

Chapter 3

The *Anopheles* vector

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Introductory remarks

Several insects are known to be vectors of human diseases. Mosquitoes in particular enjoy the questionable honour of having been the first insects to be associated with the transmission of a disease. This happened in 1878, when Manson, a British doctor practising in China, showed that mosquitoes (later identified as belonging to the genus *Culex*) transmitted human filariae. Likely, this finding influenced Ronald Ross who, in 1897, discovered oocysts on the gut wall of a mosquito fed on a malaria patient.

In 1898, the Italian zoologist G.B. Grassi and his coworkers first described the complete cycle of the human malaria parasites and pointed to a species of the genus *Anopheles* as responsible of malaria transmission. Since then we know that the epidemiology of malaria in a given environment is the result of a complex interplay among man, plasmodia and anopheline mosquitoes. These 3 elements have to be present for malaria transmission to occur in nature. *Anopheles* can be found - and actually they are - in areas where malaria parasites do not exist ("anophelism without malaria"), but malaria parasites need the vectors for perpetuating their life cycle. Artificially, human plasmodia can be transferred from infected to healthy individuals by blood transfusion or they can be kept indefinitely in culture in the laboratory, but their natural life cycle cannot dispense with the mosquito. The plasmodial infection seems to be neither advantageous nor detrimental for the mosquito, but is certainly detrimental for its human victims. The human organism is the natural shelter where malaria parasites thrive, multiply and differentiate in sexual forms. It represents at the same time the favorite source of bloodmeal for the females of several *Anopheles* species and this allows the relationship among the three "forced" partners contributing to the malaria cycle to be established.

Morphology and development

Various mosquito genera transmit plasmodia to different vertebrates, but all the vectors of human plasmodia belong to the genus *Anopheles*. A summary of mosquito taxonomy is presented in Table 1. Although taxonomy is a field for qualified specialists, the distinction between the most common mosquito genera - *Anopheles*, *Culex* and *Aedes* - is not too demanding and is of great practical importance.

The *Anopheles* egg is difficult to be seen by naked eye, being quite small (about 0.5 x 0.2 mm). It is boat-shaped and provided with two lateral floaters, filled with air (Fig. 1). The larvae hatch from the floating egg in form of small "shrimps", provided with a head, a thorax and nine abdominal segments (Fig. 2). The buccal apparatus is surrounded by brushes which create a water flow bringing food particles to the mouth. A conspicuous distinctive character is the position of the *Anopheles* larval body with respect to the water surface: parallel instead of oblique like in *Aedes*

and *Culex* (Fig. 3). This position might be related to the absence, in the *Anopheles* larva, of the respiratory siphon which in Culicine larvae brings the air to the tracheae.

The larvae undergo 3 successive moults, giving rise to organisms of a similar morphology, but of increasing sizes (from less than 1 mm for the 1st stage larvae to more than 0.5 cm for the 4th stage larvae). The fourth instar larva moults into a pupa, in which head and thorax are fused under a common envelope and bear the respiratory trumpets. The pupa is a relatively short stage during which the organism does not intake food. From it emerges the adult or *imago* (Fig. 4). The duration of the whole process, from oviposition to adult eclosion, is temperature-dependent. At the elevated temperatures of the intertropical areas it takes a minimum of 7 days.

An apparent sexual dimorphism, particularly conspicuous at the level of the antennae, allows male and female *Anopheles* adults to be easily recognized (Fig. 5). The resting position, with the 3 main body parts (head, thorax and abdomen) characteristically aligned long a straight line allows the anopheline adults to be quickly distinguished from the culicines (Fig. 6).

Reproductive biology

Like in all living organisms, the reproductive biology occupies a crucial place in the life cycle of *Anopheles* mosquitoes. A nuptial flight is one of the first activities of the newly emerged mosquitoes. Males form swarms of variable size, which aggregate around a "marker" which make a contrast with the background (Muir, 1988), e.g. the head of a person. Copulation frequently occurs within these swarms, but the significance of swarming in relation to copulation is still debated. After copulation, the female stores the sperms in a spermatheca, so that at each subsequent oviposition the eggs can be fertilized during their transit in the oviduct.

Male mosquitoes feed only on sugar-rich fluids, females may as well survive on these nutrients, but egg maturation requires the assumption of a bloodmeal by the female. Thus, "she" flies at dusk or, more usually, at night, looking for food for her progeny. Once an appropriate source is identified, the most suitable body regions of the prey are explored and a skin area where capillary blood vessels are particularly abundant and superficial is carefully and rapidly probed by the hydraulic engineering masterpiece represented by the mosquito mouth parts. When the "blood-fields" are reached, the pharyngeal pump is put into action and the formation of unwanted plugs of blood clots is avoided by anticoagulant substances contained in the saliva. The slender abdomen, characteristic of the newly emerged "empty" females, inflates like a balloon to accommodate a volume of blood which may even double the weight of the mosquito itself (1 to 3 μ l, depending on the species, Fig. 7).

In the "freshly fed" females the abdomen is almost entirely filled with blood, but quite rapidly in the ensuing hours the maturation of the eggs entails blood digestion and a substantial increase of the volume occupied by the ovaries. The process is well discernible from the outside and, depending on the relative position of blood (or blood relics) and developing ovaries, mosquitoes are defined as "subgravid" or "gravid" (Fig. 8). Usually, to lay the first batch of eggs a female mosquito requires 2 or even 3 bloodmeals, but subsequently blood taking and oviposition alternate regularly. The duration of the gonotrophic cycle, defined as the period between the bloodmeal and the subsequent oviposition, is temperature-dependent. When the average day-night temperature is above 23°C, the gonotrophic cycle takes 48 hours, which means that *Anopheles* females seek for a suitable source of blood every second to third night (Bruce-Chwatt, 1985). In areas where the proportion of *Plasmodium* gametocyte carriers is high, this implies that mosquitoes have a high likelihood of becoming infected sooner or later and, provided that the average mosquito life span is long enough

for the sporogonic cycle of the parasites to be completed, this entails in turn high rates of human infection.

In this picture other factors play an important role, namely the preferred sources of blood and the location of the most frequent biting and resting sites. These characteristics are measured by parameters called anthro(zoo-)phily, endo(exo-)phagy and endo(exo-)phily, respectively, and they are of primary importance for malaria epidemiology and for orientating malaria control strategies (see also the paragraph **Methods in malariological entomology**). Different *Anopheles* species also vary greatly in their preference for particular breeding sites. The main features of some important malaria vectors are given in the next paragraph.

Distribution and characteristics of some important *Anopheles* vectors

In the following pages the main characteristics of some *Anopheles* species and species complexes are summarized, more as an example than as an exhaustive guide which would be beyond the scope of this handbook and far beyond the competence of the authors of this chapter. The species problem in *Anopheles* is a fascinating zoological and genetic puzzle and has been reviewed by Coluzzi (1988). Here we give a brief outlook on morphology, geographical distribution, habitat and behaviour of 10 species or species complexes considered amongst the most important malaria vectors all over the world.

Anopheles gambiae complex

It includes 6 sibling species which can be identified on a cytogenetic basis or using the methods of molecular genetics. A specimen of *A. gambiae* s.s.¹ is shown in Fig. 9. This mosquito is extremely well adapted to human settings, as witnessed by its remarkable anthropophily and endophily, which make it one of the most efficient malaria vectors. This species is found all over tropical Africa and is sympatric with the less strictly anthropophilic *A. arabiensis*, which is found in South-West Arabia as well. Both species like breeding sites represented by sunny and clean water pools, devoid of vegetation. Particularly important are man made breeding sites, like wells dug to prepare mud bricks. Typically, small water pools created by cattle footprints or grooves tracked by vehicle tyres are suitable breeding sites for these malaria vectors. *A. melas* and *A. merus*, less important vectors, are adapted to brackish waters in West and East Africa, respectively.

Anopheles funestus complex

This complex of small, dark species represents the second important African vector complex (Fig. 9). Sympatric with *A. gambiae* s.s. and *A. arabiensis*, these mosquitoes succeed to the members of the *A. gambiae* complex in the colonization of the breeding sites. For reasons which are not totally clear (competitive exclusion?), towards the end of the rainy season, when the densities of the *A. gambiae* complex mosquitoes start decreasing, the grassy edges of the water pools become the favorite breeding sites for *A. funestus* mosquitoes. Anthropophilic and endophilic, they are powerful vectors which may promote the extension of the malaria transmission season to the first months of the dry season. Due to the reduced size and the dark colour, they can be easily distinguished from vectors of the *A. gambiae* complex.

The *A. minimus* complex appears to be closely related to the *A. funestus* complex. It includes a few important vector species in India and South-East Asia.

¹s.s. = *sensu stricto*, this is one of the 6 sibling species of the complex and is opposed to s.l. = *sensu lato*, meaning anyone of the 6 sibling species member of the *An. gambiae* complex

Anopheles maculipennis complex

These mosquitoes are closely related to the species which were responsible for malaria transmission in Europe (*A. sacharovi*, *A. atroparvus* and *A. labranchiae*), before the eradication of the disease. Although still present in some European countries (Greece, Italy), nowadays they may act as vectors of malaria in limited areas of Iraq and Turkey (particularly in Kurd refugee camps). Their degree of anthropophily varies, but they tend to take their bloodmeals outdoors and to rest indoors. The breeding sites are represented by sunny pools rich in vegetation, including brackish marshes.

Anopheles stephensi

An important urban and rural vector (Fig. 9), widely distributed from Afghanistan to China, India, Pakistan, Iraq, Iran, Saudi Arabia. It finds appropriate breeding sites both in urban peridomestic water collections and in rural pools. In town it is mainly anthropophilic and endophilic, while the rural sub-populations are more variable in their habits.

Anopheles culicifacies complex

As its name says (*culicifacies* = *Culex*-like), the complex includes at least 4 sibling species which in some aspects may recall culicines mosquitoes. Partly sympatric with *A. stephensi*, it extends its distribution to South-East Asia (Laos, Thailand, Vietnam) and to East Africa (Ethiopia). Its biting preference for cattle renders it sometimes an inefficient vector, which may become important when reaches very high densities. Quite flexible in biting and resting habits, it may bite indoors and outdoors and rests in human and animal shelters.

Anopheles maculatus complex

Likely, the species composing this complex have different ecological distribution and behaviour and this may explain why this mosquito is a vector in some areas and not in others (Fig. 9). Its distribution extends from China and India to South-East Asia. Its breeding sites are sunny streams, often produced by deforestation, and feeds mainly on cattle. It may be forced to bite on humans when other sources of bloodmeal are not available.

Anopheles punctulatus complex

Species of this complex are found in different habitats of Papua New Guinea, the Solomon Islands and Vanuatu. Coastal areas are colonized by *A. farauti*, the central plains of New Guinea by *A. koliensis*, and the shady forest hills by *A. punctulatus*. Mosquitoes of this complex feed on humans, but are easily diverted on cattle. The resting habits are quite flexible: a trend to endophily can easily be reverted to exophily, for instance when houses are sprayed with DDT. The numerous spots on the wings (*punctus* = spot) are characteristic of these mosquitoes.

Anopheles albimanus

This vector of malaria in Central America, the Antilles and Caribbean can be found also in Texas and Florida. It may adopt a wide spectrum of breeding sites, from artificial reservoirs to pits, marshes and lagoons. This mosquito displays a very flexible attitude with respect to feeding and resting habits. Small and dark, is characterized by pale hind legs (*albimanus* = white handed).

Anopheles darlingi

Found in practice all over Central and Southern America (Fig. 9), it likes various kinds of water collections as breeding sites, provided that they are protected with vegetation. The most endophilic fraction of the population having been eliminated by antivectorial operations, it behaves now like an "opportunistic" vector, occasionally biting humans mainly outdoors, in forest areas. Of course these characteristics have diminished the importance of *A. darlingi* as a vector of malaria, but the deforestation activities in the Amazons are causing its replacement by the much more efficient members of the *A. gambiae* complex.

Methods in malariological entomology

Because of the role of the *Anopheles* vector in the malaria cycle, a trustable analysis of the malaria situation in a given area and a sound programme of malaria control cannot dispense with entomology. Without entering the complexity of the mathematical relationships describing the rules which allow the intensity of malaria transmission to be "predicted" on the basis of measurements of several parameters, we try here to summarize the methods which allow the most important data describing the entomological component of the malaria cycle to be collected.

An apparently simple but fundamental parameter to be measured is the *Anopheles* density, which means the average number of mosquito vectors per inhabitant. The reference method for measuring vector density is the so-called landing or human bait catch. With the help of a simple aspirator (Fig. 10), mosquitoes are caught when they land on a human volunteer for taking a bloodmeal. The rationale for the choice of this method is straightforward and is based on a few considerations. The feeding and resting habits of *Anopheles* not only vary both inter- and intra-specifically, but they may also vary under the selective pressure exerted by intervention measures. Coluzzi and coworkers (1979) have shown that certain karyotypes of *A. arabiensis* are found with different frequencies biting outdoors or indoors and *A. farauti* shifted from prevailing endophily to exophily after indoor spray operation were implemented in Solomon Islands (Taylor, 1975). It would be therefore risky to determine the *Anopheles* density using the results of catches performed at the presumed resting sites. In addition, the fraction of the mosquito population which actually "counts" in terms of malaria transmission is constituted by the females who come for biting, thus it seems very appropriate to target the catch method to these mosquitoes. Practical and ethical considerations however argue against the human bait catches. From the practical point of view, since most malaria vectors bite at night (sometimes late at night), landing catches are very labour demanding in terms of execution and supervision. From the ethical point of view, the deliberate exposure of human volunteers to mosquito-borne diseases (not only malaria) is definitely questionable. Alternatives have been proposed, like the human baited double bednet: a first bednet is partly uprisen or purposely torn and works as a trap, while the second is properly located and protects the volunteer from being bitten.

In recent years, a light trap designed at the Center for Diseases Control in Atlanta, USA, has become increasingly popular (Fig. 11). Located near a volunteer sleeping under a bednet, at a height of about 0.5 m from the ground, the trap exploits the attractiveness of the human bait "from far". When the mosquito approaches to bite, the light of a small battery operated bulb may exert an attraction "from near" and bring the insect close to the fan which aspirate it into the trap bag. Practical and independent on the collector skill, the CDC light traps are definitely less efficient than the human bait catch. This may lead to an underestimation of the real amount of transmission in baseline epidemiological studies, but does not represent a major disadvantage in control programmes where usually either historical or parallel reference data for measuring the impact are available.

For special purposes, the human bait (in landing or in CDC trap catches) can be substituted by animal baits, for instance when sampling the least anthropophilic or the zoophilic fraction of the population is required.

When the *Anopheles* populations thriving in a certain area are quite stable in their habits, and their resting places are well known and easily accessible, other methods can be used. The same kind of aspirators previously shown can be used to collect living mosquitoes at their resting places (Fig. 12). For strictly endophilic vectors another practical and widespread method consists in spraying a pyrethrum suspension in a closed room/house. The knocked-down mosquitoes can then be collected on a white sheet previously laid on the floor (Fig. 13).

The sampled mosquito specimens, whatever catching method is used, can be sorted by sex, species (e.g. *A. gambiae* s.l. and *A. funestus*), stage of the gonotrophic cycle (empty, freshly fed, subgravid, and gravid) depending on the study aim, and stored for further analyses.

In case the mosquito sample is not obviously fed on humans, it may be interesting to determine the origin of the bloodmeal to evaluate host preferences. This parameter is largely influenced by host availability, however clear-cut differences for host-specific tendencies have been demonstrated. When humans and cattle were equally made available to *A. merus* and *A. gambiae*, the former took the majority of meals on animals and the latter on humans (Gillies, 1967), indicating that a primarily ecological adaptation had become genetically fixed. Bearing in mind that these habits can be partially or totally reverted by host availability, various *Anopheles* species can be ranked according to their degree of anthropophily, measured by the human blood index, i.e. the proportion of mosquitoes fed on humans (Table 2).

Various methods can be applied to examine the midgut content of freshly fed mosquitoes (Pant *et al.*, 1990). We briefly outline here (Fig. 14) a simple immunoenzymatic (ELISA) method described by Habluetzel and coworkers (1988).

The abdomens of freshly fed mosquitoes, which can be stored dry for months at room temperature, are crushed in a proper buffer. The resulting homogenate is transferred in a 96-well microplate. Among the proteins sticking to the wells are the immunoglobulins (Ig) of the animal on which the bloodmeal was taken. By using animal-specific antisera directed against the Ig fraction, it is possible to identify the bloodmeal source. When only the determination of the anthropophilic fraction of the population (i.e. the human blood index) is of interest, which is most frequently the case, an antiserum anti-human Ig can be used. In these conditions, the ratio positive-over-tested represents directly the human blood index of the studied sample. The importance of this parameter resides in the fact that it is present at the square power in the formula quantifying the "power" of a vector (vectorial capacity) for transmitting malaria (Macdonald, 1957). This means that the vectorial capacity of a fully anthropophilic vector is 4-fold that of an *Anopheles* population which takes only one half of the bloodmeals on humans. This can be easily understood by observing that the alimentary preference enters twice in the determination of the efficiency of a vector: once when it takes a bloodmeal infecting itself, and a second time when it takes a bloodmeal infecting the human host. By applying the multiplicative rule of the probability, it appears that a mosquito having a 50% probability of biting on humans, has a probability of 25% of accomplishing two "successful", independent events (i.e. of biting twice on humans), compared to the 100% of a strictly anthropophilic vector.

A third important parameter in malariological entomology is the survival time of the mosquito. Under favorable environmental conditions, an *Anopheles* mosquito can live for more than 3 - 4 weeks, but the mean survival in nature is much shorter (6 - 9 days). Thus, only a relatively small fraction of the population lives long enough for the sporogonic cycle to be accomplished. The

duration of the sporogonic cycle depends on the parasite species and on the environmental temperature, its minimum being of 10 days. This stresses the importance of age-grading a mosquito population, in order to estimate the proportion of females who live long enough to transmit malaria. Age-grading is crucial in malaria control programmes targeted at the vector (e.g. indoor spraying or impregnated bednet/curtains). The result of these control activities - if successful - is a shortening of the vector population mean survival or, in other words, an increase of its daily mortality. We have seen before that most anophelines, in the absence of limiting factors and in favorable climatic conditions, take a bloodmeal and accomplish a gonotrophic cycle every 2nd to 3rd day. If the relevant vector is an endophilic one and if a malaria control programme using e.g. insecticide-impregnated curtains is at work, each time the mosquito enters the house it has a certain chance of getting in contact with the insecticide and of being killed. As a consequence, the "life expectancy" of that mosquito is more or less dramatically shortened. If the control measure is effective, this shortening may have an impact on the sporozoite rate, i.e. on the proportion of mosquitoes harbouring *Plasmodium* sporozoites in their salivary glands. This has been the case in a malaria control programme which is currently run in Burkina Faso (Fig. 15).

The determination of the sporozoite rate was classically done by dissection of the salivary glands and microscopic examination of their content. This time consuming and skill demanding method, which could be applied only on freshly collected (preferably living) samples, has been replaced by an immunological method developed by Zavala and coworkers (1982). The method, now widespread in its ELISA variant (Fig. 16), is based on monoclonal antibodies detecting a peculiar antigen of the sporozoite surface - the circumsporozoite (CS) protein - and allow dried specimens to be used years after their collection and the infecting *Plasmodium* species to be identified. It has been pointed out that the presence of the CS antigen does not necessarily coincide with the presence of living sporozoites and that the ELISA results may therefore represent an overestimate of the true sporozoite rate (Lombardi *et al.*, 1987). The formal objections can be overcome by expressing the results as the proportion of infected mosquitoes (rather than infecting) and the discrepancy between ELISA and dissection can be minimized by processing in the assay the thorax of the mosquito rather than its whole body.

The sporozoite rate determination gives only an *a posteriori*, indirect and rough idea of the age structure of the vector population: indeed it tells us how many mosquitoes lived at least 10 days. Methods exist which directly assess morphophysiological characteristics directly related to the mosquito age (Muir, 1988). The unravelling of the tracheoles of the ovaries associated with follicle growth allows the nulliparous females, who never laid eggs, to be distinguished from the parous ones (Fig. 17), who underwent already one or more gonotrophic cycles. The ratio parous/nulliparous is obviously related to the probability of vector survival, as parous specimens are certainly older than nulliparous, but requires repeated measures on large samples, being strongly influenced by short term meteorological variables. The almost simultaneous emergence of young adults from the breeding sites, as frequently occurs during the rainy season, may suddenly, i.e. from one day to the next, increase the nulliparous fraction. In addition, the partition between the 2 age categories - parous and nulliparous - occurs at an age (4 - 5 days) which is not really crucial in relation to malaria transmission. More detailed and useful information can be gathered from the dissection and microscopical examination of the dilatations of the ovarioles (Fig. 18). These dilatations are thought to represent the "footprints" of previously laid eggs. Assuming a constant (and known) time interval between subsequent ovipositions, by multiplying this time by the number of dilatations the age of the mosquito could be estimated. Applying this method, Gillies (1988) found for a Tanzanian population of *A. gambiae* that 65% of the mosquitoes died within the first week and 92% within a fortnight. Doubts on the interpretation of the dilatations have been raised by Hoc and Charlwood (1990). In addition, the method is very elaborate and to process a limited number of specimens substantial amount of time and remarkable skills are required. We estimate that for most practical purposes the determination of the sporozoite rate may be adequate.

Vector density, human blood index and sporozoite rate allow the entomological inoculation rate (EIR) to be calculated. The EIR is given by the density (number of vectors per habitant) multiplied by the human blood index and by the sporozoite rate. If we assume for instance that the catches provided a number of 15 *Anopheles* per inhabitant and per night, that 78% of them were human fed and that 10% of the vectors showed to be sporozoite infected, the EIR will be $15 \times 0.78 \times 0.1 = 1.17^2$. This means that each individual receives 1.17 infective bites per night. The EIR is an useful parameter, since in a single figure it resumes the intensity of malaria transmission.

Conclusion and acknowledgements

In this chapter we tried to outline some practical aspects of entomology for those who, not being specialists, are interested in malaria epidemiology and control. We are fully aware that we could not provide all the details required to convert the notions we tried to illustrate in practical activities. Our laboratory is ready to provide further assistance to all the interested users of this handbook³.

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²These are real figures from data collected by the authors in Burkina Faso (West Africa)

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Table 1. Classification scheme of Culicidae.

ORDER	FAMILY	SUBFAMILIES	GENERA
		Anophelinae 3 genera	<i>Anopheles</i> <i>Chagasia</i> <i>Bironella</i>
Diptera	Culicidae	Culicinae 33 genera	<i>Aedes, Culex,</i> <i>Mansonia,</i> <i>Culiseta, Haemagogus,</i> <i>Wyeomya, etc.</i>
		Toxorhynchitinae 1 genus	<i>Toxorhynchites</i>

Table 2. Human blood index (HBI) of various *Anopheles**

HBI > 0.5	0.1 < HBI < 0.5	HBI < 0.1
<i>A. farauti</i>	<i>A. culicifacies</i>	<i>A. algeriensis</i>
<i>A. funestus</i>	<i>A. messeae</i>	<i>A. aconitus</i>
<i>A. gambiae s.l.</i>	<i>A. quadrimaculatus</i>	<i>A. annularis</i>
<i>A. koliensis</i>	<i>A. rufipes</i>	<i>A. mascarensis</i>
<i>A. nili</i>	<i>A. sergentii</i>	<i>A. multicolor</i>
	<i>A. sinensis</i>	<i>A. subpictus malayensis</i>
	<i>A. sundaicus</i>	

* Modified from Garret-Jones (1964)

FIGURE LEGENDS

- Fig. 1. *Anopheles* eggs showing the characteristic lateral floaters
- Fig. 2. Fourth instar larvae of *Anopheles*
- Fig. 3. The distinctive position of Anophelinae and Culicinae larvae with respect to the water surface (redrawn from Bruce-Chwatt, 1985)
- Fig. 4. Eclosion of an *Anopheles* adult from the pupa envelope (courtesy of Swiss Tropical Institute)
- Fig. 5. Sexual dimorphism in *Anopheles* adults: a female on the left and a male on the right
- Fig. 6. The distinctive position of Anophelinae and Culicinae adults with respect to the substrate
- Fig. 7. A female taking the bloodmeal
- Fig. 8. The morphology of the abdomen in relation with the gonotrophic cycle development: lateral view of the abdomen of a) empty, b) freshly fed, c) and d) subgravid, e) gravid female.
- Fig. 9. Some important malaria vector species
- Fig. 10. Three models of mosquito-collecting aspirators: the simplest mouth-operated model, the paper cup aspirator of Coluzzi & Petrarca (1973), and its modified electric-powered version
- Fig. 11. Scheme illustrating the mechanism of action of the CDC light trap
- Fig. 12. Catching mosquitoes at suspected resting places, the "canari" where drinking water is stored
- Fig. 13. Recovering mosquitoes on the sheet after a pyrethrum spray catch
- Fig. 14. Scheme of the ELISA test for identifying the origin of the mosquito bloodmeal
- Fig. 15. Impact of insecticide impregnated curtains on the sporozoite rate: results obtained in 2 intervention villages and in 2 control villages are shown, respectively. The grey columns refer to the baseline year, before the intervention, and black columns to the intervention or control year (unpublished data of A. Habluetzel and coworkers)
- Fig. 16. Scheme of the ELISA test for detecting the sporozoite antigen in malaria vectors
- Fig. 17. The skeins of the ovariole tracheoles (indicated by the arrow) are characteristic of nulliparous females, and allow them to be distinguished from parous specimens (lower part), where tracheoles appear stretched
- Fig. 18. The ovariole dilatations (arrows) are thought to represent previous oviposition events

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