Seasonal change in species composition and target-site mutations in *Anopheles gambiae* s.l. in the severe drought area of Kandi, North-eastern Benin

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Abstract

The persistence of malaria transmission in areas with very arid environmental conditions remains enigmatic. The present study investigated seasonal variation of mosquito species composition and Kdr and Ace-1 mutations in *Anopheles gambiae* s.l. in the very arid district of Kandi in North-eastern Benin. Adult mosquitoes were sampled over 1 year using both human landing catches (HLC) and pyrethrum spray catches in 4 villages belonging to 2 areas of different levels of aridity. The collections were carried out on a bi-monthly basis in the wet season, and once every month in the dry season to better capture the entomological situation in drought period. Females *An. gambiae* s.l. specimens were kept aside and analysed by PCR for species identification. Presence of *kdr* and *Ace-1* mutations was also assessed in the *An. gambiae* s.l. collection.

A total of 2,211 host-seeking mosquitoes belonging to 15 species were collected in the study area. *An. gambiae* s.l. was the most abundant species and represented 67% of the collection. Other *Anopheles* species were found at very low frequency as detected in the collection.

Introduction

Malaria remains a serious obstacle for development in Africa. It represents 9% of the total disease burden and results in more than 600,000 deaths every year globally, most of them children under 5 years of age [1]. The control of *Anopheles* vectors has been marked by intensive deployment of insecticide-based tools such as long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) which have contributed to a substantial reduction of malaria burden [2]. Unfortunately, the emergence and spread of insecticide resistance in malaria vector is stalling the progress made [3]. *Kdr* mutation conferring resistance to pyrethroid and DDT and Acetyl cholinesterase (Ace-1) mutation causing resistance to organophosphates and carbamates are the most distributed mechanisms in malaria mosquitoes. They are results of mutation in some genetic points that alter the site that insecticides are supposed to bind to [4]. The *Anopheles* mosquitoes transmitting malaria in Sub-Saharan Africa have a wide distribution in most countries and are able to survive in different environments [5, 6]. Thus, they are found in vast dry savannahs and in semi-desert areas where they are able to ensure the maintenance of malaria transmission during periods of drought [7].
The survival of the major vectors of malaria and their adaptation to the extreme meteorological situations which rage in the form of long and severe droughts in certain regions of Africa, particularly in the semi-desert zones, remain incomprehensible and enigmatic [8, 9]. Indeed in these regions, surface water necessary for the development of *Anopheles* larvae is absent for 6-8 months [10]. Nevertheless in such conditions, many malaria cases are recorded in health facilities [11].

The rapid repopulation of larvae observed from the start of the rainy season in *Anopheles* larval habitats remain poorly documented (Adamou et al., 2011). Based on the relatively high malaria prevalence in a sedentary human population in locations with extreme arid conditions, two historical assumptions have been being explored. As *Anopheles* eggs hardly tolerate desiccation, it has been suggested that malaria vectors estivate in severe droughts and afterwards reemerge as soon as climatic conditions improve [8, 12, 13]. The paradoxical persistence of malaria in areas with very arid environmental conditions was also associated to the fact that some species of *Anopheles* mosquito are capable of long-distance migration [14].

In the northern part of the Republic of Benin, the district of Kandi is a particularly severe drought lasting 6 months during which significant cases of malaria are diagnosed in health facilities [15]. Even if these unexpected cases could be linked to relapses or imported cases of malaria, the possibility of recent infections cannot be ruled out given the magnitude of the prevalence. In view of the harmful effects of climate change marked by increasingly high temperatures and longer droughts, it is important to study the characteristics of the vectors that maintain malaria transmission in arid ecosystems for the development of a strategy adapted to their control. The current study investigated mosquito species composition and seasonal variation as well as frequency of *Kdr* and *Ace-1* mutations in *Anopheles gambiae* s.l. in the very arid district of Kandi over one year of entomological surveillance. This allowed a better understanding of the continued malaria transmission in the extreme drought conditions.

**Methods**

**Study area**

The study was conducted in the commune of Kandi (11° 07’ N2° 56’ E) in the northeast of Benin. Kandi is 290 meters above sea level and extends over an area of 3,421 km². The relief of this commune is mainly composed of sandstone plateaus to which are added some granite hills and quartzites. Two permanent watercourses water the commune: the Sota (250 km long) and the Alibori (338 km long). Kandi has a Sudanian-type climate with a dry season from November to April and a rainy season from May to October. In Kandi, the average monthly rainfall is around 84 mm for values between 0 and 271 mm of rain. In the dry season, temperatures are very high and can reach up to 45 °C. Drought is severe in Kandi with an absence of rain for nearly six months and significant sunshine. Despite the inadequate environmental conditions of this long dry season, malaria rifes throughout the year with epidemic-type outbreaks in the rainy season [16].

Mosquitoes were collected in 4 villages of Kandi of which Thui (11° 5’N2° 45’E) and Kossarou (11° 7’N2° 56’E) located in the driest area (D + +) without water within 10 km radius, while the other 2 locations Sonsoro (11° 5’N 2° 45’E) and Pèdè (11° 11’N2° 57’E) are located in the area surrounding the semi-permanent watercourses of the commune of Kandi (here referred as D +).

**Mosquito sampling**

Adult mosquitoes were collected between May 2012 and April 2013 using human landing catches (HLC) and pyrethrum spray catches. Mosquito collections were carried out on a bi-monthly basis in the wet season (May 2012 to October 2012), and once every month (November 2012 to April 2013) to better capture the entomological situation in drought period.

Per time point, the mosquito sampling were performed in four houses per village (2 for each collection method) for 2 consecutive days. HLCs were conducted indoors and outdoors, from 6:00 pm to 6:00 am. PSC was performed in the morning at 7 am. Before spraying houses, white fabrics were laid, thereafter windows and doors were closed. Rambo® aerosol containing 0.25% transfluthrin and 0.20% permethrin was used and 15 minutes post spraying, mosquitoes were collected by from the white sheets the means of forceps.

**Mosquito processing**

Female mosquitoes were kept aside, counted and morphologically identified to species using taxonomic keys of Gillies & De Mellon [17]. In each location, a sub-sample of *An. gambiae* s.l. were randomly selected and processed for species identification following the protocols described by Santolamazza et al. [18]. L1014F Kdr and G1195S Ace-1 mutations were sought in *An. gambiae* s.l. in accordance with methods developed by Martinez-Torres et al. [19] and Weill et al. [20] respectively.

**Ethical consideration**

Approval for this study was granted by the institutional ethics review board of Centre for Research in Entomology of Cotonou located in the Benin Ministry of Health. Before collection of mosquitoes in randomly selected households, consents were sought from the heads.

**Data management and analysis**

Data generated were initially recorded by hand on standardised record forms and double entered into predesigned databases. Data of molecular species composition, Kdr and Ace-1 mutations were organised to assess the potential impact of severe drought. Confidence intervals of proportions were determined using the exact binomial test. All statistical analyses were performed using Stata version 15.0 (Stata Corp., College Station, TX).

**Results**

**Mosquito species composition**

Figure 1 and Table 1 summarize the mosquito species composition and results of molecular identification. A total of 2,211 host-seeking mosquitoes were caught in the study locations using both HLC and PSC methods (Table 1). The mosquito samples belonged to four genera (*Anopheles, Culex, Aedes, mansonia*) and 15 species. *An. gambiae* s.l. was the most abundant mosquito species in the study area and represented 67% (1,486 /2,211) of the collection (Figure 1). The proportions of *An. gambiae* s.l. in the different locations were 94% in Sonsoro, 66% in Pèdè and Thui, and 12% in...
Kossarou. Other Anopheles species were found in lower density among which An. funestus and An. pharoensis in Sonsoro and Kossarou, An. brohieri in Pèdè and Thui and An. coustani found in only Sonsoro (Figure 1).

Molecular species identification conducted on 600 samples of An. gambiae s.l. from the 4 study locations showed that there was a significantly higher frequency of An. coluzzii than An. gambiae s.s. (59% vs 40%; p<0.001). The seasonal trend looked similar since in the less dry area, An. coluzzii was predominant over An. gambiae s.s. in both dry season (70% vs 29%; p<0.001) and rainy season (53% vs 45%, p = 0.153). In the driest area, An. coluzzii was also the most abundant species in both dry (70% vs 30% and p = 0.034) and rainy (62% vs 38%, p = 0.012) seasons.

![Fig 1: Mosquito species composition in the study area](https://www.dipterajournal.com)

### Table 1: Results of molecular species identification

| Type  | Location | Species               | Dry season | Rainy season | Total    |
|-------|----------|-----------------------|------------|--------------|----------|
|       |          |                       | N  | %   | 95% CI    | N  | %   | 95% CI    | N  | %   | 95% CI    |
| Area D+ | Sonsoro  | An. coluzzi           | 56 | 82  | 73-91     | 125 | 57  | 50-64     | 181 | 63  | 57-69     |
|        |          | An. gambiae           | 11 | 16  | 5-25      | 90  | 41  | 35-47     | 101 | 35  | 29-41     |
|        |          | An. Coluzzi / gambiae | 1  | 1   | 0-4       | 5   | 2   | 0-4       | 6   | 2   | 0-4       |
|        | Pèdè     | An. coluzzi           | 28 | 54  | 40-68     | 54  | 46  | 37-64     | 82  | 48  | 40-56     |
|        |          | An. gambiae           | 24 | 46  | 32-60     | 63  | 53  | 44-62     | 87  | 51  | 43-51     |
|        |          | An. Coluzzi / gambiae | 0  | 0   | 0         | 1   | 1   | 0-3       | 1   | 1   | 0-2       |
| Total  |          | An. coluzzi           | 84 | 70% | 62-78     | 179 | 53% | 48-58     | 263 | 57% | 52-62     |
|        |          | An. gambiae           | 35 | 29% | 21-57     | 153 | 45% | 40-50     | 188 | 41% | 36-46     |
|        |          | An. Coluzzi / gambiae | 1  | 1   | 0-3       | 6   | 2   | 1-3       | 7   | 2   | 1-3       |
| Area D++ | Kossarou | An. coluzzi           | 12 | 92  | 78-106    | 17  | 45  | 29-61     | 29  | 57  | 43-71     |
|        |          | An. gambiae           | 1  | 8   | 0-22      | 21  | 55  | 39-71     | 22  | 43  | 29-57     |
|        | Thui     | An. coluzzi           | 7  | 50  | 24-76     | 54  | 70  | 60-80     | 61  | 67  | 57-77     |
|        |          | An. gambiae           | 7  | 50  | 24-76     | 23  | 30  | 20-40     | 30  | 33  | 23-43     |
|        |          | An. Coluzzi / gambiae | 19 | 70% | 53-87     | 71  | 62% | 53-71     | 90  | 63% | 55-69     |
| Total  |          | An. coluzzi           | 193| 70% | 53-87     | 250 | 55% | 56-64     | 353 | 59% | 51-67     |
|        |          | An. gambiae           | 43 | 29% | 12-46     | 197 | 43% | 34-52     | 240 | 40% | 32-48     |
|        |          | An. Coluzzi / gambiae | 1  | 1   | 0-3       | 6   | 1   | 0-3       | 7   | 1   | 0-3       |

**Seasonal trend of L1014F kdr mutation in Anopheles gambiae s.l.**

Table 2 shows seasonal variation of L1014F kdr mutation in An. gambiae s.l. in the study area. Through the less dry area, frequency of L1014F kdr mutation was significantly higher in dry season than in rainy season (93% vs 84%; p<0.001). In the dry season, frequency of L1014F kdr was 95% and 90% respectively in Sonsoro and Pèdè, against 85% and 81% in the rainy season (p<0.05). Conversely, the overall frequency of L1014F kdr mutation was similar between seasons (93% vs 88%; p = 0.45) and the trend was the same in both Kossarou (92% vs 89%; p = 0.594) and Thui (95% vs 88%; p = 0.597).
The present study investigated seasonal variation in malaria vector species composition and frequency of target-site mechanisms of insecticide resistance to understand the persistence of malaria in the severe drought district of Kandi, North-eastern Benin. In the study area, An. gambiae s.l. was the predominant as reported in several areas of the Republic of Benin [21, 22]. An. funestus, another major malaria vector and some secondary vectors such as An. pharaohensis, An. brohieri and An. coustani were found at very low frequency (< 1%). Culex quinquefasciatus was also collected in low, moderate and high proportion in Sonsoro (4%), Thui (32%) and Pèdè (33%), and in Kossarou (86%), respectively. The abundance of this mosquito species in the most urbanised study site of Kossarou as observed in Southern Benin, suggests a high risk of transmission of lymphatic filariasis [23, 24]. The mosquito composition looked similar across seasons in the study area regardless of the level of aridity.

### Table 2: Seasonal trend of L1014F kdr mutation in Anopheles gambiae s.l.

| Level of aridity | Location | Species | Dry season | Rainy season | p-value |
|-----------------|----------|---------|------------|--------------|---------|
|                 |          |         | N          | %           | N          | %      |
|                 |          |         | % F(R) (95% CI) |              | % F(R) (95% CI) |         |
| Area D+         | Sonsoro  | RR      | 61         | 90           | 144        | 72     |
|                 |          | RS      | 7          | 10           | 52         | 26     |
|                 |          | SS      | 0          | 0            | 4          | 2      |
|                 | Pèdè     | RR      | 41         | 80           | 80         | 67     |
|                 |          | RS      | 10         | 20           | 33         | 28     |
|                 |          | SS      | 0          | 0            | 6          | 5      |
|                 | Total    | RR      | 102        | 86           | 224        | 70     |
|                 |          | RS      | 17         | 14           | 85         | 27     |
|                 |          | SS      | 0          | 0            | 10         | 3      |
| Area D++        | Kossarou | RR      | 11         | 85           | 29         | 78     |
|                 |          | RS      | 2          | 15           | 8          | 22     |
|                 |          | SS      | 0          | 0            | 0          | 0      |
|                 | Thui     | RR      | 6          | 86           | 61         | 80     |
|                 |          | RS      | 1          | 14           | 12         | 16     |
|                 |          | SS      | 0          | 0            | 3          | 4      |
|                 | Total    | RR      | 17         | 85           | 90         | 80     |
|                 |          | RS      | 3          | 15           | 20         | 18     |
|                 |          | SS      | 0          | 0            | 3          | 3      |

### Table 3: Seasonal trend of G119S Ace-1 mutation in Anopheles gambiae s.l.

| Level of drought | Location | Species | Dry season | Rainy season | p-value |
|-----------------|----------|---------|------------|--------------|---------|
|                 |          |         | N          | %           | N          | %      |
|                 |          |         | % F(R) (95% CI) |              | % F(R) (95% CI) |         |
| Area D+         | Sonsoro  | SS      | 68         | 100          | 223        | 112    |
|                 |          | RS      | 0          | 0            | 0          | 0      |
|                 |          | RR      | 0          | 0            | 0          | 0      |
|                 | Pèdè     | SS      | 52         | 100          | 121        | 100    |
|                 |          | RS      | 0          | 0            | 0          | 0      |
|                 |          | RR      | 0          | 0            | 0          | 0      |
|                 | Total    | SS      | 120        | 100          | 344        | 100    |
|                 |          | RS      | 0          | 0            | 0          | 0      |
|                 |          | RR      | 0          | 0            | 0          | 0      |
| Area D++        | Kossarou | SS      | 13         | 100          | 38         | 100    |
|                 |          | RS      | 0          | 0            | 0          | 0      |
|                 |          | RR      | 0          | 0            | 0          | 0      |
|                 | Thui     | SS      | 7          | 100          | 78         | 100    |
|                 |          | RS      | 0          | 0            | 0          | 0      |
|                 |          | RR      | 0          | 0            | 0          | 0      |
|                 | Total    | SS      | 20         | 100          | 116        | 100    |
|                 |          | RS      | 0          | 0            | 0          | 0      |
|                 |          | RR      | 0          | 0            | 0          | 0      |
coluzzii is associated with permanent and semi-permanent surface water while An. gambiae s.s. is dependant to temporary breeding sites [26]. The presence of An. coluzzii in the driest area of Kandi where no permanent and semi-permanent breeding sites suggests at least an ecological plasticity of the species and its capacity to colonize environments with long and severe droughts. According to Longo-Pendy et al. [27], An. coluzzii is able to breed in a large range of aquatic conditions and tolerate higher ions concentration as compared to An. gambiae s.s. Another assumption under the predominance of An. coluzzii in the driest sector of Kandi is the ability to migrate as previously reported in situation of surprisingly persistence malaria [28, 29].

Presence of Kdr mutation was detected in An. gambiae s.l. population with significantly higher frequency in dry season as compared to rainy season. This suggests higher pyrethroid and organochlorine resistance in the study area and most importantly in the vector sub-population responsible of residual transmission in drought periods. Pyrethroid-resistance is widely spread across all regions in Benin [30-34]. In fact, this situation is associated with the recent massive deployment of LLINs across endemic countries for control of malaria, which led to selection pressure on mosquito vectors [35, 36]. In addition, the use of pyrethroids in agriculture in the study area as reported in many other rural settings in Benin [37, 38] might have contributed to the high frequency of kdr mutation recorded. Regarding the Ace-1 mechanism, no specimens with resistant genotypes was observed suggesting the absence of resistance to organophosphates and carbamates at the time of sampling. Recent studies conducted in Northern Benin, showed presence of Ace-1 mutations resistance at very low frequency (<6%) in Kandi, Gogounou, Segbana, Djougou, Copargo, and Ouake districts in Northern Benin [39]. This data imply that Ace-1 mutation will be spreading in malaria vectors across different regions. Thus, it is important to design a good strategy for monitoring and managing insecticide resistance in general for efficient control of malaria vectors.

Conclusion

Predominance of An. coluzzii and higher frequency of Kdr mutation were observed in the driest period in the district of Kandi. In the current context of climate change marked by increasingly high temperatures and longer droughts, suitable vector control should be designed taking into account characteristics of the vector population maintaining malaria transmission in such arid environmental conditions for better results after onset of rainy seasons.

Competing interests

The authors declare that they have no competing interests.

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Authors’ contributions

RG and MA wrote the main study protocol and design the study. RO, AS and RG supervised the study data collections. RG performed data analysis. RG wrote the initial draft of the manuscript, which was revised by AS. MA provided administrative and logistics support. All authors read and approved the final manuscript.

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