International Journal of Mosquito Research 2022; 9(6): 38-43

International Journal of Mosquito Research

ISSN: **2348-5906**
CODEN: **IJMRK2**
IJMR 2022; 9(6): 38-43

www.dipterajournal.com
Received: 19-09-2022
Accepted: 22-10-2022

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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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DOI: https://doi.org/10.22271/23487941.2022.v9.i6a.639

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 among which *An. funestus*, *An. pharoensis*, *An. broheri* and *An. coustani*. Molecular species identification showed in dry season a significantly higher frequency of *An. coluzzii* over *An. gambiae s. s.* in both less dry (70% *vs* 29% with $p < 0.001$) and driest (70% *vs* 30% and $p = 0.034$) areas of the district of Kandi. In the rainy season, there was similar frequency of *An. coluzzii* and *An. gambiae s. s.* in the less arid area (53% *vs* 45%; $p = 0.153$), while *An. coluzzii* remained significantly predominant (62% *vs* 38%; $p = 0.012$) in the driest zone. The frequency of *kdr* mutation was significantly higher in dry season than in rainy season (93% *vs* 84%; $p < 0.001$), while no *Ace-1* mutation was detected in the collection.
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.

Keywords: Arid; *An. gambiae sl¸ An. coluzzii, Kdr, Ace-1*

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 carbonates 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 remerge 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 watercourse 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 G119S *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. broheri* 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.

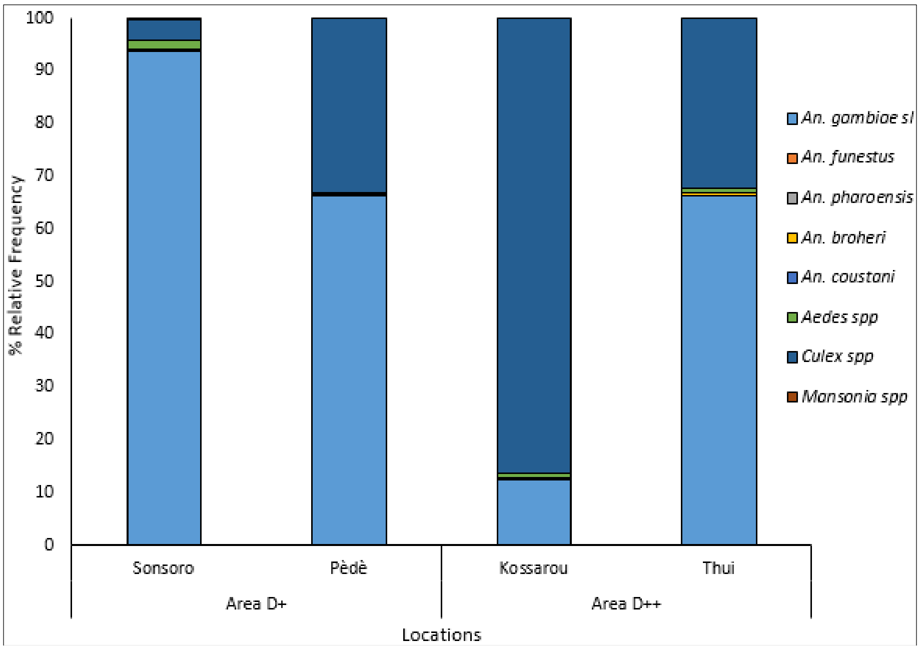

Fig 1: Mosquito species composition in the study area

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		1	1	0-3	1	1	0-2
	Total	*An. coluzzi*	84	70^a	62-78	179	53^a	48-58	263	57^a	52-62
		An. gambiae	35	29^b	21-37	153	45^a	40-50	188	41^b	36-46
		An. Coluzzi / gambiae	1	1^c	0-3	6	2^b	1-3	7	2^c	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
	Total	*An. coluzzi*	19	70^a	53-87	71	62^a	53-71	90	63^a	55-69
		An. gambiae	8	30^b	13-47	44	38^b	29-47	52	37^b	29-45
Total		*An. coluzzi*	103	70^a	53-87	250	55^a	56-64	353	59^a	51-67
		An. gambiae	43	29^b	12-46	197	43^b	34-52	240	40^b	32-48
		An. Coluzzi / gambiae	1	1^c	0-4	6	1^c	0-3	7	1^c	0-3

Seasonal trend of L1014F *kdr* mutation in *Anopheles gambiae sl*

Table 2 shows seasonal variation of L1014F *kdr* mutation in *An. gambiae sl* 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 (93% *vs* 88%; p = 0.597).

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	%	% F(R) (95% CI)	N	%	% F (R) (95% CI)	
Area D+	Sonsoro	RR	61	90	95 (90-100)	144	72	85 (80-90)	0.002
		RS	7	10		52	26		
		SS	0	0		4	2		
	Pèdè	RR	41	80	90 (82-98)	80	67	81 (74-88)	0.037
		RS	10	20		33	28		
		SS	0	0		6	5		
	Total	RR	102	86	93 (88-98)	224	70	84 (80-88)	<0.001
		RS	17	14		85	27		
		SS	0	0		10	3		
Area D++	Kossarou	RR	11	85	92 (78-100)	29	78	89 (79-99)	0.594
		RS	2	15		8	22		
		SS	0	0		0	0		
	Thui	RR	6	86	93 (74-100)	61	80	88 (81-95)	0.597
		RS	1	14		12	16		
		SS	0	0		3	4		
	Total	RR	17	85	93 (81-100)	90	80	88 (82-94)	0.450
		RS	3	15		20	18		
		SS	0	0		3	3		

Seasonal trend of G119S Ace-1 mutation in *Anopheles gambiae* s.l.

The seasonal distribution of G119S Ace-1 mutation in An. gambiae s.l. through the study locations was summarized in Table 3. There was no variation between seasons in the genotypes observed for G119S Ace-1 mutation in *An. gambiae* s.l. in all the 4 study locations. There was 100% homozygous SS individuals in the less arid area over the dry (120 / 120) and rainy (344 / 344) seasons. The trend was the same in the most arid area in both dry (20 / 20) and rainy (116 / 116) seasons.

Table 3: Seasonal trend of G119 *ace-1* mutation in *Anopheles gambiae* s.l.

Level of drought	Location	Species	Dry season			Rainy season		
			N	%	% F (R) (95% CI)	N	%	% F (R) (95% CI)
Area D +	Sonsoro	SS	68	100	0	223	112	0
		RS	0	0		0	0	
		RR	0	0		0	0	
	Pèdè	SS	52	100	0	121	100	0
		RS	0	0		0	0	
		RR	0	0		0	0	
	Total	SS	120	100	0	344	100	0
		RS	0	0		0	0	
		RR	0	0		0	0	
Area D ++	Kossarou	SS	13	100	0	38	100	0
		RS	0	0		0	0	
		RR	0	0		0	0	
	Thui	SS	7	100	0	78	100	0
		RS	0	0		0	0	
		RR	0	0		0	0	
	Total	SS	20	100	0	116	100	0
		RS	0	0		0	0	
		RR	0	0		0	0	

Discussion

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. pharoensis*, *An. broheri* 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.

Molecular species identification of the *An. gambiae* complex following the protocol described by Santomalazza *et al.* [18] showed a mixture of *An. coluzzii* (59%) and *An. gambiae s. s.* (40%), with a significantly higher predominance of *An. coluzzii* in the dry season particularly in the driest sector of the study area. This data confirms the previous observation by Ganguenon *et al.* [22] and Kpanou *et al.* [25] respectively in Kandi, Malanville and Parakou in North Benin. Originally *An.*

coluzzii is associated with permanent and semi-permenent surface water while *An. gambiae s. s.* is dependant to temporary breeding sites [26]. The presence of *An. coluzzi* 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 pyrethoids 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.

Funding
The study was part of a PhD project and was funded by Centre for Research in Entomology of Cotonou, in Benin.

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.

Acknowledgements
We thank technicians of Centre for Research in Entomology of Cotonou for their assistance. We are also grateful to populations and local authorities of Kandi for their collaboration.

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