Chapter 2
Biology of Mosquitoes

Regarding their special adaptational mechanisms, mosquitoes are capable of thriving in a variety of environments. There is hardly any aquatic habitat anywhere in the world that does not lend itself as a breeding site for mosquitoes. They colonise the temporary and permanent, highly polluted as well as clean, large and small water bodies; even the smallest accumulations such as water-filled buckets, flower vases, tyres, hoof prints and leaf axes are potential sources.

In temporarily flooded areas, along rivers or lakes with water fluctuations, floodwater mosquitoes such as *Aedes vexans* or *Ochlerotatus sticticus* develop in large numbers and with a flight range of several miles, become a tremendous nuisance even in places located far away from their breeding sites (Mohrig 1969; Becker and Ludwig 1981; Schäfer et al. 1997).

In swampy woodlands, snow-melt mosquitoes such as *Oc. cantans, Oc. communis, Oc. punctor, Oc. hexodontus*, and *Oc. cataphylla,* encounter ideal conditions for development in pools that are formed after the snow melts or after heavy rainfall.

In floodplains along coastal areas, the halophilous species (preferring brackish or salt water habitats) such as *Oc. taeniorhynchus*, *Oc. sollicitans*, *Oc. vigilax*, *Oc. caspius, Oc. detritus*, develop in huge numbers. Larvae of the *Anopheles* can be found in association with other mosquito species in fresh- or salt-water marshes, mangrove swamps, rice fields, grassy ditches, edges of streams as well as in small temporary water collections. Many species prefer habitats either with or without vegetation.

Tree-holes are the habitat of arboreal species such as *Oc. geniculatus*, *Ae. cretinus*, *Anopheles plumbeus* and *Orthopodomyia pulcripalpis*.

Species like *Cx. p. pipiens*, *Ae. aegypti* [*St. aegypti*] *Ae. albopictus* [*St. albopicta*] or *Oc. j. japonicus* can even breed in a variety of small water containers such as rain-water drums, tyres, cemetery vases, small clay pots, etc.

Furthermore, their capacity to adapt to various climatic factors or changing environmental conditions is fascinating. For instance, *Ae. albopictus*, the Asian tiger mosquito is originally a tropical species. In the course of a climate-related evolutionary adaption it developed a photoperiodic sensitivity. When days are shorter, the photoperiodically sensitive female inhabiting a temperate climate, lays eggs that are different from the eggs that she lays when days are longer. Eggs laid during shorter days, are dormant and do not hatch until the following season, ensuring the species' survival through the winter.

This ability to adapt to moderate climatic conditions and the fact that the eggs are resistant to desiccation and survive for more than a year, including the capability of adaptation to artificial breeding sites such as tyres and flower pots, make *Ae. albopictus* or *Oc. j. japonicus* successful species. This has contributed its spread globally via international trade in plants like *Dracaena* spp. ("lucky bamboo") and tyres. Within hours or days they can be transported from one country or continent to another by cars, aircrafts or trans-oceanic containers (Madon et al. 2002).

These are but a few examples, which illustrate the tremendous ecological flexibility that mosquitoes have successfully adapted to.

Like all Diptera, mosquitoes exhibit complete metamorphosis. All mosquitoes need aquatic habitats for their development, although *Aedes/Ochlerotatus* spp. can lay their eggs in moist soil. After hatching they pass through four larval instars and a pupal stage when the transformation into an adult takes place. Most species are unautogenous: following copulation, the females have to take a blood-meal to complete the egg development.

Only a few species have populations that are autogenous. They first develop egg batches without a blood-meal (*e.g. Cx. p. pipiens* biotype *molestus*).

2.1 Oviposition

Female mosquitoes lay between 50 and 500 eggs, 2–4 days (or longer in cool temperate climates) after the blood-meal. In general, the mosquitoes can be divided into two groups depending on their egg-laying behaviour (Barr 1958) and whether or not the embryos enter into a period of dormancy (externally triggered resting period) or diapause (genetically determined resting period).

In the first group, females deposit their eggs onto the water surface either singly (*Anopheles*) or in batches (*e.g. Culex, Uranotaenia, Coquillettidia, Orthopodomyia* and subgenus *Culiseta*, Fig. 2.1).

The *Culex* females lay their eggs in rafts comprising several hundred eggs locked together in a boat-shaped structure. During oviposition, the females stand on the water surface with the hind-legs in a V-shaped position. The eggs are released through the genital opening and grouped together between the hind-legs, forming a raft where the eggs stand vertically on their anterior poles attached together by chorionic protrusions (Clements 1992). The anterior pole of each egg has a cup-shaped corolla with a hydrophilic inner surface, which lies on the water surface, the outer surface is hydrophobic. The resulting surface tension helps to

Fig. 2.1 Egg raft of *Culiseta annulata* (size approx. 5 mm)

keep the egg-raft in position and when rafts drift to their aquatic boundaries they tend to remain there. Immediately following oviposition, the eggs are soft and white, but they sclerotize and darken within 1–2 h.

Anophelines lay single eggs while standing on the water surface or hovering above it. The eggs of this subfamily are adapted for floating and can easily be dysfunctioned by desiccation.

The embryos of the first group do not enter dormancy or diapause and hatch when the embryonic development is completed. Species producing nondormant eggs usually have several generations each year. Their developing stages are found for the most part in more permanent waters where one generation succeeds another during the breeding season. The number of generations depends on the length of the breeding season, as well as the abiotic and biotic conditions, and most importantly, it is the temperature which influences the speed of development.

The parameters that determine the choice of a breeding site by the females laying their eggs onto the water surface are still unknown for many species. Factors such as water quality, incidence of light, existing eggs, available food, and local vegetation are decisive factors in selecting a favourable breeding site.

For *Cx. p. pipiens,* it is known that the content of organic material in the water plays an important role in attracting the females about to lay eggs. Apparently, gaseous substances such as ammonia, methane, or carbon dioxide, which are released when organic material decomposes, create an effect of attracting the females of *Cx. p. pipiens* (Becker 1989b). They recognise that such a site has adequate food and that favourable conditions prevail for the development of their brood.

A few other examples illustrate that the egg-laying behaviour reflects the ecological conditions in the breeding site. The submerged larvae and pupae of *Coquillettidia* obtain the oxygen they need by inserting their siphon into the aerenchyma (air filled tissue) of certain plants under water. Therefore, in order to ensure the development of the larvae, the females must recognise the appropriate aquatic plants at the time they lay their eggs in order to ensure the development of the larvae.

The second group lays eggs which do not hatch immediately after oviposition (Fig. 2.2). The egg-laying behaviour of the floodwater mosquitoes (*e.g. Ae. vexans),* and the subgenus *Culicella* of genus *Culiseta,* which lay
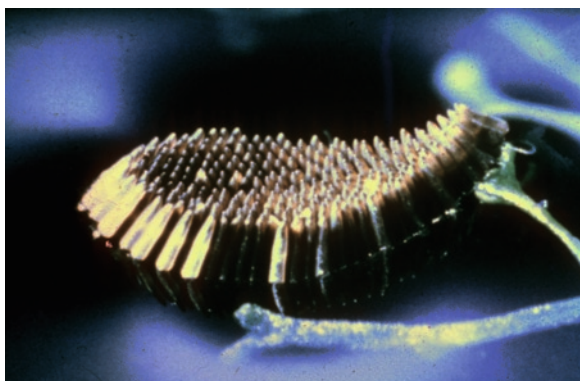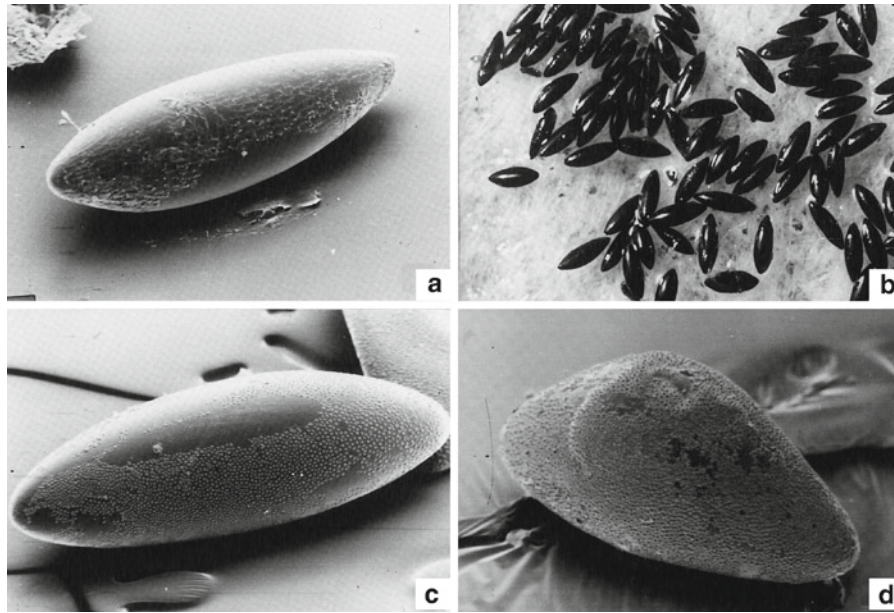
Fig. 2.2 Eggs of *Ae. vexans* (**a**: SEM-photo, 50X; **b**: light microscopical photo 8X, *Oc. cantans* (**c**, SEM, 50X), *Oc. rusticus* (**d**, SEM, 50X)

their eggs singly, not on the water surface but into the moist soil, which is subsequently flooded when water levels rise, is most interesting. The eggs are laid into small depressions or between particles of moss with a high degree of soil moisture which protect the sensitive eggs from drying-out during the embryogenesis (Barr and Azawi 1958; Horsfall et al. 1973). For *Ae. vexans* and *Oc. caspius*, which breed in flooded areas where the levels fluctuate frequently, the appropriate egg-laying behaviour is crucial to ensure successful development of the immature stages. A suitable egg-laying site for flood-water species must meet the following prerequisites:

(a) The substrate must be wet enough at the time the eggs are laid in order to ensure that the freshly laid eggs which are very sensitive to any water loss, do not desiccate before their impermeable endochorion has been tanned and the wax layer of the serosal cuticle is formed (Horsfall et al. 1973; Clements 1992);

(b) There must be a subsequent and sufficient flooding of the soil where the eggs have been laid, so that the complete process can take place from hatching all the way to the imago emergence;

(c) The water body for subsequent breeding should have as few mosquito predators as possible, to ensure that the larvae are not preyed-upon by natural enemies when they hatch.

The ability of a floodwater mosquito female to find appropriate places for egg-laying which guarantees maximum breeding success is not yet fully understood. However, respect is due to these tiny insects which have adapted their behaviour to overcome the hostile conditions in their breeding sites. If the females chose to lay their eggs in low-lying areas with almost permanent water-flow, they would encounter crucial disadvantages: low-lying areas are flooded for long periods of time and have, therefore, a very unfavourable alternating sequence of dry and flooding spells, therefore, allowing only very small populations of floodwater mosquitoes to develop. Areas with almost permanent water-flow generally have a high concentration of natural enemies, such as fish, so that the risk of being preyed-upon would be very high for the mosquito larvae.

However, flooded areas with a very short period of water-flow are also unfavourable egg-laying sites, because they entail the risk of premature drying-out. This kind of terrain becomes flooded for a short period of time and only in the years when water is abundant, thus the wet and dry sequences are not favourable for the development of several consecutive generations. These areas dry very rapidly following a flood so that the *Aedes* or *Ochlerotatus* eggs run the risks of desiccation since the developing embryos are very sensitive to water loss.

Figure 2.3, shows the preferred egg-laying sites of the floodwater mosquitoes (*e.g. Ae. vexans*). Usually, these areas consist of dense vegetation and silty soil, with the reed (*e.g. Phragmites australis*) being highly attractive to ovipositing female mosquitoes.

Usually, the reed zone precisely demarcates the mid-water level in riparian systems, since reeds need a good amount of water.

The fluctuating curve of the Rhine river, for example, makes it quite clear how important this egg-laying behaviour is for the development of the floodwater mosquitoes (Fig. 2.4). In a zone of between 4 and 5 m, there is the ideal timing of dry and wet periods, with optimum water-flow to guarantee the development of a large number of individuals in a population. Furthermore, predators, especially fish are usually low in numbers or absent.

But how do these female floodwater species find the optimum oviposition site? Gravid females obviously recognise the wet, silty, riparian clay soil as an appropriate egg-laying substrate and are attracted to it. However, these criteria alone are not enough, given that during rainy periods there would be many other places which would apparently appear adequate due to their high moisture content.

It is likely that the floodwater mosquitoes are able to differentiate between various soil types. The soil in most floodplains consists of a high percentage of clay and low percentage of humus or organic materials, in contrast to many other soils (Ikeshoji and Mulla 1970; Strickman 1980a,b; Becker 1989b).

It is also possible that flooded areas along the riverbanks produce pheromone-like odours, which may be recognised by the female mosquitoes and which induce them to lay their eggs. These odours could come from eggs, which have already been laid in the soil, or from particular associations of plants which are indicators of a specific moisture level in the soil, and the occurrence of regular floods. As a whole, the ovipositional behaviour of the floodwater mosquitoes shows an astonishing
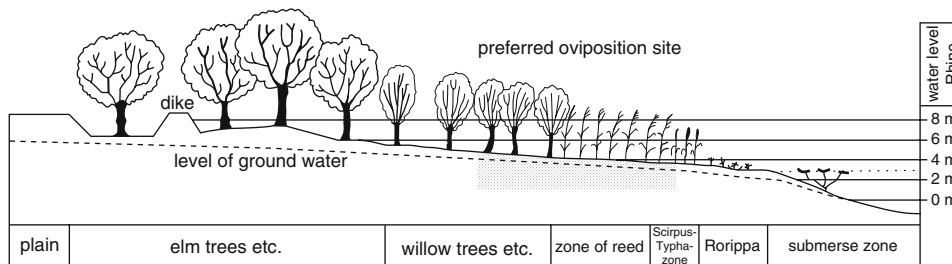

Fig. 2.3 Transsection through the Upper Rhine Valley with zones of vegetation and preferred oviposition sites of floodwater mosquitoes (*e.g. Ae. vexans*)
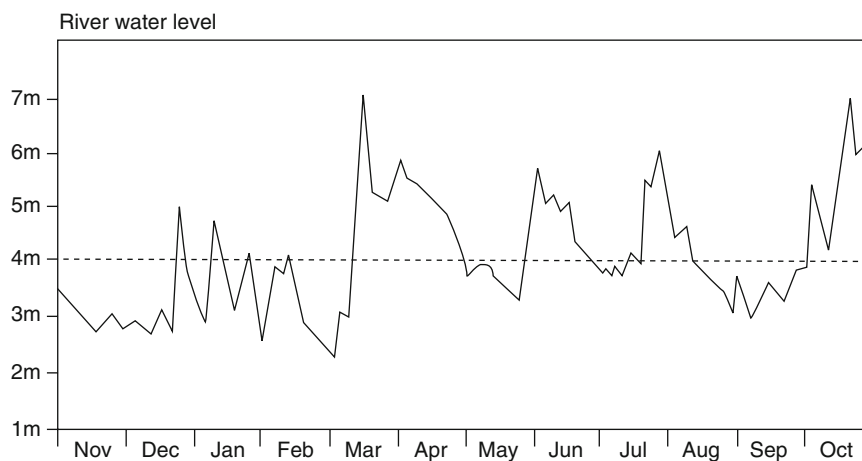

Fig. 2.4 River water levels in Central Europe favor the development of the eggs of floodwater mosquitoes

degree of adaptation to their habitat, an adaptation which has developed through the course of evolution.

2.2 Embryonic Development

Mosquito embryogenesis has been described in detail by Clements (1992). The embryonic development starts almost immediately after the eggs have been laid. Depending on the temperature, it takes about 2–7 days or more until the embryos are fully developed.

The course of embryonic development also reflects a special adaptation to various abiotic conditions in the larval habitat (Becker 1989b). The nondormant eggs of the *Culex, Coquillettidia, Uranotaenia, Orthopodomyia* and the subgenus *Culiseta,* usually hatch after a short time when the embryonic development is completed. The length of time required is dependent almost entirely on temperature. At a temperature of 30°C, the *Cx. p. pipiens* larvae hatch 1 day after the eggs have been laid, at 20°C and 10°C it takes 3 and 10 days respectively, and at 4°C, the embryonic development of the *Cx. p. pipiens* cannot be completed (Fig. 2.5).

The embryonic development of the *Aedes/Ochlerotatus* species usually takes significantly longer, for instance, larvae of *Ae. vexans* are ready to hatch in 4–8 days after oviposition, when the eggs are kept at 25 and 20°C, respectively (Horsfall et al. 1973; Becker 1989b). Hatching experiments with freshly laid *Ae. vexans* eggs kept at 20°C have shown that 8 days after the eggs had been laid, almost 50% were ready to hatch. This means that the embryonic development of *Cx. p. pipiens* usually takes only half as long as that of *Ae. vexans,* which assures a quick generation renewal of the former. The relatively slow embryonic development of floodwater *Aedes/Ochlerotatus* species can be explained by the fact that these mosquitoes lay their eggs in flooded areas where there are few ecological factors requiring rapid embryonic development. It usually takes more than one week until the next flooding occurs. Therefore, there is little ecological advantage in the fast sequence of generations that would result from rapid development of the embryos.

2.3 Hatching

Aedes and *Ochlerotatus* mosquitoes have developed a highly sophisticated mechanism which regulates the hatching process, as a direct adaptation to the greatly fluctuating abiotic conditions existing in the temporary waters where these mosquitoes breed (Gillett 1955; Telford 1963; Horsfall et al. 1973; Beach 1978; Becker 1989b). The timing of larval hatching to coincide with the presence of ideal developmental conditions, is a prerequisite for successful development in temporary water bodies.

The difference in hatching behaviour between the snow-melt mosquitoes (*e.g. Oc. cantans*, *Oc. communis*, and *Oc. rusticus*) and the floodwater mosquitoes (*e.g. Ae. vexans*) clearly illustrates the extent to which the hatching behaviour of each *Aedes/Ochlerotatus* species is adapted to the abiotic conditions in their respective breeding sites.

The breeding waters of snow-melt mosquitoes, for example depressions and ditches in marshy regions covered frequently with alder trees in central Europe, are usually flooded for long periods of time with relatively cold water.

In Fig. 2.6, the phases of development and diapause of the univoltine (monocyclic, one generation/year) snow-melt mosquitoes, are shown as a function of the water level variation of a pool in the swampy woodlands in central Europe.
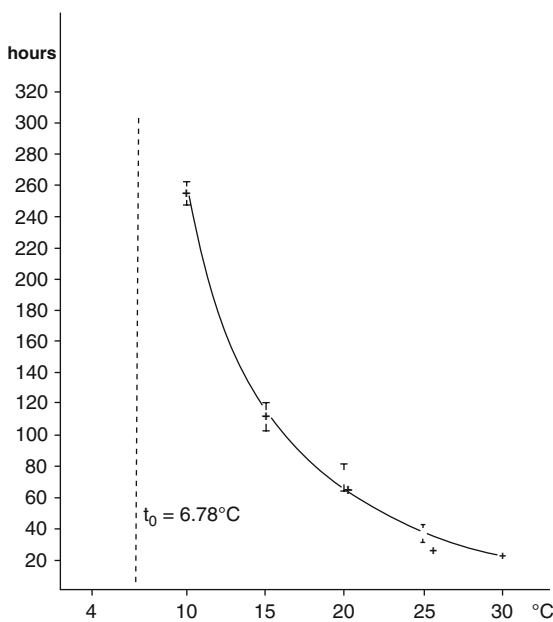

Fig. 2.5 Duration of the embryonic development of *Cx. p. pipiens* at various temperatures (t_o = no development possible according to Tischler 1984)
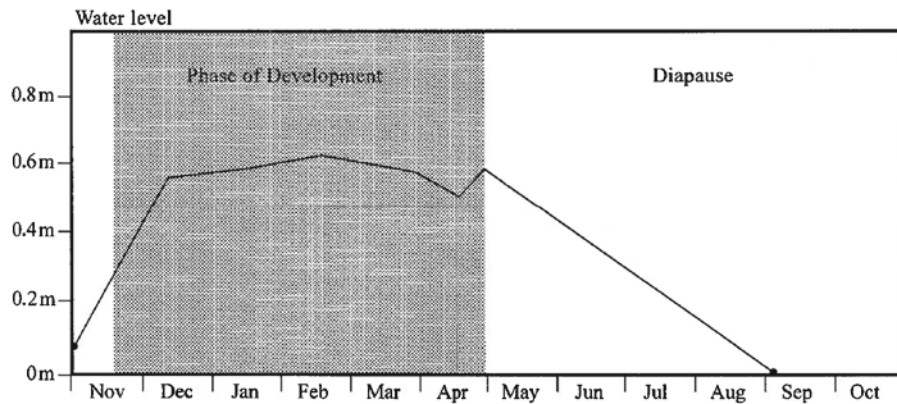
Fig. 2.6 Phases of development and diapause of univoltine snow-melt mosquitoes in a pond in Central Europe

The breeding sites of the snow-melt mosquitoes are usually flooded in the late fall and after the snow has melted. The water level usually reaches its peak in early spring. Under normal conditions it eventually recedes slowly but steadily throughout the summer until the pools are dry again.

Snow-melt mosquitoes have adapted perfectly to these conditions in their breeding sites, by their diapause patterns and appropriate reaction to the hatching stimuli. After the eggs have been laid usually in early summer, the embryos of the majority of the snow-melt mosquitoes automatically enter diapause. They are unable to hatch during the summer months, thus avoiding the risk of premature emergence during the dry spells of summer.

In central Europe, some species, *e.g.* the snow-melt mosquito *Oc. rusticus* and *Cs. morsitans*, are ready to hatch at the beginning of winter after sensing the continuous temperature decrease during autumn. The diapause of most snow-melt mosquitoes (*e.g. Oc. cantans* and *Oc. communis*) is interrupted when the temperature has dropped in autumn and the cold winter period has set-in. Consequently, these mosquitoes are ready to hatch during the snow-melt in the next spring and shortly afterwards. This factor, along with their ability to hatch in very cold and oxygen-rich waters, enables these mosquitoes to be ready to hatch at a time when favourable water level conditions are present. After hatching, the semipermanent water in forest pools provides ideal conditions for slow development. In central Europe, this usually takes place between the end of April and the beginning of May.

Even more sophisticated, is the hatching behaviour of the floodwater mosquitoes. In Fig. 2.7, the phases of

development and diapause of *Ae. vexans* are shown as a function of the water level variation (for *e.g.* the Rhine river).

Unlike the semipermanent status of the water in the breeding sites of snow-melt mosquitoes, the breeding sites of the floodwater mosquitoes are characterized by temporary water-flow caused by rapid, substantial fluctuations in the water level of the rivers following heavy rains in early and mid-summer. By contrast, late summer and winter are usually marked by extensive periods of low water levels. As a consequence of this, the best developmental conditions for larvae of floodwater mosquitoes occur between April and September, therefore, these mosquitoes diapause during autumn, winter, and early spring (Telford 1963). Due to the extremely variable nature of the water-flow, floodwater mosquitoes have to hatch during summer when high water temperatures enable rapid development to take place. Moreover, their being multivoltine (polycyclic, several generations/year), makes sense from an ecological viewpoint, since they can go through several generations coinciding with the fluctuations in the water level. This factor is mainly responsible for the huge reproduction rate of these species, often creating a tremendous nuisance.

If the hatching behaviour of the floodwater mosquito is analyzed in detail, a well-adjusted control mechanism can be seen, which is mainly influenced by the following factors:

(a) Dissolved oxygen is present during periods of high water levels, most of the flooded areas along rivers are covered with flowing and oxygen-rich water. Hatching at that time would create the risk of the
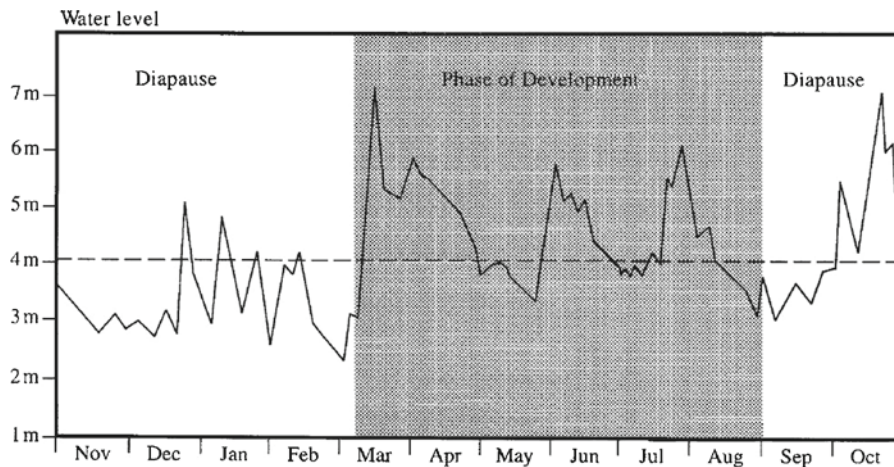
Fig. 2.7 Phases of development and diapause of the floodwater mosquito *Ae. vexans* in Central Europe

larvae being swept away. Moreover, fish usually invade deep flooded areas in search for food. In order to avoid these dangers, the floodwater mosquitoes have developed a specific hatching behaviour. The decline in dissolved oxygen when water is stagnant, usually triggers hatching of the larvae of the floodwater mosquitoes (Hearle 1926; Gjullin et al. 1941; Borg and Horsfall 1953; Travis 1953; Judson 1960; Horsfall et al. 1958, 1973; Burgess 1959; Becker 1989b). They hatch only when the flooded pools become stagnant and the oxygen content starts to decrease very rapidly (due to bacterial degradation processes). The decreasing level of oxygen in the water signals the unhatched larvae that the water will remain stagnant at the time of their hatching, thus, ensuring that the risk of being washed away, has diminished. Moreover, the bacterial action causing decomposition of organic material ensures that the larvae will have an adequate food supply. In addition, the shallow, stagnant waters are not a suitable environment for the larvae's main predator, the fish.

(b) Water temperature plays a fundamental role in the hatching process of the floodwater mosquito. Premature hatching during cold weather would greatly delay the development of the larvae, since the process is very temperature dependent.

Since floodwater mosquitoes usually diapause from autumn until springtime, the temperature-dependent hatching behaviour of *Ae. vexans* also ensures that the larvae will not hatch before the temperature of the water is warm enough to permit rapid development. This ensures that the brood will not dry out due to rapid changes in the water levels. For instance, in the Upper Rhine Valley, the *Ae. vexans* larvae hatch in springtime when the water temperature reaches 10°C or more. An interesting phenomenon is the seasonal-related hatching process. Following the cold winter phase and after the subsequent increase in temperature, a small percentage of the *Ae. vexans* larvae are ready to hatch even at 4°C. The adaptation of the temperature-dependent hatching behaviour to the climatic conditions and water-flow in an area in central Europe can be demonstrated with the example of the hatching response to water of 15°C. In springtime, the hatching rate reaches its peak at 15°C. In the months of March and April, apart from the gradually increasing water temperatures during springtime, the water level usually rises due to rainfall. This creates favourable conditions for the development of the floodwater mosquitoes. On the other hand, water of the same temperature induces a reduced hatching response during late summer and autumn. Larvae would not be able to complete their development if they were to hatch at a temperature of 15°C in October or November, since at this time of year the falling temperatures prolong the larval development, while the water level is receding.

It is remarkable that there are also differences in the hatching behaviour of the *Ae. vexans* populations in different river systems. It appears that the *Aedes/Ochlerotatus* species are adapted to the hydrological characteristics of each river system. In river systems

with a lower discharge of water, the periods of inundation are usually shorter, which means that the development of the mosquito has to be rapid. Therefore, the *Aedes* and *Ochlerotatus* mosquitoes breeding in these areas have an extended diapause until summer, to allow a faster development at higher temperatures.

Let us have a closer look at the diapause of the ready-to-hatch larvae inside the eggs of the floodwater mosquito *Ae. vexans*, especially concerning the change from a state of hatching inhibition to one of hatching readiness. This process is called "conditioning", whereas, the onset of hatching inhibition is called "deconditioning" (Horsfall 1956b; Horsfall and Fowler 1961; Clements 1963; Horsfall et al. 1973).

The factors that are most likely to have an influence on diapause, or on hatching inhibition and readiness, are temperature fluctuations, varying degrees of moisture in the air and soil as well as changes in the day-length (Brust and Costello 1969). Larvae of *Ae. vexans* are able to hatch to some extent during a flood in the same year as that in which the embryogenesis was completed, providing that the temperature remains above 20°C. Decreasing temperatures below 15°C lead to a hatching inhibition in autumn. After a cold (winter) phase, temperatures of 10°C and above have a conditioning effect, and interrupt the diapause in the next spring. However, it is worth mentioning that after a cold spell, the readiness to hatch is positively correlated with the rise in temperature. The higher the temperature during egg laying and the lower the temperature in winter, the higher is the hatching response in the following summer. The complex diapause behaviour allows the larvae of *Ae. vexans* to distinguish between favourable developmental conditions in springtime, and unfavourable conditions in late summer. It is remarkable that in winter even extreme temperatures well below the freezing point, will not kill the diapausing larvae.

Another behaviour that represents a sophisticated adaptation to the very variable water-flow in the breeding sites is what is referred to as "hatching in installments". Even within a batch of eggs laid by one female subjected to the same microclimatical conditions, not all of the larvae hatch uniformly. Without a cold phase, only a few individuals from a freshly laid egg batch of one female are ready to hatch, whereas, after a cold phase, the readiness to hatch is far greater. Apart from their inherited variability, the conditions that each egg had experienced (for instance, the location of the egg in the ovariole during maturation, the timing of the oviposition, as well as differing microclimatic factors

at the egg-laying site) determine whether a larva will hatch under certain conditions or not. Thus, the larvae hatch "in installments" (Wilson and Horsfall 1970; Becker 1989b). For instance, soil samples containing the eggs of *Ae. vexans* and kept at 25°C, were flooded several times, with dry phases of 4 weeks between each flooding step. After the first flooding, 57% of the total number of larvae hatched, 10% after the second, 25% after the third, and 8% hatched the fourth time (Becker 1989). This behaviour pattern assures long-term survival for mosquito species that develop in temporary bodies of water. If, for example, all of the larvae were to hatch at the same time under ideal hatching conditions and if, as a consequence of a sudden dry spell, all of the breeding sites were to dry-out before the brood could complete its developmental cycle. One single natural event could virtually wipe out the entire mosquito population. By "hatching in installments", the floodwater mosquito population can survive such potentially catastrophic events. There still remains a large contingent of unhatched larvae in the breeding area. At the time of the next flooding, these larvae will still have an opportunity to successfully produce a new generation without new eggs having to be laid. Incidentally, this also happens after a larvicidal treatment. It is remarkable that the unhatched larvae are able to persist for at least 4 years without losing their ability to hatch (Horsfall et al. 1973).

After the content of oxygen decreases in the breeding site, the larva initiates the shell rupture by pressing the so-called "egg tooth", an egg burster located posterio-dorsally on the head capsule of the larva, onto the egg shell. As a result, the shell splits along a particular line at the anterior end of the egg. A cap (anterior part of the egg shell) breaks away and the larva escapes by swallowing water into the gut which forces the body from the shell (Clements 1992). The whole process of hatching takes only a few minutes.

2.4 Larvae

The legless (apodous) larval body is divided into three distinct parts: (a) the head with mouth-parts, eyes and antennae; (b) the broader thorax and (c) the abdomen which is composed of seven almost identical segments and three modified posterior segments. These posterior segments bear four anal papillae to regulate the electrolyte levels. At the abdominal segment VIII, a siphon in culicines, or only spiracular lobes in anophelines, are
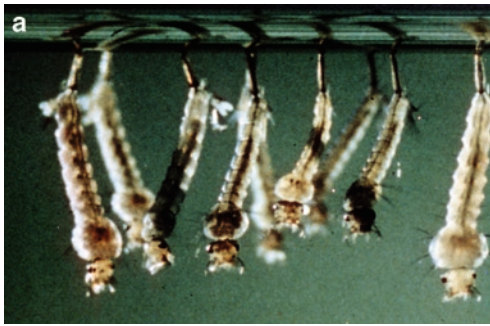
Fig. 2.8 (a) Larvae of *Aedes vexans*

Fig. 2.8 (b) Larva of *An. plumbeus*

Fig. 2.8 (c) Larva of *Coquillettidia richiardii* attached to plant tissue (photo, Hollatz)

developed where the tracheal trunks open at spiracles for the intake of oxygen. Usually the culicine larvae hang head downwards from the water surface (Fig. 2.8a). Anopheline larvae lie horizontally at the water surface. Their body is held horizontally by specialized setae (palmate setae), the notched organ located at the anterior margin of the prothorax and the spiracular lobes which are flush with the dorsal surface of the larval body and have direct contact with the air (Fig. 2.8b).

When the larvae leave the water surface, the lobes are retracted, the spiracles are closed and reach the water surface, the flaplike four or five spiracular lobes are pulled into their extended position by the surface

tension forces. A gland adjacent to the larval spiracles, secrete hydrophobic substances to avoid the influx of water into the respiratory system.

The larvae of the *Coquillettidia* and *Mansonia* live submerged. They therefore, possess a siphon which is modified for piercing submerged parts of aquatic plants to obtain oxygen from the aerenchyma. The spiracular apparatus at the distal end of the siphon contains hooks and a saw-like blade with teeth to pierce the plant tissues. These larvae have a more sessile habit, hanging head downwards whilst attached to the plant tissues and filtering the water column for food (Fig. 2.8c). They are therefore not easily recognised by predators such as fish.

The larval food consists of microorganisms, algae, protozoa, invertebrates and detritus. On the basis of their feeding behaviour they may be classified into filter or suspension feeders, browsers or predators. The filter feeders collect food particles suspended in the water column (especially larvae of the *Culex, Coquillettidia, the* subgenus *Culiseta* or to some extent the *Aedes/ Ochlerotatus* larvae). The larvae of the filter feeders, typically hang on the water surface filtering the water column beneath the surface by beating their head brushes (lateral palatal brushes) towards the preoral cavity. This generates water currents which carry food particles towards the mouth (Dahl et al. 1988). Mosquito larvae are usually not discriminatory in what they ingest. However, the size of the particles are usually less than 50 μm. Larvae can also move slowly in the water column while filter feeding. The browsers (*e.g.* most *Aedes* and *Ochlerotatus* species) collect food by re-suspension, scraping or shredding particles, microorganisms, algae, and protozoa from the surface of submerged substrates or the microbial film at the air–water interface (the *Anopheles* larvae). Even small portions of dead invertebrates and plants can be bitten-off with the mouth parts.

The anopheline larva hangs horizontally under the water surface with its dorsal side uppermost and the mouthparts directed downwards. When feeding, the larva rotates its head 180° and creates a water-current by beating its head brushes to collect the food organisms on the surface film.

Predacious larvae of the genera *Toxorhynchites, Aedes, Psorophora* and *Culex* can feed upon insects (often other mosquito larvae).

Disturbances of the water surface cause the larvae to dive for short periods of time. They dive by flexing the abdomen and moving backwards. When the larvae return to the water surface, they swim backwards until their abdomens come into contact with the surface.

Larvae moult four times at intervals, before reaching the pupal stage. At each moult, the head capsule is increased to the full size characteristic of the next instar, whereas the body grows continuously. Thus the size of the head capsule is a fairly good morphometric indicator for the larval instar. Each moult is coordinated by the relative concentrations and interactions of juvenile hormone and ecdysone, a molting hormone.
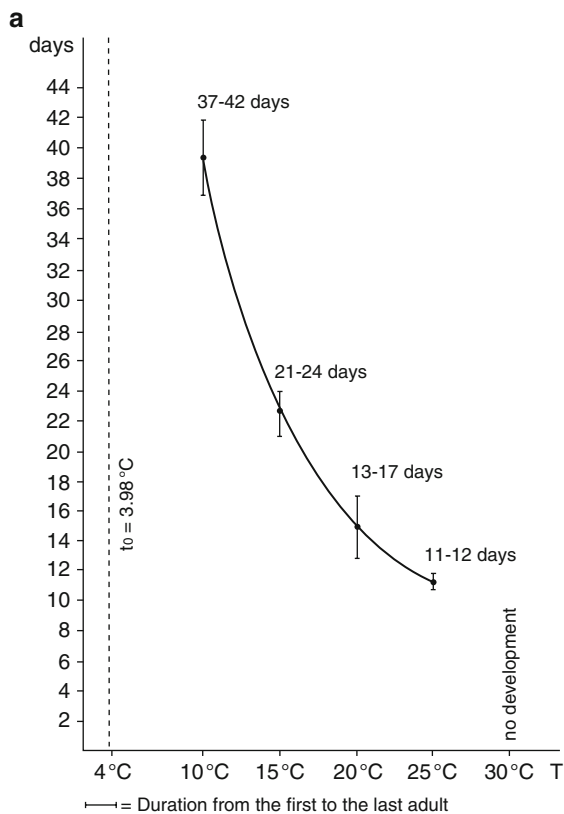
The development of larvae is temperature dependent. There are great differences in the optimum temperature for the development of different mosquito species (Figs. 2.9a–c). For instance, the snow-melt mosquitoes can complete their development at temperatures as low
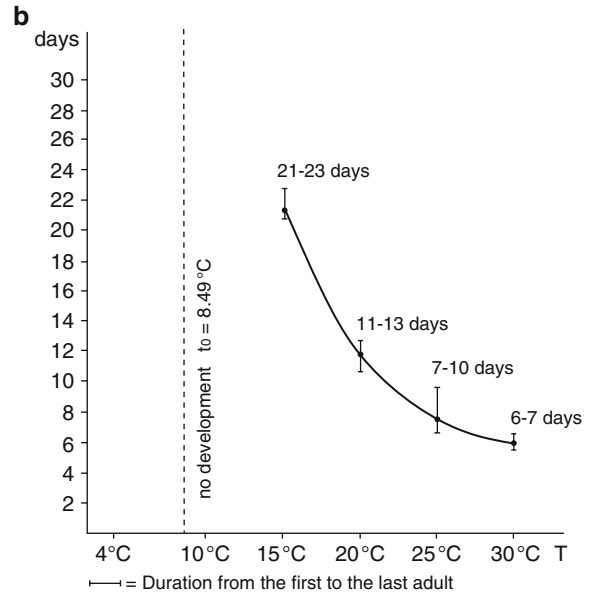
Fig. 2.9 (**b**) Larval and pupal development of *Ae. vexans* in relation to water temperature

Fig. 2.9 (**a**) Larval and pupal development of *Oc. cantans* in relation to water temperature
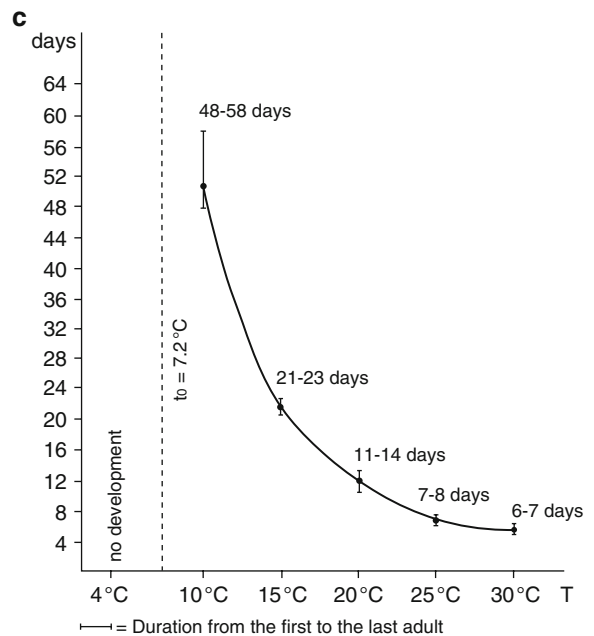
Fig. 2.9 (**c**) Larval and pupal development of *Cx. pipiens* in relation to water temperature

as 10°C, whereas they are incapable of developing successfully at temperatures above 25°C (Fig. 2.9a). Usually they hatch in southern and central Europe during February, or later in the northern parts, and the adults emerge 2–3 months later. Larvae of those species which overwinter in the larval stage such as the *Oc. rusticus* or *Cs. morsitans*, will survive in water close to freezing, or even in waters which become regularly coated with ice.

In contrast, the floodwater mosquitoes (*e.g. Ae. vexans*), develop successfully at higher temperatures within a short period of time, usually 6–7 days from hatching to emerging at 30°C (Fig. 2.9b). Larvae of *Cx. p. pipiens* can successfully develop in a wide range (10–30°C) of temperatures (Fig. 2.9c). The development of the aquatic stages also represents an adaptation to the ecological environment in breeding waters.

Mosquito larvae (*e.g. Ae. vexans*) sometimes aggregate in particular places at the breeding sites. This crowding effect may be a mechanism to reduce the chance of predation of any single larva.

2.5 Pupae

The pupae are also aquatic, the pupal stages usually lasting about 2 days, however, this period may be reduced or extended, at higher or lower temperatures respectively. During the pupal stage, the process of metamorphosis takes place. Some larval organs are histolysed, whilst the body of the adult is formed through the development of imaginal disks (cells or groups of cells that remained quiescent in the larval body until the pupal stage). In particular, the fat body of the larva is transferred to the adult stage and used as a source of vitellogenines for autogenous egg formation or as a reserve for hibernation.

Characteristically, the head and thorax of the pupae are fused into a prominent cephalothorax which carries anterolaterally two respiratory trumpets (see Chap. 5, Fig. 5.18), which are connected to the mesothoracic spiracles of the developing adults to provide oxygen. The abdomen terminates with two paddles and is kept flexed under the cephalothorax. When at rest, the pupae float motionless at the water surface, with the tip of the trumpets and the palmate setae of the first abdominal segment in contact with the water surface. The hydrophobic rims of the trumpets protrude through the water surface for respiration. An air bubble between the appendages of

the cephalothorax makes the pupa positively buoyant. Mosquito pupae are quite mobile (unlike the pupae of most other insects). When the pupa is disturbed, it dives by straightening the abdomen and spreading the paddles rapidly flexing the abdomen which has retained the larval musculature. In contrast to larvae which have to swim actively to the water surface, the pupa floats passively back to the surface after diving. Pupae of most mosquitoes are relatively tolerant of desiccation and adults can emerge successfully even if pupae have been stranded, or when the breeding sites have almost dried out. Unlike larvae, pupae do not feed.

In *Mansonia* and *Coquillettidia,* the trumpets are modified for penetrating plant tissues. The pupae, like the larvae, take the oxygen from the aerenchyma of the submerged parts of the plants.

2.6 Adults

2.6.1 Emergence

The final stage of metamorphosis is completed when gas is forced between the pupal and the pharate adult cuticle, and into its midgut. The pupa straightens the abdomen into a horizontal position, and by swallowing air it further increases the internal pressure. The cephalothoracic cuticle of the pupa then splits along the ecdysial line and the adult slowly emerges from the pupal skin (Fig. 2.10). The emerging adult moves cautiously to avoid falling onto the water surface, whilst its appendages still remain partly in the exuvia. In this phase, the emerging individual is highly susceptible to

Fig. 2.10 Emerging adult mosquito

strong winds and predators such as water striders and spiders.

The pupae of the genus *Coquillettidia* are fixed to the plant tissues in the water body. At the end of the pupal development they have to float to the water surface. Therefore, the tips of the pupal trumpets break to release the pupa from the plant before emergence (Mohrig 1969).

After emergence, the adult increases the haemolymph pressure which causes the legs and wings to stretch. It then immediately ejects droplets of fluid to empty the gut, while air is dispelled from the gut some hours later. Within a few minutes it is able to fly when the soft cuticle has sclerotized. However, 1–1.5 days more are required for males and females respectively to adjust their metabolism (Gillett 1983).

There is also a difference between male and female sexual maturity at the time of emergence. Male mosquitoes are not sexually mature at emergence as they have to rotate their hypopygium through 180° before they are ready to mate, which takes about 1 day. Therefore, the males in the population usually emerge 1–2 days before the females in order to achieve sexual maturity at the same time as the emerging females. Since the pupal stage of the two sexes appears to be about the same length, the shortening in development of males takes place primarily in the larval stage. Consequently, the male pupae and adults of a population are smaller in size than the corresponding females.

Following emergence, the adults are ready to begin their life cycle again of mating, feeding and oviposition.

The plumose antennae of the males are especially receptive to the sound generated by the female. The flagellum of the antenna starts to vibrate and stimulate the Johnston's organ which is located in the swollen second segment (pedicel) of the antenna (Clements 1963; McIver 1982). Contact pheromones may also be involved in the mating behaviour.

When a female enters a swarm it will be seized immediately by a male. Usually the male and female copulate face to face when flying outside the swarm (Fig. 2.11). The copulation requires a complex merging of the male and female reproductive structures. Usually it takes less than half a minute for the male to deposit the spermatozoa in the bursa copulatrix of the female (Clements 1963), the sperm then moves to the spermathecae. The male accessory gland secretions contain a substance known as matronae, which after copulation makes the female unreceptive for the rest of her life. The females store sufficient sperm in their spermathecae to fertilize several egg batches without further copulation. In contrast to females, male mosquitoes may mate many times. The time and preferred location of swarming is species-specific. Swarming (eurygamy) is not necessary for all species, and some species may mate without it (stenogamy). Usually, mating takes place soon after emergence, since biting females are almost invariably inseminated. After insemination the search for a host to obtain a blood-meal is the next important phase in the reproductive life of the female.

2.6.2 Mating

Mating of most mosquitoes in the palaearctic region takes place when females enter swarms of flying males. Such a swarm can consist of only a few or up to several thousands of male mosquitoes. Usually, males form swarms over a marker at low light intensities especially in the evening and morning. Markers are projecting objects which contrast with the surroundings such as bushes. When swarming, males face into the wind and fly forward and backwards, up and down over the marker. This oscillating flight pattern is often called "dancing flight". The sound produced by the male wing beat has a frequency of ~600 cs^{-1}. The frequency of the wing beat of the females is lower than that of the males at 500–550 cs^{-1} and even lesser when engorged.
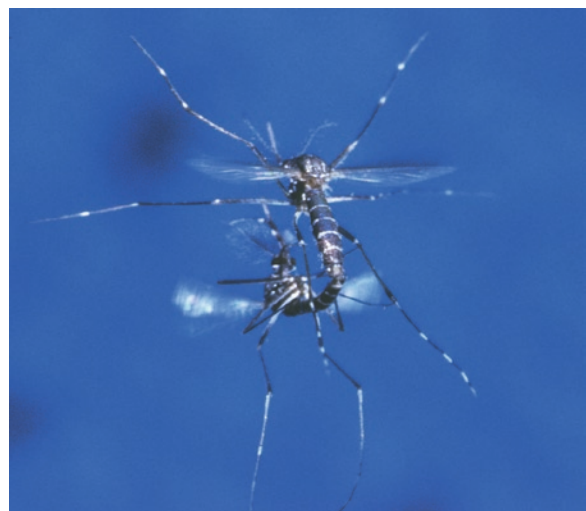

Fig. 2.11 Mating mosquitoes (Photo courtesy of Roland Kuhn, University of Mainz, Germany)

2.6.3 Dispersal and Host-Seeking Behaviour

In most mosquito species, oogenesis can only be completed when the females take a blood-meal. Therefore, they have developed a complex host-seeking behaviour to locate a potential host. Primarily, the location of the host is based on olfactory, visual and thermal stimuli. Females possess numerous antennal receptors which respond to host odours. The main olfactory stimuli are carbon dioxide, lactic acid, octenol, acetone, butanone and phenolic compounds. The host-seeking process may differ within species depending on the season and the availability of certain hosts. However, it can usually be divided into three phases (Sutcliffe 1987):

(a) Non-oriented dispersal behaviour which enhances the likelihood of the female coming into contact with stimuli derived from a potential host.
(b) Oriented host location behaviour resulting from contact with host stimuli. The strengths of the stimuli are increased as the mosquito and host come closer together;
(c) Attraction to a suitable candidate host, once the female has identified it in her immediate vicinity.

The extent of the non-oriented dispersal behaviour differs from species to species. In general, it can be separated into (a) species which usually breed and rest close to the habitat of their hosts and therefore do not fly long distances (most container breeders, *e.g. Cx. p. pipiens*); (b) species which disperse moderate distances from their breeding or resting places to the host's habitats (some species of snow-melt mosquitoes, *e.g. Oc. rusticus*); (c) species, which migrate considerably long distances to invade new habitats for biting, and/or egg-laying when suitable habitat is available (some floodwater mosquitoes, *e.g. Ae. vexans*).

The flight behaviour is influenced by temperature, humidity, illumination levels, wind velocity and the physiological stage of a female. For instance, most *Aedes/Ochlerotatus* species migrate during the twilight when the temperature is dropping and the humidity is increasing. They are usually more active on moonlit nights (Bidlingmayer 1964).

Species with a tendency for extensive flight activities usually show two different non-oriented dispersal behaviours (Provost 1953), a drift with the wind (passive migration) and an active dispersal (appetitive flight).

During passive migration, the mosquitoes ascend in swarms and use the wind to drift long distances and may occur suddenly in large numbers far away from their breeding sites. This non-oriented flight activity is especially influenced by the speed and direction of the wind and guiding landmarks. The passive migration in swarms occurs only a short time after emergence (Bidlingmayer 1985).

During the appetitive flight, female mosquitoes usually disperse actively at least 24 h after emergence. They fly upwind when the wind velocities are below mosquito flight speed (1 m/s) (Bidlingmayer and Evans 1987). The flight against the wind increases the likelihood of encountering stimuli deriving from a host. However, strong winds prevent active dispersal. This behaviour is species-specific and depends on various features of the terrain and meteorological factors. The microclimate influenced by the vegetation type, which causes increased humidity and reduced wind, strongly affects the dispersal behaviour. Therefore, females usually fly close to the ground or slightly above the top of the vegetation. According to the preferred microclimate requirements, some species occur in greatest numbers in open areas (mostly strong flyers), others in woodlands (woodland species are moderate flyers), a third group prefers edges of fields and forests and finally the fourth group comprises the urban domestic species, which are usually weak flyers (Gillies 1972; Bidlingmayer 1975). Experiments show that *Ae. vexans* migrate approximately 1 km/night during warm and humid weather periods at moderate wind speed. Increasing numbers of *Ae. vexans* females have been caught in CDC-traps at a distance of about 5 km from their breeding place 8 days after emergence, and within 2 weeks at a distance of 10 km or more. Clarke (1943a,b) recorded migration distances of marked *Ae. vexans* females of 22 km and Gjullin and Stage (1950) and Mohrig (1969) up to 48 km.

In contrast, snow-melt mosquito species stay near their breeding sites and do not regularly migrate long distances (Schäfer et al. 1997). In mark-release-recapture experiments Joslyn and Fish (1986) collected *Oc. communis* females at distances up to 1,600 m from their breeding sites. Nielsen (1957) reported a maximum flight range of about 1,600 m for *Oc. communis* and *Ae. cinereus* with an average dispersal range of less than half this distance.

In Germany, *Oc. rusticus* females have been found resting in the forest during daytime and flying to the forest edge and the adjoining fields with approaching dusk. Females preferred to disperse along rows of trees in open areas. It can be assumed that in the absence of other attractants or adverse meteorological conditions, the flight of these mosquitoes was guided by the reduced level of illumination beneath the forest canopy, the contrast in illumination with the adjoining forest edge as well as the visual image of the rows of the trees. The mosquitoes obviously follow their hosts, mostly red deer, when these animals browse in the meadows near the forest. The flight distance observed was only a few hundred meters (Schäfer et al. 1997).

Dispersal serves mostly to bring these blood sucking insects into contact with a suitable signal from a potential host animal. It is likely therefore, that species which breed in areas where few hosts are available develop a stronger tendency for migration than those which breed in the vicinity of their hosts. For instance, *Cx. p. pipiens* which breeds in human settlements migrates usually less than 500 m. It is likely that host-seeking females will find stimuli from a suitable host within a few hundred meters.

In field studies, it was shown that both, a horizontal and a vertical dispersal behaviour assists the host-seeking process. Females of *Aedes* spp. (*Ae. vexans, Ae. rossicus*, and *Ae. cinereus*) and *Ochlerotatus* spp. (*Oc. sticticus*), were most frequently captured in traps at ground level up to a height of 4 m whereas, at a height of 10 m, *Cx. p. pipiens* was by far the most abundant (99.2%) species. There is an interaction between the availability of suitable hosts and the distribution of mosquito species. For blood-seeking females of ornithophilic species (*Cx. p. pipiens* and *Cs. morsitans),* it is an advantage to search for birds in the canopy. In contrast, the *Aedes* and *Ochlerotatus* species prefer mammals as hosts, which explain the dominance of these species at ground level.

After encountering the host stimuli, the female mosquito changes its behaviour from the non-oriented flight pattern to an oriented host location. Initially, the mosquito is responsive to the host odour and then it uses this odour to track the host from a distance of >20 m. It is the release of carbon dioxide by the host, and the change in concentration of carbon dioxide in combination with other stimuli, which elicits behavioural responses. Mosquitoes are sensitive to very small changes in carbon dioxide levels. The receptors on mosquito palpi show responses to changes as small as 0.01% (Kellogg 1970). There are many other components of host breath and odour which stimulate the antenna1 receptors of female mosquitoes when mixed with carbon dioxide. For instance, lactic acid is an activating and orientating stimulus for mosquitoes, but only if carbon dioxide is also present in the air stream (Smith et al. 1970; Price et al. 1979). Interaction or synergism of the components of the host odour in attracting a given species is a very complex process which developed in the course of evolution between the insect and the target organisms. The components of the host odour only stimulate the mosquito female when they occur in a distinctive mixture typical of the host. This enables it to distinguish between different hosts and to trace the plume as a series of packets, lamellae and filaments of odour mixed and dispersed by wind (Murlis 1986). The female mosquito flies upwind in a zigzag pattern which holds the mosquito within the plume and brings it closer to the odour source. In the final stages of orientation, mosquitoes especially those which bite during daytime or at twilight, use visual contact to locate the host. The compound eyes serve to discriminate between form, movement, light intensity, contrast and colour. Mosquitoes respond particularly to blue, black and red colours, whereas least attraction is caused by white and yellow (Lehane 1991). It is unlikely that the utilization of colour information is well developed in mosquitoes active at night, but they may be particularly sensitive to intensity contrast between the background and the target. When the mosquito is in close proximity of the host, it may also distinguish between three-dimensional targets and infrared radiation may also be involved in host location.

In the immediate vicinity of the host, odour is important once again as well as body heat. Mosquitoes can easily detect temperature differences of 0.2°C. Water vapour in short-range orientation-attraction may also play a role (Lehane 1991).

2.6.4 Feeding

Mosquitoes have well-developed piercing/sucking mouthparts. Males feed on plant juices as a source of carbohydrates, the female mouthparts are developed to pierce the skin of the host to obtain blood for egg maturation (Magnarelli 1979; Clements 1992).

The mouthparts are extended into a proboscis which consists of the stylet bundle (fascicle) enclosed by the labium when the mosquito is not feeding. The stylet bundle is formed of six long, thin stylets: the labro-epipharynx, hypopharynx, and two of both maxillae and mandibles. These stylets are held tightly together by the labium. The mandibles are sharply pointed and used to rupture the skin for passage of the other stylets. The maxillae have a pointed tip and recurved teeth at their distal ends. They are the main penetrative elements of the mouthparts, which are thrust alternately by using the teeth to anchor themselves in the tissues. Thus the labroepipharynx (food channel) and hypopharynx (salivary channel) are penetrated together into the tissue (Robinson 1939). The penetration of the stylets into the tissue is caused by an alternating rotary movement of the head. Four muscles are associated with each maxilla, to protract and retract the maxillary stylets (Clements 1992).

After the females land on the host, they may probe the skin a few times with their labellae while they are searching for a capillary for the intake of blood. Thickness and temperature of the skin are probably important probing stimuli for mosquitoes, since the surface temperature of the skin is related to the number of blood vessels in the skin (Davis and Sokolove 1975). Sensilla on the ventral side of the pair of labellae and on the distal part of the labium contain receptors for stimuli that may indicate a suitable site for piercing the skin.

Having successfully punctured the skin, the female starts ingesting blood. When feeding, only the stylet bundle penetrates the skin, while the labium becomes progressively bent backwards as the mosquito probes deeper into the tissue. Two pumps, the cibarial pump below the clypeus and the pharyngeal pump, are the sucking organs which pump the blood or plant juices into the gut.

It is important for the female mosquito that the blood remains in a liquid form to enable the mosquito to complete the blood-meal. To prevent coagulation, the mosquito injects saliva into the wound which usually contains anticoagulants similar to hirudin produced by blood-sucking leeches (Parker and Mant 1979). The introduction of saliva into the host tissue usually stimulates an immune response which can cause an inflammatory reaction of the host at the site of the wound. These wounds often cause irritation and the scratching by the host can cause bacterial infection. When the stylets reach a blood vessel, blood-associated components (*e.g.* ADP

Fig. 2.12 *Anopheles* female ingesting blood (Photo courtesy of Roger Eritja, Barcelona, Spain)

and ATP) function as phagostimulants, and the mosquito starts to take-in blood through the food channel.

The female mosquito can ingest more than three times its mean body weight (Nayar and Sauerman 1975). Larger species such as the *Oc. cantans* can ingest more than 6 μl and smaller species such as the *Ae. cinereus* only 3.7 μl. The blood and especially its protein ingredients are essential for egg-production by the anautogenous females. Only a few autogenous species such as the *Cx. p. pipiens* biotype *molestus* are able to produce their first egg-batch without a blood-meal (Weitzel et al. 2009). When they originate from the eutrophic breeding sites (*e.g.* septic tanks) where the larvae can develop a prominent fat body due to the high level of nutrition, the fecundity is quite high, but still lower than after a blood-meal by the female. The fat body is obviously enough to complete ovarial development without a further blood-meal. The blood is used more for egg production and less as a source of energy. Both sexes of mosquitoes require plant juices as an energy source, mostly for flight. Plant sugars such as floral nectar, damaged fruits and honeydew are the main energy source during the adult life of both sexes (Briegel and Kaiser 1973).

Mosquitoes differ in their feeding and resting behaviour. Species which preferably feed indoors are called endophagic (endophagy), and those which feed mainly outdoors, are called exophagic (exophagy). The females which rest after feeding or during the day outdoors are called exophilic (exophily), and those which rest indoors are called endophilic (endophily). Ornithophily is expressed when females prefer to feed

on birds (ornithophilic species), zoophily when they feed on other animals (zoophilic species) and the term anthropophily is used when they prefer to feed on humans (anthropophilic species).

2.7 Survival During Dry Seasons and Hibernation

In tropical areas, the dry season survival mechanisms of mosquitoes, such as the *Anopheles gambiae*, is one of the most vexing deficiencies in the understanding of the biology of major malaria vectors. Two survival strategies could be observed: continuous reproduction throughout the year and embryo dormancy in moist soil for at least several days (Minakawa et al. 2001).

Mosquitoes in temperate zones have developed efficient overwintering mechanisms in the egg, larval or adult stages. Some species such as *Oc. rusticus* and *Cs. morsitans* can overwinter in more than one stage, *e.g.* in the larval as well as the egg stage. Several factors, especially the latitude (cold) and hydrological conditions (droughts) determine the duration of hibernation and can differ within one species according to the latitude.

2.7.1 Egg Stage

Hibernation in the egg-stage is practiced by most of the *Aedes/Ochlerotatus* species in the temperate zone. Their diapause is induced in such a way that they do not hatch when unsuitable climatic and hydrological conditions prohibit successful development to the adult stage (Sect. 2.3). The occurrence of larvae of these species overwintering in the egg stage can vary greatly within the species of *Aedes* and *Ochlerotatus*. Within some species, the hatching time is closely related to snow-melt, others hatch in late spring or summer.

2.7.2 Larval Stage

Some mosquitoes are known to overwinter in the larval stage and can even survive for days in breeding sites with a frozen surface. During the cold season, their metabolism is reduced and the larval development is delayed. For instance, larvae of the *Oc. rusticus* and *Cs. morsitans,* which hatched in autumn, hibernate in the second and third larval instar. The high content of dissolved oxygen in cold water or bubbles of oxygen under the ice enable the larvae to cover their demand of oxygen for survival. However, during a severe winter, the mortality rate can be very high. Some anopheline species such as the *Anopheles claviger* and *An. plumbeus* hibernate as larvae in pools or tree-holes, respectively. Usually, hibernation takes place in the third or fourth larval stage in water bodies that do not entirely freeze or only for a short time. This is also true for the hibernating larvae of the *Or. pulcripalpis*. In contrast to the above-mentioned species, the larvae of the *Coquillettidia richiardii*, which usually hibernate in the third or fourth-instar are not sensitive to long frost periods, because they live submerged in permanent water bodies.

Larvae of the *Cx. p. pipiens* can frequently be found during winter. Whereas the females of anautogenous, ornithophilic, eurygamous *Cx. p. pipiens* are overwintering in diapause, its biotype *molestus* reproduces during the winter. Therefore, all developing stages of this biotype can be found in the breeding habitat (usually underground breeding sites) within the temperate zones during winter.

2.7.3 Adult Stage

Most mosquito species (*Culex, Culiseta, Uranotaenia and Anopheles*) overwinter as adult females. They seek hibernating shelters (locations free of frost such as caves, stables, cellars, canals and earth burrows) during autumn and leave these sites in spring when the temperatures increase. Usually, females of these species use the remaining larval fat body and feed intensively on plant juices during autumn to synthesize huge lipid reserves for diapausing. Females of some species within the Anopheles Maculipennis Complex can take occasional blood-meals during winter to withstand the long periods of starvation (Clements 1992).