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ANOPHELISM WITHOUT MALARIA IN EUROPE

A review of the ecology and distribution of the genus Anopheles in Europe

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Chapter 1

INTRODUCTION

Malaria is one of the most important diseases in the world today. Each year roughly 100 million clinical cases of malaria are reported and more than one million people die of the disease. More than 200 million people are infected, while the disease threatens approximately two billion people (Nájera *et al.*, 1992).

Before World War II endemic malaria was found throughout Europe. After a high incidence of malaria in Europe during the war, malaria slowly disappeared from the continent. The last reported focus of indigenous malaria in continental Europe in Greek Macedonia disappeared in 1975 (Bruce-Chwatt *et al.*, 1975). Various reasons can be given for the gradual decrease of malaria in northern Europe, and thereafter in the Mediterranean part of the continent. The Rockefeller Foundation programme which resulted in the WHO-malaria eradication campaign contributed very much to the disappearance of malaria in Europe. During this campaign the application of residual insecticides and epidemiological surveillance as well as active case detection have played an important role. The availability of new drugs, improved housing, land reclamation, improved agricultural techniques, improved social and economic conditions, and better sanitation further reduced the number of malaria cases. Finally, the growth of duckweed due to the use of fertilizers, clearing of vegetation, herbicide use and the effect of water pollution by industrial effluents, insecticides, sewage and detergents further diminished the number of anophelines found (Bruce-Chwatt and Zulueta, 1980). In the Netherlands the modernization of pigsties which had provided shelter for mosquitoes contributed very much to the diminishing numbers of semihibernating *Anopheles* (Van Seventer, 1969).

Malaria is transmitted by mosquitoes which belong to the genus *Anopheles* (Diptera, Culicidae). Approximately 400 anopheline species have been described (Bruce-Chwatt, 1985), of which some 60 are known to be malaria vectors under natural conditions. Species of the *An. maculipennis* (Meigen, 1818) complex were responsible for most of the malaria transmission in Europe. Other anophelines mentioned as possible vectors of malaria in Europe were *An. algeriensis* (Theobald, 1903), *An. claviger* (Meigen, 1804), *An. hispaniola* (Theobald, 1903), *An. sergentii* (Theobald, 1907) and particularly *An. superpictus* (Grassi, 1899). This species played a secondary role in malaria transmission in south-eastern Europe.

Although endemic malaria has disappeared, every year more than ten thousand registered cases of malaria are imported into Europe by tourists, immigrants and other travellers from malaria endemic areas. This has increased the concern for a reintroduction of malaria into Europe. Climate is known to affect directly and indirectly the epidemiology of malaria. A predicted temperature increase in Europe due

to the greenhouse effect might enhance the risk for reappearance of malaria due to transmission of the imported parasites by local vector species.

This paper presents a review of the ecology and distribution of the European malaria vectors, in particular of species of the *An. maculipennis* complex, in order to analyze the relationship between the particular ecology of each vector and its role in malaria transmission in Europe. The possibility of the reintroduction of malaria in Europe based on environmental factors is discussed.

The biology and epidemiology of malaria

The life cycle of human malaria parasites comprises a phase in the mosquito and a phase in man (Fig. 1). Sexual forms (gametocytes) of the parasite are formed in

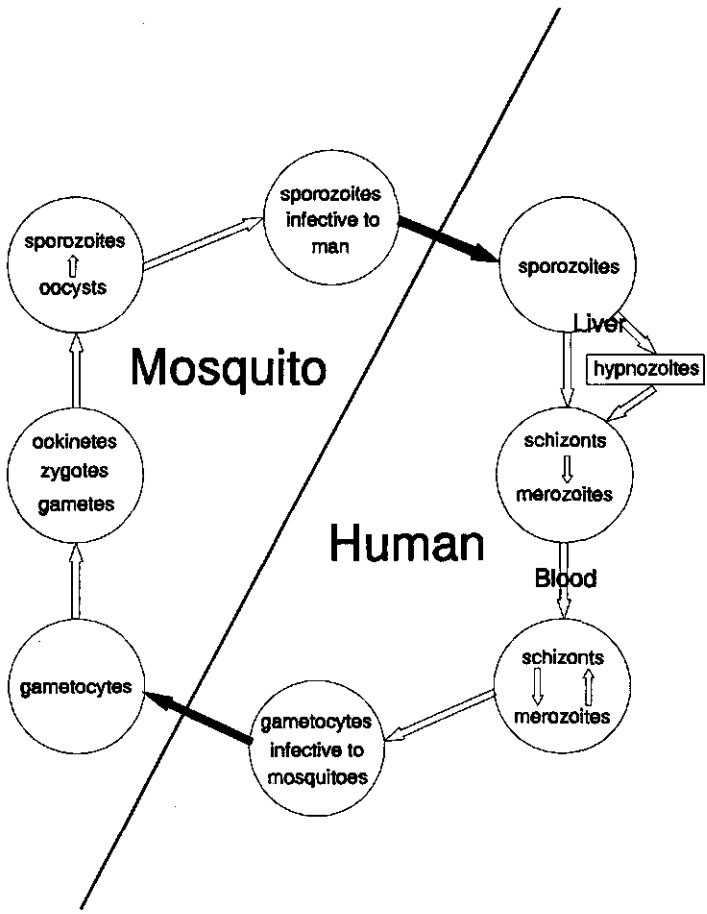

Figure 1. Life cycle of the malaria parasite *(Plasmodium spp.)*.

the human red blood cells. When a female mosquito sucks blood gametocytes are ingested. In the mosquito stomach, fusion of the male and female gametocytes creates a zygote, which in turn develops into an ookinete. The ookinete penetrates the gut wall and settles on the outer surface of the mosquito stomach to become an oocyst. Inside the oocyst, sporozoites are formed and released into the body cavity. In time, sporozoites enter the salivary glands of the mosquito and are injected into the bloodstream of the human host during feeding. The duration of the period between ingestion of parasites and formation of infectious sporozoites greatly depends on the ambient temperature. Some of the injected sporozoites enter the human parenchym cells of the liver and develop into pre-erythrocytic schizonts. After completion of the pre-erythrocytic stage, merozoites are released into the blood circulation, where they penetrate erythrocytes. The early stages of *Plasmodium* in the red blood cell are called trophozoites. The trophozoite, after a dividing process, (schizogony) develops into a schizont, which eventually bursts and depending on the species releases 8 to 32 merozoites into the blood. These can invade uninfected erythrocytes. Disease symptoms are caused by the presence of the asexual parasite stages in the human blood. Some of the merozoites give rise to sexually differentiated forms (gametocytes). These sexual forms grow to maturity and may be picked up by a mosquito. Four types of *Plasmodium* species normally occur in humans: *P. falciparum*, *P. vivax*, *P. malariae* and *P. ovale*. Humans are the only known mammalian host for the first three parasites, whereas the last one is considered to be a zoonosis, as a similar parasite is also present in higher apes. The period of development of malaria parasites in the mosquito is called the extrinsic incubation period and at 25 °C lasts from 10–20 days, depending on the species. Pre-erythrocytic development in the human host is called the intrinsic incubation period, and may last approximately 7 days for *P. falciparum*, 6–9 days for *P. vivax* or *P. ovale* and 14 to 16 days for *P. malariae*. Only *P. vivax* and *P. ovale* have the so-called 'hypnozoites' that can remain dormant up to 4 years, prior to starting their development into liver schizonts.

Plasmodium falciparum is the most pathogenic species of human plasmodia. The trophozoites of *P. falciparum* are retained in the capillaries of the internal organs, such as the brain, heart and spleen. The parasite density of *P. vivax* in the blood can reach up to 2.5–3%. *P. falciparum*, however, can reach densities of 10–20 times of that of *P. vivax* during the first infections. If it is not adequately treated, the infection is usually fatal within 2–3 weeks, sometimes already within the first week after the start of the illness. Repeated infections cause an immune response in the human host which can limit the serious effects of this disease. Without treatment *P. falciparum* parasites can persist for one or two years in the human host. In chronic infections of *P. falciparum*, parasites can persist up to 1.5 years in the human host, unless the individual is treated. Only in *P. vivax* and *P. ovale* infections, the above mentioned hypnozoites can still develop into schizonts, causing the well known relapses of tertian malaria for a period of four years without a reinfection.

The endemicity of malaria is classified according to the degree of transmission:

Hypoendemicity: Areas with little transmission.

Mesoendemicity: Varying intensity of transmission depending on local circumstances.

Hyperendemicity: Intense seasonal transmission where the immunity is insufficient to prevent the effects of malaria on all age groups.

Holoendemicity: Perennial transmission of a high degree resulting in immune responses in all age groups, particularly adults.

The malaria-risk in endemic areas is almost exclusively limited to non-immunes. In hyper- and holo-endemic areas (now also called stable malarious areas), the non-immunes consist of children ageing from 3–6 months up to 5 years, immigrants and travellers from non-endemic areas. As endemicity decreases, the potential for severe epidemics increases. In the areas of irregular malaria transmission where few people may have experienced an earlier malaria infection, all age groups are considered susceptible during an epidemic. In the case of *P. falciparum* such epidemics are often accompanied by high mortality, while in the case of *P. vivax* it causes mainly morbidity. In particular people living in areas adjacent to a malaria-endemic zone are at risk, because in years with favourable conditions for the mosquito vectors, the disease can cause an epidemic in the largely non-immune population.

More detailed information about the biology of the parasite and the epidemiology of the disease can be found elsewhere (e.g. Bruce-Chwatt, 1985; Gilles and Warrel, 1993; Wernsdorfer and MacGregor, 1988).

Malaria incidence in Europe

The impact of malaria in southern Europe and in the Balkan area has been much greater than in northern European countries. Nevertheless even in northern Europe the importance of the disease was considerable (Bruce-Chwatt and Zulueta, 1980). The differences between the various parts of Europe are well illustrated by the malaria incidence in the German army in 1917–1918 (Zieman, 1937 cited by Bruce-Chwatt and Zulueta, 1980, see Table 1).

Bruce-Chwatt and Zulueta (1980) in their book 'The rise and fall of malaria in

Table 1. Malaria incidence in the German army in 1917–1918 (after Zieman, 1937; cited by Bruce-Chwatt and Zulueta, 1980).

Area of malaria transmission	Cases per 1000
Western Europe	2.6
Eastern Europe	12.8
The Balkans	132.4
Turkey	183.7

Europe' presented an overview of the history of malaria in Europe. The most endemic malaria areas were found in the south (Table 2). Greece was probably the country in Europe with the highest incidence of malaria. During the period 1931–1935 the annual number of people in Greece infected with malaria averaged one to two million. The malaria mortality was estimated at 74 per 100.000 inhabitants. Severe malaria epidemics were reported from Rumania, where the annual number of cases was 420.000 at the beginning of this century, Yugoslavia (1942-1943: 600.000 cases), Italy (1905: 300.000 cases) and Spain (1943: 400.000 cases).

In central Europe the malaria incidence was much lower than in the southern European countries. Neither in Austria nor in Czechoslovakia or Switzerland there was any need for large scale anti-malaria operations. In the nineteenth century the malarious areas followed the course of the Danube and its tributaries from southern

Table 2. Malaria incidence in southern Europe and the Balkan states (after Bruce-Chwatt and Zulueta, 1980).

Country	Remarks
Albania	The coastal areas with its brackish marshes were highly malarious. Furthermore, malaria was reported in fertile valleys inland.
Bulgaria	Hyperendemic and mesoendemic foci were observed in the southern coastal zone at the Black Sea. The northern side of this zone was hypoendemic. The riverine plain of Thracia, the valley of the Danube and the lower valleys of other rivers were hypo- or mesoendemic. Hilly areas and higher valleys of the rivers in the south west were hypoendemic. In the mountainous areas focal malaria outbreaks were observed.
Cyprus	Hyperendemic malaria.
France	The Languedoc-Roussillon area in the south was malarious. Especially the delta of the Rhone was highly malarious. Furthermore, there have been reports from malarious areas in the south west of France (Guyenne and Gascony), Corsica was reported highly malarious.
Greece	Western Greece (Macedonia, Peloponnese and Epirus) comprised the most malarious areas due to the distribution of rainfall, topography and rice cultivation. Here the disease was everywhere hyperendemic.
Italy	Malaria was particularly severe in the south of Italy, in Latium and on the island of Sardinia (meso-hyperendemic malaria). The central part of the country was hypo-mesoendemic while the northern part had some hypoendemic foci.
Malta	Local outbreaks of fevers were reported.
Portugal	The endemic malaria zones were located in the hydrographic basins of the main rivers. Hyperendemic malaria in some areas was associated with rice cultures.
Rumania	Severe endemic malaria was reported from the valleys of the tributaries of the left bank of the Danube and on the Black Sea shore. Moderately severe endemic malaria was reported from the high valleys and plains of Moldavia, moderately and low endemic malaria areas were found in the valley and delta of the Danube.
Spain	The disease was prevalent in coastal areas in the south of Spain and along major river valleys and various endemic areas in the central part of the Iberian plateau. Rice fields were associated with malaria.
Yugoslavia	The main endemic areas were the Dalmatian coast, Macedonia, Kosovo, southern Serbia and the valleys of the tributaries of the Danube. The Dalmatian coast was hyperendemic.

Austria, extending on both sides of the river over Hungary. The main Hungarian malarious areas were in the northeast with its large flood plains and cut-off bends of rivers, and in the southwest in a region of extensive marshes and fish ponds. In the great central plain only sporadic cases of malaria were observed.

In northwestern Europe, malaria epidemics were mainly restricted to coastal areas. Intermittent and remittent fevers have been reported from England and Scotland. In the Netherlands malaria occurred in areas with brackish water in the provinces of Zeeland, Friesland, Groningen and North Holland. In Belgium malaria was reported from the coastal areas only. In Germany there were reports from Schleswig Holstein, north Rhineland and southern Württenberg. In France malaria was reported in swampy areas in the central part of the country (e.g. the Dombes plain and the Sologne area) and the northern coastal areas of the west coast (Normandy, Brittany and the Vendée). Malaria was a common disease in many parts of Denmark as well as the south of Sweden and Finland. In Sweden until about 1880 there were approximately 4000–8000 cases each year.

In Poland endemic areas were found especially in swampy lowlands. In eastern Poland the malaria incidence during 1919–1922 was 200 per 100.000. In the former Soviet Union malaria was recorded mainly in the southern Ukraine and along the lower Volga river. Small foci were found in central Russia and sporadic cases were recorded in northern Russia as far north as Arkhangelsk.

In southern Europe a continuous transmission of malaria from spring to autumn was observed while in northern European countries transmission was discontinuous and dynamic, with annual maxima. Furthermore, some vector species were able to transmit malaria inside houses and stables during autumn. This type of malaria-transmission was of particular importance in the Netherlands (Swellengrebel and De Buck, 1938).

The two main *Plasmodium* species found in Europe were *P. vivax* and *P. falciparum*. Whereas *P. vivax* occurred throughout the continent, *P. falciparum* was restricted to the south. In the Balkan states and Italy the epidemiology of malaria had a typical pattern with *P. vivax* epidemics in the spring followed by *P. falciparum* in the autumn. Transmission occurred from June to October in Rumania and from April-May to the beginning of November in Greece and southern Italy (Bruce-Chwatt and Zulueta, 1980; Hackett, 1949). In Portugal the transmission took place mainly between the end of April and October. *P. vivax* was most common in northern Portugal while *P. falciparum* was occasionally prevalent in the south (Cambournac, 1942). In France the climate restricted the transmission of *P. falciparum* to the south. A particular situation was observed in Albania and Sardinia where the vectors built up rapidly in numbers through April, May and June and then decreased (Bates, 1941[b]; Logan, 1953).

In the Netherlands and Germany two *P. vivax* peaks were noted, one in April-May, caused by an infection acquired in the autumn of the previous year and

having a long incubation period, and the second in September due to infections acquired during the summer, after a relatively short incubation period (Swellengrebel and De Buck, 1938). In Finland and Sweden a single peak in May and sometimes a second less perceptible one in October were observed. The single peak in May is characteristic for northern latitudes. The *P. vivax* infections in humans occurred in the autumn, after an incubation period of 5–13 months (Bruce-Chwatt and Zulueta, 1980).

Most of the malaria cases in Poland occurred in the summer and early autumn. In Russia two species of *P. vivax* were distinguished. The southern *P. vivax vivax* had a short incubation time of 14–20 days, while the northern *P. vivax hibernans* had a long incubation period of several months. In the northern and central parts of the former Soviet Union the important part in the annual transmission belonged to the first generation of mosquitoes bred out of eggs laid by the females after diapause. This generation had a flight in May and June and only during long, warm summers was the second generation of importance (Bruce-Chwatt and Zulueta, 1980). The transmission season in the central part of the former USSR was from the end of June to August. In the south of the former Soviet Union transmission was from May up to October with a peak in July and August (Shipitsina, 1964).

Chapter 2

TAXONOMY AND DISTRIBUTION OF FORMER MALARIA VECTORS IN EUROPE

Anophelines belong to the order *Diptera*, sub-order *Nematocera*, family *Culicidae* (mosquitoes). One of the typical characteristics of adult anophelines is their resting posture. The proboscis, head, thorax and abdomen lie on one straight axis. Furthermore, anopheline larvae lie parallel to the water surface, in contrast to other larval Culicidae (Bruce-Chwatt, 1985).

– *An. maculipennis* s.l. – The relation between malaria incidence in Europe and the presence of various members of the *An. maculipennis* complex was not discovered until 1927. Some areas were known where members of the *An. maculipennis* complex occurred without malaria. This situation was characterized as anophelism without malaria. The discovery of long-winged and short-winged varieties of *An. maculipennis* and their association with fresh and brackish water, respectively, was the first evidence for the existence of a complex of species (Van Thiel, 1927). In the early 1930's more evidence about a species complex was brought forward (Hackett and Missiroli, 1935; Martini *et al.*, 1931; Van Thiel, 1933). By the end of the 1930's most of the present species of the *An. maculipennis* complex were recognized (Bates, 1940[b]; Missiroli, 1939). The larval, pupal and adult stages of the various members of the *An. maculipennis* complex are morphologically similar to each other. However, they can be separated on the basis of egg shell patterns. More recently, larval chaetotaxy, cytotaxonomic methods, cross breeding experiments, the use of enzyme electrophoresis and analysis of cuticular hydrocarbons have provided more evidence for the existence of the different species in the complex (Bullini and Coluzzi, 1978; Bullini *et al.*, 1980; Coluzzi, 1988; Deruaz *et al.*, 1991; Guy *et al.*, 1976; Phillips *et al.*, 1990; Suzzoni-Blatger *et al.*, 1990).

The adults of the *An. maculipennis* complex are dark or medium brown. The head has a pair of antennae which are plumose in the male and sparsely feathered in the female. The palpi are about as long as the proboscis in male and female anophelines. The wings have scale clusters at the junctions of forked veins (2 and 4) and more proximally on veins 2, 3 and 4. The mesonotum is laterally darkened with a conspicuous pale frontal scale-tuft (scales thinner and sparser than in other pale-tufted species). The male gonocoxite has 2 or 3 simple parabasal spines with raised basal tubercules. The length of the four aquatic larval instars of the species increases from circa 1 to 2.5, 4 and 6 mm. The frontal hairs of the larva are plumose, the outer clypeal hairs have numerous branches. The palmate hair is reduced on abdominal segments I and II. Seta 9 of the pupa is pinnately branched on abdominal segment VIII and simple on segments III-VII (White, 1978).

White (1978) described 13 members of the *An. maculipennis* complex compris-

ing 9 Palaearctic species and 4 Nearctic ones. He based his analysis on chromosomal evidence. He proposed an identification key largely based on egg morphology (Table 3) and chromosome characteristics. The present paper focuses on the Palaearctic members of the complex as described by White (1978). The species with their synonyms and distributions are listed in Table 4. In Figs. 2 to 7 the distribution of the European species of the *An. maculipennis* complex is given. The discovery of more species within the complex is expected. Ribeiro *et al.* (1980) consider *An. subalpinus* and *An. melanoon* as two species. Cianchi *et al.* (1987) provided evidence of reproductive isolation between sympatric populations of *An. subalpinus* and *An. melanoon*. Suzzoni-Blatger and Sevin (1982) reported significant differences of the larval chaetotaxy between *An. atroparvus* populations originating from the south of France or Portugal and populations in England and Germany. An *An. maculipennis* population from the south of France was significantly different from a population in Albania.

More detailed information about the nearctic species *An. aztecus*, *An. earlei*, *An. freeborni* and *An. occidentalis* can be found elsewhere (e.g. Barr, 1988; McHugh, 1989). Barr (1988) described a fifth nearctic species, *An. hendersoni*.

Table 3. Egg determination table presented by White (1978) for the palaearctic species of the *Anopheles maculipennis* complex.

1.	Egg without floats (but rudimentary floats may develop at low temperatures); deck uniformly pale from pole to pole.	2
	Egg with floats fully formed; deck dark, barred or mottled.	3
2.		*sacharovi*
		martinius
3.	Intercostal membranes of floats smooth.	4
	Intercostal membranes of floats rough (finely corrugated).	5
4.	Upper surface of egg softly patterned with wedge-shaped black marks on a pale background; ends of deck pale almost to the tips	*atroparvus*
	Upper surface of egg entirely dark or with pattern of 2 transverse dark bars near the ends of floats, poles dark and remainder of the upper egg surface irregularly mottled.	*melanoon*
5.	Upper surface of egg marked with 2 transverse dark bars near the ends of the floats, with or without other pattern	6
	Upper surface of egg with mottled pattern but without 2 dark transverse bars near the of the floats	8
6.	Transverse dark bars on egg sharply contrasted with unmottled pale background colour of upper egg surface	7
	Transverse dark bars on upper egg surface forming part of a diffuse mottled pattern	*messeae*
7.	Eggs with tips less acutely pointed; chorion of upper egg surface relatively rough; width of egg between floats about 17% of egg length	*maculipennis* s.s.
	Eggs with tips more acutely pointed; chorion of upper egg surface relatively smooth; width of egg between floats about 12% of egg length	*beklemishevi*
8.	Upper surface of egg richly patterned with cuneiform dark marks on frosted pale background; poles narrowly dark.	*labranchiae*
9.	Upper surface of egg pale with little or no mottled pattern; poles broadly dark-capped.	*sicaulti*

Table 4. Palaearctic species of the *An. maculipennis* complex with their synonyms according to White (1978) and their distribution in Europe and along its borders.

1. *An. atroparvus* Van Thiel, 1927
   fallax Roubaud, 1934
   cambournaci Roubaud and Treillard, 1936
   Distribution: south eastern coast of Swedish mainland, Ireland, south of Great Britain, Denmark, Netherlands, Belgium, Germany, Poland, France (without Corsica), Hungary, Rumania, Bulgaria, Portugal, Spain, northern Italy and the inland areas of central Italy, former Yugoslavia, south western Russia and the coastal area of the Black Sea (Adamovic, 1980; Bruce-Chwatt and Zulueta, 1980; Collado, 1937; Hackett and Moshkovski, 1949; Jaenson *et al.*, 1986; Martini and Zotta, 1934; Pires *et al.*, 1982; Zotta, 1935; Zotta *et al.*, 1940).

2. *An. beklemishevi* Stegnii & Kabanova, 1976
   ? *lewisi* Ludlow, 1920
   ? *selengensis* Ludlow, 1920
   ? *alexandraeschingarevi* Shingarev, 1928
   Distribution: Siberia, Eastern Europe (?), Sweden above 60° N, northern Finland (Jaenson *et al.*, 1986; Korvenkontio *et al.*, 1979; White, 1978).

3. *An. labranchiae* Falleroni, 1926
   pergusae Missiroli, 1935
   Distribution: south east of Spain, Corsica, Sardinia, Sicily, coastal areas of Italy (along the Tyrrhenian coast as far north as Grosseto, along the eastern coast as far north as Foggia), Malta, Dalmatic coast Yugoslavia (Aitken, 1954; Blazquez and Zulueta, 1980; Bruce-Chwatt and Zulueta, 1980; Collado, 1937; Coluzzi and Sabatini, 1986; Missiroli, 1939; Rageau *et al.*, 1970; Weyer, 1939; Zotta, 1935).
   Morocco, Algeria, Tunis, Lybia (Collignon, 1959; Guy, 1963; Hackett, 1949; Juminer, 1959; MacDonald, 1982).

4. *An. maculipennis* s.s. Meigen, 1818
   basilei Falleroni, 1932
   typicus Hackett, 1934
   Distribution: Norway, south and central Sweden up to about 60° N, Great Britain (a few records), the Netherlands, Belgium, Germany, Poland, France (with two reports from Corsica), Switzerland, Austria, Czechoslovakia, Hungary, Rumania, Bulgaria, Portugal, Spain, Italy (one record from Sardinia), Albania, former Yugoslavia, Greece, Cyprus, European part of Russia, Turkey (Adamovic, 1980; Aitken, 1954; Bates, 1941[b]; Bruce-Chwatt and Zulueta, 1980; Jaenson *et al.*, 1986; Martini and Zotta, 1934; Postiglione *et al.*, 1973; Weyer, 1939; Zotta, 1935; Zotta *et al.*, 1940).

5. *An. martinius* Shingarev, 1926
   relictus Shingarev, 1928
   elutior Martini, 1931
   Distribution: Middle Asia (White, 1978)

Table 4 (continued).

6. *An. melanoon* Hackett, 1934
   messeae Falleroni, 1926
   subalpinus Hackett & Lewis, 1935 = *bifurcatus*
   Distribution: southern Spain, Portugal (one record from *An. subalpinus* at the eastern border and one record of *An. melanoon* at the northern border), southern France (*An. subalpinus*), Corsica (*An. melanoon*), Italy with only small numbers in Sardinia, Albania (*An. subalpinus*), Greece; foci along the black sea coast in European Turkey (*An. subalpinus*) (Aitken, 1954; Bates and Hackett, 1939; Bates, 1941[b]; Bruce-Chwatt and Zulueta, 1980; Collado, 1937; Hackett, 1934; Hackett, 1949; Logan, 1953; Postiglione *et al.*, 1973; Ramos *et al.*, 1982; Ribeiro *et al.*, 1980; Salieres *et al.*, 1978). The species *An. subalpinus* was rather similar to *An. messeae* and *An. melanoon* and has been often confused with them (Bates and Hackett, 1939).

7. *An. messeae* authors, sensu Swellengrebel and De Buck, 1933
   ? *lewisi* Ludlow, 1920
   ? *selengensis* Ludlow, 1920
   ? *alexandreaeschingarevi* Shingarev, 1928
   Distribution: Sweden and Finland up to at least 67° N, Denmark, Ireland, Great Britain, the Netherlands, Belgium, Germany, Poland, France, Austria, Czechoslovakia, Hungary, Rumania, Bulgaria, Corsica, northern Italy (up to Rieti), Albania, Greece (Macedonia), former Yugoslavia, in Siberia and eastern Europe the reported cases may be *An. beklemishevi* (Adamovic, 1980; Aitken, 1954; Bates, 1941[b]; Bruce-Chwatt and Zulueta, 1980; Hackett and Moshkovski, 1949; Jaenson *et al.*, 1986; Korvenkontio *et al.*, 1979; Martini and Zotta, 1934; Phillips *et al.*, 1990; Shannon, 1935; Suzzoni-Blatger *et al.*, 1980; Weyer, 1939; Zotta, 1935; Zotta *et al.*, 1940). Bates (1941[b]) reported that Albania was the southernmost point of this species in the Balkan. The species called by this name in Greece was probably *An. subalpinus*.

8. *An. sacharovi* Favr, 1903
   elutus Edwards, 1921
   Distribution: Never found far from the sea in Corsica, northern part of Sardinia, in coastal areas of the Italian mainland, coastal plains of Albania, former Yugoslavia, Greece, Rumania (Black Sea shore), Bulgaria, Cyprus, western and southern coastal plains of Turkey (Aitken, 1954; Aziz, 1947; Bates, 1941[b]; Bruce-Chwatt and Zulueta, 1980; Hackett, 1949; Logan, 1953; Lupascu *et al.*, 1958; Missiroli, 1939; Postiglione *et al.*, 1973; Raffaele, 1964; Rageau *et al.*, 1970; Zotta, 1935; Zotta *et al.*, 1940)

9. *An. sicaulti* Roubaud, 1935
   Morocco, Algeria (?) (White, 1978)

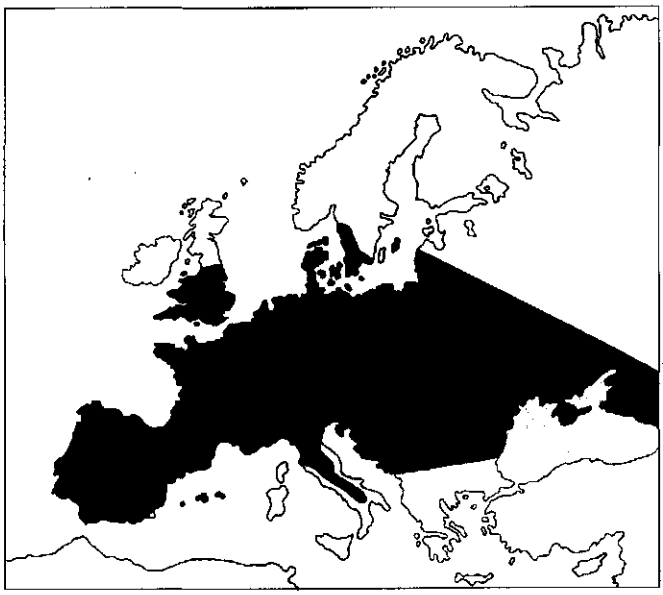

Figure 2. Distribution of *An. atroparvus* (this paper).

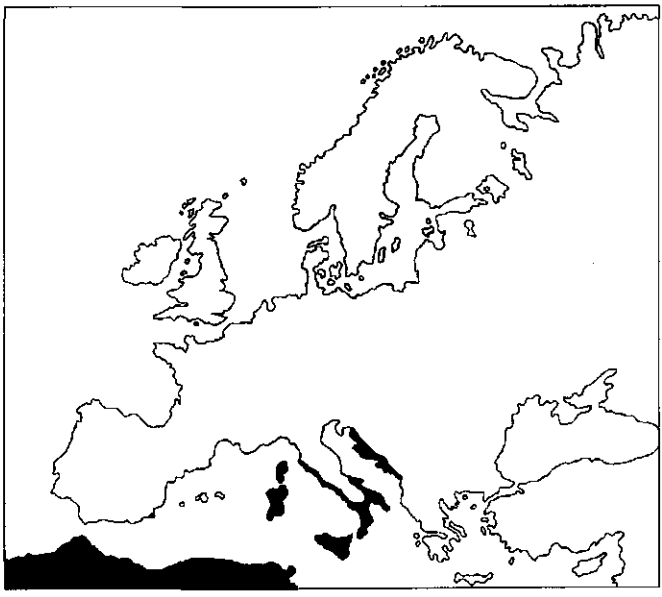

Figure 3. Distribution of *An. labranchiae* (this paper).

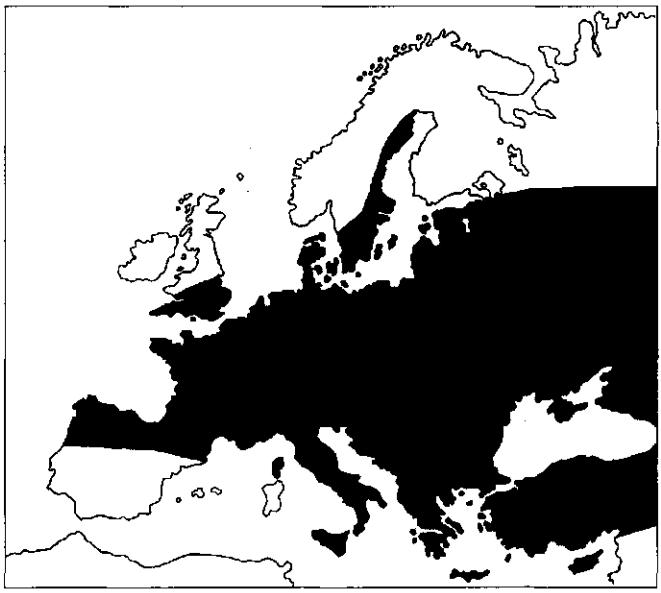

Figure 4. Distribution of *An. maculipennis* s.s. (this paper).

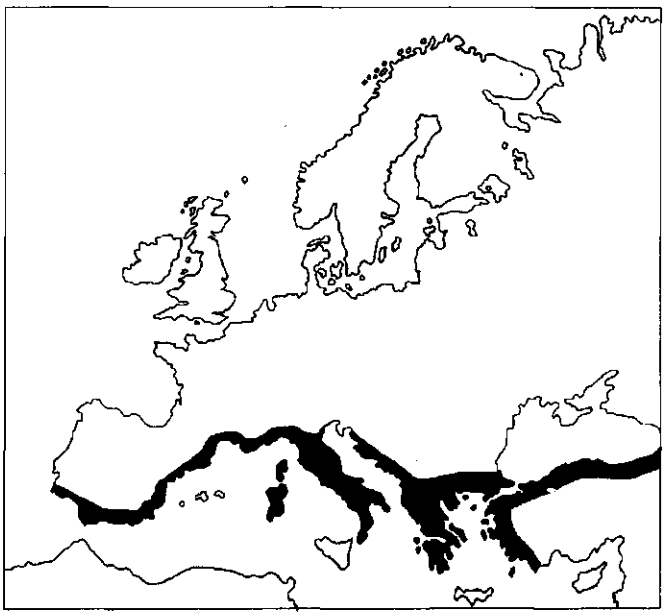

Figure 5. Distribution of *An. melanoon* (this paper).

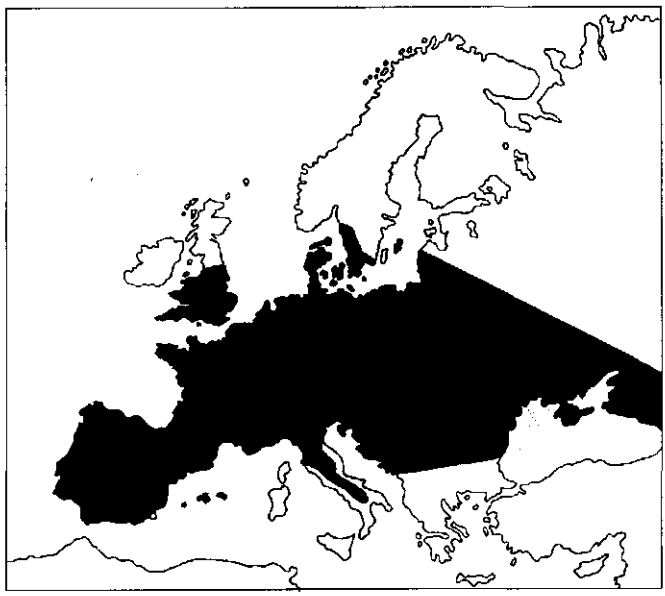

Figure 2. Distribution of *An. atroparvus* (this paper).

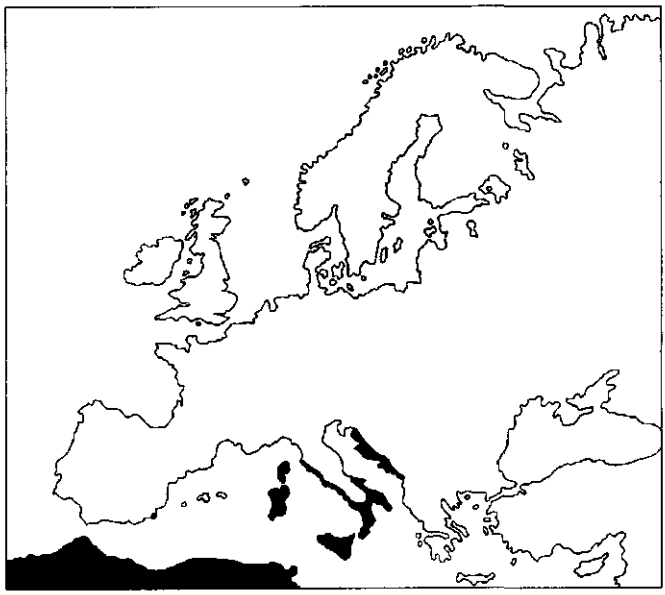

Figure 3. Distribution of *An. labranchiae* (this paper).

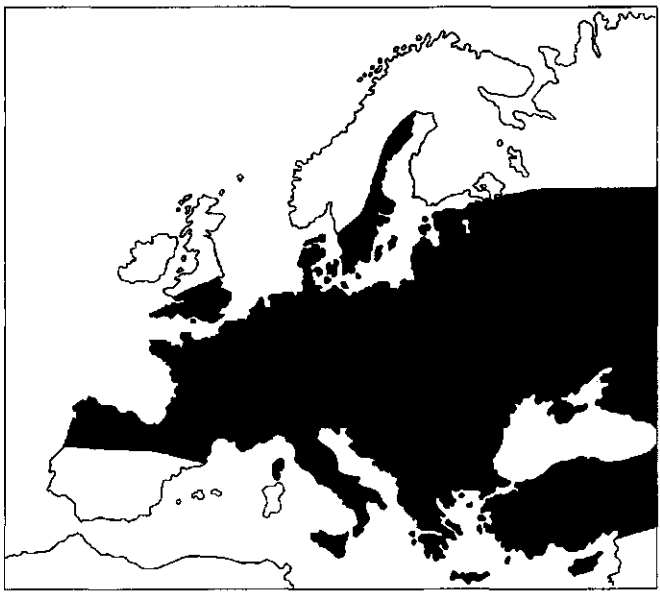

Figure 4. Distribution of *An. maculipennis* s.s. (this paper).

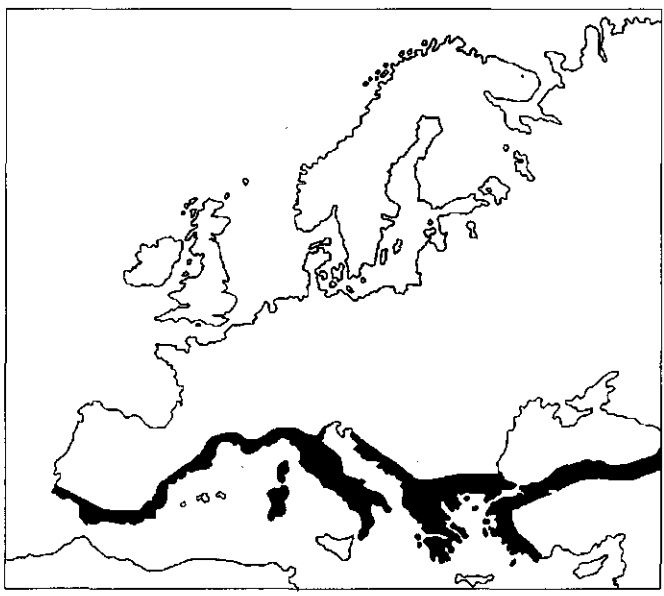

Figure 5. Distribution of *An. melanoon* (this paper).

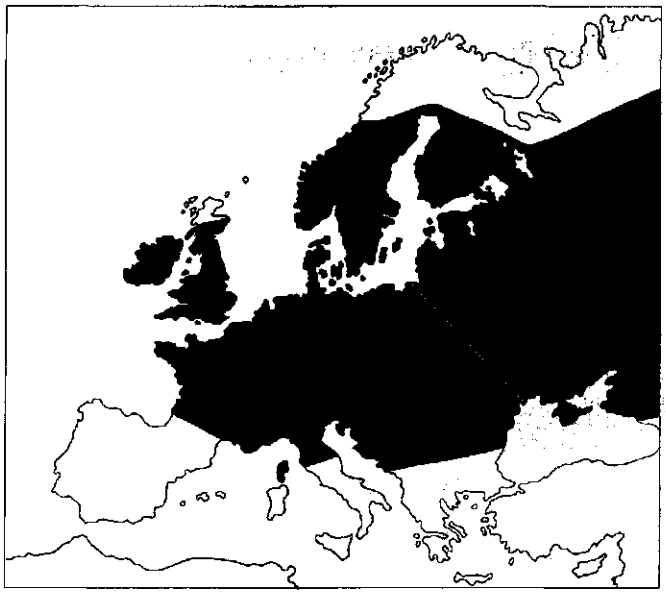

Figure 6. Distribution of *An. messeae* / *An. beklemishevi* (this paper).

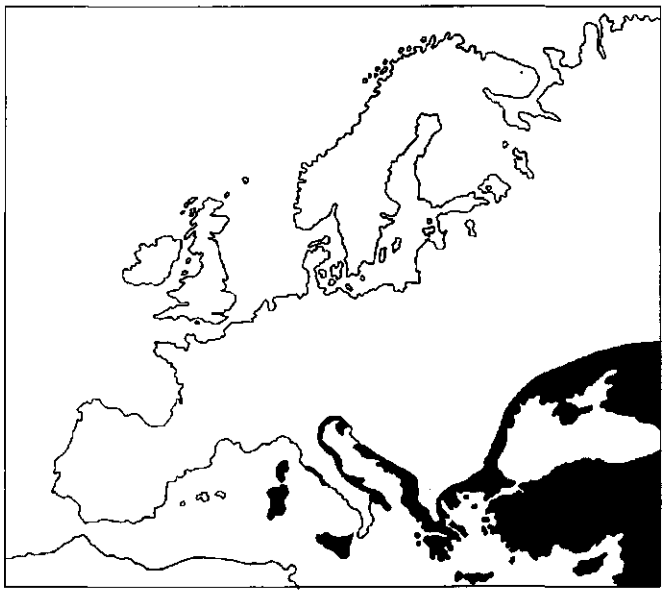

Figure 7. Distribution of *An. sacharovi* (this paper).

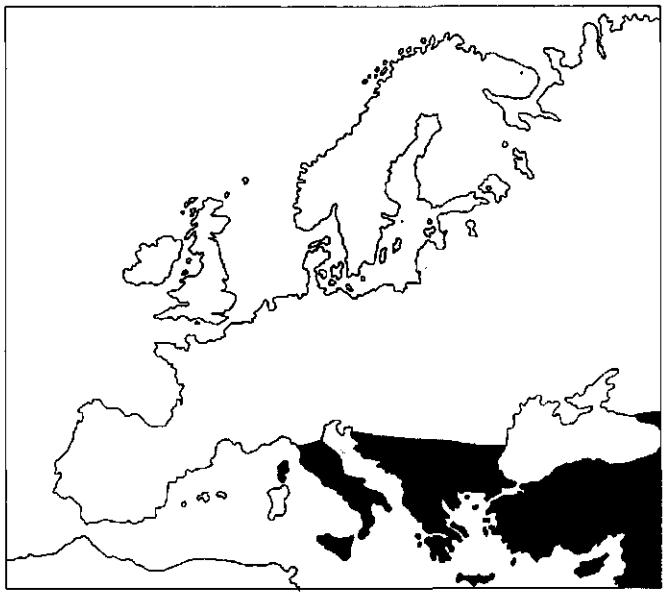

Figure 8. Distribution of *An. superpictus* (this paper).

In Europe the species *An. atroparvus*, *An. sacharovi* and *An. labranchiae* were almost invariably associated with endemic malaria. *An. atroparvus* was the most widespread vector. Particularly in the coastal lowlands of western Europe it was associated with malaria. In central and southern Italy *An. labranchiae* was the most important vector. *An. sacharovi* was the most important vector in the eastern Mediterranean region of Europe. *An. messeae* and *An. maculipennis* s.s. were reported as vectors of some significance when their densities were high, especially when a region did not have much livestock. *An. messeae* was mentioned as a vector in Eastern Europe and the Balkan area. *An. maculipennis* s.s. was mentioned as a vector in the Balkan area. *An. melanoon* played a minor role only.

Other anophelines which have been mentioned as possible vectors of malaria in Europe but which do not belong to the *An. maculipennis* complex are:

– *An. superpictus* – This species was reported to be a vector in Corsica, southern Italy, Sardinia, Sicily, Rumania, southern Bulgaria, Albania, Dalmatic coast of Yugoslavia, Yugoslavian Macedonia, Kosovo, Greece, Cyprus and Turkey (Aitken, 1954; Aziz, 1947; Barber, 1936; Bates, 1941[b]; Bruce-Chwatt and Zulueta, 1980; Hackett, 1937; Hackett, 1949; Hackett and Moshkovski, 1949; Postiglione *et al.*, 1973; Rageau *et al.*, 1970). Its presence often coincides with that of *An. sacharovi*. *An. superpictus* was reported from Spain as well but its numbers were probably very low (Weyer, 1939). The present distribution of *An. superpictus* is given in Fig. 8.

– *An. algeriensis* – Although reported by Horsfall (1955), this species is not considered an important vector in Europe. It has been reported mainly from the Mediterranean countries, but occasionally also in central and northern Europe. *An. algeriensis* typically rests and bites outdoors. Therefore, the species is only dangerous at high densities (Barber and Rice, 1935[a,b]; Hedeen, 1957; Ramos *et al.*, 1982).

– *An. hispaniola* – MacDonald (1957) mentioned this species as a potential vector of malaria. It has been recorded in Portugal, Spain, Sardinia, southern Italy and Greece (Ribeiro *et al.*, 1980). The mosquito typically rests and bites outdoors, and is considered to be a vector of minor importance only.

– *An. claviger* – This species probably played a role as a vector in the transmission of malaria in some parts of Europe (MacDonald, 1957). The species was shown to be a vector in southeastern Italy (Hargreaves, 1923). *An. claviger* is a western Palearctic species. Under the former name *claviger* two sibling species were known, namely *An. claviger* and *An. petragnanii*. *An. petragnanii* is not considered a malaria vector. Therefore, old data on *An. claviger* as a possible vector of malaria are puzzling (Ribeiro *et al.*, 1977).

– *An. sergentii* – This species has been associated with two indigenous malaria cases in Sicily. Sicily is reported as the northern distribution limit of this species (D'Alessandro and Sacca, 1967).

Chapter 3

THE BIONOMICS OF THE AQUATIC STAGES

Eggs of *Anopheles* are laid singly and they float on the water surface. Eggs gravitate towards each other, or to anything that breaks the surface of the water. The duration of the egg stages depends on the temperature of the water at the surface. Resistance to drying of the eggs varies according to their age, the variant and environmental conditions (Horsfall, 1955). The fecundity of adults is related to their size and the physiological age (Bates, 1949; Detinova *et al.*, 1963; Shannon and Hadjinicalao, 1941).

The first instar larva cuts the egg shell and emerges. The larva can emerge on a wet surface and is able to crawl to reach water. There are four larval instars and a pupal stage which rest and feed just beneath the water surface using surface tension. When a larva is disturbed it will swim downwards or sink passively. The larvae respirate by means of a pair of spiracles on the dorsal site of the eighth abdominal segment. The pupa breathes with its respiratory trumpets.

While feeding the head of the larva rotates 180 degrees and its dorsal surface remains uppermost. The mouth brushes produce a current of water from the area in front of the larva towards the maxillae. Water in breeding habitats is covered with a gelatinous film produced by various species of bacteria. Larvae feed on particles of the water film and swallow relatively large objects such as filamentous algae. In general it seems that micro-organisms, particularly bacteria and yeasts, form the basic food material of anopheline larvae (Bates, 1949; Buxton and Leeson, 1949; Horsfall, 1955). Under crowded conditions anopheline larvae can be stressed by interference due to larval autotoxins, cannibalism and exploitative competition. Under laboratory conditions *An. stephensi* took longer to develop, showed reduced survival, had an extended pupation period and produced smaller adults under crowded conditions (less than 2 cm^2 per larvae) (Reisen and Emory, 1977). Buxton and Leeson (1949) state that in nature food probably is not generally a limiting factor.

Most anopheline larvae are typically found in standing or slowly flowing waters. When anophelines are reported in running water they can be found in places such as sheltered bays or in the lee of rocks. Species reported in lake margins and in large ponds and marshes seek protection from wave action by vegetation. Species of the *An. maculipennis* complex are typically found in permanent or semi-permanent standing or slowly flowing water which is sunlit and clear. Small temporary habitats such as treeholes have been reported only very occasionally. Dense vegetation is avoided (Bates, 1949; Borob'ev, 1960; Buxton and Leeson, 1949; Hedeen, 1955; Weyer, 1939).

Chemical and physical properties of the habitat have a large effect on the development rate and mortality of the larvae. Physical factors important for the development and survival of larvae are water temperature, waves and exposure to sunlight. Chemical properties include salinity, calcium concentration, the presence of nitrogenous materials, and alkalinity.

Development rate

The development rate of the aquatic stages of *An. maculipennis* s.l. depends mainly on water temperature. The development time of the various aquatic stages is presented in Table 5. The total development rate of the aquatic stages in relation

Table 5. Mean development times of the aquatic stages of *An. maculipennis* s.l. in relation to temperature.

temp.		development time (days)					
(°C)	eggs	L1	L2	L3	L4	pupa	references
An. atroparvus							
10		----------development threshold					Artemiev, 1980
20		3.5	2.5	4.0	4.6	3.0	Mosna, 1937
15–25		2.5	2.0	3.5	3.8	2.9	Mosna, 1937
23	→	→	→	→	→	20	Corradetti, 1934
24	2	→	→	12.5–22.5		2.4	Laarman, 1955
24	→	→	→	→	→	23	Van der Kaay *et al.*, 1982
24–25	2	2	2	3	4.5	2.3	Meller, 1962
		2–2.5	1.5–3	2.5–3.5	4–8.5		
25		2.5	2.0	2.0	2.6	2.0	Mosna, 1937
20–30		2.5	2.0	2.5	2.6	2.1	Mosna, 1937
24–27	2	2	1.3	2.5	4.3	2.4	Martini, 1941
27		2	2.5	3	5		Bates, 1939
25–30		----------optimum temperature					Artemiev, 1980
20–35		2.5	1.5				Mosna, 1937
30		2.5	1.5	2.0	2.3	2.7	Mosna, 1937
30						1.6	Cambournac *et al.*, 1944
35		2.5	2.5	4.0			Mosna, 1937
35		----------upper limit					Artemiev, 1980
35						1	Cambournac *et al.*, 1944
An. labranchiae							
10–12	10–12						Kettle and Sellick, 1947
13–15	5						Kettle and Sellick, 1947
16–18	4						Kettle and Sellick, 1947
20		3.0	3.0	4.0	4.0	3.8	Mosna, 1937
15–25		2.5	2.0	3.5	4.5	3.1	Mosna, 1937
22–24	2						Kettle and Sellick, 1947
23	→	→	→	→	→	19	Corradetti, 1934
25		2.5	2.0	2.5	3.1	1.9	Mosna, 1937
20–30		2.5	2.5	3.0	4.0	2.2	Mosna, 1937
20–35		2.5	1.5	2.0	2.7	3.1	Mosna, 1937
30		2.5	2.0	1.5	2.3	2.3	Mosna, 1937
35		2.5	2.0	2.0	3.3		Mosna, 1937

Table 5 (continued)

temp. (°C)	eggs	development time (days) L1	L2	L3	L4	pupa	references
An. maculipennis s.l.							
13.9	4	→	→	→	31	10	Corradetti, 1931
14.4	4	→	→	→	27	9	Corradetti, 1931
17.1	3	→	→	→	19	5	Corradetti, 1931
19.5	3	→	→	→	15	3	Corradetti, 1931
20.0	3	→	→	→	20	3	Corradetti, 1931
23.6	2	→	→	→	11	2	Corradetti, 1931
24.5	2	→	→	→	14	3	Corradetti, 1931
24.8	2	→	→	→	10	2	Corradetti, 1931
An. maculipennis s.s.							
10		----------development threshold					Artemiev, 1980
10–12	8–10						Kettle and Sellick, 1947
13–15	5						Kettle and Sellick, 1947
19–21	3						Kettle and Sellick, 1947
22–24	2						Kettle and Sellick, 1947
23	→	→	→	→	→	19	Corradetti, 1934
25–30		----------optimum temperature					Artemiev, 1980
35		----------upper limit					Artemiev, 1980
An. melanoon							
10		----------development threshold					Artemiev, 1980
10–12	12						Kettle and Sellick, 1947
13–15	5						Kettle and Sellick, 1947
16–18	4						Kettle and Sellick, 1947
25–30		----------optimum temperature					Artemiev, 1980
35		----------upper limit					Artemiev, 1980
An. messeae							
10		----------development threshold					Artemiev, 1980
23	→	→	→	→	→	22	Corradetti, 1934
25–30		----------optimum temperature					Artemiev, 1980
35		----------upper limit					Artemiev, 1980
An. sacharovi							
10		----------development threshold					Saliternik, 1957
12–14		----------development threshold					Kligler, 1929
13–15	7	→	→	→	37		Kligler, 1930
15	→	→	→	→	→	>60	Kligler, 1929
19–21	2	→	→	→	24		Kligler, 1930
21–25		----------optimum temperature					Saliternik, 1957
23	→	→	→	→	→	22	Corradetti, 1934
22–24	2	→	→	→	12		Kligler, 1930
25	2	→	→	→	13		Kligler, 1930
25	→	→	→	→	→	11.5	Kasap and Kasap, 1983

to temperature is presented in Fig. 9. Although differences between and within species occur, the development rate of all species is positively correlated with temperature at temperatures between a lower threshold and an optimum temperature. Above an optimum temperature a sharp decline is observed. Eichler and Pagast (1948) expressed development rates for the aquatic stages of *An. messeae* in degree days above a threshold temperature (Table 6). The photoperiod had no effect on the duration of larval development (Vinogradova, 1960).

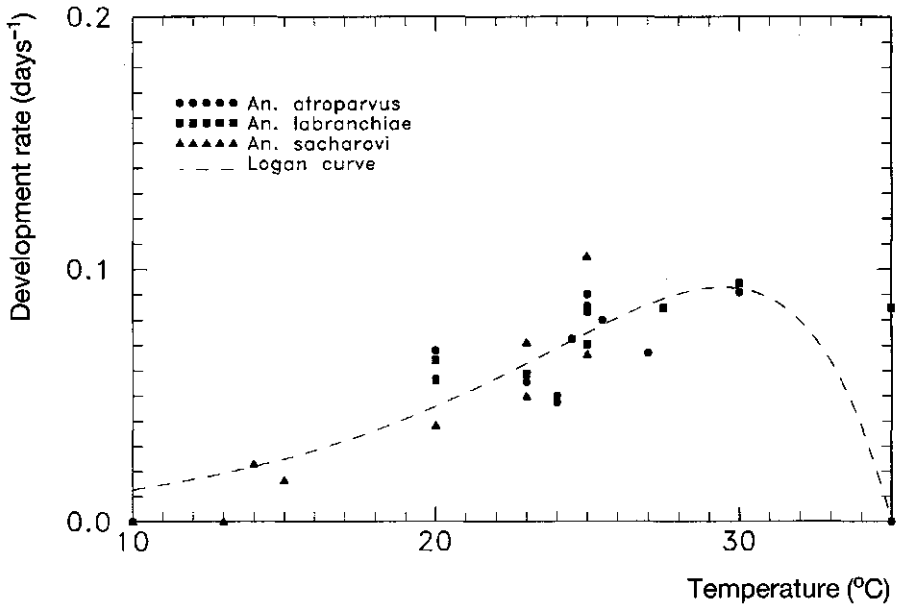

Figure 9: Development rate of larvae and pupae of *An. atroparvus*, *An. labranchiae* and *An. sacharovi* in relation to temperature (after references as listed in Table 5).

A Logan curve (dashed line) has been used to express the relation between temperature (T) and development rate (*dev.rate*) in the range between 10 and 35°C (Logan *et al.*, 1976). The development rate increases exponentially from a value A at a lower threshold of 10°C to an optimum temperature with a relative increase of B. Thereafter the development rate declines sharply until the upper lethal threshold of 35°C has been reached.

$$dev.rate = A \cdot \left(e^{B \cdot (T-10.0)} - e^{B \cdot (35.0-10.0)} \cdot \frac{(35.0-T)}{C} \right)$$

where
A: 0.021; B: 0.162; C: 5.007; R^2: 0.836

Table 6: Temperature sum needed to complete the aquatic stages of *An. messeae* (after Eichler and Pagast, 1948).

stage	temperature sum (degree days)	temperature threshold (°C)
egg	26.6	12.2
L1	18.2	14.5
L2	23.8	14.0
L3	36.8	8.9
L4	83.9	4.4
pupa	32.7	10.5
total	222.7	10.1

Mortality

Reports on mortality of the aquatic stages of the *An. maculipennis* complex in nature are sparse. Dmitriev and Artemiev (1933) reported records based on 3750 eggs in ground pools. The second instar was reached by 67% of the larvae, 40% reached the third, 25% the fourth and the 16% that pupated all became adults. Bruce-Chwatt (1985) cited Beklemishev who found that during the aquatic stage of development 90–92.5% of the anophelines died. Cambournac and Simoes (1944) observed mortality rates of the aquatic stages of *An. atroparvus* in rice fields of up to 100%.

Industrial pollution influences the mortality of *An. maculipennis* s.l. and contributed to the decrease of *An. atroparvus* in the Netherlands (Van Seventer, 1970). *An. messeae* cannot develop in polluted water (Artemiev, 1980).

Reported mortalities of the aquatic stages of *An. maculipennis* s.l. in the laboratory are listed in Table 7. The mortality of the aquatic stages is temperature-dependent and varies between species. *An. sacharovi* is the most thermophilic species of the *An. maculipennis* complex. In central Asia Artemiev (1980) reported water temperatures of the breeding sites of *An. sacharovi* rising to 38–40°C during the daytime.

Salinity also influences larval mortality. Eckstein (1936) observed larval mortality percentages for *An. atroparvus* at NaCl concentrations of 10.000, 5000, 2500, 500, 250 and 50 mg/l of 87%, 57%, 16%, 4%, 1% and 2%, respectively. The observed mortality percentages of *An. messeae* at NaCl concentrations of 10.000, 5000, 2500, 500, 250 and 50 mg/l were 100%, 83%, 42%, 5%, 4% and 0%, respectively. Corradetti (1934) reported larval mortality percentages of *An. atroparvus* at NaCl concentrations of 5000 mg/l and 10.000 mg/l of 55% and 72%, respectively. The reported larval mortality percentages of *An. messeae* at NaCl concentrations of 5000 mg/l and 10.000 mg/l were 65% and 100%, respectively.

Table 7: Reported mortalities of the aquatic stages of *An. maculipennis* s.l..

stage	temperature (°C)	mortality (%)	references
An. atroparvus			
egg	--	18	Van der Kaay et al., 1982
egg	--	20	Meller, 1962
larvae	30	14	Mosna, 1937[1]
larvae	35	100	Mosna, 1937
larvae	29	dying larvae	Danilova and Zubareva, 1932
larvae-pupae	20	0	Mosna, 1937
larvae-pupae	24	35	Van der Kaay et al., 1982[2]
larvae-pupae	25	0	Mosna, 1937
larvae-pupae	30	18	Mosna, 1937
larvae-pupae	20–35	90	Mosna, 1937[3]
pupae	24	1–11	Laarman, 1955
pupae	34	30	Cambournac and Simoes, 1944
pupae	35	78	Cambournac and Simoes, 1944
An. labranchiae			
larvae-pupae	20	0	Mosna, 1937
larvae-pupae	25	0	Mosna, 1937
larvae-pupae	30	0	Mosna, 1937
larvae-pupae	35	100	Mosna, 1937[4]
larvae-pupae	20–35	2	Mosna, 1937[5]
An. maculipennis s.l.			
larvae	4	100	Sautet, 1936
An. maculipennis s.s.			
larvae	<7.5	torpid larvae	Artemiev, 1980
larvae	>35	torpid larvae	Artemiev, 1980
An. sacharovi			
larvae	4	100	Sautet, 1936
egg	-	25	Mer, 1931
egg	25	26	Kasap and Kasap, 1983
larvae-pupae	25	79	Saliternik, 1957

[1] 14% of the population died during the fourth larval stage and 4% during the pupal stage
[2] the pupal mortality was 13%
[3] 78% of the population died during the larval stage and 12% during the pupal stage
[4] 78% of the population died during the larval stage and 22% during the pupal stage
[5] no larval mortality

Bates (1939) showed that larval survival depends on the ratio between the minerals in a medium and not on the absolute amount of one mineral. Various European anopheline species showed significant differences in the amount of calcium needed to enable larvae to survive in a medium containing an excess of magnesium sulphate.

Anopheline larvae have various natural enemies. There is not much quantitative

information about the effects of a predator or a parasite on a population of larvae under natural conditions. Predation may act on each stage of the mosquito but will usually affect the immature stages most (Service, 1976). Among the enemies of *Anopheles* larvae are fish, amphibia, reptiles, birds, and insects. The number of mosquitoes eaten by a predator depends on size and number of both predator and prey, the presence or absence of alternative prey, temperature, interaction between different predator species, and the presence or absence of shelters for the prey and predators (Buxton and Leeson, 1949; Laird, 1988). The fish species *Gambusia affinis* has been used in mosquito control. In small bodies of water containing little vegetation it can almost eliminate early stages of *Anopheles*. Protozoa, fungi and hydrachnid mites have been described as parasites of mosquito larvae (Bates, 1949). By living close to vegetation and floating materials, larvae are protected from predators. Mosquito larvae mostly form a subordinate part of the aquatic community. Consequently, their removal does not result in a radical change in the physical or biological aspects of a community (Bates, 1949).

Meller (1962) observed a density effect on the development rate and the size of *An. atroparvus* larvae and pupae. When the larval density was more than 1 larva per 3.5 cm^2, mortality as well as development time increased. Furthermore, a density dependent cannibalism was seen (Table 8).

Table 8: Density effects on development time (days) and larval mortality of *An. atroparvus* (24–25°C; after Meller, 1962).

density [cm^2/ larvae]	larval mortality (%)	cannibalism mortality (%)	development time larvae (days)
7.5	12	4	11–17
3.8	8	4	11–18
1.9	14	10	14–24
1.3	30	13	17–25
0.9	35	19	17–28

Fecundity

The fecundity of *An. maculipennis* s.l. is presented in Table 9. Shute (1936) reported up to 17 batches of eggs produced by one female *An. atroparvus* at 24°C. Those insects which survived long enough to produce more than 10 batches of eggs laid approximately 2500 eggs.

Fecundity depends mainly on the physiological age of the female. Detinova *et al.* (1963) observed a negative correlation between mean fecundity and gonotrophic cycle (Fig. 10).

Various authors found a positive correlation between the size of the female and the number of developing eggs. Since adults are larger in the cool months of spring

Table 9: Fecundity of *An. maculipennis* s.l..

number of batches	season	number of eggs per batch mean	range	references
An. atroparvus				
100		145	58–274	Van Thiel, 1933
--		212		Van der Kaay *et al.*, 1982[1]
--		260		Van der Kaay *et al.*, 1982[2]
--	spring	250	100–350	Cambournac and Hill, 1938
--			151–200	Meller, 1962
An. labranchiae				
500		132	22–295	Van Thiel, 1933
An. maculipennis s.s.				
100		170	57–291	Van Thiel, 1933
100	summer	175	106–216	Shannon and Hadjinicolao, 1941
224	summer	214	122–300	Shannon and Hadjinicolao, 1941
100	spring	305	161–412	Shannon and Hadjinicolao, 1941
An. melanoon				
100		163	64–286	Van Thiel, 1933 (*An. melanoon*)
72	summer	302	185–304	Shannon and Hadjinicolao, 1941 (*An. subalpinus*)
100	spring	353	207–515	Shannon and Hadjinicolao, 1941 (*An. subalpinus*)
An. messeae				
100		150	74–256	Van Thiel, 1933
An. sacharovi				
--	February	100		Mer, 1931
--	May	280		Mer, 1931
--	July	100		Mer, 1931
--	August	210		Mer, 1931
--	November	100		Mer, 1931
102	summer	152	83–349	Shannon and Hadjinicolao, 1941
100	spring	272	161–342	Shannon and Hadjinicolao, 1941
--		128	55–217	Kasap and Kasap, 1983

[1] after the first blood meal.
[2] after the second blood meal.

than in summer, the number of eggs per batch in spring will be higher (Detinova *et al.*, 1963; Shannon and Hadjinicalao, 1941). Detinova *et al.* (1963) reported a mean fecundity of *An. messeae* in the USSR to decrease gradually from 282 eggs in June to 235 eggs in July, 180 eggs in August and 132 in September. The average fecundity in the first gonotrophic cycle was 289, 263, 255 and 180 eggs in June, July, August and September, respectively. For the second gonotrophic cycle these numbers were 294, 255, 226 and 205, respectively. The mean fecundity of the females which have overwintered decreases from 195 eggs in April to 172 and 149 eggs in May and June, respectively (Detinova *et al.*, 1963).

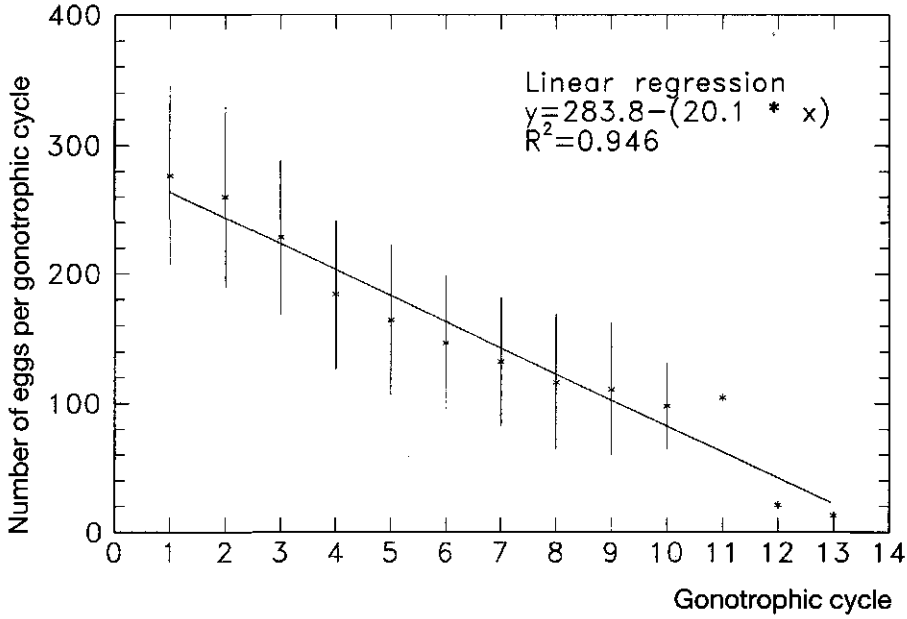

Figure 10: Fecundity of *An. messeae* in relation to the gonotrophic cycle (after Detinova *et al.*, 1963).

The distribution of the anopheline larvae is partly due to the selectivity of the females when ovipositing in certain habitats. The larva has only limited possibilities of selecting its environment. Ecological studies of the oviposition behaviour of the female adults have given puzzling results. Bates (1949) concluded that it is very likely that the adults react to both the location of the habitat and the habitat-condition through visual and chemical cues.

To determine the optimum temperature for the various species is complex. At increasing temperatures the growth rate can increase, but simultaneously mortality rates may increase too. Furthermore, at relatively high temperatures emerging adults will be smaller than at lower temperatures. Consequently the number of eggs per female decreases.

Breeding sites of *An. maculipennis* s.l.

An. atroparvus

The aquatic stages of this species are typically found in clean, sunlit, standing brackish and fresh water with horizontal submerged vegetation. The species has been found in cultivated parts of river deltas, river margins, marshes of coastal areas, lakes, brackish polders, canals and drainage ditches, rice fields, springs,

ground pools with little vegetation, wells, and cement tanks (Cambournac and Hill, 1938; Pires *et al.*, 1982; Pittaluga *et al.*, 1932; Sinton and Shute, 1943; Van Thiel, 1927; Zotta, 1938). *An. atroparvus* has been reported up to 1800 m. altitude (Cambournac, 1942).

Reported salinity of the breeding sites varies from 300–700 mg/l in Germany and Yugoslavia to 800–2500 mg/l in the Netherlands (Adamovic, 1980; Van der Torren, 1935; Weyer, 1939). Van der Torren (1935) reported *An. atroparvus* in the Netherlands at chloride concentrations of up to 8000 mg/l. Bates (1939) observed a difference in the tolerance of NaCl by *An. atroparvus* depending on whether a calcium salt was added to the medium or not. Bates (1941[a]) reported that potassium nitrate had an unfavourable effect on larvae. Under laboratory conditions De Buck *et al.* (1932) and Hackett and Missiroli (1935) reported a preference of *An. atroparvus* for oviposition in fresh water rather than in a salt solution. Bates (1940[a]) found that *An. atroparvus* in Albania under laboratory conditions showed complete indifference to the NaCl content of the water. The species showed a pronounced preference for dark background colours and for water containing calcium. In nature the species is able to breed in fresh water but is out competed by the better adapted *An. messeae*. *An. atroparvus* breeds in waters which are slightly too salt, or warm for *An. messeae* (Hackett and Moshkovski, 1949). In fresh water areas where *An. messeae* is absent, such as in parts of Italy, Spain and Portugal, *An. atroparvus* may occur (Bates and Hackett, 1939). Cambournac and Hill (1938) reported *An. atroparvus* in Portugal in water with total chlorides varying between 50 and 130 mg/l. Ramos *et al.* (1977) reported a salinity in the Algarve (southern Portugal) of 110 to 820 mg/l. However, in salt-water works the larvae tolerated a salinity of 3200-3300 mg NaCl/l. In one case the water contained 16.600 mg NaCl/l.

Pires *et al.* (1982) reported a pH of the breeding sites in the range 6.7–7.0 with extremes of 5.5 and 9.0. Ramos *et al.* (1977) observed a pH ranging from 5.5–7.0. Cambournac and Hill (1938) reported a pH between 6.0 and 8.0. Adamovic (1980) observed a pH of more than 8.

An. labranchiae

This species breeds in sunlit, stagnant fresh and brackish water containing horizontal vegetation in all coastal areas and in some inland zones. It has been reported in marshes, sluggish canals and rivers, quiet edges of flowing streams wherever a growth of algae can develop, rice fields, ponds, ground pools, grassy pools, tanks and wells (Bruce-Chwatt, 1985; Falleroni, 1926; Hackett and Moshkovski, 1949; Logan, 1953). *An. labranchiae* has been reported up to an altitude of 1720 m. (D'alessandro *et al.*, 1971).

In Europe *An. labranchiae* is normally found in brackish water. The salinity of the breeding site can reach 10.000 mg/l (Weyer, 1939). The species only breeds in

fresh waters where *An. atroparvus*, *An. melanoon* and *An. messeae* do not occur. In Sardinia, Corsica, Sicily and a small area of southeastern Spain where it is the only *maculipennis* form it has spread into all types of fresh water from hill streams to inland marshes (Hackett, 1949; Hackett and Moshkovski, 1949; Trapido and Aitken, 1953; Zulueta, 1990). In Sardinia the species has been reported in breeding habitats with a salinity of 151–1385 mg/l (Loddo, 1955). Bettini *et al.* (1978) reported *An. labranchiae* in central Italy in fresh water.

In Sardinia this mosquito has been reported in breeding sites with a pH ranging from 6.8–7.9 (Loddo, 1955), in slowly moving streams, occasionally in fast-running water, and even in turbid water (Logan, 1953). *An. labranchiae* is better adapted to warmer waters than *An. atroparvus* (Hackett and Missiroli, 1935).

An. maculipennis s.s.

An. maculipennis s.s. has been found in hilly areas at altitudes of up to 2300 m (Postiglione *et al.*, 1973) and in protected spots in hill streams, margins of rivers, (spring-fed) ditches, rice fields, ponds, springs, and (artificial) pools (Barber, 1935; Falleroni, 1926; Lewis, 1939; Rice and Barber, 1937; Zotta, 1938).

Cousserans *et al.* (1974) reported the species in a habitat with a pH of 7.8 and a chloride content of 71 mg/l. Pichot and Deruaz (1981) found a pH of 6.95. Breeding occurs in water rich in oxygen (Artemiev, 1980). *An. maculipennis* s.s. tolerates moving water better than *An. messeae* and *An. subalpinus* (Bates, 1941[b]). Missiroli (1935) associated the species with water which is subject to wide daily fluctuations in temperature.

An. melanoon

An. melanoon is typically found in sunlit stagnant water with a large surface area and some vegetation. This species has been reported in marshes, marginal zones of lakes, rice fields, ponds, and ground pools (Bates, 1941[b]; Falleroni, 1926; Hackett, 1934; Logan, 1953; Rice and Barber, 1937), *An. melanoon* (*subalpinus*) has been found up to an altitude of 1200 m. (Postiglione *et al.*, 1973).

The breeding sites have a low nitrate content and a salinity of less than 1000 mg NaCl/l (Bates, 1941[b]).

An. messeae

The aquatic stages of *An. messeae* are typically found in stagnant fresh water. Horsfall (1955) reported *An. messeae* in clear sunlit collections of water 30–50 cm deep where there is abundant growth of submerged vegetation. Plants rising above the surface of the water caused a decrease of larvae when surface and subsurface biota disappeared. The species has been reported near rivers, in marginal zones of

lakes, swamps, flood plains, pools, ponds, and ditches (Barber, 1935; Bates, 1941[b]; Hackett, 1934; Hackett and Moshkovski, 1949).

In the laboratory *An. messeae* showed little preference for fresh water for oviposition (Hackett and Missiroli, 1935). De Buck *et al.* (1932) reported no oviposition preference for fresh or brackish water (3550–7000 mg/l). In nature, saline waters are unfavourable. In many parts of its distribution area, *An. atroparvus* breeds in waters which are slightly too salt for *An. messeae* (Hackett and Moshkovski, 1949). Pichot and Deruaz (1981) reported a pH of the breeding sites of 6.95.

An. sacharovi

The aquatic stages of *An. sacharovi* are typically found in stagnant brackish waters in Europe but in Turkey this species occupies any suitable water, fresh or brackish where horizontal vegetation is present. The species has been reported in coastal marshes and lagoons, clogged mouths of streams entering the sea, irrigation canals, drainage and spring-fed ditches, rice fields, grassy pools, ponds, and seepages (Barber, 1935; Bates, 1937; Bruce-Chwatt and Zulueta, 1980; Hackett and Moshkovski, 1949; Horsfall, 1955; Kasap and Kasap, 1983; Postiglione, 1973). In Israel *An. sacharovi* has been reported in standing and slowly moving water habitats, such as swamps, fish ponds, pools, seepages, and river banks covered by floating horizontal vegetation (Pener and Kitron, 1985[b]). In the Asian part of the former Soviet Union the species was found up to an altitude of 2000 m (Chinayev, 1965).

In Sardinia *An. sacharovi* has been reported in breeding sites with a salinity of 160–19.100 mg/l and a pH ranging from 6.7–7.4 (Loddo, 1955). Saliternik (1957) reported a water depth generally greater than 30 cm and a pH range from 7 to 8.8.

Breeding sites of *An. superpictus*

The larvae of *An. superpictus* are typically found in clear gravelly beds of hill streams without vegetation which flow from the mountains into the coastal plains. The species can live in slowly flowing streams. Conditions are most favourable during the driest period when there are (almost) no floods. They are found in stony edges of streams, rice fields, swamps, irrigation channels, ditches, ponds, small pools, springs, seepage areas, cisterns, wells, pits, and drillings (Barber, 1935; Bates, 1941[b]; Bates, 1949; Hackett and Moshkovski, 1949; Postiglione *et al.*, 1973; Tshinaev, 1963; Weyer, 1939). Pener and Kitron (1985[a]) reported one larva in a polluted habitat in Israel. A gravel bed is not a prerequisite for the breeding site as Weyer (1939) also noted this mosquito in a sandy bedded site. Tshinaev (1963) observed *An. superpictus* eggs to sink to the bottom of the water where oxygen is supplied by the photosynthesis of green filamentous algae and by intake of atmospheric air in the small more rapidly flowing waters. In the Asian part of the former Soviet Union *An. superpictus* has been reported up to an altitude of 2000 m. (Chinayev, 1963).

Table 10: Fecundity of *An. superpictus*.

number of batches	season	number of eggs per batch mean	range	references
100	summer	140	93–163	Shannon and Hadjinicolao, 1941
100	spring	162	67–173	Shannon and Hadjinicolao, 1941

Table 11: Mean development times of the aquatic stages of *An. superpictus* in relation to temperature.

temp. (°C)	development time (days) eggs	L1	L2	L3	L4	pupa	references
17	>28	→	→	→	20	5	Kligler, 1929
18–21	→	→	→	→	→	22–24	Ulitcheva (cited by Tshinaev, 1963)
24–25	→	→	→	→	→	13–14	Ulitcheva (cited by Tshinaev, 1963)
25–27	→	→	→	→	→	11–12	Ulitcheva (cited by Tshinaev, 1963)

The larvae of *An. superpictus* prefer a high salinity, comparable to that preferred by larvae of *An. sacharovi* (Weyer, 1939).

The fecundity of *An. superpictus* and the development times of the various aquatic stages are shown in Tables 10 and 11. The photoperiod had no effect on the duration of larval development (Vinogradova, 1960).

Competition between species

The coastal distribution of *An. labranchiae* and *An. sacharovi* in the Balkans and Italy differs from the situation in North Africa and Turkey where these species have an inland as well as a coastal distribution. The coastal distribution in the Balkans and Italy is probably explained by the absence of other species of the *maculipennis* complex. Only in areas where other species of the complex were absent did these species penetrate inland (e.g. Sicily and Sardinia). The competition between the various members of the *An. maculipennis* complex is related to the salinity of the breeding sites. Bates (1939) tested the survival of species of the *An. maculipennis* complex in different dilutions of sea water (Fig. 11). Each of the species of the *An. maculipennis* complex has a characteristic salinity preference in its breeding sites. Resistance to dehydration in saline solutions is common for *An. labranchiae*, *An. sacharovi* and *An. atroparvus*. Hackett and Moshkovski (1949) reported *An. sacharovi* to be more tolerant to salt than *An. labranchiae*. Tolerance for NaCl seems to be identical for *An. labranchiae* and *An. atroparvus* when calcium salts are present, however, when these are absent *An. atroparvus* shows greater mortality. Eckstein (1936) and Corradetti (1934) reported *An. atroparvus* to be more tolerant to salt than *An. messeae*. Artemiev (1980) reported that the degree of

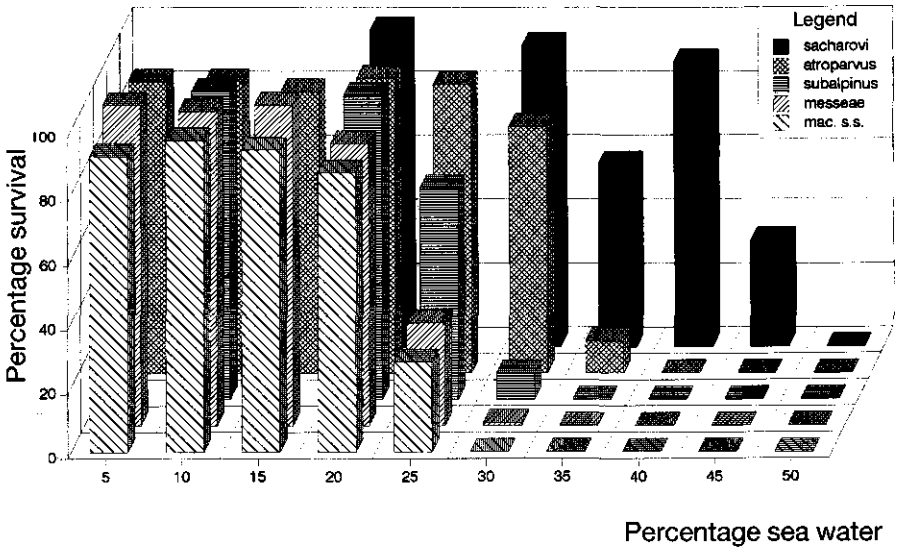

Figure 11: Average number of larvae of members of the *An. maculipennis* complex surviving for three days in different dilutions of sea water (first instar larvae kept at 27°C; after Bates, 1939).

salt tolerance increased from *An. maculipennis* s.s., *An. melanoon* (including *An. subalpinus*), *An. messeae*, *An. atroparvus* to *An. sacharovi*.

Horsfall (1955) reported that only rarely did two or more species of the *An. maculipennis* complex occupy the same site concurrently. When this was the case one or the other was usually decidedly dominant. *An. sacharovi*, *An. labranchiae* and *An. atroparvus* are able to breed in brackish water and show a predominantly coastal distribution. *An. messeae*, *An. melanoon* and *An. maculipennis* s.s. are definitely associated with fresh-water. Postiglione *et al.* (1973) observed mixtures of *An. sacharovi*, *An. maculipennis* s.s. and *An. subalpinus* in Turkey. The degree of dominance of *An. sacharovi* partly depended on the salinity of the water. Barber (1935) described a fresh water pool near a river in Greece which contained a mixture of eggs of *An. sacharovi* and *An. messeae*. In autumn the pool became brackish and *An. messeae* disappeared. Van der Torren (1935) observed the competition between *An. atroparvus* and *An. messeae* in the Netherlands depending on the chloride concentration of the breeding habitat (Fig. 12). Both larvae can be found in brackish as well as fresh water. However, *An. atroparvus* prefers brackish water above 1000 mg/l chlorides while *An. messeae* prefers water with less than 750 mg/l chlorides. He pointed out that breeding sites with a low salinity surrounded by breeding sites with a high salinity can be invaded with *An. atroparvus* while breeding sites with a high salinity surrounded by breeding sites with a low salinity can be invaded by *An. messeae*. In Rumania the fraction *An. atroparvus* found in the environs of Bucharest depends on the amount of rain. During wet seasons the percentage

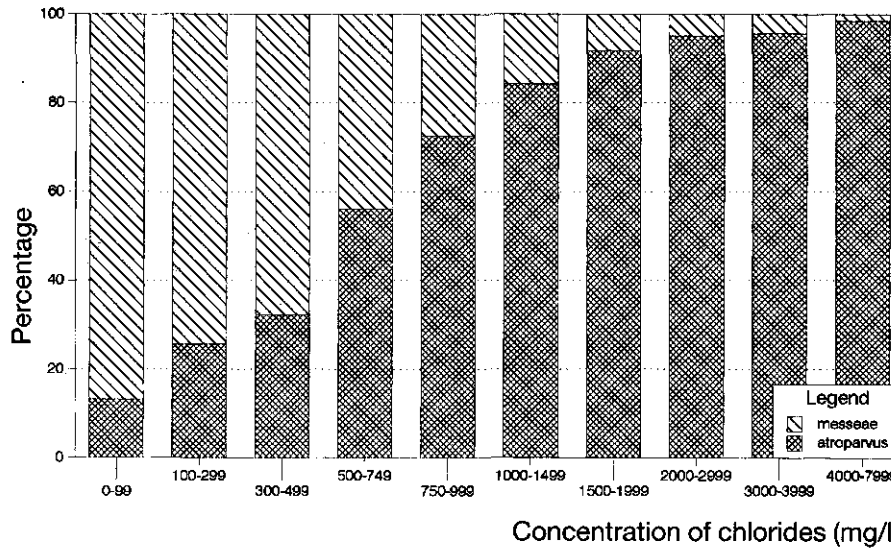

Figure 12: Competition effects depending on salinity between *An. atroparvus* and *An. messeae* (after Van der Torren, 1935).

of *An. atroparvus* found among *An. maculipennis* s.s., *An. messeae* and *An. atroparvus* decreases due to the availability of less saline waters.

The salinity of the breeding habitats partly determines the distribution of the various species of the complex. However, it is certainly not the only important factor. The temperature of the breeding site influences aquatic mortality. Laboratory experiments and an analysis of the distribution show that *An. sacharovi* is the most thermophilic species of the *An. maculipennis* complex, followed by *An. labranchiae*, *An. atroparvus* and *An. messeae*. Species of the *An. maculipennis* complex are typically found in standing or slowly moving water, however, *An. superpictus* and *An. maculipennis* s.s. show some adaptation to running water.

Chapter 4

THE BIONOMICS OF THE ADULT STAGES

Mating, blood feeding and reproduction

In nature it is unusual to find a female with an empty spermatheca. Therefore, Buxton and Leeson (1949) concluded that mating normally occurs soon after emergence and before feeding. Mating takes place only once and the amount of sperm transferred is sufficient to fertilise all subsequent batches of eggs. Shute (1936) reported numerous spermatozoa in the spermatheca of *An. atroparvus* after 15 batches of eggs had been laid.

In most anopheline species swarming of males is needed before pairing. A female flies into the swarm and mating takes place in the air. Horsfall (1955) reported *An. messeae*, *An. labranchiae* and *An. maculipennis* s.s. to swarm but doubted whether *An. atroparvus* would swarm regularly. Cambournac and Hill (1940) reported vigorous dancing of *An. atroparvus* in nature, either in stables or in the open air. Erel (1973) reported that in the field the males of *An. sacharovi* swarm above water or above any object with a height of 1.5–2 m. Light is the most important stimulus to determine the time of swarming at dusk. However, temperature and humidity influence the time of swarming to some extent (Cambournac and Hill, 1938). *An. atroparvus* does not need swarming before mating and mates almost entirely indoors. *An. labranchiae* and *An. superpictus* were found to mate in a cage of 50 cm square by 1 m in height. *An. sacharovi* can swarm in an insectary. *An. maculipennis* s.s. needs a large open air cage. *An. melanoon* and *An. messeae* mate outdoors only (Cambournac and Hill, 1940; Hackett and Bates, 1938; Horsfall, 1955).

After fertilization, the females search for a blood meal. This blood meal is required in order for eggs to mature. The main alternative food source other than blood are plant juices. The use of nectar (sugars) in nature by both males and females as an energy source for survival, reproduction and flight is common (Muir, 1989).

The mosquitoes are probably ready for their first ovipositions not later than eight days after the first blood meal. Thereafter, batches of ova will become mature in successive intervals of at least two days (Bates, 1949). Grassi (in Horsfall, 1955) reported that oviposition and ingestion of blood follow alternately after the first two blood meals. In *An. sacharovi* two blood meals were required to complete the development of the first egg batch, however, only one blood meal was needed if the pupal development occurred at high temperatures (24–30°C) or if the newly emerged females were offered sugar water or fruit juice before being given a blood

meal (Mer, 1936; Yoeli and Mer, 1938). Meller (1962) reported that *An. atroparvus*, which fed just after emergence, needed two blood meals to complete the development of the first batch of eggs, while *An. atroparvus* which fed on the second day after emergence, needed one blood meal only.

The necessity for oviposition every two (or more) days makes frequent departures from places where a blood meal has been taken necessary. Oviposition of *An. atroparvus* occurs when the air temperature ranges from 6°C to 35°C (Cambournac and Hill, 1938). *An. atroparvus* and *An. labranchiae* oviposit while hovering (Bates, 1940[a]). Dense vegetation at the height of hovering interferes with oviposition (Horsfall, 1955). Although anophelines are active between dusk and dawn, they are considered predominantly crepuscular insects. Logan (1953) and Hadjinicolaou and Betzios (1973) reported a main flight activity of female *An. maculipennis* just after sunset and during dawn. During this period the adult responded to stimuli including light, colour, sound, CO_2, odours, temperature, humidity and air movements (Bates, 1949).

The time between two blood meals is called the gonotrophic cycle. It comprises the total duration of the search for blood, digestion and oviposition. The duration of the gonotrophic cycle depends mainly on the rate of blood digestion as determined by the mosquito species, temperature and humidity. More than two thirds of the mosquitoes feed within 8 hours after oviposition. The total time needed to complete one oviposition and blood meal is about 24 hours (Detinova *et al.*, 1963). The digestion of blood by *An. maculipennis* as reported by various authors is listed in Table 12 and Fig. 13. Shlenova (1938) distinguished three humidity regimes of 30–40%, 70–80% and 90–100%, respectively. She expressed the rate of blood digestion by a temperature sum above a temperature threshold. The cumulative rate of blood digestion during a period starting at time x and ending at time $x+n$ is written as

$$RBd = \int_{t=x}^{t=x+n} \frac{(\overline{T} - N)}{TS}\, dt \qquad (1)$$

Relative humidity 30–40%: $N = 4.5$°C; $TS = 65.4$ degree-days
Relative humidity 70–80%: $N = 9.9$°C; $TS = 36.5$ degree-days
Relative humidity 90–100%: $N = 7.7$°C; $TS = 37.1$ degree-days

where

t : Time (days).
N : Temperature threshold (°C).
RBd : Rate of blood digestion (day^{-1}).
$\overline{T}$: Mean temperature during time interval (°C).
TS : Temperature sum (degree-days).

Equation 1 was suitable to express blood digestion in nature during the summer but was unsuitable during spring conditions when low temperatures occurred.

Table 12: Duration of digestion of blood by *An. maculipennis*.

month or temperature (°C)	species	time (days)	references
March	*An. maculipennis* s.l.	14	Sella; cited by Boyd, 1949
October	*An. maculipennis* s.l.	7	Sella; cited by Boyd, 1949
August	*An. maculipennis* s.l.	2.5	Sella; cited by Boyd, 1949
4	*An. atroparvus*	30	Shute, 1933[1]
15	*An. maculipennis* s.l.	>10	Nuttall and Shipley, 1902
16	*An. atroparvus*	5	Shute, 1933[1]
19–31	*An. labranchiae*	5–6	D'Alessandro *et al.*, 1971[2]
27	*An. atroparvus*	2	Shute, 1933[1]

[1] Interval between successive feedings

[2] Temperature roughly varying between 19 and 31°C; relative humidity varying between 50 and 95%; the period required for maturation of the ovaries did not vary much in nulliparous and parous females.

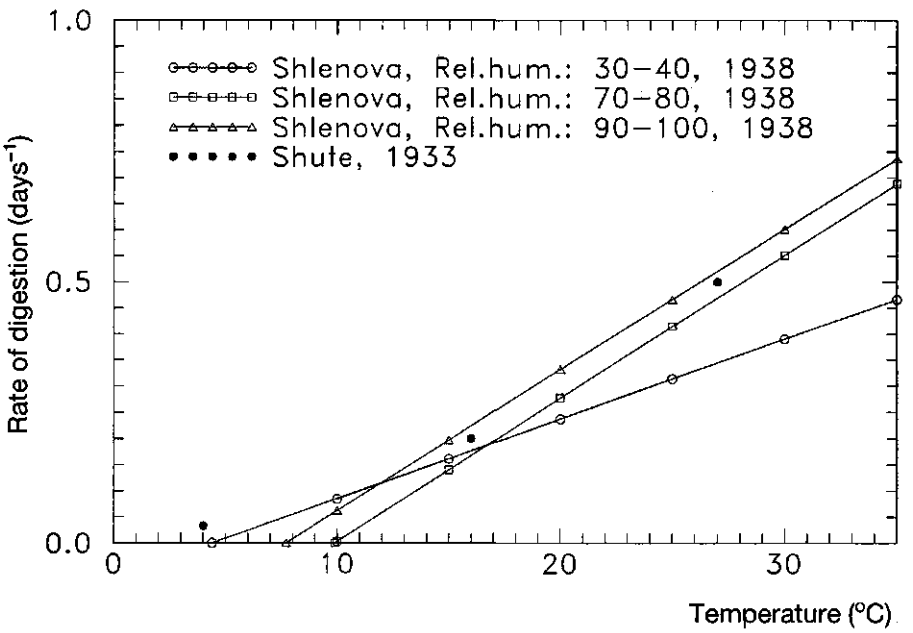

Figure 13: The rate of digestion of blood by *An. maculipennis* s.l. (after Shlenova, 1938, and Shute, 1933).

Diapause

Most anophelines in Europe go into diapause in the winter. During diapause egg laying ceases completely. Diapause is usually induced by short day length rather than low temperatures (Washino, 1977), although temperature modifies daylength response (Vinogradova, 1960; Kasap, 1987). Mosquitoes show individual variation in their reaction to daylength. The time of the first individuals undergoing diapause to one hundred percent diapause can be as long as 15–30 days. The critical daylength for the onset of diapause varies between different species and within a single species according to the geographical origin (Vinogradova, 1960). The third larval stage and successive development stages are the most susceptible stages to changes in photoperiodic conditions (Mer, 1936; Vinogradova, 1960).

Diapause can be complete or incomplete. In the first case females form a fat body. When winter approaches they seek a cold and humid shelter and remain completely inactive until spring. In the second case fat is deposited, but the insect remains active. When cold weather approaches, these insects seek a shelter in a stable or dwelling and continue to take bloodmeals, irregularly, without egg laying. In this situation (indoor biting) transmission of malaria is effected. The transmission of malaria in the Netherlands was mainly due to the presence of *An. atroparvus* in human settlements in the autumn (Verhave, 1987). The potentiality for winter survival of sporozoites will vary with the circumstances of diapause and the severity of the winter climate. Species that go into complete diapause as adults are not likely to carry the infection over into the following spring. Species that go into incomplete diapause in human dwellings can continue to transmit a *Plasmodium* infection until late autumn (Boyd, 1949; Swellengrebel and De Buck, 1938).

An. atroparvus, *An. labranchiae*, *An. sacharovi* and *An. superpictus* show incomplete diapause (Kligler and Mer, 1930; Logan, 1953; Mer, 1936; Swellengrebel *et al.*, 1936; Vinogradova, 1960). *An. maculipennis* s.s., *An. messeae* and *An. subalpinus* show complete diapause (Horsfall, 1955; Maevsky, 1963). This division is not absolute. Hackett and Missiroli (1935), Martini *et al.* (1932) and Postiglione *et al.* (1973) reported some *An. sacharovi* and *An. atroparvus* in complete diapause in caverns and attics. Nagiev (1959) reported *An. melanoon* (*subalpinus*) in Azerbaidzhan to show incomplete diapause.

Diapause of *An. atroparvus* typically starts in September in northern European countries and in October-November in southern Europe. In northern Europe, diapause usually ends in March-April depending on the ambient temperature. In southern Europe, egg laying may start already in January-February (Cambournac and Hill, 1938; Hackett and Missiroli, 1935; Martini *et al.*, 1932; Ramsdale and Wilkes, 1985; Roubaud, 1931; Swellengrebel and De Buck, 1938).

Diapause of *An. maculipennis* s.s. in Yugoslavia starts at the beginning of October. Feeding activity continues during November-December and ceases completely in January. The feeding activity is directly influenced by the temperature. Oviposi-

Table 13: Daylength needed to start onset of diapause and mass diapause of *An. messeae* (after Shipitsina, 1957; cited by Vinogradova, 1960).

latitude	onset of diapause		mass diapause	
	adults	larvae	adults	larvae
60°	19h44m	21h20m	18h10m	19h44m
46°	16h08m	16h32m	14h26m	15h11m

h = hours; m = minutes

tion ceases when the mean temperature falls below 11.5 °C. Ovarian development is completely suspended at 5-7°C. The species typically passes from a state of incomplete diapause to complete diapause in January (Guelmino, 1951).

Martini *et al.* (1932) reported the start of diapause of *An. messeae* in Germany on approximately the 16th of September. Guelmino (1951) in Yugoslavia reported a change of the resting habitat at the end of August or the beginning of September. The species continues to oviposit until the end of September. From the beginning of November until the end of February the species is to be found in a state of complete diapause. Vinogradova (1960) cited Shipitsina (1957) who reported that the onset of diapause and mass diapause depended on decreasing daylength and latitude (Table 13). Laboratory experiments conducted by Vinogradova (1960) confirmed the observations of Shipitsina on the onset of diapause.

In the Transcaucasus, diapause of *An. sacharovi* usually starts in October and ends in February. Kasap (1987) in Turkey reported that oviposition of *An. sacharovi* ceased when the photoperiod became shorter than 10 hours and the temperature was less than 18°C. At 25°C this did not occur. It was concluded that decreasing temperature coupled with decreasing daylength appeared to be important factors in initiating diapause of the adult females.

The end of diapause is determined by temperature. It is a dynamic phenomenon due to temperature differences. In Sardinia the highest number of females with a fat body was found in December (65%). In January this number decreased to 26% as the mean outdoor temperature for that month in Cagliari was 8.4°C (Logan, 1953). Solovey and Likhoded (1966) reported flight of *An. maculipennis* s.l. in the Murmansk area starting at air temperatures of 8–10°C. Oviposition of *An. maculipennis* s.s. in Yugoslavia starts when the mean outdoor temperature rises to 7.5°C at the beginning of March (Guelmino, 1951). Artemiev (1980) reported for the Moscow region a migration from winter shelters of *An. messeae* from mid-April to mid-May at mean daily temperatures of 4–8°C. The species begins to fly and feed when the temperature exceeds 7°C.

An. superpictus leaves the overwintering places later than *An. maculipennis* s.l. (Kligler, 1929; Weyer, 1939). The species stays in buildings or in outdoor shelters (Postiglione *et al.*, 1973). The lower temperature threshold for adult activity is 7.5°C (Tshinaev, 1963).

During warm winters some females of *An. maculipennis* s.l. go into diapause. D'Alessandro *et al.* (1971) reported partial diapause of *An. labranchiae* on Sicily.

Mortality is lower during diapause than during the summer. In Spain, overwintering *An. atroparvus* lived for more than 6 months, while in summer they lived for no more than 6 weeks (Hill, 1937). In summer the mortality increases due to low humidity, high temperatures and increased activity. Hill (1937) reported relative mortality rates in a cage of *An. atroparvus* during diapause of 0.012–0.013 per day. Detinova *et al.* (1963) reported total mortality during diapause to fluctuate between 70–90%. Consequently, when mean diapause takes 6 months the mean relative mortality rate varies between 0.006–0.013 per day. Detinova *et al.* (1963) reported summer gonoactive females to fly in small numbers in autumn to overwintering places, but they all died during autumn and winter. De Buck and Swellengrebel (1934) reported a relative mortality rate of 0.003 per day of *An. messeae* after starvation in winter cages for a period of 168 days.

Detinova *et al.* (1963) reported preferred temperatures of overwintering places of *An. messeae* to vary between –3 and 2°C. The optimum humidity for overwintering varies between 60% and 85%. Overwintering mosquitoes are not killed by severe winters with stable frosts but by mild winters with frequent rises in temperature alternating with frost. Maevsky (1963) referred to Beklemishev and pointed out that increased mortality during diapause is due to exhaustion of fat reserves. This is caused by high temperatures in the shelters and sharp oscillations of the temperature under dry conditions.

Eichler (1951) reported the appearance of aestivation of *An. atroparvus* and *An. messeae* during the hot season in the south of Russia related to the lack of breeding sites. The mosquitoes then cease to lay eggs but have blood meals occasionally.

Adult characteristics and the epidemiology of malaria

The malaria incidence in a certain area is determined by a range of factors such as human behaviour, the presence of infectious *Plasmodium* species and vector characteristics. In this section these latter factors are discussed in detail: the density of malaria vectors (1), the longevity (2), the duration of the extrinsic incubation period (3), the vector susceptibility to malaria parasites (4), the feeding preference (5) and the dispersal behaviour (6).

Vector density

The density of mosquitoes depends on life-history parameters (i.e. fecundity, development rate, mortality) of the aquatic and terrestrial stages of the species in relation to chemical, physical and biological properties of their habitat. The number of generations depends on the length of the season. For *An. maculipennis* s.l. it varies from two in the northern part of Russia to seven or more in Italy. Cambournac (1939) found that rice fields can produce 20.000 new adults of *An. atroparvus* daily

per hectare. Leeson (1939) reported the male:female ratio to be approximately 1:1 at a temperature of 25°C with a high humidity.

Longevity

The longevity of an adult mosquito is a point of great interest to malaria epidemiology. The mosquito must live long enough to complete the extrinsic incubation period of the *Plasmodium* species in order to be able to transfer malaria. Males have a shorter lifespan than females. Meller (1962) reported 50% mortality of
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Table 14: Reported mortalities of unfed adults (after Hundertmark, 1938).

temperature (°C)	relative humidity (%)	mean longevity (days)	
		females	males
An. atroparvus			
19	100	4.4	3.0
19	92	3.9	3.4
19	75	3.8	2.7
19	32	3.1	2.3
19	0	2.5	1.8
30	100	1.9	
30	75	2.0	
30	32	1.3	
30	0	1.1	
An. maculipennis s.s.			
19	100	3.7	3.1
19	92	3.8	3.3
19	75	3.7	2.8
19	32	3.1	2.6
19	0	2.7	2.0
An. messeae			
19	100	3.5	2.6
19	92	3.2	2.9
19	75	2.8	2.1
19	32	2.1	1.5
19	0	2.2	1.3
30	100	1.8	
30	75	1.4	
30	32	1.2	
30	0	1.0	

Table 15: Reported mortalities of female *An. maculipennis* s.l. and *An. superpictus*.

temperature (°C)	relative humidity (%)	day	surviving (%)	RMR (day^{-1})	references
An. atroparvus					
15–27	55–81(65)	15	90	0.007	Shute and Ungureanu, 1939
16–26	60–80(70)	21	60	0.024	Shute and Ungureanu, 1939
20–25	80–90(85)	15	27	0.087	Rosa, 1936
20–25	80–90(85)	20	18	0.087	Rosa, 1936
20–25	80–90(85)	25	11	0.089	Rosa, 1936
20–25	45–55(50)	5	46	0.154	Rosa, 1936
20–25	45–55(50)	10	14	0.196	Rosa, 1936
20–25	45–55(50)	15	4	0.208	Rosa, 1936
18–29	63–82(73)	14	88	0.009	Shute and Ungureanu, 1939
24	70–90(80)	11	95	0.005	Shute and Ungureanu, 1939
24	70	43	mean	0.023	Shute and Ungureanu, 1939
25	0	3.4	mean	0.294	Leeson, 1939[1]
25	30	4.9	mean	0.204	Leeson, 1939[1]
25	60	5.7	mean	0.175	Leeson, 1939[1]
25	90	8.3	mean	0.120	Leeson, 1939[1]
26–27	75–80	13	88.2	0.010	Meller, 1962[2]
26–27	75–80	21	49.0	0.034	Meller, 1962[2]

Table 15 (continued)

temperature (°C)	relative humidity (%)	day	surviving (%)	RMR (day^{-1})	references
An. atroparvus					
26–27	75–80	51	0.2	0.122	Meller, 1962[2]
26–27	75–80	13	88.2	0.010	Meller, 1962
26–27	75–80	21	77.8	0.011	Meller, 1962
26–27	75–80	36	50.0	0.019	Meller, 1962
26–27	75–80	111	0.4	0.008	Meller, 1962
22–30	63–82(73)	12	90	0.009	Shute and Ungureanu, 1939
22–30	64–94(79)	13	100	0.000	Shute and Ungureanu, 1939
An. labranchiae					
16–26	60–80(70)	21	80	0.011	Shute and Ungureanu, 1939
24	70–90(80)	11	70	0.032	Shute and Ungureanu, 1939
An. maculipennis s.s.					
15–27	55–81(65)	15	30	0.080	Shute and Ungureanu, 1939
16–26	60–80(70)	21	45	0.038	Shute and Ungureanu, 1939
18–29	63–82(73)	14	52	0.047	Shute and Ungureanu, 1939
24	70–90(80)	11	70	0.032	Shute and Ungureanu, 1939
20–34	60–80(70)	12	38	0.081	Shute and Ungureanu, 1939
22–30	63–82(73)	12	68	0.032	Shute and Ungureanu, 1939
22–30	64–94(79)	13	85	0.013	Shute and Ungureanu, 1939
An. messeae					
15–27	55–81(65)	15	4	0.215	Shute and Ungureanu, 1939
16–26	60–80(70)	21	30	0.057	Shute and Ungureanu, 1939
20–25	80–90(85)	15	15	0.127	Rosa, 1936
20–25	80–90(85)	20	6	0.138	Rosa, 1936
20–25	80–90(85)	25	2	0.155	Rosa, 1936
20–25	45–55(50)	5	34	0.216	Rosa, 1936
20–25	45–55(50)	10	8	0.253	Rosa, 1936
20–25	45–55(50)	15	2	0.261	Rosa, 1936
18–29	63–82(73)	14	6	0.201	Shute and Ungureanu, 1939
24	70–90(80)	11	50	0.063	Shute and Ungureanu, 1939
20–34	60–80(70)	12	22	0.126	Shute and Ungureanu, 1939
22–30	63–82(73)	12	44	0.068	Shute and Ungureanu, 1939
22–30	64–94(79)	13	65	0.053	Shute and Ungureanu, 1939
An. sacharovi					
20–25	80–90(85)	15	10	0.156	Rosa, 1936
20–25	80–90(85)	20	3	0.172	Rosa, 1936
20–25	45–55(50)	5	12	0.433	Rosa, 1936
20–25	45–55(50)	10	4	0.327	Rosa, 1936
25	80	11.6	mean	0.086	Kasap, 1990[3]
25	80	9.9	mean	0.101	Kasap, 1990[4]
nature		20	mean	0.050	Kasap *et al.*, 1990
An. superpictus					
25	80	16.5	mean	0.060	Kasap, 1990[3]
25	80	18.2	mean	0.055	Kasap, 1990[4]

[1] fed only once at the start of the experiment.
[2] fed with sugar water
[3] Infected mosquitoes after one blood meal fed on sugar water
[4] Uninfected mosquitoes after one blood meal fed on sugar water

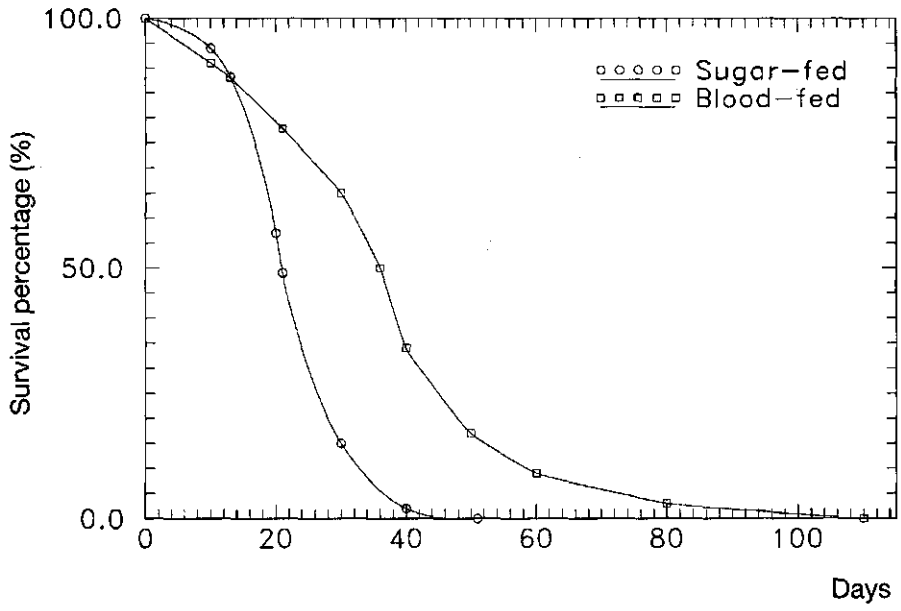

Figure 14: Laboratory mortality of female *An. atroparvus* fed on sugar water or blood (after Meller, 1962).

– *nutrition* – Longevity of females depends also on nutrition. Mosquitoes fed on blood live longer than those fed on sugar water. Without food, survival of the mosquitoes is limited. Laarman (1955) observed 50% mortality of recently emerged unfed mosquitoes after 2 days. Meller (1962) observed the difference in longevity of female *An. atroparvus* fed on sugar water and those fed on blood (Fig. 14).

– *incidence of parasites and predators* – In nature the mean longevity of a mosquito is influenced by the incidence of parasites and predators. When the adult mosquito emerges from the pupa it can be attacked by skating bugs (*Microvelia*) and several other Heteroptera. At rest or in flight the mosquitoes are caught and eaten by dragonflies, wasps, scorpion flies and a variety of predatory Diptera. At rest they are also eaten by lizards and geckoes. Bats and birds may destroy large numbers in flight (Buxton and Leeson, 1949). Barber and Rice (1935[b]) reported a parasite incidence of 4.1% in *An. maculipennis* of East Macedonia. According to Bates (1949) physical conditions play a far greater role in the mortality of adult mosquitoes than parasites or predators.

Most experiments on longevity of adult mosquitoes have been performed in an artificial situation. Experiments on adult survival with members of the *An. maculi-*

pennis complex in nature are rare. McHugh (1989) estimated daily survivorship of *An. freeborni* in nature to be 0.74 with a gonotrophic cycle of 4 days. It is not sure whether experiments in an artificial situation can be used to determine the longevity in nature. Detinova *et al.* (1963) estimated the mortality in *An. messeae* and *An. superpictus* based on data of age composition described by various authors. The data showed that among physiologically young females mortality is roughly 50% in each gonotrophic cycle. In physiologically older females mortality increases to 60–75% in each gonotrophic cycle. Daily relative mortality rates can be given depending on the duration of the gonotrophic cycle. The relative mortality rates vary between 0.17–0.35, 0.14–0.28, 0.12–0.23 and 0.10–0.20 per day for a gonotrophic cycle of 4, 5, 6 and 7 days, respectively.

There is some evidence that the development of ookinetes and oocysts affects the mosquito in different ways (WHO, 1987). However, according to Sinton and Shute (1938) heavy infections of *P. vivax* and *P. falciparum* do not increase the mortality of *An. atroparvus*.

Duration of the extrinsic incubation period

An anopheline mosquito which has taken an infected blood meal is not infectious until the extrinsic incubation period of the parasites has been completed. The duration of the extrinsic incubation period strongly depends on the temperature of the insect body. Once the mosquito becomes infectious it will remain so during most of its life. Only during diapause do mosquitoes gradually lose their infectivity. The parasite was reported unaffected by changes in temperature between 4 and 24°C for at least 80 days. In *An. maculipennis*, *P. vivax* either as oocysts or as gland sporozoites, survived three weeks of continuous exposure to temperatures ranging from 4 to 5.5 °C, or six days at temperatures below freezing point. High temperatures are lethal to sporozoites of *P. vivax*, the proportion surviving decreasing rapidly at temperatures above 32°C. (James, 1925; James, 1926; James, 1927; Roubaud, 1918).

There is a clear impact of the parasite species on the duration of the extrinsic incubation cycle. Minimum temperatures reported to complete the sporogonic development of different *Plasmodium* species in the mosquito are presented in Table 16.

Table 16: The minimum temperature needed to complete the extrinsic incubation period.

temperature (°C)	parasite species	references
15	*P. vivax*	MacDonald, 1957
16.5	*P. malariae*	Grassi, 1901
17.5	*P. vivax*	Grassi, 1901
18	*P. falciparum*	Grassi, 1901
19	*P. falciparum*	MacDonald, 1957

Moshkovsky and Rashina (1951) used Eq. 1 to express the duration of the extrinsic incubation period. They reported

P. vivax	$N = 14.5°C$	$TS = 105$ degree-days
P. falciparum	$N = 16°C$	$TS = 111$ degree-days
P. malariae	$N = 16°C$	$TS = 144$ degree-days

The development rate of *P. vivax* in various members of the *An. maculipennis* complex is shown in Fig. 15. The figure shows the trend of a reduced incubation period with increasing temperatures. No clear differences between the various members of the *An. maculipennis* complex were seen. Data found on the duration of the extrinsic incubation period in *An. atroparvus*, *An. messeae*, *An. sacharovi* and *An. superpictus* are listed in Table 17. Although the *P. falciparum* data reported by Roubaud (1918) and Cambournac (1942) appear incongruous, the data indicate a trend of a reduced incubation period of *P. falciparum* with increasing temperatures (Table 17).

Vector susceptibility to malaria parasites

The susceptibility of anopheline species for a Plasmodium infection is genetically determined. *P. vivax*, *P. falciparum* as well as *P. malariae* were all transmitted

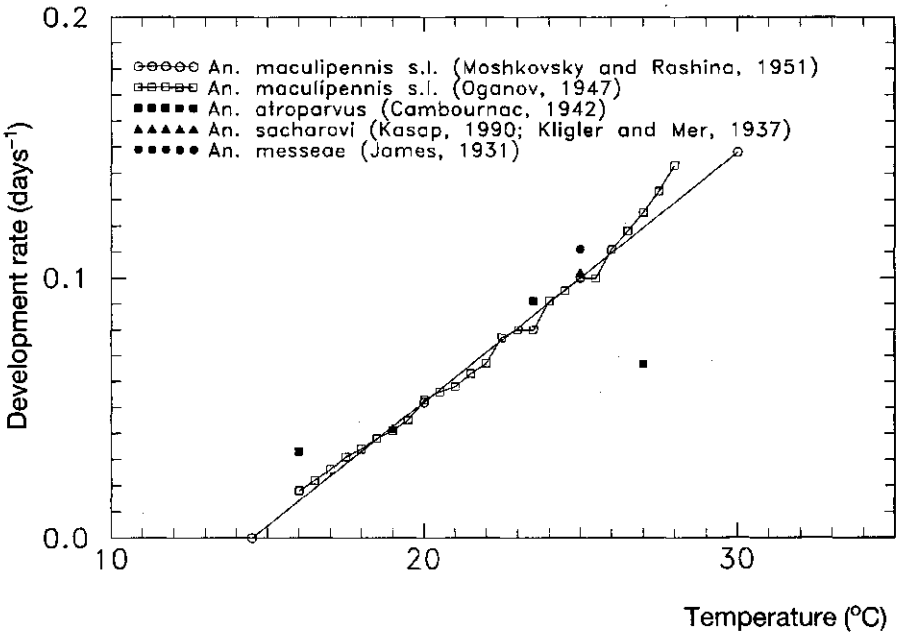

Figure 15: The development rate of *P. vivax* in *An. maculipennis*.

Table 17: Duration of the extrinsic incubation period (days) in *An. maculipennis* and *An. superpictus* in relation to temperature.

temperature (°C)	parasite species *P. falciparum*	*P. vivax*	*P. malariae*	references
An. atroparvus				
15.6			–	Cambournac, 1942
15–17	–	30		Cambournac, 1942
17–20			35–45	Cambournac, 1942
19	20			Roubaud, 1918
18–22	35			Cambournac, 1942
21.1	25			Cambournac, 1942
23–24	15	10–12	25	Cambournac, 1942
25		14		Roubaud, 1918
27		15		Cambournac, 1942
37.7	–	–		Cambournac, 1942
An. messeae				
6		44		James, 1931
25		9		James, 1931
19	20			Roubaud, 1918
25	14			Roubaud, 1918
An. sacharovi				
15.6	–	slowly		Kligler and Mer, 1937
19	35	24		Kligler and Mer, 1937
21	23			Kligler and Mer, 1937
24	18			Kligler and Mer, 1937
25	14–15			Kligler and Mer, 1937
25[1]		9.8		Kasap, 1990
An. superpictus				
25[1]		11.7		Kasap, 1990

[1] relative humidity 80%

by *An. maculipennis* s.l. and *An. superpictus* (Weyer, 1939, Barber and Rice, 1935[b]). Whereas *An. sacharovi*, *An. labranchiae* and *An. atroparvus* were susceptible to the European strain of *P. falciparum*, various reports indicate that today these species are not susceptible to tropical strains of this parasite. Experimental work carried out in England showed that *An. atroparvus* was not susceptible to strains of *P. falciparum* from India or East and West Africa, although it could be infected with an Italian and Rumanian strain (Shute, 1940). Unpublished investigations by Shute (1947) cited by Zulueta *et al.* (1975) showed that, under Nigerian conditions, the English strain of *An. atroparvus* cannot be infected with local strains of *P. falciparum*. Italian, Portuguese and Rumanian strains of *An. atroparvus* were not susceptible to infection with African *P. falciparum*. Russian strains of *An. atroparvus*, *An. messeae* and *An. sacharovi* were not susceptible to infection with African *P. falciparum*. An Italian strain of *An. labranchiae* was not susceptible to infection with *P. falciparum* from Kenya (Daskova, 1977; Daskova

and Rasnicyn, 1982; Ramsdale and Coluzzi, 1975; Ribeiro *et al.*, 1989; Teodorescu, 1983; Zulueta *et al.*, 1975).

An. sacharovi and *An. superpictus* transmit *P. vivax* in Turkey (Kasap, 1990). In the USSR *An. atroparvus*, *An. messeae* and *An. sacharovi* were susceptible to *P. vivax* from Africa and Asia. The species was refractory to infection with west African *P. malariae* and *P. ovale* (Daskova, 1977; Daskova and Rasnicyn, 1982). A Moldavian strain of *An. atroparvus* could be infected with *P. vivax* from Irak, Laos and Turkey and *P. ovale* from Nigeria but not with *P. malariae* from the Central Afican Republic, Madagascar and Gabon (Teodorescu, 1983).
Infection of mosquitoes by human *Plasmodia* seems to be effected without regard to the number of gametocytes of *P. falciparum* or *P. vivax* ingested (Kligler and Mer, 1937; Kasap, 1990).

Feeding preference

The degree to which malaria can spread in a community depends on the Human Blood Index (HBI) which expresses the association between the vector and the human hosts. The human blood index is defined as the proportion of freshly fed *Anopheles* giving a positive reaction for human blood. Garrett-Jones *et al.* (1980) suggested using a more precise definition such as: 'The proportion of feeds taken on man by the members of a specific and specified mosquito population, expressing the degree of mosquito-man biting contact exhibited by that population'. The various European species of the *An. maculipennis* complex differ greatly in their proportion of blood meals taken on humans.

Transmission of malaria is related to the resting and feeding habits of the mosquitoes. The members of the *An. maculipennis* complex may feed in a house or stable, settle down in it and spend one or more days and nights for digestion of the meal and maturation of the ovaries. Female anophelines leaving shelters are not only those leaving for purposes of oviposition but turnover effects play an important role as well (Christophers and Missiroli, 1933). The resting and feeding habits can be classified according to the following characteristics:

- The preference of mosquitoes for resting in a man made structure or outdoors (endophily or exophily)
- The preference of mosquitoes for feeding in a man made structure or outdoors (endophagy or exophagy)
- The preference of mosquitoes for feeding on humans or on (domestic) animals (anthropophily or zoophily)

Transmission particularly occurs when there is a concentration of infected mosquitoes in a site visited by humans at night. The distribution of mosquitoes depends on the distribution of the host and the microclimate of the resting places. Members of the *An. maculipennis* complex can be found during the day in loca-

tions where a considerable proportion had a blood meal during the previous night (Boyd, 1949). Christophers and Missiroli (1933) and James (1919) pointed out that mosquitoes prefer open shelters characterised as 'humid, ill-lighted and ill-ventilated'. Shannon (1935) studied the microclimate preferences of mosquitoes of the *maculipennis* complex in diurnal shelters in Greece. Relative darkness was the most important requirement for resting anophelines. However, at a temperature of less than 23°C they tolerated a stronger light intensity than at higher temperatures. At excessive temperatures (above 35°C up to 40°C) all anophelines seek even darker and consequently cooler shelters. With this behaviour the adult mosquito avoids situations of high temperatures with low humidities. An increase in humidity can compensate for an increase in temperature but compensation for the highest temperatures is impossible because the saturated vapour pressure increases non-linearly in relation to temperature.

Detinova *et al.* (1963) reported the daytime population of *An. maculipennis* to be distributed between natural shelters and indoor resting-places. Reported outdoor resting sites of members of the *An. maculipennis* complex include hollows and cavities under earth banks and bridges, caves, rock cavities, hollow trees and shaded stacks of bee-hives (Postiglione *et al.*, 1973).

The availability of humans and animals influences the feeding preference (Garrett-Jones *et al.*, 1980; Hackett, 1931). In Denmark the incidence of malaria cases decreased after the 1860s due to a change in live-stock breeding. At that time, animals which spent most of their time outdoors were moved to stables and pigsties near human settlements. Stables and pigsties near human settlements were a much more attractive place to stay than houses for the more zoophilic mosquitoes (Wesenberg-Lund, 1921). The opposite effect, when live-stock is removed, is well known. If animals are scarce more specimens of a zoophilic species will feed on humans (Bruce-Chwatt and Zulueta, 1980).

The feeding preferences of *An. maculipennis* s.l. and *An. superpictus* are listed in Table 18. Using the Human Blood Index the whole *maculipennis* complex can be considered as moderate malaria vectors. As a rule one can say that *An. sacharovi* bites humans readily; *An. atroparvus* and *An. labranchiae* less readily; *An. beklemishevi*, *An. maculipennis* s.s., *An. melanoon* (including *An. subalpinus*), *An. messeae* and *An. superpictus* reluctantly (Hackett, 1937; Weyer, 1939). Characteristics of the various species of the *An. maculipennis* complex and *An. superpictus* are given below.

An. atroparvus

This species was reported a malaria vector where it occurred. When a known population of this species in the Netherlands was permitted free choice between humans and pigs in an enclosure, the ratio of attraction was 1:12.3 in favour of the pig (Van Thiel, 1939).

Table 18: Feeding preference of *An. maculipennis* s.l. and *An. superpictus*.

place	resting place	number	HBI	references
An. atroparvus				
Spain	–	5349	6.1	Pittaluga, 1932
Spain	houses	–	40.0	Olivaria and Hill, 1935
	stables	–	7.0	Olivaria and Hill, 1935
Netherlands	houses	–	84.0	Swellengrebel and De Buck, 1938
Netherlands	houses	–	20–60[1]	Swellengrebel and De Buck, 1938
Spain	houses	56	25	Garrett-Jones *et al.*, 1980
	outside houses	203	3.0	Garrett-Jones *et al.*, 1980
	houses	50	24	Garrett-Jones *et al.*, 1980
An. labranchiae				
Sicily	domestic	436	9.9	Cefalù *et al.*, 1961
Sicily	natural	54	3.7	Cefalù *et al.*, 1961
Algeria	outside houses	142	1.4	Garrett-Jones *et al.*, 1980
Morocco	houses	64	34	Garrett-Jones *et al.*, 1980
Morocco	houses	210	34.3	Garrett-Jones *et al.*, 1980
Morocco	outside houses	206	11.7	Garrett-Jones *et al.*, 1980
Tunisia	houses	110	64.5	Garrett-Jones *et al.*, 1980
Tunisia	outside houses	219	15.1	Garrett-Jones *et al.*, 1980
An. maculipennis s.s.				
Greece	houses	1798	21.2	Barber and Rice, 1935[b]
	stables	4607	0.5	(+ *An. messeae*)
An. messeae				
Rumania	–	–	60.0	Weyer, 1934
Yugoslavia	houses	–	45.6	Kostich, 1936
	stables	–	15.4	Kostich, 1936
Greece	houses	1798	21.2	Barber and Rice, 1935[b]
	stables	4607	0.5	(+ *An. maculipennis* s.s.)
Netherlands	houses	–	63.0	Swellengrebel and De Buck, 1938

The species seeks by preference man made shelters and feeds under a cover such as a roof (Cambournac and Hill, 1938; Hill, 1937). In the Netherlands 36% of marked *An. atroparvus* remained in a stable for 2 days, 3% for 5 days and 0.5% as long as ten days (Swellengrebel and De Buck, 1938). Artemiev (1980) described the species as endophilic, feeding both indoors and outdoors. The species prefers cattle but readily feeds on humans. The feeding preference depends on the availability of cattle (Cambournac and Hill, 1938). High temperatures and low relative humidities stimulate *An. atroparvus* to bite humans more readily (Cambournac, 1978).

An. beklemishevi

Since *An. beklemishevi* is considered the eastern race of *An. messeae*, many characteristics of this species are similar to those of *An. messeae*. *An. beklemishevi* was not considered an important vector of malaria (Jaenson *et al.*, 1986).

Table 18: (continued).

place	resting place	number	HBI	references
An. sacharovi				
Former Asian Soviet Union	–	–	16–20	Khodukian and Shtrengol'd cited by Chinayev, 1965
Cyprus	houses	133	88.6	Barber, 1936
	stables	17	35.3	Barber, 1936
Greece	houses	3980	61.3	Barber and Rice, 1935[b]
	stables	2855	7.5	Barber and Rice, 1935[b]
Greece[2]	houses	304	61.5	Hadjinicolaou and Betzios, 1973
	stables	236	1.3	Hadjinicolaou and Betzios, 1973
	artificial pit	175	5.1	Hadjinicolaou and Betzios, 1973
Greece	houses	260	38.5	Hadjinicolaou and Betzios, 1973
	stables	709	0.7	Hadjinicolaou and Betzios, 1973
	artificial pit	55	1.8	Hadjinicolaou and Betzios, 1973
Greece	houses	1248	61.1	Garrett-Jones *et al.*, 1980
	houses	537	0.9	Garrett-Jones *et al.*, 1980
Italy	outside houses	127	2.4	Garrett-Jones *et al.*, 1980
Palestine	stables	543	2.8	Kligler *et al.*, 1932
Palestine	houses	133	37.6	Kligler *et al.*, 1932
Palestine	stables	1176	6.9	Kligler *et al.*, 1932
Palestine	houses	1722	28.7	Kligler *et al.*, 1932
An. superpictus				
former Asian Soviet Union	–	–	30–31	Khodukin and Shterngol'd cited by Chinayev, 1965
Greece	house	111	29.7	Barber and rice, 1935
	stable	1611	1.6	Barber and rice, 1935

[1] varying during the summer months
[2] The man-animal ratio in the village was 1:7.2 at the peak of the summer season.

An. labranchiae

Outbreaks of malaria in Italy were clearly associated with the occurrence of *An. labranchiae*. The high percentage of non-human blood as reported by Cefalu *et al.* (1961) probably underestimated the feeding preference for humans because cattle were the most abundant hosts. According to Hackett and Missiroli (1935) this species persistently tries to enter bedrooms, even in the presence of an abundance of animals. When a known population of this species in Italy was permitted the free choice between humans and pigs in an enclosure, the ratio of attraction was 2.2:1 in favour of humans (Van Thiel, 1939).

Collignon (1959) and Guy (1963) described *An. labranchiae* as endophilic in Morocco and Algeria as it is found abundantly in human habitations and animal shelters in the morning. In Sardinia, man-made shelters, especially pigsties and stables were important shelters for *An. labranchiae* females. Shelters for males included mainly grottos and bridges (Logan, 1953). Stables constitute a main resting shelter for this species as they contain dark humid corners protected from air cur-

rents. Escalar (1933) observed a decline in the number of adults retrieved from dwellings when brick pigsties increased in number. The species is not completely endophilic and Cefalù *et al.* (1961) reported facultative exophily for a population on Sicily. The females are indifferent with regard to the selection of a certain type of shelter. The choice of a shelter depends on external factors such as the availability and proximity of one type of shelter or another. Collignon (1959) reported a considerable turnover at night eventually leading to exophily. Metge (1991) reported outdoor shelters in tree cavities.

An. maculipennis s.s.

An. maculipennis s.s. was never considered very important in malaria transmission. The species has little contact with humans (Hackett, 1949; Weyer, 1939). Nevertheless, it was the common vector in Bosnia Herzegovina and a reported vector in Rumania, Greece, Austria, Hungary and Czechoslovakia (Bruce-Chwatt and Zulueta, 1980). The species is reported to be responsible for malaria transmission when there is a shortage of domestic animals (Barber and Rice, 1935[b]).

Barber and Rice (1935[b]) reported a ratio of the average number of mosquitoes caught in a house to that in a stable of 1:6.5 (east Macedonia; *An. maculipennis* s.s. and *An. messeae*). Artemiev (1980) described the species as endophilic and resting in stables and dwellings. It feeds on humans both outdoors and indoors. The proportion of females with human bloodmeals depends on the number of cattle on a given farm. Postiglione *et al.* (1973) reported outdoor resting sites including hollows and cavities under earth banks and bridges, caves, rock cavities, hollow trees and shaded stacks of bee-hives.

An. melanoon

An. melanoon was never considered important in malaria transmission (Hackett, 1949; Weyer, 1939). Barber and Rice (1935[b]) recorded this species to be 4 to 8 times as abundant in occupied stables as in dwellings in Greece. Artemiev (1980) described the species as exophilic or semi-exophilic. It feeds on humans both indoors and outdoors but prefers cattle. Postiglione *et al.* (1973) reported outdoor resting sites in Turkey for *An. melanoon* (*subalpinus*) including hollows and cavities under earth banks and bridges, caves, rock cavities, hollow trees and shaded stacks of bee-hives.

An. messeae

An. messeae is so powerfully attracted to animals that its contact with humans is largely suppressed in an agricultural area with much livestock. Therefore, *An. messeae* was only responsible for outbreaks of malaria when its densities were very high and there was a shortage of domestic animals (Barber and Rice, 1935[b]; Bruce-Chwatt and Zulueta, 1980, Hackett and Moshkovski, 1949). Nevertheless, it was reported as the most common vector in Rumania, Poland and Serbia. It was a reported

vector in Austria, Hungary, Czechoslovakia, Germany, Sweden, Finland and the former Soviet Union (Bruce-Chwatt and Zulueta, 1980; Jaenson *et al.*, 1986).

Artemiev (1980) described the species as endophilic as it was found in stables, barns and cellars as well as in human dwellings. Barber and Rice (1935[b]) reported a ratio of the average number of mosquitoes caught in a house to that in a stable of 1:6.5 (east Macedonia; *An. maculipennis* s.s. and *An. messeae*).

An. sacharovi

An. sacharovi is potentially the most dangerous vector species of the *An. maculipennis* complex. Barber *et al.* (1936) observed a density dependent malaria transmission on the plain of Philippi and Chrysoupolis in Greece. Artemiev (1980) reported the species to feed on humans but also on cattle when available. According to Hackett and Missiroli (1935) this species persistently enters bedrooms, even in the presence of an abundance of animal blood. Van Thiel (1939) observed that females in cages in Italy fed on humans in preference to pigs when given freedom of choice (ratio 2.4:1).

The occurrence of the species in dwellings and stables depends on the position of the houses in relation to breeding sites, the conditions of the dwelling and the habits of the inhabitants (cattle stabled or kept outdoors during the night, people sleeping in- or outdoors) (Kligler and Mer, 1932). Barber and Rice (1935[b]) reported a ratio of the average number of mosquitoes caught in a house to that in a stable of 1:3.4 (east Macedonia). *An. sacharovi* migrates frequently and often leaves its shelter during maturation of the ova (Kligler and Mer, 1932). Hajinicoloaou and Betzios (1972) reported *An. sacharovi* to bite humans readily indoors as well as outdoors. Gökberk (1961) reported that this species rests in caves and causes outdoor transmission. Postiglione *et al.* (1973) reported outdoor resting sites including hollows and cavities under earth banks and bridges, caves, rock cavities, hollow trees and shaded stacks of bee-hives. Kligler and Mer (1932) reported *An. sacharovi* feeding outdoors in Palestine. However, it is disturbed by the slightest breeze.

An. superpictus

This species is zoophilic but will feed on humans wherever they are encountered, in open country or in villages (Postiglione *et al.*, 1973). *An. superpictus* was a vector of some significance in south-eastern Europe during a long warm season when its densities were high, especially when a region did not have much cattle (Barber and Rice, 1935b; Postiglione *et al.*, 1973). This species is typically a late summer mosquito. Its densities increase throughout the summer and usually reach a peak in August or September (Barber and Rice, 1935[b]). There have been reports that a female has to take several blood meals to complete the development of eggs (Tshinaev, 1963, citing Kanchaveli).

The adults can be found in stables as well as dwellings (Barber and Rice, 1935[a]; Postiglione *et al.*, 1973; Weyer, 1939). *An. superpictus* is more disposed to rest in stables than *An. sacharovi*. Barber and Rice (1935[b]) reported a ratio of the average

number of mosquitoes caught in a house to that in a stable of 1:20 (east Macedonia). It is a partially exophilic mosquito. Postiglione *et al.* (1973) reported *An. superpictus* in outdoor shelters such as hollows and crevices under banks or overhanging cliffs, rock cavities, caves and nest holes.

Dispersal

The dispersal of adult anophelines is related to feeding, resting, oviposition and diapause. Direction, distance and density of flight depend on the species, season, meteorology and the availability of sites to feed, rest, oviposit or diapause (Horsfall, 1955). The movements can roughly be classified in two groups (Boyd, 1949):

- Active movement
  Local movement and mass migration are distinguished. Local movement is exhibited by summer generations in the proximity of a breeding place. Mass migration is observed when diapause starts. Pre-diapause movements involve greater distances than is the case with feeding and oviposition.
- Passive movement
  The insect is transported by means which it cannot control such as horizontal or vertical air movements, cars, ships and aeroplanes. This movement only involves a limited number of insects but can be an important way of extending the geographic range of an anthropophilic species.

Pre-feeding flight ranges of *An. atroparvus* up to 14 km have been reported (Swellengrebel and Nykamp, 1934). Swellengrebel (1929) concluded that an invasion of Medemblik (the Netherlands) by *An. atroparvus* was caused by mosquitoes of both sexes being imported downwind. Cambournac and Hill (1938) reported an effective flight range of a female *An. atroparvus* of at least 3 km. Swellengrebel and De Buck (1938) reported flight ranges of 3-5 km.

Concerning dispersal of *An. labranchiae*, Metge (1991) reported females up to 400 m from a pool and Sevenet and Andarelli (1956) a flight range of 2-5 km. Under favourable circumstances flights of 6.5 km have been observed (Missiroli, 1927).

Kligler and Mer (1930) observed flight ranges of *An. sacharovi* before diapause up to 14 km from the nearest breeding site. The numbers observed varied inversely with the distance. During the transmission season, flight ranges of 4.5 km have been observed (Kligler, 1924). Saliternik (1957) reported marked mosquitoes at 3.5 km from a release point. If the breeding places are far from a blood source, the dispersal flight can exceed 3.5 km. Soliman (1961) in Syria concluded that although *An. sacharovi* can disperse up to 7 km, its highest concentration is found within 1 km from the breeding site.

Tshinaev (1963) and Weyer (1939) reported flight ranges of *An. superpictus* over 6 km.

Chapter 5

RISK OF REINTRODUCTION OF MALARIA IN EUROPE

An increase in the prevalence and the severity of malaria in Europe probably occurred over a period from Hellenistic to early Roman Imperial times. During this period demographic and economical expansion was associated with increased travel and trade. Before these times there was only a mild prevalence of malaria in Europe due to infections by *P. malariae* and *P. v ivax*. These parasites were transmitted almost exclusively by *An. atroparvus* and *An. messeae*. The current refractoriness of *An. atroparvus* to tropical strains of *P. falciparum* probably acted in the past as a barrier preventing the penetration of *P. falciparum* into Europe. Only after a process of selection was *An. atroparvus* able to transmit tropical strains of *P. falciparum* in southern Europe. This parasite was transported to Europe by traders, slaves and soldiers. Furthermore, the population increase resulted in greater agricultural activities which caused deforestation and soil erosion. Increasing trade and navigation, irrigation, deforestation and coastal alluviation favoured the establishment of the North African species *An. labranchiae* and the Asian species *An. sacharovi* in southern Europe. The presence of *An. sacharovi* and *An. labranchiae* favoured the transmission of malaria since these two species are more effective vectors of malaria than *An. atroparvus* (Bruce-Chwatt and Zulueta, 1980; Zulueta, 1973[b]; Zulueta *et al.*, 1975).

Eradication campaigns succeeded in the eradication of malaria from continental Europe although they failed in their objective to eradicate anophelines from an area by spraying with insecticides, even in such isolated areas a islands. In 1949 *An. superpictus* was no longer observed on Cyprus. However, larvae of *An. superpictus* have been found there every year since 1950. The disappearance of *An. sacharovi* from Cyprus is a fact. The increased sea- and airborne links with other parts of the Mediterranean, however, may result in the reappearance of *An. sacharovi* on the island (Aziz, 1947; Bruce-Chwatt and Zulueta, 1980). During the eradication campaign on Sardinia in 1947 the numbers of *An. labranchiae* decreased considerably and the species was replaced by various other members of the genus *Anopheles*. In the Geremeas area (south-east side of the island) *An. labranchiae* was replaced by *An. hispaniola*. Collections from this area in 1980, however, have shown that numbers of *An. labranchiae* are now increasing again while *An. hispaniola* disappeared from *An. labranchiae* habitats (Trapido and Aitken, 1953; Aitken *et al.*, 1954; Lecis *et al.*, 1980).

High densities of *An. sacharovi*, *An. labranchiae* and *An. atroparvus* which would probably allow transmission have been reported from areas of Greece, Italy, Spain, and Portugal (Bruce-Chwatt and Zulueta, 1980; Hadjinicolaou and Betzios, 1973; Zulueta *et al.*, 1975). In two areas of central Italy where *An. labranchiae* had

practically disappeared this species has reappeared due to large scale rice cultivation (Maremma in North Latium and Grosseto in Tuscany) (Bruce-Chwatt and Zulueta, 1980; Zahar, 1990). Today *An. superpictus* is found in southern Italy near Calabria where its densities may also permit malaria transmission (Bruce-Chwatt and Zulueta, 1980).

Since vector densities still allow transmission in various regions of southern Europe, there is a chance of reintroduction of malaria from malaria endemic areas into Europe. The increase of international air travel (e.g. tourists, business, foreign students, immigrants) has resulted in an increase in the number of imported malaria cases in Europe from 1383 cases in 1971 to 6883 in 1986. Most of the imported malaria cases in Great Britain during the period 1977-1986 were due to *P. vivax* infection. The prevalence of *P. falciparum* cases increased from 17% in 1977 to 32% in 1986. Because under-reporting of malaria cases occurs, the real number of imported malaria may be much higher. Recent reports show that the number of under-reported cases can be as large as the number of reported cases (J.L.M. Lelyveld, personal communication). Fig. 16 presents the number of registered imported cases in Europe from 1971 till 1986 (Antunes *et al.*, 1987; Bruce-Chwatt *et al.*, 1974; Bruce-Chwatt and Zulueta, 1980; Cambournac, 1978; Davidson *et al.*, 1993; Gentilini *et al.*, 1981; Majori *et al.*, 1990; Phillips-Howard *et al.*, 1988; Steffen *et al.*, 1990; Zahar, 1990; Zulueta, 1973[a]).

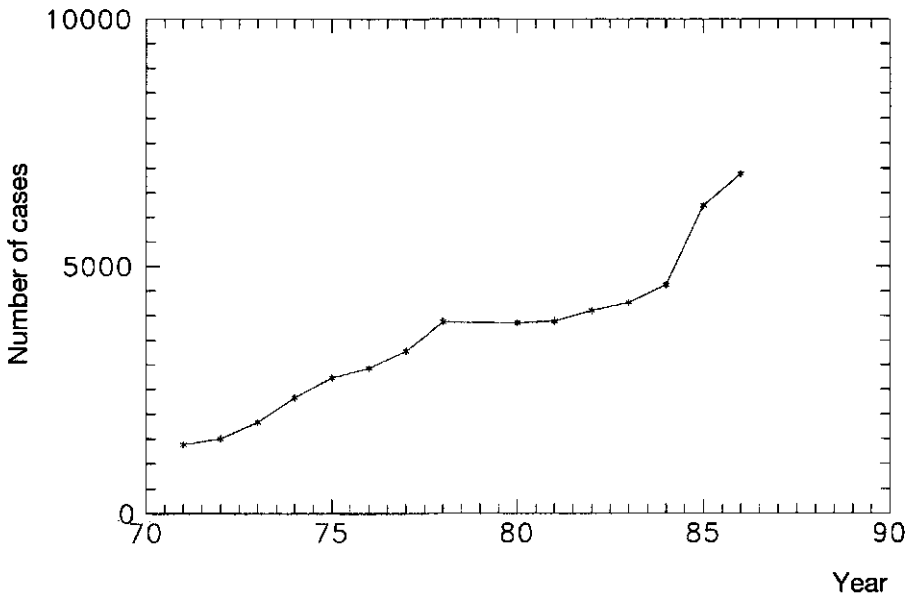

Figure 16: The number of registered imported malaria cases in Europe from 1971 till 1986 (after Bruce-Chwatt and Zulueta, 1980, and Zahar, 1990).

In this context the resurgence of *P. vivax* malaria in the Asian part of Turkey in the 1970's and 1980's needs to be mentioned as well. In 1976 and 1977 the number of cases recorded was 37.000 and 115.000, respectively. The number of cases decreased to 29.000 in 1979 and increased again to 67.000 cases in 1983. Thereafter, the number of cases decreased (Zahar, 1990). *An. sacharovi* acted as the most important vector (Ramsdale and Haas, 1978). Of particular interest is the reappearance of *An. sacharovi* in northern Israel. Densities increased up to 1983. In that year more than 10% of the larvae reported were *An. sacharovi*. At the same time the species extended its distribution. This species was previously the main vector in this area and indicates the risk of reestablishment of malaria in Israel due to accidental introduction (Pener and Kitron, 1985[a]).

The lack of co-adaptation between vector and parasites might give some protection against imported parasites. However, local transmission of imported malaria is possible as various examples in the past have shown. After World War II (1945-1947) more than 3500 introduced cases were reported in Germany (Bruce-Chwatt and Zulueta, 1980). At least 300 introduced cases were reported in 1914-1918 in France (Coutelen *et al.*, 1953). In England 330 locally contracted cases were reported due to local transmission in 1917-1918 (James, 1920). Between 1981 and 1989, 7683 cases with *P. vivax* were imported to the former USSR from Afghanistan by demobilized military personnel. There were 36 reported cases of introduced malaria (Sergiev *et al.*, 1992).

Laboratory experiments have shown that in Europe *An. atroparvus*, *An. messeae*, *An. sacharovi* and *An. superpictus* may be susceptible to *P. vivax* from Africa and Asia. However, there is no recent evidence for large scale reintroduction of malaria in Europe. All the reported autochthonous cases in Europe between 1977–1986 have been classified as congenital, transfusion or airport malaria (Phillips-Howard *et al.*, 1988). Rodhain and Charmot (1982) assume that only a mass introduction of *P. vivax* could eventually trigger a new endemic situation in France, provided the presence of a sufficiently dense vector population. Furthermore, exotic strains of *P. falciparum* malaria parasites are not easily transmitted by the European anophelines. Because control measures in Europe have proven to be effective it is clear that the chances for large-scale reintroduction of malaria in Europe should not be exaggerated. Nevertheless, Bruce-Chwatt (1971) and Zulueta (1973[a]) pointed out that any deterioration of organized services by a major catastrophe or war and movement of populations including migrant labour may bring back to Europe a series of communicable diseases of which malaria is just one. The recent war in Yugoslavia is of particular interest since war situations in the past created ideal circumstances for malaria epidemics. Because conditions for a renewal of transmission in several countries of the Mediterranean still exist, the occurrence of foci of introduced malaria from particularly *P.vivax* in some regions should not be under-estimated.

Of particular interest is the relationship between malaria and climate. Malaria is closely related to environmental factors. Changes in rainfall and temperature lead

to changes in vector distribution, density and longevity. Higher temperatures accelerate the development of the parasite in the mosquito as well as the development of the mosquito vector and cause changes of larval and adult mortality. As a possible factor in the apparent increase of epidemic potential in the last few years in the highland areas of Africa the so-called greenhouse effect is mentioned. This greenhouse effect is due to increasing concentrations of carbon dioxide, methane, nitrous oxide and CFCs in the atmosphere of the earth. The reports of the Intergovernmental Panel on Climate Change (1990, 1992) predict under the 'business-as-usual' emissions scenario an average increase during the next century of approximately 0.3°C per decade. In 2030 the forecasted mean temperature change from pre-industrial times in southern Europe is about +2°C in winter and varies from +2 to +3°C in summer (business-as-usual scenario). A mean temperature increase of even 0.5°C can increase the potential transmission period in marginal areas. Thus, a non malarious area may change into one subject to seasonal epidemics (Nájera *et al.*, 1992). There is a serious possibility that rises in temperature and rainfall would allow malaria to survive in areas immediately surrounding the current distribution limits (Parry and Carter, 1988; WHO, 1990).

A temperature increase in Europe might result in a more northern distribution limit of *An. labranchiae* and *An. sacharovi*, which are the most thermophilic species of the *An. maculipennis* complex. The species also show the highest association with humans within the *An. maculipennis* complex. A typical situation has been observed at the south-eastern coast of Spain between Alicante and Murcia where *An. labranchiae* established a bridgehead but did not penetrate inland. That particular part of the Spanish coast is characterized by low rainfall (200–300 mm annual precipitation) and high summer temperatures. Therefore conditions here are very similar to the ones encountered in northern Africa. However, recent studies showed a disappearance of *An. labranchiae* from this area due to changed agricultural practices and the application of residual insecticides (Blazquez and Zulueta, 1980).

Pearce (1992) suggests an increased malaria risk in Europe due to climate change, especially in southern Europe. We do not expect a return to a state of endemic malaria in Europe due to an increasing potential transmission intensity caused by climate changes. The socio-economic conditions in Europe, in particular the medical health system and current animal husbandry will prevent a reintroduction of endemic malaria. However, an increased risk of incidental *P. vivax* cases is expected, especially in those areas in south-eastern Europe where socio-economic conditions have deteriorated.

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