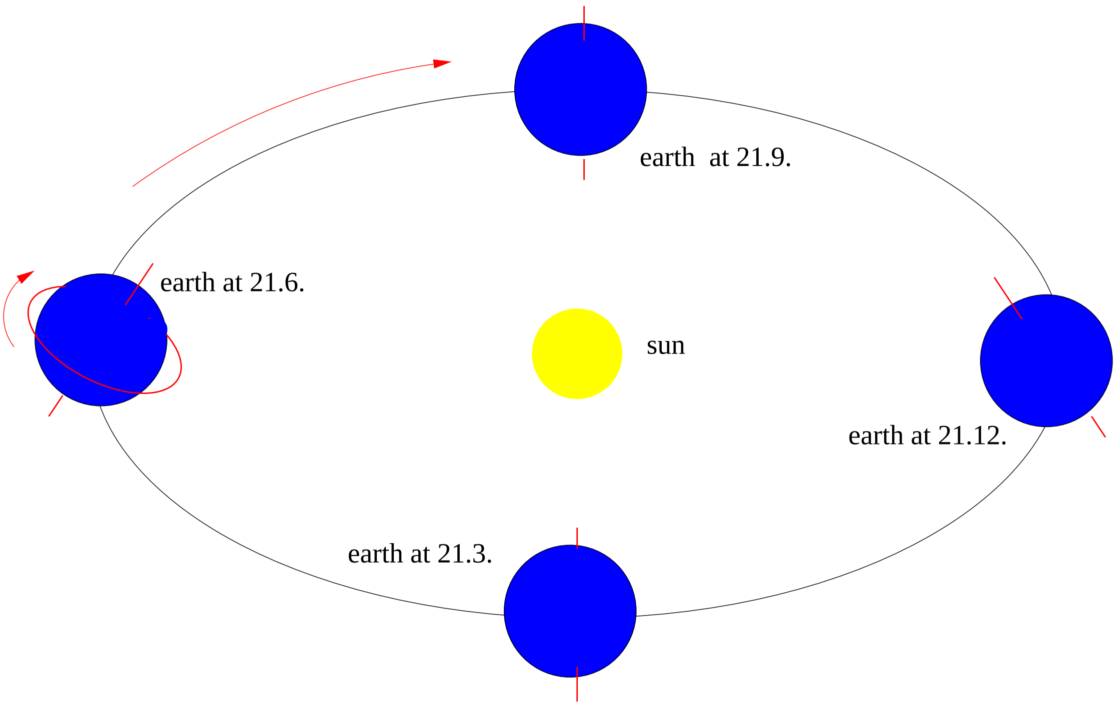

Figure 3.8: During the course of a year the globus orbits the sun once. Additionally the earth turns each day once around its axis. Since the axis of the earth is inclined by 23°, the northern hemisphere receives on summer days (July 21, left) longer light as on winter days (December 21, right). The same is true for the southern hemisphere, but there the summer occurs during the months at which winter occurs in the northern hemisphere. At the time of equinoxes (March 21, front, and September 21, back) day and night have the same length. Of course, the earth is in reality much smaller and the distance to the sun much larger as shown here

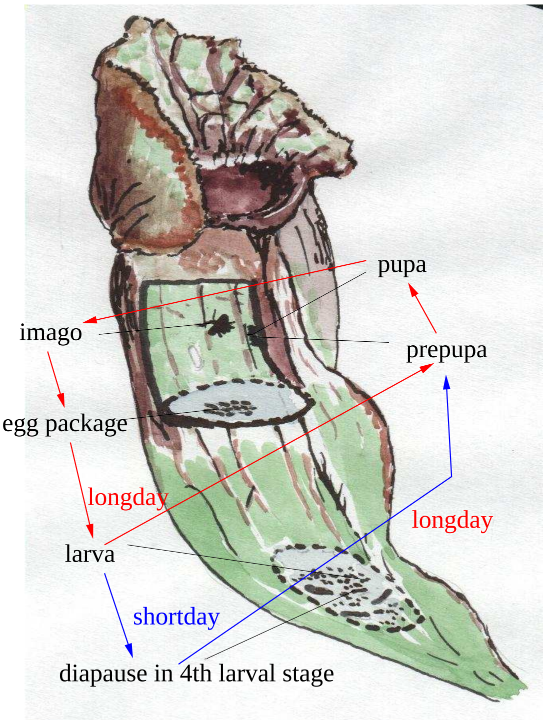

Figure 3.6: *The* larvae *of the pitcher plant midge* Metriocnemus knabi *live in water of the pitcher* leaves of Saracenia purpurea. *They feed on the drowned insects and moult four times.* Under longday *(summer) the larvae of the last larval stage crawl up the inner wall of the pitcher leaf and produce a jelly as a kind of cradle. These prepupae metamorphose in 2-3 days (at* $23^0 C$*) into* pupae *and afterward into an adult, winged, legged* imago. *It ecloses from the jelly cocoon, flies out of the pitcher plant leaf and mates. The females deposit an* egg package *on the surface of the water in a new pitcher leaf. Under* shortday *the larvae stay in the fourth stage in the water and enter* diapause *for hibernation (whereby they might totally freeze). Not before* longdays *occur they crawl out of the water, pupate and transmute* into an imago. *The development mit diapause is shown blue. After Paris and Jenner (1959)*

in Europe, for example Stockholm in Sweden, will be seen for a longer time in the light of the window (the sun) as compared to a place at the southern hemisphere, for example Fireland at the southern tip of South America. If, however, the axis of the globe is pointing away from the window (=sun; it is winter time at the northern hemisphere), Stockholm will be seen for a shorter time in the light of the window (= sun) as compared to a place at the southern hemisphere. In the summer the length of the light period is longer as in the winter, and this is the case also on the southern hemisphere. But there the summer is in the months, in which on the northern hemisphere winter prevails. The further to the poles at the northern or southern hemisphere the locations are, the more pronounced are these differences. Figure 3.9 shows, that the summer days in Stockholm are longer as the summer days in Tübingen.

Now back to the Chironomids. If the daylength shortend in the fall and reaches 13.5 hours (figure 3.10, Paris and Jenner (1959)), diapause is induced. In the fall, winter and spring we have shortdays, in the summer longdays. 10 to 14 shortdays are necessary to induce diapause in the animals. It does not matter whether the temperature of the water in the pitcher leaves is high or low. Decisive is only the daylength. The light conditions of the environment can vary a lot. But again its only the length of the day which is important. Even a light intensity of 0.00025 Lux (the fullmoon would be dazzling bright in comparision: its intensity is at clear days up to 0.5 lux) is still received by the larvae and induces diapause, once the shortdays have begun. This is probably an adaptation to the very low light conditions in the interior of the pitcher leaves, which are additionally covered by a leaf lid and surrounded by peat and other plants. Diapause is found also in other pitcher plant-midges. They were studied by Bradshaw and his coworkers (Bradshaw (1972), Bradshaw and Lounibos (1972), Hard et al. (1993)).

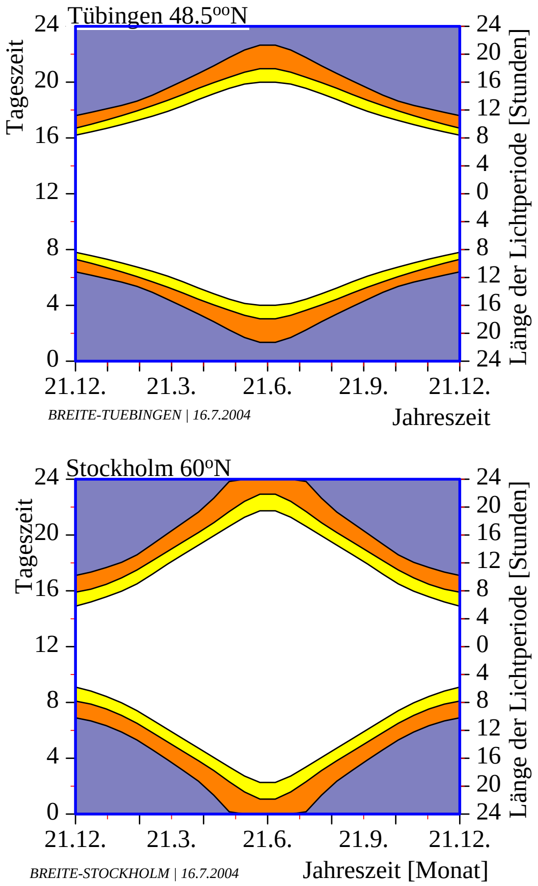

Figure 3.9: *The further polward a place, the longer are the days there in summer and the shorter in winter. Tübingen (top) lies at the 48.5th latitude, Stockholm (bottom) at the 60th. The white space inside represents daylength (indicated at the right) at various times of the year (on the horizontal axis) for the time between sun rise and sun set. Before sun rise and after sun set it is, however, pretty bright. Therefore the 'civil twilight' is additionally included in yellow and the astronomic twilight in red. At civil twilight the sun is 3°, at the astronomic twilight 9° below the horizon*

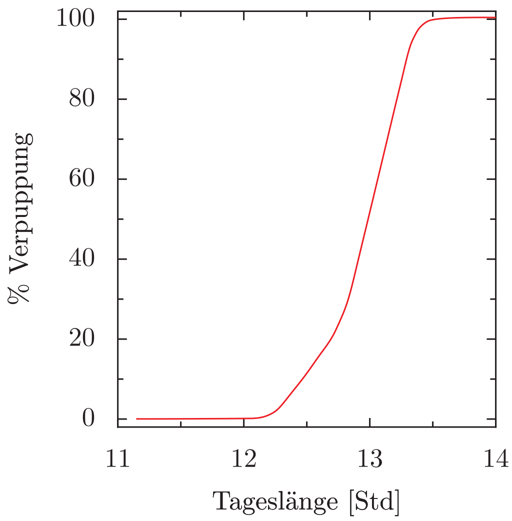

Figure 3.10: *Photoperiodic reaction in* Metriocnemus*: It was studied in the laboratory, how the pupa formation (that is, no diapause) depends on daylength. After 40 days of 12 hours light period all animals were still in diapause, whereas at 13.5 hours all animals had pupated (diapause broken). The critical daylength lies thus between 12 and 13.5 hours. After Paris and Jenner (1959)*

3.2 When the potatoes go in the cellar, the Colorado beetle goes in the soil

Beetle represent the largest Order of insects and of all animals generally. There are at least half a million species. In only 10% the larval stages and the way of life are known. They are spread all over the earth and found on every continent. Even in the water, on glaciers, in caves and desserts they make a living.

The Colorado beetle *(Leptinotarsa decemlineata)* is easily recognized at its 10 black lateral stripes (figure 3.11). About 120 years ago it began to feed on potatoes. Before it fed on other nightshade plants. Even today the Colorado beetles do not eat many wild potatoes. The reason is, that they contain a poisonous substance, *demissin*, which deterres the animals. In cultured potatoes this poison is absent or present in low concentrations only. Therefore our potatoes are attacked by the Colorado beetle. If the Colorado beetle protected wild potatoe *Solanum demissum* is crossed with our cultivar *Solanum tuberosum*, the bastard is partly resistent against the Colorado beetle.

What is the life cycle of the Colorado beetle? The females lay eggs in the spring, out of which new generation develops. The larvae and beetles eat potato leaves. If the days become shorter in the late summer and early fall (shortday), the beetles stop feeding, their gonads regress and they are unable to reproduce. Finally they crawl into the soil and begin the diapause. The animals respire poorly. Reserve substances such as fat and glycogen[1] are accumulated in the body to a larger amount as normally.

After hibernation the Colorado beetle comes back to the surface of the soil and searches for food plants. Feeding, growing, reproduction and diapause are synchronized with the development of the food plants.

Figure 3.11: *Colorado beetle* (Leptinotarsa decemlineata*, very top*) *and larvae eat leaves of a potato plants*

Because the Colorado beetle leads to much dammage in potato fields, its way of life is intensively studied. It was alo tried to find out what happens during diapause. To understand it we have to look at the head and the brain of the Colorado beetle in more detail (figure 3.12), because the brain controls the diapause. At the outside of the head capsule [2] the eyes, antenna and the mouth tools are apparent. The interior of the head is shown in the right part of

[1] eine Art Stärke

[2] remind you: Insects do not possess a skeleton made of bones as in our body but an outer skeleton, which surrounds the body like an armour. The head too is protected by a helmet-like capsule out of chitin

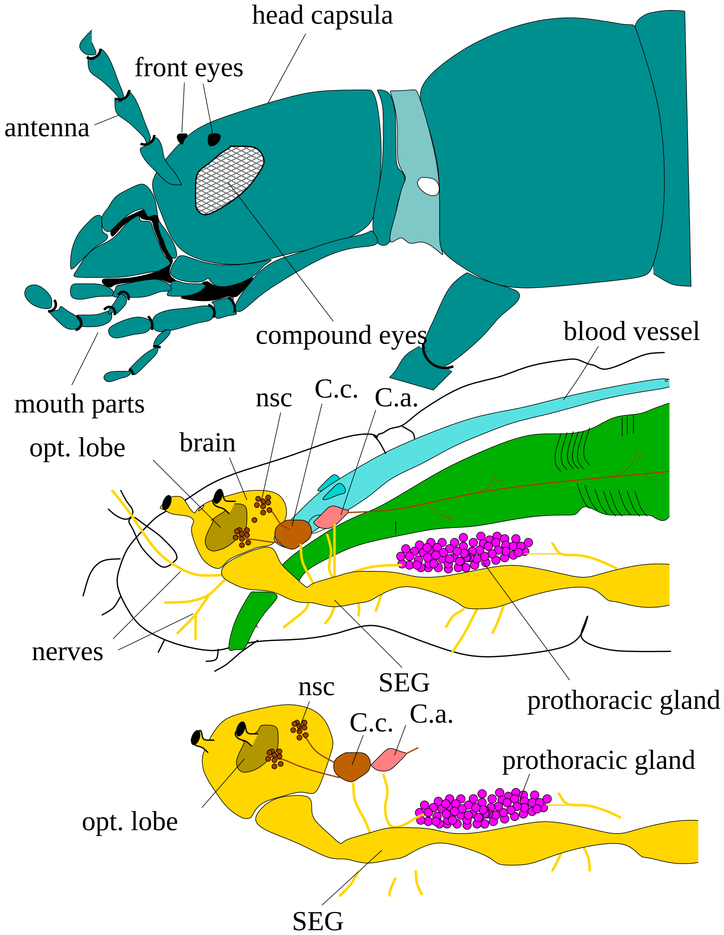

Figure 3.12: *Lateral view at (top) and* in *the head (centre) of a Colorado beetle: Compound eye consisting of many small individual ommatidia. On top of it two of the three forehead eyes (Einzelaugen). In front left antenna with members. The antenna touches, feels, smells the surrounding and measures its temperature. In front of the head the complicated mouth parts. In the head capsule (center) the brain (marked yellow) with nerves (yellow) leading to the antenna, the forefront eyes and to other parts. Lateral of the brain the optic lobes (darker yellow) with nerves from the compound eyes. They transmit the images of the eyes to the brain. Two branches of the brain (yellow) pass laterally the esophagus (green) and go to the subesophagal ganglion (SEG), from where the nervous system runs to the thorax and the abdomen. In the brain groups of larger cells (neurosecretory cells nsZ, brown), which are not only neurons, but produce also hormones. Some appendices of the brain play a decisive role during the development of the larva to the adult animal: Behind the central brain and on top of the gut* the Corpora cardiaca (*light brown) and* the Corpora allata (*reddish brown). In addition the* prothoracic glands *(PTD, magenta) in the thorax. The open blood system is shown in bright blue. Lower part: Most important parts in and at the brain which are important for the development, the moult and the metamorphosis of the larva into the adult insect. Here it is also taken care of, that the animals stop developing when diapause has started (see figure 3.14)*

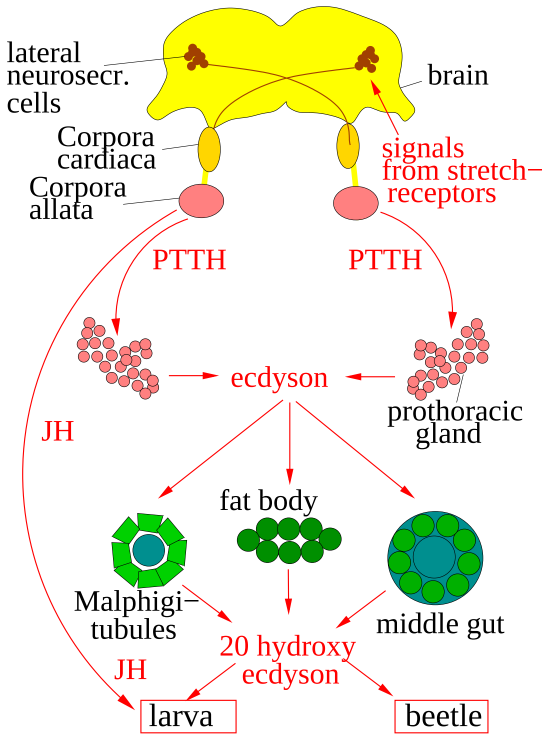

Figure 3.13: *Hormones control the development of the Colorado beetle: If the larva have eaten so much, that nothing fits any more in their chitinous armour, it is signalled by stretch receptors at the anterior gut to the neurosecretory cells nsC in the brain. The nsC than produce* the prothoracotropic hormone *PTTH. It stimulates the prothoracic glands to produce the eclosion hormone* ecdyson. *For this purpose the PTTH is transported via the neurons of the group of the left nsC and via the right* Corpus cardiacum *Cc to the* right Corpus allatum *Ca and there secreted to the hemolymph. The PTTH of the right nsC is transported to the* left C*a. Thus the nerves cross each other. Via the hemolymph the PTTH reaches the prothoracic gland. It induces the synthesis of ecdyson from cholesterine, which is transported via the hemolymphe to the fat body, the central gut and the* Malphigian tubulues *(kind of kidney) where it is converted to a much more effective hormone, the 20-hydroxy-ecdyson. It induces in the epithelium cells the moult. Whether a larva moults to a larger larva (left) or via a pupa to a beetle (right) is determined* by the juvenile hormone *JH*, which is made in the Corpora allata *and secreted into the hemolymphe. The larva will in this case moult into a new larva. If JH is lacking, the larva transforms into a pupa and an adult animal*

figure 3.12. This seems to be quite complicated. However, in order to understand how the Colorado beetle develops and how development is interrupted during diapause it suffices to concentrate on a few parts of the brain and its appendices. This is done in figure 3.13 for development and in figure 3.14 for the diapause.

In the females beetle the egg production is stimulated by the juvenile hormone. With the fall approaching and the leaves of the potatoes turning brown the time for the Colorado beetle has come. The egg production is terminated and the animals crawl into the earth for hibermation.

What is now going on in the brain and with the various hormones (figure 3.14)? As is the case in the pitcher plants-midges, the approaching winter is signalled to the Colorado beetle by the days getting shorter. The neurosecretory cells in the brain send a signal to the *Corpora allata* to terminate the production of juvenile hormone. Without juvenile hormone the ovaries do not produce eggs, although the ecdyson-production is normal. The animals stop reproducing. At the same time the behaviour of the animals changes under shortday conditions. The beetles stop feeding and crawl into the soil. The diapause begins.

That is is indeed the juvenile hormone which is needed for egg production and that without the hormone diapause begins was experimentally shown. If the head capsule of a Colorado beetle is opened and *the Corpora allata* removed under the binocular microscope, the animals begin to diapause even under longdays, that is, summer conditions. If the *Corpora allata* are implanted again, diapause is terminated and the animals behave as in the summer.

Figure 3.15 shows, at which daylength the diapause is induced. At the x-axis of the diagram the daylength was plotted, at the y-axis the percentage of animals in di-

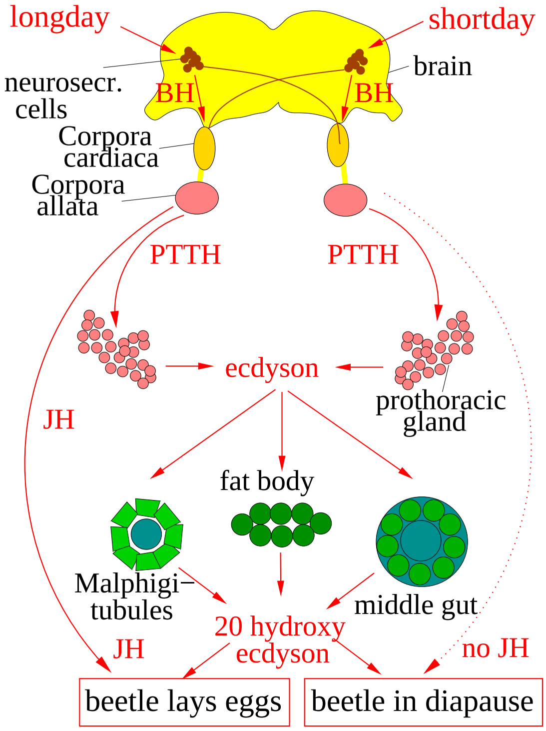

Figure 3.14: Hormones control the diapause of the Colorado beetle: In female beetles during the summer (longday, left side) the egg production is stimulated by the juvenile hormone (the brain-hormone BH stimulates the JH-production). In the fall the egg production is terminated and the animals crawl into the soil where they hibernate. Under shortday (right side) the neurosecretory cells in the brain send a signal to the *Corpora allata* to stop juvenile hormone production. Without juvenile hormone the ovaries do not produce eggs, although the ecdyson-production is normal. At the same time the behaviour of the animals changes under shortday conditions. The beetles stop feeding and crawl into the soil. The diapause begins.

apause. The critical daylength, at which about half of the animals enter diapause, is around 16 hours.

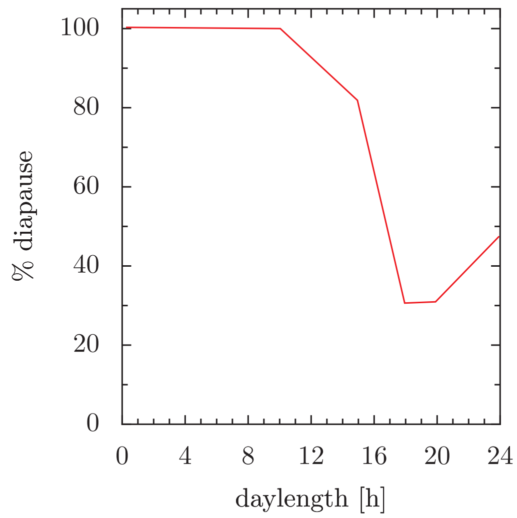

Figure 3.15: Shortday induces diapause in the adult stage of the Colorado beetle. The critical daylength lies at a light period of about 16 hours. Under shortdays, that is fall, neurosecretory cells in the brain inhibit the *Corpora allata*. The inactive *Corpora allata* do not make any juvenile hormone and therefore the ovaries can not produce eggs anymore. The animals can not reproduce and diapause begins. After Wilde et al. (1959)

In measuring daylength by insects or other animals and plants there is another problem: In the experiments, in which it was tested, how many of the animals enter diapause at various light periods (for example in figure 3.15), a timer was used which switches light on and off. In nature, however, the day begins with dawn and ends with dusk. The light is quite weak first and reaches higher intensities after a certain time only. But at the time of twilight (morning and evening) the differences are enormous, namely in one hour 100 000 lux. That is shown in figure 3.16. At about 100 Lux the changes per time are strongest.

When does for the Colorado beetle (and for other organisms, which react photoperiodically) the light period and when does it end? We saw already in figure 3.9, that the light periods of the day differ, depending on whether the time between sunrise and sunset or the civil twilight or even the astronomic twilight is taken into account. In the experiments mit Colorado beetles the daylength in the laboratory was first varied by switching artificial light on and off by using a timer. This led to the curve of the photoperiodic reaction in figure 3.15. We do not know, however, how it is in nature, since for the animals the day could start with the begin of the astronomic twilight or with the begin of civil twilight. The curve would shift correspondingly. It was found, that in reality in many photoperiodic reactions in animals and plants the civil twilight is used as onset and end of the day. There is a simple reason for it:

The changes in light intensity in the morning and evening are marked in figure 3.16 by vertical lines. They correspond to a value of the curve, which belongs to one power of the light intensity. Where the vertical lines lie close together, the changes in brightness are especially pronounced. And this is just the range in which the civil twilight lies. If this brightness is used as onset or end of light, the time point can be determined very precisely, because the changes are so steep. Daylength can therefore in this range of light intensity be determined very accurately, even if the sky should be clouded, or on another day clear wheather should prevail.

Besides the Colorado beetle there are a number of other vermints known in agriculture which possess diapause. One of them is the boll worm *Pectinophora gossyp-*

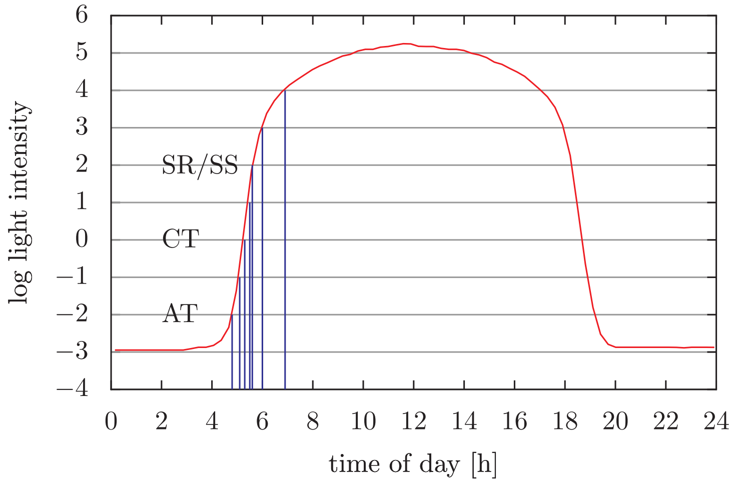

Figure 3.16: *The light intensity changes during the course of a day from about 100 lux at sun rise to 100 000 lux at noon (close to the equator even 500 000 lux), declines afterward in the same way as it increased, until it reaches in a night without moon 0.001 Lux. The y-axis is plotted logarithmically, that is, between two dashes of the scale a whole power is given (for example from 0 to 100 to 1000 to 10000 upward, or from 0 to 0,01, 0.0001 downward. If we would have used a normal scale, the differences would be even more dramatic. Indicated are also the intervals from sunrise to sunset, the daylength including the civil twilight and the daylength including the astronomic twilight. Lower figure: In the range of the civil twilight light intensity changes are strongest between 10 and 100 Lux. That is shown by the blue lines which are plotted at one power distances at the y-axis. The values were recorded on April 2, 1966 in Tübingen* ($48^0 32' N$, $9^0 3.5' O$) *at clear weather and new moon. After Erkert (1969)*

iella, a vermint of cotton plants, the corn borer *Ostrinia nubilalis*, and the Pine Lappet Moth *Dendrolimus pini*, which damages pine trees (figure 3.17).

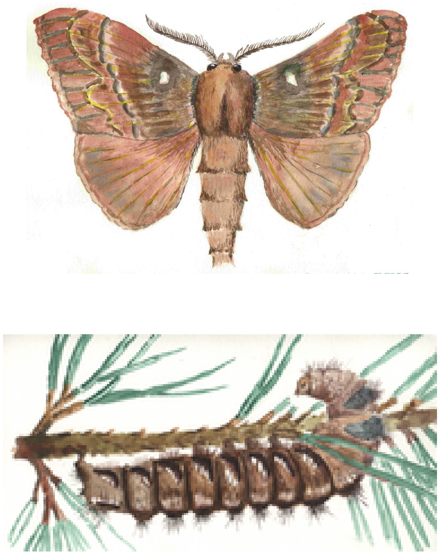

Figure 3.17: *The Pine Lappet Moth* Dendrolimus pini *is a vermint in the woods. The caterpillars (bottom) feed on pine needles. Water colour of the author after an image in Novak et al. (1982)*

3.3 How the silkmoth babies survive the winter

As a final example for diapause the Chinese silk moth *Bombyx mori* is presented. It belongs to the insect order of butterflies (*Lepidopteren)* and there to the family of true spinner (*Bombycidae*). It is native to the tropical and and subtropical regions especially of Asia. In China the silk with which the oldest larva spins its pupal cocoon, was used for producing cloths. The cocoons are thrown in hot water. This solves the adhesive and the silk thread can be spooled off.

The life cycle of the animals and the photoperiodic control of diapause are shown in figure 3.18. In *Bombyx mori* the diapause occurs in the egg stage. In contrast to many other insects with diapause the silkmoths are longday animals: The females lay eggs in the spring, that is in shortdays. They develop without diapause. Females in the summer, that is in longdays, however, lay diapause-eggs (Meenal et al. (1994)). The photoperiodic signal *longday* is percieved by the mother. Diapause begins in the middle of the embryo development (*blastokinese*), when the egg in the egg shell has developed to a kind of baby larva. During diapause no cell division occurs. Development is holded until the environmental temperature has been below 5^0C for at least 14 days. The low temperature terminates diapause. If the temperatures become favorable again, the embryo ecloses from the egg. Different larval stages are passed until in the last stage a cocoon is spun. In it the larva metamorphoses to a butterfly.

Like in the Colorado beetle it was tried also in the silk moth to clarify, how diapause is brought about.

The first step is to recognize daylength. This is done by light-sensitive cell groups in the brain. To perceive enough light in the pupal cocoon, the head capsule of the pupa of the silk moth contains a translucent triangle. It is situated on top of the light-sensitive cells (Bounhiol and Moulinier (1965)). Longday induces in *Bombyx mori* females a signal in the neurosecretory cells of the brain. From there it is tranferred via nerves to the subesophageal ganglion. There a diapause-hormone is produced and secreted (figure 3.19). Via

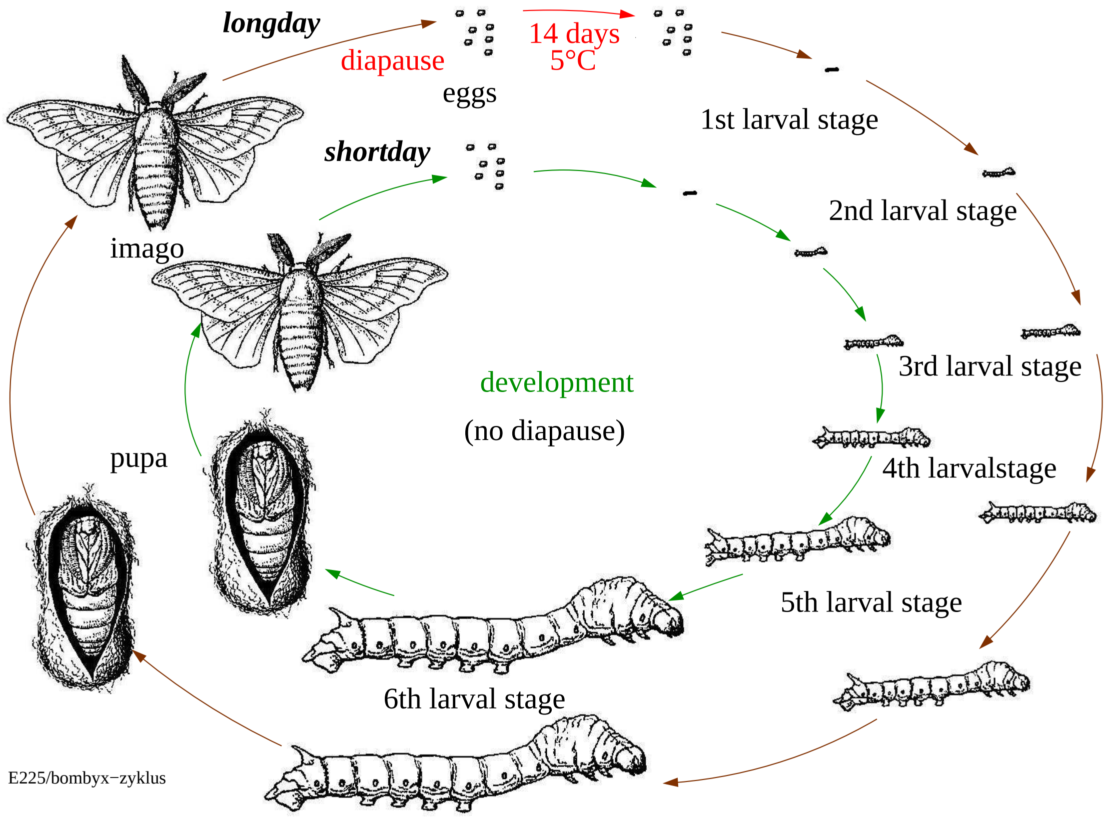

Figure 3.18: *The females of the silk moth lay their eggs* under shortday *conditions and they develop without diapause (internal circle in the figure). Out of the eggs eclose larvae, which moult four times. After the last moult the animals metamorphose in a cocoon spun out of silk. Under* longday *females* develop, *which produce diapause-eggs (outer circle in the figure). They survive the winter. Diapause occurs in a certain embryo stage. For the embryo to develop, they have to be chilled for at least twelf to fourteen days to* 5^0 *C or lower. This sets in motion a kind of 'alarm clock' which terminates at higher temperatures diapause. The animals develop via different larval stages and the pupal stage to the silkmoth. After Isobe and Goto (1980)*

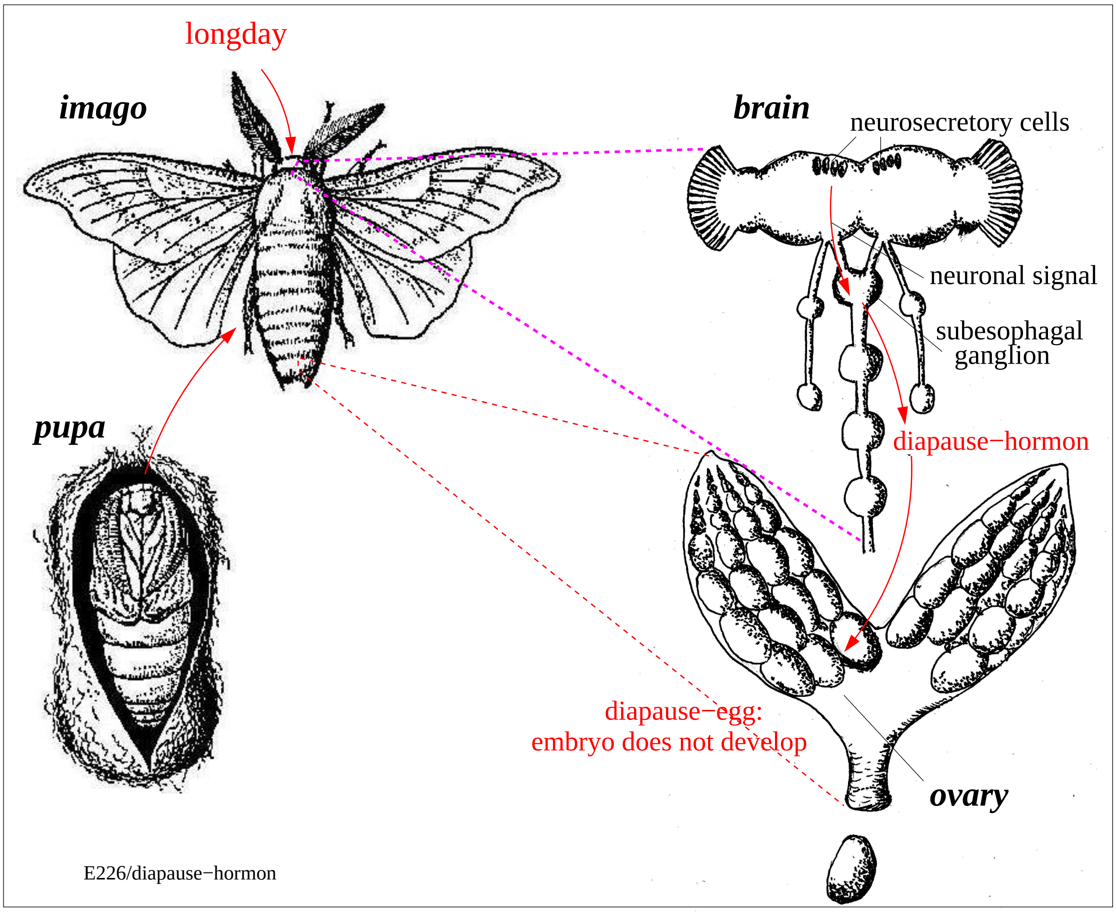

Figure 3.19: *Longdays during the summer induce females of the silk moth (*Bombyx mori*) to lay diapause eggs: A signal is transmitted from the neurosecretory cells in the brain via nerves to cells in the subesophageal ganglion, which produce diapause hormone. It is secreted into the hemolymph and reaches the ovary. There it holds embryo development in the egg (Isobe and Goto (1980))*

the hemolymph it reaches the ovaries and causes the embryos to diapause (Nakagaki et al. (1991)). The diapause-hormone is a substance consisting of 24 amino acids and is a neuropeptide[3]. Since its chemical structure is exactly known, it has been synthesized. If injected into an animal, it enters diapause even under conditions, where there is normally no diapause (shortdays). This shows clearly, that it is indeed a hormone which induces diapause.

The diapause-hormone is produced in twelf neurosecretory cells in the subesophageal ganglion of the pupae. The cells are arranged in three groups, lie at the lower side of the subesophageal ganglion and are connected with the *Corpus cardiacum*. A gene[4] takes care, that the diapause-hormone is expressed in the subesophageal ganglion only. In other tissues it is inactive. The diapause-hormone activates a gene, which is responsible for the production of trehalase. This enzyme is needed to make glycogen. Glycogen is a kind of chemical storage for animals, like starch is for plants. To allow the ovaries to develop, glycogen has to be *degraded*. If glycogen is *formed*, the ovaries are not able to develop and the animals stay in diapause.

The diapause of the embryos in the eggs is terminated, if a chilling period of twelf to forteen days of 5^0C or below is experienced (figure 3.20). This activates an enzyme, the esterase A4. During the winter such low temperatures are found in one row in the habitat of the silk moths. If the temperature in the spring is favorable again, the embryo in the egg continues to develop after the coldness in the winter. Cell divisions occur, the embryo grows and finally ecloses out of its egg shell.

With the onset of low temperature an enzyme, the esterase A4, is changed. If the temperature stays low, the enzyme is activated after twelf to fourteen days. A Japanese group found out, how the chilling period activates the enzyme. Esterase A4 is normally connected with a peptid called PIN. In this connection the esterase A4 is inactive. At low temperature, however, the connection between PIN and the esterase A4 is dissolved. This takes about 14 days at 5^0C 14. If the temperature increases, the esterase A4 changes its structure and it becomes active for a short while. Glycogen can now be dismantled and the embryos are able to continue development.

We are thus dealing with a molecular timer which is able to measure long periods (14 days) like a stopwatch. In the case of the diapause of the silk moth embryo the low temperature could set in motion the stop watch. Twelf to fourteen cold days are needed to free enough esterase A4 from PIN. The esterase would than become active at 25°C and allow the embryons to continue development.

3.4 Diapause is better than freezing to death

In the last three sections three examples for diapause in insects were presented, namely a Chironomid, the Colorado beetle and the silk moth. In all these cases the unfavourable time would be budgeted in their timetable for development. A sudden spell of frost in the fall would therefore not come as a fatal surprise, because they are already in the safe diapause stage.

Some insects must, however, use an other strategy. If the environmental conditions do not change in a predictable way the diapause-strategy does not work. In this case the animals have to react directly with an emergency program to the unfavourable conditions. In contrast to diapause this

[3] Proteins consist of amino acids. A peptide is a protein which -in contrast to proteins- consists of a few amino acids only. Neuropeptides are produced in nerve cells and are used like other neurotransmitters to send messaged from one nerve to another

[4] A gene is a region of genomic sequence and corresponds to a unit of inheritance

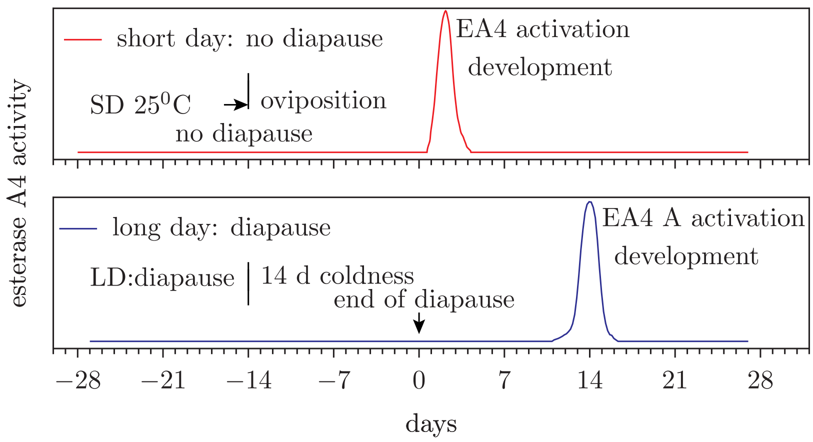

Figure 3.20: *Top: Under shortday conditions females of the silk moth lay eggs, the embryos of which develop without diapause. They need in a particular stage an active enzyme, esterase A4 (red curve, top). At* 25^0C *they develop via larval stages to the adult (arrow).*
Bottom: Under longday the esterase A4 is not active, because it is connected with the peptide PIN. Therefore the embryos are held in a particular stage and undergo diapause (lower curve, days -28 to -14). A chilling period of at least 5^0C *is necessary (lower curve, days -14 to 0), allowing the PIN-peptide to detach from the esterase A4. At a favorable temperature (for example 25°C) the esterase A4 is activated for a short time and diapause terminated. The embryos are not able to develop. Larvae eclose 14 days after the end of the low temperature period. This happens not only in the life animal, but also in the test tube (in vitro) containg PIN and the esterase A4 first chilled and later transferred to a higher temperature. The esterase EA4 is thus in combination with the PIN peptide a molecular timer and alarm clock which wakes up the embryo out of its diapause sleep. After Kai et al. (1995)*

is called *quiescence* (resting period). It is terminated as soon as the conditions become favorable again. This is important for those insects, which are overtaken by the unfavourable conditions in a developmental stage, in which they can not fly or run away. An example for quiescence is the chironomid *Polypedilum vanderplanki* (figure 3.21). The larvae in water puddles in indentations of rocks of strongly glared rocks in parts of West- and East-Africa (figure 3.22 top). During the dry period the water disappears and the larvae dry out completely (Hinton (1953)). During dessication the body and its metabolism is changed in such a way, that the animals are able to survive for many years. It was shown in the laboratory that they can endure all kinds of brutal treatments such as brief heating somewhat above 100^0C, liquid helium, one day in absolute alcohol, one week in glycerol. So much concerning quiescence. It begins not before the conditions become unfavourable, which is in the case of the african chironomid the loss of water from the rock pools.

In contrast to quiescence the *diapause* begins already before the environmental conditions become unfavourable. And the diapause ends often already before the environmental conditions are favorable again for some time (figure 3.22 bottom).

In areas of the temperate and higher latitudes winter is unfavourable for the development of insects. Many of them enter therefore winter diapause (figure 3.23). In other regions of the earth heat and draught is a problem. This applies for example for desserts. Here insects undergo often a diapause in the summer. The photoperiodic conditions leading to winter diapause are shortdays, those leading to summer diapause longdays (Saunders (1982)). Although there are quite a number of envi-

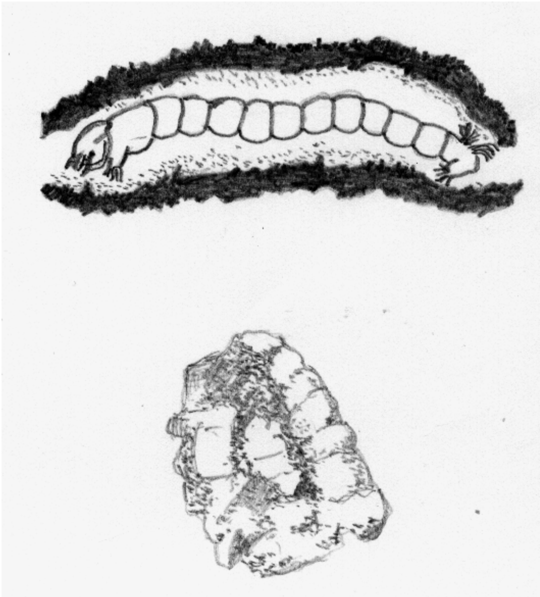

Figure 3.21: *The larvae of the african chironomid* Polypedilum vanderplanki *lives in tubes in water puddles in indentations of rocks. During the dry period the water dissapears completely and the larvae dry out almost completely (bottom). During dessication the body and its metabolism is changed in such a way, that the animals are able to survive for many years (*quiescence*). They can endure all kinds of brutal treatments such as brief heating somewhat above* $100^0 C$*, liquid helium, one day in absolute alcohol, one week in glycerol. After rain the larvae are reanimated in a very short time (top). Drawn by the author according to a sketch (top) and photography (bottom) of Takashi Okuda, Ibaraki (Japan)*

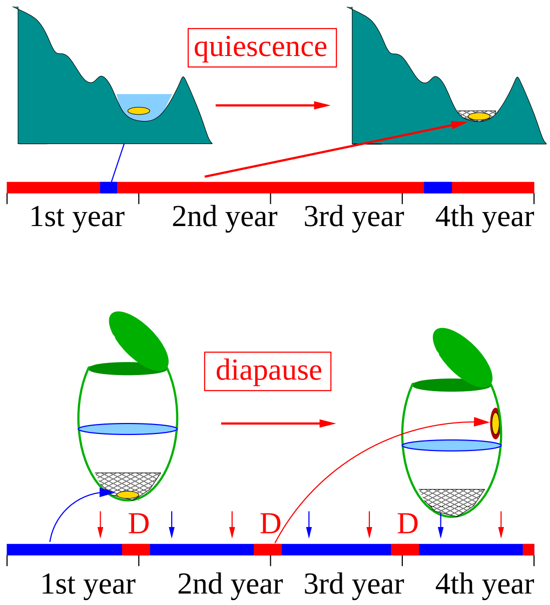

Figure 3.22: *Upper example:* Quiescence *in the African Chironomid larva* Polypedilum. *It lives in water-filled rockpools (left). If the water dries out, the larvae enter* quiescence *(right): Drought can be endured for a longer period of time without harm. In the case of* quiescence *the insects react directly to unfavourable conditions of the environment, which can not be predicted, because they occur eventually and irregularly and might stay unfavourable for a long time. The time axis shows twice rain in four years (blue), once in the fall, two years later in the spring.*
Lower example: Diapause in *pitcher plant midges. They live in the water of pitcher plant leaves. During the summer the animals develop without a resting period via larvae and pupae to an adult animal, which lays eggs again (left). In the fall (right) diapause is induced by environmental factors (days are getting shorter). Although the shortdays signal the unfavourable conditions (winter is coming soon), they themself are not directly harmful. The time axis shows the normal occurrance of winter (red line) in each of the four years and the regular onset of diapause in the fall (red arrow) and likewise the regulare end of it (blue arrow) in the spring of each year*

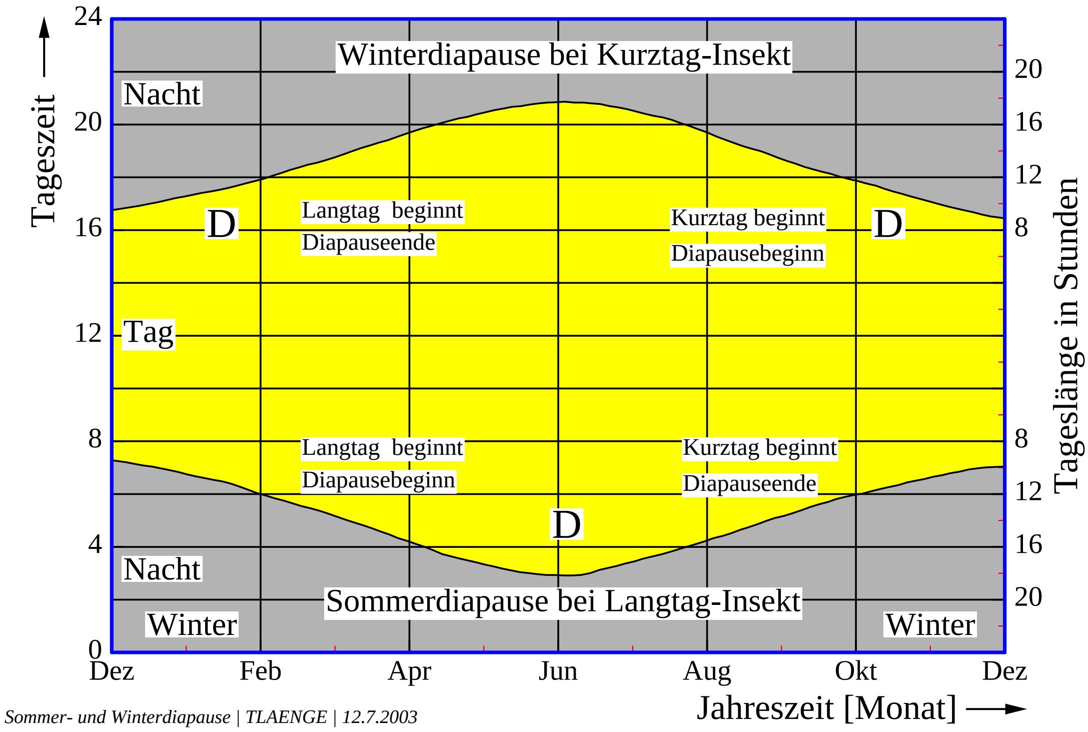

Figure 3.23: *In the winter many insects are not able to develop. Therefore they undergo a winter diapause (D). It begins in the fall and ends in spring. These insects are called longday insects, because they are active under longday conditions and develop (upper lettering). In some regions of the earth drought is, however, the limiting factor (lower lettering). This applies for example to desserts. Here summer diapause (D) is often found. It begins in the summer and ends in the fall, when the dry period finishs. Animals with summer diapause are called shortday animals, because they are active under shortday conditions (at the end and begin of the year) and develop*

ronmental factors such as humidity, temperature and quality of the food which signal the unfavourable season, but photoperiod is the most reliable and exact informant and is therefore used by many insects as an environmental factor. Already 10 to 15 minutes differences in daylength can decide whether diapause begins or whether development continues. Only in those cases where light is not able to penetrate to the insect as is the case with the leafcutter bees (see page 33), the duration of lower temperature of the day is used instead of the daylength.

What must an insect be able to do, to enter diapuse at the right time of the year? Let us put ourself for a moment in the situation of an insect. To recognize the time of the year we need a calendar. The length of the daily light period would be suitable, since it varies regularly with the season. This calendar is external, to start with. We must, however, recognize it. Since it is light, we need eyes for it. They signal in the morning, when light is bright enough (civil twilight, figure 3.9), the onset of the light period, and in the evening, when it is dark enough, the end of the light period. To find out. how long the light period is, we need also a clock. With its help we can recognize, whether the light period is short enough as to enter diapause.[5] We would, however, not restrict ourself to just *one* photoperiodically effective day. It is safer, to enter diapause, if we have seen and measured *several* shortdays in sequence. We need therefore also a counter, which adds up the days with shortdays. A switch has to be triggered, which stops development and starts diapause (figure 3.24).

All these devices, photoperiodic eyes (light receptors), a timer (circadian clock), a photoperiodic counter and a switch (hormonal system) are located in insects in the brain. Light receptors receive the photoperiodic signals and distinguish between light and darkness. The length of the night (daylength is seldom used) is determined by a timer which uses a circadian clock. Once the photoperiodic counter has added up the photoperiodically effective cycles and enough of them are encountered, the informations are signalled to a center. It works up the integrated information and controls via a photoperiodic switch the events at the target organs which are needed for the onset of diapause.

Diapause can occur in all stages of an insect, that is in the egg-, larval-, pupal- and adult stage (for the various stages of development of an insect see figure 2.31). It is, however, specific for each species. Each species contains a program in its genome which determines, in which stage diapause occurs. However, before that diapause has to be induced by the daylength (*photoperiodically*). In most cases the photoperiodically sensitive stage is before the stage, in which diapause occurs. If diapause occurs in the last larval stage, often the early larval stages are photoperiodically induced. In the silk moth *Bombyx mori* it is the egg and the first larval stage which is photoperiodically sensitive. In the giant silk moth *Philosamia cynthia* the larvae in the 4th and 5th stage are sensitive for shortday.

Only in a few cases the diapause is also photoperiodically *terminated*. This is the case in the giant silk moth *Antheraea pernyi* (figure 3.25). The diapause is in-

[5] At the critical daylength half of the population of animals is induced photoperiodically. In the case of the diapause induction of the corn borer it amounts to 14.2 hours (figure 3.26). At longer light periods less or none, at shorter more or all animals would enter diapause. The diapause of the corn borer is terminated, if the critical daylength reaches or surpasses 14.2 hours. It is thus the same length as in inducing diapause.

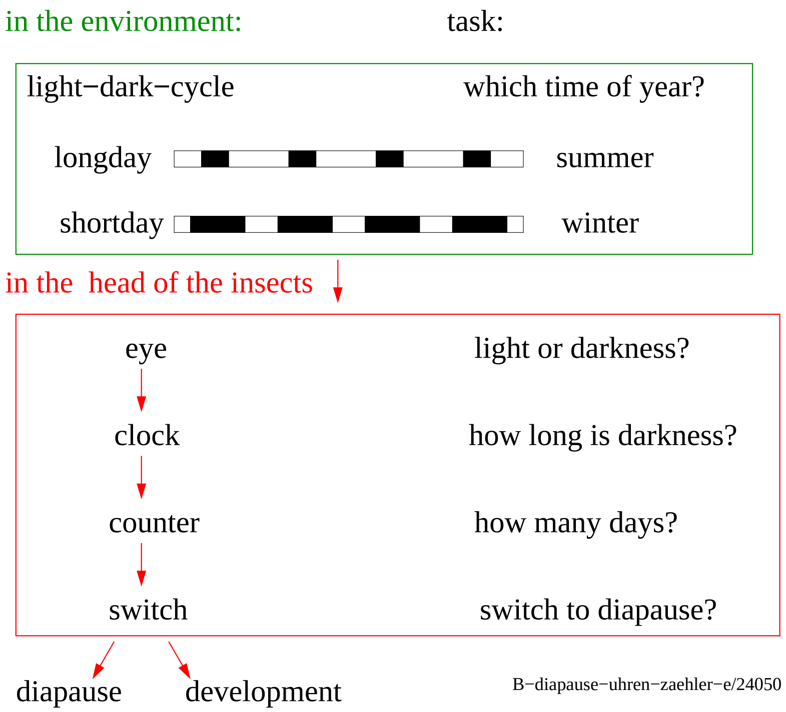

Figure 3.24: *To control diapause photoperiodically, the animals need: Eyes to recognize the length of the light period*, a clock *to measure the daylength, a photoperiodic* counter *which adds up the days with a daylength being short (in shortday animals) or long (in longday animals) to induce diapause, and a switch, which takes care of stopping development and inducing diapause. All these devices are in insects in the brain*

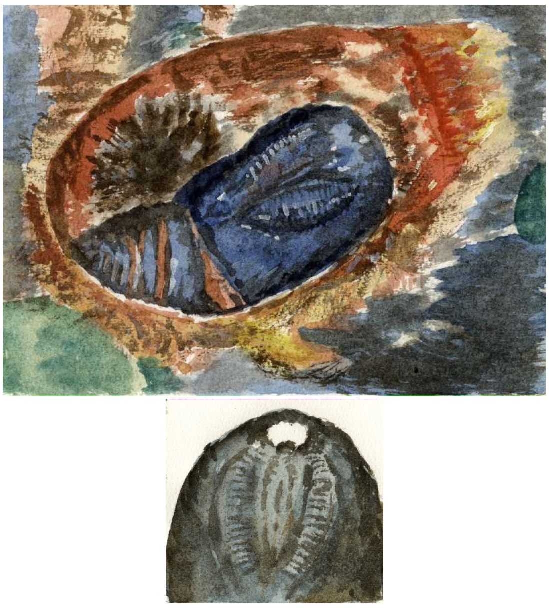

Figure 3.25: *Pupa of a giant silk moth* Antheraea pernyi *in the cocoon, which has been cut open here (top), with a window for light in the cuticle on top of the brain (bottom) and the last larval skin top left. The cuticle of the pupa is coloured dark and transmits only little light. The translucent window on top of the neurosecretory cells of the brain allows light to enter the brain*

duced *and* terminated at the same critical daylength (figure 3.26). Normally, however, other conditions such as for example a certain period of low temperatures (like in the silk moth, page 54) or internal processes are necessary to terminate diapause

During diapause the metabolism is low, the water content scarce and behaviour has changed. Neither sperms nor eggs are produced.

In insects, which need for their development from the egg to the adult animal a year or even several years (*univoltine* species), diapause occurs in each animal always in the same stage. This diapause is called *obligate*. In *multivoltinen* species with several generations per year the diapause is *facultative*: It occurs only in that generation, in which the external conditions induce diapause (for example shortday in the fall, figure 3.27). The other generations develop without diapause. In some insects such as *Bombyx mori* strains are known with obligatory diapause and others with facultative diapause (Isobe and Goto (1980)).

3.4.1 In regions of the earth with early winter diapause starts earlier

Unfavourable conditions begin in the various regions at different times. The closer they are to the poles, the earlier they start. Daylength is also varying. Therefore we find in varieties of a species from different latitudes divergences in the critical daylength, at which diapause is induced. In the various geographic varieties of the cutworm *Acronycta rumicis* (figure 3.28) the differences in the critical photoperiod are gradual. In the Cabbage Butterfly *Pieris brassicae*, however, only

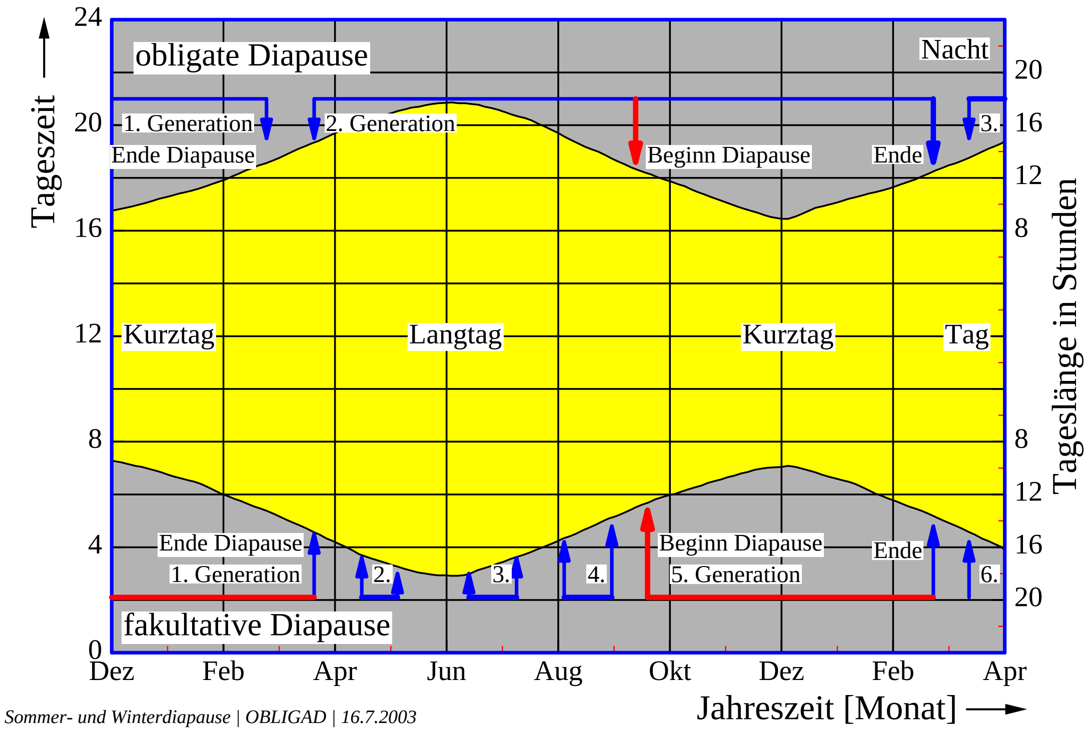

Figure 3.27: Obligate *diapause is found in one generation per year (or in several years) only in a certain developmental stage (upper part of the figure). They are called univoltine species. In the example shown the first generation (the parents) terminates diapause at the end of February (1st blue arrow). The eggs are deposited in March (2nd blue arrow). The second generation (children) develop slowly over the year and start to diapause in September (red arrow). It ends in February (3rd blue arrow. In multivoltine species with several generations per year (lower part of the figure) diapause* is facultativ*e: There are several generations per year (blue arrows) and diapause occurs only in the generation in which the external conditions induce diapause (for example shortday in the fall, red arrow, 5th generation). In the figure the length of the light period is shown yellow during the course of the year (day), the length of the dark period gray (night)*

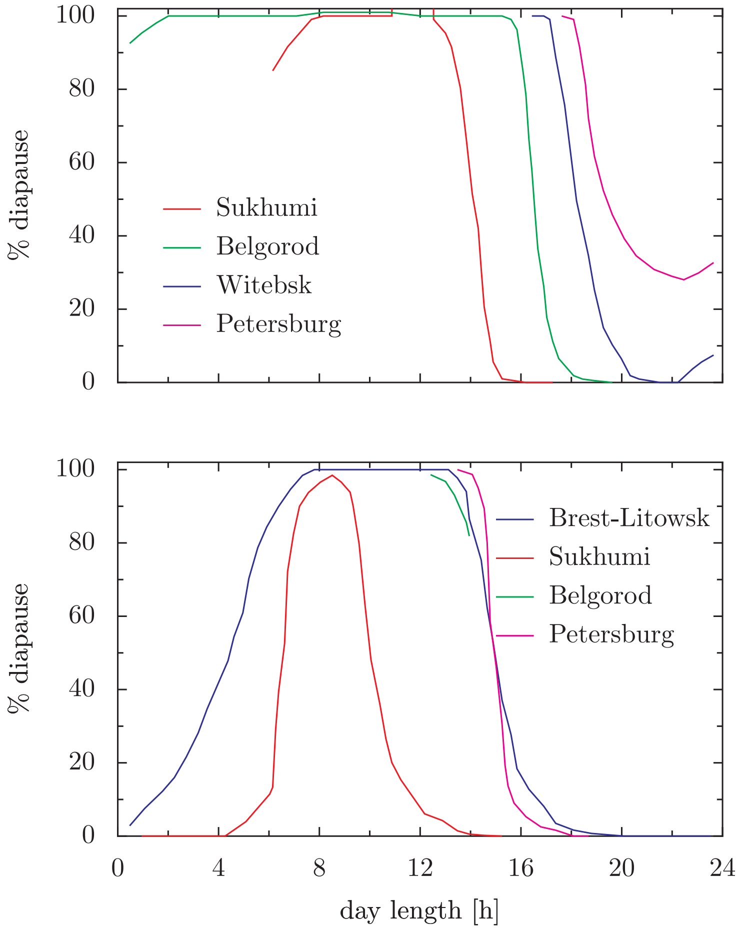

Figure 3.28: *Geographic varieties of the cutworm* Acronycta rumicis *(top, Petersburg light blue 60°N, Witebsk dark blue 55°N, Belgorod green 50°N, Sukhumi red 43°N) and of the Cabbage Butterfly* Pieris brassicae *(bottom, Petersburg light blue 60°N, Brest-Litowsk dark blue 52°N, Belgorod green 50°N, Sukhumi red 43°N) of various latitudes in Russia. Percent diapause as a function of the daylength. After Danilevskii (1965)*

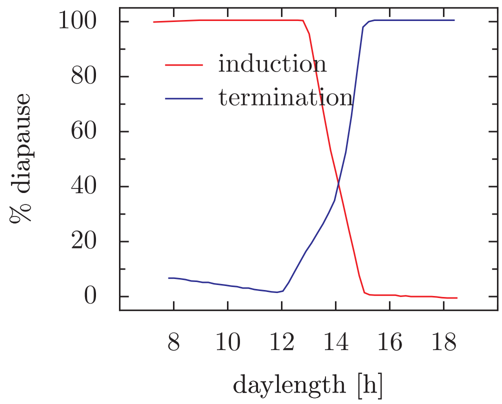

Figure 3.26: *The giant silkmoth* Antheraea pernyi *begins to diapause in the fall, when days shorten. This resting stage occurs in the pupal stage (see figure 3.25). The red curve shows, at which daylength diapause is induced. In days consisting of 16 hours of light and 8 hours of darkness (summer) no diapause occurs (red curve at 0%), in days with 12 hours light and 12 hours darkness (middle of September) diapause is induced in all animals (red curve at 100%). During spring with increasing light periods of the days the diapause of the animals is terminated (blue curve). At days with 14 hours of light and 10 hours of darkness the red and blue curve mirror each other. The critical daylength (at which about half of the animals react photoperiodically) for inducing diapause and terminating diapause is the same. Photoperiodically sensitive are the last larval stages and the pupal stage. After Saunders (2002), Williams and Adkisson (1964)*

two geographic varieties exist (figure 3.28, Danilevskii (1965)).

3.4.2 Diapause-eyes

To recognize the daylength, eyes or other light-sensitive devices (*light receptors*) are needed. The photoperiodic sensitivity of these devices begins mostly during the twilight at values between 10 and 100 lux. They do not react at weaker light. In this range the light intensity outdoors changes maximally (figure 3.16). The photoreceptors can, however, be protected from the light to different amounts. If the animals are for example enclosed at the time of photoperiodic sensitivity in a cocoon, weaker light suffices to stimulate them.

Possible photoperiodic light receptors are the compound eyes, the ommatidia (*ocelli*) and light-sensitive structures of the brain. Which of them are used in reality differs in the various insects possessing diapause (figure 3.29). Different colours of the light can act in different strength.

3.4.3 Diapause-clocks

Once an insect has received the photoperiodic stimulus, a clock has to measure the daylength. Depending on whether the daylength lies below or beyond a critical value, the insect will develop or enter the diapause stage.

If a scientist studies something quite complicated, he often uses modells. Or, with other words, he puts forward a hypothesis. A detective would likewise put forward hypotheses in trying to solve the case he is trying to solve. He will ponder, how the theft might have had occurred and who might be the thief. Using a model one can play through the situation (theft or diapause) and try to find out whether every-

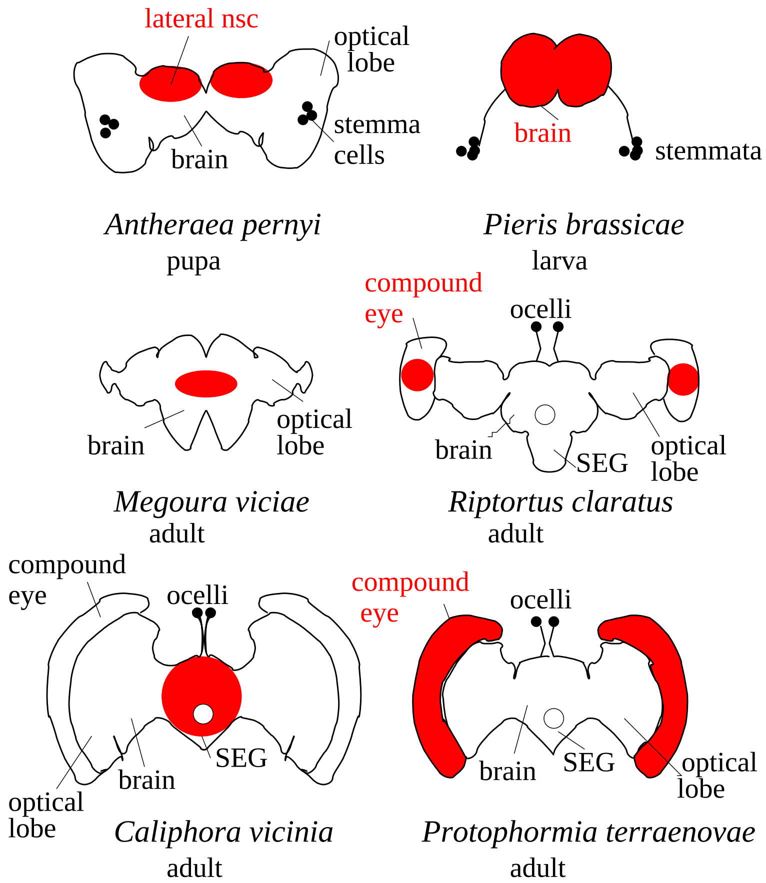

Figure 3.29: *Photoperiodic receptors were localized in the red marked areas of six arthropods (frontal view of the brain, the optic lobes and the eyes). Top left: Giant silkmoth* Antheraea pernyi. *Light receptors are the lateral neurosecretory cells (laterale nsC, red). Top right: Cabbage butterfly* Pieris brassicae. *Brain is sensitive. Center left: Aphid* Megoura viciae. *Brain sensitive.* Center right: Riptortus claratus. *Compound eyes for photoperiodically operating light sensitive. Bottom left: Fly* Caliphora vicinia *contains photoperiodic receptors in the central part of the brain. Bottom right: Fly* Protophormia terraenovae *reacts with compound eyes to photoperiodically effective light. After Numata et al. (1997)*

thing makes sense. If there are contradictions (the assumed thief was at the time of the theft not at the place), the modell has to be changed. A good detective as well as a good scientist will use not just one, but several hypotheses, because the case will be clarified faster this way.

For the clocks, which are used to measure the daylength in photoperiodically induced diapause various modells were proposed. One of it was put forward by Lewis and Saunders (1987). It is especially interesting, because it uses a clock in a feedback modell (figure 3.30) and takes at the same time care of the number of cycles needed to induce diapause. According to this modell the oscillation would damp out without light. With light the concentration of the substance X increases, wheras at the same time some X is lost. If the light is not constantly applied but for example 8 hours per day, the damped curve gets a push each day. If the push occurs at the right time (that depends on the length of the light period), the damped curve becomes an undamped one (upper blue curve). Now the curve is at all the days beyond the horizontal line. During this time X is produced and accumulates therefore. After a certain number of days sufficient X has accumulated to induce diapause (figure 3.31).

The factor X could be a substance which under photoperiodic treatment *promotes* the reaction actively which triggers the switch for diapause. It might, however, be as well a substance which, depending on the photoperiodic tretment, *inhibits* the reaction. Both cases where found in the diapause of various insects and in both cases neurosecretory cells are involved. Afterward a mechanism is effective which transforms the signals into reactions which lead finally to the diapause .

3.4.4 Diapause-counter

In some cases just one single photoperiodically effective day suffices to induce diapause as for example in *Chaoborus americanus* (Bradshaw (1969)). In most cases, however, several *inductive cycles* are necessary. How many cycles are needed does not depend on the environmental temperature. Thus, at 15°C and at 20°C the same number of cycles are required to induce diapause.

How the diapause-counter works can be seen nicely in the parasitic wasp (*Nasonia vitripennis*). This parasitic wasp deposits its eggs in the pupae of flies. They eclose and eat up the fly residing in the pupa. In the fall with its decreasing day lengths the mother deposits eggs which do not develop, but enter diapause. Since these parasitic wasps oviposit their eggs daily, one can collect them and observe, whether new parasitic wasps will develop or whether they stop developing because they have entered diapause. In this way one can determine, how many shortdays are required inorder to induce diapause in all animals (figure 3.32).

3.4.5 What happens before, during and after diapause in the insects?

If diapause does indeed protect the insects from the rigors of the weather, quite a number of processes have to occur in their body. The metabolism adapts to the unfavourable conditions in the environment. The cells of the body should not freeze, because they would be destroyed. Therefore insects secret substances such as glycerol and sorbitol as antifreeze in the hemolymph and protect it (the blood) from frost. The insects do thus the same as we do when we protect the cooling water of our car motors

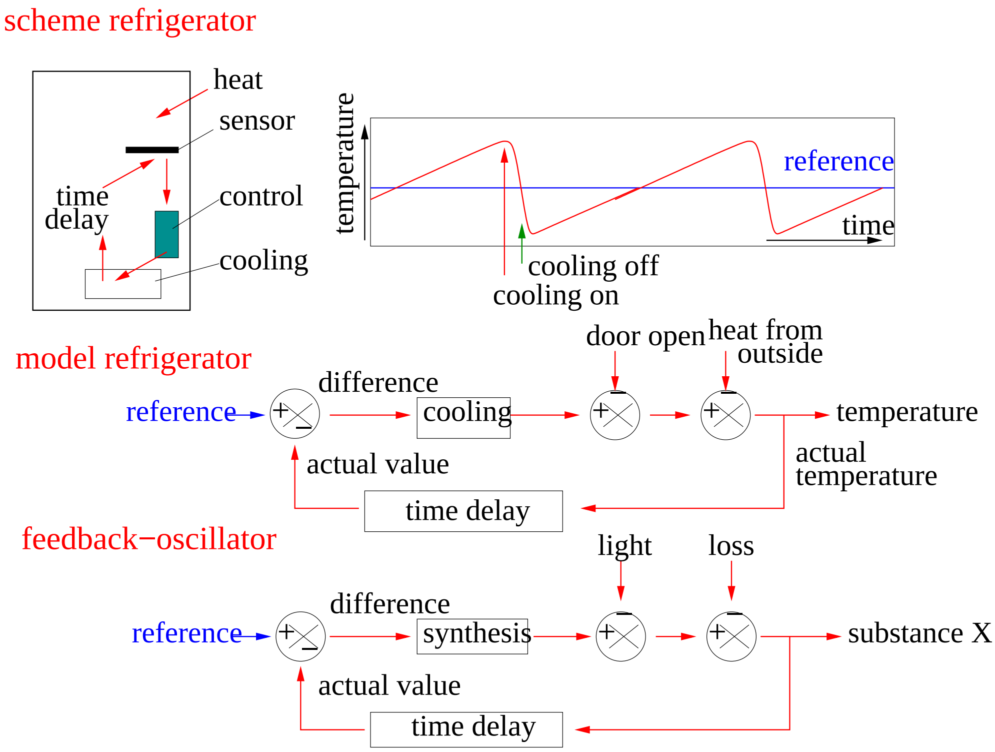

Figure 3.30: *Modell of a refrigerator and photoperiodic timing with a feedback-oscillator (clock). Left: Scheme of a refrigerator. A temperature sensor ascertains that the temperature in a refrigerator has exceeded 6°C. Since the set point was put to 6°C by means of the controller, the compressor is started and chills the refrigerator. It takes, however, a while (*time delay*), until the set point is reached and usually the temperature sinks somewhat below the set point. The red curve at the top right shows the temperature course with increasing temperature, cooling on (red arrow), cooling off (green arrow). All this repeats periodically. Center: Modell of a refrigerator. The set point (6°C) of the temperature in the refrigerator is compared with the actual value. In case of a positive difference (for example 8°C) cooling starts. The temperature decreases and is time delayed compared again with the set point, until the difference is gone. Cooling stops. Since, however, the refrigerator is not ideally insulated, the temperature increases again. The process reiterates and leads to oscillations, as we have seen allready in the upper right part of the figure. If the door of the refrigerator is opened, additional heat enters and the curve would shift (not shown). Bottom: Modell of an oscillator (a clock), in which the concentration of a substance X is compared with a set point. If the actual value is below the set point, more X is synthetized. Since some X is constantly lost (loss), and since the feedback to the set point is time delayed, oscillations are induced. How light works, is explained in the text. It is comparable with the opening of the door of the refrigerator. After Lewis and Saunders (1987), Saunders and Lewis (1987a), Saunders and Lewis (1987b)*

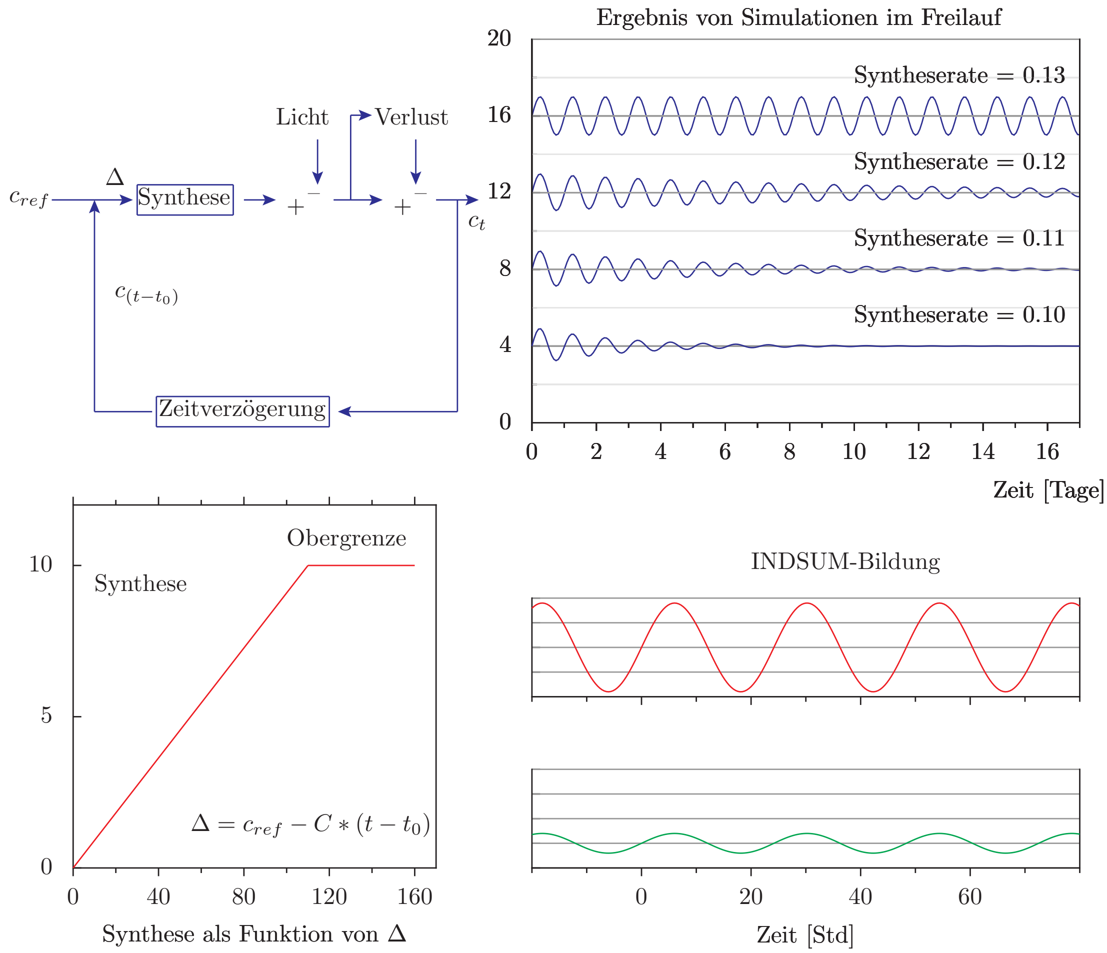

Figure 3.31: *Modell of the photoperiodic timing and its photoperiodic counter. Left modell of an oscillator as described already in figure 3.30. Without light the oscillation would damp out, as shown at the right lower blue curve. Light increases the concentration of the substance X, whereas constantly a part of the substance is lost. If light is not applied constantly, but for example 8 hours per day, the damped curve gets a push each day. If the push occurs at the right time (this depends on the length of the light period), the damped curve becomes an undamped one (upper blue curve). Now the curve is at all the days beyond the horizontal line. During this time X is produced and accumulates therefore. After a certain number of days sufficient X has accumulated to induce diapause. After Lewis and Saunders (1987), Saunders and Lewis (1987a), Saunders and Lewis (1987b)*

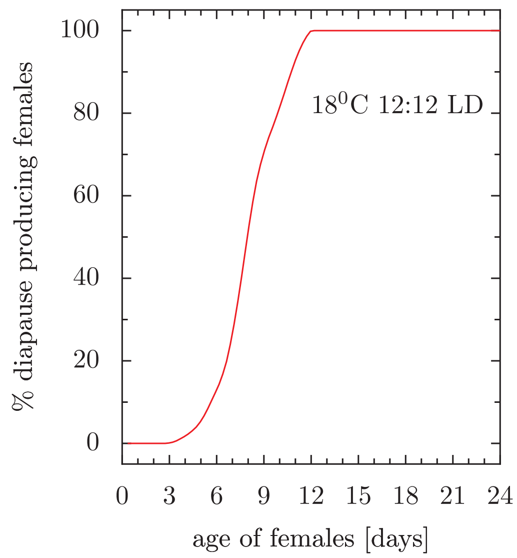

Figure 3.32: *Photoperiodic counter in the parasitic wasp* Nasonia vitripennis. *In the fall, when the daylengths decreases (in this figure days with 12 hours light and 12 hours darkness), the mother lays eggs which develop to the pupal stage and enter diapause. Since these parasitic wasp lay their eggs daily, one can collect them and simply observe, whether new parasitic wasp will develop or whether they stop developing because they have entered diapause. In this way one can determine, how many shortdays are required inorder to induce diapause in all animals. With five shortdays only a few animals enter diapause, wheras the remaining of the Geleges develop. Twelf days of shortday induce diapause in all animals. After Saunders (1966)*

with glycerol from freezing. These liquids reduce the freezing point at which water normally freezes. In the same way freezing is prevented in insects. Furthermore reserve substances such as fat, proteins and carbohydrates are produced in the body. The animals are thus able to draw for a long time on their reserves even without food supply. To reduce loss of water, the cuticle of the insect armour is protected by additional wax against drying out. All these changes and preventions have to occur, before diapause begins. How is this induced by the daylength?

We have seen a scheme already (figure 3.24), which shows how light is perceived by photoperiodic eyes, how its length is determined by an internal clock and how the number of effective days is summed up by a photoperiodic counter and how, after everything is alright, a photoperiodic switch in the body stops development of the insects in a fixed stage: Diapause begins. We got to know also some examples in which the processes and backgrounds of diapause were described (page 51, 56). Now we will be concerned with the processes going on in the brain and its accessory glands before diapause can begin. Before we must, however, dip into the brain of insects (see figure 3.12, 3.13 and 3.14).

In the brain and in the subesophageal ganglion neurosecretory cells are found in various regions. They produce hormones, which are important for development and diapause. Additionally accessory glands of the brain such as the Corpora cardiaca, the Corpora allata and the prothoracic glands play an important role.

In the *diapause of adult insects* such as the Colorado beetle (have a look again at figure 3.14 in subsection 3.2) the neurosecretory cells in the brain stimulate the prothoracic glands to produce ecdyson. Furthermore they cause the Corpora allata to produce juvenile hormone. Under shortday conditions ecdyson is still synthetized in the prothoracic glands and secreted, but the Corpora allata are set to stop juvenile hormone production. Without juvenile hormone the ovaries are, however, not able any more to produce eggs. In parallel to it the behaviour changes. The adults stop feeding and crawl into the soil. If the *Corpora allata* of the Colorado beetle are removed, diapause is induced. If the *Corpora allata* are implanted into a diapausing animal, it resumes development. Shortday thus inhibits the production and secretion of juvenile hormone, reproduction is prevented and diapause begins. Ecdyson production is, however, completely normal.

If the diapause does not occur in the adult stage, but in the larval, pupal or nymphal stage, again lack of hormone is the cause of it. However, it is not the juvenile hormone, which is lacking, but ecdyson (figure 3.33). Examples are the giant silkmoth *Hyalophora cecropia,* the corn borer *Ostrinia nubilalis*, the Cabbage Butterfly *Pieris rapae*, and the fleshfly *Sarcophaga.* When the neurosecretory cells in the brain experienced shortdays for some time, they do not produce any brain hormone. Without brain hormone the prothoracic does not produce ecdyson. As a consequence development is stopped. Under longday the neurosecretory cells are stimulated to produce brain hormone. It activates the Corpora cardiaca and the Corpora allata (two appendix glands of the brain) and furthermore the prothoracic gland in the thorax (chest of the insects). The moulting hormone ecdyson can now be produced again in the prothoracic gland and secreted and the animals are able to continue development and to moult.

This is, however, not always the case (na-

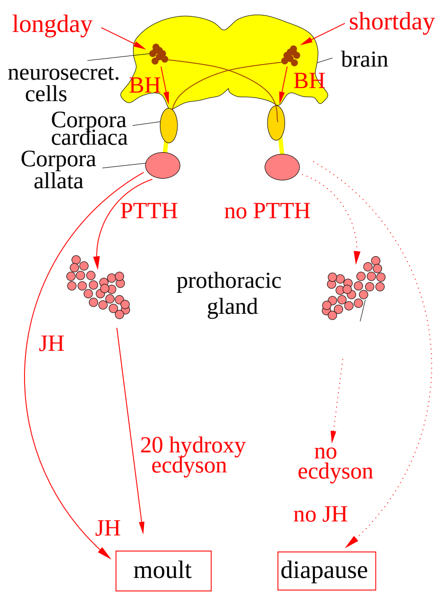

Figure 3.33: *Diapause in the larval, pupal and nymphal stage is induced by a lack of hormone. Examples are the giant silkmoth* Hyalophora cecropia, *the corn borer* Ostrinia nubilalis, *der Cabbage butterfly* Pieris rapae, *and die fleshfly* Sarcophaga. *Left part: When the neurosecretory cells in the brain experienced shortdays for some time, they do not produce any brain hormone. Without brain hormone the prothoracic does not produce ecdyson. As a consequence development is stopped. Right side: Under longday the neurosecretory cells are stimulated to produce brain hormone. It activates the Corpora cardiaca and the Corpora allata (two appendix glands of the brain) and furthermore the prothoracic gland in the thorax (chest of the insects). The moulting hormone ecdyson can now be produced again in the prothoracic gland and secreted and the animals are able to continue development and to moult. After Williams (1952)*

ture plays). In other cases, in which diapause occurs in the *larvae*, the endocrine system stays *active* (figure 3.34). The larvae are able to moult, but pupation is prevented. The brain causes *the Corpora allata* to produce juvenile hormone and to secret it. But for the larvae to moult the prothoracic glands must be functional and secret ecdyson. That is, however, not the case here. An example is the corn borer *Diatraea grandiosella*. During its *larval diapause* the metabolism is low, the body contains little water, fat reserves are present, the metamorphosis into the adult insect is inhibited, the movement of the animals strongly restricted. Partly continuous larval moults take place. This kind of larval diapause is terminated by higher environmental temperatures or by the adequate photoperiod (in most of our examples in our latitudes by longday).

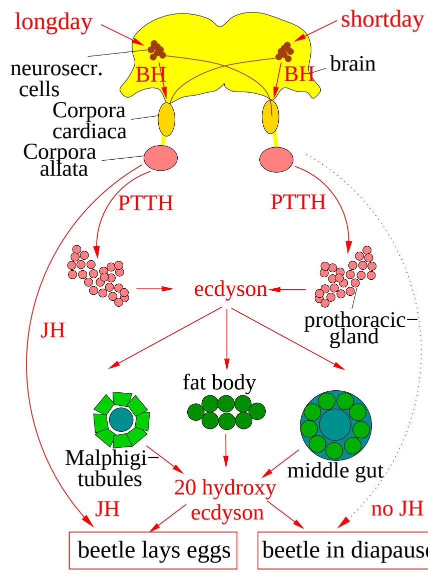

Figure 3.34: *Right part: Shortday induces diapause in the* corn borer Diatraea grandiosella *in the larval stage: The brain causes* the Corpora allata *to produce juvenile hormone and to secrete it. The prothoracic gland does not secret ecdyson (because no brain hormone BH is made). Therefore the animals can not moult into a pupa. The larvae enter diapause. Left: Under longday the neurosecretory cells of the brain make the* Corpora allata *to produce and secret juvenile hormone as is the case under shortday. Additonally, however, the prothoracic gland is stimulated to secrete ecdyson. Therefore the animals are able to moult and to develop up to the pupal stage (and imago). After Yin and Chippendale (1973)*

Diapause is found in most cases in the *pupal stage*. This applies especially to butterflies and flies. Here too the metabolism is reduced. The power plants of the cells (mitochondria) are less active. The metamorphosis into the winged adult insect is hamstrung.

The pupae are usually not photoperiodically sensitive anymore (the photoperiodic induction of pupal diapause occurs in a larval stage). But here too exceptions exist: In the case of the giant silkmoths *Hyalophora cecropia* and *Antheraea pernyi* the pupal stages do react to the daylength. Diapause can be prolonged by shortday during the pupal rest and terminated by longday.

3.4.6 Diapause: A topic with variations

As often seen in nature, things are not following obstinately fixed rules, but there are exceptions and modifications. That applies also for the induction of diapause by photoperiod. It can be modified by environmental temperature, food offer and quality of food. In most cases high temperature prevents diapause and promotes it at low temperatures. In some cases the critical daylength shortens with increasing temperature. Usually, however, it is independent of temperature, at least in a certain temperature range. That is the case also in the fleshfly *Sarcophaga*, as shown in figure 3.35. Between 15 and 18° C the critical daylength stays constant. But if the temperature rises to 20^{0}C or above, the animals in the pupal stage do not enter diapause anymore, but continue development without developmental rest.

On the other hand there exist also cases in which diapause occurs at *higher* temperatures, for example in *Abraxas miranda* (Masaki (1980)). The animals develop under shortday and at low temperatures. At higher temperatures they enter diapause. Often optimal temperatures exist for the photoperiodic induction of diapause. In tropical species this temperature is mostly higher as compared to insects from moderate latitudes (for example in *Oedipoda miniata* 27-28°C).

The kind and amount of food can also influence diapause. In the bollworm *Pectinophora* a high oil content of the cotton seeds facilitates diapause. The chironomid *Chaoborus* enters usually diapause under shortday conditions. However, if plenty of food is available, diapause is surpressed (Bradshaw (1970)).

3.4.7 Diapause for the descendants

Diapause of insects is genetically programed. This can be seen if we compare the diapause of animals of the same species,

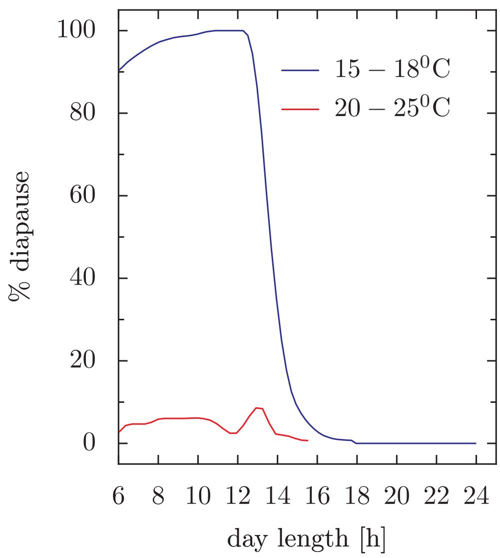

Figure 3.35: *The fleshfly* Sarcophaga *stops development in days with short light periods (shorter than 13.5 hours) and diapauses in the pupal stage. That is shown by the course of the blue curve: The percentage of animals in diapause is high under shortdays. It does decline somewhat at very short light periods per day (very left part of the blue curve), but in the latitudes, in which the studies were made, such short light periods do not occur. No diapause occurs under longer light periods* in Sarcophaga *(low values of the blue curve in the right part of the figure). The course of this curve is independent of the environmental temperature between 15 and 18° C. However, at* 20^0 *C and higher temperatures (red curve) almost no diapause occurs in the population. After Saunders (1971)*

but from different latitudes of the earth. Those animal populations are called *geographic varieties*. The fruitfly *Drosophila littoralis* (figure 3.36) has a number of geographical varieties from Northern Scandinavia to the Caucasus (figure 3.37). The animals from Oulu stay in diapause, as long as the light period of the day is 19 hours or less. Remember, that Oulu is quite up in the north and that in the summer the light period of the day is very long. Diapause is terminated at still longer light periods in the summer, for example at 21 hours light per day and the animals development continues. This is shown by the blue curve in figure 3.38. High values stay for all animals in diapause. The value at 21 hours light period is the only one which is low. At this daylength almost all animals have terminated diapause and develop.

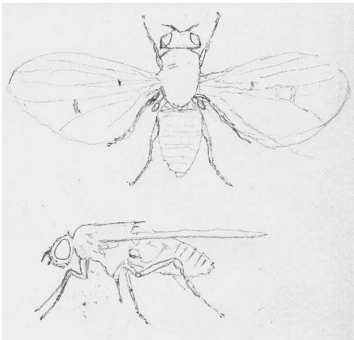

Figure 3.36: *Females of the fruitfly* Drosophila littoralis

If we compare the blue curve with the red one which applies to the animals of the southern variety from the Caucasus, a big difference is obvious: In these animals diapause is terminated already at 13.5 hours light per day. At 12 hours light period per day almost all animals are in diapause.

The length of the light period, at which about half of the animals has terminated diapause (or in cases, where diapause is in-

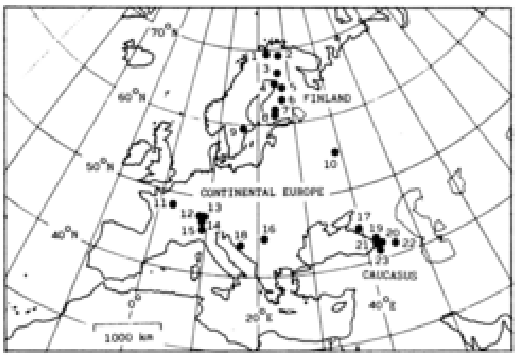

Figure 3.37: *The habitat of the fruitfly* Drosophila littoralis *(figure 3.36) reaches from North Scandinavia (for example Oulu, Finnland,* 65^0*northern latitude, Nr. 4) to the Caucasus (for example Kutaisi, 42° northern latitude, Nr. 20) and is found in the different regions as geographic varieties. 1 Kilpisjärvi, 2 Inari, 3 Rovaniemi, 4 Oulu, 5 Paltamo, 6 Kuopio, 7 Padasjoki, 8 Hollola, 9 Strängnäs, 10 Moskau, 11 Paris, 12 Dietikon, 13 Zürich, 14 Gersau, 15 Tessin, 16 Baile Herculane, 17 Krasnodar, 18 Biograd, 19 Khobi, 20 Kutaisi, 21 Udoti, 22 Tbilisi, 23 Batumi. From Lankinen (1985)*

duced: about half of the animals begin diapause), is the *critical daylength*. It amounts to 19 to 20 hours in the *Oulu*-variety and 12.5 to 13 hours in the *Kutaisi*-variety.

If females *of the Oulu* variety are mated with males of the southern variety from the Caucasus, the critical daylength of the offsprings is around 17 hours, that is in the middle between the critical daylength of the parents. The offsprings are said to show an *intermediary* behaviour.

Geographic varieties which adapt the critical daylength to the daylight conditions of the latitudes have a decisive advantage: They are able to use that daylength for terminating diapause at which no danger exists anymore to freeze to death while the animals have started to continue the development. The situation is completely different with animals in the surrounding of Oulu as compared to the animals in the Caucasus. There the days have to have at least 19 hours light, here in the Caucasus 13 hours of light per day are already sufficient.

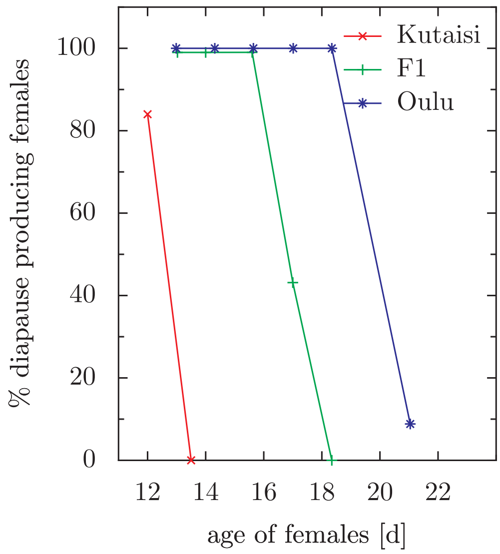

Figure 3.38: *The blue curve shows the percentage of animals of a northern variety of* Drosophila littoralis *from Oulu, Finnland (65^0 northern latitude) at varying light periods of the day which are still in diapause (high values) or have terminated it (low values). The red curve shows the situation in the variety from* Kutaisi *in the south (Caucasus). The critical daylength (explained in the text) of the* Oulu *variety is 19 hours and 42 minutes. In the Caucasus-variety* Kutaisi *it is only 12 hours and 36 minutes. Crossing both varieties with each other leads to a critical daylength of the first generation intermediate between the one of the parent, namely at 16 hours and 18 minutes. After Lumme (1982)*

4 The sun compass of a beach hopper

Beach hopper are able to orient themself at the beach of the sea with the help of an internal sun compass and to find the way back to the sea quickly if it is getting too dry. If the sun is covered by clouds, but some blue sky is still visible, the polarization pattern of the sky helps them to orient. During the night the moon is used.

Perhaps you have been already at a beach of the sea (figure 4.1). There you find quite diverse zones, depending on how close to the sea or further inland they lie. The wettness and the salt content differ, wind influences the sand differently depending on whether it is dry or wet. These zones are shifting regularly due to the tides (ebb and flood) and irregularly due to storms. The fauna on and in the sand has to adapt continuously to the changing life conditions. Some animals stay all the time in a certain zone or try to reach this zone. Other animals migrate through these zones and behave thereby quite differently.

Figure 4.1: *Beach-biotope of the beach hopper* Talitrus saltator *under conditions which change constantly due to the tides and storms and high tides. Italian coast close to Florence. Drawn by Mareike Förster after a photography in Pardi and Scapini (1987)*

An Italian scientist, Leo Pardi, and his colleague, Felicita Scapini, studied at the Italian East coast a beach hopper (*Talitrus saltator*). It belongs to the amphipods (*Malacostracae*, figure 4.2). That is an order of the crustaceans. Normally we imagin a crustacean as a larger animals, but there are also very small ones among them. The slater for example belong to the crustacean. The beach hopper is relatively small. It is found frequently at the european coasts at the beach close to the high water line (figure 4.1). During the day it is sheltered in the (not too) wet sand. During the night it undergoes excursions up to 100 meters toward the inland. If during the day it becomes too dry, it returns in the direction to the sea. It does not need to see the sea, but can make use -like bees- of a sun compass. The heights of the sun plays no role for orientation. The sun is so to speak projected down to the horizon and the angle between sun and direction to the sea used as a compass. In the same way as in the sun compass-orientation of bees the beach hopper needs also a day clock which informs about the course of the sun during the day.

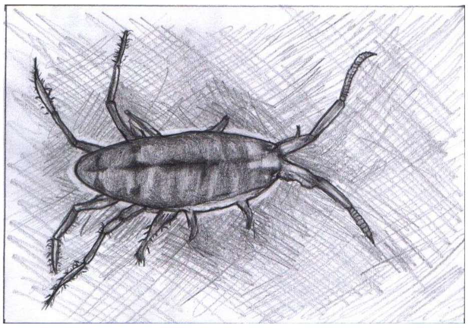

Figure 4.2: *Beach hopper* Talitrus saltator *(amphipode), a crustacean. Drawn by Mareike Förster after a photography in Pardi and Scapini (1987)*

Depending on the position of the coast, the escape direction differs in the different populations. If the coast lies in the north, the animals escape to the north. If it is in the west, they try to find salvation in that direction. The flight direction is fixed for each population. If parent animals with different flight directions are mated, the offsprings use an intermediate flight direction.

If the sun can not be seen direcly because it is behind clouds, the beach hopper -like bees (see page 20)- can use the pattern of the polarized light at the sky for orientation. During the day further orientation aids are used such as land marks and the inclination of the beach. Even a magnet compass is used. Without these additional aids the astronomic direction finding would be less precise. During the night the moon is used for orientation (Papi and Pardi (1953), Papi (1960)).

How was it shown that the animals use the sun for orientation? Pardi and Scapini (1987) captured beach hoppers and positioned them in the center of a glass vial (figure 4.3). This vial was surrounded by an opaque plastic screen. It allowed the animals to see the sun, but not the sea and the land. As expected, they jumped in the usual flight direction towards the sea. A translucent sheet of paper was put on top of a mirror which was mounted underneath the bottom of the vial. The positions of the animals were drawn on the paper. One can, of course, also use a digital camera to take picture and evaluate the images later. If the sun is in the west, the image would look like shown in figure 4.4.

Now the two scientist did not only block the landscape by using a cardboard as a blind, but also the sun. The sun was, however, reflected with a mirror from another direction onto the animals. The animals escape now in such a way as if the mirror sun is the real one (figure 4.5).

These beach hoppers are thus able to find out the current angle to the sun and use this information for their flight direction. If the sun *is* used by the animals for orientation, they must change the angle slowly, since the sun is traveling from east to west. And this is indeed what they do: The path of the sun is taken care of by the animals. That is, they must possess -like bees- an internal clock which is used as a time reference and to determine the current sun direction.

If they do possess an internal clock which allows to take into account the course of the

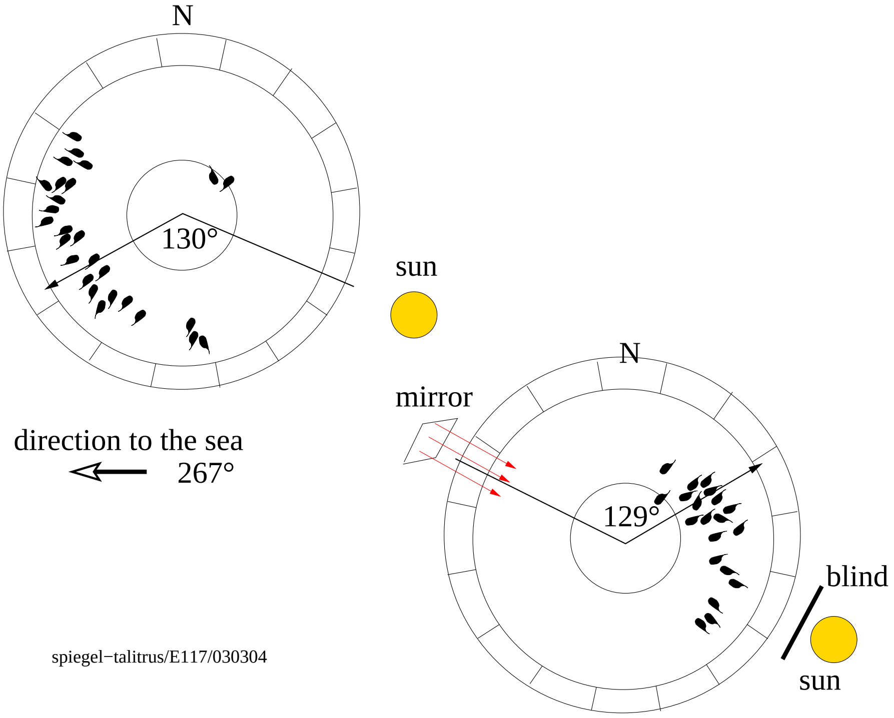

Figure 4.5: S*un compass orientation of the beach hopper* Talitrus saltator. *They orient themself during the escape towards the sea (large arrow, 267°) according to the sun (top right, 130° to the sun). If the sun direction is faked with a mirror and the direct view of the sun prevented by a blind*, Talitrus *uses the mirror-sun (bottom left, 129° to the mirror-sun). N north. After Pardi and Scapini (1987)*

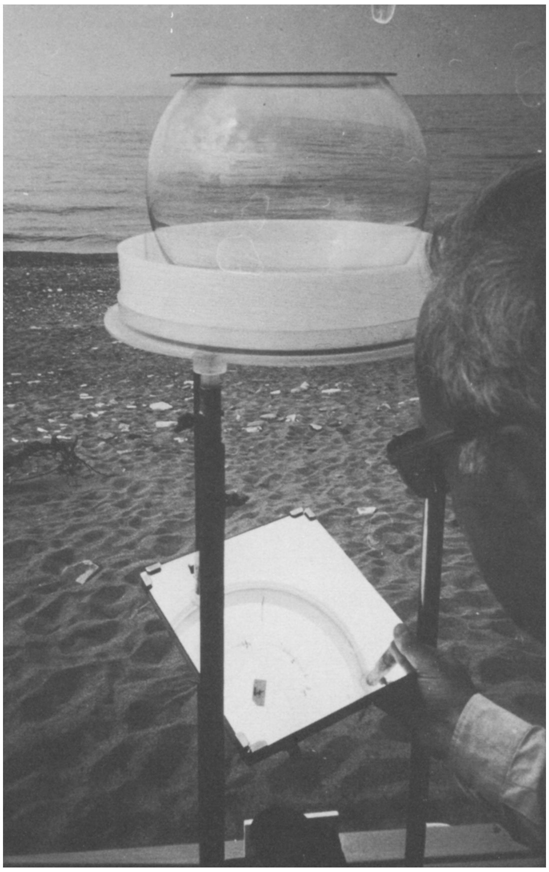

Figure 4.3: *Beach hoppers were cought at the beach and put in the center of the bottom of a glass vial. They escaped from there in the direction of the sea, although an opaque plastic screen prevented the animals from seeing the landscape. A translucent sheet of paper was layed on a mirror which was mounted underneath the bottom. At the paper the positions of the animals were drawn with a pencil (see figure 4.4). After Pardi and Scapini (1987)*

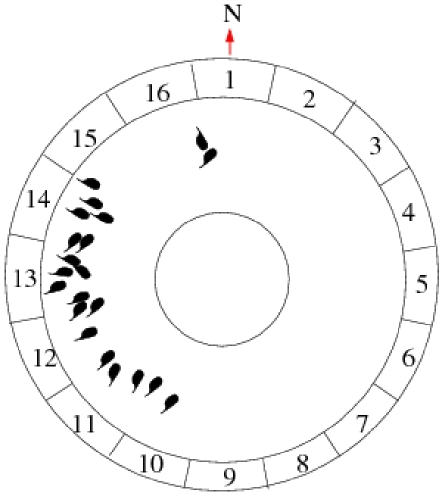

Figure 4.4: *The flight direction of beach hoppers in a glass vial (figure 4.3) is shown by the animals on the image. They escape towards the west in the direction of the sea. After Pardi and Scapini (1987)*

sun, they should take a wrong direction if the internal clock is shifted. For testing this the scientist transferred the animals in an artificial light-dark cycle. This cycle did not coincide with the natural day but began 6 hours earlier and ended 6 hours earlier (that is, a day advanced by 6 hours). If the animals are now brought back in natural conditions and their flight direction is tested, it was shifted by 90°. In a further experiment the day was delayed by 6 hours. Here too the flight direction was shifted by 90° in respect to the control in a normal day, but now in the other direction. The flight direction of the animals had thus changed after the internal clock was regulated (figure 4.6).

Figure 4.7: *The sand wolf spider* Arctosa cinerea *(left close to its whole, right from the front, females 12 to 14 mm long) uses sun compass orientation. It is not only found at the rivers of the mediterranean countries, but also at Scandinavian ones. Spiders of Italian populations, which where transferred in the summer to Scandinavia were not able to orient themself at sun times unknown to them. Animals of the local scandinavian populations orient themself also at the time of midnight sun. Water colors by the author using images for example under http://www.jki.bund.de/nn_806766/DE/pressestelle/Spinne___j/Spinne2007.html, http://www.deutschewildtierstiftung.de/aktuelles/natur_des_jahres/2007/spinne.php?-PHPSESSID=ab00b...*

4.1 Further examples for sun compass orientation

In ants (Santschi (1911), Brun (1914), newer publication Wehner (1998)) and spiders (Papi (1955), see figure 4.7) sun compass orientation was verified. The sand wolf spider *Arctosa cinerea* is found at European rivers from Finnland to the Mediterranean countries. Italian populations were not able to orient themself in the Scandinavian summer at sun times unknown to them. The local Scandinavian population, however, was able to do so even at the midnight sun.

Migratory locusts are incessantly migrating and do not return to the regions they came from. There are ten typical migratory locust species. *Locusta migratoria* is the most common one. Its swarms are able to migrate several thousand kilometers (up to 5000 kilometer were verified in one swarm). They follow the wind, but orient also according to the sun and moon.

Under the butterflies the Monarch (*Danaus plexippus*) migrates up to 3000 kilometers (figure 4.8). In late summer and fall each day thousands of butterflies migrate from Canada and the northeastern and northwestern states of the USA to the countries around the Gulf of Mexiko, California and the northern Central America (figure 4.9). In the spring they fly from their winter quarters back to the summer quarters. Individuals were labelled in the summer quarters and recollected in the winter quarters, which are often restricted to small areas. They had covered distances between 1700 and 3000 kilometers. That verified, that one and the same individual had survived and covered that distance. It disproofed the possibitity, that the animals stopped on the way, layed eggs and the descendants continued the journey. On the journey back to the north, however, the *offsprings* of the migrators to the south make

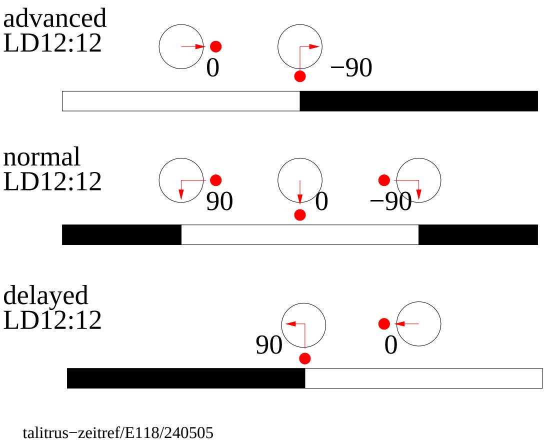

Figure 4.6: *If the light-dark cycle of beach hoppers is shifted by an artificial illumination which does not coincide with the natural day, the time reference of the animals is also shifted and the orientation shifts correspondingly: A light-dark cycle (top) advanced by 6 hours changes the synchronization of the day clock of the animals in such a way that they escape in the morning at 6 o'clock (their internal clock tells them it is noon) in the direction of the sun and at 12 o'clock in a direction the controls (center) would choose in the evening (their internal clock tells them it is evening). A light-dark cycle delayed by 6 hours (bottom) changes the synchronization of the day clock of the animals in such a way, that they escape at 12 o'clock (their morning) to the East instead of south. At 18 o'clock they escape in the same way as the controls (center) would do at noon (and it is their noon). The orientation uses thus an internal clock. After Pardi and Scapini (1987)*

Figure 4.8: *Thousands of Monarch-butterflies (*Danaus plexippus*) fly each year from their northern summer quarters to the southern winter quarters. Here a swarm has settled down on a twig of a conifer (see figure 4.9). Aquarell from the author after a black/white photography in Günther et al. (1989)*

it finally to the northern summer quarters. Here too the animals use a sun compass. The caterpillars feed on milkweed (*Asclepias*) only and the animals are therefore restricted to areas, in which these plants grow.

Other butterflies migrate also over long distances such as the Cabbage Butterfly (see Johnson (1969)).

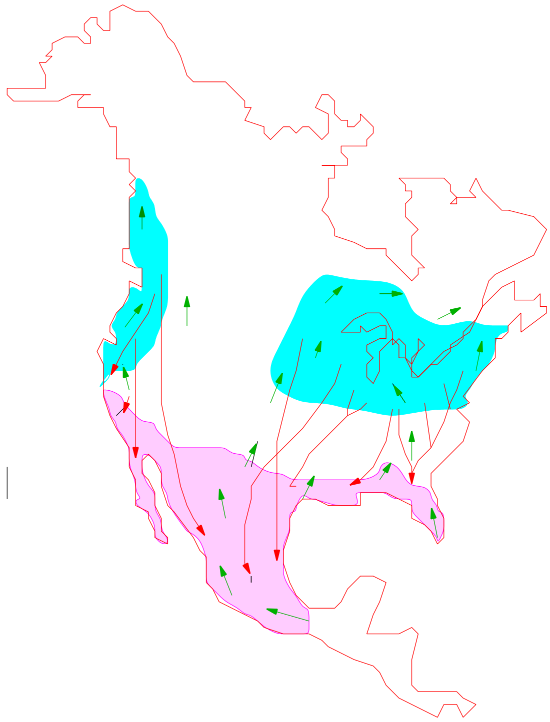

Figure 4.9: *The Monarch-butterfly (*Danaus plexippus*) flies each year in late summer and fall from its summer quarters in Canada and the northeastern and northwestern States of the USA (light blue areas, red arrows) to the winter quarters in den countries around the Gulf of Mexiko, California and the northern Central America (magenta areas, green arrows). In the summer- and winter quarters milkweed (*Asclepias*) grows, for which the caterpillars have specialized. They are not able to live on other plants. After Schmidt-Koenig (1975)*

5 Our head clock

Man seems to have also a time sense. Some people with a 'head clock' wake up at night at a certain time without using a clock. Perhaps you belong to these people? In this chapter you will see how to find out by using a slightly modified alarm clock.

Earlier in this book it was shown, that organisms, animals and plants, possess internal clocks which help them to adapt to the time structure of the environment.

Thus bees are able to remember certain times, at which they can obtain nectar or pollen from plants. Are humans also able to decide to do something at a certain time without using external aids?

There is a book by Clauser (1954), in which it is reported, that some people are able to wake up at certain times of the night, if they intended to do so in the evening. They might, for instance, tell themself: I intend to wake up this night at 3 o'clock. Often certain rites are used additionally. They might for example knock three times at the edge of the bed in the evening. These people fall asleep quite normal, sleep deeply and well and wake up briefly before or after the intended time (figure 5.2). Clausen ascribed this ability to a *head clock*, which functions in man perhaps in a similar way as in bees and can be used as an alarm clock.

I have once proposed to pupils of the Gesamtschule in Tübingen to try to find out whether modern man does still possess this head clock. I distributed battery driven alarm clocks to the pupils. They were asked to intend in the evening, to wake up at a certain time of the night. The alarm clocks were all set to 12 o'clock and stayed at that time, because a piece of paper was inserted between the contact of the battery and the battery holder (figure 5.1). Therefore current was unable to flow. The pupils were told to just remove the piece of paper after waking up in the night at the intended time. They should not look at their own clock, but simply continue to sleep.

On the next day they brought their alarm clocks, which were now running, to the school. Looking at the alarm clock and counting the hours back allowed to calculate the time at which the clock was started, when the contact to the battery was made. If a pupil for example had intended to wake up at 4:30 and his alarm clocks showed in the morning 3 o'clock, when he arrived at school at 8:00, he and his classmates knew that he woke up three hours before, that is at 5:00 o'clock. Thus he woke up half an hour too late. In this experiment there were indeed some students who met the intended time of waking up quite well. In figure 5.2 some examples of people are shown, which hit the intended time (0 in the figure) quite precisely.

It is not known, whether the head clock is based on the circadian clock in man. Perhaps you might like to try to find this out in your school.

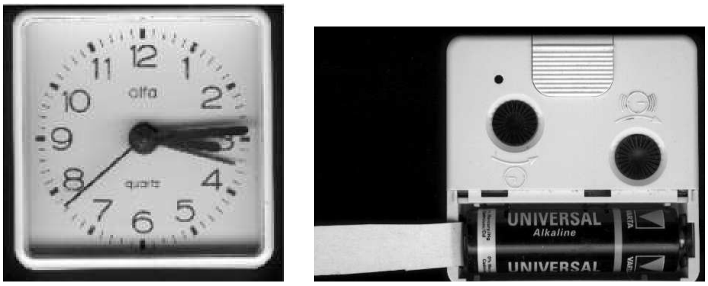

Figure 5.1: *An alarm clock is reset to 12 o'clock in the evening and a stripe of paper wedged between the battery and the holder. If the paper is pulled out at the time of waking up in the night (intended: 3:30 o'clock), the clock starts to run, because it has contact again to the battery. On the next day you need only to look at the alarm clock (for example at 8:00 in the morning) and count back the time (here: 3 hours 14 minutes) to know at which time during the night the clock was set in motion, that is, the time of waking up (8:00 minus 3:14 = 4:46 clock, that is 1 hour and 18 minutes after the intended waking up. In this case there is apparently no precise head clock at work. Anyway, the train would have left if one would have relied on this clock*

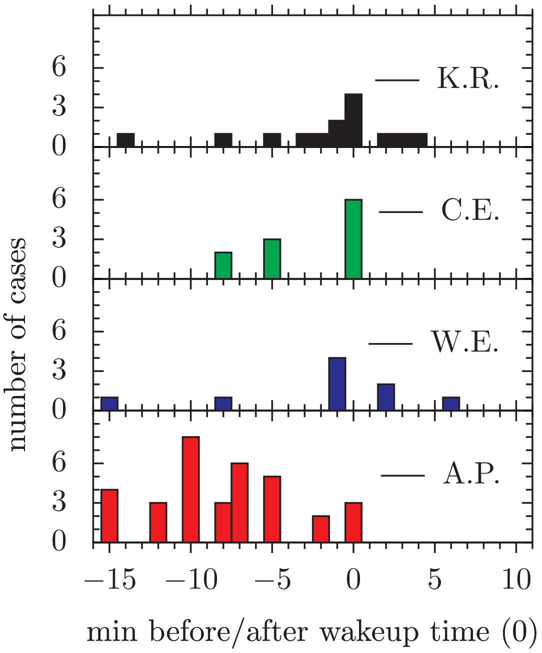

Figure 5.2: *Some people possess a head clock. It allows them to wake up at night at a certain intended time without alarm clock. Here are examples shown, in which the four persons A. P., W. E., C. E. and K. R. used their head clock quite effectively. On the horizontal axis the time is plotted at which the person woke up (0 is the intended time of waking up, for example 4:00 in one person, 2:30 in another). The vertical axis shows, how often this person did wake up in reality and at what time. C. E. for example woke up in one night 15 minutes before the intended time. In another night it was 8 minutes before the intended time. In four nights she woke up one minute before, in two nights one minute after the intended time. In two further trial nights she woke up 6 respectively 10 minutes later. After Clauser (1954)*

6 What you need for the experiments and where to obtain it

Here I compile, what you need and have to take into account, if you want to perform the mentioned observations and experiments.

6.1 Experiments

In the following the various observations and experiments are compiled and what you need for them. Read again the text to the various topics which are mentioned.

Observing Forsythia-flowers: On page 5 it is described, how the movement of *Forsythia* flowers can be observed and photographed. If you have a video camera or a digitale camera at disposal or if you can borrow one, you should make sure it has a safe stand. The best would be a tripod. Using a cable release or an infrared release is advisable (prevents bluring of the shots).
The images can be transferred to a computer (read the instructions or ask somebody to show you how to do it). Measure the distance between the tips of the petals on the screen. Under Linux a program (kruler) is available, which simulates a millimeter ruler on the screen. The values can be transferred into a spreadsheet program and afterward plotted as a diagram.

Flaming Katy *Kalanchoe:* The *Kalanchoe* petal movement is described on page 7. *Kalanchoe*-seeds are tiny. It is added to the CD cover. Fill sand mixed with garden soil in a flower pot and use at the uppermost layer very fine sieved soil. Strew seeds on it, but do not cover it with soil*:* *Kalanchoe* is a light germinator: The seed needs light for germinating. The best time for sawing is June. If the seedlings are to dense, take them out with a tooth pick and plant them in small flower pots (gardeners call it ‘to prick out´). The plants grow during the summer without flowers. Not before fall, when the days become shorter, flower buds are induced, and by December you will have an interesting and nice-looking christmas present. Those of you, who do not want to wait for such a long time, should offer the plants already in the summer winter days: You have to darken the plants over night for 13 hours. If you have done this for about two weeks, they start to form flower buds after some time.
How the movement of the petals can be observed is described on page 7.
For cross- and longitudinal sections through the petals you need elder pith (brake off the twig of an elder tree and cut it in peaces). Cut out the pith and wetten with water. Cut a slit with a new razor blade and buckle a petal in it. Depending on whether you want to make a cross section or a longitudinal section, the petals have to be oriented correspondingly. For observing

the structure you need a binocular or a normal microscope. Ask for it at school (books concerning microscoping see page 97). Microscope slides and coverslips you can borrow at school or buy in a shop for laboratory supply. A fine brush helps to transfer the sections in a drop of water on the microscope slide.

If you want to observe the petal movement under constant conditions, a dark room in the cellar is suited. Normally the temperatures in the cellar are quite regular. Green light is obtained, if you wrap a white fluorescence tube with a green foil (e.g. Cinemoid or Rosco, Dedo Weigert movie GmbH, Karl Weinmair Str. 10, 80807 München). You could also build an air conditioned box, as described in Engelmann (2004b).

Smaller amounts of chemicals (for example sugar for the *Kalanchoe*-flowers) can be weighted with a letter balance.

Morning glory begins to flower: This is described on page 12. Seeds of the morning glory *Pharbitis nil* is added to the CD cover. Soak the seeds in water over night and put three to four of them in pots. Grow the plants on the balcony or in the garden or plant them direcly in the garden. They need a sunny place and a long stick for twining. Watch the flowers early in the morning, while still folded like a bag and during the course of the morning while they open and during the evening while wilting.

A flower clock: The flower clock is described on page 12. In the following the plants are listed, which open at various times of the day. It is also described when they flower (roman numbers, month), where they are found and/or how they are reared (still missing). After Hess (1990) page 217 (only opening times) and after Zander, cited after Beling (ZVP 9 259 1929) in Jores (1937), some notes by Bünning, also corrections of the data (with opening times o and closing times z):

- 4-5 Wild rose *Rosa canina* VI, light woods and shrubbery, edge of the woods, hedges, pastures. Especially frequent in mean mountain ranges
- 5 Red Puppy *Papaver rhoeas* V-VII, cereal fields, debris, common
- 5-6 Blue Flax *Linum perenne* VI-VIII?
- 6 Cichory *Cichorium intybus* VII-VIII, waysides, margin of fields, pastures, widespread. On clay
- 6-7 Rose Bay *Epilobium angustifolium* VII-VIII, wood glades, clear cuttings, wood- and waysides, heathland, debris, recurrent.
- 7 Coltsfoot *Tussilago farfara* III-IV, clear cuttings, debris, railroad embankments, widespread
- 7-8 Lesser Bindweed *Convolvulus arvensis* VI-X, fields, vineyard, debris, waysides, common
- 8-9 Stengelloser Gentium *Gentiana clusii*
- 9-10 Tulip *Tulipa* spec
- 10-11 Lesser Centaury *Centaurium pulchellum*
- 11-12 Common Tormentil *Potentilla erecta* VI-X, light woods, clear cuttings, heathland, meagre meadows, on acid soil widespread. Meagre indicator.

- 18 Perfoliate Honeysuckle *Lonicera caprifolium* VI-VIII?
- 18-19 Common Evening Primrose *Oenothera biennis* VI-VIII, waysides, railroad embankments, sand fields, quarries, river banks
- 19-20 Thorn Apple *Datura stramonium* VI-IX, debris at ways, scattered. Poisonous!
- 20-21 Nodding catchfly *Silene nutans* III-IX, sunny, dry hills, light shrubbery and woods, widespread
- 21-22 Queen of the night *Selenicereus grandiflorus*
- o 3-5 z 9-10 Goatsbeard *Tragopogon pratensis* V-VII, dry meadows, waysides, recurrent
- o 3-4 Threecoloured Bindweed *Convolvulus tricolor* VI-X?
- o 4-5 z 11-12 Hawks-beard *Crepis tectorum*
- o 4-5 z 10 Cichory *Cichorium intybus* VI-VIII, waysides, margin of fields, pastures, widespread. Especially on clay soil
- o 5-6 z 8-10 Dandelion *Taraxacum officinale* IV-V, meadows, gras places, drifts, common
- o 6-7 z 10 Corn Sowthistle *Sonchus arvensis* VII-X, fields, recurrent. Loam indicator
- o 7 z 17 White Sea lilly *Nymphaea alba* VI-VIII, nutrient-free, standing waters, widespread
- o 7-8 z 15-16 *Anthericum ramosum*
- o 7-8 z 15 *Mesembryanthemum barbatum*
- o 8 z 15-16 Scarlet Pimpernell *Anagallis arvensis* VI-X, fields, gardens, common
- o 9 z 12 Marygold *Calendula arvensis* VI-X, fields, vineyards in Southern Germany, likes warmth
- o 17 *Nyctago hortensis*
- o 9-10 z 15-16 *Mesembryanthemum cristallinum*
- o 10-11 z 14-15 Pink Sandwort *Arenaria rubra*
- o 10-11 Yellow Daylilly *Hemerocallis flava*
- o 10-11 Star of Bethlehem *Ornithogalum umbellatum* IV-V, meadows, gardens, vineyard, fields, scattered
- o 10-11 Mallow, almost all species *Malva* spec., for example *Malva neglecta* VI-IX, ways, walls, debris, village plant
- z 16-17 *Mirabilis dichotoma*
- z 17-18 Evening Primrose, different species *Oenothera* VI-VIII, waysides, railroad embankments, sand fields, quarries, banks of rivers
- z 18-19 Cranesbill *Geranium daucifolium*
- z 21-22 Nightflowering Catchfly *Silene nocturna*
- o afternoon White Cambion *Melandrium album* V-X, shrubbery, cultured land, debris, widespread

Butterfly clock Begin or highest flight activity of various day- and night butterflies. Noted is also where and at which time of the year the butterflies occur in Germany. After Hess (1990) S.219

- 2 Segelfalter *Iphiclides podalirius*
- 3 Gammaeule *Autographa bractea*
- 4 Blaukernauge
- 5 Widderchen *Lycastes zygaena exulans*

- 8 Bläuling *Cyanius* (*Lycaenidae*)
- 9 Grosser Kohlweissling *Pieris brassicae*
- 10 Taubenschwänzchen *Macroglossum stellatorium*
- 11 Admiral *Vanessa atalanta*
- 18 Wolfsmilchschwärmer *Celerio euphorbiae*
- 19 Totenkopfschwärmer *Acherontia atropos*
- 20 Tigermotte *Spilosoma monthastri*
- 21 Ligusterschwärmer *Sphinx ligustri*

Flowers of the Evening-Primrose open:
On page 15 the opening of the Evening-Primrose is described. You can obtain seeds of *Oenothera biennis* from a plant. Flower stands with flowers on top might have already seeds in the husks further down. You just saw it in the garden. They do not flower, however, in the first year, but in the second. Seeds have been added to the cover of the CD. It is better to look in a garden for flowering plants. Perhaps you can ask somebody whether you might dig out a plant. Once they are in the garden, they propagate quite strongly.
For rearing them normal garden soil is sufficient, The plant flowers from June to the end of October. Each evening between 20 o'clock and 22 o'clock you can observe the opening of the flowers.

Training Bees for colors: On page 18 it is described, how bees can be trained for colours. You need coloured paper (stationary shop or printout on the printer with the PC), glass disks for covering and some honey.
A movie is available from the Institut for den wissenschaftlichen Film in Göttingen, Nonnenstieg 72, about the dance in bees (page 19). It is named *Entfernungs- and Richtungsweisung bei der Honigbiene- Rund- und Schwänzeltanz* and was produced by Karl von Frisch and Martin Lindauer. Order number IWF C 1335.
How bees recognize the polarization pattern of the sky is described on page 20. Polarization foiles out of plastic can be purchased at www.edmundoptics.de (linear polarizing sheet, 2*2 inch, two squares, order number M43-781). How to build a 'bee spectacle' is described in the following. It shows you how a bee sees the sky at various times of the day. (missing)

Pollination of the Grass of Parnassus:
On page 23 the pollination of the Grass of Parnassus is described.You find the plant in fenland and on wet fenmeadows, but also at wet locations of dryer meadows.

Fragrance of flowers: From page 29 onward some observations and experiments are described concerning the fragrance of flowers. The crown of daffodils can be stained with neutral red by solving a small amount of stain in water and putting the stalks of the flowers into it. At the locations where the flowers give off scents, the neutral red accumulates, because the cell walls give off fragrance and water.
In the Gentianaceae *Exacum affine*, the Persian Violet, the fragrance intensity varies in a daily fashion (see page 29). The plants can be purchased in flower shops and at gardeners. You can observe them at home.

As a control scent you can for example use spirit, which has been denaturated by a smelling substance, to discourage drinking.
On page 30 the Soapwort *Saponaria officinalis* is mentioned. You will find the plant at waysides and watersides. Occassionaly it is also kept as an ornamental in the garden. It growth 30 to 60 cm heigh and flowers from June to September.
The Wax Plant *Hoya carnosa* can be purchased in flower shops or ask somebody who owns the plant to give you a young shoot with leaves. In water in a vase it forms roots after a while. Plant it in a pot with garden soil and wait until it flowers, in order to study the fragrance and the daily changes in intensity.

Alfalfa-flowers: On page 33 the pollination mechanism of Alfalfa is described. You can study the pollination mechanism of the flowers and trigger with a match the mechanism. With luck and patience you might also observe how a bee visits a flower and how it activates this mechanism. The leafcutter bee *Megachile rotundata* is found in Germany only at the Kaiserstuhl, but there are numerous other wild bees which fly on the flowers of alfalfa.
Leafcutter bees *Megachile rotundata* can be purchased in the USA. They are in the breeding tubes consisting of cut leaf pieces and eclose some time after taken out of the refrigerator.

Globe and season: To see, how summer and winter come about with the differences in the length of the light periods, you can use a globe (described on page 44). But a ball is also sufficient. You have to hold and turn it in such a way that the axis is inclined to the light source (window in dark room) by 23°.

Colorado beetle: Colorado beetles (see page 48) you find on potatoe fields. The larvae as well as the beetles are showy coloured. If you find animals in the fall, collect them and put them in a high jar half filled with sand. Cover the jar with a net and feed daily with potatoe leaves until the bettle crawls into the earth. Keep the jar at a chilly place, for example in the cellar. In the spring the beetle comes back to the surface.

Head clock: Prepare an alarm clocks powered with a battery in such a way that it can be used as a 'stop watchuhr'. This is described on page 87. You can use it to test yourself or others whether you have a well functioning head clock.

6.2 More to the experiments

Here are some more items you need for the experiments and measurements.

Chemicals: Sugar, neutral red (drugstore), spirit (denaturated alcohol with pyridine).

Glasware: Glass disks (glazier), microscope slides for microscopic observations, cover slip to cover microscopic sections (both in shops for laboratory supply)

Equipment: Letter balance to weight small amounts of chemicals

Animals: Honey bees can be observed easily on flowers. A bee keeper will gladly show you what it looks like in the interior of a bee hive. There are also a number of movies on bees and their behaviour. Leafcutter bees *Megachile rotundata* can be sent from the USA, e.g. from www.jwmleafcutters.com. The Chironomid *Metriocnemus knabi* is found in the Northern States of the USA and Canada. In Europa is a related species, *Metriocnemus martinii*, which lives in the water of tree holes. I do not know how it hibernates. Perhaps you can find out. Pine Lappet Moth *Dendrolimus pini* an Kiefern. Silk Moth *Bombyx mori.* The giant silk moth *Philosamia cynthia, Hyalophora cecropia* and *Antheraea pernyi* can be purchased at insect börsen in the pupal stage. The butterflies mentioned for the butterfly clock (figure **??** and page 6.1) can be observed outdoors. There are well illustrated books on butterflies available. Parasitic Wasp (*Nasonia vitripennis*). Fleshfly *Sarcophaga.* Fruitfly *Drosophila litoralis.* Beach hopper *Talitrus saltator.* Sand wolf spider *Arctosa cinerea.* Monarch-butterfly *Danaus plexippus.*

Plants: Alfalfa *Medicago sativa, Stephanotis floribunda,* Wax plant *Hoya carnosa,* Limeplant *Citrus auranthium,* Orchid *Odontoglossum constrictum,* Persian Violet *Exacum affine,* Hammerstrauch *Cestrum nocturnum,* Daffodil *Narcissus,* Grass of Parnassus *Parnassia palustris,* Flaming Katy *Kalanchoe blossfeldiana*, twig of Elder, Morning Glory *Pharbitis,* american Evening Prime Rose *Oenothera biennis,* butterfly flowers, bee flowers, bird flowers, bat flowers, plants listed for the flower clock (page 12 and page 92).

Miscellaneous: Millimeter paper, centimeter ruler or triangle with millimeter scale, coloured cardboard, black paper/cardboard, tweezers, razor blades, cuvette with plastic disk, flask, thermometer, mirror.

6.3 Measurements, evaluations, computer

The Internet is your friend if you need informations on companies, where you can buy items which you need for the experiments and for many other informations.

Concerning measurements, evaluations and the use of computers you find more in Engelmann (2004b). I use the operating system Linux on my PC. It is cost-efficient, reliable and there is helpful support by Linux groups (check in the Internet for Linux-User-Groups) in case of problems[1]. If you work with another operating system such as Microsoft and you do not want to change completely to Linux, you can download or puchase a Life Knoppix CD (by Hans Knopper or bookshop Lehmanns in various city in Germany). It contains a condensed Debian-distribution with numerous programs. You insert the CD in your CD-ROM and you can work under Linux without affecting your original system. You can also install Linux besides another operating system on the computer.

[1] for example the Linux-User Group Tübingen, which I own much help: lug-tuebingen@jura.uni-tuebingen.de

7 Epilogue

7.1 For the bookworms: Further books. Movies

There are two books on movements in plants, Hensel (1981) and Simons (1994), which contain many additional informations on the topics covered here. In both books experiments are described. A book has been published by Engelmann and Klemke (1983), in which experiments on rhythmic events in plants and animals are presented. It is out of print, but perhaps still available in libraries. A more recent book on experiments *Rhythms in organisms* is available in the Internet under Engelmann (2004b). Also in the Internet is the book *Rhythms of Life* (Engelmann (2007)). This is, however, written for students and specialists. Some books on interesting outdoor excursions for children are by Paatz (1938), Paatz (1947), and Baer and Mühlbauer (1990), but unfortunately all out of print.

To get to know the microscope see the following books: Drews (1992), Gerlach (1987), Jung (1998), a thorough introduction in the die microscopy and its technique, Kremer (2002), a new book on microscopy, well and nicely illustrated, with precise instructions and a rich source for microscopic ecercises and hints into the micro-world, Nachtigall (1998) with practical instructions and proposals for selecting objects. I recommend especially Wanner (2004).

I have written some more books or am in the process of writing. They are also concernded with topics which have to do with rhythmic processes in organisms - my special field as a scientist: *How plants grow and move:* Engelmann (2004a), *Biocalendar: The year in the life of plants and animals:* Engelmann (2009a), *Rhythms in structures of organisms: Engelmann (2004c), Flying clocks - The clocks of Drosophila:* Engelmann (2009c), Clocks which run according to the moon - Influence of the moon on the earth and its life: *Engelmann (2009b), Our internal clocks - Biological timing in humans and other mammals: Engelmann (2009d), Rhythms of life - An introduction using selected topics and examples: Engelmann (2007)*, Rhythms in organisms - Observing, experimenting, recording and analyzing: Engelmann (2004b)

Several movies on bees are available, which can be borrowed by schools and other institutions from the Institut für den wissenschaftlichen Film in Göttingen. I list some of them in the following[1]:

- *Colour sense of bees:* Frisch and Lindauer (1977)
- *Language of bees:* Frisch (1936)
- *Development of the honey bee:* Frisch (1950a)

[1] the designation C3 or the like are the order numbers of the movies. Catalogues of the Göttingen Institution are available in most schools (at least in Germany, Austria and Swizzerland) or can be obtained from: Institut für den wissenschaftlichen Film, Nonnenstieg 72, 37075 Göttingen

- *Distance- and direction instructions in the honey bee -round- and wag-dance:* Frisch and Lindauer (1978)
- *Verification of colour sight in the honey bee:* Frisch and Lindauer (1977)
- *Pollen- and nectar collecting of the honey bee:* Frisch (1950b)

7.2 Some remarks regarding deficiencies and further plans

I have written this book gladly, but noticed during the work, how much more difficult it is to write a specialized book for young readers as compared to a scientific paper. I know the many deficiencies and beg the ‘Guinea pigs’, which are the first one to read it, parents, children, teachers on feedback (errors, forgotten, unintelligible or mistakable items, ...). This is the only way to improof it.

It is planned in a later version to have certain illustrations as short animations (for example the *Kalanchoe*-petal movement in time lapse). Parallel to it I plan also to make the book available as a CD with a cover large enough to contain some seeds, chemicals and aids (for example polarization foils for demonstrating, how a bee sees the sky).

Finally I am planning to produce a movie together with a specialist about the topics treated in this book.

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