World Health Organization
REGIONAL OFFICE FOR Africa

N V A R
African Network on Vector Resistance

Atlas
of insecticide resistance in malaria vectors of the WHO African region

---

ANVR
(African Network for Vector Resistance)

Harare, October 2005

Acknowledgement
It is thanks to the active participation of more than 20 African countries and several research institutions members of ANVR that data summarized in this Atlas have been collected. These data remains ownership of these countries and research institutions.

---

1. Introduction

This Atlas is presenting recent data on distribution of insecticide resistance in malaria vectors of the African region. It contains data that have been collected between 1999 and 2004 by countries themselves and by research institutions in the framework of the **African Network for Vector Resistance (ANVR)**. It does not contain other data that have been generated outside ANVR.

Launched in 1999 by the WHO Regional Office for Africa, the African Network for Vector Resistance has been supporting countries of the region in monitoring insecticide resistance in malaria vectors. So far, a total of 63 national technicians from 24 countries have been trained to monitor resistance, using standardized protocols and methods that are recommended by WHO. ANVR is benefiting from active participation of several renowned scientific institutions, most based in Africa.

The objectives of this Atlas are:

- To retrocede to African countries mapped data that can be easily used.
- To update countries and the international community on the current status of insecticide resistance in Africa, especially pyrethroid resistance.
- To ensure vector resistance status is taken into consideration when selecting vector control interventions and insecticides.
- To share available information at regional and global levels.
- To stimulate and assist national malaria control programs, partners and funding agencies in the adoption of locally adapted tactics for management of vector resistance in the context of integrated vector management.

This document is targeting policy makers, national program managers as well as all partners involved in malaria control in Africa.

2. Distribution of malaria vectors in Africa

Malaria is characterized by its biological diversity. This diversity is conditioned mostly by the vector species that are involved in transmission (including their distribution, behavior and vectorial capacity). It is also conditioned, among others, by seasonality of transmission, pathogenicity of parasites species and by immune response of human hosts.

A regional data base on the geographical distribution of vectors belonging to the *Anopheles gambiae* complex has been developed. As seen from **Map 1**, the four main vector species (*An. gambiae s.s*. *An. arabiensis*, *An. melas* & *An. merus*) belonging to this complex have quite different distribution patterns.

Anopheles gambiae s.s., globally the most important vector, is widely distributed in low lands throughout inter-tropical Africa. Commonly associated with *An. funestus*, this species is responsible for intense transmission either seasonal or perennial depending on local climatic conditions and opportunities for larval breeding. In some areas, two other important vectors of local importance can also be found (*Anopheles nili* and *Anopheles moucheti*), especially in Central Africa.
Anopheles arabiensis has a wide distribution but is found predominantly in fringes and highlands: Southern and Eastern Africa, highlands, Sahelian areas of Western and Central Africa. In these areas, it is commonly associated with *Anopheles funestus* and, to a lower extent, to *Anopheles gambiae s.s.* These areas are characterized by very seasonal transmission, most commonly of low intensity and by occurrence of outbreaks whose frequency and intensity are closely related to climatic conditions.

Anopheles melas in the Western Africa and *An. merus* in the East are mostly found in costal areas where they can be locally important vectors, especially when associated with *An. gambiae* s.s.

Malaria patterns are closely related to transmission. In areas of intense transmission, (rural areas in most lowlands of inter-tropical Africa) young children usually develop a protective immunity before the age of 5. Most related malaria deaths occur during this period, malaria being essentially a childhood disease. On the contrary, in areas of low to very low transmission (urban areas, fringes, highlands, epidemic prone areas), people usually do not develop strong protective immunity and malaria related deaths can occur at any stage of the life.

3. ANVR framework

All national malaria control programmes are *de facto* members of ANVR. ANVR is a network managed by the WHO Regional Office for Africa. It included the following core scientific institutions:

- Centre Muraz, Bobo Dioulasso, Burkina Faso.
- Centre de Recherches Entomologiques de Cotonou (CREC), Bénin.

- Institut Pierre Richet (IPR), Bouaké, Côte d'Ivoire.
- Institut de Recherches pour le Développement en Coopération (IRD), Montpellier France.
- Liverpool School of Hygiene and Tropical Medicine (LSHTM), Liverpool, England.
- National Institute for Communicable Diseases, Johannesburg, South Africa.
- OCEAC, Yaoundé, Cameroon

Additional regional institutions have joined the network. ANVR institutions are involved in training of national staff, development and standardization of protocols and new tests methods. They also provide when and where needed technical assistance (identification of biological material, biochemical and molecular assays, consultantships..).

4. Test procedures

WHO test tubes for adult mosquitoes have been the main method used for bioassays. Test protocols for biological, biochemical and molecular assays have been fully standardized, following WHO existing recommended methods and summarized in an ANVR standard operating procedure manual. Most bioassays have been carried out using young female mosquitoes emerged from field collected larvae and pupae or from F1 progeny of wild caught blood-fed females. Insecticide treated papers used for bioassays have been produced in Malaysia and provided through ANVR.

The following criteria have been used for interpretation and classification of results, based on WHO recommendations:

	At least 80 mosquitoes tested per bioassay	Twenty* to 79 mosquitoes tested per bioassay
Susceptible	Mortality 98 – 100 %	Mortality 98 – 100 %
Resistance suspected, to be confirmed	Morality 95 – 97 %	Mortality 80 – 97 %
Resistance	Mortality < 95 %	Mortality < 80 %

* Tests carried out with less than 20 mosquitoes have not been considered

Test mosquitoes have been identified morphologically. When possible, species, forms, and resistance mechanisms have been identified using molecular markers. The *kdr* mutation responsible for pyrethroid and DDT cross-resistance has been detected using specific primers (Martinez-Torres *et al*., 1998). Modified acethylcholinesterase (AchE), a major mechanism for organophosphate and carbamate resistance, has been identified using both biochemical (Hemingway *et a*l., 1998) and molecular assays (Weill *et al*., 2004). Results of molecular assays (*kdr* and AchE) have been mapped using allelic frequencies (%) of the genes responsible for the mutation. Results obtained through the network based on biochemical assays for mono-oxygenases and esterases (according to Hemingway, 1998) were not considered reliable enough to be included in this document.

Priority has first been given to DDT and pyrethroids which are most in use for malaria control, especially pyrethroids. Since very few tests have been carried out with other insecticides (carbamates and organophosphates (OPs)), results have not been reported in

this document. Data have been mapped per insecticide (DDT, permethrin, deltamethrin, and lambdacyhalothrin) and per vector species. Much fewer tests have been carried out with *An. funestus* because this species is far more difficult to collect and to breed than species of the *An. gambiae* complex.

5. Results

In total, 473 tests over 24 countries covering 196 different sites have been reported through ANVR (**Map 2**). All data have been verified and validated before being introduced in the data base and mapped. General comments are provided below on resistance status. *For detailed information by country, readers should refer to the corresponding map(s) displayed by major vector species, insecticide, and sub-region for West Africa.*

5.1 *Anopheles gambiae s.s.*

Results are summarized in **maps 3 to 13**.

5.1.1. Resistance to DDT and pyrethroids

Summary of tests carried out with *Anopheles gambiae s.s.*

	Number of tests	Proportion of tests showing *confirmed resistance*	Proportion of tests showing *high resistance level*
DDT	87	64.4 %	23 %
Permethrin	138	49.3 %	11.6 %
Deltamethrin	118	31.4 %	0.8 %
Lambdacyhalothrin	45	26.7 %	0 %

In the majority of surveyed localities in West, Central, and Eastern Africa, *An. gambiae s.s.* has bee found **resistant to DDT** (**maps 3 & 4**). **Pyrethroid resistance** is widespread, especially in West Africa (**maps 5 to 10**). Occurrence of deltamethrin and lambdacyhalothrin resistance is apparently lower than that of permethrin. However, this difference is likely due to the relative "strength" of the discriminative concentrations used than a lower resistance to these specific insecticides. In West Africa at least, the presence of the *kdr* mutation is clearly associated with cross-resistance between DDT and all public health pyrethroids. *Kdr* is widely distributed and allelic frequencies of the gene in several areas are very high, commonly higher than 80 % (**map 11**). A slightly different mutation (*kdr*-type) has been detected in East Africa (Kenya, Uganda, Burundi...) (Ranson *et al*., 2000). However, data related to this mutation were not generated through ANVR. Although the two *kdr* mutations are responsible for DDT resistance, the West African one is responsible for higher resistance to pyrethroids than the East African one.

It can be safely deducted from existing data that the *kdr* mutation is present in almost all countries west of Cameroon. It has been found in both the S and M molecular forms of *An. gambiae s.s.* Frequency within the S form is much higher and distribution more widespread than within the M form, except on the coastal areas of Côte d'Ivoire (**maps 11 & 12**). The

kdr mutation has not been found so far in *An. arabiensis*. The Eastern Africa mutation is likely responsible for DDT and pyrethroid resistance that has been found e.g. in Uganda. In Ethiopia, *An. gambiae s.sl.* is resistant to DDT but susceptible to pyrethroids. A resistance mechanism different from *kdr* is likely involved, that is specific to DDT (e.g. glutathione transferase).

Although data available for Eastern and Southern Africa have been so far limited, they suggest that situation of DDT and pyrethroid resistance of *An. gambiae s.s.* in these areas is much less critical than in Central and West Africa.

5.1.2. Resistance to carbamates. Resistance to carbosulfan (carbamate) has recently been detected in Côte d'Ivoire (Chandre *et al.*, 2003). The mechanism involved is a modified acethylcholinesterase (AchE). A molecular diagnostic test has been recently developed. AchE has been found more widespread than expected (**map 13**) with relatively high allelic frequencies (over 40 %) already observed in different localities. AchE is a major mechanism responsible for organophosphate (OP) and carbamate resistance (LIN/IRD unpublished data). Its implication in OP resistance in the concerned areas has not yet been established. There has been no recent evidence for OP resistance in malaria vectors from Africa.

5.2. *Anopheles arabiensis*

Insecticide resistance has been found much less frequent in *An. arabiensis* than in *An. gambiae s.s.* (**maps 14 & 15**). In several countries of Southern Africa, this species is fully susceptible to DDT and pyrethroids (Botswana, Namibia, Swaziland, Zambia, Zimbabwe). However, DDT resistance has been reported in South Africa (**map 15**). There is also evidence of DDT resistance in Eritrea and Ethiopia (reported in **map 3** under *An. gambiae s.l.*) and of cross resistance between DDT and pyrethroids in *An. arabiensis* from northern Cameroon.

5.3. *Anopheles funestus s.l.*

Only few data on susceptibility of *An. funestus s.l.* have been collected through ANVR. Except in Uganda where a possible resistance to lambdacyhalothrin has been detected that needs to be confirmed, full susceptibility to DDT and pyrethroids has been found in all tested localities (**map 16**). However, these data do not include tests carried out in South Africa and Mozambique where resistance to deltamethrin has been found that has got important operational consequences. On the basis of the usually dramatic impact that residual spraying and ITNs have got on *An. funestus s.l.* populations throughout Africa (published data and grey literature), it is reasonable to assume that outside Southern Africa, this species is mostly susceptible to insecticides, including DDT and pyrethroids. However, more detailed information on resistance status of this species is needed.

6. Overall situation analysis, potential impact of insecticide resistance, and selection of interventions

Although there are important gaps in this initial ANVR resistance mapping, some general conclusions can be already drawn and practical recommendations made. Detailed analysis country by country should be made by readers themselves on the basis of maps presented in this document.

6.1. Resistance in major vectors

An. gambiae s.s. DDT and Pyrethroid resistance are already widespread throughout Western and Central Africa. According to other sources of information, it is also present in several parts of Eastern Africa. Carbamate resistance has been detected in West Africa involving a major resistance mechanism that has been found already spread over several countries. The situation of "**multiple-resistance**" observed in West Africa most likely results from the intensive use of agricultural insecticides which induce a selection pressure on *An. gambiae s.s.* populations, especially in the "cotton belt" of Western and Central Africa. It can be safely assumed that *kdr* resistance is also present in south-eastern Mali, Ghana and Nigeria.

An. arabiensis. DDT resistance in *An. arabiensis* has already been found in different parts of Africa. The *kdr* mutation has not yet been detected in this species and DDT resistance is likely due to a specific mechanism. Pyrethroid resistance in *An. arabiensis* has been found in Northern Cameroon.

An. funestus s.l. At continent level, *An. funestus* remains globally susceptible to insecticides except in Southern Africa (South Africa & Mozambique) where it is resistant to pyrethroids (but susceptible to DDT). This resistance is due to a mechanism other than the *kdr* mutation (detoxification). A possible case of resistance to lambdacyhalothrin has been suspected in Uganda but has to be confirmed.

6.2. Resistance is an evolving process. Significant changes in resistance patterns have been observed over the past 10 years in West Africa. The situation presented in this document will likely evolve in the near future because of the massive use of pyrethroids for malaria control. When planning any vector control intervention, it is essential to assess the resistance status of local vector populations in order to select a suitable insecticide. It is also essential to ensure subsequent regular monitoring. When possible, the potential of resistance on the efficacy of intervention(s) should be assessed.

6.3. Resources for resistance monitoring. Monitoring of insecticide and drug resistance should be considered as integral component of any malaria control program. Financial resources for insecticide resistance monitoring can be obtained from funding partners on condition it has been included in national action plans and funds have been requested. ANVR now provides technical assistance to National Programs for this planning.

6.4. Operational consequences of resistance.

Insecticide treated nets (ITNs). Fortunately, when pyrethroid resistance is induced by the *kdr* mutation, it does not dramatically reduce efficacy of ITNs. Even in areas with very high prevalence of this resistance, ITNs still efficiently prevent malaria. The potential impact of resistance mechanisms other than *kdr* has not yet been fully assessed.

Residual spraying. Very little information is available on the potential impact of the *kdr* mutation on indoor residual spraying of DDT and pyrethroids. However, there is a fear that *kdr* resistance might reduce its efficacy since it relies largely on mass killing of vectors. Potential impact of resistance mechanisms other than *kdr* on residual spaying has already been documented in several occasions. In South Africa e.g., the development of a non-*kdr* pyrethroid resistance in *An. funestus* has dramatically reduced efficacy of the spraying program and resulted in a sharp increase in the number of malaria cases.

The way forward. Further extension of DDT and pyrethroid residual spraying in Africa would most likely face difficulties because of widespread insecticide resistance. In any case, spraying operations should be planned based on a detailed assessment of resistance (distribution, intensity, and mechanisms involved) and the adoption of resistance management tactics. Massive deployment of ITNs might further exacerbate pyrethroid resistance and worsen the current situation. A possible scenario could be that personal protection provided by ITNs will be maintained despite resistance while the community protection expected from high coverage of this intervention might be reduced because of resistance. As far as residual spraying is concerned, an impact of resistance should *a priori* be expected unless absence of such impact has been shown. Only insecticides to which local vectors are susceptible should be selected for residual indoor spraying. Resistance management policies should be progressively adopted by all residual spraying programs to prolong the use-life of existing insecticides. The arsenal of insecticides that are currently available for residual spraying is already very limited.

6.5. Choice of malaria vector control interventions.

ITNs. In most lowlands of Africa with intense transmission, an interruption or a dramatic reduction of transmission through residual spraying or ITNs interventions would be technically difficult to achieve because of intensity of transmission, widespread insecticide resistance or the absence of operational vector control services. It would also be financially difficult to sustain. On the contrary, a significant reduction in malaria incidence can be achieved through personal protection of vulnerable groups by mass distribution of ITNs, *including in areas where vectors are resistant to pyrethroids*.

ITNs are effective also in low transmission areas (unstable malaria). However, to benefit from the full potential of this intervention, programs should target the highest possible coverage in order to protect the whole community through a mass impact on the vector population. It is yet unclear to which extent pyrethroid resistance may reduce the impact of ITNs on vector populations.

Residual spraying and resistance management. In areas of less intense transmission (unstable malaria) which include most parts of Eastern and Southern Africa (highlands and fringes) as well as northern parts of West and Central Africa, *indoor residual spraying* remains a an effective intervention on condition that local vectors are susceptible to insecticides sprayed and vector control services are able to deliver the intervention properly. However, given the current distribution of resistance in Africa, prolonged use of insecticides for residual spraying should be planned and implemented *in the framework of a resistance management policy*. Rotation between different insecticides over time offers a practical solution for resistance management in residual spraying programs. Residual spraying is also a choice intervention in epidemic prone areas, when a rapid impact on transmission is needed.

ITNs are a viable option in unstable malaria areas as well, especially in areas with high mosquito nuisance and where mosquito nest are already widely used by communities. In this case, ITNs will provide personal protection or, eventually, community protection depending on coverage rates, vector resistance status, and potential impact of resistance

Combination of interventions and integrated vector management. Although being commonly opposed, ITNs and residual spraying can be *combined* at country or eventually district level depending on local contexts thus benefiting of respective advantages of both methods. ITNs can be used e.g. low lands while targeting IRS to highlands or areas of special economic interest where a rapid and dramatic impact is needed). In some situations (e.g. urban environment), other interventions can also be used in combination with IRS and/or ITNs such as the use of larvicides.

Sound vector control interventions should preferably be based on a combination of interventions and insecticides, adapted to specific contexts. Reducing reliance on a single intervention or a single insecticide is a major objective of any resistance management policy in the overall context of *integrated vector management.*

Recommendations

To countries

- To initiate and/or strengthen insecticide resistance monitoring as a component of the national malaria control plans. The necessary resources (human and financial) should be made available, eventually obtained from funding partners.
- To fill gaps in the current knowledge of resistance in malaria vectors (distribution, mechanisms involved) and to start testing susceptibility to insecticides other than DDT and pyrethroids (carbamates, organophosphates).
- To share and disseminate information on insecticide resistance. The present Atlas offers opportunity for rapid dissemination of information. It will easily be updated.
- To select vector control interventions and insecticides taking into account, among other important factors, the resistance status of local vector populations.
- To ensure continuous resistance monitoring.
- To adopt insecticide resistance management as part of national policies for vector control.

To funding partners

- To ensure resistance assessment and monitoring is included in requests for funding related to malaria vector control and is adequately funded.
- To support the adoption of insecticide resistance management policies.

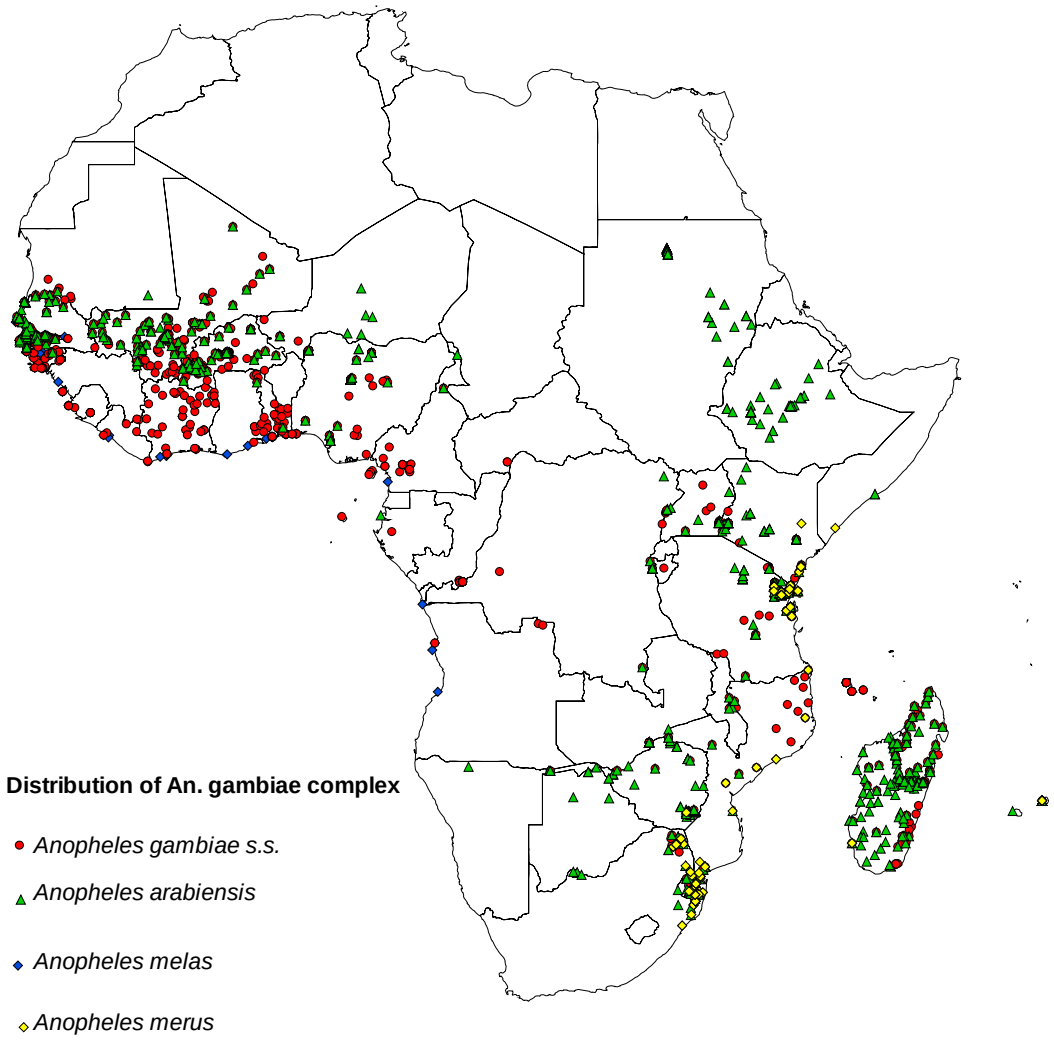
To WHO

- To further build capacity for resistance monitoring at country level and coordinate resistance monitoring activities in the African region, in the framework of ANVR..
- To develop regional guidelines for insecticide resistance management and promote adoption and implementation of resistance management tactics.
- To update and complete the present document, collecting and incorporating data obtained at country level as well as published information.
- On request of national programs, to review country by country the situation of insecticide resistance and to provide technical advice on the selection of insecticides and implementation of locally adapted vector control strategies.
- To further stimulate research on the operational impact of insecticide resistance on the efficacy of vector control interventions.
- In view of the situation of insecticide resistance in malaria vectors, to further promote the adoption of integrated vector management principles, with the objective to further reduce reliance on single insecticide and intervention.

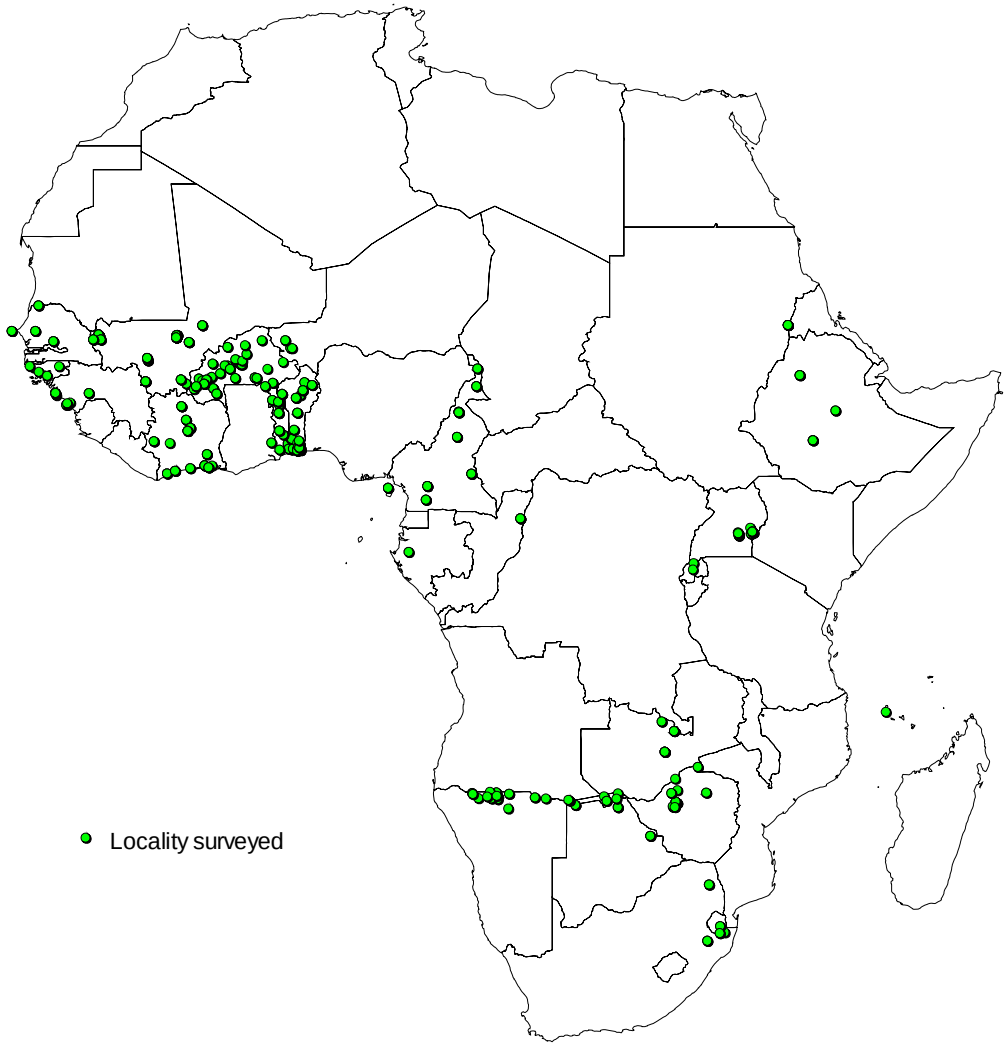
To WHO and Industry

- To promote the search for new insecticides alternative to DDT and pyrethroids.

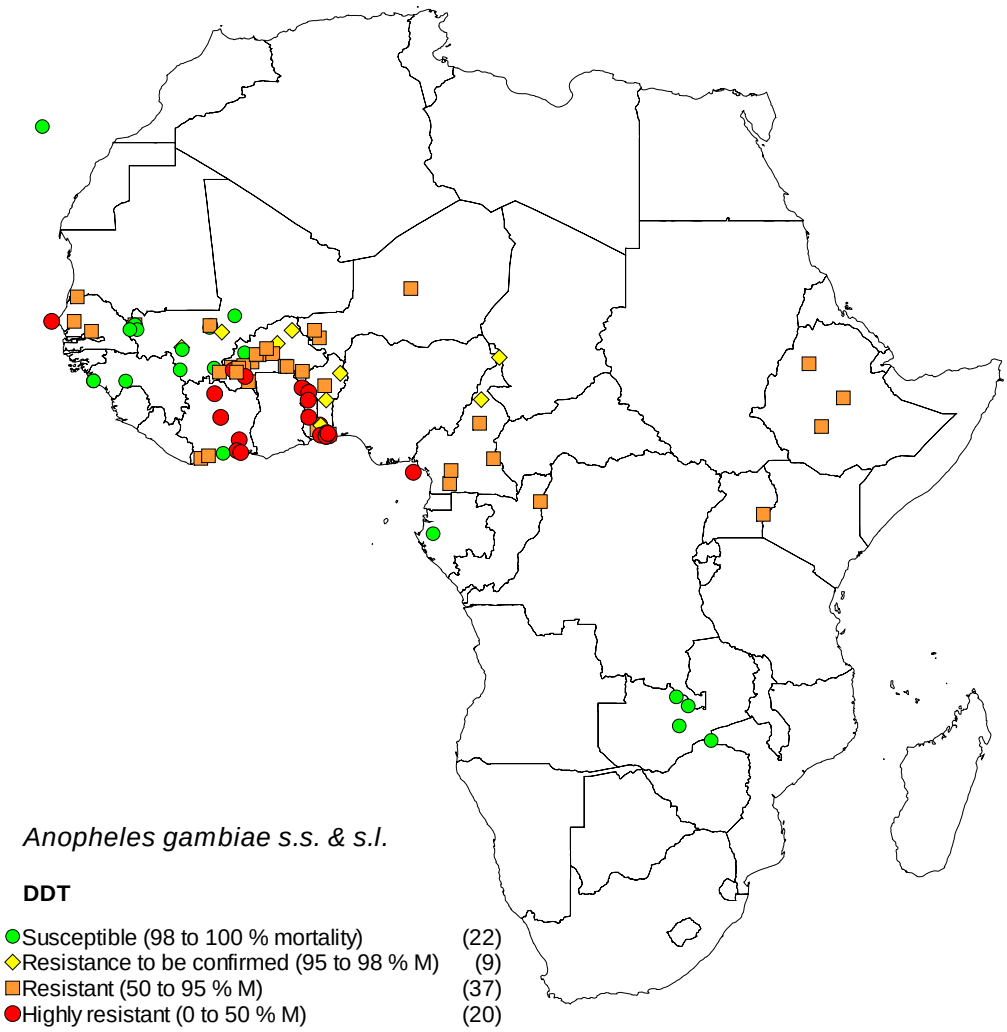
Map 1. Geographical distribution of vectors belonging to the *Anopheles gambiae* complex (ANVR Data Base)



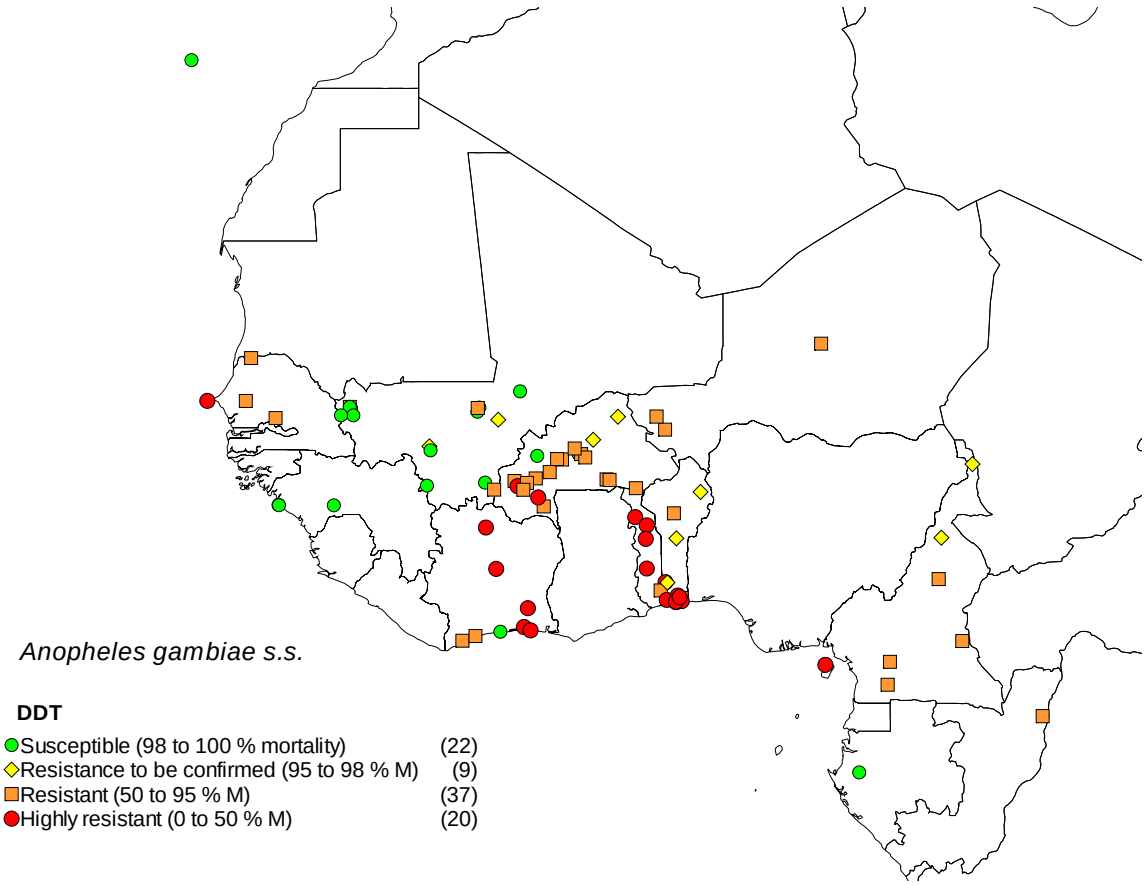
Map 2. Distribution of sentinel sites and surveyed locations
(24 countries, 196 sites, 473 tests)



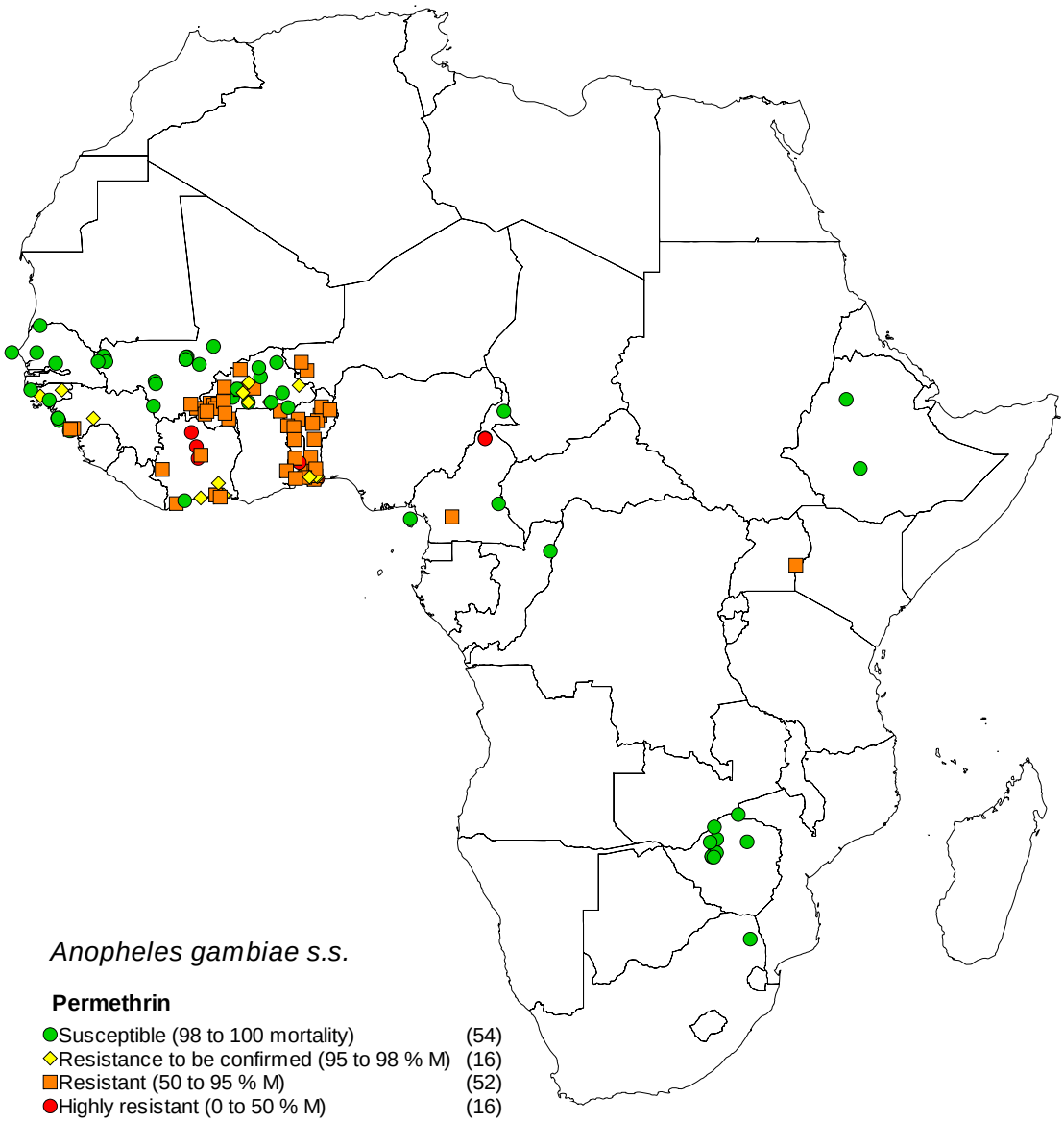
Map 3. Distribution of DDT resistance in *Anopheles gambiae s.s.* and *s.l.*
NB: data from Ethiopia reported as *An. gambiae s.l.* are most likely *An. arabiensis*



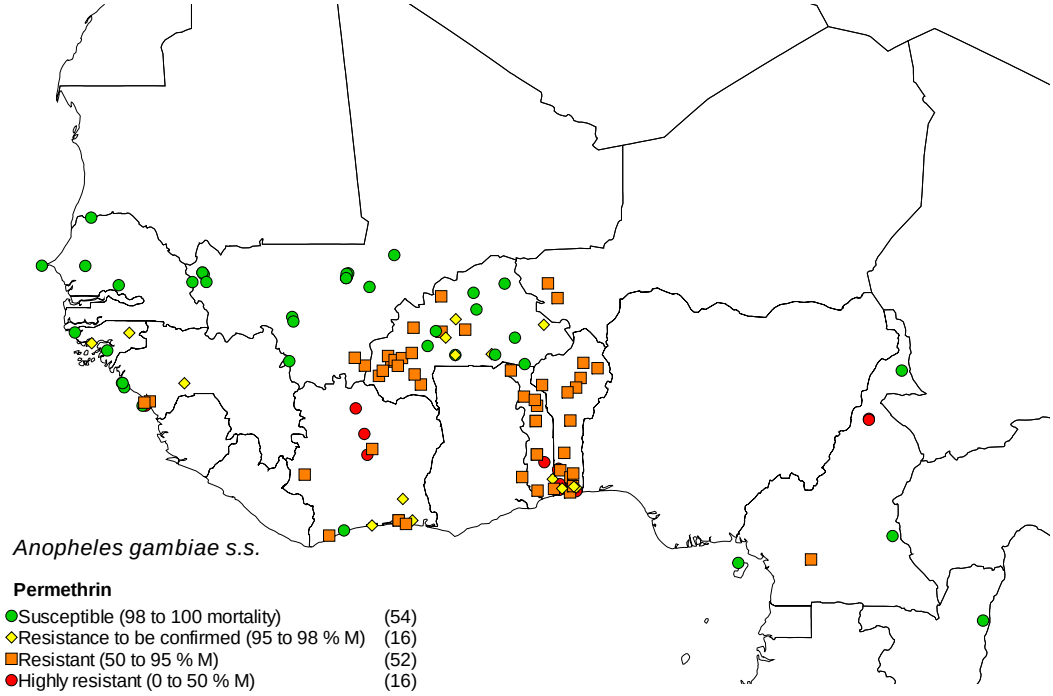
Map 4. Distribution of DDT resistance in *Anopheles gambiae s.s.*, West Africa



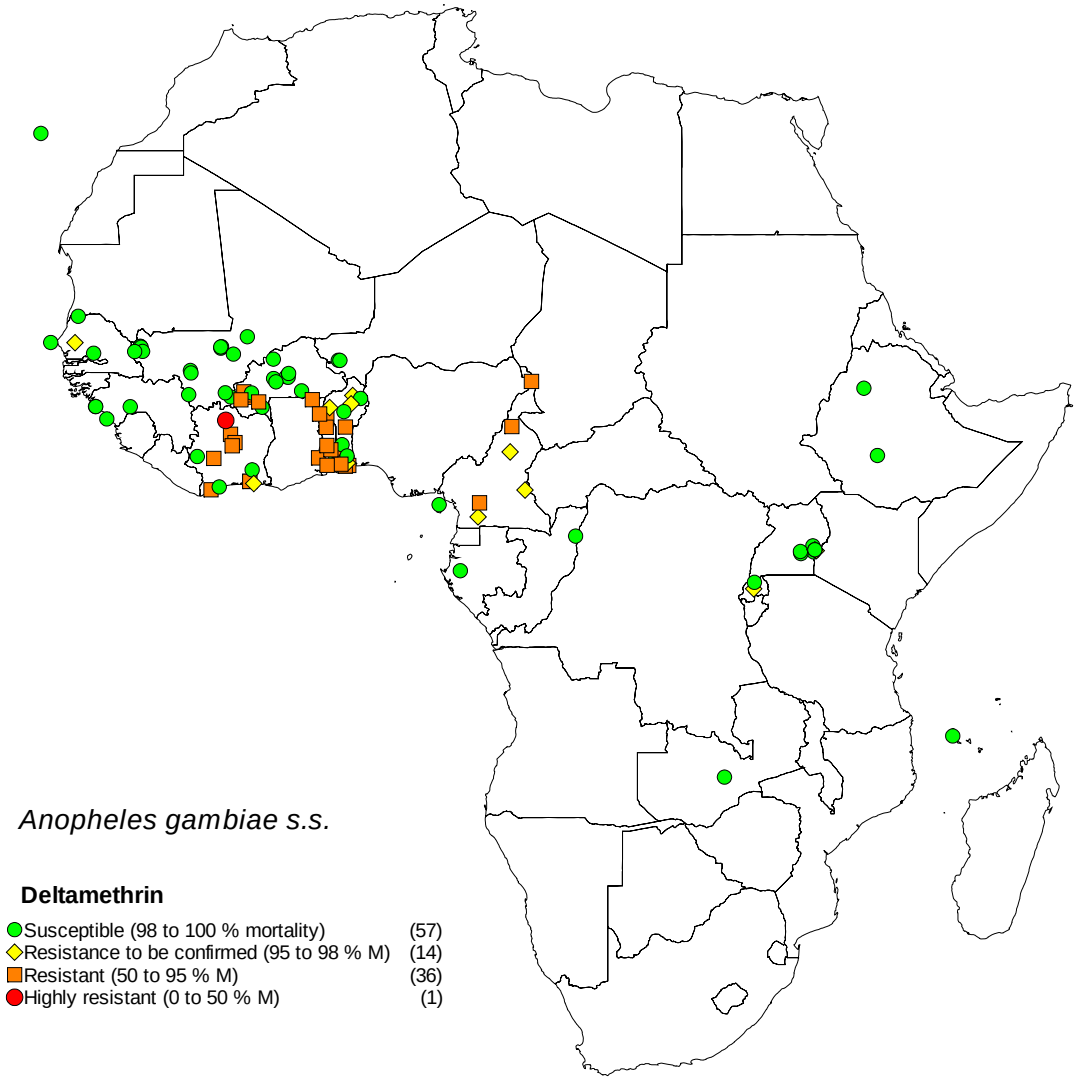
Map 5. Distribution of permethrin resistance in *Anopheles gambiae s.s.* and *s.l* .
NB: data from Ethiopia reported as *An. gambiae s.l.* are most likely *An.arabiensis.*



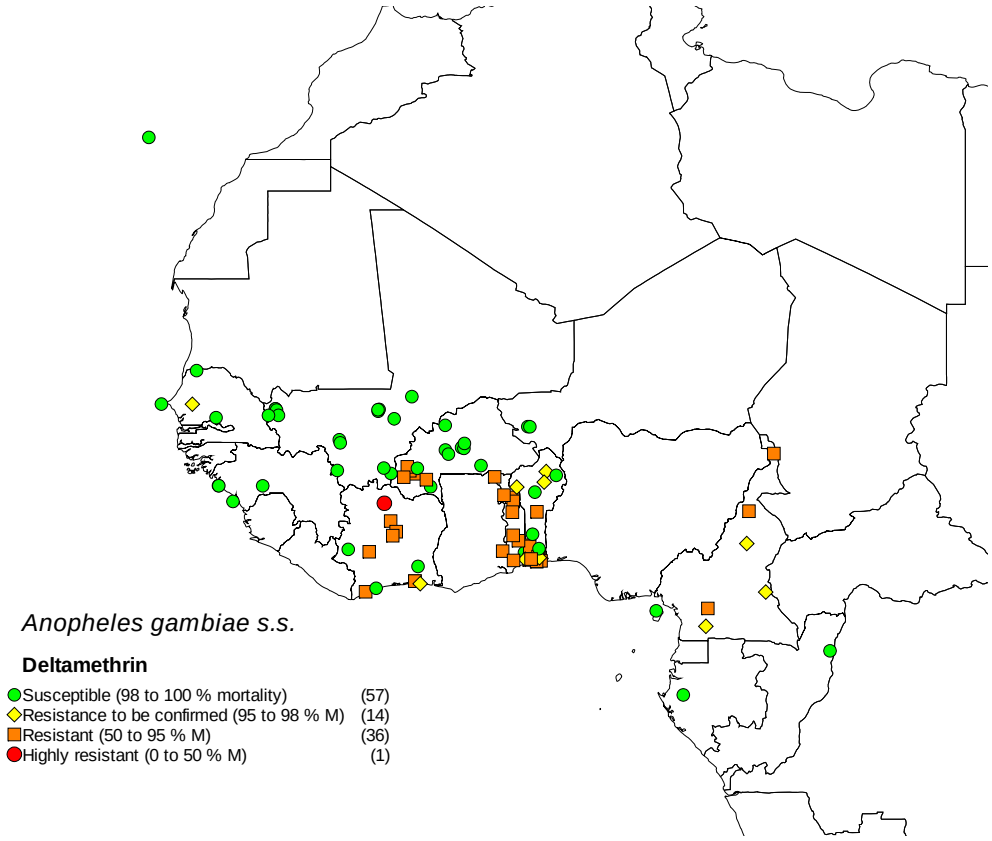
Map 6. Distribution of permethrin resistance in *Anopheles gambiae s.s.*, West Africa



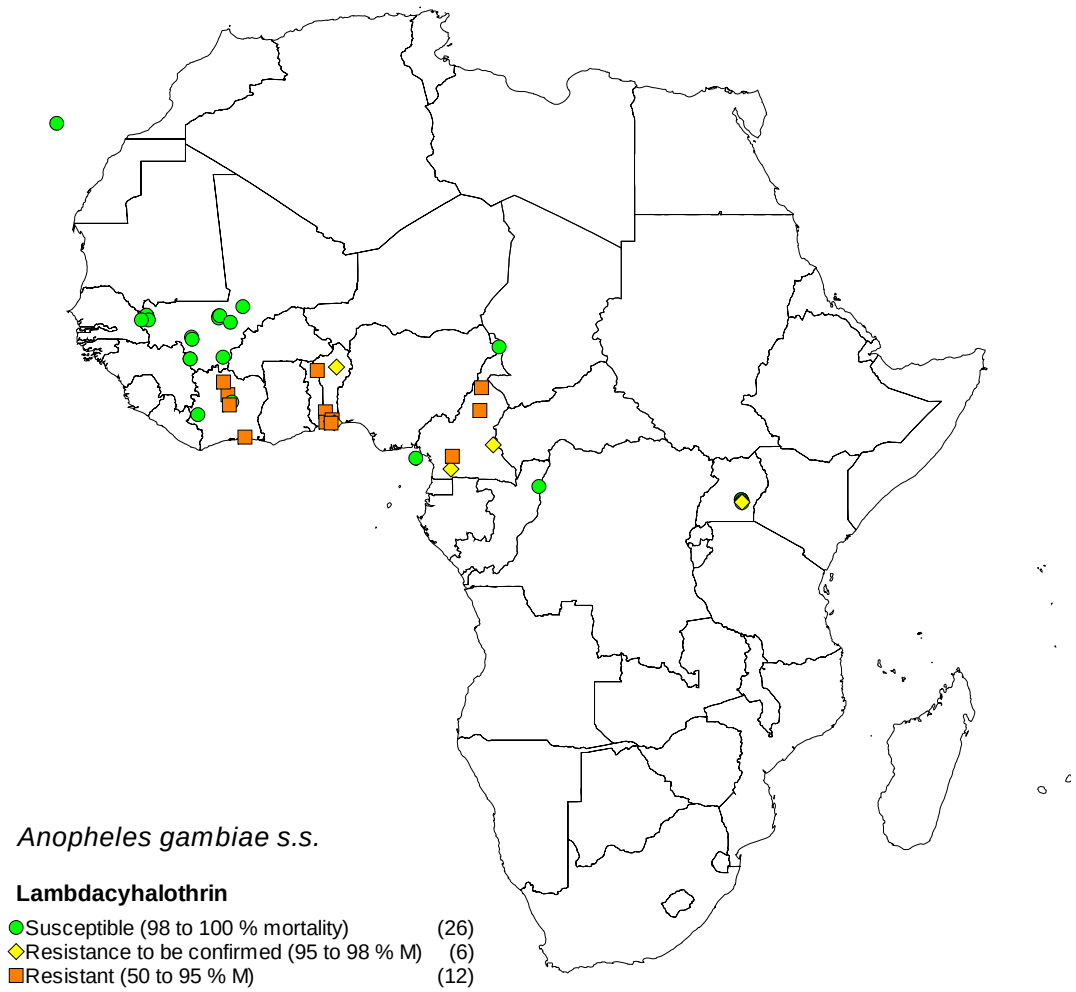
Map 7. Distribution of deltamethrin resistance in *Anopheles gambiae s.s.*and s.l.
NB: data from Ethiopia reported as *An. gambiae s.l.* are most likely *An.arabiensis.*



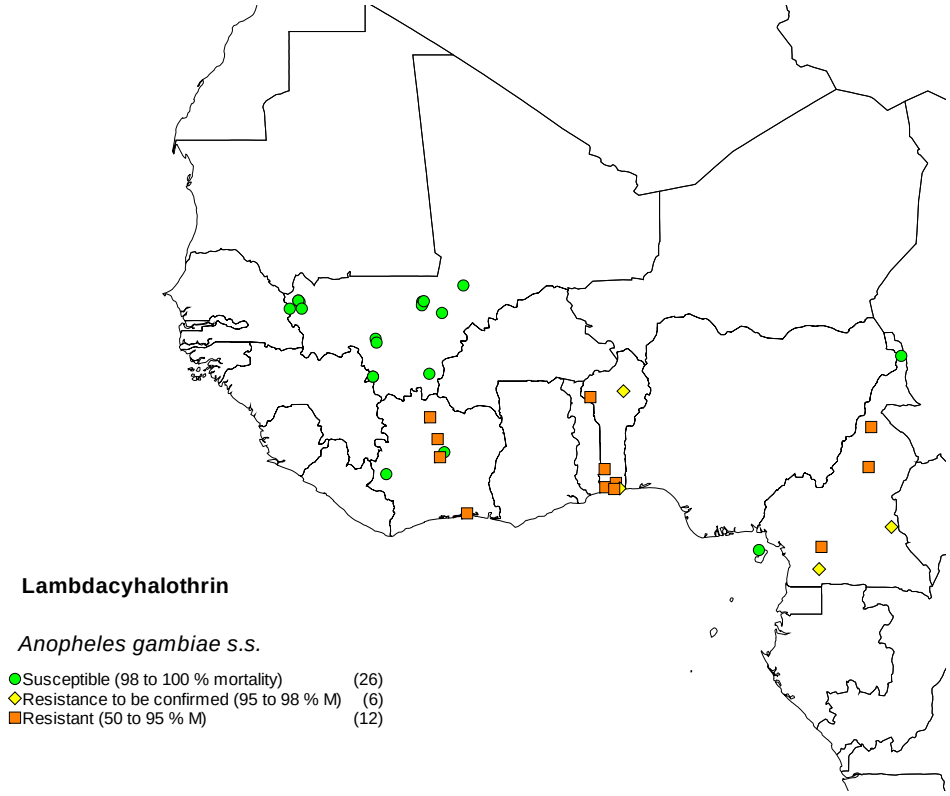
Map 8. Distribution of deltamethrin resistance in *Anopheles gambiae s.s.*, West Africa



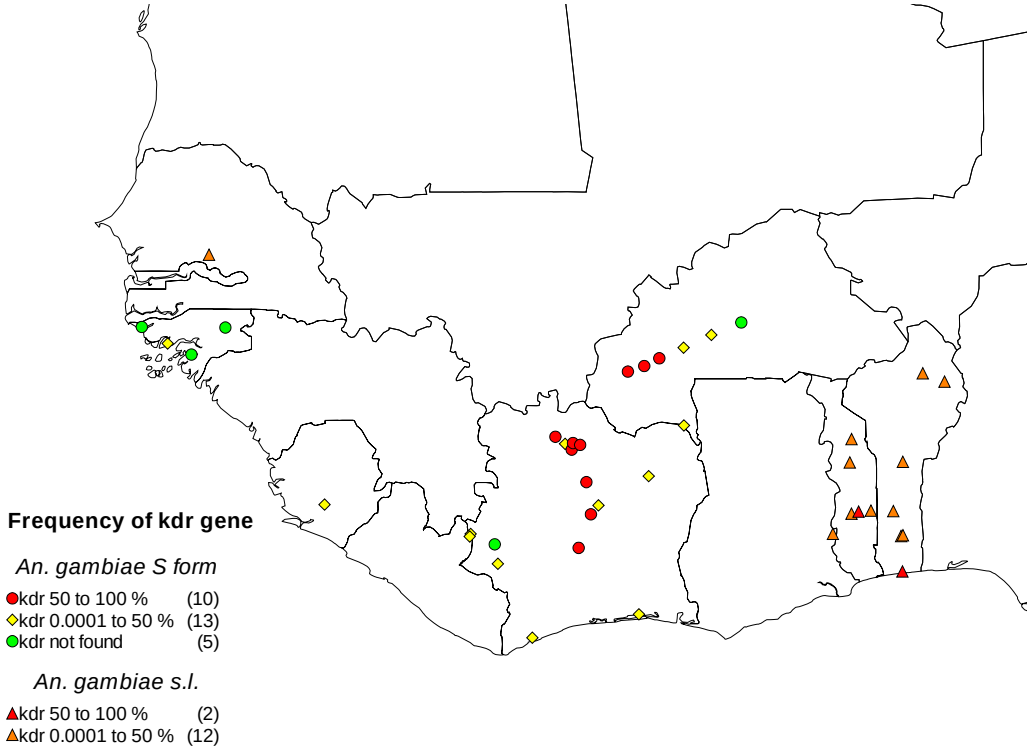
Map 9. Distribution of lambdacyhalothrin resistance in *Anopheles gambiae s.s.*



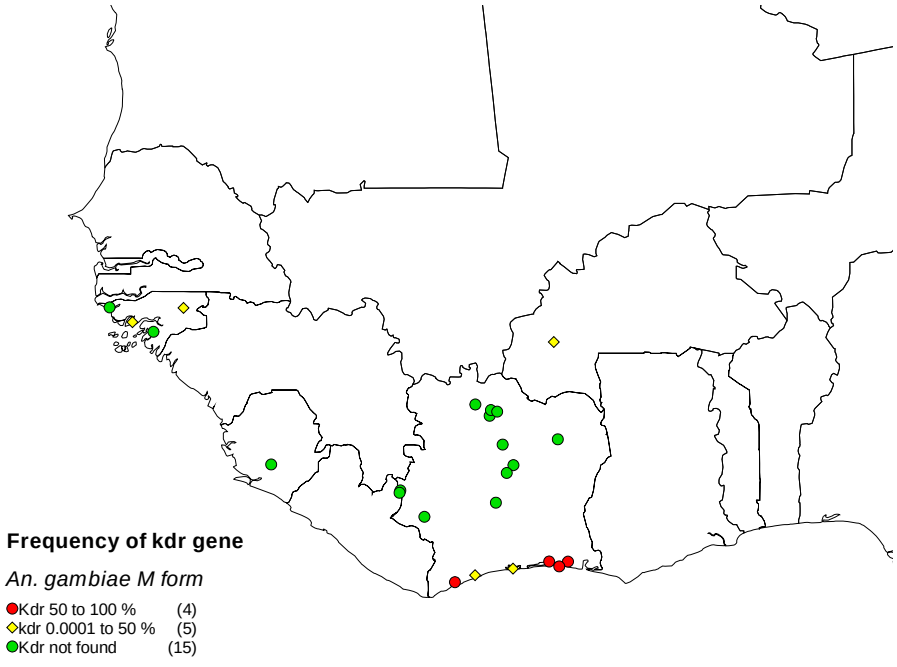
Map 10. Distribution of lambdacyhalothrin in *Anopheles gambiae s.s.*, West Africa



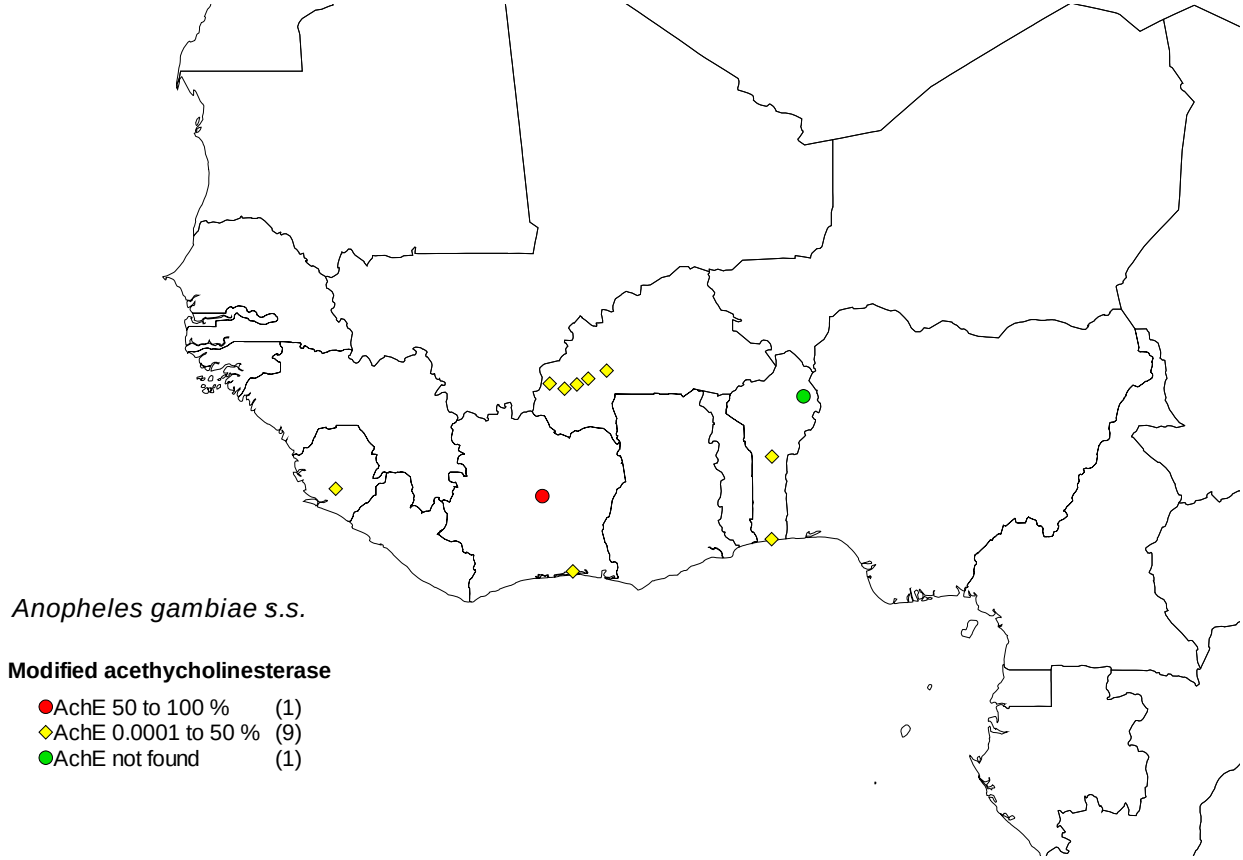
Map 11. Frequency of *kdr* gene in *Anopheles gambiae s.l.* and the S form of *Anopheles gambiae s.s.* in West Africa



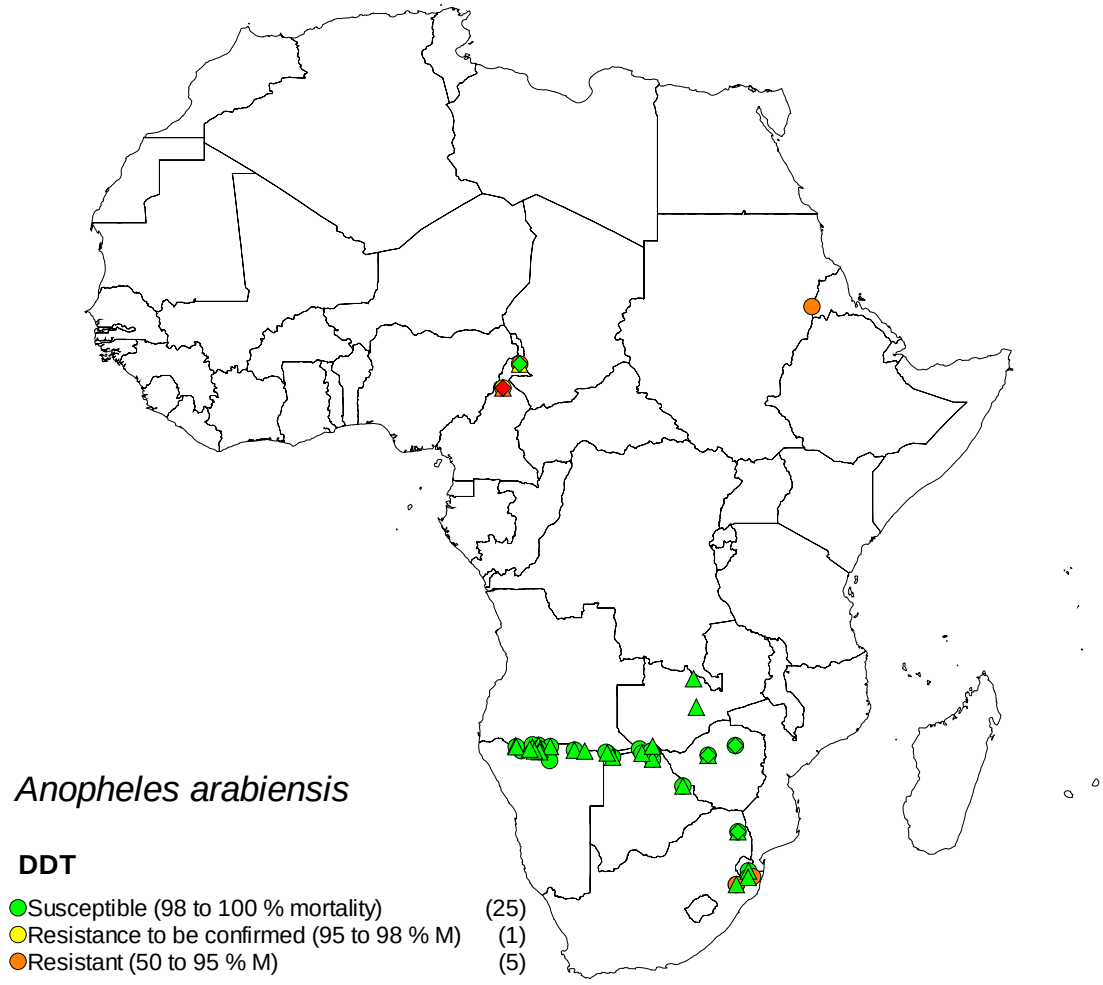
Map 12. Frequency of *kdr* gene in the M form of *Anopheles gambiae s.s.* in West Africa



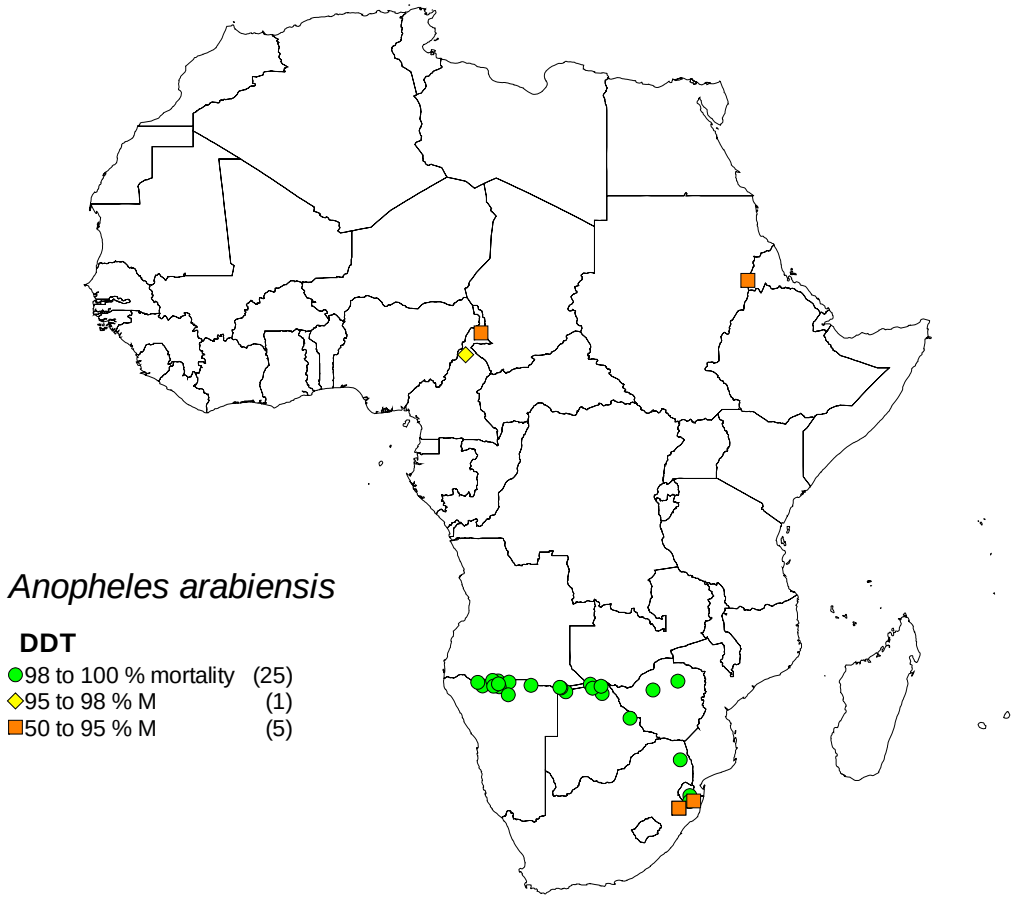
Map 13. Preliminary data on distribution of modified acethylcholinesterase resistance in *Anopheles gambiae s.s.* of West Africa (normally conferring resistance to carbamate and organophosphate insecticides)



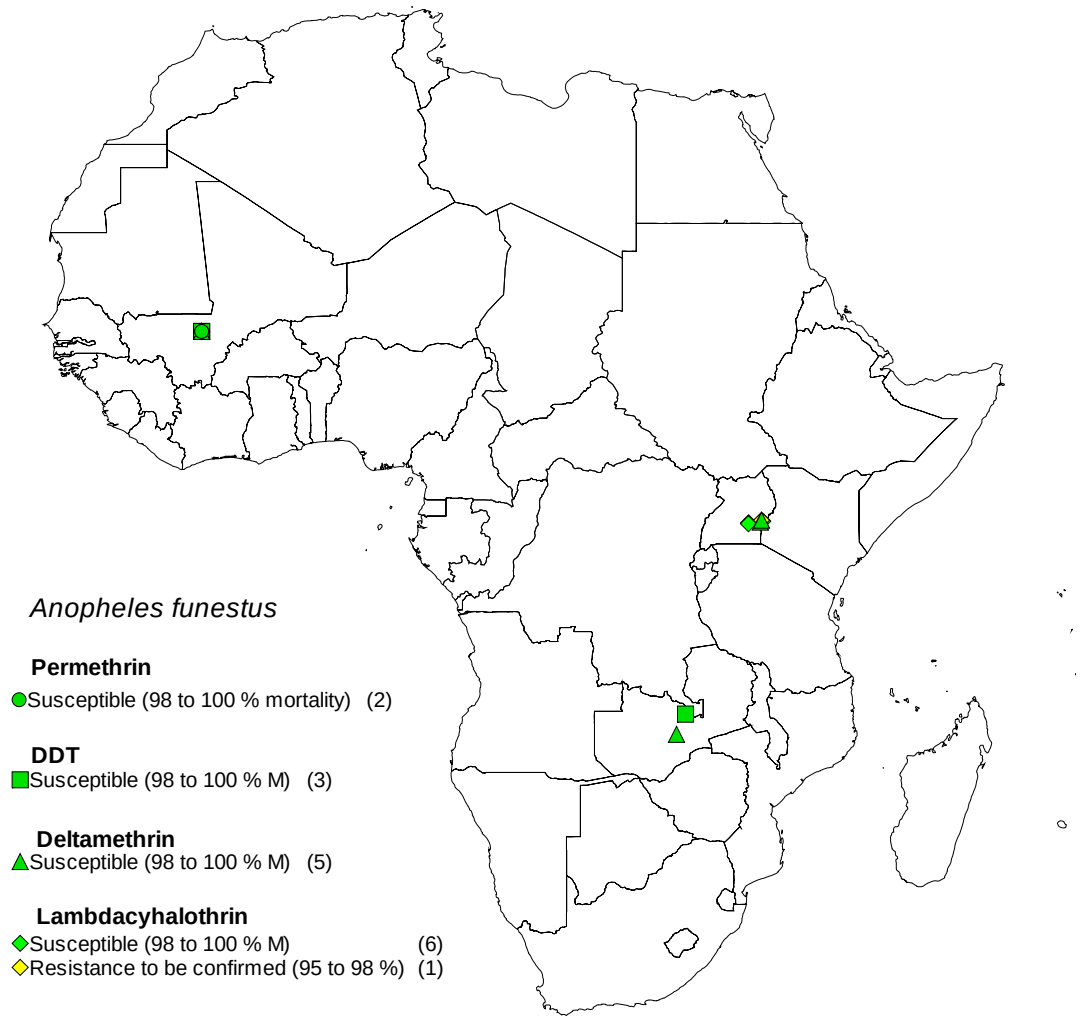
Map 14. Distribution of insecticide resistance in *Anopheles arabiensis*
(for Ethiopia data, see maps 3, 5 and 7)



Map 15. Distribution of DDT resistance in *Anopheles arabiensis*
(for Ethiopia data, see maps 3)



Map 16. Distribution of insecticide resistance in *Anopheles funestus s.l.*



References

- Hemingway J. (1998) Techniques to detect insecticide resistance mechanisms (field and laboratory manual). *Document WHO/CDS/CPC/MAL/98.6* World Health Organization, Geneva.
- Martinez-Torres D., Chandre F., Williamson, M.S. Darriet, F. Bergé, J.B., Devonshire A.L., Guillet P., Pasteur N. & Pauron D. (1998) Molecular characterization of pyrethroid knockdown resistance (*kdr*) in the major malaria vector *Anopheles gambiae* s.s. *Insect Molecular Biology*, **7**, 179-184.
- N'Guessan R., Darriet F., Guillet P., Carnevale P., Traore-Lamizana M., Corbel V., Koffi, A.A. & Chandre, F. (2003) Resistance to carbosulfan in field populations of *Anopheles gambiae* from Côte d'Ivoire based on reduced sensitivity of acetylcholinesterase. *Medical and Veterinary Entomology*, **17**, 19-25.
- Ranson H., Jensen, B., Vulule J.M., Wang X., Hemingway J. & Collins F.H. (2000) Identification of a point mutation in the voltage-gated sodium channel gene of Kenyan *Anopheles gambiae* associated with resistance to DDT and pyrethroids. *Insect Molecular Biology*, **9**(5), 491-497.
- Weill M., Malcolm C., Chandre F., Mogensen K., Berthomieu A., Marquine M., Raymond M. (2004) The unique mutation in ace-1 giving high insecticide resistance is easily detectable in mosquito vectors. *Insect Mol Biol*. **13(1)**:1-7
