Flower clocks
Time memory
Time forgetting

University Tübingen 2015

Wolfgang Engelmann
Bernd Antkowiak

Flower clocks, time memory and time forgetting

Wolfgang Engelmann and Bernd Antkowiak
Universität Tübingen 2016

This book consists of 189 pages. It was written by using LyX, a professional system for typesetting documents http://www.lyx.org. It uses the typesetting system LaTeX. Vector grafic illustrations were produced with xfig and Inkscape under Linux. For diagrams PyxPlot was used. An older English and German Version is available under http://nbn-resolving.de/urn:nbn:de:bsz:21-opus-38007 and http://nbn-resolving.de/urn:nbn:de:bsz:21-opus-38017.

Dedicated to Karlheinz Baumann in admiration
of his nature movies

Contents

List of Figures

List of Tables

Introduction

This book was published for the first time in 1998 and revised in 2002, 2004 and 2007. In the meantime more is known about the treated topics. Therefore I have asked Bernd Antkowiak to join me in a new edition; not only to update the text, but also to revise the illustrations. In addition, we have produced and added a number of short videos to demonstrate movements in plants (page 149 and following pages).

The overall format of the book was maintained, but the sequence of Chapters 1-5 were changed. In chapter 1 some examples are presented of plants, the flowers of which open and/or close at a certain time of the day. This might occur just once, when the flowers open and stay open until they wilt after some time and form seeds (eg various morning glories such as *Pharbitis nil,* video on page 152, the grass lilly *Anthericum ramosum*, video on page 149, *Cichorium intybus*, video on page 150). But there also exist flowers which open and close for several days before wilting (eg *Kalanchoe blossfeldiana*, video on page 151, numerous Asteraceae such as *Bellis perennis*, video on page 150 *Taraxacum officinalis*, video on page 152, *Tragopogon pratensis*, *Crepis biennis*, *Calendula officinalis*, *Centaurea cyanis*, video on page 150, Mesembryanthemum such as *Delosperma sutherlandii*, video page 150, water lilies such as *Nymphaea alba*, video page 151, Ranunculaceae such as *Ranunculus aconitifolius*, *Ranunculus ficaria,* video on page 152).

In chapter 2 you will get to know the interplay between flowers and insects, by which the flowers are pollinated and these insects obtain food. For bees their time memory is important in this connection, by which they are able to use the offer of pollen and nectar by the flowers at times when available.

A special kind of time memory also exists in humans, the head clock. This time sense allows some people to undergo certain actions at certain times of the day or night without the help of clocks or other time cues, as reported in chapter 3.

Besides this time memory there also exists a time forgetting: Insects are able to survive unfavorable periods by entering a diapause. In this stage they are able to outlast coldness, heat or dryness. It can persist for years. Four different examples are presented in chapter 4.

Chapter 5 shows the example of a sandhopper which is able to orientate itself in its environment by using the sun, in order to escape in a certain direction. Further examples for this sun compass are given.

Finally we propose some experiments (chapter 7). We mention books which help the reader to deepen the treated topics, and movies which illustrate subjects of this book.

1. Flower clocks

Here we will talk about flowers, the petals of which open and close rhythmically which can last for several days as in the case of the flaming katy Kalanchoe blossfeldiana. *Other plants open their flowers at certain times of the day or night, and wilt afterward. The underlying mechanisms are partly known. A flower clock can be laid out in a school garden.*

1.1. Opening, closing and wilting of flowers

In a review article of **?** numerous examples are presented for the opening, closing and wilting of flowers. The authors describe the various kinds of opening and closing eg in *Oenothera* (see page 17). Rapid opening also occurs, if the edges of calyces are held together by special structures, until the petals unfold and the developing pressure causes the petals to separate. Various types can be distinguished (eg the cap of poppy flowers).

The flaming katy *Kalanchoe blossfeldiana*[1] is native to the

[1] animals and plants are named in such a way that the first part (here: *Kalanchoe*) stands for the genus (the genus human would be *Homo*) and starts with a capital letter. The second part of the name stands for the species (here: *blossfeldiana* after a seed trader Blossfeld; the species of the present humans would be *sapiens*; the full names are therefore *Kalanchoe blossfeldiana* and *Homo sapiens*). The species name has no capitals. Other species of the same genus are *Kalanchoe daigremon-*

island of Madagascar east of Africa. There it grows on dry places. To prevent drying out during the hot season it develops thick fleshy leaves, in which water can be stored. This property and how the seasons can induce succulent leaves has been studied intensively in Göttingen (**??**).

After the winter in Madagascar the *Kalanchoe* begins to flower. Up to 300 of the small deep red flowers can develop on one plant. Each flower produces numerous seeds, which are tiny and very light.

The plants are often sold in flower shops and by gardeners around the Christmas time for two reasons. Firstly, they look pretty, and secondly, they belong to the shortday plants. These plants flower during short days, that is during our winter time. Flower shops can therefore sell the blooming plants during Advent, Christmas and end of the year.

1.1.1. 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. This cycle is repeated each day for up to two weeks, until finally the flowers wilt and the numerous tiny seeds ripen. Opened and closed flowers are shown in figure 1.1 and the right figure shows the opening and closing schematically. Two videos (see page 151) show the opening and closing of the flowers at the inflorescence of a plant in a light-dark change with 12 h light and 12 h green light. The latter equals darkness for the

tianum (devils backbone, see *Wikipedia*) for our plant example and *Homo heidelbergensis* for an extinct human species.

plant: Light is not absorbed in the green range of the spectrum –therefore the leaves look green.

Figure 1.1.: *Flowers of the flaming katy* Kalanchoe blossfeldiana *are open during the day (left), during the night they close (center). The flowers possess four tips above the flower tube. At the lower end of the flower is the green calyx and the peduncle. How the petals open during the day and close during the night is schematically shown in two hour intervals on the right.*

Figure 1.2: Kalanchoe *flowers are mounted in holes of a plastic disk which floats on a water-filled cuvette. They can be photographed for instance every three hours from above. The flowers open during the day (as shown here) and to close during the night in the same way as in intact plants.*

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 of a thin plastic disc. The disc floats on a cuvette filled with water (figure 1.2). If we watch the flowers, we will notice that they continue to open in the morning and to close during the night.

Now the detective in us rises up and asks: How are the petals able to move? And how does the flower sense that it is night and it has to close or that it is day and it has to open?

To answer the first question, we have to look at the flowers more closely. 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 1.3). The tips of the petals are the ones which move. They bend outward during the day: The flowers open. During the night the petals move upward, resulting in closed flowers. In order to understand how the movement is accomplished, we have to look at these petals more closely.

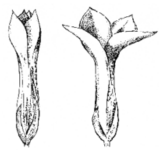

Figure 1.3: Kalanchoe *flowers during the night (left) and during the day (right). In the night the petals are bend upward and therefore the flowers are closed. During the day the petals bend outward: The flowers open. Details and how it works is explained in figure 1.5, 1.6 and 1.7.*

We cut the petals into thin sections with a razor blade and put them in a drop of water on a microscope slide[2] and observe them under the microscope[3] (figure 1.4).

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

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

For this purpose we prune the pith of an elder twig with a knife. It is moistened with water and notched with a new razor blade at the top center (be careful, very sharp). The tip of a petal is clamped in this slit in such a way that a bit of the tip shows above the pith. With the razor blade we cut very thin cross sections perpendicular to the longitudinal direction of the pith (do not push, but pull). The sections are transferred with the tip of a wet brush and placed in a drop of water on a microscope slide. The elder pith sections can be thrown away. We make a lot of sections, because we need some practice in order to obtain real thin sections. With a pair of forceps the edge of a cover slip is positioned close to the drop of water and slowly lowered on the sections in the drop. Under the microscope you can now look for the best section.

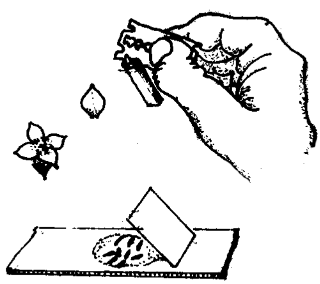

Figure 1.4: *In the pith of an elder twig the tip of a petal of a* Kalanchoe *is cut with the razor blade in very thin sections and observed on a microscope slide under the microscope.*

Under the microscope cells in the various tissues of the petal are recognizable. There is an upper cell layer (*epidermis*) with papillae like cells (kind of half a balloon, see figure 1.5). They are colored red. Below are about 15 layers of so called *parenchyma cells*. They form a loosely arranged cell accumulation with much air space between the cells. The lowest cell layer is a *cobble epithelium* with interlocked cells (lower right part of the figure).

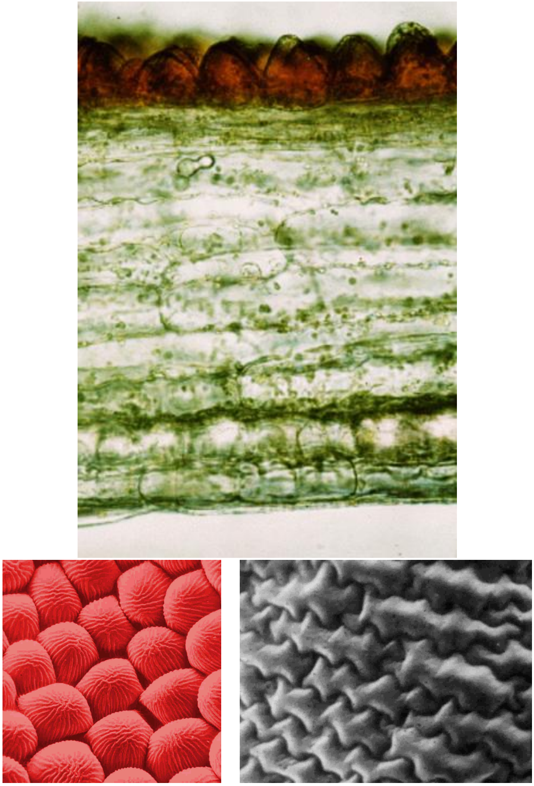

Figure 1.5.: *Top: Longitudinal section through a petal shows an upper layer of so called* epidermis cells *which are structured like papillae. They are colored red. Underneath are several so called* parenchyma cells*. The lower epithelium is a cobble epithelium: The cells are interlocked with each other. Scanning electron microscope picture of upper (bottom left) and lower epidermis (bottom right).*

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 such a cell in more detail (figure 1.6). The motor cells shrink and swell, depending on the 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 probably serves as a bendable end trestle for the extending or shrinking motor cells in the parenchyma. The upper epidermis with its papillae cells is 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 1.7.

Similarly the papilla cells can shrink and swell (Figure 1.8).

1.1.2. An internal clock opens and closes the *Kalanchoe* flowers

Now the detective in us awakens again 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 a weak green light shines all the time[6]. This is shown in the second part of the video, in which the green light was also on during the day and in the following night (page 151). What a surprise: Even

[4] salt here doesn't mean table salt (sodium chloride), but salts in a chemical sense such as potassium chloride.

[5] if table salt is dissolved in water, ions will be formed: Sodium ions are positive, chloride ions 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 g of sugar to 1 l of water or 6.8 g of sugar to 100 ml of water. The flowers now move much longer compared to pure water.

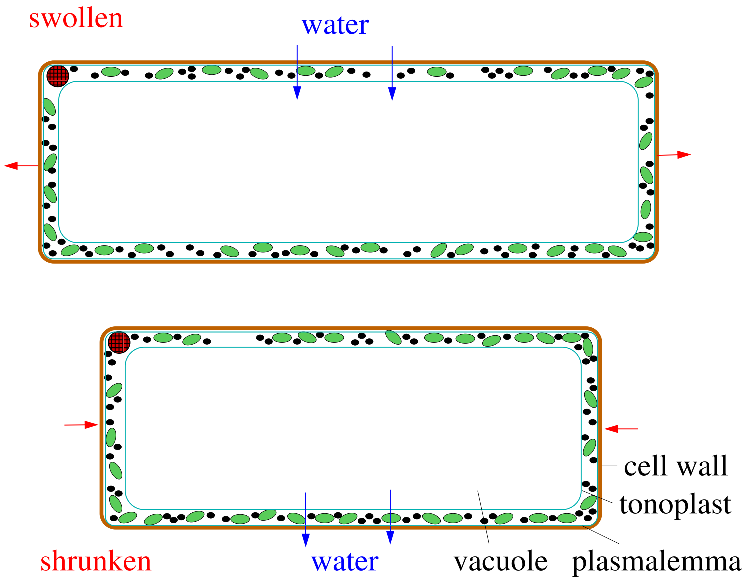

Figure 1.6.: *A motor cell of* Kalanchoe *consists of a wall (thick), the* cytoplasm *and a central bubble (*vacuole*). Between cytoplasm and cell wall is a membrane called* plasmalemma*. Between cytoplasm and vacuole is a membrane called* tonoplast*. The cytoplasm contains a nucleus (larger blob top left) and organelles with diverse tasks. Mitochondria (small items, drawn black) support the cell with energy, in the chloroplasts (oval green) carbon dioxide is converted to sugar with the aid of light. The vacuole is a bag filled with water and dissolved salts. If there are plenty of salts in the vacuole, water is sucked (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.*

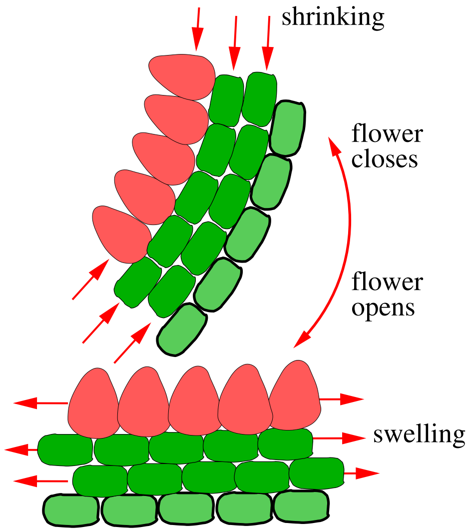

Figure 1.7.: *The motor cells shrink and swell depending on the salt content of their vacuoles (see figure 1.8). If they swell, the parenchyma cells extend (lower part of the figure, red arrows. In the scheme only two cell layers are shown instead of 15). The lower epidermis serves as a bendable end trestle. 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).*

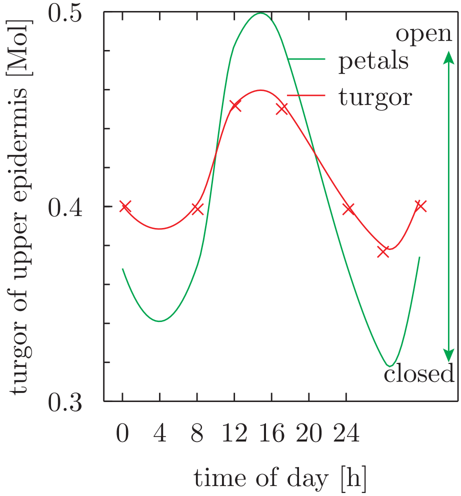

Figure 1.8.: *The opening and closing widths of flowers were measured (red x with curve) and the turgor determined at the given times (green curve). For this purpose the upper epidermis, that is the papilla cell layer, of a flower was removed with a pair of forceps at the indicated time and its turgor determined (how this is done is described in* **?***). With increasing turgor (green curve turns upward) the flowers open, with decreasing turgor they close.*

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 due to temperature cycling.[7] If we determine now the period length of an oscillation[8] of this petal movement under continuous green light (and at a constant temperature), it is not exactly 24 h anymore, but only 22 h. Thus the flowers open each day two hours earlier as compared to a normal day with light-dark cycling. Apparently the flowers possess an internal clock which controls the opening and closing. This clock is faster by 2 h as compared to the length of a natural day which its 24 h. It is therefore called circadian clock (from Latin *circa* = about and *dies* = the day).

1.1.3. The circadian clock runs at different temperatures 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 special mechanisms into their clocks which ensure that they run with the same speed independent of the temperature. Circadian clocks also possess a mechanism which makes sure the period is independent of the environmental temperature. If *Kalanchoe* flowers are observed under weak green continuous light at 15° C and at 20° C, the period length differs only slightly (see

[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.

[8] that is the time between fully opened flowers until the next point in time of fully opened flowers and it is called period length or period for short.

figure 1.9). That is not self-evident, because most processes in

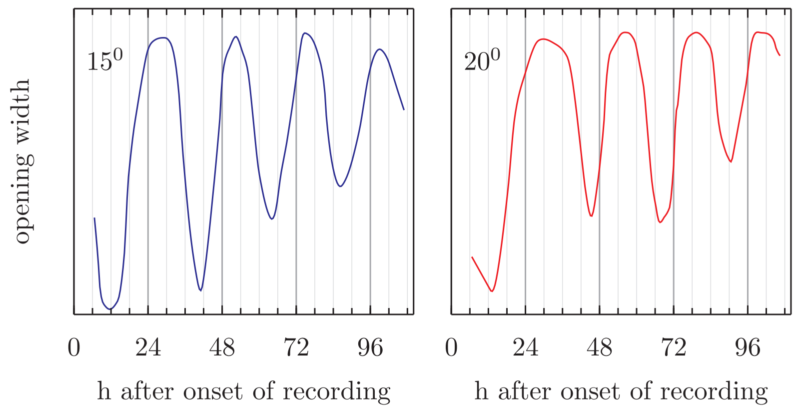

Figure 1.9.: *The movement of* Kalanchoe *flowers was recorded at a temperature of 20° C (red) and of 15° C (blue) at different times and plotted as diagrams. At both temperatures the flowers open (increasing values) and close (decreasing values) rhythmically. Maximal opening is somewhat earlier at 20° C as compared to 15° C, but that is also true for the maximal opening in the following three days. The periods between the maximal opening are, however, almost identical. Thus, the clock in the motor cells runs with the same speed. After* **?**.

organisms proceed faster at higher temperatures. 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.

1.2. A flower clock in the school garden

We have just seen that the flaming katy *Kalanchoe* opens and closes its flowers each day.[9] Only after the flowers are pollinated and the seeds have formed, does the movement stop and 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 wilts in the evening (figure 1.10, **?** and two videos on page 152). In *Pharbitis* the opening and closing of the flowers is due to bending of the midrib of the petals. It is caused by turgor changes of the inner epidermis cells of the midrib (**?**).

Some plants have even been named according to the time of day at which their 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 pm[10].

Linné (figure 1.11) constructed a flower clock in 1751: Various plants are planted as a kind of clock-circle in a round garden

[9] Unfortunately *Kalanchoe* plants were bred so that the flowers do not move any more. They were thought to look more attractive if the flowers do *not* close. But wouldn't it be much more interesting, if one could observe these movements? You should have a look at the plant in the evening and check whether the flowers have closed, before buying it, or you cultivate the plant from seeds obtained from a Botanical Garden.

[10] 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 color of the flowers (EMail of February 4th, 2002).

Figure 1.10.: *The flowers of the morning glory* Pharbitis *blossom out in the morning by unfolding the petals of the flower bud. 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. Images from a video, see page 152.*

bed in such a way, that their flowers open or close at the corresponding day- and night-times (figure 1.12).

Figure 1.11: *The Swedish botanist Carl von Linné. Drawn by Mareike Förster after an image in* **?**.

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 throughout the day, but at distinct times of the day. Therefore one can also establish a butterfly clock (see figure 1.13).

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 1.14). It flowers from June until the end of October. Each evening between 8 and 10 pm one or several flowers open so rapidly, that one can observe it (figure 1.15). You should absolutely watch that spectacle. The petals unfold quite jerkily, because the calyces are connected with each other by a kind of zip fastener mechanism. The locking mechanism between petals ruptures suddenly by the pressure exerted by the unfolding petals, and after a few minutes the flower is completely unfolded. At the

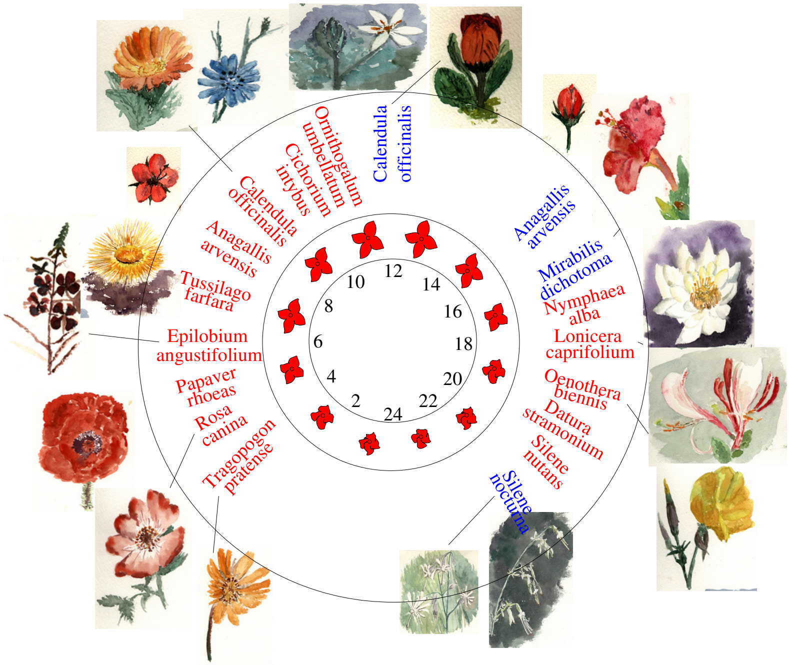

Figure 1.12.: *Between 1 o'clock and 24 o'clock flowers open (o, names red) or close (z, names blue). Illustrations at the margin, in the center* Kalanchoe *flowers at various times of the day (see figure 1.1). Names of the plants clockwise: o 8* Anagallis arvensis, *o 9* Calendula arvensis, *o 6* Cichorium intybus, *o 10-11* Ornithogalum umbellatum, *z 12* Calendula arvensis, *z 15-16* Anagallis arvensis. Mirabilis dichotoma, *z 17* Nymphaea alba, *o 18* Lonicera caprifolium, *o 19-20* Datura stramonium, *o 20-21* Silene nutans, *z 21-22* Silene nocturna, *o 3-5 Tragopogon pratense, o 4-5* Rosa canina, *5* Papaver rhoeas, *o 6-7* Epilobium angustifolium, *7* Tussilago farfara. *After* **???***. Water colored by the author WE. At page 155 these and further plants are also specified with the common English names, partly with video links.*

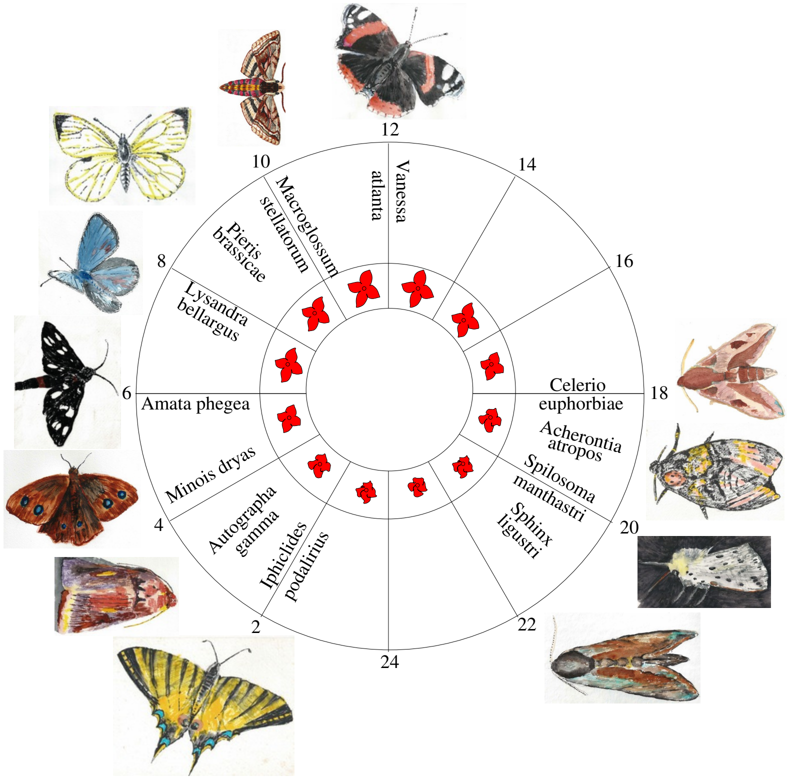

Figure 1.13.: *A butterfly clock shows the time of day (numbers) at which the butterflies become active. From 8 clockwise: Eros blue* Lysandra bellargus*, 9 cabbage butterfly* Pieris brassicae*, 10 hawk moth* Macroglossum stellatorium*, 11 red admiral* Vanessa atalanta*, 18 Mediterranean hawk-moth* Celerio euphorbiae*, 19 death's head hawk-moth* Acherontia atropos*, 20 dogbane tiger moth* Spilosoma monthastri*, 21 privet hawk moth* Sphinx ligustri*, 2 swallowtail* Iphiclides podalirius*, 3 gold spangle* Autographa bractea*, 4 dryad* Minois dryas*, 5 nine-spotted moth* Amata phegea*. Images water colored by WE after* **?** *and other templates.*

Figure 1.14: *The evening primrose* Oenothera biennis *flowers between June and the end of October. Each evening between 8 and 10 pm one or several flowers open (see the series of images in figure 1.15). At the same time the flower emanates a sweet fragrance, which attracts moths.*

same time the flowers emit 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 circadian clock (**?**).

In *Oenothera lamarkiana* blue light (400 to 510 nm) inhibits the opening of the flowers. It affects the lower third of the calyces (**?**).

Figure 1.15.: *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 8 (top left) and 9.15 pm (bottom right). The first three images were taken in intervals of 20 min, the last five in a few minutes. Photographed by the author WE.*

2. Flowers and insects

Many flowering plants are pollinated by insects. Cross pollination is of advantage for the plants. The insects are attracted by fragrance, color 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 themselves and the hive. They use an internal daily 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 pollinate the flowers of plants of the same species (see e.g. figure 2.1). This prevents flowers from being fertilized with their own pollen. For organisms cross pollination is more advantageous than self pollination, since the genome will differ to a higher degree. 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 pharaohs, problems arise easily: Often the children suffer under hereditary diseases (**??**), http://de.wikipedia.org/wiki/Inzest.

The flowers adapted during the course of evolution of life on earth to certain insect groups and are pollinated by them. Butterfly flowers are pollinated by butterflies (figure 2.2), bee flowers (figure 2.3) by bees.

Parallel to this adaptation the insects also adapt to flowers.

Figure 2.1: *In the flower of the Meadow sage* Salvia pratensis *the stamen (brown) is pressed against the back of the thorax by a lever and the pollen is wiped off, if a bee tries to reach the nectar source. Image by the author WE after* **?***; more there.*

For them it is important to find the flowering plants, to remember good nectar- and pollen- donators and also the time at which food is offered by the plants. For more detailed information in the context of flowers and insects see the book by **?**.

Insects are mainly attracted by pollen and nectar of the flowers as food, whereby color, shape, pattern and fragrance are important. In bumblebees (*Bombus terrestris*) it was shown, that electric fields around the flowers play a role (**?**). These fields also vary in pattern and structure, partly on a flower in a matter of seconds, an advertisement, which acts rapidly and dynamically upon the pollinators.

Flying insects such as bees and bumblebees are normally positively charged, whereas flowers often possess a negative potential. In this way the pollen can be transferred more easily and it sticks better. Depending on the condition of the pollen the potential of the flowers changes. Bumblebees, which are

Figure 2.2.: *Phlox as an example of a butterfly flower. Moths fetch only nectar from the flowers and use a long proboscis which they roll out and insert into 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.*

Figure 2.3.: *Left: Bee wipes off pollen from a willow catkin and forms pollen panties at the hind legs. Center: Bee licks nectar on a borage flower (borage* Borrago officinalis, *flowers from June to the fall). Right: Bee visitor at thyme. Bee flowers are often blue, yellow or white colored (often additional UV-markings which we can not see in contrast to the bees). Besides nectar they offer plenty of pollen. Fragrance is of intermediate strength and often honey-like. Bee flowers are open during the day and the bees must be able to land on the flowers. Photographed by author WE.*

themselves charged, are able to sense these fields and their changes and recognize in this way the condition and the kind of the flower. Similar observations were made in bees (*Apis melifera,* references in **?**).

Flowers and peduncles of *Petunia* and other plants (*Gerbera*, *Digitalis*, *Geranium* and *Clematis*) possess an electric field, which is influenced by approaching and landing bumblebees. It can be made visible by electrostatically colored powder, whereby the density of the powder reflects the field strength at the surface of the flowers (see experiment on page 161). A 30 cm sized plant has an electric field in the atmosphere with a potential difference of 30 V as compared to the earth.

The potential already changes before landing (see video Electric field), it is thus directly provoked by electrostatic induction between the charge of the bee and the flower. Pollinators are able to influence flower signals by leaving odor marks on the petals or by initiating flower signals such as color, form and humidity, which occurs in periods of minutes and hours. The potential changes, however, occur in seconds. Due to the electric fields the bees can learn faster and more precisely, which flowers offer good food (references in **?**).

Some flowers also offer protection and warmth.

Flowers open and close often rhythmically or at certain times. We have heard about it already in the first chapter. Other rhythms are found in flowers: Pollen- and nectar-offering, fragrance, and heat production. It is thus advantageous 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 the insects to get to know the flowers better and find pollen and honey faster and more effectively. This behavior 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 which guarantees 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 **?** while having his breakfast on the veranda of his vacation home. The bees helped themselves 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 assumed 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 be available.

Later, von Frisch and his students did numerous training experiments with bees (**?**). They offered the bees concentrated sugar water and at the same time specific signals such as color, fragrance, and 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 2.4). 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. They are effective already after a single trial. For colors they needed three to four trials, for learning the time of offer, six to ten, 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 h. Because pollen and nectar and the connected signals such as fragrance are often offered by the flowers only at certain times of the day, bees use special collecting hours.

The proficiency of bees in searching for pollen and nectar is even more admirable. Once a forager bee has found a new source of food, it is able to tell its co-workers in the hive, in which direction and how far they have to fly to reach the plant. In this way the bees find food faster thereby saving energy. How this is done is explained in figure 2.5 and e.g. under Bienentanz.

A bee flies 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 finds a remunerating cherry tree. It collects plenty of pollen from the flowers and strips it as panties to its hind legs. Furthermore it sucks nectar from the flowers and stores it in its honey stomach. Being full it flies back to the hive. There it lands at the entrance board and enters the dark hive to the combs where breed is reared.

At the comb it performs a dance, which is shown schematically in figure 2.6 in the interior of the hive: It runs upward (long red arrow) and wags with its abdomen. Then it turns, runs downward (short red arrow) and repeats the same dance. This round dance (short red arrow) and waggle dance (long red arrow) is repeated several times. During the dance other forager bees follow the dancing bee. In doing so they obtain a lot of information: whether the forager found either pollen or

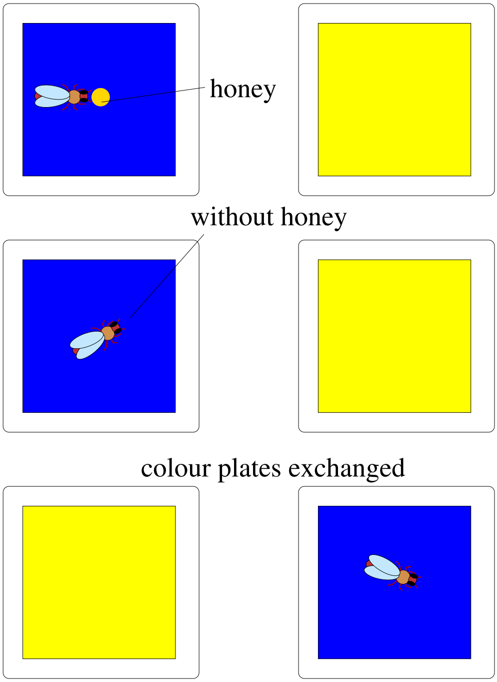

Figure 2.4.: *Bees are trained for certain colors, here blue, by offering some honey on a glass plate. A colored cardboard was placed underneath the glass. The glass plate on top of the yellow color was left empty. After the training period a glass plate without honey is placed on the colored cardboard. 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 memorize the location where honey was offered.*

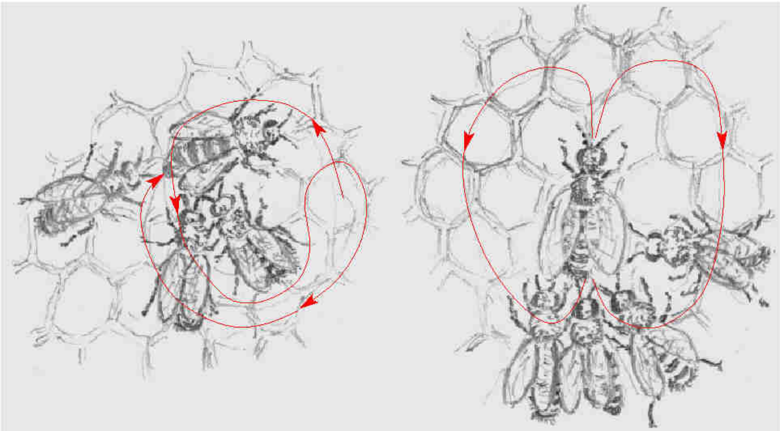

Figure 2.5.: *Forager bees are able to communicate to their coworker bees in the hive, in which direction a source of food can be found and how far away it is. Furthermore fragrance and quality of the food is signaled. Left: Round dance. The forager bee dances at the vertically hanging comb in the hive 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: Waggle dance. If food is 250 m or further away, the foragers in the hive are informed about the food by a waggle dance. It looks like a flat 8 and informs about direction and distance of the food. How this works is shown in figure 2.6 and explained under Bienentanz.*

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 upward in the dark hive. Since our bee found food in direction of the sun, its waggle dance will point upward (long red arrow). Another 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 2.6). To transmit the direction, the angle between the food position and the sun is coded in the waggle dance. This *sun compass orientation* also works under overcast sky. Small patches of blue sky still allow the bees orientation by using the polarization pattern of the sky to determine the suns direction (see figure 2.7 and Polarisation pattern). You might like to observe this typical polarization of the sky and how it turns during the day with the sun's orbit by looking through a circular polarization star-foil[1] (see figure 2.8).

If individual bees are caught on the way from the food source to the hive and kept in darkness for some time, they are still able to transmit the direction of the food correctly to other foragers, if brought back into the hive (figure 2.9). Since they were unable to observe the way of the sun during their confinement, they must possess an internal clock with the 24-h-rhythm of the orbit of the sun. This clock allowed the bees to remember the time interval since transferred into the hive. They have therefore taken into account the course of the

[1] Obtainable in photo shops for camera lenses, but also in the Internet trade as cheap foils. Sun glasses can also be used (try out, whether they do indeed polarize by looking into the sky before buying).

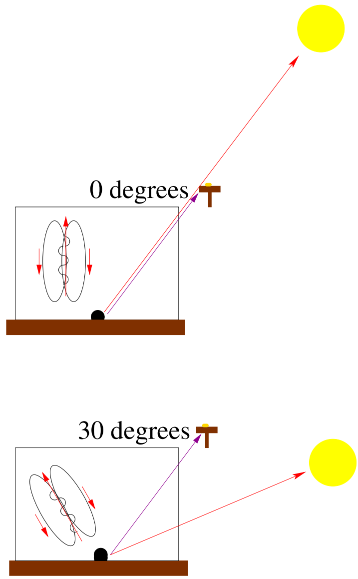

Figure 2.6.: *Sun compass orientation of the honey bee and waggle dance. In the upper sketch food (F) is in the direction of the sun. The forager projects this direction upward in the vertically hanging comb of the dark hive. It informs other foragers about the direction by performing a waggle dance as described before. In a figure-eight pattern the bee runs down at the side and then 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 is* 30^0 *left of the sun. The waggle dance is consequently inclined by* 30^0 *to the left. After* **?**.

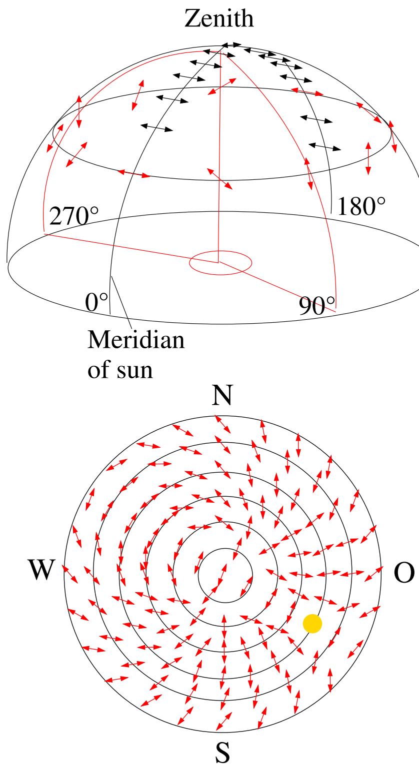

Figure 2.7.: *The polarization pattern of the sky changes in a characteristic way during the day. It can be recognized by the bees and with it the time of day, provided a small piece of blue sky can be seen. Top: Hemisphere of the sky showing the direction of the polarization of light, indicated by arrows 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* **?***.*

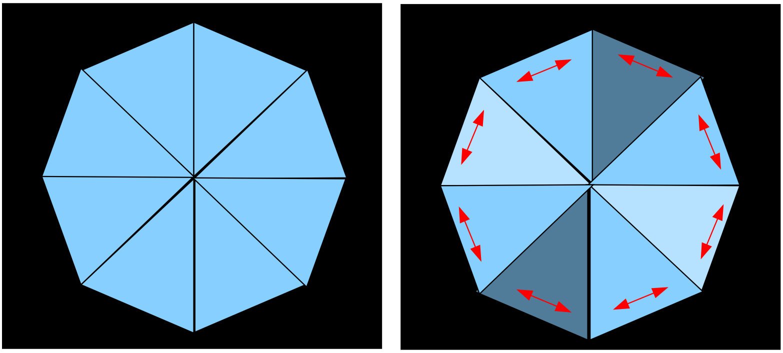

Figure 2.8.: *Cut 8 triangles out of a polarization foil in such a way, that the direction of polarization (red double arrow) runs parallel to the outer margin of each triangle. Insert them between two glass plates and put an adhesive at the edges to prevent the foils from getting out of place. Watch 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 (illustration on the right). 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 compound 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 2.7) and in a way as you see it with your star foil. After* **?**.

sun (15° per h) and made provisions for it when displaying the waggle dance.

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

The distance from the hive to the food is also signaled. If the food source is far away, they waggle less frequently with their abdomen, if the to food is closer to the hive, they waggle more frequently (figure 2.10).

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 bee dispatches the nectar into a cell of a *honey comb*. The pollen is transferred to a *comb cell*, which was prepared for the laying an egg by the queen (figure *2.11*). Out of the egg emerges a larva which feeds on the previously deposited pollen. Finally a young bee will hatch and take over the necessary work in the hive allowing the population to survive.

2.2. Other tricks of plants to become pollinated by insects

In bogs and infrequently used wet agriculture such as swampy meadows and the wet parts of dryer meadows you might find grass of Parnassus *(Parnassia palustris)* (figure 2.12). The flowers contain five filamented stamen arranged in a circle around the ovary (figure 2.12). One of the five stamens is

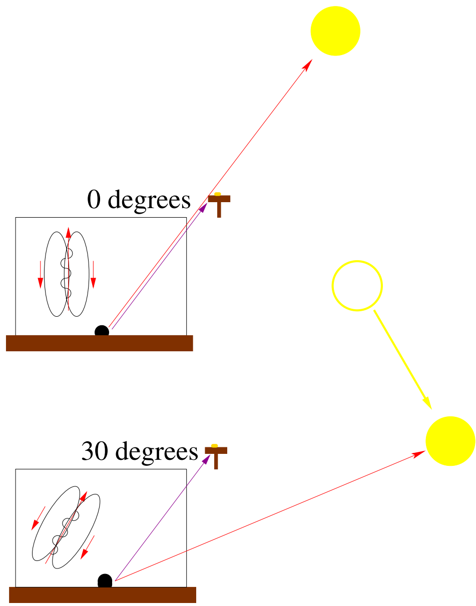

Figure 2.9.: *Upper image: A bee displaying a waggle dance in the hive: It found food in direction of the sun and transmitted this direction to other foragers by running upward on a comb. Lower image: Bee displaying a waggle dance after having been confined for 2 h in a dark box on its way from the food 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 30° more westward after two h, 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 during the confinement was before being returned to the hive. The bee took the path of the sun (15° per h) into account and indicated the correct direction during its waggle dance.*

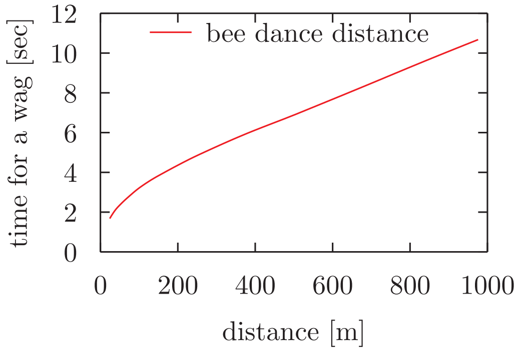

Figure 2.10.: *During a waggle dance the distance of the food source from the hive is signaled to other forager bees by wagging the abdomen more frequently at shorter distances and less frequently at longer distances. The curve shows how many seconds the forager needed for one completed wag turn at different distances of the food. For food further away it needs more time. After* **?***.*

Figure 2.11.: *View at cells with pollen (right, yellow) and with covered honey (gray lids) in the comb of a bee hive at the time of honey yield. Photos by the author WE, thanks to our colleague and bee keeper Walter Mayer.*

shifted over the top of the ovary by extension of the filament. The anther[2] 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, 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.

[2] a stamen consist typically of a stalk called the filament and an anther.

Figure 2.12.: *Grass of Parnassus* Parnassia palustris *is found in bogs and in wet, swampy meadows and the wet parts of dryer meadows. Blooms from July until September. Flowers contain five filamented stamen arranged around the ovary. One of it is shifted over the top of the ovary by extension of the filament. The anther opens face up, flies are landing on the flowers, getting brushed with pollen at their belly and distribute it. On the next day the filament bends outward and the anther is later shed. A new anther is shifted over the top and opens. This continues for five days. All anthers are now shed, only the stubs are left. Now the stigma with its three to four lobes 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. In this way the flowers are cross pollinated from plants of the same species and self pollination is prevented. Meanly the flies do not get any nectar from these flowers. They are deceived by small yellow-green knobs which shine like honey. They are made up by stamen which do not form anthers (recognizable as small structures in front of the petals). Unexperienced flies are indeed bluffed. Photographed by the author WE. See also* **?**.

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 am and noon. *Anguria triphylla* offers its nectar from noon to 7 pm (figure 2.13). The butterfly has

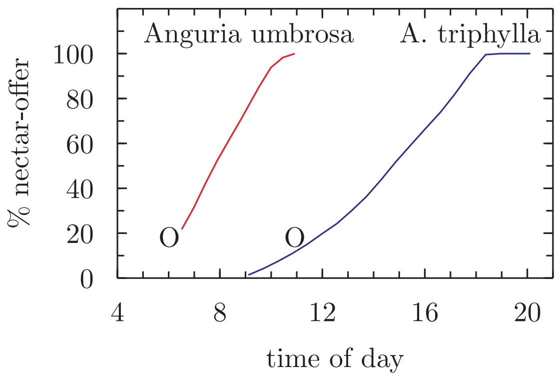

Figure 2.13.: *Nectar secretion of* Anguria umbrosa*, a pumpkin plant from the jungle of Trinidad, occurs between 7 am and noon (green), pollen a bit earlier (bottom left). In* Anguria triphylla *the nectar is secreted between noon and 19 pm (red), the pollen (bottom center) between 10 am and noon. After* **?***.*

adapted to it: There are two different species, one of which pollinates *Anguria umbrosa* and the other one *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.

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 often possess 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 have a smell (or also stink). Roses, spices such as lavender and thyme, or the evening primrose (we talked about it on page 17) are examples. At least 30% of all higher plants produce substances, which vaporize easily (are volatile) and therefore have a smell or fragrance. 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 one 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 to the water before. Neutral red is a color 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 be seen nicely in daffodil which possess a crown between the petals and the stamen and ovaries. It serves as a fragrance and color structure and furthermore possesses special markings which can easily be seen under ultraviolet light[3]: If daffodil flowers are stained with neutral red, the yellow crown turns red (figure *2.14*). The crown attracts the pollinators.

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. After* **?**.

Nectar guides 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 colors 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 plenty of nectar throughout the year. Bird flowers are found in the tropics and subtropics. More than a hundred of plant species possess bird flowers. Plants with bat flowers are also found in the tropics

[3] we are not able to see ultraviolet light with our eyes, but insects and especially bees can. Often these flowers show patterns under ultraviolet light 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).

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 colored white, 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 perfume industry by extracting them from the flowers with special solvers or with water vapor. However, nowadays many scents are also artificially produced (‘synthesized), because the chemical composition is known.

In the gentian family *Exacum affine* (figure 2.18), the Persian violet, flowers are continuously open, 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 observing them at home and to smell them. If it smells strongly, we enter a 3 in table 2.1 under the observation time, 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 are plotted in a diagram (see the red curve in figure 2.19).

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* **?**.

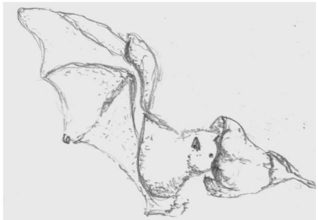

Figure 2.16.: *Left: As an example of a bat flower* Campsis radicans *is shown. Bat flowers occur in the tropics and subtropics, possess tough structured flowers with a lot of pollen and a slimy nectar. The flowers sit on long stalks elevated over the leaves. Photography of the author. Right: Bat flower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

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 tomato and potato to the nightshade family (*Solanaceae*). Photographed by author WE in the Botanical Garden Tübingen.*

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 spirit in a small flask (number 1) and dilute it. Spirit is denatured alcohol and smells of pyridine. If we pour 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 unable 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.

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.*

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* (**?**) and the soapwort, *Saponaria officinalis* (figure 2.20, curve of fragrance of *Saponaria*: figure 2.21). In

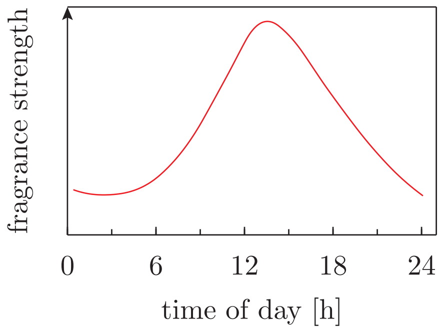

Figure 2.19.: *Diagram of the fragrance rhythm of the Persian violet* Exacum affine*. Clock time at x-axis, fragrance strength at y-axis. The red curve shows the fragrance strength over the day (maximum at early afternoon).*

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.

the same way the intensity of fragrance changes in the wax plant *Hoya carnosa* (**?**). In this and most other plants fragrance consists of several components. In *Hoya carnosa* the various components reach their strongest fragrance at the same time.

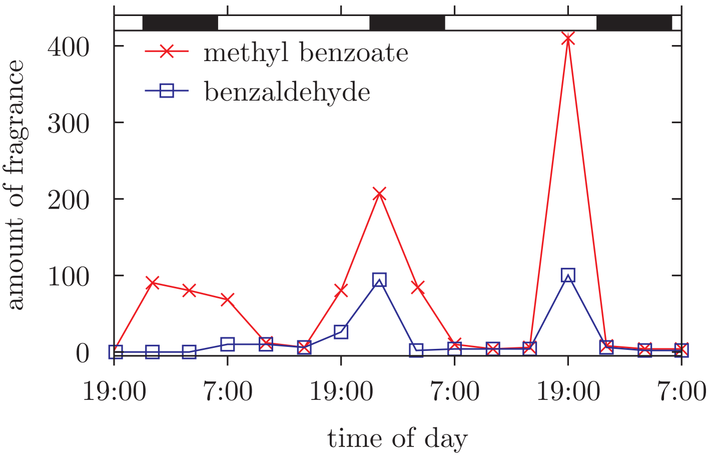

Figure 2.21.: *Fragrance production of the soapwort* Saponaria *was measured. The two compounds methyl benzoate (red curve with red values) and benzaldehyde (blue curve with blue values, actual values multiplied by ten, since the curve would have been too small) are both secreted in the evening, but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

In other plants the times of strongest fragrance can differ. In *Stephanotis floribunda* the maxima of 1-nitro-2-phenylethane and methyl benzoate are displaced by 12 h from each other (**?** 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

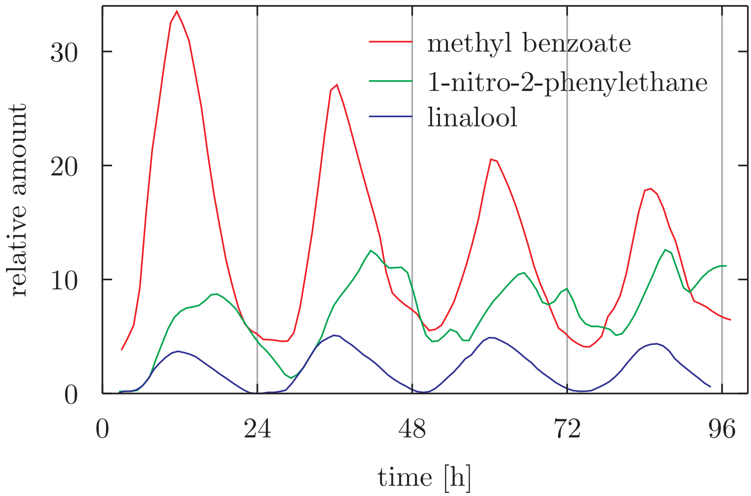

Figure 2.22.: *The fragrance production in* Stephanotis floribunda *was recorded with a gas chromatograph. The two fragrant substances methyl benzoate (red curve) and linalool (blue curve) are emitted at the same time of day, but in different amounts. The fragrant 1-nitro-2-phenylethane (green) has,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

flowers which scent during the day such as the bitter Seville orange *Citrus aurantium* or *Odontoglossum constrictum* do not display an endogenous rhythm of fragrance (figure 2.23).

Figure 2.23.: *Day fragrant plants such as the bitter Seville orange* Citrus aurantium*, a citrus plant, or the orchid* Odontoglossum constrictum *(constricted Odontoglossum) does not possess an endogenous rhythm of fragrance. Water colored by the author WE.*

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 parasitic

Figure 2.24.: *Fragrances of plants attract enemies of varmint insects as for example parasitic wasps. They oviposit the eggs in the caterpillar. The larvae of the wasps eat the caterpillars from inside. Water colored by the author WE, after* **?**.

wasps. The parasitic wasp females pinch the animals with their ovipositor and lay their eggs in their body. From the eggs larvae eclose which begin to eat the caterpillars or aphids from within. Ichneumon wasps are also attracted by scents of the excrements of caterpillars (**?**, figure 2.24).

2.4. How to earn money with leafcutter bees

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

Alfalfa[4] (*Medicago sativa* ssp. *varia*) is an interesting example (figure 2.25, **?**). 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 alfalfa fields, which are often huge. 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. A bee inserting its proboscis into the flower for obtaining nectar sets a flap mechanism in motion which ruptures the suture of the shuttle and as a result this opens. Stamen and stigma which where held by the shuttle flip upward and hit the underside of the head (figure 2.27). Honey bees do not like it. Older bees

[4] Spanish alfalfez, derived from the arabic al-fisfisa, “fresh food”.

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 2.26.: *Leafcutter bee* Megachile rotundata, *an important pollinator of alfalfa-flowers. Photography by Theresa Pitts-Singer (left, see acknowledgements, on page 176) and water colored by WE (right) after* **?**.

have learned to steal the nectar on the side of the flower, but as a result no pollen is transferred to the next flower.

The leafcutter bee *Megachile rotundata*, however, is not bothered by the 'hook to the chin' of the stamens of the alfalfa. Therefore it pollinates this food clover successfully. It belongs to the *Hymenoptera* like other bees (figure 2.26, see **?** and www.ars.usda.gov/npa/beelab).[5] It originates from Eastern Europe and Western Asia. In contrast to the honey bees it does not live in colonies, but solitary.

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, then upholstered with small oval pieces which they cut out of leaves from alfalfa- or other plants with their mouth parts (figure 2.28 and 2.29). The bee roles the leaf cut to a bag and flies with it to the bore hole (figure 2.29 right). There the leaf is pushed in the bore hole 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 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

[5] Leafcutter bees are distributed allover the earth. The genus *Megachile* contains almost 1500 species. Among them is the worlds 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.

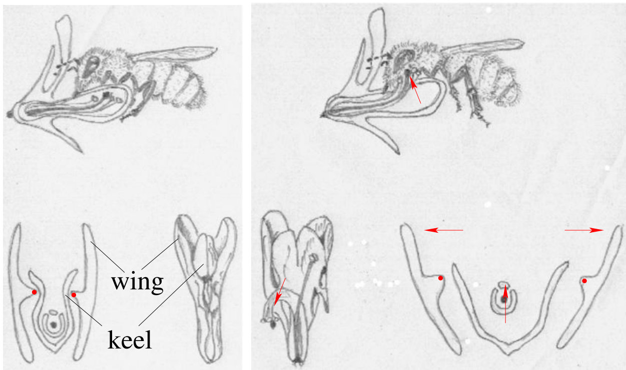

Figure 2.27.: *Pollination mechanism of alfalfa: A leafcutter bee tries to suck nectar from the flower of an alfalfa (top left). By doing so its head hits the flap mechanism which flaps the stamens of the flowers on its chin (top right). Stamens and pistil are kept down by the inner lip of the keel (marked 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 as the bee visits them. Drawn by the author WE after illustrations in* **?**.

Figure 2.28.: *If you find punched leaves like that, you have found the work of a leafcutter bee. Water colored by the author WE after* **?**.

Figure 2.29.: *Left: A leafcutter bee cuts a piece out of a leaf and uses it to coat the tube for its breed. Right: 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 colored by the author WE after a photography in* **?** *and* **?**.

thin layer on top of the dough. In less than a minute an egg is laid 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. It is filled again with pollen/nectar mixture, nectar and an egg. 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 stretched so much, that it would burst, if the larva would continue to feed. It stops feeding and the skin does indeed burst. However, before bursting a new larger and softer skin had formed. Now the larva can continue to feed, until it has to moult again. That occurs four times and takes ten days altogether. The gut is closed during the larval stages, thus preventing the food from becoming spoiled by the feces. 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 again growing 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, the eyes turn then pink to black, and finally the whole pupa turns grayish black. The pupal skin is stripped off, the

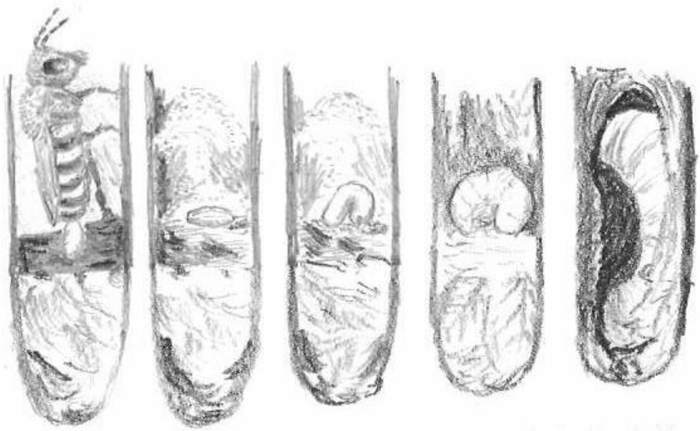

Figure 2.30.: *The leafcutter bees use tube-like structures as for example stalks as breeding chambers. With their mouth parts they cut pieces out of leaves (see figure 2.29) and cover the inner walls with it. Pollen is collected and deposited, and the final layer covered with nectar. An egg is deposited on it (left) and the chamber closed with several pieces of leaves. After oviposition the larva stays in its chorion (nr 2), ecloses in the second larval stage (nr 3), feeds on the supply in the breeding chamber, grows and moults several times (nr 4) until it reaches the prepupal stage (nr 5), which enters diapause. Sketched by WE after figures in* **?** *and* **?***.*

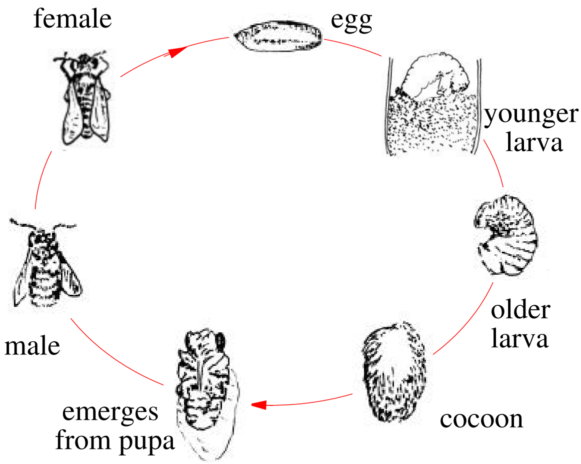

Figure 2.31.: *Life cycle of an insect with metamorphosis shown in the example of the leafcutter bee: From the egg ecloses a larva which lives in a breeding cell with food. It grows until the larval skin can not extend anymore. A new skin is formed underneath the old one, the old skin ruptures and the new larva ecloses out of the old skin. Two more larval stages follow until finally a prepupa is formed. This prepupa spins a cocoon, in which it hibernates or, in the summer, it metamorphoses directly 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 WE after* **?**.

wings unfold and the soft surface of the body hardens. The larva has turned into a bee. It chews a hole in the cocoon and pushes the lid of the breeding cell off in order to escape into freedom.

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 (chest of insects) 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 hectare of an alfalfa field[6] 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 rears 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 pollen and nectar. How effective *Megachile* works, is shown in the following comparison: For 100 kg seeds 100 000 honey bees have to work 6 h, 318 bumble bees 2 h, 75 wild bees and 1 *Megachile* 1 h. One female pollinates 9 to 40 flowers per min. In the case of large fields huts are build, in which the nest tubes of the leafcutter bees are placed (figure 2.32). It is worthwhile for the farmers to buy the leafcutter

[6] a hectare is an area of 100 times 100 m, that is 10000 m^2.

bees, since they can harvest 1 300 kg alfalfa seeds instead of 150 kg without them.

But how do the insects know that winter is approaching and that it is time to enter diapause? To wait until the first frost falls is too risky. The body cannot 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 the daylength for this purpose: In the fall the days become shorter and in the winter they are much shorter than they are in the summer. If a certain daylength is reached, as for instance 12 h at the 21st of September, development is ceased in a certain stage until the winter has passed and the environmental conditions are favorable again. More about it in chapter 4.

In the case of the leafcutter bee it is, however, not the daylength which is used for triggering diapause. It would be worthless anyway, since the larvae are inside the breeding tubes and surrounded by several layers of leaves which are additionally 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 if the nights become longer in the fall, it is also colder for a longer period of the day. If the animals would measure the length of the cold period of the day instead of the length of the dark period, they would have a kind of annual calendar at their disposal and would realize, that it 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*.

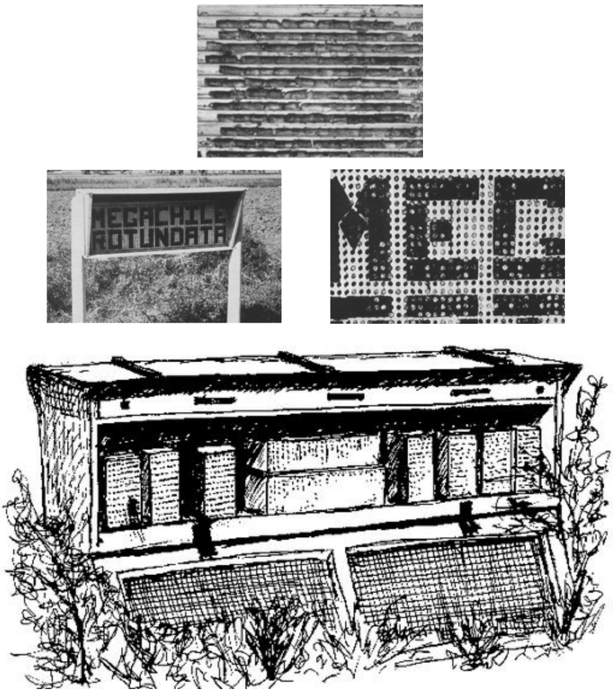

Figure 2.32.: *Breeding help made of wood with long holes (top, opened and showing the numerous breeding cells). Many of these breeding helps are hosted in a shelter on the experimental field (center, the entrance has been brushed with 'MEGACHILE ROTUNDATA'). Huts (bottom) for hundreds of rearing tubes 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 pollinate the alfalfa flowers. In this way the farmers obtain more than ten times as many seeds as they would without the bees. From* **?** *(top and center) and after* **?** *(bottom).*

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 to the left. To the right a breeding cell (picture taken by Theresa Pitts-Singer, see appendix A.3). 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.*

If the breeding chambers are kept in the refrigerator at 7° C, diapause can last up to two years. Only after several days of temperatures beyond 17° C suffices to brake diapause. The prepupae transmute in a bee and leave the winter quarter trying to find a partner, to mate and to start a new generation of leafcutter bees.

3. Our head clock

Man also seems to have 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 had obtained 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 **?**, 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 themselves: 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 normally, sleep deeply and well, and wake up briefly before or after the intended time (figure 3.2). 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.

Already in the 18th century **?** reported, that out of 200 respondents more than half of them claimed to wake up at a certain hour determined before going to sleep without waking

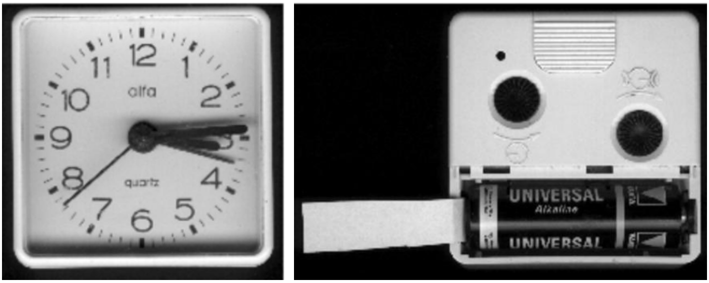

Figure 3.1.: *An alarm clock is reset to 12 o'clock in the evening and a strip of paper wedged between the battery and the holder. If the paper is pulled out at the time of waking up in the night (intended: 3:30 o'clock), the clock starts to run, because it has again contact to the battery. On the next day you only need to look at the alarm clock (for example at 8:00 in the morning) and count back the time (here: 3 h 14 min) 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 o'clock, that is 1 h and 18 min 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 had relied on this clock.*

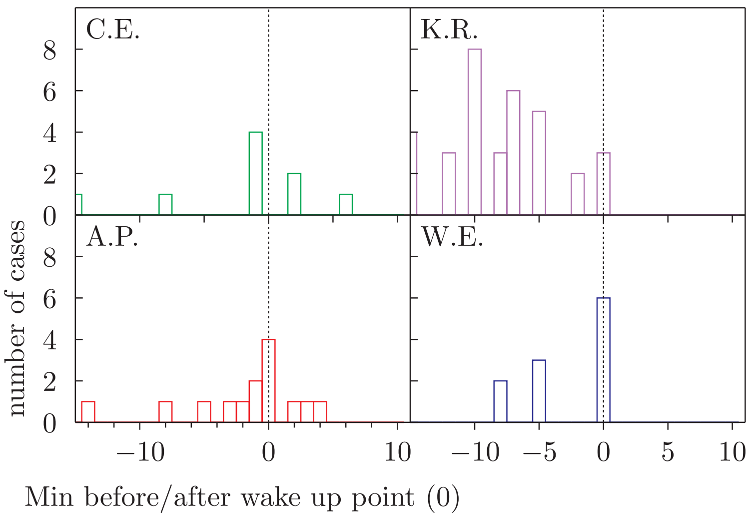

Figure 3.2.: *Some people possess a head clock. It allows them to wake up at night at a certain intended time without an alarm clock. Here examples are shown, in which the four persons A. P., W. E., C. E. and K. R. used their head clock quite effectively. On the horizontal axis the time is plotted at 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. W. E. for example woke up in one night 15 min before the intended time. In another night it was 8 min before the intended time. In four nights she woke up 1 min before, in two nights 2 min after the intended time. In two further trial nights she woke up 6 min later. After* **?**.

up before too often. Later studies of the head clock of humans were performed by **???**. Which time cues were used for this self awakening during sleep is under discussion. An internal clock or other possible mechanisms of time estimation such as REM-sleep[1] are candidates.

One of the authors (WE) has once undertaken an experiment with pupils of the Gesamtschule in Tübingen to try to find out whether modern man does still possess this head clock. We distributed battery driven alarm clocks to the pupils and asked them to try 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 3.1). Therefore current was unable to flow. The pupils were told to just pull out 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 am and his alarm clocks showed 3 am, when he arrived at school at 8 am, he and his classmates knew that he woke up three hours before, that is at 5 am. Thus he woke up half an hour late. In this experiment there were indeed some students who met the intended time of waking up quite well. In figure

[1] Rapid Eye Movement or paradoxical sleep is a sleep phase characterized by rapid eye movements and other properties such as 4 to 8 Hz theta waves and slow alpha waves in the EEG which are normally only found during the waking stage.

3.2 some examples of people are shown, which hit the intended time (0 in the figure) quite precisely.

Nowadays it is easier to activate a digital camera or the camera of a mobile phone at the time of waking up after having set to display date and clock time in the shot. One has to simply call the picture in the morning and read the time.

It is not known, whether the head clock is based on the circadian clock in man. Perhaps you might like to try to find out in your case or in the case of friends and acquaintances (proposal: If somebody with a reliable head clock travels through time zones, the waking up should still occur according to the home time, because the circadian clock takes some days until it is reset. Likewise one could test during the change to or from daylight-saving time, whether the head clock runs according to the old time).

4. 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 for them 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 the winter somehow. How these animals do it and what happens in detail shall be demonstrated in a few examples in this chapter.

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 unfavorable times.

4.1. How a midge fools the tricky pitcher plant

One of the authors (WE) went for two years to Ann Arbor as a post-doc and worked there at the University of Michigan with David Shappirio. As a zoologist he worked on the development of insects. He introduced him to the pitcher plant midge *Metriocnemus knabi* and its strange life.

Tramping through the bogs and swamps of Michigan one often finds plants with pot-like leaves and therefore called pitcher plants by the Americans. At the top the pitcher leaves are open, but a kind of collar surrounds the opening. Looking into a leaf you will notice that it is partly filled with water (figure 4.1).

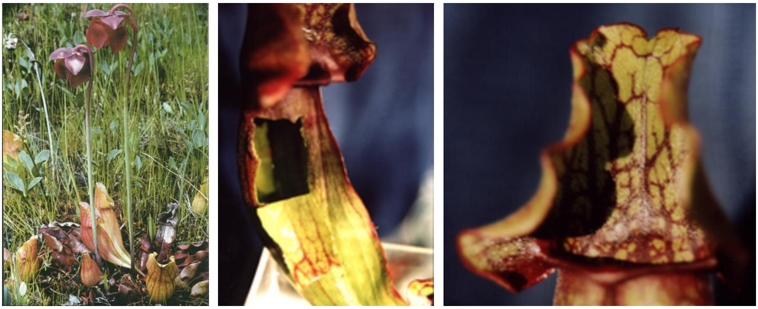

Figure 4.1.: *Pitcher plant* Sarracenia purpurea *with pitcher leaves, in bloom (left). Center: pitcher leaf with a cut window. Right: Opening of the pitcher leaf. Left figure from home page Dr. Bradshaw and Dr. Holzapfel (with permission). The other figures from the botanical garden in Tübingen (WE).*

If the water of such a leaf is poured into a flat dish, one understands the strange name of this plant: The fluid contains hundreds of drowned insects or parts of them. These insects landed on the lid of the pitcher plant leaf and fell into the pitcher. This is usually not fatal for most insects. They paddle to the fringe of the water and crawl out. However, this is not possible in the case of the pitcher plant. The 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 such a leaf and look carefully at the inner surface, we realize why the insects have no chance to escape this deadly pitcher: The upper layer (*epidermis*) consists of cells, the surface (*cuticle*) of which is arranged like tiles on a roof. Furthermore the cuticle 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 4.1).

Recently it was found, that the pitchers of carnivorous plants such as *Sarracenia*, *Nepenthes* and *Dionaea muscipula* catch their prey also by attracting them with blue light (430–480 nm), the emission of which is induced by UV-light (**?**). The catch will be drastically reduced, if the luminescent part of the peristoms is covered or cut off. During the day this light can not be seen, only in the dark. Mainly nocturnal insects are caught, and the weak scattered UV light, which reaches the earth during the night, is sufficient to induce the luminescence. Insects first see the peristoms glimmer blueish. If they land there, it is shining blueish out of the interior. Even the fluid in the interior of the pitcher leaves is fluorescent. The insects are thus caught by the plants in the same way that humans use with blue light traps. Phenoles seem to be responsible for the lighting.

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 biotope on top of each other, especially peat moss (*Sphagnum*). If the older ones do not get enough light any more, they decay and turn into peat after some time. The upper mosses thus have no contact to the soil and the minerals. They have to obtain these substances from the dead plants. A plant which is able to retrieve minerals and nitrogen from the dead insects thus has 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*). Its life cycle is described in figure 4.2. After about 4 weeks (at 23°C) the fourth larval stage is terminated. The larva crawls out of the water and produces a jelly like cocoon with its salivary glands, which adheres closely above the surface of the water to the wall of the pitcher. In this pupal cradle the larva becomes a prepupa. It transforms into a pupa and after 2 to 3 d (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 pitcher plant midges have to enter a *diapause*-stage for hibernation (figure 4.3). From September onwards no prepupae are found anymore in Michigan (35° northern latitude). Instead, the animals stay in the last (fourth) larval stage in the water of the pitcher leaves. The metabolism is reduced and the larvae become insensitive against frost. In the same way 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

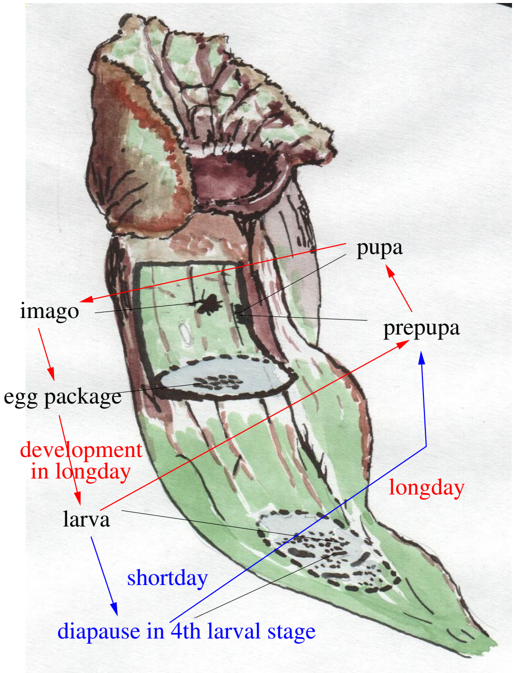

Figure 4.2.: *Life cycle of* Metriocnemus *in Saracenia pitcher: The females deposit an egg package on the surface of the water. The larvae eclose and feed on the drowned insects. They moult four times and under* longday *(summer) the larvae of the last larval stage crawl up the inner wall of the pitcher, turn in a jelly cocoon into a* prepupa *and a* pupa*, out of which the* imago *ecloses. It flies out of the pitcher, mates and again deposits an egg package. Under* shortday *the larvae stay in the fourth stage in the water and hibernate in* diapause*. Not before* longdays *occur they crawl out of the water, pupate and transmute into an* imago*. After* **?***.*

freeze completely without killing the larvae. In February/March diapause is terminated. The larvae crawl out of the water and

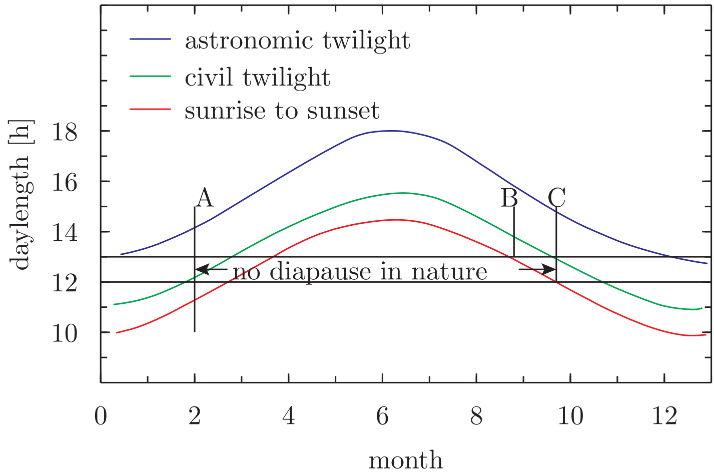

Figure 4.3.: *From end of February/begin of March the first pupae of the pitcher plant midge* Metriocnemus knabi *can be found in nature (A with vertical marking). That is the time at which diapause is terminated. Pupae are found until the middle of September (B with vertical marking). At the end of September (C with vertical marking) no pupae are found anymore. Between B and C thus lies the date at which diapause is induced. After* **?***.*

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 bee, diapause is not induced in the pitcher plant midge 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 length of the daylight in the winter is shorter than during the summer.

During the course of a year the earth orbits the sun. Additionally the earth turns once around its axis each day. Because the axis of the earth is not perpendicular to the earth-sun-line, but instead inclined by 23°, the northern hemisphere receives longer light on summer days than on winter days. This is shown in figure 4.4. 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 in Europe, for example Stockholm in Sweden, will be seen for a longer time in the light of the window as compared to a place at the southern hemisphere, for example Tierra del Fuego 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 than in the winter, and this is also the case on the southern hemisphere. But there the summer is in the months, in which winter prevails on the northern hemisphere.

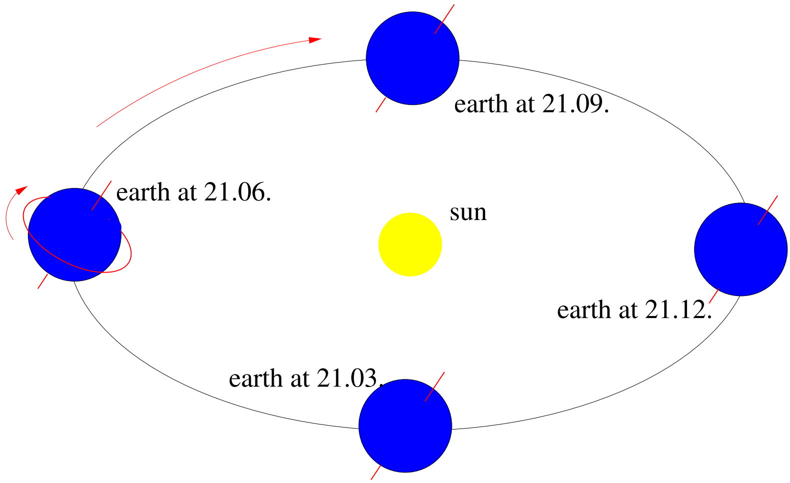

Figure 4.4.: *During the course of a year the globe orbits the sun once. Additionally the earth turns each day once around its axis. Because the axis of the earth is inclined by 23°, the northern hemisphere receives longer light on summer days (July 21, left) than on winter days (December 21, right). 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 than shown here.*

The closer to the poles at the northern or southern hemisphere the locations are, the more pronounced are these differences. Figure 4.5 shows that the summer days in Stockholm are longer than the summer days in Tübingen (Figure 4.6).

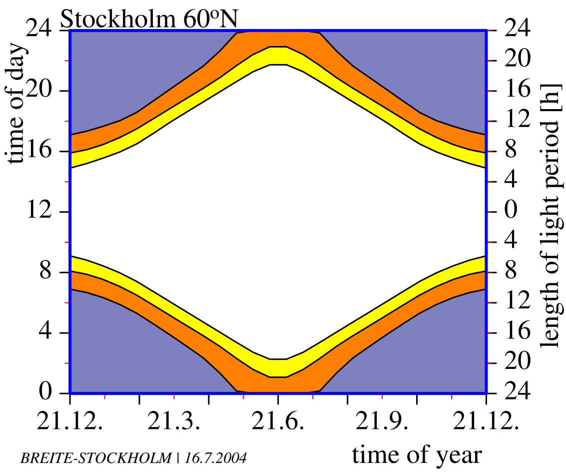

Figure 4.5.: *The further poleward a place, the longer are the days in summer and the shorter in winter. Stockholm lies at the 60th latitude. The white space inside represents daylength (indicated at the right) at various times of the year (on the horizontal axis) for the time between sun rise and sun 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.*

Now back to the chironomids. If the daylength shortens in the fall and reaches 13.5 h (figure 4.7, **?**), diapause is induced.

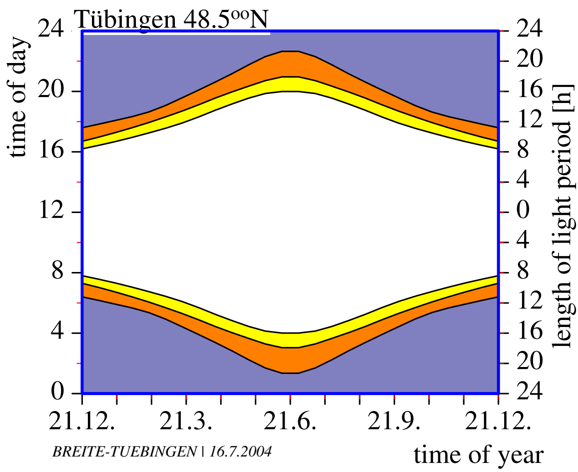

Figure 4.6.: *Tübingen lies at the 48.5th latitude. Otherwise as in figure 4.5.*

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. Only daylength matters. The light conditions of the environment can vary a lot. But again it is only the length of the day that is important. Even a light intensity of 0.00025 lux (the full moon would be dazzling bright in comparison: 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 also found in other pitcher plant midges. They were studied by Bradshaw and his coworkers (**???**).

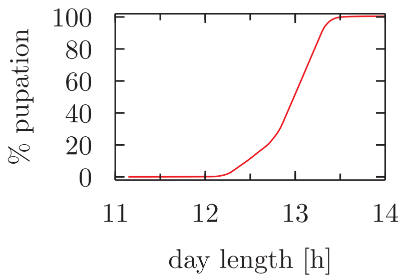

Figure 4.7.: *Photoperiodic reaction in* Metriocnemus*: In the laboratory it was studied, how the formation of pupae (that is, no diapause) depends on the day length. After 40 days with 12 h of daily light periods all animals stayed in diapause, with 13.5 h light periods all animals had pupated (diapause broken). The critical day length is thus between 12 and 13.5 h. After* **?***.*

4.2. When the potatoes come in the cellar, the Colorado beetle goes in the soil

Beetles 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 deserts they are found.

The Colorado beetle *Leptinotarsa decemlineata* is easily recognized by its 10 black lateral stripes (figure 4.8). 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 deters the animals. In cultured potatoes this poison is absent or present in low concentrations only. Therefore our potatoes are attacked by this beetle. If the wild potato *Solanum demissum* is crossed with our cultivar *Solanum tuberosum*, the bastard is partly resistant against the Colorado beetle.

What is the life cycle of the Colorado beetle? The females lay eggs in the spring, out of which a 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 respiration is reduced. The reserve substances fat and glycogen[1] are accumulated in the body to a larger amount than normally.

After hibernation the Colorado beetle occurs again at the

[1] kind of starch

Figure 4.8.: *Colorado beetle (*Leptinotarsa decemlineata, *left) and larvae (right) feed on leaves of a potato plant. Egg laying and larvae of the beetle on the right.*

surface of the soil and searches for new food plants. Feeding, growing, reproduction and diapause are synchronized with the development of the food plants.

Because the Colorado beetle leads to much damage in potato fields[2], its way of life has been intensively studied. It was also 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 4.9), because the brain controls diapause. From outside the head capsule[3] the eyes, antenna and the mouth tools are apparent. The interior of the head is shown in figure 4.10. This seems to be quite complicated. However,

[2]In "Pupils experiment" the 12 year old Daniel Schütz performed in 1997 a feeding experiment with the colorado beetle. 100 beetles fed more than 1 kg of potato leaves in their life, a larva in one week almost 1 g and a beetle in one week 1.63 g.

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

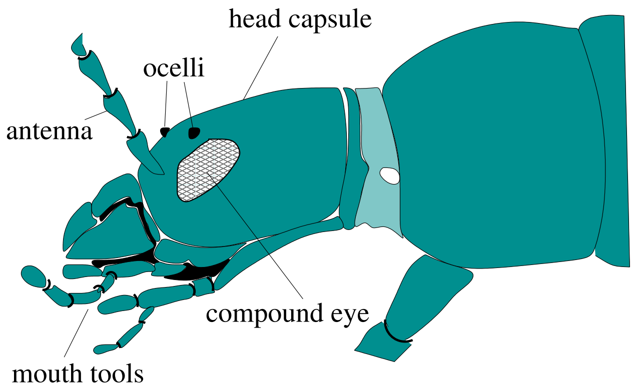

Figure 4.9.: *Lateral view at the head of a Colorado beetle: Compound eye consisting of many small individual ommatidia. On top of it two of the three forehead eyes (ocelli). In front the left antenna with members. The antenna touches, feels, smells the surrounding and measures its temperature. In front of the head are the complicated mouth parts.*

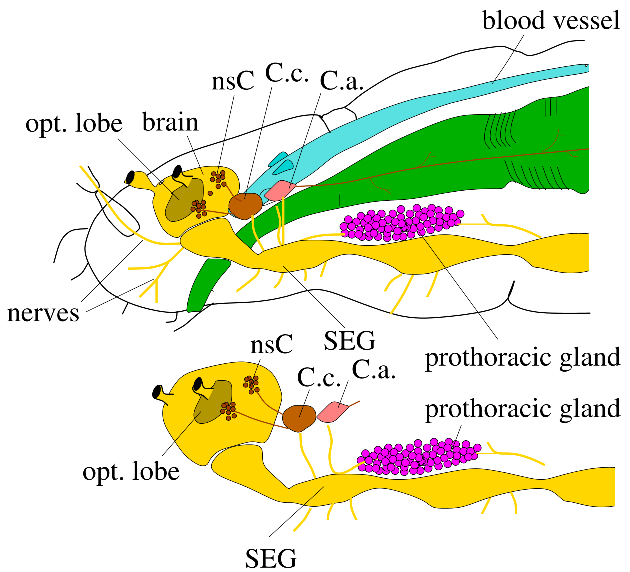

Figure 4.10.: *In the head capsule is the brain (yellow) with nerves (yellow) to the antennae, ocelli and other parts. Laterally optic lobes (dark yellow) are shown with nerves from the compound eyes. They send visual informations to the brain. Two branches of the brain (yellow) surround the esophagus (green) running to the sub-esophageal ganglion (SEG), from where the nervous system leads to thorax and abdomen. In the brain neurosecretory cells nsC (brown), which also produce hormones. Some brain appendices are important for the development of the larva to the imago: Behind the central brain and above the gut the Corpora cardiaca (light brown) and the Corpora allata (reddish brown). In the thorax the prothoracic glands (PTG, magenta). Open blood system light blue. Bottom figure: Important parts in and at the brain, which are responsible for the development of the larva into the adult insect.*

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 4.11 for development and in figure 4.12 for the diapause.

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

What is happening now in the brain and with the various hormones (figure 4.12)? As in the case of the pitcher plants midges the approaching winter is signaled to the Colorado beetle by the days getting shorter. The neurosecretory cells in the brain send a signal to the Corpora allata (an appendix gland of the brain) to stop the production of juvenile hormone. Without juvenile hormone the ovaries do not produce eggs, although ecdyson production continuous. The animals stop reproducing. In addition the behavior of the animals changes under shortday. 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 4.13 shows, at which daylength the diapause is induced. On the x-axis of the diagram the daylength is plotted, and on the y-axis the percentage of animals in diapause is

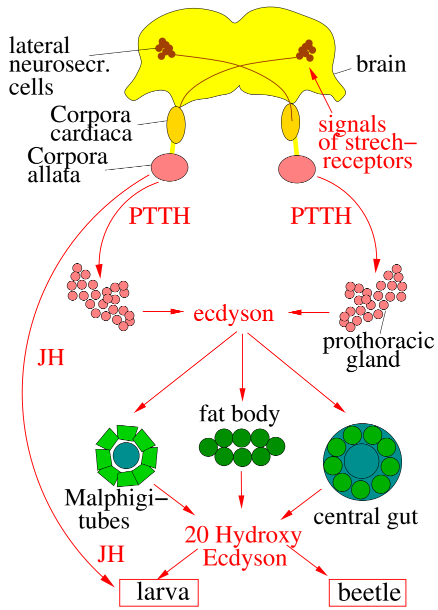

Figure 4.11.: *If the larvae have eaten so much that they do not fit in their armor anymore, stretch receptors at the anterior gut send a message to the nsC in the brain. They respond by producing the* prothoracotropic hormone *PTTH, which reaches the prothoracic gland via the blood-lymph. There ecdyson is made from cholesterol. The blood lymph is transported to the fat body, the middle gut and the kidneys (*Malphigi-*vessels) and there converted to the much more effective 20-hydroxy-ecdyson. It induces the moult in the epithelium cells. Whether the larva moults to the next larval stage (left) or to a beetle (right) is determined by the* juvenile hormone *JH. Without it the larva turns into a beetle.*

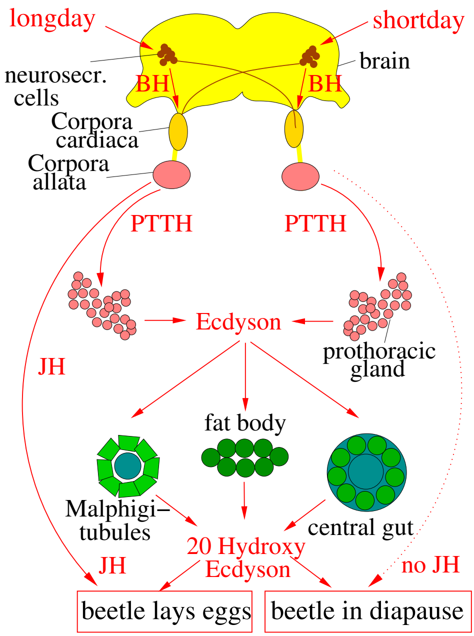

Figure 4.12.: *Hormones control diapause: Under longday (summer, left) the brain-hormone BH stimulates the juvenile hormone production (JH) and this the production of eggs. This is stopped in the fall. Under shortday (right) the neurosecretory cells in the brain send a signal to the* Corpora allata *to stop JH production. Without JH the ovaries do not produce eggs, although the ecdyson production is normal. At the same time the behavior of the animals changes. The beetles stop feeding and crawl into the soil for hibernation. The diapause begins.*

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

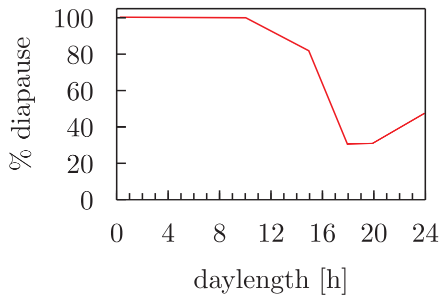

Figure 4.13.: *Shortday induces diapause in the adult stage of the Colorado beetle. The critical daylength lies at a light period of about 16 h. Under shortdays, that is fall, neurosecretory cells in the brain inhibit the* Corpora allata*. The inactive Corpora allata do not make any juvenile hormone and therefore the ovaries can not produce eggs anymore. The animals can not reproduce and diapause begins. After* **?***.*

In measuring daylength by insects or other animals and plants there is another problem: In the experiments in which the number of animals entering diapause was tested at various light periods (for example in figure 4.13), a timer was used to switch the light on and off. In nature, however, the day begins with dawn and ends with dusk. The light is quite weak at 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 4.14. At about 100 lux the changes per time are strongest.

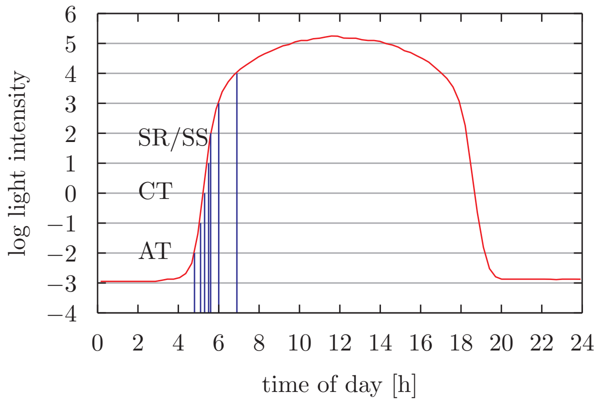

Figure 4.14.: *The light intensity changes during the course of a day from about 100 lux at sun rise to 100 000 lux at noon (close to the equator even 500 000 lux), declines afterward in the same way as it increased, until it reaches 0.001 lux in a night without moon. The y-axis is plotted logarithmically, 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 used a normal scale, the differences would be even more dramatic. Indicated are also the intervals from sunrise (SR) to sunset (SS), the daylength including the civil twilight (CT) and the daylength including the astronomic twilight (AT). Lower figure: In the range of the CT 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 ($48^0 32' N$, $9^0 3.5'$) at clear weather and new moon. After* **?**.

When does the light period begin and end for the Colorado beetle (and for other organisms, which react photoperiodically)? We already saw in figure 4.5, 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 with Colorado beetles the daylength in the laboratory was first varied by switching artificial light on and off using a timer. This led to the curve of the photoperiodic reaction in figure 4.13. We do not know, however, how it is in nature, because for the animals the day could start with the beginning of the astronomic twilight or with the beginning 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 4.14 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 be determined very accurately in this range of light intensity, even if the sky should be clouded, or on another day clear weather should prevail.

Besides the Colorado beetle there are a number of other varmints known in agriculture which possess diapause. One of them is the boll worm *Pectinophora gossypiell*a, a varmint of cotton plants, the corn borer *Ostrinia nubilalis*, and the pine lappet moth *Dendrolimus pini*, which damages pine trees (figure 4.15).

Figure 4.15.: *The pine lappet moth* Dendrolimus pini *is a varmint in the woods. The caterpillars (right) feed on pine needles. Water colored of the author after an image in* **?**.

4.3. How the silkmoth babies survive the winter

As a final example for diapause the Chinese silkmoth *Bombyx mori* is presented. It belongs to the insect order of butterflies (*Lepidoptera)* 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, is used for producing cloths. The cocoons are thrown in hot water. This dissolves 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 4.16. 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 (**?**). The photoperiodic signal *longday* is perceived by the mother. Diapause begins in the middle of the embryo development (*blastokinesis*), when the egg in the egg shell has developed to a kind of baby larva. During diapause no cell division occurs. Development is held until the environmental temperature has been below 5° C for at least 14 d. 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.

As was done for the Colorado beetle, we try and clarify how diapause is brought about for the silkmoth. 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 silkmoth contains a translucent triangle. It is situated on top of the light-sensitive cells (**?**). Longday induces in *Bombyx mori* females a signal in the neurosecretory cells of the brain. From there it is transfered via nerves to the sub-esophageal ganglion. There a diapause-hormone is produced and segregated (figure 4.17). Via the hemolymph it reaches the ovaries and causes the embryos to diapause (**?**). The diapause-hormone is a substance consisting of 24 amino acids and is a neuropeptide[4]. Since its chemical structure is exactly known, it has been synthesized. If injected, the animal enters diapause even under conditions, where there

[4]Proteins consist of amino acids. A peptide consists of a few amino acids only. Neuropeptides are produced in nerve cells and are used like other neurotransmitters to send messages from one nerve to another

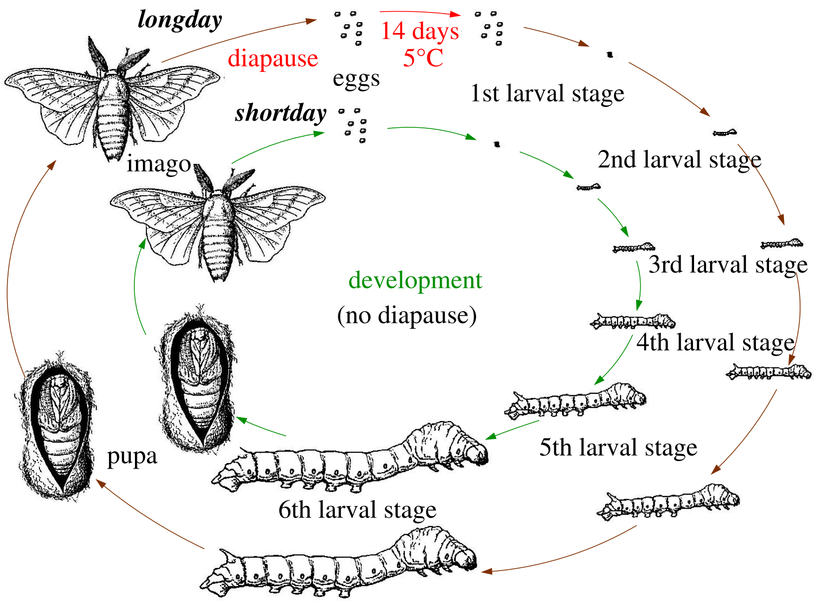

Figure 4.16.: *The females of the silkmoth lay their eggs under* shortday *conditions and they develop without diapause (internal circle in the figure). Out of the eggs eclose larvae, which moult four times. After the last moult the animals metamorphose in a cocoon spun out of silk. Under* longday *females 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 12 to 14 d to 5°C or lower. This sets a kind of 'alarm clock' in motion which terminates diapause at higher temperatures. The animals develop via different larval stages and the pupal stage to the silkmoth. After* **?**.

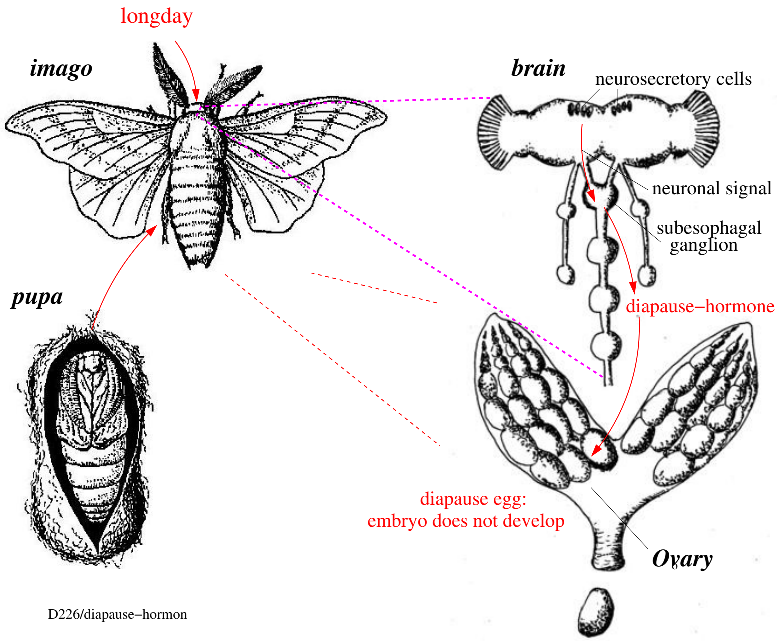

Figure 4.17.: *Longdays during the summer induce females of the silkmoth (*Bombyx mori*) to lay diapause eggs: A signal is transmitted from the neurosecretory cells in the brain via nerves to cells in the sub-esophageal ganglion, which produces diapause hormone. It is segregated into the hemolymph and reaches the ovary. There it impedes embryo development in the egg (**?**).*

normally is no diapause (shortdays). This shows clearly, that it is indeed a hormone which induces diapause.

The diapause-hormone is produced in twelve neurosecretory cells in the sub-esophageal ganglion of the pupae. The cells are arranged in three groups, lie at the lower side of the sub-esophageal ganglion and are connected with the Corpus cardiacum. A gene[5] takes care, that the diapause-hormone is expressed in the sub-esophageal 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 twelve to fourteen days of 5^0 C or below is experienced (figure 4.18). This activates an enzyme, the esterase A4. During the winter such low temperatures occur for this period of time in the habitat of the silkmoths. 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 12 to 14 d. 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 d at 5° C. If the temperature increases, the esterase A4 changes

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

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 d) like a stopwatch. In the case of the diapause of the silkmoth embryo the low temperature could set the stop watch in motion. Twelve to fourteen cold days are needed to free enough esterase A4 from PIN. The esterase would then become active at 25° C and allow the embryos to continue development.

4.4. Diapause is better than freezing to death

In the previous three sections three examples for diapause in insects were presented, namely a chironomid, the Colorado beetle and the silkmoth. In all these cases the unfavorable 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 another 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 unfavorable conditions. In contrast to diapause this 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 unfavorable conditions in a developmental stage, in which they can not fly or run away. An example for quiescence is the chironomid *Polypedilum*

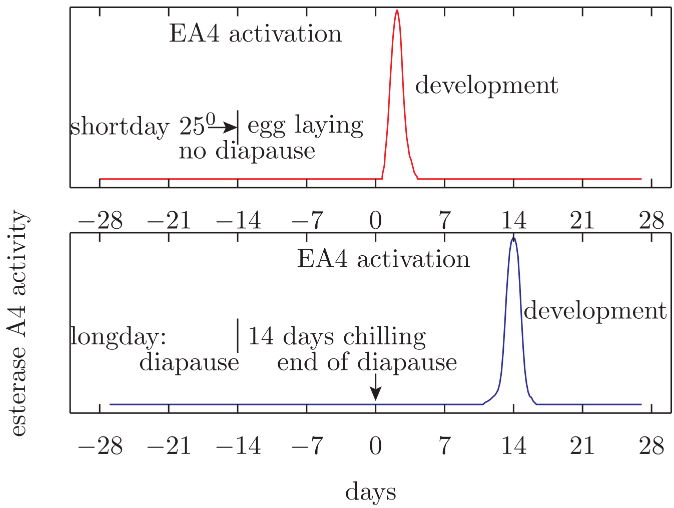

Figure 4.18.: *Top: Under shortday conditions females of the silkmoth lay eggs, the embryos of which develop without diapause. They need in a particular stage an active enzyme, esterase A4 (red curve, top). At 25°C they develop via larval stages to the adult (arrow).*
Bottom: Under longday the esterase 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°C 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 is terminated. The embryos are now able to develop. Larvae eclose 14 d after the end of the low temperature period. This happens not only in the life animal, but also in the test tube (in vitro) containing 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 the embryo out of its diapause sleep. After **?**.

vanderplanki (figure 4.19). The larvae live in water puddles

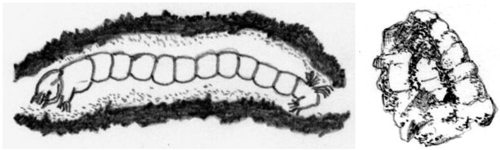

Figure 4.19.: *The larvae of the African chironomid* Polypedilum vanderplanki *lives in tubes in water puddles in indentations of rocks. During the dry period the water disappears completely and the larvae dry out almost completely (bottom). During dessication the body and its metabolism is changed in 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°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 WE according to a sketch (left) and photography (right) of Takashi Okuda, Ibaraki (Japan). Video on the homepage of Takashi Okuda: Homepage Takashi Film Polypedilum.*

in indentations of rocks of strongly glared rocks in parts of West- and East-Africa (figure 4.20 top). During the dry period the water disappears and the larvae dry out completely (**?**). 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°C, liquid helium, one day in absolute alcohol, one week in glycerol. So much concerning quiescence. It does not begin before the conditions become unfavorable, which is in the case of the African chironomid the loss of water from the rock pools. If the dried out larvae are moistened, they return to their normal shape in about 20 min. There exists a movie in the Internet on the Homepage of Takashi Okuda: Homepage Takashi movie Polypedilum, and the Kosmos-publisher in Stuttgart (Germany) is going to sell an experimental kit with dried larvae, allowing to observe the reanimation by wetting. In addition there is a movie on a DVD, which also shows the biotope in Africa. The protection mechanisms of the anhydrobiotic stage[6] have been studied (**?**). Thereby trehalose and G3LEA proteins[7] play a role. Other animals such as the water bear *Milnesium tardigradum*, a tardigrade, furthermore a nematode of the *Plectidae* family and the enduring eggs of the brine shrimp *Artemia franciscana*[8], inhabitants of salty inland waters, are able to sustain the drying out in the stage of anhydrobiosis (**??**).

In contrast to quiescence the *diapause* begins already before the environmental conditions become unfavorable. And the diapause often ends already before the environmental conditions are favorable again for some time (figure 4.20 bottom).

[6] life without water

[7] Group 3 late embryogenesis abundant proteins.

[8] of the family Artemiidae and the genus Anostraca.

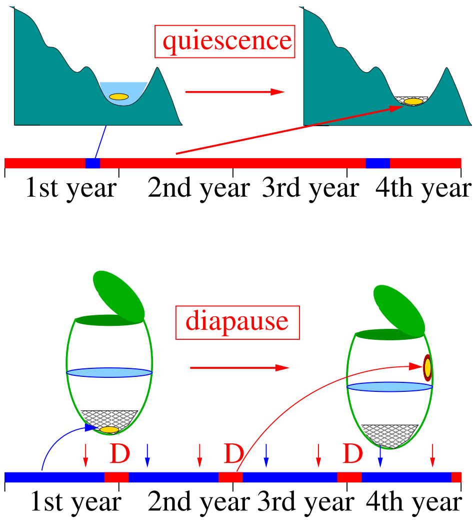

Figure 4.20.: *Upper example:* Quiescence *in the African chironomid larva* Polypedilum. *It lives in water-filled rock pools (left). If the water dries out, the larvae enter* quiescence *(right): Drought can be endured for a longer period of time without harm. In the case of* quiescence *the insects react directly to unfavorable conditions of the environment, which can not be predicted, because they occur eventually and irregularly and might stay unfavorable 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 unfavorable conditions (winter is coming soon), they themselves are not directly harmful. The time axis shows the normal occurrence of winter (red line) in each of the four years and the regular onset of diapause in the fall (red arrow) and likewise the regular end of it (blue arrow) in the spring of each year.*

In areas of the temperate and higher latitudes winter is unfavorable for the development of insects. Therefore many of them enter winter diapause (figure 4.21). In other regions of the earth heat and drought is a problem. This applies for example for deserts. Here insects often undergo a diapause in the summer. The photoperiodic conditions leading to winter diapause are shortdays, those leading to summer diapause longdays (**?**). Although there are quite a number of environmental factors such as humidity, temperature and quality of the food which signal the unfavorable season, the photoperiod is the most reliable and exact informant and is therefore used by many insects as an environmental factor. Already 10 to 15 min 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 56), the duration of lower temperature of the day is used instead of the daylength.

What must an insect be able to do, to enter diapause at the right time of the year? Let us put ourselves in the situation of an insect for a moment. 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 4.5), 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 also need a clock. With its help we can recognize, whether the light period is short enough to enter diapause.[9] We would,

[9] At the critical daylength half of the population of animals is induced

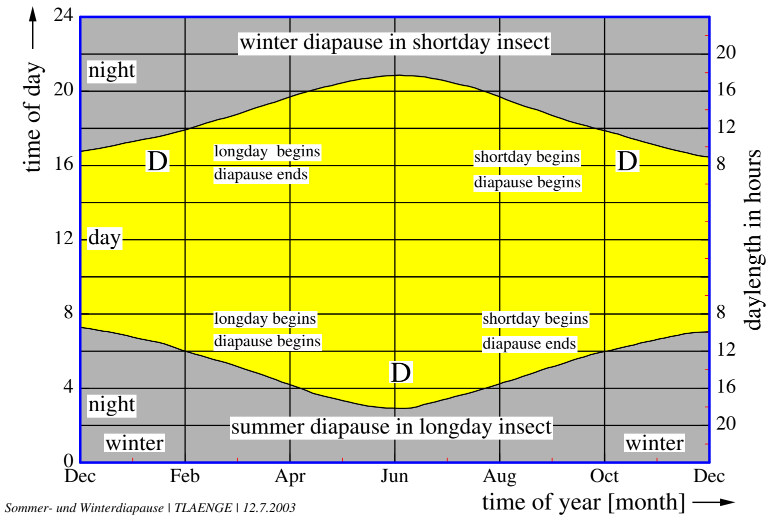

Figure 4.21.: *In the winter many insects are not able to develop. Therefore they undergo a winter diapause (D). It begins in the fall and ends in spring. These insects are called longday insects, because they are active under longday conditions and develop (upper lettering). In some regions of the earth drought is, however, the limiting factor (lower lettering). This applies for example to deserts. Here summer diapause (D) is often found. It begins in the summer and ends in the fall, when the dry period finishes. Animals with summer diapause are called shortday animals, because they are active and develop under shortday conditions (at the end and begin of the year).*

however, not restrict ourselves to just *one* photoperiodically effective day. It would be safer, to enter diapause, if we saw and measured *several* shortdays in sequence. Therefore we need also a counter, which adds up the days with shortdays. A switch has to be triggered, which stops development and starts diapause (figure 4.22).

All these devices, photoperiodic eyes (light receptors), a timer (circadian clock), a photoperiodic counter and a switch (hormonal system) are located in the insects 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 signaled to a center. It works up the integrated information and controls the events via a photoperiodic switch 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

---

photoperiodically. In the case of the diapause induction of the corn borer it amounts to 14.2 hours (figure 4.24). 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.

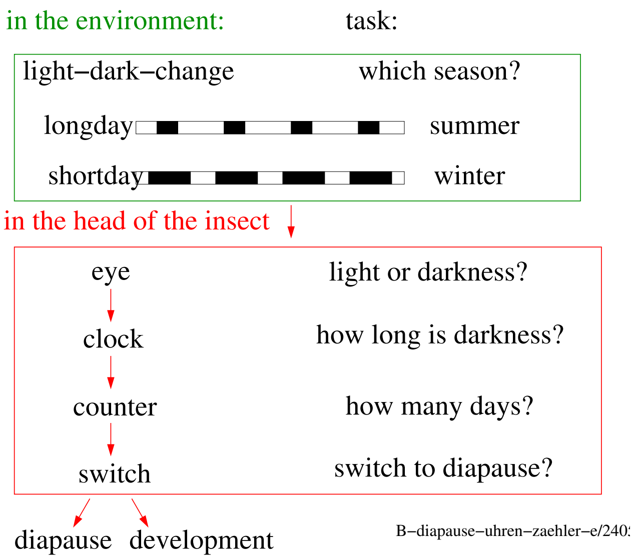

Figure 4.22.: *To control diapause photoperiodically, the animals need:* Eyes *to recognize the length of the light period*, a clock *to measure the daylength, a photoperiodic* counter *which adds up the days with a daylength being short (in shortday animals) or long (in longday animals) to induce diapause, and a* switch, *which takes care of stopping development and inducing diapause. All these devices are in the brain of the insects.*

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 silkmoth *Bombyx mori* it is the egg and the first larval stage which is photoperiodically sensitive. In the giant silkmoth *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 silkmoth *Antheraea pernyi* (figure 4.23). The diapause is induced *and* terminated

Figure 4.23.: *Pupa of a giant silkmoth* Antheraea pernyi *in the cocoon, which has been cut open here and shows also the last larval skin (left). Right: A window for light in the cuticle on top of the brain. The cuticle of the pupa is colored dark and transmits only little light. However, the translucent window on top of the neurosecretory cells of the brain allows light to enter the brain.*

at the same critical daylength (figure 4.24). Normally, however, other conditions such as for example a certain period of low temperatures (like in the silkmoth, page 96) or internal processes are necessary to terminate diapause.

During diapause the metabolism is low, the water content

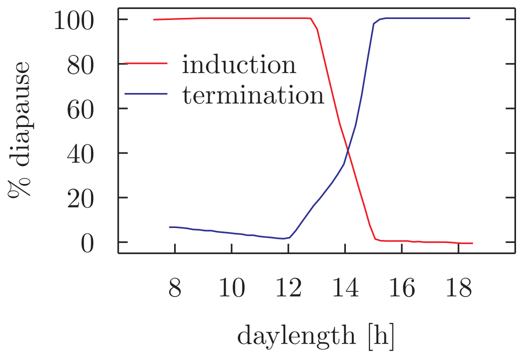

Figure 4.24.: *The giant silkmoth* Antheraea pernyi *begins to diapause in the fall, when days shorten. This resting stage occurs in the pupal stage (see figure 4.23). The red curve shows at which daylength diapause is induced. In days consisting of 16 h of light and 8 h of darkness (summer) no diapause occurs (red curve at 0%), in days with 12 h light and 12 h 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 h of light and 10 h 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* **???**.

scarce and behavior 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 always occurs in each animal in the same stage. This diapause is called *obligate*. In *multivoltine* 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 4.25). The other generations develop without diapause. In some insects such as *Bombyx mori* strains are known with obligatory diapause and others with facultative diapause (**?**).

4.4.1. In regions of the earth with early winter diapause starts earlier

Unfavorable 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 4.26) the differences in the critical photoperiod are gradual. In the cabbage Butterfly *Pieris brassicae*, however, only two geographic varieties exist (figure 4.26, **?**).

4.4.2. Diapause-eyes

To recognize the daylength, eyes or other light-sensitive devices (*light receptors*) are needed. The photoperiodic sensitivity of these devices mostly begins during the twilight at values

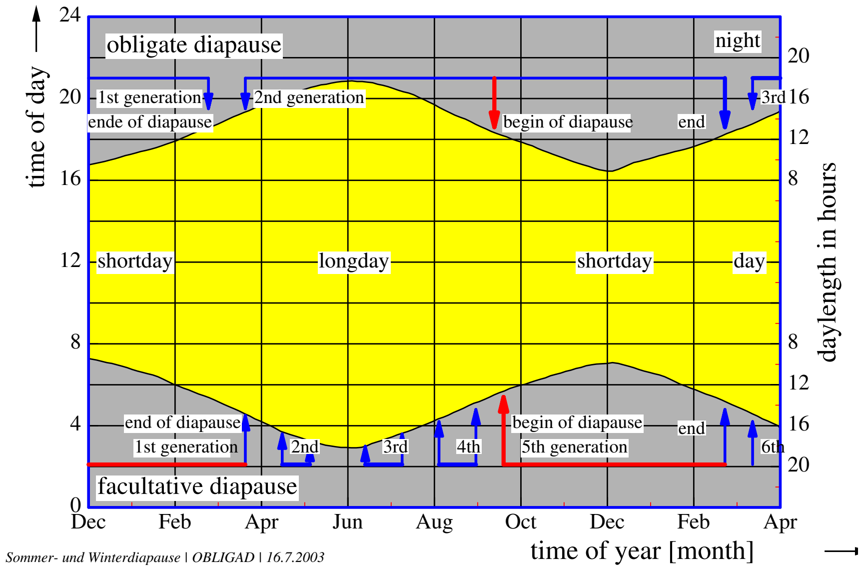

Figure 4.25.: Obligate *diapause is found in one generation per year (or in several years) only in a certain developmental stage (upper part of the figure). They are called univoltine species. In the example shown the first generation (the parents) terminates diapause at the end of February (1st blue 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).*

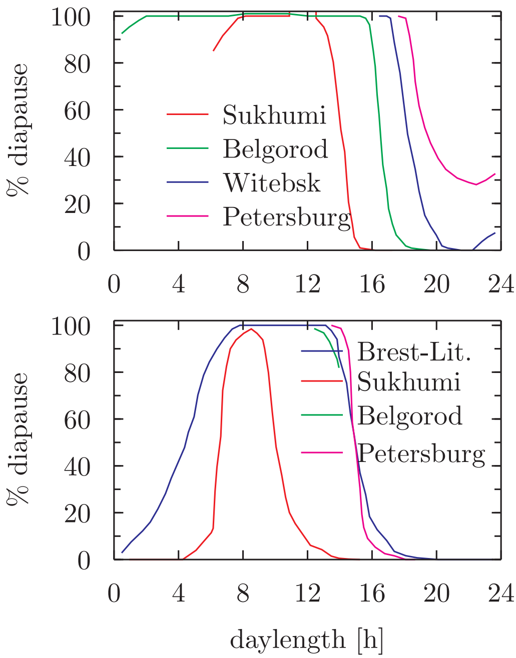

Figure 4.26.: *Geographic varieties of the cutworm* Acronycta rumicis *(top, Petersburg light blue 60°N, Witebsk dark blue 55°N, Belgorod green 50°N, Sukhumi red 43°N) and of the cabbage Butterfly* Pieris brassicae *(bottom, Petersburg light blue 60°N, Brest-Litowsk dark blue 52°N, Belgorod green 50°N, Sukhumi red 43°N) of various latitudes in Russia. Percent diapause as a function of the daylength. After* **?**.

between 10 and 100 lux. They do not react to weaker light. In this range the outdoors light intensity changes maximally (figure 4.14). The photoreceptors can, however, be protected from the light to different amounts. If the animals are for example enclosed in a cocoon at the time of photoperiodic sensitivity, weaker light suffices to stimulate them.

Possible photoperiodic light receptors are the compound eyes, the ocelli and light-sensitive structures of the brain. Which of them are used in reality differs in the various insects possessing diapause (figure 4.27). Different colors of the light can act in different strength.

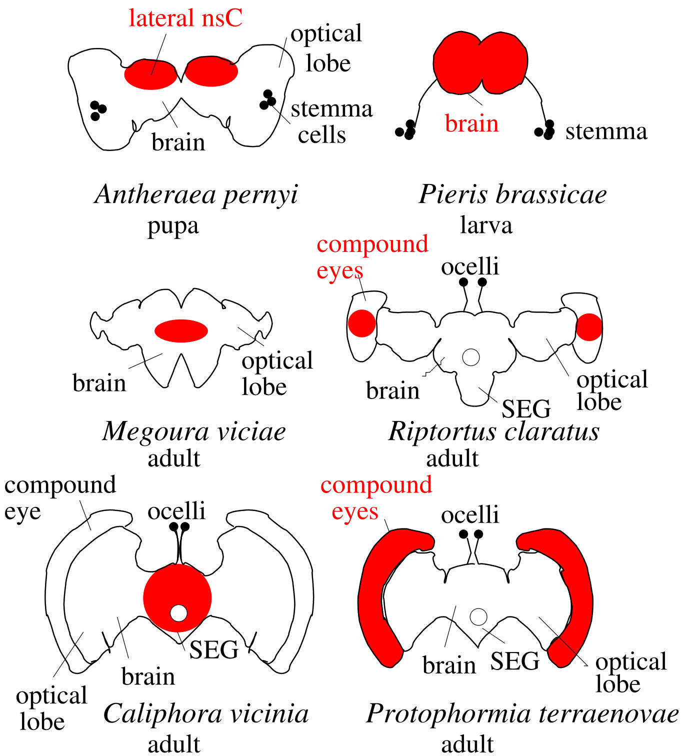

Figure 4.27.: *Photoperiodic receptors were localized in the red marked areas of six arthropods (frontal view of the brain, the optic lobes and the eyes). Top left: Giant silkmoth. Light receptors are the lateral neurosecretory cells (lateral nsC, red). Top right: cabbage butterfly* Pieris brassicae. *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* ?.

4.4.3. 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 models. 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 everything makes sense. If there are contradictions (the assumed thief was at the time of the theft not at the place), the model 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 models were proposed. One of it was put forward by **?**. It is especially interesting, because it uses a clock in a feedback model (figure A.1) and at the same time it takes care of the number of cycles needed to induce diapause. It is described in the Appendix (page 169).

4.4.4. Diapause-counter

In some cases just one single photoperiodically effective day suffices to induce diapause as for example in *Chaoborus americanus* (**?**). 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*. It deposits its eggs in the pupae of flies. They eclose and eat up the fly while residing in the pupa. In the fall with its decreasing day lengths the mother deposits eggs which do not develop, but enter diapause (figure 4.28). Since these parasitic wasps oviposit their

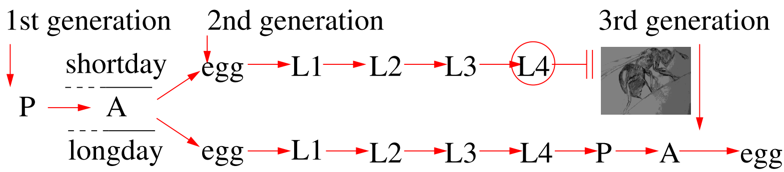

Figure 4.28.: *The parasitic wasp* Nasonia vitripennis *deposits its eggs in pupae of flies. They eclose and eat up the fly in the pupa. Under longday, that is summer, eggs are deposited, which develop to larvae. They moult several times. After metamorphosis into an adult parasitic wasp the animals mate, lay again eggs into pupae and a new summer generation grows up (lower part of figure). In the fall with days becoming shorter, the mother lays eggs, which develop up to the last larval stage and afterward enter diapause (upper part of figure). After* **?**.

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 in order to induce diapause in all animals (figure 4.29). More informations in http://www.werrenlab.org/nasonia/.

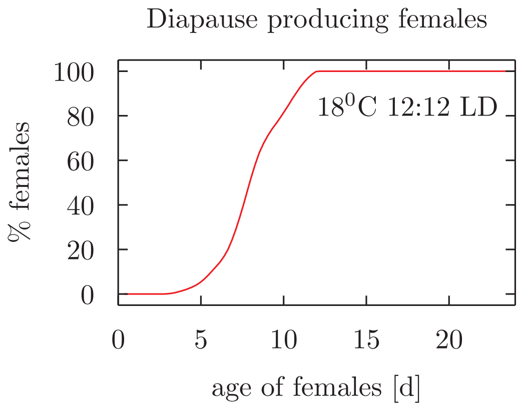

Figure 4.29.: *Photoperiodic counter in the parasitic wasp* Nasonia vitripennis. *In the fall, when the daylength decreases (in this figure days with 12 h light and 12 h darkness), the mother lays eggs which develop to the pupal stage and enter diapause. Since these parasitic wasps lay their eggs daily, one can collect them and simply 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 in order to induce diapause in all animals. With five shortdays only a few animals enter diapause, whereas the remaining of the clutch develop. Twelve days of shortday provoke that all the animals developing from the deposited eggs enter diapause in the pupal stage. After* **?**.

4.4.5. 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 unfavorable 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 thus do the same as we do when we protect the cooling water of our car motors 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 on their reserves for an extended time even without food supply. To reduce loss of water, the cuticle of the insect armor 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 4.22), 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 certain stage: Diapause begins. We also got to know some examples in which the processes and backgrounds of diapause were described (page 92, 99). Now we will be concerned with the processes going on in the brain and its accessory glands before diapause can begin. Before that we must, however, dip into the brain of insects (see figure 4.9, 4.11 and 4.12).

In the brain and in the sub-esophageal 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 4.12 in subsection 4.2) 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 synthesized 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 to produce eggs any more. Simultaneously the behavior 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 nymph stage, again lack of hormone is the cause of it. However, it is not the juvenile hormone, which is lacking, but ecdyson (figure 4.30). 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 gland 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 and furthermore the prothoracic gland in the thorax. 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 (nature plays). In other cases, in which diapause occurs in the *larvae*, the endocrine system stays *active* (figure 4.31). 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).

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.

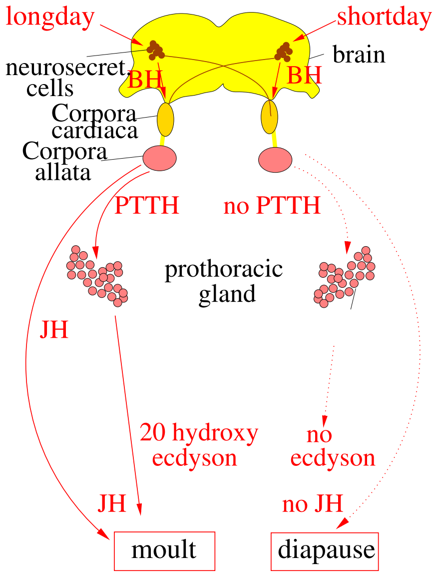

Figure 4.30.: *Diapause in the larval, pupal and nymphal stage is induced by a lack of hormone. Examples are the giant silk-moth* Hyalophora cecropia, *the corn borer* Ostrinia nubilalis, *the cabbage butterfly* Pieris rapae, *and the fleshfly* Sarcophaga. *Left part: When the neurosecretory cells in the brain experienced shortdays for some time, they do not produce any brain hormone BH. Without BH the prothoracic gland does not produce ecdyson. As a consequence development is stopped. Right side: Under longday the neurosecretory cells are stimulated to produce BH. It activates the Corpora cardiaca and the Corpora allata and furthermore the prothoracic gland in the thorax. 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* **?**.

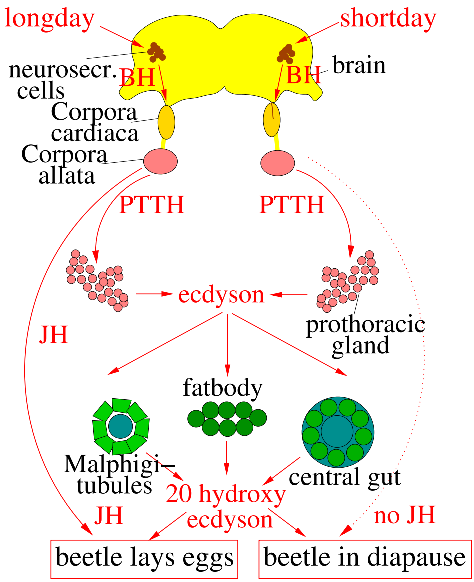

Figure 4.31.: *Right part: Shortday induces diapause in the corn borer* Diatraea grandiosella *in the larval stage: The brain causes the Corpora allata to produce juvenile hormone and to secrete it. The prothoracic gland does not secret ecdyson (because no brain hormone BH is made). Therefore the animals can not moult into a pupa. The larvae enter diapause. Left part: Under longday the neurosecretory cells of the brain stimulate the Corpora allata to produce and secret juvenile hormone as is the case under shortday. Additionally, 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* **?**.

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.

4.4.6. Diapause: A topic with variations

As often seen in nature, things are not following obstinately fixed rules, but there are exceptions and modifications. That also applies for the induction of diapause by photoperiods. 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 also the case in the Fleshfly *Sarcophaga* as shown in figure 4.32. Between 15 and 18° C the critical daylength stays constant. But if the temperature rises to 20° 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 also exist cases in which diapause occurs at *higher* temperatures, for example in *Abraxas miranda* (**?**). 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

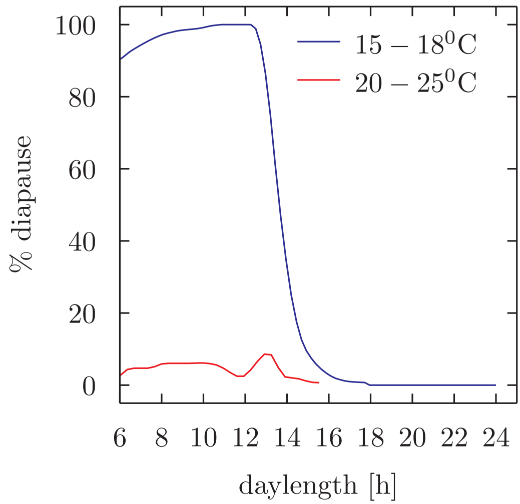

Figure 4.32.: *The fleshfly* Sarcophaga *stops development in days with short light periods (shorter than 13.5 h) and diapauses in the pupal stage. That is shown by the course of the blue curve: The percentage of animals in diapause is high under shortdays. It does decline somewhat at very short light 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° C and higher temperatures (red curve) almost no diapause occurs in the population. After* **?**.

as compared to insects from moderate latitudes (for example in *Oedipoda miniata* 27 to 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 diapause usually under shortday conditions. However, if plenty of food is available, diapause is suppressed (**?**).

4.4.7. 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, but from different latitudes of the earth. Those animal populations are called *geographic varieties*. The fruitfly *Drosophila littoralis* (figure 4.33) has a number of geographical varieties

Figure 4.33.: *Females of the fruitfly* Drosophila littoralis. *Left: Photography of WE, animals kindly supplied by Charlotte Förster, Würzburg. Right: Females drawn by WE after images by Hilary Burn.*

from Northern Scandinavia to the Caucasus (figure 4.34). The animals from Oulu stay in diapause, as long as the light period

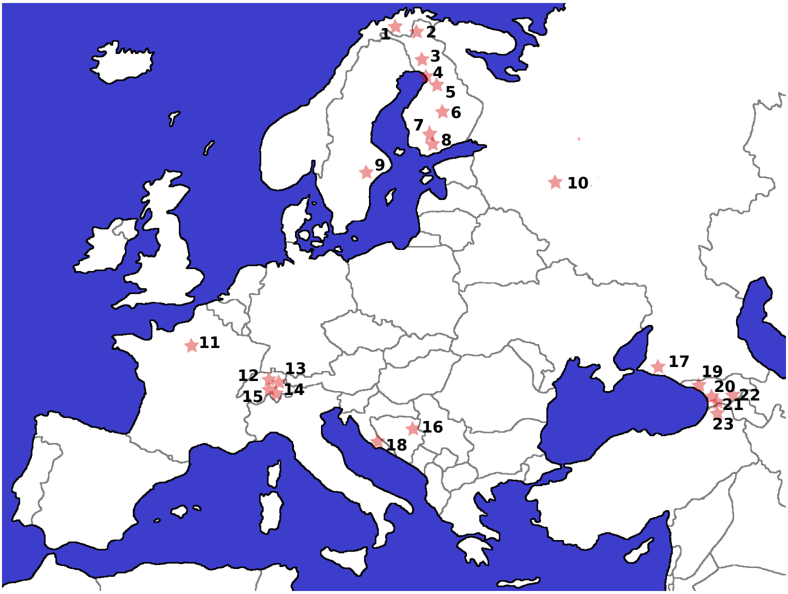

Figure 4.34.: *The habitat of the fruitfly* Drosophila littoralis *(figure 4.33) reaches from North Scandinavia (for example Oulu, Finnland, 65° northern latitude, Nr. 4) to the Caucasus (for example Kutaisi, 42° northern latitude, Nr. 20) and is found in the different regions as geographic varieties. 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* **?**, *basis map: digitale-europakarte.de, Impressum Timo Keil Zehlendorfer Str. 20 24111 Kiel Deutschland, Tel.: +49-431-3103330 info@keil-media.de.*

of the day is 19 h 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 h light per day and the animals development continues. This is shown by the blue curve in figure 4.35. High values stay for all animals in diapause. The value at 21 h light period is the only one which is low. At this daylength almost all animals have terminated diapause and develop.

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 h light per day. At 12 h 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 induced: about half of the animals begin diapause), is the *critical daylength*. It amounts to 19 to 20 h in the *Oulu*-variety and 12.5 to 13 h 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 offspring is around 17 h, that is in the middle between the critical daylength of the parents. The offspring are said to show an *intermediary* behavior.

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 h light, here in the Caucasus 13 h of light per day are already sufficient.

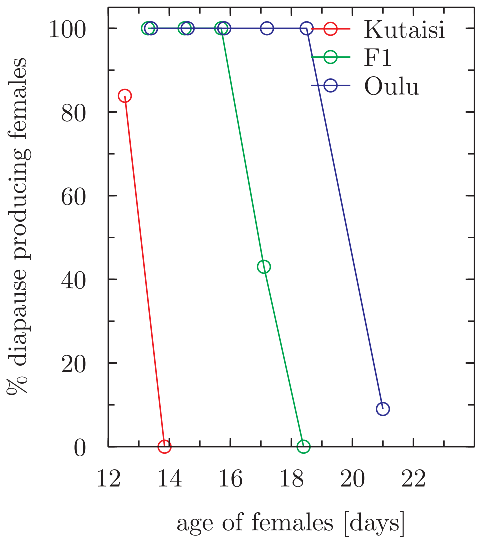

Figure 4.35.: *The blue curve shows the percentage of animals of a northern variety of* Drosophila littoralis *from Oulu, Finnland (65° northern latitude) at varying light periods of the day which are still in diapause (high values) or have terminated it (low values). The red curve shows the situation in the variety from* Kutaisi *in the South (Caucasus). The critical daylength (explained in the text) of the* Oulu *variety is 19 h and 42 min. In the Caucasus-variety* Kutaisi *it is only 12 h and 36 min. 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 h and 18 min. After* **?**.

5. The sun compass of a beach hopper

Beach hopper are able to orient themselves 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 already been at a beach of the sea (figure 5.1). There you find quite diverse zones, depending on how close to the sea or further inland they lie. The wetness 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.

An Italian scientist Leo Pardi and his colleague Felicita Scapini studied the beach hopper *Talitrus saltator* at the Italian East coast. It belongs to the amphipods (*Malacostracae*, figure 5.2). That is an order of the crustaceans. Normally we imagine a crustacean as a larger animal, but there are also very small ones among them. The later for example belongs to the

Figure 5.1.: *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* **?**.

crustaceans. The beach hopper is relatively small. It is found frequently at the European coasts at the beach close to the high water line (figure 5.1). During the day it is sheltered in the (not too) wet sand. During the night it undergoes excursions up to 100 m 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 of a sun compass, like bees do. 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 also needs a day clock which informs about the course of the sun during the day.

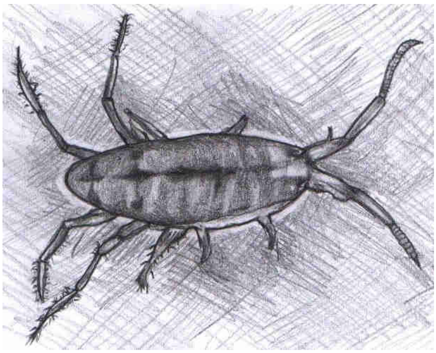

Abbildung 5.2.: *Beach hopper* Talitrus saltator *(amphipode), a crustacean. Drawn by Mareike Förster after a photography in* **?***.*

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 offspring uses an intermediate flight direction.

If the sun can not be seen directly because it is behind clouds, the beach hopper – like bees (see page 32) – 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 (**??**).

How was it shown that the animals use the sun for orientation? **?** captured beach hoppers and positioned them in the center of a glass vial (figure 5.3). 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 pictures and evaluate the images later. If the sun is in the West, the image would look like shown in figure 5.4.

Now the two scientists 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 5.5).

These beach hoppers are thus able to find out the current

Figure 5.3.: *Beach hoppers were caught 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 laid on a mirror which was mounted underneath the bottom. At the paper the positions of the animals were drawn with a pencil (see figure 5.4). Drawn by WE after* **?**.

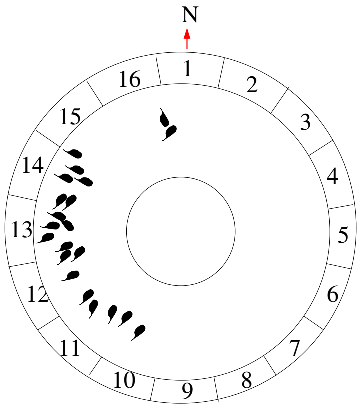

Figure 5.4: *The flight direction of beach hoppers in a glass vial (figure 5.3) is shown by the animals on the image. They escape towards the West in the direction of the sea. After* **?**.

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 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 h earlier and ended 6 h earlier (that is, a day advanced by 6 h). 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 h. Here too the flight direction was shifted by 90° in respect to the control on a normal day, but now in the other direction. The flight

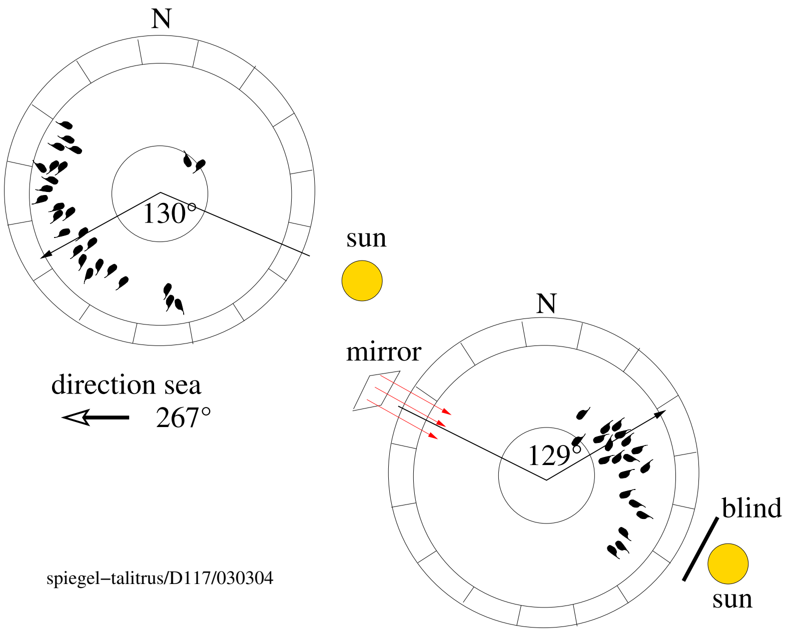

Figure 5.5.: *Sun compass orientation of the beach hopper* Talitrus saltator. *They orient themselves during the escape towards the sea (large arrow, 267°) according to the sun (top right, 130° to the sun). If the sun direction is faked with a mirror and the direct view of the sun prevented by a blind,* Talitrus *uses the mirror-sun (bottom left, 129° to the mirror-sun). N North. After* **?**.

direction of the animals had thus changed after the internal clock was regulated (figure 5.6).

5.1. Further examples for sun compass orientation

In ants (**??**, newer publication **?**) and spiders (**?**, see figure 5.7) 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 themselves 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 km 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*), which weights less then 1 g, migrates up to 3000 km (figure 5.8, http://de.wikipedia.org/wiki/Monarchfalter). 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 5.9). In the spring they fly from their winter quarters back to the summer quarters. Individuals were labeled 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 km. That

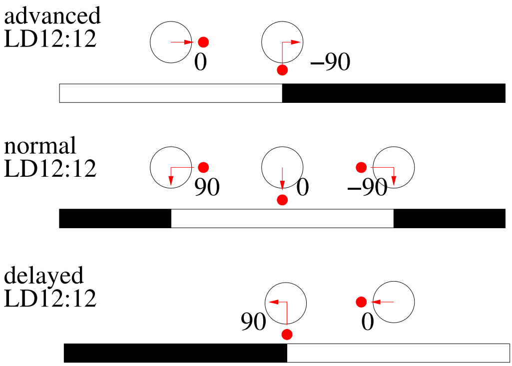

Figure 5.6.: *If the light-dark cycle of beach hoppers is shifted by an artificial illumination which does not coincide with the natural day, the time reference of the animals is also shifted and the orientation shifts correspondingly: A light-dark cycle (top) advanced by 6 h changes the synchronization of the day clock of the animals in such a way that they escape at 6 am (their internal clock tells them it is noon) in the direction of the sun and at noon 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 h (bottom) changes the synchronization of the day clock of the animals in such a way, that they escape at noon (their morning) to the East instead of South. At 6 pm they escape in the same way as the controls (center) would do at noon (and it is their noon). The orientation thus uses an internal clock. After* **?**.

Figure 5.7.: *The sand wolf spider* Arctosa cinerea *(left: close to its hole, 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 themselves at sun times unknown to them. Animals of the local Scandinavian populations also orient at the time of midnight sun. Water colored by the author using images for example under wolf spider, wolf spider closeup.*

Figure 5.8.: *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 5.9). Photographies in the Santuario de la Mariposa Monarca, Michoacán, Mexiko by Adrina Abad, thanks to Rodrigo Mendoza Gutiérrez and Alejandra Olivo for the mediation.*

verified that one and the same individual had survived and covered that distance. It disproved the possibility that the animals stopped on the way, laid eggs and the descendants continued the journey.

So far it was assumed that for the migration a sun compass in combination with an internal map was used. In order to find out whether this is correct, a flight simulator was used. In the center of a cylinder a butterfly was fixed on a thread. An air draft makes them fly in place. The chosen flight direction can be measured and the virtual path reconstructed (**??**). It turned out that the animals would have flown in a South West direction. The scientist tested afterward again the butterflies from Ontario in the flight simulator, but now 2500 km west of Calgary. A *true navigator* would have used a compass and a map, thus always knowing where it is. A *false navigator* does have a compass, but does not know his position. The animals tested in Calgary had not noticed that they were displaced westward. They owned only a sun compass and not a magnet compass (**?**).

In spite of it they find their destination just by not crossing mountain ranges and water. They finally reach the Rocky Mountains and turn south. The perpendicular highlands of Michoacán in central Mexico stop the migration. Thus, there are geographical conditions which act like a funnel conducting millions of butterflies each year in the closely confined area of their winter quarters.

For the flight back fits again the view of a funnel, now in the reversed direction with butterflies dispersing over a wide area. On the journey back the animals use again a sun compass, this time to a northern and northeastern direction.

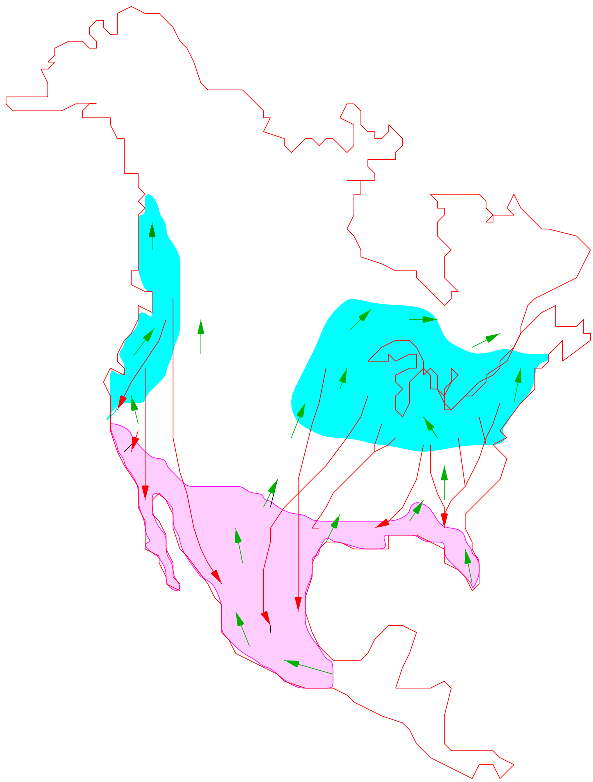

Figure 5.9.: *The Monarch-butterfly (*Danaus plexippus*) flies each year in late summer and fall from its summer quarters in Canada and the northeastern and northwestern States of the USA (light blue areas, red arrows) to the winter quarters in den countries around the Gulf of Mexico, California and the northern Central America (magenta areas, green arrows; see the animation in Monarch). In the summer- and winter quarters milkweed (*Asclepias*) is growing, for which the caterpillars have specialized. They are not able to live on other plants. After* **?**.

The caterpillars feed on milkweed (*Asclepias*) only and the animals are therefore restricted to areas in which these plants grow. The plants contain heart glycosides and protect in this way the caterpillar and butterflies against enemies, because they taste unpleasant and they are poisonous. The eye-catching color of the caterpillars and butterflies warns potential enemies.

Other butterflies also migrate over long distances such as the cabbage Butterfly (see **?**).

6. Observations, movies

We hope that we have motivated you with this book to start with your own observations. As a further challenge we have taken some time lapse movies which are accessible via the Internet (page 149). These movies deal mainly with rhythmic movements of petals, leaves and stems of plants. Furthermore a few rhythmic movements in minute pace are shown (*Codariocalyx motorius* on page 150, *Lathyrus niger* on page 151). There are also some short videos on the eclosion rhythm of *Drosophila pseudoobscura* on page 150, and the movements of a nematode on page 151. Most of the plant pictures serve to illustrate this book. In the following we describe first, how the pictures were taken and converted into movies. Then we offer a tabular overview of the various movies on page 149 and add some remarks to it.

6.1. Video pictures

Modern digital cameras or Smart phones nowadays possess rather effective means for taking short videos or time lapse movies.

For high quality images good cameras are recommended such as a Nikon reflex camera D700.

Taking pictures with the Nikon D700 the battery was constantly charged via a power supply during the partly long recording time. A remote control (Remote Cord MC-36) was connected by a cable with the camera. It allowed to determine the duration between shots (eg every 5 Min). The pictures were stored on a 15 GB storage card in the camera. In the case of very long recordings a 2 GB storage card was used for bridging, when the first card was full, but further pictures had to be taken.

For the recordings the time automatic of the camera was set for a preset diaphragm (eg 11). If during the night the light intensity was reduced, the exposure time was automatically prolonged in order to always get the same depth of focus.

In many cases the objects were illuminated with a white light panel, the intensity of which could be set by turning a knob. The panel consists of a large number of white light emitting diodes and is better than using white fluorescent tubes.

One should make test pictures before starting the time lapse recording to find the correct illumination (that is, incidence of light, direct or indirect light, use of several mirrors etc).

For recording and focusing it is advantageous to use the monitor of the camera as a *Live-View*. With the first *slight* pressure on the trigger the object to be recorded appears as an image on the monitor of the camera and can be enlarged with the zoom knob which helps to focus the lens. During the following shots this enlarged image is still displayed on the monitor.

The lowest image quality was used for storing in the TIF format (about 9 MB per image). At the end of a time lapse series the storage card was inserted into the microdrive of a PC and the images stored on a large external USB-hard disk (2TB).

There are nowadays budget-priced cameras such as the Ricoh CX5 in which the facility to produce time lapse movies is already built into the camera. For longer recordings it is recommended to use a better lithium battery and a larger storage card.

To produce a time lapse movie from the single images programs can be purchased. Alternatively such movies can also be prepared on the command line under Linux. For this, the images have first to be reduced in size and afterward converted into a movie. We will not list all the single steps and commands, but the interested reader can ask the author WE who will supply these informations.[1]

6.2. List of movies

Anagallis arvensis flower opening http://nbn-resolving.de/urn:nbn:de:bsz:21-opus-66480

Anemone nemorosa stalk and leaf movement http://nbn-resolving.de/urn:nbn:de:bsz:21-opus-66491

Anthericum ramosum petal movement http://nbn-resolving.de/urn:nbn:de:bsz:21-opus-66505

Arabidopsis thaliana circumnutation of hypocotyl http://nbn-resolving.de/urn:nbn:de:bsz:21-opus-66520

Averrhoa bilimbi leaflet movement after mechanical stimulation http://nbn-resolving.de/urn:nbn:de:bsz:21-opus-66532

Averrhoa carambola leaflet movement and mechanical

[1] Links and further informations:
ffmpeg: https://ffmpeg.org
ffmpeg Manpage: https://ffmpeg.org/ffmpeg.html

stimulation http://nbn-resolving.de/urn:nbn:de:bsz:21-opus-66549

Bellis perennis flower opening and closing http://nbn-resolving.de/urn:nbn:de:bsz:21-opus-66516

Biophytum sensitivum leaflet movements during course of a day http://nbn-resolving.de/urn:nbn:de:bsz:21-opus-66554

Centaurea cyanis flower opening and closing http://nbn-resolving.de/urn:nbn:de:bsz:21-opus-66563

Cichorium intybus flower opens and wilts http://nbn-resolving.de/urn:nbn:de:bsz:21-opus-66585

Cichorium intybus flower opening http://nbn-resolving.de/urn:nbn:de:bsz:21-opus-66570

Codariocalyx motorius terminal leaflet movements show day-night rhythm
http://nbn-resolving.de/urn:nbn:de:bsz:21-opus-66610

Codariocalyx motorius lateral leaflet movements http://nbn-resolving.de/urn:nbn:de:bsz:21-opus-66626

Codariocalyx motorius lateral leaflet movements with periods in the minute range http://nbn-resolving.de/urn:nbn:de:bsz:21-opus-66598

Codariocalyx motorius lateral leaflet movements, leaf inverted http://nbn-resolving.de/urn:nbn:de:bsz:21-opus-66602

Convolvulus arvensis flowers http://nbn-resolving.de/urn:nbn:de:bsz:21-opus-66634

Delosperma sutherlandii flower opening and closing http://nbn-resolving.de/urn:nbn:de:bsz:21-opus-66652

Drosophila pseudoobscura eclosion out of the puparium http://nbn-resolving.de/urn:nbn:de:bsz:21-opus-66660

Drosophila pseudoobscura eclosion rhythm http://nbn-resolving.de/urn:nbn:de:bsz:21-opus-66676

Galanthus nivalis opening and closing of flowers http://nbn-resolving.de/urn:nbn:de:bsz:21-opus-66680

Kalanchoe blossfeldiana petal movement of cut flower http://nbn-resolving.de/urn:nbn:de:bsz:21-opus-66695

Kalanchoe blossfeldiana daily petal movement at inflorescence http://nbn-resolving.de/urn:nbn:de:bsz:21-opus-66709

Lathyrus niger ultradian leaflet movement http://nbn-resolving.de/urn:nbn:de:bsz:21-opus-66713

Lonicera xylosteum flower opening http://nbn-resolving.de/urn:nbn:de:bsz:21-opus-66735

Maranta leuconeura petal movement http://nbn-resolving.de/urn:nbn:de:bsz:21-opus-66748

Maranta leuconeura joint with midrib of the leaf as a hand: movement http://nbn-resolving.de/urn:nbn:de:bsz:21-opus-66759

Nematodes at dead beetle http://nbn-resolving.de/urn:nbn:de:bsz:21-opus-66766

Nymphaea alba flower closing http://nbn-resolving.de/urn:nbn:de:bsz:21-opus-66785

Nymphaea alba flower opening in the morning http://nbn-resolving.de/urn:nbn:de:bsz:21-opus-66771

Ornithogalum umbelliferum: Daily closing and opening of a flower http://nbn-resolving.de/urn:nbn:de:bsz:21-opus-66792

Oxalis acetosella movements http://nbn-resolving.de/urn:nbn:de:bsz:21-opus-66806

Papaver rhoea petal movement http://nbn-resolving.de/urn:nbn:de:bsz:21-opus-66817

Phacelia viscida nutations of inflorescence, flower opening http://nbn-resolving.de/urn:nbn:de:bsz:21-opus-66827

Pharbitis nil flower opening http://nbn-resolving.de/urn:nbn:de:bsz:21-opus-66857

Pharbitis nil flower opening http://nbn-resolving.de/urn:nbn:de:bsz:21-opus-66847

Ranunculus ficaria flower opening http://nbn-resolving.de/urn:nbn:de:bsz:21-opus-66863

Rosmarinus officinalis flower opening http://nbn-resolving.de/urn:nbn:de:bsz:21-opus-66877

Taraxacum officinale flower closing and opening http://nbn-resolving.de/urn:nbn:de:bsz:21-opus-66881

Tulipa tarda petal movement and circumnutation of peduncles http://nbn-resolving.de/urn:nbn:de:bsz:21-opus-66899

Tussilago farfara flower opening and closing. Nutations of peduncles http://nbn-resolving.de/urn:nbn:de:bsz:21-opus-66900

7. 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 observations and experiments mentioned. You should read again for the various topics the text referred to.

7.1. Flaming Katy *Kalanchoe*

How the movement of the petals can be observed is described on page 5.

Kalanchoe seeds are tiny. For rearing and care see Kalanchoe Anzucht Pflege. 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, since *Kalanchoe* germinates in light only (light germinator). The best time for sowing 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 h. If you have done this for about two weeks, they start to form flower buds after some time.

How the movement of the petal tips can be observed is described on page 5.

For cross- and longitudinal sections through the petals you need elder pith (brake off the twig of an elder tree and cut it in pieces). Cut out the pith and wet 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 the use of the microscope see page 174). Microscope slides and cover slips 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 will be 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 **?**.

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

7.2. Morning glory begins to flourish

This is described on page 15. Seeds of the morning glory *Pharbitis nil* are available in the seed trade or in botanical gardens. 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 directly 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.

7.3. A flower clock

The flower clock is described on page 15. In the following the plants are listed, which open at various times of the day. It is also described when they flower (roman numbers, months), where they are found and/or how they are reared. After **?** page 217 (only opening times) and after Zander, cited after **?** in **?**, 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 median mountain ranges

5 Red puppy *Papaver rhoeas* V-VII, cereal fields, debris, common. Video: on page 152

5-6 Blue flax *Linum perenne* VI-VIII

6 Cichory *Cichorium intybus* VII-VIII, waysides, margin of fields, pastures, widespread. On clay. Video: on page 150

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 Trumpet 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 lily *Nymphaea alba* VI-VIII, nutrient-free, standing waters, widespread

o 7-8 z 15-16 Spider plant *Anthericum ramosum*

o 7-8 z 15 Bearded Fig-marigold *Mesembryanthemum barbatum*

o 8 z 15-16 Scarlet pimpernel *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 *Ice plant Mesembryanthemum cristallinum*

o 10-11 z 14-15 pink sandwort *Arenaria rubra*

o 10-11 yellow daylily *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

7.4. A 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 **?** pg. 219

2 Swallowtail *Iphiclides podalirius*
3 dryad *Autographa bractea*
4 Eros blue *Lysandra bellargus*
5 mountain burnet *Lycastes zygaena exulans*
8 gold spangle *Cyanius* (*Lycaenidae*)
9 cabbage butterfly *Pieris brassicae*
10 hawk moth *Macroglossum stellatorium*
11 red admiral *Vanessa atalanta*
18 Mediterranean hawk-moth *Celerio euphorbiae*
19 death's head hawk-moth *Acherontia atropos*
20 dogbane tiger moth *Spilosoma monthastri*
21 privet hawk moth *Sphinx ligustri*

7.5. Flowers of the Evening-Primrose open

On page 17 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 already have 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. 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 8 pm and 10 pm you can observe the opening of the flowers.

7.6. Training bees for colors

On page 28 it is described how bees can be trained for colors. You need colored paper (stationary shop or printout on the printer with the PC), glass disks for covering and some honey. A movie is available about the dance in bees (page 29). It is named *Entfernungs- and Richtungsweisung bei der Honigbiene-Rund- und Schwänzeltanz* and was produced by Karl von Frisch and Martin Lindauer (see also youtube Schwänzeltanz).

How bees see the polarization pattern of the sky is described on page 32. Polarization foils can be purchased at Polarisations-folien or at www.edmundoptics.de (linear polarizing sheet 2*2 inch, two squares, order number M43-781, or circular polarizing film).

7.7. Pollination of the grass of Parnassus

On page 36 the pollination of the grass of Parnassus is described. You find the plant in fenland and on wet fen meadows, but also at wet locations of dryer meadows.

7.8. Fragrance of flowers

From page 49 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 49). 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 denatured by a smelling substance to discourage drinking.
On page 50 the soapwort *Saponaria officinalis* is mentioned. You will find the plant at waysides and watersides. Occasionally it is also kept as an ornamental in the garden. It growth 30 to 60 cm height 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.

7.9. Alfalfa-flowers

On page 55 the pollination mechanism of alfalfa is described. You can study the pollination mechanism of the flowers and trigger the mechanism with a match. 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.

7.10. Electrical potential of flowers

The electrical potential of flowers (see 24) can be made visible with an electrostatic powder. It is produced by Electrostatic-Magic™ (Nottingham, Great Britain). Colored plastic particles with a diameter of about 30-50 µm are charged by a high voltage of an electrode (see Internet Powder Coating Machine) and sprayed in the vicinity of the flowers by air pressure. The peduncle is earthed with an electrode, as is also the case in nature. The flowers are photographed immediately before and after the spraying. Locations with high powder density possess the strongest electric fields (see Elektrische Felder von Blüten).

7.11. 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 81). 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°.

7.12. Colorado beetle

You find Colorado beetles (see page 86) on potato fields. The larvae as well as the beetles are showy colored. 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 potato leaves until the beetle 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.

7.13. Parasitic wasp *Nasonia vitripennis*

Nasonia vitripennis is a parasitic wasp (Hymenoptera: Pteromalidae). It deposits its eggs in pupae of various flies. Larvae eclose from the eggs which eat up the pupa, moult several times and finally leave the pupal case as wasps (see the movie Nasonia Schlüpfen). At 25° C it takes 14 d until the *Nasonia* adults eclose[1]. The males eclose before the females. If one wants to see the developing larvae, one has to open the pupal cases of the house flies.

Catch house flies (*Musca domestica*) and put them in a translucent vial with old soft cheese, on which the females can deposit their eggs. Close the vial with a foamed plug or a dense net. Once the pupae have formed, release the flies and deposit the vial outdoors, in order to attract parasitic wasps (*Nasonia vitripennis*). A net of the right mesh covering the vial hinders flies from entering.

The mentioned times are for long days, that is, light conditions as during the summer. In the fall with shorter days eggs

[1] At 25° C larva hatch after 1.5 – 2 d from the eggs and start to feed on the pupa. Until the 7th day the 4th stage is reached. Afterward the prepupa and a white pupa forms, which becomes yellowish. Between the 9th and 10th day the eyes, head and thorax pigment and until the 13th day the whole body is pigmented. The males eclose before the females on the 13th and 14th day. At this temperature the first eclosing *Nasonia* animals can thus be expected after 14 days.

are are deposited, which develop only until the last larval stage and enter diapause to survive the winter.

If the animals are kept in a room in the cellar under shortday conditions (eg 8:16 h LD, timer for the switch between light and dark), they stay in the last larval stage and do not develop further. If groups of the animals are exposed to different numbers of short days, one can determine how many days are needed for all animals to enter diapause (see figure 4.29).

7.14. Head clock

Prepare an alarm clocks powered with a battery in such a way that it can be used as a 'stopwatch'. This is described on page 69. You can use it to test yourself or others whether you have a well functioning head clock. It is, however, easier to take a shot at night after the intended waking up by using a digital camera or a mobile phone camera after adjusting it to record date and clock time in the image. In the morning the time can be read and compared with the intended wakeup time.

7.15. Pitcher plants: Attraction of insects, UV

On page 77 it was described, how carnivorous plants attract their prey, especially insects, by blue light (430-480 nm) which was induced by UV light (366 nm). For these wavelengths low cost UV lamps with LEDs are available (e.g. Graphiteq Zellfusion 5 € plus shipping costs). Illuminate the peristom and the interior of a *Sarracenia* pitcher plant leaf and observe the blueish loom. Determine the number of attracted insects with and without UV-induced blue light. Compare with other pitcher plants.

7.16. More to the experiments

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

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

Glassware: 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 behavior. 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* on pine. Silkmoth *Bombyx mori*. The giant silkmoth *Philosamia cynthia, Hyalophora cecropia* and *Antheraea pernyi* can be purchased at insect exchanges in the pupal stage. The butterflies mentioned for the butterfly clock (figure 1.13 and page 7.4) 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.* Sandwolf 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,* night-blooming jasmine *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 15 and page 155).

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

8. 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 **?**. One can use the operating system Linux. 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 purchase a Life Knoppix CD (by Hans Knopper or bookshop Lehmanns in various cities in Germany). It contains a condensed Debian-distribution with numerous programs. You insert the CD in your CD-ROM and can now work under Linux without affecting your original system. You might also install Linux besides another operating system on the computer.

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

A. Appendix

A.1. Diapause-model for *Sarcophaga*

On the basis of the feedback model of **??** **?** proposed a model, which simulates the locomotor activity of the Weta[1] (*Hemideina thoracica*). This Anostostomatide from the Northern Island of New Zealand is up to 40 mm long and night active. It lives in holes of tree barks and feeds on plant parts and small insects.

The model works like a refrigerator (see figure A.1). It was used by **?** to model the photoperiodic behavior of *Sarcophaga argyrostoma*. This flesh fly hibernates in its pupal stage, but the signal for the induction of diapause is received already by the embryo in the uterus of the mother and in the young larvae. In the longdays of the summer the larvae develop to adults (flies), but in the fall the animals stop developing in the pupal stage. In this stage they stay in diapause for several months. The critical daylength amounts to 14.5 h[2]. However, as in most insects with diapause, the length of the night is measured; thus the critical dark period amounts to 9.5 h. Two things are decisive: The length of the night is measured with the circadian

[1] Weta is derived from the Maori word Wetapunga, meaning the *god of ugly things*

[2] the critical daylength depends on the geographical latitude of the population. The one given here is for the population around Edinburgh in Scotland

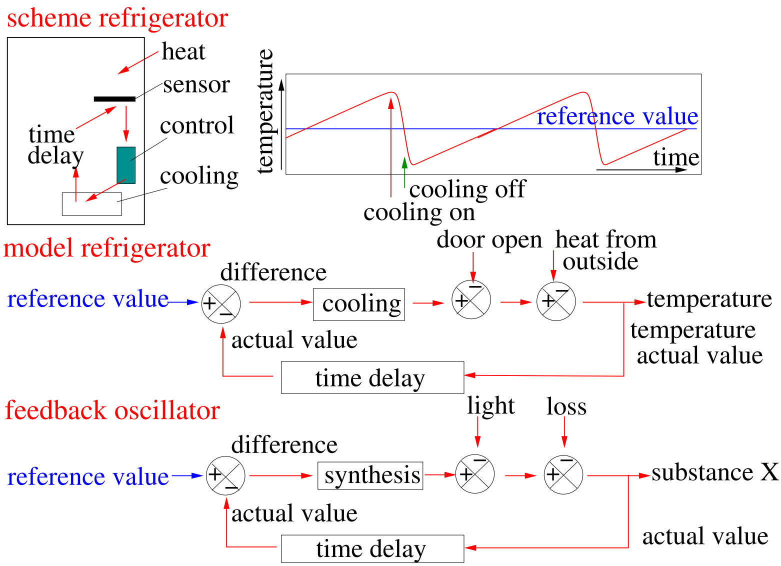

Figure A.1.: Top: *Scheme and temperature course of a refrigerator. A sensor ascertains that, if the temperature has exceeded the set point, the compressor chills. After a* time delay *the set point is reached and the temperature sinks somewhat below the set point, as shown by the red curve (cooling on: red arrow, cooling of: green arrow).* ***Center:*** *Model of refrigerator. Its set point is compared with the actual value, until there is no difference any more. Cooling stops. Since, however, the refrigerator is not ideally insulated, the temperature increases again and this results in oscillations (curve upper right).* ***Bottom:*** *Oscillator model: The concentration of a substance X is compared with a set point. If the actual value has fallen below it, more X is synthesized. Due to some loss of X and due to the time delay in the feedback, oscillations occur. The effect of light corresponds to the opening of the refrigerator door. After* **???***; see also* **?***.*

clock and a counter adds something up during inductive dark periods (INDSUM), which after a sufficient number of days induces finally diapause.

Light plays a double role: It synchronizes in an LD the oscillator, and it controls the diapause/non-diapause switch. In a shortday (with a dark period shorter than the critical one) a light sensitive phase Φ_i falls in the dark period, but in a longday it receives light. A certain phase relationship between the oscillator and the external LD in the environment thus decides, whether diapause occurs or not. However, additionally a specific number of shortdays has to be received in order to reach a sufficiently high *diapause titer*. This substance inhibits directly or indirectly the secretion of the neuropeptide prothoracotrophic hormone PTTH in the brain of the pupa.

Depending on the synthesis rate of an (unknown) substance X the oscillation in DD is undamped or it damps out (see figure A.3). The authors assume a damped oscillation e.g. at a synthesis rate of 0.10. If light is given daily for 8 h (LD 8:16), the damped curve gets each day a kick. If the kick occurs at the right time (depending on the length of the light- and thus also on the dark period), the damped curve turns into an undamped one. At a proper length the curve surpasses the horizontal limit line and Φ_i lies in the dark. During this time X is synthesized and after a certain number of days a sufficient amount has accumulated, which leads to diapause (figure A.3 with INDSUM indicated). At a by one h shorter dark period (15:9 h LD) the maximum of X formation falls into the light period (lower part of figure A.3) and X will be destroyed. Incidentally, the period length is changed by the *time delay* element.

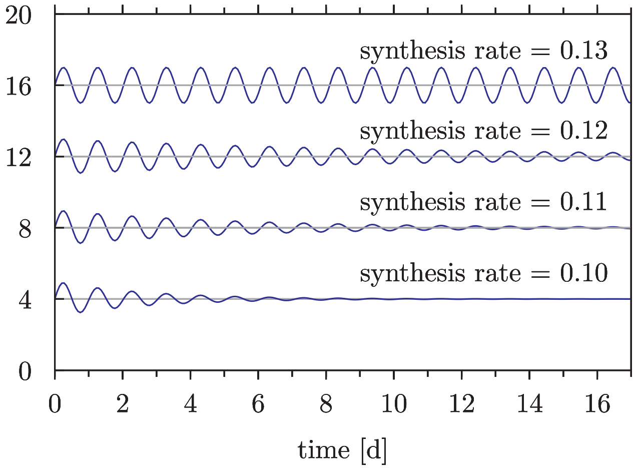

Figure A.2.: *In the Lewis-Saunders oscillator model (see also figure A.1) the damping in DD depends on the synthesis rate. At a value of 0.13 there is no damping, at 0.10 the oscillation is completely damped after a few cycles, as shown in the lowermost blue curve. If at a damping of 0.10 the oscillator is simulated under short day (LD 14:10, see figure A.3), diapause is induced. After* **???**.

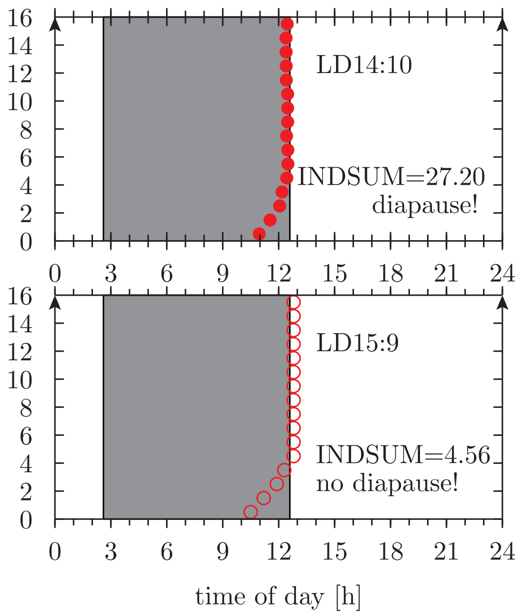

Figure A.3.: *A damped oscillation (synthesis rate 0.10) receives by a LD 14:10 h each day a kick. If it occurs at the right time –which depends on the length of the light period, the damped curve becomes an undamped one and the maximal values lie above the threshold (see figure A.2). Φ_i (red circles) are now located in the dark (gray area). X is synthesized and accumulates, so that after a certain number of days enough is available (INDSUM=27.20) to induce diapause.*
If, however, the LD is 15:9 h, that is a by 1 h longer light period, the maxima of the oscillations lie still above the threshold, but in the light. Light destroys X and the values have to be subtracted, which leads to an INDSUM of 4.56 only. After **???***.*

The factor X could be a substance which, depending on the 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 treatment, *inhibits* the reaction. Both cases were found in the diapause of various insects and in both cases neurosecretory cells are involved. Afterward a mechanism becomes effective which transforms the signals into reactions which finally lead to the diapause.

A.2. Further books, movies

There are two books on movements in plants, **?** and **?**, which contain many additional informations on the topics covered here. In both books experiments are described. A book has been published by **?** 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 **?**. Also in the Internet is the book *Rhythms of Life* **?**. This is, however, written for students and specialists. Some books on interesting outdoor excursions for children are by **???**, but unfortunately all out of print.

To get to know the microscope see the following books: **???**, a thorough introduction in the microscopy and its technique, **?**, a new book on microscopy, well and nicely illustrated with precise instructions and a rich source for microscopic exercises and hints into the micro-world (**?**) with practical instructions and proposals for selecting objects. I recommend especially **?**.

There are some more books by Engelmann which are also concerned with topics related to rhythmic processes in organisms:

- *Rhythms in organisms - Observing, experimenting, recording and analyzing* http://nbn-resolving.de/urn:nbn:de:bsz:21-opus-37910
- *Rhythms of life - An introduction using selected topics and* examples http://nbn-resolving.de/urn:nbn:de:bsz:21-opus-37998
- *How plants grow and move* http://nbn-resolving.de/urn:nbn:de:bsz:21-opus-37764
- *Biocalendar: The year in the life of plants and animals* http://nbn-resolving.de/urn:nbn:de:bsz:21-opus-37626
- *Flying clocks - The clocks of* Drosophila http://nbn-resolving.de/urn:nbn:de:bsz:21-opus-37965
- *Lithium ions against depression: Is the daily clock involved in depression? Experiments in Spitzbergen* http://nbn-resolving.de/urn:nbn:de:bsz:21-opus-53405
- *Rhythms in structures of organisms* http://nbn-resolving.de/urn:nbn:de:bsz:21-opus-37943
- *Clocks which run according to the moon - influence of the moon on the earth and its organisms* http://nbn-resolving.de/urn:nbn:de:bsz:21-opus-37674
- *Our internal clocks - biological time measurement in man and other mammals* http://nbn-resolving.de/urn:nbn:de:bsz:21-opus-37740

- *Cellular clocks:* http://nbn-resolving.de/urn:nbn:de:bsz:21-opus-39937

Several teaching movies on bees are available:[3]:

- *Color sense of bees:* **?**
- *Language of bees:* **?**
- *Development of the honey bee:* **?**
- *Indication of distance and direction in the honeybee – round and waggle dance:* **?**
- *Verification of color sight in the honey bee:* **?**
- *Pollen- and nectar collecting of the honey bee:* **?**

A.3. Thanks, requests, addresses

Claudia Holt and Serge Roy corrected the English text, Dirk Engelmann, the Lyx-User-Group and the Linux-User-Group Tübingen were often helpful with technical questions. Thanks also to the employees in the Botanical Garden Tübingen, especially Herr Franz, Frau Fiebig, Frau Dr. Kehl, Herr Lauterwasser, for advice and help with pictures of plants. Gerhard Mickoleit helped to take pictures of the butterfly collection in the zoological collection of the University of Tübingen. Mareike

[3]The Institut für den wissenschaftlichen Film in Göttingen had to close down in 2010. The movies can now be checked out from the 'Kompetenzzentrum für nicht-textuelle Materialien (KNM)' of the Technische Informations-Bibliothek in Hannover (see TIB).

Förster, Tübingen, produced a number of drawings after originals. Our special thanks to her. James H. Cane, USDA-ARS Pollinating Insect Research Unit Utah State University, Logan, UT 84322 USA, has kindly supplied the picture of a breeding cell (figure 2.33) and of a *Megachile* bee (figure 2.26) of his coworker Theresa Pitts-Singer.

We would appreciate hints regarding errors, shortcomings, deficits or unclear parts in the book. Please feel free to contact the author WE if you have questions. Check also the publication site TOBIAS-lib - of the Tübingen University library Publication site for further publications and movies. Our addresses are

Wolfgang Engelmann, Schlossgartenstrasse 22, 72070 TÜBINGEN, Tel. 07071-68325, EMail: engelmann@uni-tuebingen.de

Bernd Antkowiak, Experimentelle Anaesthesiologie an der Medizinischen Fakultät der Universität Tübingen, 72070 TÜBINGEN, Tel. 07071/2981121 Fax 07071/2925073, EMail: bernd.antkowiak@uni-tuebingen.

Abbreviations

BH	brain hormon
C	Celsius
cm	centimeter
EEG	electroencephalogram
eg	for instance, example given
etc	and so on
g	gram
GB	gigabyte
h	hour
Hz	Hertz
km	kilometer
l	liter
LD	light-dark-cycle
m	meter
ml	milliliter
mm	millimeter
N	North
nm	nanometer
REM	Rapid Eye Movement
UV	ultraviolet
V	volt

Index

Name index