The Distribution of Two Major Malaria Vectors, *Anopheles gambiae* and *Anopheles arabiensis*, in Nigeria

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The distribution of Anopheles gambiae *and* An. arabiensis *across the ecological zones of Nigeria (arid savanna in the north gradually turns into humid forest in the south) was investigated. Results of the present study were compared to the distributions determined from samples of indoor-resting females reported by an earlier study over 20 years ago. Larvae were sampled in the rainy seasons of 1997 and 1999 from 24 localities, 10 of which were sampled in both years. Specimens were identified by the polymerase chain reaction method. Results showed that species composition changed significantly among the 10 localities in both years (χ^2=13.62, P = 0.0002), but this change was significant in only four of the 10 localities. The identity of the prevalent (more abundant) species changed between 1997 and 1999 in only three of 10 localities.* An. arabiensis *was prevalent in several localities in the southern Guinea savanna, an area where it was virtually absent over 20 years ago. The data suggest that* An. arabiensis *has extend its range, although differences in sampling technique (larval sampling versus adult collection) can not be ruled out as a possible explanation.*

Key words: *Anopheles gambiae* - *Anopheles arabiensis* - distribution - forest - savanna - Nigeria

Members of the *Anopheles gambiae* complex are the most important vectors of malaria in sub-saharan Africa. The complex consists of about seven species that vary in their ability to transmit malaria (White 1974, Hunt et al. 1998). Two species of the complex, *An. gambiae* and *An. arabiensis*, are both the most broadly distributed and the most efficient vectors of malaria (White 1974, Coetzee et al. 2000).

The range and relative abundance of *An. arabiensis* and *An. gambiae* appear to be strongly influenced by climatological factors, especially total annual precipitation (Lindsay et al. 1998). Generally, *An. arabiensis* tends to predominate in arid savannas, whereas *An. gambiae* is the dominant species in humid forest zones (White 1974, Lindsay et al. 1998, Coetzee et al. 2000). Moreover, where the two species occur in sympatry, large changes in species composition often occur, with *An. arabiensis* predominating during the dry season and *An. gambiae* becoming more abundant during the rainy season (Di Deco et al. 1981). However, *An. gambiae* may sometimes be more abundant than *An. arabiensis* during the dry season or vice-versa (Service 1970, White & Rosen 1973).

Based on collections of indoor-resting females, Coluzzi et al. (1979) concluded that the distribution of *An. gambiae* and *An. arabiensis* across the ecological zones of Nigeria was "puzzling". They found that *An. gambiae* was prevalent in forest zones but was also abundant in several localities in the savanna. Conversely, *An. arabiensis* was predominant in several Sudan, Sahel and northern Guinea savanna localities, was absent from the intervening southern Guinea savanna, but reappeared in forest zones farther south.

We have begun a population genetic study of both species in Nigeria and, as a necessary first step, we determined the distribution of both species across the country. We then addressed the following question: has the distribution of the two species changed since the study by Coluzzi et al. (1979)?

MATERIALS AND METHODS

Study area - As one travels from north to south in Nigeria, mean annual rainfall increases and the number of dry season months decreases (Davies 1977). Thus, Nigeria can be divided into seven major ecological zones (Figure) (Coluzzi et al. 1979), such that arid savanna in the north gradually turns into humid forest in the south. Mean annual rainfall across the ecological zones ranges from more than 2500 mm in the forests to less than 500 mm in the Sudan and Sahel savannas (Coluzzi et al. 1979).

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Mosquito collection - Mosquitoes were collected from 24 localities across Nigeria (Figure) during the rainy season between June and August 1997 and between May and June 1999. Ten of the 24 localities were sampled in both years (Table). Giwa, Zaria, Lafia, Bida, Jebba, Otukpo and Benin were each sampled over two or more days, whereas all other localities were each sampled in one day. Samples were not collected from two ecological zones, Mangrove forest and Sahel savanna, due to logistic difficulties.

In each locality, larvae of all available instars, or pupae, or both were collected from at least seven shallow, sunlit, temporary rain pools (Figure) within a radius of approximately 1 km. These pools were usually devoid of vegetation except in Bida (locality 8, Figure), where they were also collected from fields of rice seedlings. Sample size per pool was not determined and samples from all pools in each locality were mixed. Sample size per locality was at least 200 except in Garki, where only 65 specimens were collected. Similarly, the sample size of the 1997 collections from Zaria, Ogbomoso, Lagos and Sapele ranged between 30-40 mosquitoes.

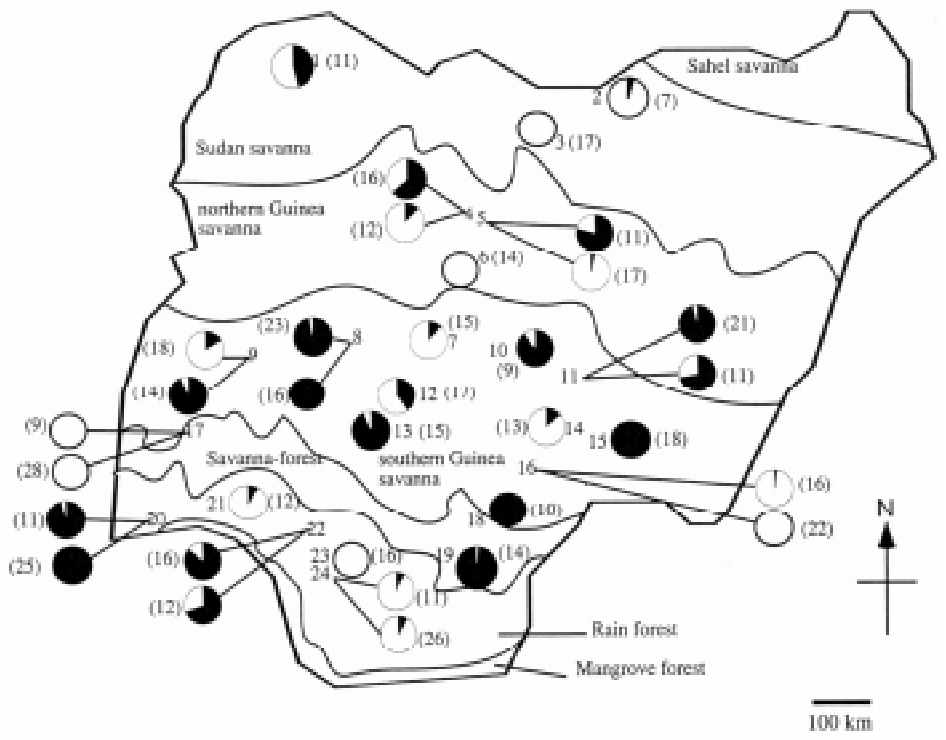
Larvae were reared on location in paper cups to adults because they were more easily distinguished as *An. gambiae sensu lato* by the keys of Gillies and Coetzee (1987). Rearing success was at least 95%, and emerging adults and occasional dead larvae or pupae were fixed in 95% ethanol. In Sapele (1999 collection only), Garki, and Enugu, however, larvae and pupae were killed and preserved in 95% ethanol. All specimens were then hand-carried to the University of Vermont.

DNA extraction and species identification - Following DNA extraction (Collins et al. 1987), each of at least 30 randomly selected individuals per locality (Table) was identified to species by the polymerase chain reaction (PCR) method (Scott et al. 1993). However, the sample sizes employed from the 1997 collections from Zaria, Ogbomoso, Lagos and Sapele were 29, 20, 28 and 27, respectively (Table).

Statistical analyses - We employed chi-square analyses to test the significance of changes in species composition over two years, first, among all 10 localities and second, in each locality. The statistical analyses were performed using the software JMP® version 3.1 (SAS Institute Inc., Cary, NC).

RESULTS

Each of *An. arabiensis* and *An. gambiae* was prevalent over the other in at least one locality in each of five ecological zones (Figure). The two spe-



Species composition and distribution of *Anopheles gambiae sensu lato* across Nigeria. Number of pools sampled per locality is shown in parenthesis. Localities are numbered from 1-24 and are defined in the Table. In localities with two pie charts, the upper chart represents data for 1997 and the lower chart is for 1999. ○*An. arabiensis;* ●*An. gambiae*

TABLE

Species composition in 1997 and 1999 in 24 localities across Nigeria

Locality name (code) [a]	1997 *Ag:Aa* [b]	1999 *Ag:Aa*	χ^2	*P*
Sokoto (1)	60:50	-	-	-
Garki (2)	-	2:43	-	-
Kano (3)	-	0:30	-	-
Giwa (4)	31:18	6:43	29.05	0.001
Zaria (5)	23:6	2:48	51.25	0.001
Kaduna (6)	0:50	-	-	-
Abuja (7)	6:44	-	-	-
Bida (8)	29:1	30:0	1.40	0.236
Jebba (9)	8:42	45:3	68.79	0.001
Keffi (10)	-	27:3	-	-
Lafia (11)	48:2	36:14	11.84	0.001
Lokoja (12)	-	20:30	-	-
Okene (13)	28:2	-	-	-
Makurdi (14)	-	4:26	-	-
Kwenev (15)	-	50:0	-	-
Otukpo (16)	1:49	0:50	1.39	0.237
Ogbomoso (17)	0:20	0:50	-	NS
Enugu (18)	-	50:0	-	-
Okigwe (19)	-	49:1	-	-
Lagos (20)	27:1	50:0	2.07	0.150
Kajola (21)	-	6:64	-	-
Benin (22)	43:7	23:10	3.18	0.074
Oghara (23)	-	0:70	-	-
Sapele (24)	2:25	4:46	0.01	0.926

a: codes are as in Figure; *b*: *Anopheles gambiae* : *An. arabiensis*; NS: not significant

cies coexisted in several localities but in very disproportionate numbers. In fact species composition reached a 1:2 ratio or greater in only three of the 24 localities: Sokoto, Giwa (1997 collection only) and Lokoja (localities 1, 4 and 12, respectively, Figure).

Species composition changed significantly among the 10 localities sampled in both 1997 and 1999 ($\chi^2 = 13.62$, $P = 0.0002$). Further analyses of data between 1997 and 1999 revealed that species composition changed significantly in four of 10 localities: Giwa, Zaria, Jebba, and Lafia (Table). However, the identity of the prevalent (more abundant) species changed over two years in only three of 10 localities: localities 4, 5 and 9 (Figure).

DISCUSSION

An. arabiensis prevailed in some localities in arid savanna zones, as expected (White 1974, Lindsay et al. 1998, Coetzee et al. 2000), but it also was the prevalent species in some forest zones. Similarly, *An. gambiae* was the more abundant species in some localities in the forest zone as well as in arid savanna. The observations above are in agreement with those of Coluzzi et al. (1979), who collected indoor-resting females. However, the present results differ from those of Coluzzi et al. (1979) in two ways. First, *An. arabiensis* was the prevalent species in several southern Guinea savanna localities, such as Abuja, Lokoja, Jebba (1997 collection only), Makurdi and Otukpo (localities 7, 12, 9, 14 and 16, respectively, Figure). In contrast, Coluzzi et al. (1979) found virtually no *An. arabiensis* in eight localities, two of which were sampled in both the dry and rainy seasons, in the southern Guinea savanna. This observation suggests that *An. arabiensis* probably has extended its range during the interval between the two studies. Moreover, *An. arabiensis* from the southern Guinea savanna have significantly fewer alleles and lower heterozygosity per locus at 10 microsatellite loci than those from the Sudan and northern Guinea savannas (Onyabe & Conn, unpublished observations).

Second, in the present study, *An. gambiae* was the prevalent species in Benin City (locality 22, Figure) in both 1997 and 1999 (86% and 70%, respectively). Coluzzi et al. (1979), in contrast, observed an island of *An. arabiensis* and the virtual absence of *An. gambiae* in this locality.

Differences in sampling technique may also explain the disparities between the present study and that of Coluzzi et al. (1979). We employed larval

sampling whereas Coluzzi et al. (1979) collected indoor-resting females. Adult female collections may be biased against *An. arabiensis* because this species is thought to be more zoophilic and less endophilic than *An. gambiae* (White & Rosen 1973, Molineaux & Gramiccia 1980, Coluzzi 1984) (but see Diatta et al. 1998). It is not clear how these different sampling methods affect estimates of species composition. However, alongside larval collections in 1999 in Kwenev (locality 15, Figure), we collected 204 indoor-resting females, all of which, like collected larvae, were identified as *An. gambiae*. In this case at least, larval and adult collections gave identical results.

The shifts in species composition after two years in four of 10 localities in this study have also been observed in other studies (Faye et al. 1997, Toure et al. 1998). Large fluctuations in species composition are also known to occur seasonally (Service 1970, White & Rosen 1973, Di Deco et al. 1981), but the present results can not be attributed to seasonal effects because our samples were collected in the rainy seasons of 1997 and 1999.

Given that the identity of the prevalent species was stable over at least two years in most localities, the changes in distibutions between the present study and that of Coluzzi et al. (1979) can not entirely be attributed to random temporal fluctuations. Further population genetic data should confirm whether there has indeed been a change in the range of, in particular, *An. arabiensis*.

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