Current status of pyrethroid resistance in African malaria vectors and its operational significance

1. Introduction

Malaria vector control in Africa relies predominately on the use of residual insecticides in the domestic environment [1]. Insecticide Treated Nets (ITNs) and Indoor Residual Spraying (IRS) are the cornerstone of malaria control programmes [2] and high coverage with either of these interventions can result in a dramatic reduction in malaria associated morbidity and mortality [3-5]. The success of these tools has contributed towards the optimism that elimination of malaria as a public health problem in the African continent is a feasible objective [6]. However the lack of insecticide classes available for public health and the emergence of resistance to the majority of existing insecticides, is in danger of substantially reducing the contribution of vector control to the malaria elimination agenda.

The public health pesticide products market is perceived as minor and risky compared to the market for agricultural insecticides and has suffered from massive under investment with the result that there have been no new active ingredients with wide scale public health applications for over 30 years. The may be largely attributed to the dramatic increase in the cost of developing and registering new insecticides which has increased by 500% in the last 20 years. It is estimated that the costs of developing new active ingredients now total $300-$400M and development and registration takes at least ten years to complete [7].

Vector control is very dependant on a single class of insecticides, the pyrethroids. These insecticides are safe and fast acting and are the only class approved for use on insecticide treated materials [8]. Pyrethroids are also increasingly deployed in IRS programmes in Africa [9] and are widely used in the control of agricultural pests worldwide [10]. The dramatic increase in reports of pyrethroid resistance in malaria vectors [11, 12] over the past decade is therefore a great cause for

concern. However few studies have addressed the impact of pyrethroid resistance on malaria control and controversy still remains about the epidemiological significance of current levels of resistance in Sub-Saharan Africa. The purpose of this review is to outline our understanding of the mechanisms responsible for pyrethroid resistance in malaria vectors and to critically appraise alternative methods of resistance monitoring. The current distribution of pyrethroid resistance in malaria vectors in Africa and the available evidence on the entomological and epidemiological impact of this resistance are also reviewed.

2. Resistance Mechanisms

Typically two major mechanisms are assumed to be responsible for insecticide resistance: changes in the target site that reduce the binding of insecticides, and increases in the rate of insecticide metabolism that lower the amount of insecticide reaching the target site. Both of these resistance mechanisms are known to contribute to pyrethroid resistance in malaria vectors and are subjects of extensive research to determine their distribution and impact, and to develop improved methods of detection. Of these, target site resistance is best understood and molecular diagnostics to detect this resistance mechanism are now integrated into insecticide resistance monitoring strategies in some malaria control programmes [13, 14]. Metabolic resistance is more complex but recently advances have been made in identifying the key enzymes responsible for insecticide detoxification, paving the way for the development of molecular markers for this resistance mechanism. Although these two mechanisms clearly play a major role in conferring pyrethroid resistance, it is also important to consider other physiological or behavioural changes in the mosquito population that may impact on the efficacy of pyrethroid insecticides. Two potential mechanisms that have been long recognised, but whose importance in malaria vectors has been largely overlooked are cuticular and behavioural resistance.

Target site resistance

The pyrethroid insecticides (and the organochlorine insecticide DDT) target the voltage-gated sodium channel on the insects' neurones [15, 16]. Their binding delays the closing of the sodium

channel prolonging the action potential and causing repetitive firing, paralysis and eventual death of the insect. Alterations in the target site that cause resistance to insecticides are often referred to as *knockdown resistance* or *kdr* alleles in reference to the ability of insects with these alleles to withstand prolonged exposure to insecticides without being 'knocked-down'. Several mutations in the sodium channel have been associated with resistance to pyrethroids in a variety of insects [17]. One of the most common amino acid replacements, and so far the only residue associated with pyrethroid resistance in malaria vectors, is a substitution of the leucine residue found at codon 1014 with either phenylalanine (1014F) or serine (1014S). The putative insecticide binding site of the insect sodium channel has recently been predicted [18]. This major breakthrough helped confirm the importance of several candidate resistance mutations and predict other regions of the transmembrane protein where amino acid substitutions might reduce insecticide binding. Interestingly residue 1014 does not appear to interact directly with the insecticide but is predicted to alter channel activation kinetics [18].

The relationship between the *kdr* genotype and the resistance phenotype in malaria vectors has recently been extensively reviewed and will not be discussed in detail here [19]. In summary it is very clear that *kdr* is associated with resistance to pyrethroids and DDT but it is not evident that the presence of this resistance allele alone is sufficient to result in control failure.

Metabolic resistance

Metabolic resistance occurs when elevated activities of one or more enzymes results in a sufficient proportion of the insecticide being sequestered or detoxified before it reaches the target site to impair the toxicity of the insecticide. The cytochrome P450s are the primary enzyme family responsible for pyrethroid metabolism [20]. There are 111 P450 enzymes in *An gambiae* [21] and, as in other insects, only a small number of these enzymes are capable of detoxifying insecticides. Identifying the enzymes responsible has been facilitated by microarray based approaches to detect detoxification genes that are over expressed in resistant mosquitoes compared to susceptible populations from the same region [22]. This approach has identified three 'candidate' P450 enzymes that were found to be repeatedly over expressed in pyrethroid resistant populations:

CYP6M2, CYP6P3 and CYP6Z2 [23-25]. Functional characterisation of these enzymes has shown that they are all able to bind to pyrethroid insecticides but only two of these, CYP6P3 and CYP6M2 can metabolise the insecticide [24, 26]. It is possible that elevated expression of an enzyme that can bind but not detoxify the insecticide may result in resistance by reducing the total bioavailability of insecticide (as is seen with over expression of carboxylesterases in organophosphate resistant populations of Culex mosquitoes [27] provided that the enzymes are expressed in an appropriate tissue. Functional genomics approaches to demonstrate gain or loss of the resistance phenotype resulting from the over expression or silencing of these enzymes are needed to conclusively demonstrate their role in resistance. Nevertheless the observation that the two enzymes whose expression most strongly correlates with resistance are both capable of detoxifying pyrethroids strongly implicates these enzymes in conferring the resistance phenotype. Interestingly recent studies in *An funestus* have identified the putative ortholog of *An gambiae* CYP6P3, CYP6P9, as being the prime candidate for conferring pyrethroid resistance in this species [28, 29].

Other enzyme families may also play a secondary role in pyrethroid resistance by, for example protecting from the oxidative stress induced by pyrethroid exposure [30], detoxifying secondary products of P450 based metabolism [31], or by binding insecticides to lower the total *in vivo* concentration of insecticide [32]. The contribution that these enzymes make towards the pyrethroid resistance phenotype in malaria vectors is yet to be elucidated.

The absence of molecular markers for metabolic resistance makes it difficult to directly assess the impact of this resistance on control. The situation is complicated by the co-occurrence of *kdr* and metabolic resistance in many vector populations. However the population of malaria vectors that has proved most intransigent to control with pyrethroid insecticides is *An funestus* from Southern Mozambique [33]. As mentioned above, there is no evidence of target site resistance in this population and instead resistance appears to be caused by the massive over expression of a small number of P450 enzymes [29, 34]. Extensive efforts are underway to identify the causal mutations associated with metabolic resistance to pyrethroids in the major malaria vectors [35].

Cuticular resistance

Reduced uptake of insecticide, often referred to as cuticular resistance, is frequently described as a minor resistance mechanism [36]. Certainly for pests where the major route of insecticide delivery is via ingestion, this is likely to be the case. However for malaria control, where insecticides are typically delivered on bednets or on wall surfaces, uptake of insecticides is primarily through the appendages. Earlier experiments with DDT have shown that the hind legs make the greatest contact with insecticide treated surfaces and removal of this pair of legs reduces mortality in DDT susceptibility tests [37]. Hence an increase in the thickness of the tarsal cuticle, or a reduction in its permeability to lipohilic insecticides, could have a major impact on the bioavailability of insecticide *in vivo*. Intriguingly microarray experiments have identified two genes, *cplcg3* and *cplcg4,* encoding cuticular proteins that are up-regulated in pyrethroid resistant strains of Anopheles mosquitoes from three populations and two species [25, 38, 39]. Clearly much more work needs to be done to identify the significance of cuticular resistance. A better understanding of the processes involved in insecticide uptake could be translated into improvements in insecticide formulations to help overcome pyrethroid resistance.

Behavioural resistance

There have been several anecdotal reports of mosquitoes changing their behaviour as a result of intensive indoor use of insecticides but there is currently insufficient data to assess whether these behavioural avoidance traits are genetic or adaptive responses [40, 41]. Genetic changes in the malaria vector population that shifted feeding or resting behaviour to minimise contact with insecticides in the indoor environment could have a very dramatic impact on the efficacy of current malaria vector control interventions, potentially exceeding the impact of physiological resistance. Scale-up of ITNs and/or IRS could increase the importance of outdoor transmission of malaria and necessitate new tools to target exophagic or exophilic malaria vectors. There is a clear need for robust controlled studies to assess the importance of behavioural resistance.

Interactions between resistance mechanisms

Understanding patterns of cross resistance caused by alternative mechanisms is vital to the implementation of effective resistance management strategies. It is generally assumed that resistance renders the selecting insecticide, and all others with a similar mode of action, ineffective. For example, the high frequency of *kdr* mutations in malaria vectors is often attributed to extensive past use of DDT to control agricultural pests in Africa [42]. This assumption may hold true for target site resistance; it is clear that selection with DDT can cause cross resistance to pyrethroids and *vice versa*. However this may not necessarily be the case for metabolic resistance mechanisms. CYP6P3 and CYP6M2 are efficient at detoxifying pyrethroids but do not metabolise DDT (Paine and Stevenson, personal communication). Furthermore, some P450 enzymes show specificity for either type I or type II pyrethroids [43]. In the alternative scenario, metabolic resistance may confer resistance to more than one class of insecticides. For example, a pyrethroid resistant strain of *An funestus* from Mozambique shows cross resistance to carbamate insecticides and synergist data suggests that cytochrome P450s are responsible for both phenotypes [34]. Whether the same enzyme is capable of metabolising both insecticide classes, is currently unknown but this information is clearly of key importance if rotations or mixtures are to be employed as part of a resistance management programme. Bioassays using synergists should be utilised to elucidate the full spectrum of cross resistance prior to implementation of any resistance management strategies.

3. Detecting and reporting resistance

Regular monitoring for insecticide resistance is essential in order to react proactively to prevent insecticide resistance from compromising control. If the frequency of resistance alleles is allowed to build up unchecked, resistance may eventually become 'fixed' in the population as initial detrimental effects on the insect's fitness are overcome by compensatory mutations. Once resistance reaches very high levels, strategies to restore susceptibility are unlikely to be effective.

Bioassays

Currently most resistance monitoring is dependent on bioassays, using fixed insecticide concentrations and exposure times, and the data is reported as percentage mortality. The World Health Organisation (WHO) has defined diagnostic doses for most of the insecticides used in malaria control, and produces susceptibility test kits consisting of exposure chambers and insecticide treated filter papers. Guidelines for the test procedures and interpretation of results are available from WHO [44]. It is important that the mosquitoes used for the bioassays are standardised for age, sex and physiological status as all of these can affect the outcome of the tests. Typically either adults raised from isofemale lines or F1 progeny from field collected blood fed females are used. The limitations and advantages of these two alternatives have recently been discussed [45].

These diagnostic dose assays are simple to perform and provide standardised data sets that, assuming the guidelines are followed, can be readily compared to identify temporal and/or geographical variations in the resistant status of malaria vector populations. However it is important to recognise some of the limitations of these susceptibility tests. As only a single concentration of insecticide is used, the results do not provide any information about the level of resistance in a population. For example if 50 % of population A and 20 % of population B were killed after exposure to the diagnostic dose of permethrin, it cannot be concluded that population B is *more* resistant than population A. The results only indicate that both populations are resistant (according to WHO definitions if there is < 80 % mortality the population is defined as resistant) and that, subject to tests of significance, there is a higher frequency of resistant individuals in population B than in A. Dose response assays would be needed to compare the levels of resistance in two populations (e.g. by measuring the Resistant Ratios and their 95% confidence intervals). For pyrethroids, median knock down time (MKDT) is also a useful quantifiable variable [46]. Similarly, the results of these tests cannot be used to compare the levels of resistance to two different insecticides. If 50 % mortality was observed after exposure to the diagnostic dose of permethrin (0.75 %) whereas mortality was 70 % after exposure to the diagnostic dose of deltamethrin (0.05%), it is not correct to state that the population is more resistant to permethrin

than deltamethrin. Again, all that can be stated is that the population is resistant to both insecticides.

Partly due to the limitations of the diagnostic dose assays described above and partly due to the difficulties that are sometime incurred in obtaining a regular supply of the insecticide impregnated papers from WHO, an alternative bioassay methodology has been developed [47] and is being adopted by some monitoring programmes. This method, known as the CDC bottle bioassay, uses glass bottles coated with a known concentration of insecticide. As these test kits are assembled in the users own laboratory the concentration of insecticide can be readily adjusted enabling dose response curves to be developed to compare two or more strains. A caveat to this is that the flexibility, and the potential variation in the insecticide grade used in the tests, impairs comparison of results between two separate studies.

Both WHO diagnostic doses and CDC bottle bioassays can be modified to incorporate synergists. Synergists such as piperonyl butoxide, that block the activity of two major detoxification enzyme families, can be used to explore the role of different resistance mechanisms [48]. If resistance is due to increased metabolism, exposure to an appropriate synergist prior to insecticide bioassays should increase the level of mortality observed.

Biochemical tests

Biochemical tests to detect alterations in activities of enzyme families associated with insecticide resistance have been available for over two decades and are sometimes used in combination with insecticide bioassays [49]. These assays employ model substrates to record the overall activity of glutathione transferases, carboxylesterases or cytochrome P450s in individual insects. Biochemical assays are also available to detect target site resistance to organophosphate and carbamate insecticides caused by insensitive acetylcholineseterase. The enzymatic reaction produces a colour change that is generally visible to the naked eye and hence these assays do not require access to expensive equipment. However, it is important that the mosquitoes are kept on ice from the point of collection to the performance of the assay and this can often pose logistical challenges. Furthermore there are sensitivity and specificity issues that limit the utility of some of

these assays. For example, with over 100 different cytochrome P450 enzymes in malaria vectors, an assay that measures the total level or activity of this enzyme family may not have the sensitivity to detect over expression of the single or small number of P450 enzymes that are thought to be involved in pyrethroid metabolism. This may explain the lack of significant correlation observed in many studies between cytochrome P450 activity and bioassay mortality results [50, 51]. In addition not all members of the enzyme family will have the same affinity for the model substrates used in these assays (e.g. CDNB (1-chloro 2-4, dinitrobenzene) is the substrate typically used to assess glutathione transferase activity but the Epsilon class of GSTs which are responsible for DDT resistance have relatively low activity with this substrate).

Knowledge of the enzymes responsible for insecticide detoxification should be translated into improvements in these biochemical assays. Indeed several new substrates for GSTs and P450 enzymes specifically implicated in resistance are showing promising results in laboratory trials (Paine and Vontas, personal communication)

Molecular tests

A multitude of molecular assays have been developed to detect *kdr* alleles in malaria mosquitoes, several of which were recently compared in a study by Bass et al [52]. These are routinely used by research laboratories monitoring for insecticide resistance and are gradually being incorporated into some national malaria control resistance monitoring programmes. Unfortunately, despite the recent identification of the key enzymes responsible for metabolic resistance to pyrethroids in *An gambiae* and *An funestus,* there are currently no simple DNA based assays to detect these resistance mechanisms. Detection of these genes is presently dependent on RNA based approaches using relatively sophisticated equipment (e.g. Real Time PCR).

Assays to detect the genetic mutation(s) responsible for the resistance phenotype in individual insects can provide an early warning of the emergence of resistance which may not have been detectable by bioassays that can only record the population response. The presence of a single individual with an allele known to confer resistance should be cause for concern as experience dictates that resistance can spread very rapidly in a population unless the selection pressure is

eased and/or the genetic cost associated with the resistant allele is high. Conversely, a negative result from a molecular assay should not lead to complacency. As discussed above, molecular assays are presently only available for target site resistance and the failure to detect *kdr* clearly cannot be interpreted as an absence of resistance in a population. Hence molecular assays should be seen as a complement rather than a substitute for bioassays.

Standardisation and reporting

In order to incorporate data from resistance monitoring into evidence based decisions on appropriate insecticide based interventions for malaria control it is clearly essential that the data is both reliable and accessible. There are guidelines already in place for minimum sample size per assay but there is little guidance on the number of sites and frequency with which resistance monitoring should occur [53]. It is clear that resistance is a dynamic trait, and wide fluctuations in resistance levels throughout the malaria transmission season have been reported [45, 54]. Resistance can also be very focal, particularly when vector composition differs between sites [55], hence a minimum number of sampling sites should be established, taking into account patterns of vector distribution and insecticide usage.

The WHO/AFRO African Network for Vector Resistance was established in 2000 and amongst its objectives was the important goal of improving the dissemination of resistance data. Accordingly a database was established to store the results of resistance monitoring activities by ANVR members but until recently, this database was not readily accessible by outside users. The recent establishment of IRBase [56] as an online centralised resource for collating data on insecticide resistance in disease vectors, and the integration of this with the ANVR database, will hopefully ensure that both published and unpublished data on resistance in malaria vectors is more readily available to all interested parties.

4. Current status of pyrethroid resistance in malaria vectors

The maps in Figure 1 summarise the current published literature on the distribution of pyrethroid resistance and their underlying mechanisms in malaria vectors in Africa. Resistance data was available for 33 out of 49 African countries investigated (Figure 1A). As pointed out previously [12], there are clusters of data corresponding to locations where well trained groups of entomologists play a major role in resistance monitoring. West Africa is the most documented part of the continent, whereas there is a severe lack of information from central Africa. In West Africa, pyrethroid resistance was first reported in Cote d'Ivoire in 1993 [57] and later in Benin [58], Burkina Faso [59], Ghana [60], Mali [61], Niger [62], Nigeria [63], Sierra Leone [64] and Togo [65]. Susceptibility to pyrethroids is still found in *An gambiae s.l.* in Senegal [66], Guinea Bissau [67] and Guinea Conakry [68] although the L1014F mutation has been detected in these three countries (fig 1b) . In Central Africa, pyrethroid resistance is found in *An gambiae s.l* in Cameroun [69, 70], Chad [45, 71], Gabon [72] and Equatorial Guinea [14]. In the East, *An gambiae s.s.* and *An arabiensis* populations were found mostly susceptible in Tanzania [73], Mozambique [74] and Madagascar [75] but pyrethroid resistance has been reported in eastern Uganda [76, 77], the Gwave region of Zimbabwe [50] and in Sudan [78, 79].

There is a paucity of published data on insecticide resistance in *An funestus.* With respect to pyrethroid resistance*,* most of the available literature originates from South Africa [80-82] and Mozambique [81, 83, 84]. In other places, suspected resistance was reported in Obusi and Kassena-Nankana regions from Ghana [85, 86] whereas full susceptibility to permethrin and deltamethrin was found in Southern Burkina Faso [87] and Tanzania [73]. There is also a deficit of data on resistance status in secondary malaria vectors in Africa. Full susceptibility to pyrethroids has been reported in *An labranchiae* in Morocco [88] and in *An pharoensis* in Egypt [89] and Ethiopia [90] but there is little information available on resistance levels in other vectors such as *An moucheti* and *An nili*.

The current understanding of the distribution of *kdr* alleles is shown in Figure 1B. The 1014F mutation was first detected in *An gambiae* from Cote d'Ivoire [91]. Subsequently 1014S was

detected in *An gambiae* from Kenya [92]. This somewhat misleadingly led to these two mutations being referred to as *kdrWest* and *kdrEast*. It is now clear however that these alleles are not restricted to either side of the Continent. In *An gambiae s.s.* the 1014F mutation has been detected as far East as Uganda [93] and the 1014S mutation has been found in Angola [94] and several countries in central Africa [14, 72, 95] (Figure 1B). There have also been several reports of 1014F/1014S hybrids [69, 96, 97] mainly in the molecular S form [77, 97-99].

It is thought that there have been at least four independent origins of 1014F in the *An gambiae* S-form [96]. The appearance of the L1014F mutation in the M form may be a result of introgression from the S form [100, 101] or from independent mutation events, as recently suggested in Bioko island [102]. The L1014S allele is predominately found in the S form [94] but has been recently found in the M form in Guinea Equatorial [103] and Cameroon [97].

Both 1014F and 1014S alleles have been detected in the sister taxa, *An arabiensis* [66, 79, 104]. To date *kdr* does not appear to have arisen in *An funestus* with both genetic mapping and direct sequencing of the sodium channel in pyrethroid resistant populations failing to find any evidence of target site resistance in this species [105]. Outside of Africa, *kdr* has been found in several malaria vectors including *An stephensi* and *An culicifacies* [106, 107]

The absence of simple genetic markers for metabolic resistance means that far less is known about the distribution of the responsible alleles. Biochemical assays, and in some cases microarray studies, have implicated metabolic resistance In *An gambiae s.l.* in Kenya [22, 108], Cameroun [109, 110], Benin [25], Nigeria [25], Ghana [23], Mozambique [74], South Africa [111] and Zimbabwe [50]. Over expression of CYP6P3 and/or CYP6M2 has been found in pyrethroid-resistant *An gambiae* populations from Benin, Nigeria and Ghana [23, 25], mainly in co-association with the *kdr* L1014F allele. This co-occurrence of resistance genes may constitute an additional threat to malaria vector control as epistasis between these two types of resistance conferred extremely high level of pyrethroid resistance in *Culex quinquefasciatus* [112].

5. Entomological and epidemiological impact of pyrethroid resistance

There have been very few studies that have assessed the epidemiological impact of insecticide resistance and interpreting data from such studies is complicated by the large number of confounding factors. Probably the clearest example of control failure being directly attributed to pyrethroid resistance was reported from the borders of Mozambique and South Africa. In 1996 the malaria control programme in KwaZulu Natal switched from using DDT to deltamethrin for indoor spraying [113]. Within four years, notified malaria cases had increased approximately four fold and *An funestus,* which had previously been eradicated, had re-appeared and was observable emerging alive from pyrethroid sprayed houses. Bioassays showed that this species was resistant to pyrethroids but susceptible to DDT [33]. The decision to revert to IRS with DDT was accompanied by a decline in malaria cases by 91% [114, 115].

On the island of Bioko on the West African coast, an IRS campaign with lambdacyalothrin failed to curtail an increase in the population density of pyrethroid resistance *An gambiae* M form although a modest but significant reduction in transmission index and malaria reported cases was observed [14, 116]. High frequencies of the L1014F *kdr* allele were observed in the local *An gambiae* population (M form). Only after pyrethroids were replaced with the carbamate bendiocarb did the mosquito population decline [14]. Nevertheless, in an operational scale programme such as this, the possible contribution of other factors to the failure of pyrethroid IRS to control mosquito population density cannot be overlooked so the direct consequence of the high *kdr* frequency is uncertain.

Another programmatic study was conducted in the highland provinces of Burundi, where a vector control programme combining IRS with pyrethroids and ITNs was initiated in 2002 one of the most malaria affected island provinces, Karuzi. Here the interventions significantly reduced Anopheles density by 82% and transmission intensity by 90% and occurrence of clinical episodes by 43% in children despite high frequencies of the L1014S allele in the local S form of *An gambiae* s.s. [117-119]. Since the authors reported an increase in *kdr* allele frequency in both treated and untreated groups [118], the origin of the selection pressure is difficult to appraise.

There have been extensive randomized controlled trials (phase III) in Africa aiming at investigating the efficacy of ITNs for malaria prevention [120], but very few have assessed how pyrethroid resistance might affect the effectiveness of such intervention. In the Korhogo area in the north of Côte d'Ivoire where the 1014F *kdr* allele frequency is >90% [121] and malaria is endemic, lambdacyhalothrin-treated nets had a significant impact on the entomological inoculation rate (55% reduction) [122] and on malaria incidence in children < 5 (56% reduction of clinical attacks) compared to a control group having no nets [123]. This was the first clear-cut evidence of ITNs continuing to provide effective personal protection against malaria in an area with a very high frequency of *kdr* in the vector population. However, the absence of a physical barrier in the control group may have overestimated the impact of pyrethroid treated nets against *kdr* mosquitoes in this study.

In southern Benin, a randomized controlled trial was carried out in a mesoendemic area to assess the impact of long lasting ITN scale-up on malaria morbidity in children <5. In this area, where the *kdr* frequency is around 50 % in *An gambiae,* transmission increased during the rainy seasons but was not followed by a seasonal variation in parasite infection and clinical incidence, suggesting that ITNs still keep certain level of efficacy in moderate pyrethroid resistance area [124].

Other smaller scale studies have assessed the impact of resistance on entomological parameters, using experimental huts, with very variable results. An early experimental hut trial of ITNs in Côte Ivoire showed no apparent difference in the effectiveness of ITNs between two adjacent sites with resistant and susceptible populations of *An gambiae* [125, 126]. By contrast, a comparative study of the efficacy of lambdacyhalothrin used for IRS or net treatment in Benin indicated a major loss of efficacy associated with pyrethroid resistance in *An gambiae* at Ladji, in southern Benin compared to Malanville in the north where the vectors remain largely susceptible to pyrethroids [127].

One of the problems associated with many of these studies is that, due to the lack of molecular markers for alternative resistance mechanisms, the frequency of *kdr* alleles is frequently used as a proxy for resistance. This can be misleading if metabolic or other resistance mechanisms are the predominant resistance mechanism. There is an urgent need for properly controlled large-scale

trials to assess the impact of pyrethroid resistance on IRS and ITNs. Such studies should use both entomological and epidemiological indices and should be conducted in areas where alternative resistance mechanisms are known to be responsible for pyrethroid resistance. Furthermore, these studies must consider the possibility of behavioural resistance and monitor for changes in key traits such as location of resting and feeding which may impact on the efficacy of current insecticide based interventions.

6. Conclusions

Pyrethroid resistance, as measured by conventional bioassays, is clearly widespread in malaria vectors across Africa. Molecular studies tracking the frequency of insecticide resistance alleles have shown very dramatic increases in the frequency of these alleles in *An gambiae* in recent years presumably reflecting the increased selection pressure on malaria vectors.

However, the primary source of this selection pressure is still unclear although evidence is accumulating to incriminate the agricultural use of insecticides in selection for insecticide resistance in malaria vectors. For example, seasonal fluctuations in results from WHO bioassays in major agricultural regions of Sudan and Cameroon reflect the timing of application of insecticides to the key crops in the region [45, 54]. In addition several reports have documented a higher incidence of insecticide resistance in areas of intensive agriculture than found in comparable non-agricultural sites [58, 59, 128]. There are also examples where use of pyrethroids for malaria control appears to be the primary factor in selecting for resistance [62, 129, 130]. Other sources of selection pressure including the use of consumer products such as coils or aerosols, or selection due to other toxins permeating mosquito breeding sites may also be contributing to the resistance phenotype [25, 131].

Uncertainty over the major causes of insecticide resistance is an impediment to the introduction of resistance management strategies. Enthusiasm for resistance management may also be hindered by the paucity of reliable data on the impact of resistance on current interventions, as discussed above, and this must clearly be a priority for further research. Nevertheless, the rapid increase in pyrethroid resistance and the increasing selection pressure from a wide range of sources

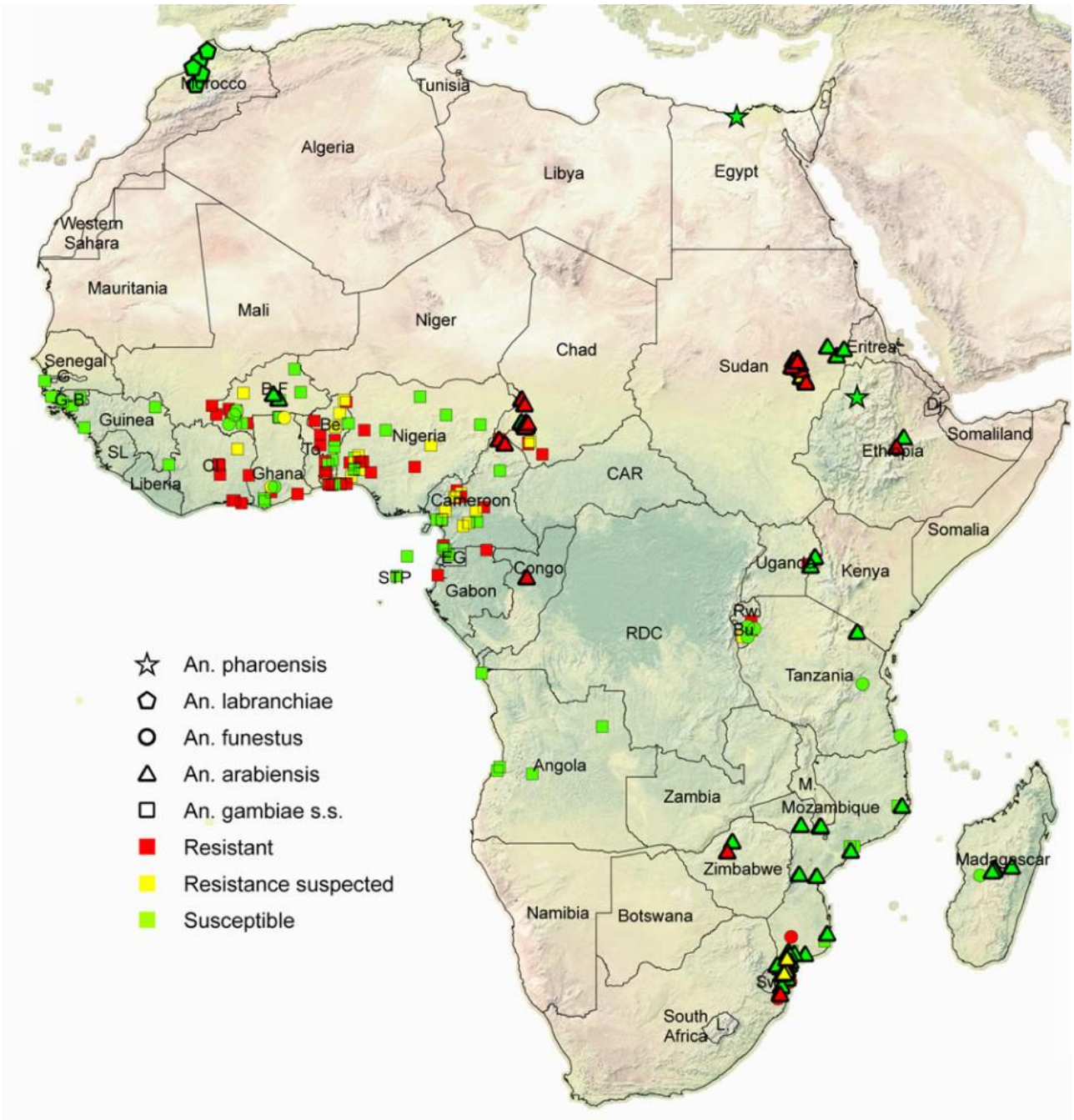
necessitates an immediate proactive response to resistance management to avoid compromising existing effective interventions. Resistance management is challenging when only a single chemical class is recommended for a particular application, such is the case of bednets. Even for IRS, with only four insecticide classes currently available and resistance reported to all four of these in some populations of *An gambiae* [45], the options for managing resistance and providing sustainable vector control with existing chemicals are limited.

Alternative insecticide classes (e.g. pyrroles, oxadiazines) used to control agricultural pests have recently proven effective in laboratory and field trials against pyrethroid-resistant mosquitoes but unfortunately these are not currently available in suitable formulations and some of them may never be mass-produced for malaria control [132-134]. Initial results from studies combining unrelated insecticides on mosquito nets [126, 135, 136] or insecticide with synergists or repellents [137-139] to delay the appearance of resistance or further prevent its development are encouraging. At larger scale, a randomized controlled trial carried out in an area with insecticide resistance in southern Benin (REFS-MAE initiative), showed a significant reduction of transmission and malaria incidence in children <5 by combining spatially different interventions in the household (e.g. ITN plus carbamate treated plastic sheeting) compared to the control group (i.e. selective coverage of ITN to populations at risk) [124]. Nevertheless, more work is needed on the durability of these approaches and their acceptability by populations before the additional cost can be justified to control programme managers.

The future is not all bleak. The Innovative Vector Control Consortium, established in 2005, is a public private partnership established to stimulate the search for alternative active ingredients or improved formulations of insecticides for vector control and several promising leads are now being evaluated in laboratory and field trials [140]. However, given the protracted regulatory procedures, it is likely to be many years before these new chemicals are an option for malaria control programmes. Hence it is vital that policy makers and programme implementers recognise the growing threat posed by insecticide resistance and strive to integrate resistance management into all control programmes. In addition, alternative, non-insecticidal methods should be encouraged, wherever feasible, to help reduce the reliance on pyrethroid insecticides.

Figure 1. Map of Africa showing the distribution of pyrethroid resistance (1A) and their underlying mechanisms (1B) in malaria vectors. To build these maps, an Insecticide Resistance (IR) database was generated using data colleted from 2000-2010. Sites were located using publications and the Geonames database of the National Geospatial-Intelligence Agency, and spatialized using the ESRI ArcGis Software. Resistance was defined according to standard WHO definitions, as described by WHO [44]. Metabolic resistance data were incorporated when the field populations showed significantly higher enzyme activity and/or gene expression level than that of the reference susceptible strain (using biochemical assay and/or detox chip microarrays). Target site resistance is recorded wherever the alleles 1014F and 1014S *kdr* are found although it is recognised and do not reflect allelic frequency.

Figure 1A


Morocco
Tunisia
Algeria
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Egypt
Western Sahara
Mauritania
Mali
Niger
Chad
Sudan
Eritrea
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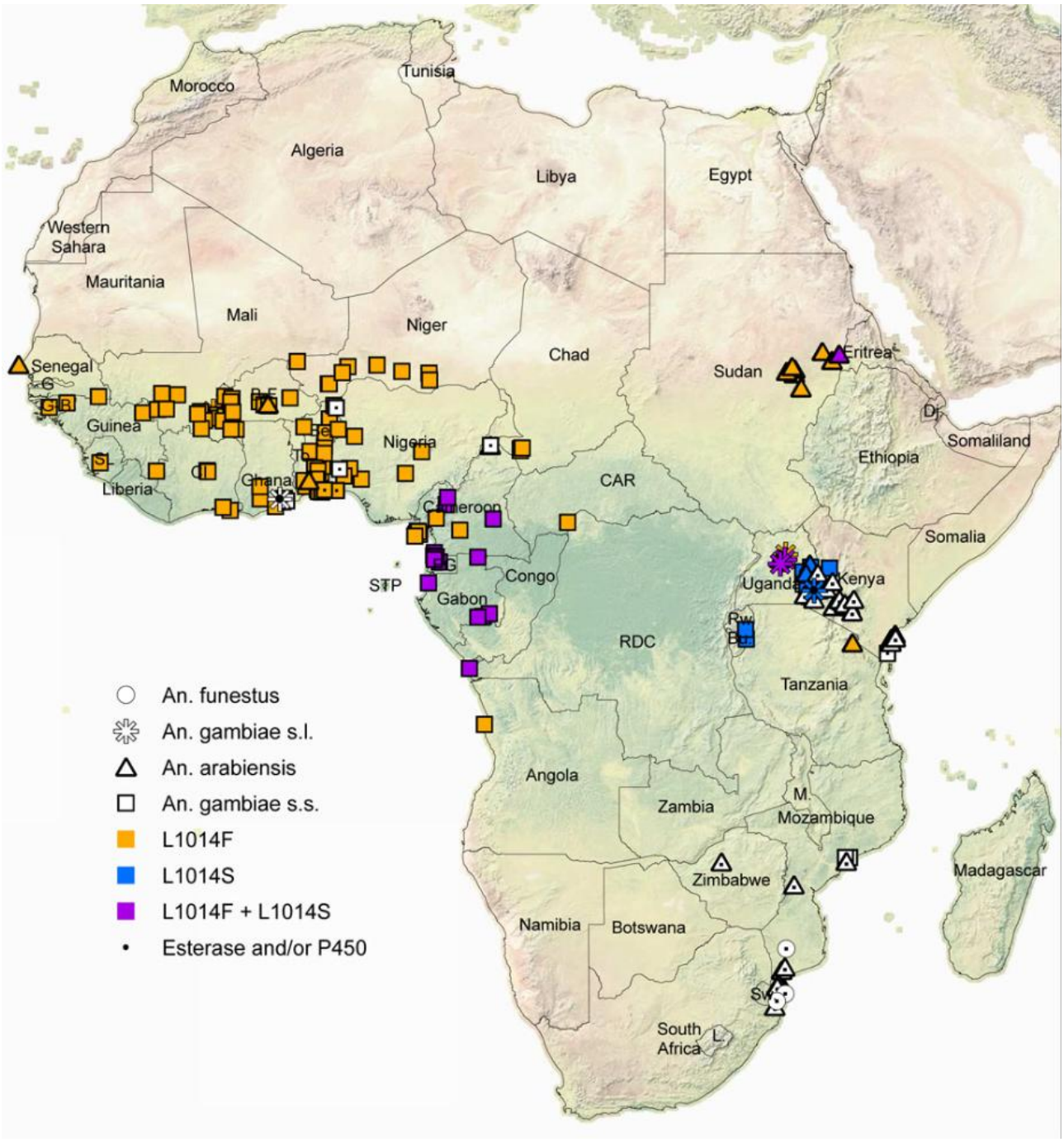
Nigeria
Ethiopia
Somaliland
Liberia
Ghana
CAR
Cameroon
Somalia
Congo
Kenya
Gabon
RDC
Tanzania
Angola
Zambia
Mozambique
Zimbabwe
Namibia
Botswana
South Africa
Madagascar
An. pharoensis
An. labranchiae
An. funestus
An. arabiensis
An. gambiae s.s.
Resistant
Resistance suspected
Susceptible

Figure 1B


Morocco
Tunisia
Algeria
Libya
Egypt
Western Sahara
Mauritania
Mali
Niger
Chad
Sudan
Eritrea
Senegal
Guinea
Nigeria
Ethiopia
Somaliland
Liberia
Ghana
CAR
Cameroon
Somalia
STP
Congo
Gabon
Uganda
Kenya
RDC
Tanzania
Angola
Zambia
Mozambique
Zimbabwe
Madagascar
Namibia
Botswana
South Africa
An. funestus
An. gambiae s.l.
An. arabiensis
An. gambiae s.s.
L1014F
L1014S
L1014F + L1014S
Esterase and/or P450

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