Distribution and Frequency of \textit{kdr} Mutations within \textit{Anopheles gambiae} s.l. Populations and First Report of the \textit{Ace.1G119S} Mutation in \textit{Anopheles arabiensis} from Burkina Faso (West Africa)

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**Abstract**

An entomological survey was carried out at 15 sites dispersed throughout the three eco-climatic regions of Burkina Faso (West Africa) in order to assess the current distribution and frequency of mutations that confer resistance to insecticides in \textit{An. gambiae} s.l. populations in the country. Both knockdown (\textit{kdr}) resistance mutation variants (L1014F and L1014S), that confer resistance to pyrethroid insecticides, were identified concomitant with the \textit{ace-1} G1195 mutation confirming the presence of multiple resistance mechanisms in the \textit{An. gambiae} complex in Burkina Faso. Compared to the last survey, the frequency of the L1014F \textit{kdr} mutation appears to have remained largely stable and relatively high in all species. In contrast, the distribution and frequency of the L10145 mutation has increased significantly in \textit{An. gambiae} s.l. across much of the country. Furthermore we report, for the first time, the identification of the \textit{ace-1} G116S mutation in \textit{An. arabiensis} populations collected at 8 sites. This mutation, which confers resistance to organophosphate and carbamate insecticides, has been reported previously only in the \textit{An. gambiae} S and M molecular forms. This finding is significant as organophosphates and carbamates are used in indoor residual sprays (IRS) to control malaria vectors as complementary strategies to the use of pyrethroid impregnated bednets. The occurrence of the three target-site resistance mutations in both \textit{An. gambiae} molecular forms and now \textit{An. arabiensis} has significant implications for the control of malaria vector populations in Burkina Faso and for resistance management strategies based on the rotation of insecticides with different modes of action.

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**Introduction**

The pyrethroid class of insecticides have become a mainstay for vector control since the ban of DDT due to off-target toxicity and the development of resistance. They have been most widely used to treat bed nets (ITNs) dedicated to personal and community protection [1,2,3]. Unfortunately, knock down resistance (\textit{kdr}) to pyrethroids, which also confers cross-resistance to DDT, was first reported in \textit{Anopheles gambiae} populations from Côte d’Ivoire [4]. Resistance likely resulted from the earlier intensive use of DDT and selection from pyrethroid use in crop protection particularly in cotton areas [5,6]. \textit{kdr} was initially shown to result from a point mutation (L1014F) in the pyrethroid target protein the voltage-gated sodium channel [7]. Based on a simple PCR diagnostic developed in the first report of the \textit{kdr} mutation [7] several studies have been carried out on the distribution and the frequency of this mechanism throughout Africa. Initial studies showed that L1014F \textit{kdr} was most widely distributed in West African \textit{An. gambiae} s.l. populations [6,8,9]. This mutation was observed initially in the S molecular form of \textit{An. gambiae} s.s. reaching high frequency but was not found either in sympatric mosquitoes of the M molecular form or \textit{An. arabiensis} populations [5]. This provided further evidence of reproductive barrier between the M and S molecular forms [10,11] and the two molecular forms of \textit{An. gambiae} s.s. were recently confirmed as two distinct species termed \textit{Anopheles coluzzii} for the M form and \textit{Anopheles gambiae} for the S form [12]. However, a few years after the initial finding of the \textit{kdr} mutation in the S molecular form, this mutation was also reported in the M form from the littoral of Benin and Côte d’Ivoire [13]. In-depth investigations carried out later in these geographic regions confirmed that this phenomenon was frequently observed in littoral but was rare inland [11]. DNA sequencing of these mosquitoes suggested that the mutation emerged in the M form by
genetic introgression from the S form [14,15]. In contrast, the emergence of the Leu-Phe kdr mutation within Anopheles arabiensis resulted from a de novo mutation event [13]. An extensive monitoring program in Burkina Faso has revealed that the L1014F kdr mutation initially detected in low frequency in the An. gambiae M molecular form and An. arabiensis [11,13] has spread throughout the country and is observed in mosquito populations at relatively high frequency [16,17]. Recently the L1014S kdr, which initially predominated in East Africa [18,19], was reported in West Africa, first in Benin and then Burkina Faso within An. arabiensis populations [20,21]. More recently this mutation was reported in a small number of individuals of the M and S forms of An. gambiae in Burkina Faso [22]. Taken together these results provide fundamental insight into the evolutionary processes underlying resistance in Anopheles gambiae s.l. Furthermore from an applied perspective, the emergence of resistance has significant implications for vector control programmes, especially those focused on the use of ITNs/Long-Lasting Insecticidal Nets (LLINs) or indoor residual sprayings (IRS). Although LLINs had shown good control of certain pyrethroid resistant populations [23] reduced efficacy of treated nets against An. gambiae populations with kdr resistance has since been reported [24].

Other insecticides belonging to the organophosphate (OP) and carbamate (CM) classes have been investigated to be used in mosaic, or in combination, with pyrethroids for bednet impregnation [25]. In addition to the use of LLINs, bendiocarb was recently used in IRS applications in West Africa through the President's Malaria Initiative (PMI) roadmap [26]. Initially described in Culex populations from Côte-d'Ivoire [27] reduced susceptibility to OPs and CMs was observed in An. gambiae populations in the North of Côte d'Ivoire and related to the domestic use of insecticide [28]. An. gambiae populations from Benin with resistance to the CM bendiocarb were reported after just three year of IRS use [29]. A common mechanism of resistance to OP and CM insecticides results from a single point mutation (termed ace-1R) in the target protein the acetylcholinesterase enzyme [30]. This mutation results in a glycine to serine replacement at amino acid position 119 and can be detected by a simple PCR-Restriction Fragment Length Polymorphism (RFLP) diagnostic [31]. This approach has been used to examine the frequency and distribution of this mutation in Burkina Faso where it was found predominately in the An. gambiae S form and in low frequency in the M form [9,16,32]. A recent study suggested that the mutation had introgressed from one form to the other but the precise origin of the introgression could not be determined due to the small sample size [33]. Since then, extensive country-wide surveys were performed in Burkina Faso from 2008 to 2010 and no case of An. arabiensis carrying this mutation was reported, although sample sizes for this species were sometimes small [16,17]. However insecticide resistance may also occur by other physiological mechanisms such as metabolic detoxification through increased enzyme activities (monooxygenases, esterases, or glutathione S-transferases) [34,35].

Burkina Faso is composed of three agro-climatic areas which exhibit different patterns of insecticide use especially in relation to crop protection. The present study provides an update on the distribution and the prevalence of the kdr L1014F and L1014S and ace-1R mutations in An. gambiae s.l. populations throughout the 13 health regions dispersed across these different agro-climatic areas. We report here, for the first time, the occurrence of the ace-1R mutation at remarkably high frequencies in An. arabiensis.

Materials and Methods

Study sites

Burkina Faso covers three ecological zones, the Sudan savannah zone in the south and west where rainfall is relatively heaviest (5–6 months), the arid savannah zone (Sudan-sahelian) which extends throughout much of the central part of the country and the aridland (Sahel) in the north. The northern part of the country has a dry season of 6–8 months. The varied ecological conditions are reflected in the different agricultural systems practiced throughout the country, from arable to pastoral lands. The western region constitutes the main cotton belt extending to the south where some new cotton areas have been cultivated since 1996. All ecological zones support the existence of Anopheles species that vector malaria and the disease is widespread throughout the country. Larvae were sampled from 15 sites dispersed throughout the three ecological zones (Table 1). The GPS coordinates were incorporated in Table 1.

Mosquito sampling

Larvae of An. gambiae s.l. were collected from at least 10 breeding sites dispersed throughout each sampling site mainly comprising pools of standing water and other small water collections. Larvae were pooled to constitute a colony, which was reared in the inactivity to adulthood. A sample of 100 adult females were randomly sorted, killed and kept on silica gel in 1.5-ml tubes and stored at −20°C prior to PCR analysis. Anopheline species were identified morphologically using the standard identification keys of Gillies and Coetzee [36].

PCR analyses

An average of 30 mosquitoes was sampled per site by PCR analysis. Genomic DNA was extracted from single specimens and used as template for PCR to determine the species within the An. gambiae complex using the protocol SINE 200 of Santalomazza et al. [37] that allows the concomitant identification of An. gambiae M and S (respectively known as Anopheles coluzzii and Anopheles gambiae) and An. arabiensis. The same individuals were then tested for both the L1014F and L1014S kdr mutations using the protocols of Martínez-Torres et al. [7] (using specific primers Agd1, Agd2, Agd3 and Agd4) and Ranson et al. [18] (using Agd1, Agd2, Agd4 and Agd5) respectively:

- Agd1: 5'-ATAGATTCCCCGACGATG-3';
- Agd2: 5'-AGACAAGGATGATGAAACC-3';
- Agd3: 5'-AATTTGGATTACTTACGACA-3';
- Agd4: 5'-CTGTAGTGATAGGAAATTTTA-3';
- Agd5: 5'-TTTGCATTACTTACGACTG-3'.

The ace-1R mutation was detected from the same samples by PCR according to the protocol of Weill et al. [31] using specific primers Ex3Agdir (GATCGTGCCACCCGTGTTCCG) and Ex3-AGre (AGGATGCCGGCCTGGAAACACAG). Then the PCR products were digested using Alu 1 enzyme at 37°C for 3 hours.

Statistical analysis

Data were compared between ecological zones and pooled for each species to compare the genotypes frequency between An. gambiae species by Chi² tests. The genotypic frequencies of L1014F and L1014S and ace-1R in mosquito populations were compared to Hardy-Weinberg expectations using the exact test procedures implemented in GenePOP (ver.3.4) software [38].
Table 1. Distribution of *Anopheles gambiae* s.l. from 15 sites in Burkina Faso.

| Study sites     | Geographic references | Social environment | Climatic areas                     | Agricultural practices                     | Date of collection | *An. gambiae* s.l. | *An. gambiae* | *An. coluzzii* | *An. arabiensis* |
|-----------------|------------------------|--------------------|-----------------------------------|-------------------------------------------|-------------------|-------------------|---------------|---------------|-----------------|
|                 |                        |                    |                                   |                                           |                   | N                | n1 | %     | n2 | %     | n3 | %     |
| Gaoua           | 10°40'N; 3°15'W        | sub-urban          | Sudanian                          | cereals, cotton, old area                | 30/10/2012        | 43               | 39 | 90,69 | 1  | 32,33 | 3  | 6,98  |
| Banfora         | 10°40'N; 3°15'W        | sub-urban          | Sudanian                          | cereals, cotton, old area                | 09/07/2012        | 30               | 24 | 80,00 | 6  | 20,00 | 0  | 0     |
| Sindou          | 10°40'N; 3°15'W        | rural              | Sudanian                          | cotton, old area                        | 01/10/2012        | 35               | 24 | 68,57 | 6  | 17,14 | 5  | 14,29 |
| Orodara         | 10°40’N; 3°15’W        | sub-urban          | Sudanian                          | fruits, cotton, old area                | 23/19/2012        | 28               | 23 | 82,14 | 4  | 14,29 | 1  | 3,57  |
| Dioulassoba     | 10°40’N; 3°15’W        | traditional-urban  | Sudanian                          | swamp                                   | 23/11/2012        | 29               | 4  | 13,79 | 5  | 17,24 | 20 | 68,97 |
| Soumousso       | 10°40’N; 3°15’W        | rural              | Sudanian                          | cotton, old area                        | 30/12/2012        | 30               | 20 | 66,67 | 3  | 10,00 | 7  | 23,33 |
| Boromo          | 10°40’N; 3°15’W        | sub-urban          | Sudan-sahelian                    | cotton, old area                        | 08/10/2012        | 33               | 16 | 48,48 | 0  | 0     | 17 | 51,52 |
| Dédougou        | 10°40’N; 3°15’W        | sub-urban          | Sudan-sahelian                    | cotton, old area                        | 06/10/2012        | 30               | 12 | 40,00 | 2  | 6,67  | 16 | 53,33 |
| Koudougou       | 10°40’N; 3°15’W        | urban              | Sudan-sahelian                    | cotton, since 1996                     | 07/11/2012        | 37               | 19 | 51,35 | 5  | 13,51 | 13 | 35,14 |
| Nanoro          | 10°40’N; 3°15’W        | rural              | Sudan-sahelian                    | cereals                                | 09/07/2012        | 32               | 4  | 12,50 | 24 | 75,00 | 4  | 12,50 |
| Koupela         | 10°40’N; 3°15’W        | sub-urban          | Sudan-sahelian                    | cotton, since 1996                     | 06/10/2012        | 30               | 14 | 46,67 | 8  | 26,67 | 8  | 26,67 |
| Fada            | 10°40’N; 3°15’W        | sub-urban          | Sudan-sahelian                    | cotton, since 1996                     | 25/08/2012        | 60               | 19 | 31,67 | 27 | 45,00 | 14 | 23,33 |
| Kaya            | 10°40’N; 3°15’W        | sub-urban          | Sahelian                          | cereals, vegetables                    | 03/10/2012        | 32               | 15 | 46,88 | 5  | 15,63 | 12 | 37,50 |
| Ouahigouya      | 10°40’N; 3°15’W        | sub-urban          | Sahelian                          | cereals, vegetables                    | 08/10/2012        | 31               | 20 | 64,52 | 10 | 32,26 | 1  | 3,23  |
| Dori            | 10°40’N; 3°15’W        | sub-urban          | Sahelian                          | cereals, vegetables                    | 01/10/2012        | 33               | 12 | 36,36 | 5  | 15,15 | 16 | 48,48 |

N: number total of mosquitoes.

n1: number of *An. gambiae*.

n2: number of *An. coluzzii*.

n3: number of *An. arabiensis*.

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Ethical issues

Ethical approval was not required in this study. This study was not carried out on private land. For each, no permission was required for our study does not degrade the environment. No permission was required for these locations/activities as the field activities did not involve damaged of protected species. We did not use any vertebrate during this study.

Results

Out of 516 mosquitoes analysed in PCR, 513 successfully scored (less than 5% failure rate). Overall species composition of the collected mosquitoes comprised a higher proportion of An. gambiae (51.7%) than An. coluzzii (21.6%) and An. arabiensis (26.7%) (Table 1). The species repartition across the three ecological regions revealed that An. gambiae was the predominant species in all regions including, in the Sahel where it comprised more than 49% of the An. gambiae s.l. population. Anopheles arabiensis was the second most predominant vector found in samples collected from the three regions. Somewhat An. coluzzii was found at a relatively low proportion of less than 15%. The central areas were characterised by an overlapped repartition of the three species 38.4%, 27.81% and 33.75% for An. gambiae, An. coluzzii and An. arabiensis respectively and proportions did not differ significantly ($\chi^2 = 1.95$, df = 1, $P > 0.05$). In the Sahel region, An. gambiae also predominated (49.75%) and the proportions of the two other species did not differ significantly at 21.01% and 29.74% for An. coluzzii and An. arabiensis respectively ($\chi^2 = 4.88$, df = 1, $P > 0.05$).

The overall frequency of the L1014F mutation averaged 50% and did not significantly differ between species (Figure 1A) whatever the ecological zone (Figure 1B) ($\chi^2 = 0.14$, df = 1, $P > 0.05$) even though the highest values were observed in the sudan zone (Figure 2). However some deviation from Hardy-Weinberg expectations was observed within the An. arabiensis populations in Dedougou and Dori and within An. coluzzii populations in Fada.
Kaya, Ouahigouya and Dori with an excess of resistant homozygous alleles (Table 2). The same patterns were found in seven sites for *An. gambiae* (Gaoua, Banfora, Sindou in the West, Dedougou, Koudougou and Koupela in the central region and Ouahigouya in the Sahel) (*P* < 0.05).

The overall allele frequency of the L1014S *kdr* mutation (Figure 3) was relatively higher in *An. gambiae* (48%) followed by *An. coluzzii* (38%) and *An. arabiensis* populations (37%) with no significant difference between the last two (*χ^2* = 3.24, df = 1, *P* > 0.05) (Figure 1C). Comparing between ecological regions, L1014S *kdr* frequency did not differ significantly between species, except in the Sahel where it was significantly higher in *An. coluzzii* (*χ^2* = 3.24, df = 1, *P* = 0.04) and *An. gambiae* (*χ^2* = 9.79, df = 1, *P* < 0.001) in the Sudan and Sudan-sahelian savannah (with respectively *χ^2* = 6.89, df = 1, *P* < 0.008 and *χ^2* = 17.34, df = 1, *P* < 0.0003) (Fig. 1F). In the Sahel no significant difference was observed between the three species (*χ^2* = 0.89–0.021, df = 1, *P* > 0.05). The observed genotypic frequencies were significantly different from Hardy-Weinberg expectations at the 95% confidence level (Table 2) in *An. gambiae* population from Orodara, Soumousso, Koudougou, Fada, Ouahigouya, Dori and Dioulassoba, Koudougou and Kaya for *An. arabiensis* where a heterozygote deficit was observed (*P* = 0.005). Furthermore, the percentage of homozygous resistant individuals was significantly higher in *An. arabiensis* (25%) than in *An. gambiae* (6.25%). No homozygous resistant individual was recorded in *An. coluzzii* from any site.

**Discussion**

This study provides current information on the distribution of three members of the *Anopheles gambiae* complex across Benin and the frequency and distribution of three important target-site resistance mechanisms in these populations. In regards to the distribution of *An. gambiae* species throughout the country, the most significant finding is that *An. arabiensis* appears to be spreading in the Sudan whereas in the past it comprised only...
### Table 2. Allelic and genotypic frequencies at the kdr 1014F and 1014S locus in *An. gambiae* s.l. populations.

| Species          | Sites      | N  | Genotypes | Genotypes | Genotypes |
|------------------|------------|----|-----------|-----------|-----------|
|                  |            | 1014L | 1014L | 1014F | 1014F | f(L1014F) | [95%CI] | p(HW) | 1014L | 1014L | 1014F | f(L1014F) | [95%CI] | p(HW) |
| *An. arabiensis* | Gaoua      | 5   | 1     | 0     | 2     | 0.66  | 0.66 | [8.5–9.82] | -   | 0     | 2     | 0.66 | [8.5–9.82] | -   | 0.2000 |
|                  | Banfora    | 0   | 0     | 0     | 0     | -    | -   | -          | -   | 0     | 0     | 0.9  | -          | -   | -      |
|                  | Sindou     | 10  | 5     | 0     | 0     | 0    | -   | -          | -   | 1     | 4     | 0    | [7.38–9.18] | -   | -      |
|                  | Oroduara   | 1   | 1     | 0     | 0     | 0    | 0.4678 | 0    | 0     | 0     | 0.45 | -          | -   | -      |
|                  | Dioullassoba | 30  | 1     | 5     | 14    | 0.82 | 0.2308 | 2    | 8     | 0.42  | [2.34–3.38] | -   | 0.0033 |
|                  | Soumousso  | 11  | 1     | 1     | 5     | 0.78 | 0.0956 | 2    | 2     | 0.37  | [4.37–5.21] | -   | 0.2914 |
|                  | Boromo     | 17  | 2     | 3     | 12    | 0.79 | [3.42–5.00] | 0.000 | 6    | 3     | 0.28 | [2.31–3.35] | -   | 0.3405 |
|                  | Dédougou    | 23  | 6     | 0     | 10    | 0.62 | 0.1652 | 5    | 2     | -     | -    | 0.3213 |
|                  | Koudougou   | 13  | 2     | 3     | 8     | 0.73 | [3.9–5.36] | -   | 0     | 0     | 0.5  | [6.41–7.41] | -   | -      |
|                  | Nanoro      | 6   | 4     | 0     | 0     | 0    | 0.4406 | 0    | 2     | 0.5   | [4.39–5.39] | -   | 0.0857 |
|                  | Koupela     | 13  | 2     | 3     | 3     | 0.56 | [4.61–5.73] | -   | 2     | 3     | 0.53 | [4.39–5.39] | -   | 0.1795 |
|                  | Fada        | 25  | 6     | 5     | 3     | 0.39 | [2.87–3.65] | 0.0933 | 8    | 3     | 0.57 | [3.42–4.48] | -   | 0.9035 |
|                  | Kaya        | 17  | 4     | 3     | 5     | 0.54 | [3.61–4.69] | -   | 1     | 4     | 0.37 | [3.07–3.81] | -   | 0.0061 |
|                  | Ouashigouya | 1   | 0     | 1     | 0     | 0.5  | [3.32–4.32] | 0.0031 | 0    | 0     | 0    | [18.5–20.5] | -   | -      |
|                  | Dori        | 22  | 6     | 2     | 8     | 0.56 | [3.1–4.22] | -   | 2     | 4     | 0.26 | [2.32–2.84] | -   | 0.2260 |
| *An. coluzzii*   | Gaoua      | 1   | 1     | 0     | 0     | 0    | [18.5–20.5] | -   | 0     | 0     | 0    | [18.5–20.5] | -   | -      |
|                  | Banfora    | 7   | 0     | 1     | 5     | 0.91 | [6.69–8.51] | -   | 0     | 1     | 0.16 | [3.04–3.36] | -   | 0.0909 |
|                  | Sindou     | 12  | 1     | 1     | 4     | 0.75 | [6.15–9.35] | 0.2727 | 1    | 5     | 0.91 | [6.69–8.51] | -   | -      |
|                  | Oroduara   | 5   | 2     | 1     | 1     | 0.37 | 5.58–6.32 | 0.4286 | 1    | 0     | 0.12 | [3.27–3.51] | -   | -      |
|                  | Dioullassoba | 9   | 1     | 1     | 3     | 0.7  | [6.61–8.01] | 0.3333 | 1    | 3     | 0.7   | [6.61–8.01] | -   | 0.3333 |
|                  | Soumousso  | 4   | 2     | 1     | 0     | 0.16 | [4.36–4.68] | -   | 0     | 1     | 0.33 | [6.16–6.82] | -   | 0.2000 |
|                  | Boromo     | 0   | 0     | 0     | 0     | -    | -   | -          | -   | 0     | 0     | -    | -          | -   | -      |
|                  | Dédougou    | 3   | 1     | 1     | 0     | 0.25 | [6.67–7.17] | -   | 0     | 1     | 0.5  | [9.28–10.28] | -   | 0.6190 |
|                  | Koudougou   | 7   | 0     | 3     | 2     | 0.7  | [6.6–8.01] | 1    | 2     | 0     | 0.2  | [3.72–4.12] | -   | -      |
|                  | Nanoro      | 39  | 1     | 5     | 18    | 0.85 | [2.82–4.52] | 0.3983 | 12   | 3     | 0.37 | [2.06–2.8]  | -   | 0.3333 |
|                  | Koupela     | 9   | 3     | 5     | 0     | 0.31 | [3.54–4.16] | 1    | 1     | 0     | 0.06 | [1.64–1.76] | -   | 0.7446 |
|                  | Fada        | 46  | 7     | 7     | 13    | 0.61 | [2.33–3.55] | 0.4186 | 17   | 2     | 0.38 | [1.94–2.71] | -   | 0.0817 |
|                  | Kaya        | 8   | 2     | 3     | 0     | 0.6  | [6.17–7.37] | 0.0476 | 2    | 1     | 0.4   | [5.13–5.93] | -   | 0.3333 |
|                  | Ouashigouya | 17  | 4     | 0     | 6     | 0.6  | [4.19–5.39] | 0.017 | 2    | 5     | 0.6   | [4.19–5.39] | -   | 1      |
|                  | Dori        | 9   | 3     | 0     | 2     | 0.4  | [5.13–5.93] | 0.0476 | 1    | 3     | 0.7   | [6.61–8.01] | -   | -      |
| *An. gambiae*    | Gaoua      | 74  | 14    | 8     | 17    | 0.53 | [3.15–2.81] | 0.0002 | 0    | 35    | 0.92 | [2.12–3.96] | 1   | -      |
|                  | Banfora    | 29  | 7     | 7     | 10    | 0.56 | 2.43–3.55 | 0.0434 | 3    | 2     | 0.14 | [1.36–1.64] | 1   | 0.1518 |
|                  | Sindou     | 46  | 8     | 3     | 13    | 0.6  | [2.49–3.69] | 0.0003 | 5    | 17    | 0.81 | [2.78–4.44] | -   | 0.0611 |
| Species | Sites       | N   | Genotypes | Genotypes |       | Genotypes | Genotypes |
|---------|-------------|-----|-----------|-----------|-------|-----------|-----------|
|         |             |     | 1014L     | 1014L     | 1014F | f(L1014F) | [95%CI]   | p(HW)     |     | 1014L     | 1014L     | 1014F | f(L1014F) | [95%CI]   | p(HW)     |
|         |             |     | 1014F     | 1014F     |       |           |           |           |     | 1014F     | 1014F     |       |           |           |           |
|         |             |     | f(L1014F) | p(HW)     |       |           |           |           |     |           |           |       |           |           |           |
| Orodara | 33          | 5   | 7         | 11        | 0.63  | [2.6–3.86] | 0.0904    | 1         | 9   | 0.41      | [2.2–3.02] | 0.0420 |
| Dioulassoba | 8        | 0   | 1         | 3         | 0.87  | [823–997] | -         | 2         | 2   | 0.75      | [771–921]  | 0.3257 |
| Soumousso | 29         | 8   | 9         | 3         | 0.37  | [2.29–3.63] | 0.5690    | 5         | 4   | 0.32      | [2.16–2.8]  | 0.0000 |
| Boromo   | 25         | 8   | 7         | 1         | 0.28  | [2.31–2.87] | 0.7912    | 4         | 5   | 0.43      | [2.78–3.64] | 0.1201 |
| Dedougou | 19         | 5   | 0         | 7         | 0.58  | [3.72–4.88] | 0.0004    | 7         | 0   | 0.29      | [2.75–3.33] | 0.0150 |
| Koudougou | 26        | 9   | 2         | 8         | 0.47  | [2.61–3.55] | 0.0005    | 4         | 3   | 0.26      | [2.03–2.55] | 1      |
| Nanono   | 5          | 1   | 0         | 3         | 0.75  | [7.71–9.21] | 0.1429    | 0         | 1   | 0.25      | [4.64–5.14] | 0.1429 |
| Koupela  | 24         | 7   | 1         | 6         | 0.46  | [3.08–4.00] | 0.0013    | 4         | 6   | 0.57      | [3.37–4.51] | 0.0003 |
| Fada     | 30         | 3   | 9         | 7         | 0.6   | [2.87–4.07] | 0.6254    | 5         | 6   | 0.44      | [2.54–3.42] | 0.0473 |
| Kaya     | 19         | 5   | 7         | 3         | 0.43  | [2.88–3.74] | 0.5785    | 3         | 1   | 0.16      | [1.86–2.18] | 0.0000 |
| Ouahigoya | 30        | 10  | 3         | 7         | 0.42  | [2.84–3.25] | 0.0020    | 2         | 8   | 0.45      | [2.48–3.38] | 0.0632 |
| Dori     | 18         | 4   | 4         | 4         | 0.5   | [3.49–4.49] | 0.2300    | 1         | 5   | 0.55      | [4.03–5.13] | 0.0520 |

N: number of mosquitoes.
f(1014F): frequency of the kdr W resistant allele.
f(1014S): frequency of the kdr E resistant allele.
p(HW): probability of the exact test for goodness of fit to Hardy Weinberg equilibrium.
-: not determined.
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around 5% of the An. gambiae complex species [6]. Furthermore, this species is now present in Sindou at 14.29% (nearest the frontier of Cote-d’Ivoire) where it was absent a decade ago [9]. The reason for this is not clear but could be related to climatic changes, such as irregularities in rainfall observed in the boundaries of the Sudan region that may make the landscape more favourable to the establishment of this species.

Across sampling covering 15 sites we identified the L1014F and L1014S kdr mutations concomitant with the ace-1 G119S mutation confirming the presence of multiple resistance mechanisms in the An. gambiae complex in Burkina Faso [16,17]. The distribution and the prevalence of the L1014F kdr mutation in An. gambiae species including An. gambiae, An. coluzzii and An. arabiensis, has been well documented in Burkina Faso for over a decade [9,16]. Many studies reported this mutation at high frequency within An. gambiae and An. coluzzii populations especially in An. gambiae populations from the Sudan area where mutation frequency was approaching fixation [9,15,16]. Over recent years the frequency of this mutation has increased within both An. coluzzii and An. arabiensis. In this study although the L1014F mutation remains widespread in all three ecological regions and is present at relatively high frequency within the three species (averaging 50%), the frequencies reported in this current study were lower in the Sudan ecological regions (West and South West covering the old cotton belt) than those from previous studies [9,16,22]. For the other climatic zones i.e. central and northern regions the allele frequencies of L1014F varied within the three species with particularly high frequencies in An. arabiensis. The reason(s) for the reduction of L1014F frequency in An. gambiae populations in the Sudan area is not known, however, a similar trend was recently observed in the Western region of Burkina Faso where transgenic and biological control practices have been implemented for crop protection of cotton over the last four years (a long side conventional crop protection approaches) (Nammentougou, unpublished). These alternative cotton-growing practices would be expected to reduce the quantity and frequency of insecticide use in agriculture and this may in turn reduce the selection pressure experienced by local mosquito populations. The analysis of observed genotypic frequencies revealed a heterozygote deficit for the L1014F mutation in the three species of An. gambiae s.l. from many sites especially in the Sahel for An. coluzzii and An. arabiensis and in the Sudan and Sudan-Sahel for An. gambiae which deviated significantly from Hardy-Weinberg expectations. This finding is not surprising as the same patterns were observed in the West (Orodara and Soumouso) four years ago [9] in combination with a novel mutation, N1575Y, in the voltage-gated sodium channel, recently reported in An. gambiae s.l. populations in Soumouso [39].

The L1014F kdr mutation was recently recorded at highest frequency in An. arabiensis populations in the centre of the country [21] and in Bobo-Dioulasso at frequencies averaging 38% [40]. Previous studies have recorded only a few individuals of An. gambiae and An. coluzzii from the Centre-East part of the country [17] carrying this mutation in the heterozygous form. The present
study reveals that this mutation has since spread across the whole country and is now observed at relatively high and similar frequencies (40%) between the three species. The comparison of the observed genotypic frequencies of this mutation with that expected for Hardy-Weinberg equilibrium indicated, depending on the site, a deficit or excess of heterozygotes, mainly for An. gambiae populations. The occurrence of the L1014F kdr mutation in An. coluzzii had been suggested to have occurred by introgression from An. gambiae and via a de novo mutation event in An. arabiensis [15], however, the origin of the L1014S mutation in An. gambiae, An. coluzzii and An. arabiensis species in West Africa is not so clearly understood. The proximity of Burkina Faso from the Benin frontier where the L1014S mutation was first reported in An. arabiensis populations [20] suggests that it arrived in Burkina Faso via migration of An. arabiensis carrying the mutation from Benin, however, the origin of this mutation in An. gambiae and An. coluzzii populations in Burkina Faso remains to be elucidated.

In this study we report, for the first time, the presence of the ace.1 G119S mutation in An. arabiensis populations from eight sites: Dioulassoba, Soumouso in the West, Boromo, Dédougou, Koudougou, Nanoro and Fada in the Centre-North and East and Kaya in the North. In these sites An. arabiensis was observed as the second major vector after An. gambiae except at Fada and Nanoro where the proportion of An. arabiensis was lower than that of An. coluzzii. To confirm this finding, we repeated the PCR amplification of ace.1R for our An. arabiensis specimens and used, as a control, 30 specimens of An. Arabiensis which we had confirmed in a previous study do not have this mutation. No false positives were observed in these samples suggesting our data is robust. The ace.1R allele was observed in this study in An. arabiensis at varying frequency reaching a maximum value of 78% in populations from Dioulassoba and the lowest value in Kaya at 8%. Except for samples from Soumouso and Nanoro where the sample size was not sufficient (n<10) to compare genotype frequencies, deviations from Hardy-Weinberg equilibrium were observed at three sites (Dioulassoba, Koudougou and Kaya) as a result of a high heterozygote deficit. The same pattern was observed in An. gambiae from Orodara, Soumouso, Koudougou, Fada, Ouahigouya and Dori. The deficit of heterozygous genotypes observed in Orodara and Soumouso is not new as Dabiré et al. [41] reported similar results from the these areas from which the duplicated allele (ace.1D) was reported by Djogbenou et al. [33]. It is possible that this duplicated allele ace.1D is also present within An. arabiensis especially in Dioulassoba where the proportion of homozygous mutants was atypically high (60%). The high frequency of this mutation in Dioulassoba populations is intriguing as recent studies failed to find any L1014F kdr or ace.1R in An. arabiensis population from this site [40,42]. As for the L1014S mutation, additional sequence analysis of the region flanking the ace.1 locus are necessary to confirm whether the ace.1 mutation in An. arabiensis has evolved along the same pathway as kdr e.g. as a de novo mutation or introgression from An. gambiae or An. coluzzii. Unfortunately our PCR data is not backed up by

Figure 4. Distribution the ace-1R allele frequency from 15 sites dispersed across Burkina Faso.
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Table 3. Allelic and genotypic frequencies at the ace-1 locus in *An. gambiae* s.l populations from 15 sites in Burkina Faso.

| Species       | Sites  | N   | Genotypes | f(119S) | [95%CI]  | p(HW)  |
|---------------|--------|-----|-----------|---------|----------|--------|
| *An. arabiensis* |        |     |           |         |          |        |
| Gaoua        | 3      | 3   | 119G 119G | 0       | 0        | -      |
| Banfora      | 0      | 0   | 119G 119S | 0       | -        | -      |
| Sindou       | 5      | 5   | 119S     | 0       | 0        | -      |
| Orodara      | 1      | 1   | 119S     | 0       | 0        | -      |
| Dioulassoba  | 20     | 4   | 119S     | 12      | 0.7      | [2.95–7.13] | 0.0264 |
| Soumouso     | 7      | 1   | 119S     | 5       | 0.78     | [5.74–7.57] | 0.2308 |
| Boromo       | 15     | 5   | 119S     | 9       | 0.36     | [2.67–5.42] | 0.9488 |
| Dedougou     | 14     | 4   | 119S     | 6       | 0.5      | [3.19–7.25] | 0.0444 |
| Koudougou    | 12     | 5   | 119S     | 7       | 0.58     | [3.72–9.1] | 0.0004 |
| Nanoro       | 3      | 2   | 119S     | 1       | 0.33     | [6.16–17.45] | 0.2000 |
| Koupeia      | 8      | 8   | 119S     | 0       | 0        | -      |
| Fada         | 13     | 4   | 119S     | 1       | 0.38     | [2.96–6.26] | 0.9449 |
| Kaya         | 12     | 11  | 119S     | 1       | 0.08     | [1.52–2.27] | 0.0435 |
| Ouahigouya   | 1      | 1   | 119S     | 0       | 0        | -      |
| Dori         | 14     | 14  | 119S     | 0       | 0        | -      |
| *An. coluzzii* |        |     |           |         |          |        |
| Gaoua        | 1      | 1   | 119S     | 0       | 0        | -      |
| Banfora      | 6      | 6   | 119S     | 0       | 0        | -      |
| Sindou       | 6      | 6   | 119S     | 0       | 0        | -      |
| Orodara      | 4      | 4   | 119S     | 0       | 0        | -      |
| Dioulassoba  | 5      | 4   | 119S     | 1       | 0.1      | [2.67–4.71] | -      |
| Soumouso     | 3      | 3   | 119S     | 0       | 0        | -      |
| Boromo       | 0      | 0   | 119S     | 0       | 0        | -      |
| Dedougou     | 2      | 0   | 119S     | 2       | 0.5      | [9.28–34.65] | 1      |
| Koudougou    | 5      | 2   | 119S     | 3       | 0.3      | [4.49–10.78] | 1      |
| Nanoro       | 23     | 17  | 119S     | 6       | 0.13     | [1.34–2.04] | 1      |
| Koupeia      | 8      | 6   | 119S     | 2       | 0.12     | [2.28–3.9] | 1      |
| Fada         | 27     | 27  | 119S     | 0       | 0        | -      |
| Kaya         | 5      | 5   | 119S     | 0       | 0        | -      |
| Ouahigouya   | 9      | 6   | 119S     | 3       | 0.16     | [2.64–4.39] | 1      |
| Dori         | 5      | 5   | 119S     | 0       | 0        | -      |
| *An. gambiae* |        |     |           |         |          |        |
| Gaoua        | 36     | 22  | 119S     | 11      | 0.23     | [1.33–2.2] | 0.2811 |
| Banfora      | 24     | 20  | 119S     | 4       | 0.08     | [1.05–1.46] | 1      |
| Sindou       | 24     | 21  | 119S     | 3       | 0.06     | [0.92–1.23] | 1      |
| Orodara      | 23     | 22  | 119S     | 0       | 0.04     | [0.74–0.99] | 0.0222 |
| Species      | Sites          | N  | 119G | 119G  | 119S | 119S | f(119S) | [95%CI]       | p(HW)   |
|--------------|----------------|----|------|-------|------|------|---------|---------------|---------|
|              |                |    | 119G | 119G  | 119S | 119S |
| Dioulassoba  |                | 4  | 4    | 0     | 0    | 0    | -       | -             | -       |
| Soumousso    |                | 20 | 18   | 0     | 2    | 0.1  | [1.29–1.88] | 0.0021     |
| Boromo       |                | 15 | 9    | 4     | 2    | 0.26 | [2.32–4.31] | 0.2260     |
| Dedougou     |                | 12 | 8    | 4     | 0    | 0.16 | [2.1–3.59]  | 1          |
| Koudougou    |                | 18 | 14   | 1     | 3    | 0.19 | [1.82–3.07] | 0.0029     |
| Nanoro       |                | 4  | 3    | 1     | 0    | 0.12 | [3.27–6.29] | -          |
| Koupela      |                | 12 | 12   | 0     | 0    | 0    | -       | -             |
| Fada         |                | 19 | 18   | 0     | 1    | 0.05 | [0.96–1.27] | 0.0270     |
| Kaya         |                | 15 | 11   | 4     | 0    | 0.13 | [1.69–2.62] | 1          |
| Ouahigouya   |                | 19 | 14   | 2     | 3    | 0.21 | [1.85–3.16] | 0.0096     |
| Dori         |                | 11 | 10   | 0     | 1    | 0.09 | [1.68–2.59] | 0.0476     |

N: number of mosquitoes.

f(119S): frequency of the 119S resistant ace.1 allele.

p(HW): probability of the exact test for goodness of fit to Hardy Weinberg equilibrium.

- not determined.

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insecticide susceptibility bioassays and so we cannot assess the correlations between kdr and ace-1 mutations and the phenotypic expression of resistance.

The emergence of the ace-1R mutation in An. gambiae s.l. population from the cotton-growing areas may be linked to the agricultural use of OP and CM insecticides used for crop protection. Other sources of selection pressure outside the cotton belt include insecticide use for vegetable growing and domestic use of insecticide in public health. Bioassays performed in 2012 on An. gambiae populations from sites located in the cotton belt of the West of Burkina Faso revealed the development of resistance to CMs and OPs especially to benidocarb (Dabiré, unpublished) correlating with the prevalence and frequency of genetic resistance revealed in the present study. However, further bioassays on a wider scale are now required in order to understand the implications of the current status of the ace-1R mutation for the efficacy of OP and CM insecticides in vector control in Burkina Faso. The information provided by such studies combined with the genetic data presented here is a prerequisite for the informed use of CM and OP based-combinations for bednet impregnation and/or indoor residual spraying.

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Author Contributions

Conceived and designed the experiments: RKD AD PC. Performed the experiments: DDS JB HKT. Analyzed the data: RKD MN. Wrote the paper: RKD CB. Supervised field work: MN. Revisied the manuscript: MN AD CB. Performed PCR analyses: DDS JB HKT. Assured the financial support of the study through the Ministry of Health: CB. Read and approved the final version of the manuscript: RKD MN AD DDS JB HKT CB PC.

References

1. Carnevale P, Robert V, Boulin C, Halma JM, Pzaar L, et al. (1998) La lutte contre le paludisme par des moustiquaires impregnées de pyroside in Burkina Faso. Bull Soc Pathol Exot 91: 832–846.
2. D’Alessandro U, Olaye RO, McGuire W, Langerok P, Bennett S, et al. (1995) Mortality and morbidity from malaria in Gambian children after introduction of an impregnated bednet programme. Lancet 345: 479–483.
3. Binka FN, Kab бюджей A, Adjou M, Williams LA, Lengeler C, et al. (1996) Impact of permethrin impregnated bednets on child mortality in Kassen-Nankana district, Ghana: a randomized controlled trial. Trop Med Int Health 1: 147–154.
4. Elissa N, Mouquet J, Riviёre F, Meunier JY, Yao K (1993) Resistance of Anopheles gambiae s.s. to pyrethroids in Cote d’ivoire. Ann Soc Belg Trop Med 73: 291–294.
5. Chandre F, Darrier F, Manga L, Akogbeho M, Faye O, et al. (1999) Status of pyrethroid resistance in Anopheles gambiae s.s. evaluated by World Health Organ. Bull 72: 230–234.
6. Diabate A, Baldet T, Chandre F, Akogbeho M, Guingomde TR, et al. (2002) The role of agricultural use of insecticides in resistance to pyrethroids in Anopheles gambiae s.l. in Burkina Faso. Am J Trop Med Hyg 67: 617–622.
7. Martinez-Torres D, Chandre F, Williamson MS, Darriet F, Berge JB, et al. (2004) The molecular M and S forms of Anopheles gambiae s.l. from Burkina Faso (West Africa). Bull World Health Organ 82: 136–137.
8. Weill M, Malcolm C, Chandre F, Mogensen K, Berthomieu A, et al. (2004) The emergence of the ace-1R mutation in Anopheles gambiae sensu lato. Trans R Soc Trop Med Hyg 103: 1113–1120.
9. Dabiré KR, Diabate A, Namountougou M, Toe KH, Ouari A, et al. (2009a) Combined pyrethroid and carbamate ‘twist’-treated mosquito nets: field efficacy against pyrethroid-resistant Anopheles gambiae and Culex quinquefasciatus. Med Vet Entomol 15: 105–112.
10. N’Guessan R, Aikpon R, Akogbeho M, Rowland M (2007) Reduced efficacy of insecticide-treated nets and indoor residual spraying for malaria control in pyrethroid resistant area, Benin. Emerg Infect Dis 13: 199–206.
11. Guillet P, N’Guessan R, Darriet F, Traore-Lamizana M, Chandre F, et al. (2003) Identification of a point mutation in the voltage-gated sodium channel gene of Kenyan Anopheles gambiae associated with resistance to DDT and pyrethroids. Insect Mol Biol 9: 491–497.
12. Verhaeghen K, Van Bortel W, Roelants P, Backeljau T, Coosemans M (2006) Detection of the East and West African kdr mutation in Anopheles gambiae and Anopheles arabiensis from Uganda using a new assay based on FRET/Melt Curve analysis. Malaria J 5: 16.
13. Djebel I, Bousaari O, Sidiki A, Martin T, Ramon H, et al. (2011) Dynamics of insecticide resistance in malaria vectors in Benin: first evidence of the presence of L1014S kdr mutation in Anopheles gambiae from West Africa. Malaria J 10: 261.
14. Babolo A, Traore A, Jones CM, Sano M, Flood L, et al. (2012) Three years of insecticide resistance monitoring in Anopheles gambiae in Burkina Faso: resistance on the rise? Malaria J 11: 232.
15. N’Gueiran M, Simonard F, Baldet T, Diabate A, Ouedraogo JB, et al. (2012) Multiple insecticide resistance in Anopheles gambiae s.l. populations from Burkina Faso, West Africa. PLoS One 7: e48102.
16. Henry MC, Assi SB, Rogier C, Dossou-Yovo J, Chandre F, et al. (2005) Protective efficacy of lambda-cyhalothrin treated nets in Anopheles gambiae pyrethroid resistance areas of Cote d’Ivoire. Am J Trop Med Hyg 73: 859–864.
17. N’Guessan R, Goebel V, Akogbeho M, Rowland M (2007) Reduced efficacy of insecticide-treated nets and indoor residual spraying for malaria control in pyrethroid resistant area, Benin. Emerg Infect Dis 13: 199–206.
18. Verhaeghen K, Van Bortel W, Roelants P, Backeljau T, Coosemans M (2006) Detection of the East and West African kdr mutation in Anopheles gambiae and Anopheles arabiensis from Uganda using a new assay based on FRET/Melt Curve analysis. Malaria J 5: 16.
19. Verhaeghen K, Van Bortel W, Roelants P, Backeljau T, Coosemans M (2006) Detection of the East and West African kdr mutation in Anopheles gambiae and Anopheles arabiensis from Uganda using a new assay based on FRET/Melt Curve analysis. Malaria J 5: 16.
20. Dabiré KR, Diabate A, Namountougou M, Toe KH, Ouari A, et al. (2009a) Combined pyrethroid and carbamate ‘twist’-treated mosquito nets: field efficacy against pyrethroid-resistant Anopheles gambiae and Culex quinquefasciatus. Med Vet Entomol 15: 105–112.
21. Aikpon R, Agossa F, Ouse R, Oussou O, Yadouleton A, et al. (2012) Evaluation of the efficacy of bendiocarb in indoor residual spraying against pyrethroid resistant malaria vectors in Benin: results of the third campaign. Parasit Vectors 5: 163.
22. Chandre F, Darriet F, Do onnio JM, Riviёre F, Pasteur N, et al. (1997) Distribution of organophosphate and carbamate resistance in Culex pipiens quinquefasciatus (Diptera: Culicidae) in West African. J Med Entomol 34: 664–671.
23. N’Guessan R, Darriet F, Guillet P, Carnevale P, Traore-Lamizana M, et al. (2003) Resistance to carbosulfan in Anopheles gambiae from Ivory Coast, based on reduced sensitivity of acetylcholinesterase. Med Vet Entomol 17: 19–23.
24. Aikpon R, Agossa F, Ouse R, Oussou O, Aizoun N, et al. (2013) Bendiocarb resistance on the rise? Malaria J 11: 232.
25. Verhaeghen K, Van Bortel W, Roelants P, Backeljau T, Coosemans M (2006) Detection of the East and West African kdr mutation in Anopheles gambiae and Anopheles arabiensis from Uganda using a new assay based on FRET/Melt Curve analysis. Malaria J 5: 16.
26. Osse R, Aikpon R, Padonou GG, Oussou O, Yadouleton A, et al. (2012) Reduced efficacy of insecticide-treated nets and indoor residual spraying for malaria control in pyrethroid resistant area, Benin. Emerg Infect Dis 13: 199–206.
27. Guillet P, N’Guessan R, Darriet F, Traore-Lamizana M, Chandre F, et al. (2003) Combined pyrethroid and carbamate ‘twist’-treated mosquito nets: field efficacy against pyrethroid-resistant Anopheles gambiae and Culex quinquefasciatus. Med Vet Entomol 15: 105–112.
28. N’Guessan R, Goebel V, Akogbeho M, Rowland M (2007) Reduced efficacy of insecticide-treated nets and indoor residual spraying for malaria control in pyrethroid resistant area, Benin. Emerg Infect Dis 13: 199–206.
29. Aikpon R, Agossa F, Ouse R, Oussou O, Aizoun N, et al. (2013) Bendiocarb resistance on the rise? Malaria J 11: 232.
30. Verhaeghen K, Van Bortel W, Roelants P, Backeljau T, Coosemans M (2006) Detection of the East and West African kdr mutation in Anopheles gambiae and Anopheles arabiensis from Uganda using a new assay based on FRET/Melt Curve analysis. Malaria J 5: 16.
31. N’Guessan R, Goebel V, Akogbeho M, Rowland M (2007) Reduced efficacy of insecticide-treated nets and indoor residual spraying for malaria control in pyrethroid resistant area, Benin. Emerg Infect Dis 13: 199–206.
35. Hemingway J, Karunaratne SH (1998) Mosquito carboxylesterases: a review of the molecular biology and biochemistry of a major insecticide resistance mechanism. Med Vet Entomol 12: 1–12.
36. Gillies MT, Coetzee M (1987) A supplement to the Anophelinae of Africa south of the Sahara. Puh. South Afr. Inst Med Res 55: 143.
37. Santolamazza F, Calzetta M, Eyang J, Barrese E, Dia I, et al. (2008) Distribution of knock-down resistance mutations in Anopheles gambiae molecular forms in west and west-central Africa. Malar J 7: 74.
38. Raymond M, Rousset F (1995) GENEPOP Version 1.2 A population genetics software for exact tests and ecumenicism. J Hered: 248–249.
39. Jones CM, Toe HK, Sanou A, Namountougou M, Hughes A, et al. (2012a) Additional selection for insecticide resistance in urban malaria vectors: DDT resistance in Anopheles arabiensis from Bobo-Dioulasso, Burkina Faso. PLoS One 7: e45995.
40. Jones CM, Liyanapathirana M, Agossa FR, Weetman D, Ranson H, et al. (2012) Footprints of positive selection associated with a mutation (N1575Y) in the voltage-gated sodium channel of Anopheles gambiae. Proc Natl Acad Sci U S A 109: 6614–6619.
41. Dabire KR, Diabate A, Namountougou M, Djogbenou L, Kengue P, et al. (2009b) Distribution of insensitive acetylcholinesterase (ace-1R) in Anopheles gambiae s.l. populations from Burkina Faso (West Africa). Trop Med Int Health 14: 396–403.
42. Dabire RK, Namountougou M, Sawadogo SP, Yaro LB, Toe HK, et al. (2012) Population dynamics of Anopheles gambiae s.l. in Bobo-Dioulasso city: bionomics, infection rate and susceptibility to insecticides. Parasit vectors 5: 127.