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Heterogeneity in distribution of *An. gambiae* s.l and 2La chromosomal inversion in western Kenya

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Abstract

Environmental heterogeneity determines the distribution of disease vectors. Understanding vector distribution is important in evaluating malaria control measures. An assessment of distribution of *An. gambiae* was undertaken in low- and high-elevation zones of western Kenya based on 2La inversion. *An. gambiae* s.s. comprised 90.3% in high-elevation zones while *An. arabiensis* was 76% in low-elevation zone. The 2La inversion which facilitates local adaptation to degree of aridity showed significant variations in distribution of the two alternative arrangements. Frequency of inverted arrangement (2La⁻) was 14.3% in high-elevation and 52% in low-elevation zones. Frequency of standard arrangement (2La⁺) was 57.1% in high-elevation and 25% low-lying zones. Observations were that vector species are differentially distributed and adapted to environmental heterogeneities in study areas. Heterogeneous vector system complicates malaria control targeting vector as observed elsewhere in Africa. *An. gambiae* s.s. along altitudinal gradient deviated significantly from Hardy-Weinberg equilibrium suggesting restricted gene flow, which could relate to differences in microclimatic conditions.

Keywords: Malaria; adaptation; 2La inversion; *Anopheles gambiae*

Introduction

An. gambiae sensu lato is an important malaria vector in sub-Saharan Africa and has the largest geographical distribution. This is linked to ability of this vector to rapidly adapt to anthropogenic environmental changes [1, 2] which allows it to successfully exploit heterogeneities in the environment. The rapid adaptation is related to chromosomal inversions and different inversion combinations show non-random association with environmental heterogeneities [3, 4]. This contributes to more efficient exploitation of the environment and ultimately increases in malaria transmission [3]. Inversions are also distributed non-randomly in the *An. gambiae* genome, which suggests that the inversions or genes captured within them are targets of selection.

Understanding vector species distribution is vital to designing effective malaria control measures [5]. Studies in West Africa show that the distribution range of *An. gambiae* is strongly influenced by climatic factors. In Nigeria, strong and clear clines in 2La inversion frequencies exist in *An. gambiae* [4] and correspond to a clear aridity cline from the moist tropical forest in south to near-desert like conditions in north. Studies in Cameroon show that chromosome-2 inversions promote ecological divergence leading to spatial and /or temporal isolation between ecotypes [6]. Such ecological partitioning by this species is not well investigated in the Western Kenya region where malaria is both endemic and epidemic. This region is divided into two epidemiological zones, low-elevation malaria-endemic zones [7, 8] and high-elevation zones where transmission is moderate and prone to epidemics [9]. These two zones exhibit ecologic and climatic variation related to topography and altitude. High elevation zones (> 1,400m) have high moisture content because of high rainfall [7] while low-elevation (< 1400m) zones are drier with less rainfall.

The ecological adaptation and geographic distribution of *An. gambiae* s.l., an important malaria vector in Western Kenya region has not been well described. A Study undertaken in Western Kenya showed that populations of *An. gambiae* had inversion frequencies similar to those found in West Africa [8]. In West Africa, the forest zone is nearly monomorphic for the standard chromosome arrangement (2La⁺) while in the Savanna, the inverted arrangement (2La⁻) is frequent [3, 10]. Currently, Insecticide Treated Nets (ITNs) and Indoor Residual Spray

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(IRS) are essential component in malaria control programs in Kenya [11]. Such programs have previously been complicated by heterogeneous vector system [4]. Therefore understanding the distribution of *An. gambiae* s.l. mosquitoes and their adaptation in malaria prone areas of western Kenya is important in the evaluation of the continued success of current vector control measures. It is also important in the determination of how current global climate change has affected geographical distribution of *An. gambiae* mosquitoes. This study aimed at determining the distribution of *An. gambiae* s.l. and the 2La chromosome inversion in western Kenya. The observed differential distribution of vector species and 2La chromosomal inversions between the low- and high-elevation zones suggests that *An. gambiae* and *An. arabiensis* and carriers of different 2La inversion arrangements experience differential selection related to environmental differences in Western Kenya.

Materials and Methods

Study sites: Two high-elevation villages and one low-elevation village were selected for *An. gambiae* sampling between January 2007 and August 2008. Kisian (07° 68'2 S, 34° 67'814 E, altitude: 1161 – 1200m) in Kisumu district, western Kenya is low-lying site located in Lake Victoria basin. Its topography is mostly flat terrain, with the area being extensively deforested and swamps reclaimed for farming. The area receives annual rainfall <1000mm and temperature range is 16.8 - 31.5 °C. Larval habitats in Kisian are mainly irrigation trenches and hoof prints. Iguhu (0° 17' N, 34° 74' E, altitude: 1450-1580m) is a highland site in western Kenya with hill and valley topography. Forest cover has extensively been cleared and the area receives an average rainfall of 2000mm per year [12]. Most swamps in this area have been reclaimed for farming and gold mining also takes place in some swamps. The most abundant larval habitats in Iguhu are irrigation trenches, abandoned gold mines and hoof prints. Emutete (0° 32' N, 34° 64' E, altitude: 1,453-1,632m) is highland site also with hill and valley topography. Swamps have been reclaimed for agriculture with maize being the main crop in this area. The most abundant larval habitats in this area are permanent ditches that store water for irrigation and irrigation trenches. The low-elevation village Kisian is separated from high-elevation villages by ~ 52 km. The working hypothesis was that no differences would be seen in distribution of the 2La inversion in *An. gambiae* s.s. along a altitudinal gradient in western Kenya and that there was no differential adaptation by *An. gambiae* s.l. mosquitoes to ecological differences related to differences in microclimate conditions.

Mosquito collection: Surveys were undertaken for a 20-month period in three villages. Anopheline larvae were collected once every month and all available larval habitats were prospected. The reason for sampling larvae was to ensure unbiased sampling of *An. gambiae* s.l. in study areas since adults have differential resting behaviour encompassing both indoor and outdoor resting. Once taken to the laboratory,

fourth and third instar larvae sampled were morphologically identified and those identified as to *An. gambiae* s.l. were preserved in 100% ethanol for rDNA-polymerase chain reaction identification. First and second instar larvae were allowed to mature to the third instar stage before identification and preserved for molecular analyses.

Climate data collection: HOBO data loggers were placed outside selected homesteads to record outdoor temperature and outdoor absolute humidity in one highland village (Ighu) and low land village (Kisian) during study period. The data loggers were placed in Stevenson screen at 2m above the ground. Three loggers were used to collected climate data in each village.

Molecular Methods: A proportion of larvae morphologically identified as *An. gambiae* s.l. were randomly picked for molecular identification. About 200 from the low-elevation village and similar number from the high-elevation villages were assayed. Genomic DNA was extracted from individual larvae and identification done based on ribosomal DNA PCR [13]. Thermal cycling condition were initial hold at 94 °C for 4 minutes, followed by 30 cycles of 94 °C for 30 seconds, 55 °C for 30 seconds and 72 °C for 30seconds and final step at 72 °C for 7minutes. Mosquitoes identified as *An. gambiae* s.s. were karyotyped for the 2La inversion following the PCR protocol [14] the observed banding pattern for 2La inversion is shown in plate in result section.

Statistical Analysis: Chi-square tests were used to determine differences in abundance of *An. gambiae* s.s. and *An. arabiensis* between the low-elevation and the high-elevation villages and to compare the frequencies of the 2La inversion arrangements. Conformance to Hardy-Weinberg equilibrium was tested using Wright's *F* statistic [15, 16]. Where $F = (4ac - b^2) / (2a+b)(2c+b)$ and a and c are absolute frequencies of the two homozygous classes and b the frequency of heterozygote. A t-test was used to compare mean outdoor absolute humidity and temperature between high- and low-elevation villages.

Results

Abundance of *An. gambiae* and *An. arabiensis*: A significant difference was observed in the abundance of *An. gambiae* s.s. and *An. arabiensis* between the low-elevation and high-elevation villages (Pearson, $\chi^2 = 133.4$, DF = 2, $P < 0.001$; Table 1). *An. gambiae* s.s. was the predominant species in high-elevation villages (Ighu and Emutete) while *An. arabiensis* was predominant in the lowland village (Kisian). There was a positive association between *An. gambiae* s.s. abundance and increase in altitude while *An. arabiensis* showed inverse relationship with increase in altitude. This suggests that environmental heterogeneities related to differences in altitude play a crucial role in determining the spatial distribution of malaria vectors in western Kenya. Significant difference was observed in outdoor absolute humidity between low and high elevation villages ($t = 94.5$, $df = 46$, $P << 0.0001$).

Table 1: Abundance of *An. gambiae*.s.s and *An. arabiensis* and outdoor temperature and absolute humidity in low-and high-elevation villages in Western Kenya

Elevation (m)*	Mean temperature (Range)	Mean outdoor absolute humidity (Range)	N	<i>An. arabiensis</i>	<i>An. gambiae</i> s.s.
1450 - 1632	20.1 (11.7 - 34.8)	9.7 (2.6 - 17.1)	159	11.3% (18)	88.6% (141)
1161 - 1200	23.8 (16.4 - 41.2)	14.2 (5.8 - 26.5)	134	76% (102)	24% (32)

* data from high-elevation villages are pooled. Elevation is in meters above sea level. Temperature in in °C and humidity in gm/M³

2La inversion frequency and conformance to Hardy-Weinberg equilibrium: Similarly, a significant difference in distribution of inverted and standard chromosomal arrangements between low- and high-elevation villages (Pearson, $\chi^2 = 12$, DF = 4, P = 0.017) was observed. There was 3-fold difference in frequency of inverted inversion and 2-fold difference in frequency of standard inversion between low- and high-elevation villages. The inverted arrangement (2La) decreased with increase in altitude, from 52% in lowland to 14.3% in highland (Table 2). The standard arrangement (2La⁺) frequency increased with increase in altitude from 24% in lowland to 57.1% in highland.

Table 2: Frequency of 2La inversion arrangements and conformance to Hardy-Weinberg equilibrium.

Village	N	% Inversion frequency			F	P
		2La	2La+	2La/+		
Emutete	28	14.3	57.1	28.6	0.229	ns
Iguhu	104	25	53.9	21.1	0.539	< 0.05
Kisian	25	52	24	24	0.269	ns
Total*	157	27.4	49.2	22.9	0.522	< 0.05

F is a measure of Hardy-Weinberg: when its absolute value is < 1.96√N equilibrium is verified (P = not significant) otherwise there is significant deficiency of heterozygotes. *total represent pooled population, ns = not significant

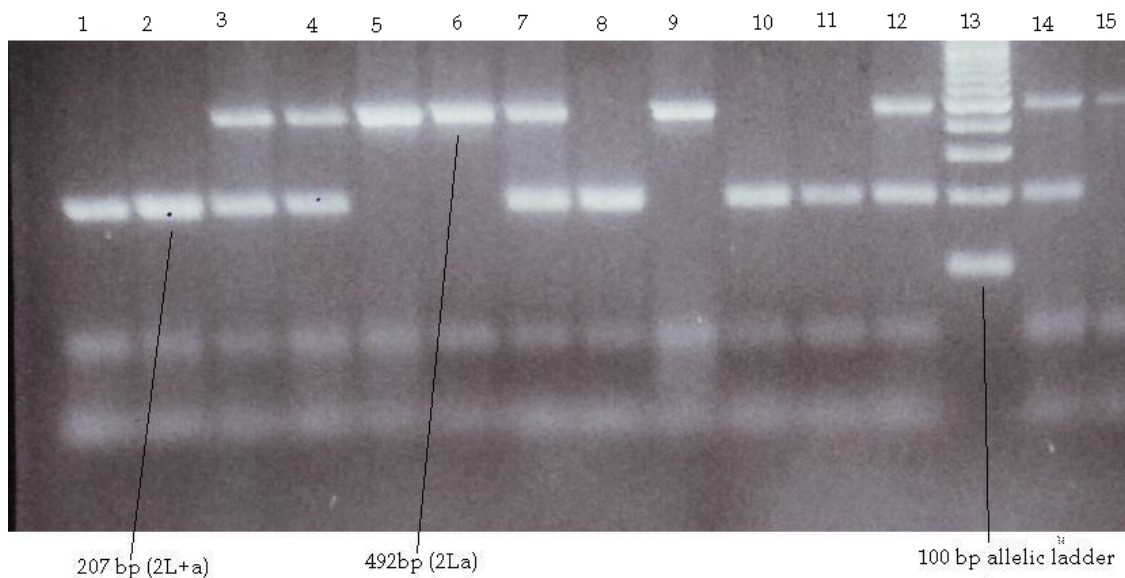


Plate: The observed banding pattern for 2La inversion

Deviation from Hardy-Weinberg equilibrium was found in Iguhu population due to heterokaryotype deficit (Table 2). There was no deviation from HW equilibrium in Emutete and Kisian populations suggesting that each of the two populations was at a state of panmixia. When the three populations were pooled a significant deviation from HW equilibrium was observed due to heterozygote deficiency. Suggesting that *An. gambiae* s.s. populations along an altitudinal gradient in western Kenya are structured and gene flow is restricted between low- and high-elevation populations.

Discussion

Distribution heterogeneity of *An. gambiae* s.l along altitudinal gradient: Understanding the distribution of malaria vectors is important in strategic planning of vector control measures [5, 17-19]. Geographical distribution of malaria vectors has been found to be sensitive to climatic conditions [3, 20]. In this study *An. gambiae* s.s. and *An. arabiensis* were

the only members of *An. gambiae* complex found, a finding that is consistent with previous studies in Western Kenya [7, 21-23]. *An. gambiae* s.s. was the predominant species in high-elevation villages (1,450 – 1,632m) whereas *An. arabiensis* predominated in the low elevation village (1,100 – 1,200m). Significant differences were observed in mean outdoor absolute humidity between high- and low-elevation villages. Low elevation zones experience less rainfall and high temperatures while high elevation zones are cool and wet, suggesting that ecological differences in western Kenya are related to differences in elevation and climatic factors. Several studies [4, 5, 15, 24-27] have shown that *An. arabiensis* is more abundant in dry zones while *An. gambiae* is more common in wet zones. Similar observations were made in this study, whereby *An. gambiae* predominated in the wetter and cold high-elevation villages of Iguhu and Emutete while *An. arabiensis* was the prevalent species in dry and hot low-elevation Kisian.

An interesting observation made in this study was presence of

An. arabiensis in high elevation areas (1,450 – 1,632m) of western Kenya contrary to previous studies by Minakawa *et al* [7] and Shililu *et al* [22], working in western Kenya, did not record any *An. Arabiensis* in areas above 1,400 m. This study found that 11.3% (18.9) of *An. gambiae* s.l. mosquitoes specimens analyzed from high elevation sites were *An. arabiensis*. This observation corroborate another study undertaken in Kenya and Uganda at altitudes of 1,670 – 2,290m that reported presence of *An. arabiensis* at those altitudes [28]. The presence of *An. arabiensis* above 1,400m can be attributed to effect of climate change related ecological changes that have enabled *An. arabiensis* to adapt and expand its geographical range. Study in Kenya [29] showed that deforestation changed the microclimate of mosquito larval and adult habitats and enhanced the survivorship and reproduction of *An. arabiensis* in the highland region. The existence of this species in high-elevation zones could lead to stable malaria transmission and increased malaria prevalence in highlands. This could lead to high mortality and morbidity since at high altitudes malaria transmission is low and human population has poorly developed immunity to malaria because exposures are infrequent [30].

2La chromosomal inversion frequency: This study compared the distribution of various 2La inversion arrangements between low and high-elevation western Kenya villages. The 2La inversion system in *An. gambiae* has been shown to affect malaria transmission. A stable, significant 2-fold difference in *Plasmodium* infection rates have been detected among carriers of different inversion karyotypes on chromosome 2 [8]. The infection rates in standard inversion were higher than in inverted inversion. Hence studies aimed at describing the distribution of 2La inversion in natural population are of great epidemiological importance [31]. Studies suggest that inversions on second chromosome of *An. gambiae* are involved in ecological specialization [6] and hence are strongly exposed to environmental selection. Observed heterogeneous distribution of 2La inversion arrangements in this study could reflect a corresponding variation in microclimatic situation related to altitudinal and ecological differences and confirms great ecological flexibility of *An. gambiae* s.s. [32].

The inverted (2La) inversion arrangement decreased 3-fold from 52% in the dry low-elevation village to 14.3% in the wet less humid high-elevation villages. This suggests that differences in microclimatic conditions related to altitudinal differences in western Kenya exert selection pressure on *An. gambiae* s.s. leading to differential adaptation and distribution of chromosomal variants. The minimum absolute outdoor humidity was 2-fold higher in low-elevation village than high-elevation villages. Therefore monitoring of 2La inversion frequencies along climatic clines could provide an early warning system for climatic effects on distribution of *An. gambiae* s.s. particularly if inversions carry genes that influence traits under selection [33].

Ecological flexibility of *An. gambiae* mosquitoes associated with inversion polymorphisms is not only relevant to the epidemiology of malaria but also impact on the efficacy of vector control strategies. As was shown in Nigeria, different degrees of endophily associated with inversion polymorphism led to a non-uniform exposure of different inversion karyotype carriers to insecticides [4]. The differential

distribution of *An. gambiae* s.l. and 2La chromosomal inversions observed in this study could affect current vector control measures in Western Kenya, which are based primarily on insecticide treated bed net (ITN) and indoor residual spray (IRS). To be able to predict the continued success of these vector control measures, it is crucial that vector species distribution and adaptation patterns are understood. Studies suggest that use of treated nets may lead to selection for *An. gambiae* s. s. 2La karyotypes that preferentially feed outdoors [34]. It is therefore significant to monitor frequencies of 2La inversion to effectively evaluate control measures.

Deviations from Hardy-Weinberg expectations observed in this study were due to heterozygote deficiency. This is likely due to selection against heterozygotes which could be less adaptable to the environmental conditions. A study by Lehmann and Diabete [35] indicated that selection has a key role in shaping inversion frequencies. The results of this study differ from others based on microsatellite markers, which have suggested no population differentiation over vast geographical areas up to 700km [36]. The current study used markers that are subject to selection to analyze population structure of *An. gambiae* s.s. populations on a spatial scale. Studies on chromosomal inversions in other regions have led to distinction of *An. gambiae* s.s. into several chromosomal forms [37], hence a better understanding of the population structure of this important malaria vector.

This study begins to address the association between chromosomal inversions and the distribution and local adaptation of *An. gambiae* s.s., an important malaria vector in Western Kenya and the Eastern Africa region. The findings suggest that *An. gambiae* s.s. is differentially distributed and is not a panmictic population in Western Kenya. In view of importance this species has on malaria transmission extensive studies are needed to establish whether the *An. gambiae* s.s. population in the Eastern Africa region is a mosaic of populations distinguished by different combinations of chromosomal inversion [4]. Since understanding population structure is central to attempts to reduce malaria by genetic manipulation of vector populations [38].

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