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Dynamics of Anopheline vector species composition and reported malaria cases during rain and dry seasons in two selected communities in Kwara State

Oduola, A. O.,[1] Adelaja, O. J.,[1] Aiyegbusi, Z. O.,[1] Tola, M.,[2] Obembe, A.[3] Ande, A. T.[1] and Awolola, S.[2]

[1] Department of Zoology, University of Ilorin, Ilorin, Nigeria
[2]Molecular Entomology and Vector Control Research Laboratory
Nigerian Institute of Medical Research, Lagos, Nigeria
[3]Department of Biosciences and Biotechnology, Kwara State University, Malete, Nigeria

Abstract

Indoor resting mosquitoes were collected between November 2013 and May 2014 using Pyrethrum spray collection (PSC). The mosquitoes were identified using morphological keys and species specific polymerase chain reaction (PCR) assays. The species composition of members of the *An. gambiae complex* and reported malaria cases in the selected study communities were spatially mapped using Arc GIS software. A total of 645 mosquitoes were collected from the study sites and morphologically identified. This comprised 598 (92%) *Anopheles* species, 42 (7%) *Culex* species and 5 (1%) *Aedes* species. One hundred and ninety-six *An. gambiae s. l.* samples were randomly selected and successfully identified by PCR. The composition of the mosquitoes species collected in Gaa Bolohunduro showed that a higher proportion 53 (64.6%) were *An. arabiensis* compared to lower proportions 5(6.1%) of *An. coluzzii* and 24 (29.3%) *An. gambiae*. In Osin, only 12 (10.5%) were *An. arabiensis* compared to 13 (11.4%) and 89 (78.1%) identified as *An. coluzzii* and *An. gambiae* respectively. The variation in the composition of members of the *An. gambiae s. l.* identified in Gaa Bolohunduro was not significant (p=0.070) compared to Osin (p=0.043). A weak correlation coefficient showed that the predominance of *Anopheles* in both communities was not dependent on rainfall, though variation in the members of the complex within the wet and dry season was significant (p<0.01). The sympatric occurrence and availability of *An. gambiae s. s* and *An. arabiensis* during the dry and wet seasons corroborated the high malaria prevalence identified in Ilorin South. This suggests that both vectors will sustain malaria transmission in the study sites. Hence, a vector control strategy in these localities should be informed by the individual behaviour of each vector species identified.

Keywords: *Anopheles arabiensis*, *Anopheles coluzzii*, *Anopheles gambiae*, distribution, composition, malaria cases.

Introduction

Malaria is a major public health problem in Nigeria and most parts of the world [1]. In Nigeria, malaria is generally endemic in rural and urban communities [2]. Out of the 124-283 million people who contracted the disease globally, between 367,000-755,000 were reported to have died [3] with 40% of the estimated total cases of malaria deaths from Africa occurring in Nigeria and The Republic of Congo [4].

An. gambiae and *An. funestus* (Giles) are two predominant mosquito species which belong to complexes and are reported vectors of malaria parasites [5, 6, 7, 8]. In sub-Saharan Africa, the intensity of malaria transmission varies from one location to another and is greatly influenced by peculiarities such as mosquito species composition, vector competence [9] and seasonality related fluctuations in abundance [10]. High levels of malaria transmission have been reported as a result of these peculiarities and the vector's ability to exploit different breeding habitats, peaking at different times, to prolong the transmission period [8, 11]. In order to implement a successful malaria vector control programme, there is a need to have a good knowledge of these mosquito species and the dynamics influencing these peculiarities. Despite

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the massive net campaign programme by the National Malaria Elimination Programme [12] and other stake-holders in north-central Nigeria, very little effort have been channeled towards identifying the members of the major vector groups responsible for malaria transmission in this region. Although, there is a body of information on mosquito abundance of *An. gambiae s. l.* in north-central Nigeria [13, 14], very little has been done [15] to provide an updated information on the spatial abundance of members of the *An. gambiae* group in this region. The *An. gambiae s. l.* is primarily a major vector of malaria in sub Saharan Africa. It comprises seven morphologically indistinguishable species that play different roles in malaria transmission [5]. Despite the widespread distribution and established roles of *An. gambiae s. l* in malaria transmission in Nigeria, information on the identity, distribution and spatial variation of members of the complex remain scanty and unavailable to guide vector control activities in Kwara State, north-central Nigeria.

This study was carried out to investigate the relative abundance, species composition of endophilic mosquitoes, and the spatial relationships of members of the *Anopheles gambiae* complex with reported malaria cases in two local government areas in Kwara State, north-central Nigeria.

Materials and methods

Study area

The study was carried out in Gaa Bolohunduro (08.27^{0}148N and 04.38^{0}087E) and Osin (8.25^{0}080N and 4.34^{0}127E) both located in Ilorin South and Ilorin East Local Government Areas of Kwara State (Figure 1). The two distinct seasons in this area are the rainy season (March to October) and the dry season (November to February). The mean temperature is 30^{0}C during the dry season and 24^{0}C in the rainy season while the mean annual rainfall is about 1,150 mm. The yearly average minimum and maximum relative humidity is 65% and 85% [16]. Study-sites are rural communities and are located in the guinea savanna and semi-arid ecosystem. Ten houses were randomly selected for mosquito collections. Houses selected were built with mud, bamboo and roofed with straw and corrugated iron sheets. Most of the houses were without ceiling and so provided an unlimited access for host seeking mosquito entry. Both communities were sited close to a river with marsh lands along its banks. While the inhabitants of Gaa Bolohunduro are Nomadic Fulanis, those in Osin are predominantly Yorubas who are hunters and farmers.

Mosquito sampling and identification

Adult mosquitoes were sampled once every month between November 2013 and May 2014. Collection started late in Osin due to delayed consent from household and collection of indoor resting mosquitoes was done using pyrethrum spray catch method [5]. Prior to identification, all knocked down mosquitoes were collected following standard procedures [33] in properly labeled Eppendorf tubes. With the aid of a binocular microscope, the Anopheline mosquitoes were identified using morphological keys [17,18]. The species specific PCR assays were carried out at the Molecular Entomology and Vector Control Research Laboratory, Nigerian Institute of Medical Research, Yaba, Lagos. Mosquito samples identified morphologically as *An. gambiae* s.l were characterized to species level using Polymerase Chain Reaction (PCR) [19].

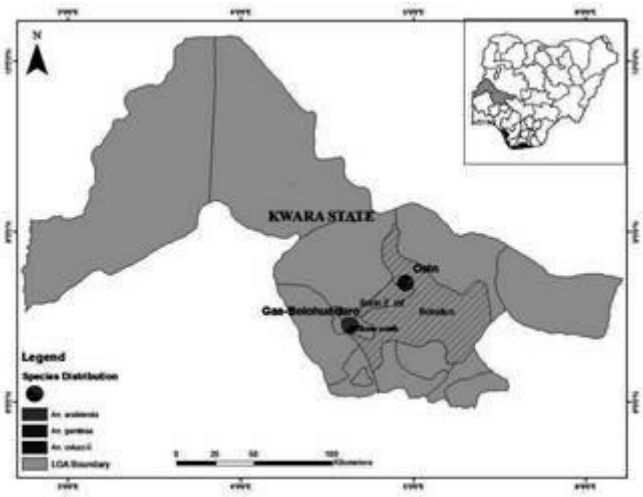


Figure 1. Map of Kwara State showing study communities and the spread of *Anopheles gambiae* sibling species.

Reported malaria cases

Reported malaria data cases for the study locations during the period of mosquito sampling were obtained from the Roll Back Malaria (RBM) Office at the Kwara State Ministry of Health, Ilorin.

Map processing

Maps were geo-referenced and digitized for Kwara state using Arc GIS software (version 3.29 ESRI CA, USA). Separate layers were created for the malaria cases and PCR-identified *An. gambiae* sibling species from each site which were displayed and classified using Arc View Spatial Extension.

Data analysis

Chi-square test was used to test for the significant difference in the species composition and the proportion of sibling of Anopheline species collected at different season. Also, Pearson correlation coefficient was used to compare the relationship between rainfall and Anopheline abundance using SPSS version 16.0 (SPSS Inc. Chicago Illinois).

Ethical consideration

Informed consent was obtained from the communities and household head before mosquito sampling commenced. Ethical approval for this study was obtained from the

University of Ilorin Ethical Review Committee (UERC/ASN/195).

Results

Composition of endophilic mosquitoes in the study communities

A total of 645 mosquitoes comprising 3 genera namely; *An., Culex* and *Aedes* were collected from the study communities (Table 1). The abundance of *Anopheles* species in Gaa Bolohunduro 307 (93.3%) and Osin 291 (91%) was significantly higher compared to that of *Culex* and *Aedes* species throughout the period of study in both communities (x^2= 51.2, *df* =5, *p*=0.050; x^2 = 40.3, *df*=4, *p*=0.046) (Table 1). All the Anopheline mosquitoes sampled were morphological identified to be *An. gambiae s. l.* and a higher average indoor resting density (IRD) was recorded in Osin compared to Gaa Bolohunduro (Table 1). However, the difference between the monthly IRD from both communities were not significant (x^2=4.50, *df*=8, *p*=0.366). Out of the 196 that were randomly selected and subjected to molecularly analysis, 65 (33.2%) were *An. arabiensis,* 18 (9.2%) were *An. coluzzii* while 113 (57.6%) were *An. gambiae* (Table 2). The percentage of *An. arabiensis* 53 (64.6%) sampled in Gaa Bolohunduro was higher compared *to An. gambiae* 24 (29.3%) and *An. coluzzii* 5 (6.1%) found in the same locality. The variation in the composition of members of the *An. gambiae s. l.* in Gaa Bolohunduro was not significant (*p*=0.078) compared to Osin (*p*=0.043). However, a strong association (*r*=0.975) was observed in the monthly distribution of two members of the *An. gambiae s. l*, *An. arabiensis* and *An. gambiae* (Table 3).

Table 1. Species composition of mosquito genera and average IRD of *Anopheles* species collected in study areas.

Study areas	No. of rooms surveyed	*Anopheles* species No. (%)	*Culex* species No. (%)	*Aedes* Species No. (%)	Average IRD of *Anopheles*	Total No. (%)
Gaa Bolohunduro	60	307 (93.3)	21 (6.4)	1 (0.3)	0.85Ç1.63	329 (51)
Osin	50	291 (92.1)	21 (6.6)	4 (1.3)	0.97Ç2.41	316 (49)
Total	110	598 (92.7)	42 (6.5)	5 (0.8)	0.91±3.12	645

Table 2. Summary of species composition of *An. gambiae* from the study sites.

	Study areas		
Species composition	Gaa Bolohunduro (%)	Osin (%)	Total (%)
An. arabiensis	53 (64.6)	12 (10.5)	65 (33.2)
An. coluzzii	5 (6.1)	13 (11.4)	18 (9.2)
An. gambiae	24 (29.3)	89 (78.1)	113 (57.6)
Total	82	114	196

Table 3. Species composition of *An. gambiae s. l* from the study sites.

	Communities							
	Gaa Bolohunduro				Osin			
Months	Number	*An. arabiensis* (%)	*An. coluzzii* (%)	*An. gambiae* (%)	Number	*An. arabiensis* (%)	*An. coluzzii* (%)	*An. gambiae*(%)
Nov.	3	0 (0)	1 (33.3)	2 (66.7)	-	-	-	-
Dec.	6	5 (83.3)	0 (0)	1 (16.7)	-	-	-	-
Jan.	16	14 (87.5)	0 (0)	2 (12.5)	10	1 (10)	2 (20)	7 (70)
Feb.	12	6 (50)	1 (8.3)	5 (41.7)	13	0 (0)	3 (23.1)	10 (76.9)
Mar.	12	8 (66.7)	1 (8.3)	3 (25)	8	2 (25)	1 (12.5)	5 (62.5)
Apr.	33	20 (60.6)	2 (6.1)	11 (33.3)	53	8 (15.1)	4 (7.5)	41 (77.4)
May.	-	-	-	-	30	1 (3.3)	3 (10)	26 (86.7)
Total	82	53 (64.6)	5(6.1)	24 (29.3)	114	12(10.5)	13 (11.4)	89 (78.1)

Table 4. Distribution of the members of the *An. gambiae* sibling species during different seasons.

Seasons	Communities									
	Gaa Bolohunduro					Osin				
	No.	*An. gambiae* (%)	*An. coluzzii* (%)	*An. arabiensis* (%)	Malaria cases (×1000)	No.	*An. gambiae* (%)	*An. coluzzii* (%)	*An. arabiensis* (%)	Malaria cases (×1000)
Late Dry (Nov.-Feb.)	37	10 (27)	2 (5.4)	25 (67.6)	1.49-3.15	29	22 (75.9)	6 (20.7)	1 (3.4)	0.99-1.68
Early Wet (Mar.-May)	45	14 (31.1)	3 (6.7)	28 (62.2)	1.12-2.39	85	67 (78.8)	7 (8.3)	11 12.9)	1.54-2.98
Total	82	24 (29.3)	5 (6.1)	53 (64.6)		114	89 (78.1)	13 (11.4)	12 (10.5)	

N= Numbers analysed by PCR.

Seasonal abundance of members of the Anopheles gambiae s.l. *in relation to rainfall*

The abundance of *An. gambiae s.l* peaked in April 2014 in both communities as rainfall increased. However, the predominance of the *Anopheles* species was not strongly dependent on rainfall in both Gaa Bolohunduro (r= 0.560) and Osin (r = 0.583) (Figure 2 and 3)

Seasonal abundance of Anopheles gambiae mosquitoes in relation to reported malaria cases

The percentage composition of *An. gambiae* and *An. coluzzii* was significant (p<0.01) and this coincided with higher reported malaria cases in Osin (1.54-2.98 x 1,000) during the wet season compared to the dry season (0.99-1.68 x 1,000) (Table 4).

Though, the difference in the proportion of *An. arabiensis*, the predominant vector species during the wet and dry season in Gaa Bolohunduro was not significant, the number of reported malaria cases was higher during the dry season (1.49-3.15 x 1000) compared to the wet season (1.12-2.39 x 1,000) (Figure 4 and 5).

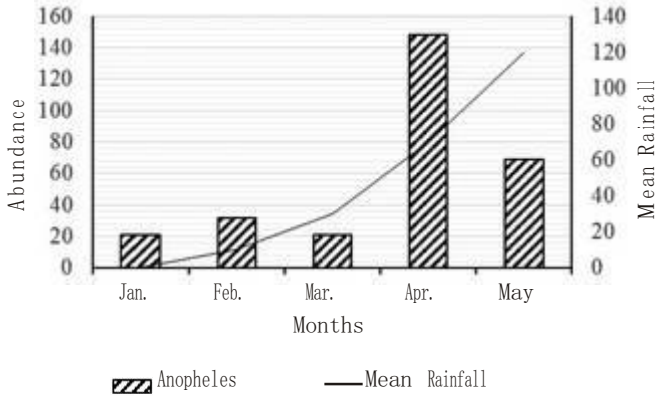


Figure 2. Relationship between *Anopheles* mosquito abundance in Osin and mean rainfall.

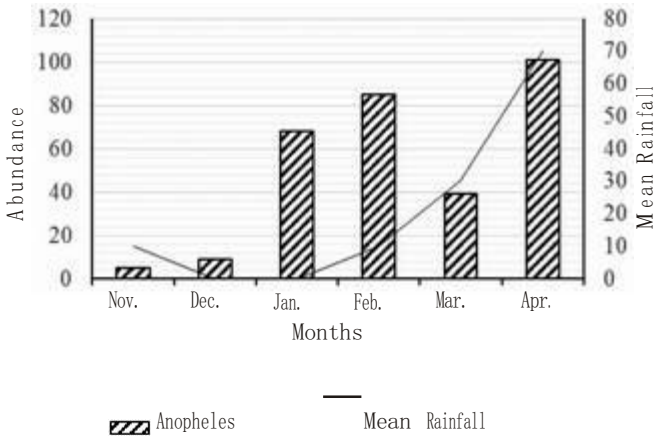


Figure 3: Relationship between *Anopheles* mosquito abundance in Gaa Bolohunduro and mean rainfall.

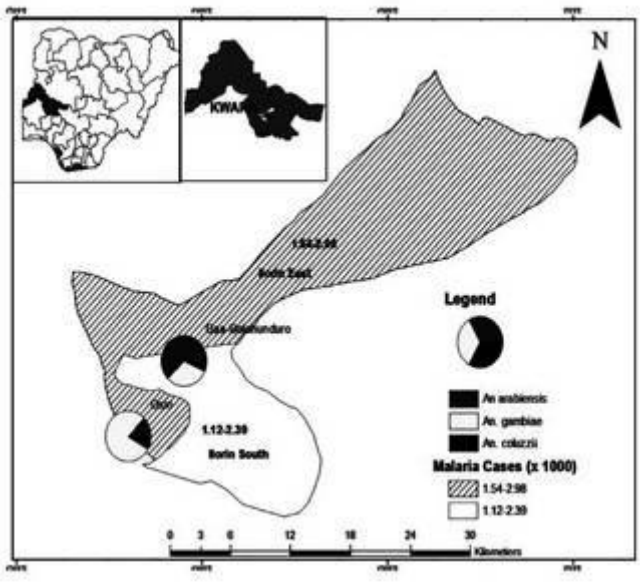


Figure 4: Spatial distribution of members of the *An. gambiae* complex and reported malaria cases during the wet season.

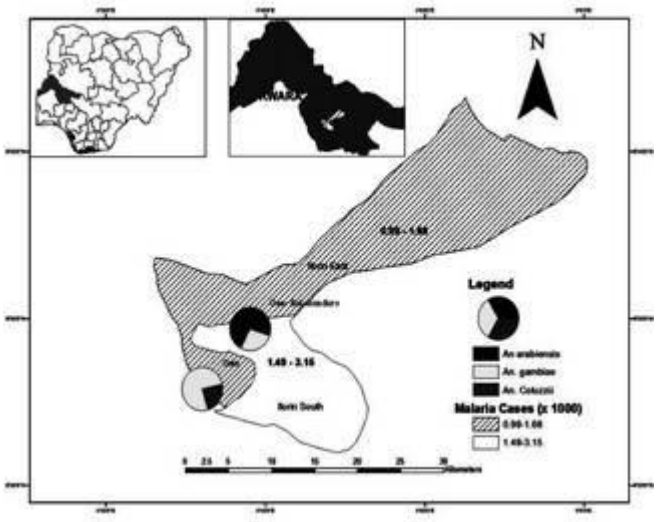


Figure 5: Spatial distribution of members of the *An. gambiae* complex and reported malaria cases during the wet season.

Discussion

This study reports the co-occurrence of three indoor resting mosquito vectors (*Aedes aegypti*, *An. gambiae* and *Culex quinquefasciatus*) of public health importance in Gaa Bolohunduro and Osin. The correct identification of these species within a community is a step towards identifying suitable control strategies. Here, we report *Anopheles gambiae s. l* as the predominant mosquitoes species found indoors compared to Culicines such as *Aedes aegypti* and *Culex quinquefasciatus* which are non vectors of malaria. The endophagic behaviour of Anophelines is a basic pre-requisite for the implementation of the indoor residual spray intervention. Similar results from other studies have shown that the total mosquitoes collected by pyrethrum spray catch had higher densities of Anophelines compared to the Culicines [11, 20].

The lower density of *Culex* and *Aedes* species found in the predominantly rural community were not surprising considering that the preferential polluted habitats of *Cx. quinquefasciatus* and the container habitats of *Ae. aegypti* usually associated with urban environments [21, 22] were less abundant in the rural study communities. However, the occurrence of both *Culex* and *Anopheles* species indoors is an indication of the possibility of the community dwellers being co-infected with malaria and lymphatic filariasis as Nigeria is one the most endemic country for filariasis in the world [23]. The co- occurrence of these three major vectors in both study communities is also an indication of suitable breeding sites in the community and the need to incorporate environmental management strategies as a component of the integrated vector management. *Anopheles gambiae s. l.* identified in this study belong to a species complex and has been severally reported as the principal vector of malaria in Nigeria [18, 24, 25, 26].

This study for the first time characterized three members of this major group and indicated the seasonal fluctuations arising in the composition of these species in Kwara State, north-central Nigeria. The three members of the *An. gambiae* complex characterized in both Gaa Bolorunduro and Osin communities are *An. gambiae* (formerly *An. gambiae* 'S'form), *An. coluzzii* (formerly *An. gambiae* 'M' form) and *An. arabiensis* [27].

The sympatric occurrence of these vectors; *An. gambiae* and *An. arabiensis* had previously been reported in the three states: Niger [15]; Oyo [8] and Osun [11] neighbouring to Kwara State. However, this is a first report showing the differential dominance of the members of the *An. gambiae* complex under the new nomenclature [27] according to season and location in Kwara state Nigeria. *Anopheles arabiensis* was the predominant vector species identified in Gaa Bolohunduro compared to *An. gambiae* in Osin. The difference in the composition of members of the *An. gambiae* complex in Gaa Bolohunduro was not significant compared to Osin where *An. gambiae* was significantly higher than other members. This makes correct identification in every locality a necessary requirement to implement a control strengthened by the understanding of the behavior of each of the vector species.

Though the density of the malaria vectors were observed to be on an increasing trend in April in both communities, the monthly density of *Anopheles* species found in the study areas was not significantly different in both the late dry season and early wet season. This suggests that mosquito density in these communities may not be directly dependent on rainfall. There is however a possibility that a longitudinal study covering the 12 (twelve) months of the year may clearly show this dependence. The density of Anopheline species in Ilorin, north-central of Nigeria had been reported to be influenced by mean rainfall [13]. Another explanation for the non-dependence of mosquito density on rainfall could be as a result of the closeness of the study communities to rivers with marsh lands which retain water suited for *Anopheles* mosquito breeding during the dry season. The implication of this non-dependence of Anopheline density on rainfall is that competent malaria vectors are available to sustain malaria transmission all through the year, whether dry or wet. A higher proportion of *An. arabiensis* identified in this study compared with other studies carried out in forest regions of southwestern Nigeria. This reflects the greater preference of this species for drier ecological conditions [18, 24] which abounds in the savannah of North Central Nigeria. The study location in Kwara is largely savannah and it is a transition between the forest and the arid region. The prevalence of *An. arabiensis* when compared to *Anopheles coluzii* in Gaa Bolohunduro and Osin during the rainy season seem to conflict with the preference of this species for drier ecological conditions. An explanation of the abundance of *An. arabiensis* during the rainy season could be as a result of the massive hatching of eggs that had been aestivating in breeding sites that became dry during the dry season or

possibly the recent report of the existence of two phenotypically distinguishable forms of *An. arabiensis*: A normal form and a heavily melanized form best adapted to arid hot environment [28].

However, there is a need to further investigate and establish if we are dealing with two different forms with specific ecological preferences or just one form. A variation in these species may possibly have an effect on their behaviour in malaria transmission. Despite the co-existence of *An. gambiae, An. coluzzii* and *An. arabiensis* in both communities, the predominance of *An. arabiensis* in Gaa Bolohunduro may have also been influenced by the species preference for blood meal from animal sources. Majority of the inhabitants in Gaa Bolohunduro are nomadic and pastoral cattle rearers living in close proximity with their animals. The opportunistic feeding behaviour of *An. arabiensis* species [18] may have attested to their biting of humans indoors when most animals had been moved away in search of pasture. *Anopheles gambiae* and *An. coluzzii* are highly endophilic, anthropophagic and wet season vectors [7]. *Anopheles arabiensis* has been reported to have a behavioural plasticity to survive the harsh conditions of the arid environment [28]. Again, the positive association observed between *An. arabiensis* and *An. gambiae* in this study supported previous claim that both species are more common during the wet season and disappear during the dry season [29]. The proportions of *An. gambiae* and *An. coluzzii* was significantly higher during the wet season collections in Osin. Similarly, an earlier study in Oyo State had reported a higher proportion of *An. gambiae* compared to *An. arabiensis* [8].

An increased reported case of malaria in Gaa Bolohunduro during the dry season is in contrast with most reports of significant malaria prevalence pattern during the rainy season [30, 31]. However, these higher numbers of malaria cases in this locality is consistent with available data on the influence of vector variation and availability of competent vectors during the dry season on the heterogeneity of malaria transmission [32]. Though the sporozoite infection status of the vectors was not determined, the density of *An. gambiae* and *An. arabiensis* found in the human dwellings strongly suggests their involvement in malaria transmission when compared to *An. coluzzii*.

Conclusion

The dynamics of strong indoor resting behaviour, significant availability of *An. arabiensis* in the wet season and higher reported malaria cases in the dry season calls for a further investigation and the review of management strategies based on emerging new knowledge in these localities.

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