8

Malaria and dengue vector biology and control in West and Central Africa

Didier Fontenille[#] and Pierre Carnevale[#]

Abstract

In West and Central Africa endemic malaria and epidemic yellow fever are still main causes of morbidity and mortality. From Dakar in Senegal to Kinshasa in the Democratic Republic of Congo the pattern of malaria transmission shows a huge variability, in term of dynamics (rhythm and intensity) of transmission, as well as in terms of the vector species involved. The *Plasmodium* annual entomological inoculation rates (EIR) vary from less than one to more than 1000 infectious bites per person and *P. falciparum* represents more than 90% of malaria infections. In most settings south of the Sahara, several vector species are sympatrically involved in malaria transmission either simultaneously or replacing each other seasonally (Coluzzi 1984; Fontenille and Simard 2004). These vectors differ greatly in terms of density and vector efficiency.

Despite an efficient vaccine, deadly outbreaks of Yellow Fever (YF) virus, which circulates among monkeys and sylvatic vectors, still occur occasionally from Cameroon to Senegal (Mutebi and Barrett 2002). Although *Aedes aegypti*, the local vector, is abundant, and Dengue 2 virus is present in the forest, human dengue remains very rare and localized in Western Africa. But the situation could worsen with the recent introduction and spreading of *Ae. albopictus*, a potential dengue vector, in Central Africa.

Any vector control strategy, whether based on traditional (insecticides and impregnated/treated nets) or genetic control strategies (sterile-male releases or introduction of transgenic mosquitoes), aiming at significantly reducing malaria burden or yellow fever/dengue occurrence in Africa, will have to account for such entomological heterogeneity added to ecological and socio-economic diversities.

This paper provides an update on the bionomics and genetics of the four major African malaria vector systems (the *Anopheles gambiae* complex and the *An. funestus*, *An. nili* and *An. moucheti* species groups) and of the *Ae. aegypti* and *Ae. albopictus* species. It also reviews current vector control measures against malaria and yellow-fever vectors.

Key words: vectors; malaria; yellow fever; dengue; Africa

The vectors

The biology of the main African malaria vectors has been known, in general terms, for more than 50 years. The description and identification of vector species were

[#] LIN, IRD, BP 64501, 911, Avenue Agropolis, 34394 Montpellier cedex 5, France. E-mail: Didier.Fontenille@mpl.ird.fr; pjcarnevale2001@yahoo.fr

traditionally based upon morphological characteristics and sub-divisions (taxa) called sub-species, forms, varieties, races, etc. These have been described not only according to slight morphological differences, but also in terms of distribution, biology, ecology, behaviour, etc. In West and Central Africa 5 different species are considered major malaria vectors: *An. gambiae*, *An. arabiensis*, *An. funestus*, *An. nili* and *An. moucheti*. At least 4 or 5 other species are considered secondary or locally important vectors, such as *An. paludis*, *An. hancocki*, *An. melas*, etc. (Hamon and Mouchet 1961)

***Anopheles gambiae* complex**

Three species of the complex, among seven, are present in West and Central Africa: *An. gambiae sensu stricto*, *An. arabiensis* and *An. melas*. *An. gambiae* is usually predominant in humid environments while *An. arabiensis* is found in drier areas, but they coexist widely over much of their range of distribution. The salt-water species *An. melas* breeds in mangrove swamps along the west coast of Africa south till Namibia (Coetzee, Craig and Le Sueur 2000). Identification of species is based on fixed paracentric inversions or on the recently developed and very convenient PCR-based diagnostic tool detecting species-specific sequence differences in the ribosomal-DNA intergenic spacer (rDNA-IGS) region (Scott, Brogdon and Collins 1993).

Furthermore, extensive studies of karyotype distributions in natural *An. gambiae* populations often revealed strong and persistent deviations from Hardy-Weinberg equilibrium due to a deficit, or even complete absence, of certain heterokaryotypes. These results led to the designation, in West Africa, of five 'chromosomal forms' named under the non-Linnaean nomenclature Forest, Savanna, Mopti, Bamako and Bissau (Coluzzi, Petrarca and Di Deco 1985).

Recently, analysis of the rDNA-IGS region revealed fixed sequence differences between sympatric and synchronous chromosomal forms of Savanna/Bamako and Mopti populations in Mali and Burkina Faso, leading to the designation of two non-panmictic molecular forms named S and M. Both molecular forms are found throughout West and Central Africa (Favia et al. 2001; Della Torre et al. 2001). All Mopti specimens identified so far belong to the M molecular form; however, outside Mali and Burkina Faso, the M form may exhibit chromosomal arrangements typical of the Bissau, Forest or Savanna forms. The S molecular form may also carry standard chromosomes, indicative of the Forest form, or typical Savanna and Bamako karyotypes. While some very rare M/S hybrids have been found in Sierra Leone, Mali and Cameroon, evidence for reproductive isolation between molecular forms has accumulated to the point that incipient speciation is being suggested (Della Torre et al. 2002). For example in South Cameroon, a population-genetic study based on microsatellite DNA markers demonstrated significant genetic differentiation between sympatric M and S populations, both within the standard Forest chromosomal form of *An. gambiae* (Wondji, Simard and Fontenille 2002). The biological and vectorial significance of this genetic subdivision is currently under investigation.

Insecticide resistance has long been recorded in almost all West-African countries. Pyrethroid resistance due to *Kdr* mutation has recently been observed in S, and then also in M forms in every country in which this was investigated (i.e. Senegal, Sierra Leone, Burkina Faso, Mali, Côte d'Ivoire, Ghana, Benin, Cameroon, etc.). One *An. arabiensis* specimen from Burkina Faso was also found to carry the resistance allele (Diabate, pers. comm.). Other resistance mechanisms (resistant AChE, esterases, oxydases, Rdl, GST) have also been recorded in West- and Central-African populations of *An. gambiae* (Weill et al. 2003).

***Anopheles funestus* group**

An. funestus is widespread throughout sub-Saharan West Africa. Since the 1930s this group is known as being composed of several species closely resembling each other that can only be differentiated by very small morphological characters at larval or adult stages (Gillies and De Meillon 1968), or by a recently developed PCR assay (Koekemoer et al. 2002; Coetzee and Fontenille 2004). *An. funestus*, *An. leesoni*, *An. rivulorum* and *An. brucei* have been recorded in West and Central Africa. Their biology and vectorial capacity are very different. With the exception of *An. funestus*, these species are mainly zoophilic and therefore not considered malaria vectors. In 2003, Cohuet et al. have described a new taxon c1osely related to *An. rivulorum*, based on biological, morphological and genetic characteristics. This taxon, provisionally called "*An. rivulorum*-like", is present at least in Burkina Faso and Cameroon, is clearly different from the South African *An. rivulorum*, and does not seem to play any role in malaria transmission.

An. funestus itself is highly polymorphic, both biologically and genetically, showing at least 11 paracentric chromosomal inversions on chromosomes 2 and 3. In populations of Burkina Faso, huge Hardy-Weinberg disequilibrium and linkage disequilibrium between inversions led Costantini et al. (1999) to describe two chromosomal forms called 'Kiribina' and 'Folonzo', based on the presence and association of paracentric inversions, and then to hypothesize incipient speciation within *An. funestus*. In Senegal, 3 chromosomal populations exhibiting different anthropophilic demeanours were recognized, sometime in sympatry (Dia, pers. comm.). In Cameroon a cline of inversion frequencies is observed from the humid forest in the South (with 'Folonzo'-like inverted populations) to the dry savannas in the North (with 'Kiribina' standard populations), with strong heterozygote deficiency in areas where both forms occur. All these data suggest restricted gene flow between chromosomal forms of *An. funestus*. However, on the other hand, several observations from Cameroon (and East Africa) did not detect any evidence for reproductive isolation between 'Folonzo' and 'Kiribina', with heterokaryotypes observed at the expected frequencies within populations. Recent use of microsatellite markers in Senegal and Cameroon showed that gene flow is permitted between chromosomal forms, and showed isolation due to geographic distance between populations. These results strongly suggest that heterozygote deficits at chromosomal loci are mostly locus-specific and reflect environmental selection on the inversions themselves (or the genes they contain) (Cohuet et al. 2005). No pyrethroid resistance has yet been observed in West-African populations of *An. funestus*, in contrast to findings in Mozambique and South Africa, which seriously complicates vector control (see chapter 9).

***Anopheles nili* and *An. moucheti* groups**

Anopheles nili has a wide geographic distribution, spreading across most of West and Central Africa. Larvae of *An. nili* are typically found in vegetation or in dense shade along the edges of streams and large rivers. Extensive morphological, ecological and ethological variations among *An. nili* populations have been reported demonstrating that *An. nili* actually represents a group consisting of at least 4 species: *An. nili s.s.*, *An. somalicus*, and *An. carnevalei* and the recently described new malaria vector *An. ovengensis* (Awono-Ambene et al. 2004). Based on fixed nucleotide differences between ITS2 haplotypes, primers were designed to develop an allele-specific PCR assay for rapid identification of species within the *An. nili* group (Kengne et al. 2003).

An. moucheti is a group of very efficient forest vectors, whose larvae breed in slow-running streams and large rivers of equatorial Africa. Morphological and behavioural variations suggest that at least three taxa may belong to the *An. moucheti* group: *An. moucheti moucheti*, *An. moucheti nigeriensis* and *An. bervoetsi*. However, comparison of DNA sequences of specimens from several populations and countries strongly suggests that possibly only two truly different species, both vectors, exist (Kengne et al., unpublished results). All the populations tested were sensitive to insecticides.

Aedes aegypti* and *Ae. albopictus

Aedes aegypti, the domestic vector of the YF virus, is present in every West-African country, all specimens belonging to the black *formosus* form in both sylvatic and domestic populations. In spite of the fact that West-African populations of *Ae. aegypti formosus* are experimentally able to transmit dengue-2 virus, very few occurrences of dengue have been observed (Burkina Faso, Senegal) (Failloux, Vazeille and Rodhain 2002).

Recently *Aedes albopictus*, an Asian potential vector of dengue, has been discovered in some West-African countries: Nigeria, Cameroon and Equatorial Guinea. In the south of Cameroon this invasive species tends to replace *Ae. aegypti* in many locations (Fontenille and Toto 2001; Toto et al. 2003) and its spreading is a matter of concern.

Vector control

More than 120 years after the discovery of *Plasmodium* by Laveran, malaria remains one of the major public-health problems in Africa south of the Sahara.

From 1955 to 1968 the goal was to achieve global eradication of malaria through Indoor Residual Spraying (IRS) of every house with residual insecticides (DDT, then DLN, HCH, various organophosphates). This programme did not involve Africa south of the Sahara, which remained in the 'pre-eradication stage'. Due to different constraints (lack of funds, technical and operational issues, etc.) this programme was abandoned in 1969 and transformed to 'malaria control' with 4 technical variants dealing primarily with diagnosis and treatment. The 1992 WHO Global Strategy recommended not only case management but also selective and sustainable vector control for malaria prevention. Two main methods are available for such vector control: insecticide-impregnated mosquito bednets (ITNs) and other materials, and IRS, which is still effective and widely used in several countries, mainly in Southern Africa, (Mabaso, Sharp and Lengeler 2004), Burundi, etc. This approach was able to stop malaria epidemics such as the 1987 deadly outbreak in Madagascar and in KwaZulu Natal.

During the African Summit on Roll Back Malaria held in Abuja (25 April 2000) it was agreed to initiate appropriate and sustainable action to strengthen the health systems. Among other things, a decision was reached to ensure that by the year 2005 at least 60% of those at risk of malaria, particularly children under five years of age and pregnant women, will be able to benefit from the most suitable combination of personal and community protective measures such as ITNs and other accessible and affordable interventions to prevent infection and suffering. For a variety of reasons, these goals have not been met (see below).

In West-African countries *Anopheles* control is now mainly based on the large-scale use of ITNs and other impregnated materials supplied by national vector control

programmes or NGOs and private initiatives. Experimental surveys have confirmed efficacy of these methods in terms of reduction of incidence of malarial disease (Carnevale et al. 1991; Lengeler 1998), and overall infant mortality in Ghana (Binka et al. 1996), Kenya (Nevill et al. 1996), Burkina Faso (Habluetzel et al. 1997) and The Gambia (D'Alessandro et al. 1995). Moreover, recent trials showed a mass effect of permethrin-impregnated nets in Ghana (Binka, Indome and Smith 1998), in Kenya (Howard et al. 2000) and with impregnated curtains in Burkina Faso (Diallo et al. 2004) with no rebound mortality even after several years of ITN usage (Binka et al. 2002; Hawley et al. 2003; Maxwell et al. 2003). It was noticed that when some coverage of the population (60 to 80%) was maintained, even people not covered by ITNs can be protected from malaria if they are living inside 'treated' compounds or in their vicinity (less than 300m). These observations open a new field of research and offer hope in terms of public-health outcome of this type of intervention.

These positive results concerning efficacy and effectiveness led to the recommendation and promotion of ITNs for malaria control. Unfortunately, according to the recently (2003) published WHO Africa malaria report, the proportion of children under 5 years sleeping under nets is low – about 15% across 28 countries surveyed. Even fewer children (less than 2%) sleep under ITNs. Only two countries, The Gambia and Sao Tomé and Principe, reported user rates of more than 10% even if the availability of nets has increased noticeably over the last 10 years. However, more and more countries are engaged within the Abuja initiative, and recent unpublished information from different West-African countries (such as Burkina Faso, Ghana and Mali) suggest that coverage rates are increasing. In North Cameroon it was also noticed that mobile teams treating the nets of users directly inside villages were well received and dramatically increased the percentage of ITNs and therefore their actual efficacy (Manga et al., pers. comm.). There are several well-known approaches to increase the affordability of ITNs for people and therefore scaling-up the coverage, and hence the efficiency of ITNs: social marketing, highly subsidized prices, tax-free, as a gift to pregnant women during antenatal clinic visit, given free of charge by companies to their employees and families, 'do-it-yourself kits', 'centre for impregnating mosquito nets', mobile teams doing re-treatment free of charge, ITNs given free of charge during EPI vaccination campaigns, etc. Every method has its advantages and limitations, the crucial point being that it needs to be adapted, tailored, suited to the targeted population in terms of price, size, shape, colour of the nets and cultural behaviour of the population

The main drawbacks in the large-scale use of ITNs are human behaviour (resistance to use), as well as the cost of nets, the need for their regular re-impregnation and their widespread availability and distribution. Recently, prices have decreased; promotion, delivery and affordability have improved with social marketing programmes. Nets are now produced in African countries and are more adapted to human needs in terms of quality, size, shape, colour, opacity, etc. It has been proposed to provide them free of charge (Curtis et al. 2003) for example during the EPI vaccination programme, or as a 'kit for pregnant women' (Guyatt et al. 2002) or a gift for birth through local health systems or NGOs. A technical solution to the re-treatment issue was recently found with the development of 'long-lasting nets' (LLNs) (Guillet et al. 2001), wash-resistant nets such as the Olyset Net® (with permethrin incorporated into the polyethylene fibre) or Permanet2® (with deltamethrin stuck onto the polyester fibre), which sustains their efficacy even after several years of use in the field (N'Guessan et al. 2001).

Another potential drawback in the efficacy of ITNs is pyrethroid resistance of several *An. gambiae* populations recently noticed in several West-African countries (Chandre et al. 1999), attributed to large-scale use of insecticides for agricultural purposes. Resistance to carbamates, organochlorines and organophosphates have also been recorded for a long time in *An. gambiae* populations from several countries of West and Central Africa. Trials of ITNs in experimental huts against pyrethroid-resistant *An. gambiae* showed that they still confer protection to users through a reduction of entry rates, an increase of exit rates, a decrease of man–vector contact and an increase of the mortality rate of resistant specimens (Darriet et al. 2000). Moreover the large-scale use of lambdacyhalothrin-treated nets in the Korhogo area (northern Ivory Coast) where *Kdr* allelic frequencies are > 0.90 among *An. gambiae* populations, induced not only a sharp reduction of entomological parameters (inoculation rate, vectorial capacity, etc.) but also a ~50% reduction of incidence rate of malaria morbidity among children of less than 5 years of age (Carnevale et al. 2001). On the other hand, combination of different classes of insecticides tested in experimental huts in Ivory Coast showed that they might be a potential tool for resistance management. The mixture might also have an advantage in terms of lower cost and toxicity (Hougard et al. 2003). Insecticide resistance of *Culex quinquefasciatus* is also a major drawback for the use of ITNs because 'mosquito control' at household level is mainly directed against nuisance and, therefore, protection conferred by nets must be as comprehensive as possible to gain actual use and participation of the community. The positive 'collateral effect' of ITN (against lice, bugs, ticks, non-vector mosquitoes, etc.) has actually been noticed several times and put forward in their regular use. Mixtures of insecticides could be a solution in such circumstances.

For the time being there is no control of *Aedes aegypti* in West Africa. The management of domestic larval breeding sites was obligatory in most of the West-African countries in the 1960s, but it was gradually stopped and larval indices (Breteau, container, house indices) slowly increased. Currently, control is rarely carried out, by emptying or treating domestic breeding sites, or by ULV pulverization of insecticides only during epidemic episodes.

References

Awono-Ambene, H.P., Kengne, P., Simard, F., et al., 2004. Description and bionomics of *Anopheles* (*Cellia*) *ovengensis* (Diptera: Culicidae), a new malaria vector species of the *Anopheles nili* group from south Cameroon. *Journal of Medical Entomology,* 41 (4), 561-568.

Binka, F.N., Hodgson, A., Adjuik, M., et al., 2002. Mortality in a seven-and-a-half-year follow-up of a trial of insecticide-treated mosquito nets in Ghana. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 96 (6), 597-599.

Binka, F.N., Indome, F. and Smith, T., 1998. Impact of spatial distribution of permethrin-impregnated bed nets on child mortality in rural northern Ghana. *American Journal of Tropical Medicine and Hygiene,* 59 (1), 80-85.

Binka, F.N., Kubaje, A., Adjuik, M., et al., 1996. Impact of permethrin impregnated bednets on child mortality in Kassena-Nankana district, Ghana: a randomized controlled trial. *Tropical Medicine and International Health,* 1 (2), 147-154.

Carnevale, P., Dossou-Yovo, J., Henry, M.C., et al., 2001. Disease control trial with lambdacyhalothrin treated nets against malaria transmitted by pyrethroid resistant *An.gambiae* at Korhogo, Ivory Coast. *In: Vector control workshop novel insecticides for insecticides treated nets: new tools and methods for targeted and selective vector control*. Gates Malaria Programme, London School of Hygiene and Tropical Medicine, London.

Carnevale, P., Robert, V., Snow, R., et al., 1991. L'impact des moustiquaires imprégnées sur la prévalence et la morbidité liées au paludisme en Afrique sud-saharienne. *Annale de la Société Belge de Médecine Tropicale,* 71 (Suppl. 1), 127-150.

Chandre, F., Darrier, F., Manga, L., et al., 1999. Status of pyrethroid resistance in *Anopheles gambiae sensu lato*. *Bulletin of the World Health Organization,* 77 (3), 230-234.

Coetzee, M., Craig, M. and Le Sueur, D., 2000. Distribution of African malaria mosquitoes belonging to the *Anopheles gambiae* complex. *Parasitology Today,* 16 (2), 74-77.

Coetzee, M. and Fontenille, D., 2004. Advances in the study of *Anopheles funestus*, a major vector of malaria in Africa. *Insect Biochemistry and Molecular Biology,* 34 (7), 599-605.

Cohuet, A., Dia, I., Simard, F., et al., 2005. Gene flow between chromosomal forms of the malaria vector *Anopheles funestus* in Cameroon, Central Africa, and its relevance in malaria fighting. *Genetics,* 169 (1), 301-311.

Cohuet, A., Simard, F., Toto, J.C., et al., 2003. Species identification within the *Anopheles funestus* group of malaria vectors in Cameroon and evidence for a new species. *American Journal of Tropical and Medical Hygiene,* 69 (2), 200-205.

Coluzzi, M., 1984. Heterogeneities of the malaria vectorial system in tropical Africa and their significance in malaria epidemiology and control. *Bulletin of the World Health Organization,* 62 (Suppl.), 107-113.

Coluzzi, M., Petrarca, V. and Di Deco, M.A., 1985. Chromosomal inversion intergradation and incipient speciation in *Anopheles gambiae*. *Bollettino di Zoologia,* 52, 45-63.

Costantini, C., Sagnon, N., Ilboudo-Sanogo, E., et al., 1999. Chromosomal and bionomic heterogeneities suggest incipient speciation in *Anopheles funestus* from Burkina Faso. *Parassitologia,* 41 (4), 595-611.

Curtis, C., Maxwell, C., Lemnge, M., et al., 2003. Scaling-up coverage with insecticide-treated nets against malaria in Africa: who should pay? *Lancet Infectious Diseases,* 3 (5), 304-307.

D'Alessandro, U., Olaleye, B.O., McGuire, W., et al., 1995. Mortality and morbidity from malaria in Gambian children after introduction of an impregnated bednet programme. *Lancet,* 345 (8948), 479-483.

Darriet, F., N'Guessan, R., Koffi, A.A., et al., 2000. Impact de la résistance aux pyréthrinoïdes sur l'efficacité des moustiquaires imprégnées dans la prévention du paludisme: résultats des essais en cases expérimentales avec la deltaméthrine SC. *Bulletin de la Société de Pathologie Exotique,* 93 (2), 131-134.

Della Torre, A., Costantini, C., Besansky, N.J., et al., 2002. Speciation within *Anopheles gambiae:* the glass is half full. *Science,* 298 (5591), 115-117.

Della Torre, A., Fanello, C., Akogbeto, M., et al., 2001. Molecular evidence of incipient speciation within *Anopheles gambiae s.s.* in West Africa. *Insect Molecular Biology,* 10 (1), 9-18.

Diallo, D.A., Cousens, S.N., Cuzin-Ouattara, N., et al., 2004. Child mortality in a West African population protected with insecticide-treated curtains for a period of up to 6 years. *Bulletin of the World Health Organization,* 82 (2), 85-91.

Failloux, A.B., Vazeille, M. and Rodhain, F., 2002. Geographic genetic variation in populations of the dengue virus vector *Aedes aegypti*. *Journal of Molecular Evolution,* 55 (6), 653-663.

Favia, G., Lanfrancotti, A., Spanos, L., et al., 2001. Molecular characterization of ribosomal DNA polymorphisms discriminating among chromosomal forms of *Anopheles gambiae s.s. Insect Molecular Biology,* 10 (1), 19-23.

Fontenille, D. and Simard, F., 2004. Unravelling complexities in human malaria transmission dynamics in Africa through a comprehensive knowledge of vector populations. *Comparative Immunology, Microbiology and Infectious Diseases,* 27 (5), 357-375.

Fontenille, D. and Toto, J.C., 2001. *Aedes (Stegomyia) albopictus* (Skuse), a potential new Dengue vector in southern Cameroon. *Emerging Infectious Diseases,* 7 (6), 1066-1067.

Gillies, M.T. and De Meillon, B., 1968. *The Anophelinae of Africa south of the Sahara (Ethiopian Zoogeographical Region).* 2nd edn. South African Institute for Medical Research, Johannesburg. Publications of the South African Institute for Medical Research no. 54.

Guillet, P., Alnwick, D., Cham, M.K., et al., 2001. Long-lasting treated mosquito nets: a breakthrough in malaria prevention. *Bulletin of the World Health Organization,* 79 (10), 998.

Guyatt, H.L., Gotink, M.H., Ochola, S.A., et al., 2002. Free bednets to pregnant women through antenatal clinics in Kenya: a cheap, simple and equitable approach to delivery. *Tropical Medicine and International Health,* 7 (5), 409-420.

Habluetzel, A., Diallo, D.A., Esposito, F., et al., 1997. Do insecticide-treated curtains reduce all-cause child mortality in Burkina Faso? *Tropical Medicine and International Health,* 2 (9), 855-862.

Hamon, J. and Mouchet, J., 1961. Les vecteurs secondaires du paludisme humain en Afrique. *Médecine Tropicale,* 21, 643-660.

Hawley, W.A., Phillips-Howard, P.A., Ter Kuile, F.O., et al., 2003. Community-wide effects of permethrin-treated bed nets on child mortality and malaria morbidity in western Kenya. *American Journal of Tropical Medicine and Hygiene,* 68 (4 Suppl.), 121-127.

Hougard, J.M., Corbel, V., N'Guessan, R., et al., 2003. Efficacy of mosquito nets treated with insecticide mixtures or mosaics against insecticide resistant *Anopheles gambiae* and *Culex quinquefasciatus* (Diptera: Culicidae) in Cote d'Ivoire. *Bulletin of Entomological Research,* 93 (6), 491-498.

Howard, S.C., Omumbo, J., Nevill, C., et al., 2000. Evidence for a mass community effect of insecticide-treated bednets on the incidence of malaria on the Kenyan coast. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 94 (4), 357-360.

Kengne, P., Awono-Ambene, P., Antonio-Nkondjio, C., et al., 2003. Molecular identification of the *Anopheles nili* group of African malaria vectors. *Medical and Veterinary Entomology,* 17 (1), 67-74.

Koekemoer, L.L., Kamau, L., Hunt, R.H., et al., 2002. A cocktail polymerase chain reaction assay to identify members of the *Anopheles funestus* (Diptera: Culicidae) group. *American Journal of Tropical Medicine and Hygiene,* 66 (6), 804-811.

Lengeler, C., 1998. Insecticide treated bednets and curtains for malaria control (Cochrane review). *The Cochrane Library* (3).

Mabaso, M.L., Sharp, B. and Lengeler, C., 2004. Historical review of malarial control in southern African with emphasis on the use of indoor residual house-spraying. *Tropical Medicine and International Health,* 9 (8), 846-856.

Maxwell, C.A., Chambo, W., Mwaimu, M., et al., 2003. Variation of malaria transmission and morbidity with altitude in Tanzania and with introduction of alphacypermethrin treated nets. *Malaria Journal,* 2 (1), 28/1-9. [http://www.malariajournal.com/content/2/1/28]

Mutebi, J.P. and Barrett, A.D., 2002. The epidemiology of yellow fever in Africa. *Microbes and Infection,* 4 (14), 1459-1468.

Nevill, C.G., Some, E.S., Mung'ala, V.O., et al., 1996. Insecticide-treated bednets reduce mortality and severe morbidity from malaria among children on the Kenyan coast. *Tropical Medicine and International Health,* 1 (2), 139-146.

N'Guessan, R., Darriet, F., Doannio, J.M., et al., 2001. Olyset Net efficacy against pyrethroid-resistant *Anopheles gambiae* and *Culex quinquefasciatus* after 3 years' field use in C te d'Ivoire. *Medical and Veterinary Entomology,* 15 (1), 97-104.

Scott, J.A., Brogdon, W.G. and Collins, F.H., 1993. Identification of single specimens of the *Anopheles gambiae* complex by the polymerase chain reaction. *American Journal of Tropical Medicine and Hygiene,* 49 (4), 520-529.

Toto, J.C., Abaga, S., Carnevale, P., et al., 2003. First report of the oriental mosquito *Aedes albopictus* on the West African island of Bioko, Equatorial Guinea. *Medical and Veterinary Entomology,* 17 (3), 343-346.

Weill, M., Lutfalla, G., Mogensen, K., et al., 2003. Comparative genomics: insecticide resistance in mosquito vectors. *Nature,* 423 (6936), 136-137.

WHO, 2003. *The Africa malaria report*. [http://www.rbm.who.int/amd2003/amr2003/amr_toc.htm]

Wondji, C., Simard, F. and Fontenille, D., 2002. Evidence for genetic differentiation between the molecular forms M and S within the Forest chromosomal form of *Anopheles gambiae* in an area of sympatry. *Insect Molecular Biology,* 11 (1), 11-19.