Original article

Chromosomal inversion polymorphisms of Anopheles arabiensis from some localities in Ethiopia in relation to host feeding choice

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Abstract: Indoor resting blood-fed anopheline mosquitoes were collected from five localities in Ethiopia. Cytogenetic and bloodmeal determinations were conducted. *Anopheles arabiensis* was the only sibling species identified in all of the study localities. Paracentric chromosomal inversion polymorphisms were observed on 2R and 3R arms. The highest cattle-fed percentage was from those mosquitoes collected from cattle sheds (47.5%) followed by mixed dwellings(44.2%) and human dwellings (3.9%). Statistically significant correlation (r=0.94, P<0.001) was observed between the frequencies of 3Ra inversion and cattle feeding. [*Ethiop. J. Health Dev.* 1998;12(1):23-28]

Introduction

Chromosomal rearrangements in the *A. gambiae* complex that occur as paracentric inversions are used reliably for the identification of the six sibling species. In this regard those rearrangements that occur as fixed homozygote are especially useful (1). Similarly, inversions that occur as intraspecific polymorphisms are known to have significant adaptive values (2). Interrelationships between inversion polymorphisms and seasonality in *A. arabiensis* have been demonstrated (3). Additionally the significance of intraspecific inversion polymorphisms in relation to biting and resting habits, climatic and ecological conditions have been also documented (4). In comparison very few investigations have been carried out that view paracentric inversions relative to parameters pertaining to vector potential such as human blood index and sporozoite rate (5). In this study data is presented on the interspecific inversion polymorphism of *A. arabiensis* in relation to host choice pattern.

Methods

Study Area: Five localities were selected from different malarious regions in the east (Alibete and Ledi), south (Sille and Erbore) and southwestern (Jawe) parts of Ethiopia.

The vegetation cover of the study sites ranges from mixed tropical thorn bush and woodland in Alibete, Ledi and Sille to Savannah grassland and woodland in Erbore and Jawe, respectively. The human activity of all the study localities comprises of subsistence agriculture and animal husbandry, including nomadic pastoralism with the exception of Jawe, where the indigenous inhabitants practise hunting, fishing and honey gathering.

The inhabitants of all the localities are settled in scattered small villages. Cattle are sometimes kept for the night in a section of the house partitioned from the rest. These dwellings are thus called "mixed dwellings".

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Mosquito Collection: Blood-fed, indoor resting female *Anopheles* mosquitoes were collected from 0500-0900 hours early in the morning from each study locality following the peak mosquito breeding season. Collection was carried out from May to June 1995 in Alibete, Ledi, Sille and Erbore; and from Mid September to October 1995 in Jawe. Mosquitoes were collected with mouth

Table 1: **Percentage frequencies of chromosomal inversions in *Anopheles arabiensis* from indoor resting collections in selected localities in Ethiopia**

No. of	Alibete	Erbore	Ledi	Sille	Jawe	Total
specimens(N	43	45	49	50	31	218
1.96√N	0.30	0.29	0.28	0.28	0.35	0.13
			2Rb			
+b/+b	16.3	15.6	18.4	26	32.3	21.1
+b/b	55.8	48.9	49.0	40.0	38.7	46.8
b/b	27.9	35.6	32.7	34.0	29.0	32.1
Freq.	55.0	60.0	57.1	54.0	48.4	55.5
F	-0.14	-0.01	0	0.19	0.22	0.05
			3Ra			
+a/+a	69.8	68.9	79.6	48.0	64.5	66.1
+a/a	30.2	31.1	20.4	52.0	35.5	33.9
a/a	0	0	0	0	0	0
Freq.	15.1	15.6	10.2	25.0	17.7	17.0
F	-0.18	-0.18	-0.11	-0.34	-0.21	-0.20

operated aspirators from human dwellings, cattle shelters and mixed dwellings whenever these biotopes are available and kept separately. The pictorial keys of Verrone (6) and the morphological descriptions of Gilles and Coetzee (7) were used to distinguish the *A. gambiae* s.l. from other anopheline species. Each mosquito collection was then divided into two groups. In the first group specimens were killed and dried for bloodmeal detection, the second group were kept under humid conditions until the majority reached the semi-gravid stage. Gravid and pregravid specimens were excluded from each sample.

Chromosomal Preparation: Semi-gravid *A. gambiae* s.l. were transferred to screw-caped vials filled with Carnoy's fixative (three parts absolute ethanol and one part glacial acetic acid) and transported to the laboratory and stored at -20ºC until chromosomal analysis was carried out. Polytene chromosomes were obtained following the method of Hunt (8). Briefly, ovaries from mosquito specimens were dissected out on a microscope slide and stained with Orcein for five minutes. After washing with 50% propionic acid, a cover slip was placed over the ovaries and a gentle pressure was applied by tapping. The giant polytene chromosomes from the ovarian nurse cells were scored for inversions following the technique of Coluzzi (4).

Karyotype frequencies were tested for deviation from the Hardy-Weinberg equilibrium (5) using Wright's F statistics following the method of Brown (21), where $F=(4ac-b^2)/[(2a+b)(2c+b)]$, a and c are the frequencies of the two homozygotes and b is the frequency of the heterozygotes. When $|F|>1.96/\sqrt{N}$, (N is the number of samples), there is a significant departure from expected values of the Hardy-Weinberg equilibrium. Positive and negative values for F indicate deficiency and excess of heterozygotes, respectively. A P-value of 0.05 was taken as a cut off point.

Blood meal Identification: A direct ELISA described by Beier (9) with slight modification was used to test mosquitoes for the presence of human and bovine immunogloblins. Each mosquito abdomen was crushed in 125µl phosphate buffer saline (PBS). 50µl of the triturate was added to flat bottomed 96 well microtitre plate. Plates were held at room temperature for three hours after which they were washed three times with PBS containing 0.5% Tween 20(Sigma Chemicals, USA). 50µls of peroxidase conjugated anti-human IgG A-8419 and anti-bovine IgG , A-8917,(Sigma Immuno Chemicals, USA) were then added into respective plates which were previously labelled for the respective anti-IgG's. Both the anti-human and anti-bovine IgG's were initially diluted 1:2000 in 0.5% boiled casein containing 0.025% TW20. After 30 minutes wells were washed four times. 100µl of peroxidase substrate, ABTS (2,2' azino-di [3-

Table 2: **ELISA bloodmeal results of *Anopheles arabiensis* from some areas in Ethiopia. (Numbers in parenthesis are percentages in relation to total tested).**

Biotope	Village	Result of bloodmeal test				others
		Total Tested	Human (H)	Bovine(B)	Mixed(H+B)	
Human	Ledi	24	12(50)	0	0	12
Dwellings	Alibete	19	5(26.3)	2(10.5)	2(10.5)	10
	Sille	138	69(50)	6(4.3)	0	63
	Jawe	55	30(54.5)	1(1.8)	0	24
Mixed dwellings	Sille	163	22(13.5)	72(44.2)	8(4.9)	61
Cattle	Sille	50	0	22(44)	1(2)	27
shelters	Erbore	89	3(3.4)	44(49.4)	0	42

ethylbenzthiazoline sulfonate (6)]), was then added to each well.

Absorbance at 405 nm was recorded with a reader (Labsystems Multiscan MCC 1340, Finland) 30 minutes after the addition of substrate. Human and bovine blood dried on filter paper was used as positive controls while male anopheline mosquitoes served as negative control. Positive and negative controls were included on each plate. Samples were considered positive when their absorbance values were greater than the mean plus three times the standard deviation of four negative controls.

Results

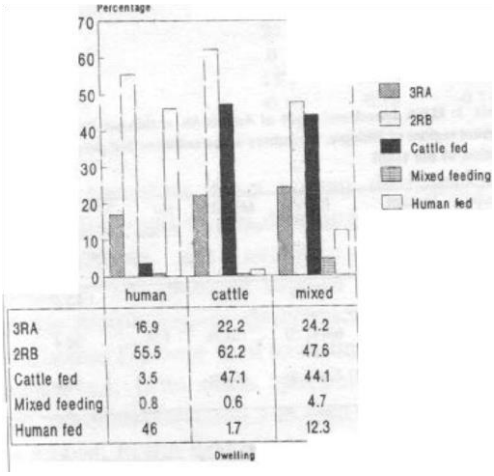
Overall, 218 mosquito specimens were scored for inversion polymorphisms. In all of the study localities, *A. arabiensis* was the only cyto species identified. Polymorphism was observed for paracentric inversions on chromosome 2R and 3R. The heterozygote and the inverted homozygote were observed for 2R (Table 1). On the other hand, only the heterozygotes were observed in the case of chromosome arm 3R. The mean karyotype frequencies for the 2Rb and 3Ra inversion are 55.5% and 17%, respectively.

Additionally, a statistically significant difference was observed in the frequencies of inversion 3Ra between the study localities ($X^2 = 11.73$, for 2 x n, n>2. P=0.02). In contrast, the difference in the frequency of 2Rb between the localities was not statistically significant ($X^2 = 4.69$, P>0.05).

Table 3: **ELISA bloodmeal reult of *Anopheles arabiensis* Pooled from different regions of Ethiopia, (Numbers in parentheses are percentage in relation to the total)**

Biotope	Human (H)	Bovine(B)	Mixed(H+B)	No Tested	% identification
Human dwellings	116(50.2)	9(3.9)	2(0.9)	231	55.0
Cattle	3(2.2)	66(47.5)	1(0.7)	139	50.4
Sheds					
Mixed	22(13.5)	72(44.2)	8(4.9)	163	62.6

Out of 538 *A. arabiensis* collected from different biotopes 299 (55.6%) gave positive reaction to either human, bovine or both mixed blood. The results are presented in Table 2 and summarized in Table 3. The highest human blood proportion came from human dwellings followed by mixed dwellings and cattle sheds (Table 3). The differences among the three biotopes in relation to the human blood percentage were statistically significant (P< 0.0001). The human blood proportion from human dwellings from Alibete was lower than the other three villages. The human blood percentage was significantly higher (P< 0.01) in the mixed dwellings than in the cattle shed. Mixed feeding was recorded only in two localities, Alibete and Sille (Table 1). The highest mixed feeding proportion was recorded in Alibete, (10.5%), from human dwellings, followed by 4.9% and 2% from mixed dwellings and cattle sheds collections, respectively, in Sille. When the results are pooled from the different localities (Table 3) the highest mixed feeding proportion came from the mixed dwellings followed by the human dwellings and cattle sheds. The differences in the mixed proportions from the three biotopes were not significant except between mixed and human dwellings (P>0.05). Within the mixed dwellings the



	human	cattle	mixed
3RA	16.9	22.2	24.2
2RB	55.5	62.2	47.6
Cattle fed	3.5	47.1	44.1
Mixed feeding	0.8	0.6	4.7
Human fed	46	1.7	12.3

Figure 1: **Percentages of inversion polymorphisms and feeding choice of *A. arabiensis* in three biotopes**

bovine proportion was more than three times higher than the human-fed proportion.

A statistically significant correlation (r=0.94, P<0.001) (Fig.1) was observed between those *A. arabiensis* carrying the 3Ra inversion and those that have fed on cattle when the inversion polymorphisms and feeding choice from all the localities were pooled together and seen in relation to the three biotopes.

Discussion

In agreement to previously done studies (10,11), *A.arabiensis* was the only sibling species identified in all the study localities.

The overall inversion polymorphism picture indicated a panmictic (randomly mating) population of indoor resting *A. arabiensis*. In Sille, however, in those *A. arabiensis* possessing the 3Ra inversion, a significant excess of heterozygotes was observed. This condition could have been the result of a differential endophily and/or anthropophily of heterozygotes or the propensity of both the homozygotes to be exophagic and exophilic. Investigations carried out on *A. arabiensis* in West Africa (4) have shown carriers of different 2R inversions that have the tendency to occur more frequently in indoor and outdoor enviornments. Similar studies in the Gambia (22) have asserted that carriers of different karyotypes of the 2Rn inversion in *A. melas* have a non-random migration pattern into houses.

Inversion polymorphisms on 2Ra, 2Rb, 2Rd and 3Ra have been reported from selected localities in the east, south and southwestern Ethiopia (9,10). In the present study 2Rb and 3Ra were the only polymorphisms identified. Higher inversion polymorphisms are recorded from the Northern Savannah populations (West Africa and Sudan) of *A. arabiensis* (4,5,12) of which the western part of Gambella is included (11). Both 2Ra and 2Rd are recorded from Itang and Gambella, these localities are further west from Jawe, nearer to the Sudanese boarder. It, therefore, seems highly likely that Jawe and the surrounding areas are the boundary limits for the 2Ra and 2Rd inversions in western Ethiopia.

The presence of 2Rb and 3Ra polymorphic inversion in this study is in concordance to the fact that *A. arabiensis* populations within and south of the Rift Valley commonly possess these inversion (10,12,13).

The common occurrence of 2Rb and 3Ra could very well imply that these inversions may have an adaptive role in this species preference for arid conditions (13,14,15,16).

The higher human blood proportions from human dwellings and lower human blood proportions from the mixed dwellings and cattle sheds is expected. White *et al.* (17) have reported a human blood index of 100 % for *A. gambiae* s.l in Jimma collected from houses. Krafsur (18) has also

reported a similar result in Gambella. In another study which involved different biotopes around Jimma, the highest human blood positive proportion came from human dwellings followed by mixed dwellings, animal sheds and pit shelters (19).

Bovine-fed proportions were also higher in cattle sheds, showing catholic feeding behaviour tendency of *A. arabiensis*. The lower human blood percentage and the higher bovine blood percentage from human dwellings in Alibete could be due to the high number of cattle kept around houses in the open. Mosquitoes fed outside on cattle could seek shelter inside houses, reducing the proportion of the human fed mosquitoes. The presence of bovine-fed mosquitos in human dwellings and human fed mosquitos in cattle sheds is possibly due to mosquitoes which feed outdoors on either of the hosts and later rest either in cattle sheds or human dwellings.

Within the mixed dwelling the bovine-fed proportion was more than three times higher than the human-fed proportion, showing *A. arabiensis*' preference for cattle. This finding is in accordance with previous studies (13,14,20).

When the paracentric inversion polymorphisms of *A. arabiensis* are seen in relation to the host feeding of this vector species, those possessing the 3Ra inversion were seen to be highly prone to feed on cattle (Fig. 1).A similar study conducted by Petrarca and Beier (5) has shown that the majority of the *A. arabiensis* carrying the standard homozygote form of 2Rb have fed on humans. The investigators of this study, however, have concluded that this situation was rather due to the differential post-feeding endophily of this malaria vector species and not a situation whereby the carriers of the 2Rb standard homozygote form to preferentially feed on humans. In this study, the frequency of cattle feeding in *A. arabiensis* was highest whenever cattle was available (cattle shelters and mixed dwellings). Similar to this, the frequency of 3Ra was also higher when cattle were around. However, the frequency of 3Ra in human dwellings was not significantly lower in comparison to cattle shelters and mixed dwellings as expected to be. It is, therefore, difficult to conclude that those *A. arabiensis* carrying the 3Ra inversion feed on cattle preferentially. On the other hand, the correlation between the frequencies of 3Ra and cattle feeding was significant. This finding could instil the notion that the gene/genes within the segment designated "a" of chromosome arm 3R could play a decisive role in the feeding choice of *A. arabiensis*. An indepth study on the possible relationship of intraspecific inversion polymorphisms with host feeding choice in *A. arabiensis* is however required to further substantiate this hypothesis.

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