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HOST BLOOD MEALS AND CHROMOSOMAL INVERSION POLYMORPHISM IN *ANOPHELES ARABIENSIS* IN THE BARINGO DISTRICT OF KENYA

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ABSTRACT. Studies were carried out in the villages of Kapkuikui and Maji-Ndege in the Loboi area of Baringo District, Kenya, to obtain baseline data on species identification of the *Anopheles gambiae* group, their feeding and resting behavior, and their frequencies of chromosomal inversions. This was carried out towards predicting the effect of introducing permethrin-impregnated cloths or other intervention measures. In this study, *Anopheles arabiensis* was identified as the only species of the *An. gambiae* group. This species contained 2 inversions, 2Rb and 3Ra, occurring at frequencies ranging from 55 to 60%, and from 5 to 11%, respectively. There was no evidence for nonrandom mating. Indoor- and outdoor-collected samples were significantly different in respect of inversion 3Ra in one village and in the distribution of the different sources of blood meals in both areas. In these villages, 37% of indoor-resting mosquitoes fed outside before entering houses to rest.

INTRODUCTION

Members of the *Anopheles gambiae* group of species are important vectors of malaria, bancroftian filariasis, and certain arboviral infections in tropical Africa (White 1974). Most of these species are morphologically indistinguishable, but their identification may be important for both epidemiological purposes and better management of control operations.

Fixed paracentric chromosomal inversions provide a method for identifying these species (Coluzzi et al. 1979). Polymorphisms for such chromosomal inversions have been utilized to study the relationships between certain inversion karyotypes and variation in the behavior and ecology of the vectors (Coluzzi et al. 1977, 1979, 1985) and to malaria sporozoite indices (Petrarca and Beier 1992).

Mosquito feeding and resting behavior is important in relation to adulticidal methods of vector control. For example, the tendency of some of the species of the *An. gambiae* group to feed indoors and to rest outdoors (exophily) was considered to be responsible for the failure of house spraying to interrupt transmission of malaria in the Garki district of Nigeria (Molineaux and Gramiccia 1980). On the other hand, Sharp and le Sueur (1991) have shown that the human blood index in *Anopheles arabiensis* Patton varies depending on whether a particular area is subject to house spraying.

Recently, Mutinga et al. (1992, 1993) have evaluated the use of wall cloths treated with permethrin for the control of malaria and leishmaniasis in Marigat, Baringo District, Kenya. Following initial encouraging results, extension of the use of the wall cloth to other untreated areas was proposed. Our study collected baseline data in these areas on the sibling species of the *An. gambiae* group, frequencies of chromosomal inversions, and feeding and resting behavior of the vector species. Studies such as these form an essential component of our understanding of the control of this group of species.

MATERIALS AND METHODS

The villages of Kapkuikui and Maji-Ndege in the Loboi area of Baringo District, Kenya, served as study sites. The area lies along the eastern arm of the Rift Valley. For a detailed description of the area see Mutinga et al. (1993). In these villages residents own large numbers of cattle and goats that are normally kept in cattle sheds.

The villages were visited near the end of the long rainy season during June and July 1991, and August 1992. On each monthly visit, mosquitoes were collected from the same 3 houses for 5 consecutive days, using battery-operated aspirators for 30 person-minutes per house. Mosquitoes were also collected from 3 outdoor pit traps (Muirhead-Thomson 1958) that were dug about 6 m away from the houses being sampled for indoor-resting mosquitoes. The indoor- and outdoor-resting catches were done in the morning hours and in parallel.

The mosquitoes were brought to the ICIPE field station in Marigat where they were sorted. All freshly bloodfed mosquitoes of the *An. gambiae* group of species were either held at room temperature to allow ovarian development to reach the appropriate stage for chromosomal preparation or had their abdomens smeared im-

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Table 1. Frequencies (%) of chromosomal inversions 2Rb and 3Ra and tests for heterogeneity between indoor and outdoor samples in *Anopheles arabiensis*. *n* represents number of mosquitoes with 2Rbqr arrangement.

			2Rb			3Ra			2Rbqr
Locality		*N*	%	χ^2	*P*	%	χ^2	*P*	*n*
Kapkuikui	Indoor	666	57.3	0.5	0.48	11.9	6.4	0.01[1]	15
	Outdoor	78	60.3			5.1			2
Maji-Ndege	Indoor	600	55.8	0.0	0.99	10.4	0.9	0.32	11
	Outdoor	126	55.6			8.3			

[1] Differences between indoor and outdoor are significant at $P < 0.05$.

mediately on white filter papers for subsequent blood meal analysis by the ELISA test.

The blood meal tests were done at the Robert von Ostertag Institute, Berlin, Germany. The mosquitoes were identified to species by microscopic examination of the specific banding patterns on chromosome X (Coluzzi 1966) and the chromosomal inversion karyotypes were scored following the nomenclature of Coluzzi et al. (1979).

The inversion frequencies and proportions feeding on humans were compared between indoor- and outdoor-resting populations using chi-square tests. Wright's fixation index (F) (Brown 1970) was used to identify significant deviations of the observed frequencies from those expected according to Hardy-Weinberg ratios. Deviations are considered to be significant ($P < 0.05$) from the Hardy-Weinberg expectations if F is numerically greater than $1.96/\sqrt{N}$, where N = number of specimens. $F > 0$ indicates a deficiency and $F < 0$ an excess of heterozygotes. The bias of the test with regard to the deviations from the Hardy-Weinberg ratios is negligible for samples above 20 (Brown 1970).

RESULTS

Of 1,470 mosquitoes identified by the chromosomal technique, all were *An. arabiensis*. This constituted 96% of all the specimens processed, less than 4% could not be identified. The majority of mosquitoes identified were collected resting indoors (86%). Both indoor- and outdoor-collected samples were polymorphic for inversions 2Rb, 3Ra, 2Rbr, and 2Rq. The frequency of inversion 2Rb ranged from 55 to 60% and that of 3Ra from 5 to 11%. The chromosomal arrangements 2Rbr and 2Rq, which were always found in association, only occurred with a frequency of approximately 2%.

Comparison of the frequencies of occurrence of the 2 inversions between indoor and outdoor samples showed that in Kapkuikui village, the 2 samples were not homogeneous based on the 3Ra inversion ($\chi^2 = 6.4$, $P = 0.01$, Table 1). However, there were no significant differences between the 2 areas in terms of the inversions 2Rb and 3Ra for indoor samples ($\chi^2 = 0.74$, $P = 0.39$; $\chi^2 = 1.33$, $P = 0.25$) and outdoor samples ($\chi^2 = 0.87$, $P = 0.35$; $\chi^2 = 1.50$, $P = 0.22$), respectively. Similarly, there were no significant differences between years (i.e., between 1991 and 1992).

The observed karyotype frequencies were all in agreement with Hardy-Weinberg ratios suggesting conditions of random mating between indoor and outdoor samples (Table 2) except for the 2Rb indoor samples from Kapkuikui in which the F value was numerically greater than $1.96/\sqrt{N}$.

For both inversions 2Rb and 3Ra from indoor-collected samples, the F value was less than 0, suggesting an excess of heterozygotes. In the other outdoor-collected samples, with the exception of the 2Rb inversion from Kapkuikui village, the F values all suggested a deficit of heterozygotes.

Table 3 lists the different sources of blood meals in indoor- and outdoor-collected samples. Of the total number processed (1,302 and 291), 26.2 and 15.1% could not be identified from both indoor- and outdoor-collected samples, respectively. The sources of blood meals identified included man and domestic and wild animals. Out of 10 different hosts identified, 84% had fed on either man or bovine and only 4 cases were mixed feeds, 3 of man and bovine and one of man and Suidae. The proportion of blood meals from humans differed highly significantly between indoor and outdoor samples ($\chi^2 = 246$, $P < 0.001$ (1 df).

DISCUSSION

In this study, *An. arabiensis* was the only species of the *An. gambiae* group identified. As in other studies in eastern Africa, 2 common inversions, 2Rb and 3Ra, were encountered (Mosha

Table 2. Observed and expected karyotype frequencies according to Hardy-Weinberg ratios in *Anopheles arabiensis.*

Locality		N	$1.96/\sqrt{N}$	F-value 2Rb	F-value 3Ra
Kapkuikui	Indoor	666	0.08	−0.126[1]	−0.016
	Outdoor	78	0.22	−0.008	0.115
Maji-Ndege	Indoor	600	0.08	−0.089	−0.021
	Outdoor	126	0.17	0.002	0.006

[1] Differences between observed and expected karyotype frequencies are significant at $P < 0.05$.

and Subra 1982, Mnzava and Di Deco 1986, Petrarca and Beier 1992). Furthermore, as the study area lies along the Rift Valley, the recording of inversions 2Rbr and 2Rq (2Rbqr) was not surprising (Coluzzi et al. 1979, Mnzava and Di Deco 1986, Petrarca and Beier 1992).

The heterogeneity for inversion 3Ra between indoor and outdoor samples from Kapkuikui village ($P = 0.01$) and the significant deviation from Hardy-Weinberg ratios of the indoor-collected samples on the 2Rb inversion may be due to combining samples for 1991 and 1992 "Wahlund effect" (Wallace 1968). These differences may not be due to nonrandom mating. Differences between 1991 and 1992 samples were not statistically significant.

Blood meal analysis was carried out against a wide range of potential hosts and although a small number were found to have fed on all 10 potential sources, human and bovine blood meals accounted for 84%. Given that there was no significant variation in the frequencies of the 2 inversions reported, significant differences in the distribution of blood meal sources between indoor and outdoor samples ($\chi^2 = 246$, $P < 0.001$) may not necessarily reflect genetic variation, but rather host availability.

As indicated above, in these villages domestic animals are kept in specially built sheds surrounding the houses. It was assumed that most of the mosquitoes found resting indoors would have fed on humans and those outdoors on other sources. To the contrary, an overall human blood index of 63.1% was recorded from the indoor samples.

Sampling of mosquitoes was carried out in parallel from both indoors and outdoors. Though results of blood meal analysis on outdoor-collected samples only showed a human blood index of 4.9%, it must be borne in mind that Sharp et al. (1990) reported a high proportion of mosquitoes leaving huts in window traps. This behavior was not investigated in this study.

Of the indoor-resting *An. arabiensis,* 37% had fed outdoors on both domestic and wild animals. Such postprandial endophily (White 1974) has been encountered elsewhere, for example, in Tanzania (Smith 1958, White 1971), in Jimma, Ethiopia (White 1974), in southwestern Arabia (Gillies and Coetzee 1987), and in northern Natal, South Africa (Sharp and le Sueur 1991).

Sharp and le Sueur (1991), reviewed the behavioral variation in the human blood index in indoor-resting catches in *An. arabiensis* from Africa and concluded that the presence or absence of insecticide deposits on the walls influenced the human blood index. In unsprayed areas, a human blood index of between 60 and 100% from indoor-resting catches was always recorded. This finding agrees very well with our results from an unsprayed area that had an average human blood index of 63.1%.

One of the purposes of this study was to establish baseline data on the indoor/outdoor feeding and resting behavior of the vector species and the potential effect of introducing permethrin-

Table 3. Identification of blood meals from *Anopheles arabiensis* collected from 2 villages in Kenya.

Source of blood meal	Numbers Indoor	Numbers Outdoor
Man	606	11
Bovine	240	162
Small ruminant	55	35
Hippopotamus	18	22
Suidae	19	6
Canidae	10	1
Wild ruminant	6	5
Buffalos	0	4
Felidae	1	1
Avian	2	0
Man/bovine	3	0
Man/Suidae	1	0
Nonhuman total	355	236
Unidentified total	341	44
Total identified	961	247

treated wall cloths or other intervention measures. Indoor resting of bloodfed mosquitoes does occur in our study area. This is in line with other studies on *An. arabiensis* from unsprayed areas (Sharp and le Sueur 1991). In the short term, therefore, permethrin-treated wall cloths or other intervention measures would have an effect.

However, in the long term, if impregnated wall cloths or residual house spraying with irritating insecticides were to be used continuously on a large scale, they may select for the house-leaving component of *An. arabiensis*, as has been the case in the Garki areas of Nigeria (Molineaux and Gramiccia 1980) and in northern Natal, South Africa (Sharp et al. 1990). Should chemical intervention be introduced, this study will form an essential baseline in gauging whether there is any change in the feeding and resting behavior of this species that will result in erosion of efficacy of control.

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