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Polymerase Chain Reaction Used To Describe Larval Habitat Use by *Anopheles gambiae* Complex (Diptera: Culicidae) in the Environs of Ifakara, Tanzania

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ABSTRACT Larvae of the *Anopheles gambiae* complex were collected in and around the town of Ifakara, southern Tanzania during the wet season of 1994 and identified to species by polymerase chain reaction. All but 1 surface pool contained mixed populations of *An. gambiae* and *An. arabiensis* larvae. The 2 species varied among locations rather than types of water. *An. arabiensis* predominated in pools close to cattle. The numbers of identified early instars of both species were similar, but more *An. gambiae* 4th instars were identified, perhaps indicating that *An. gambiae* were able to survive heavy rainfall better than *A. arabiensis*.

KEY WORDS *Anopheles gambiae* complex, Tanzania, larvae distribution, polymerase chain reaction

LARVAE OF THE 2 most widely distributed members of the *Anopheles gambiae* Giles complex, *An. gambiae* Giles and *An. arabiensis* Patton, tend to occur in temporary, sunlit pools such as rain puddles (Gillies and DeMeillon 1968, Gillies and Coetzee 1987). Differences in larval ecology may explain adult sympatry. In an area where both species were common in a variety of water bodies, White et al. (1972) found that *An. arabiensis* larvae predominated in water in cattle footprints where temperatures may reach 39 or 40°C. Thus, *An. arabiensis* may occupy a more specialized habitat than *An. gambiae*. Because specialists generally do better than generalists in their chosen habitat, it follows that the relative densities of the 2 species may differ according to the microhabitat occupied. However, both species generally have been found in the same habitat (Service 1970, White et al. 1972, White and Rosen 1973, Service et al. 1978). Larval sites are even shared among incipient species of *An. gambiae* from West Africa (Coluzzi et al. 1985). In the Kilombero valley of Tanzania, adult *An. arabiensis* are most common during the short and at the start of the long rains when the only breeding sites are puddles. Numbers of *An. gambiae* increase during and at the end of the long rains when greater variety of larval habitats are available (Kilombero Malaria Project, unpublished data). A similar pattern was described for Segera, Tanzania, by White et al. (1972). It is possible that *An. gambiae* use of a variety of water bodies in addition to the more usual puddles, resulting in the increase in adults at such times.

Studies on the larval ecology of *An. gambiae* have been limited compared with those on the adults. Previously, it has only been possible to identify larvae by examination of salivary gland chromosome banding patterns or by electrophoresis. Chromosome preparation of larval salivary glands is a specialized technique, is restricted to 4th instars, and material cannot be preserved before preparation. Young instars either were not identified or larvae were reared through to 4th instar for identification. Consequently, effects such as competition could not be investigated. More recently enzyme-linked synthetic oligonucleotide probes have been used to identify larval *An. arabiensis* (Githeko et al. 1993). Using appropriate primers (Paskewitz and Collins 1990) it is now possible to identify all stages of *An. gambiae* and *An. arabiensis* using polymerase chain reaction (PCR) techniques.

Materials and Methods

During 15–18 May 1994 nonmoving bodies of fresh water in and around Ifakara town were examined by J.D.C. and 5 assistants for the presence of larvae. If none was seen after a minimum period of 3 min, 3 dips with a shallow tray were taken. If no larvae were collected, the site was considered negative. When larvae were found, a 10-min collection using a pipette was taken by J.D.C. Care was taken to include all instars when present. Larvae were returned to the laboratory in tubes partially filled with water, where they were identified to instar, placed on dry filter paper, and the following day identified to species using the PCR technique.

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Table 1. Number of *An. gambiae* complex larvae identified to species according to location of collection

No.	Location	Habitat	No. larvae	
			An. gambiae	*An. arabiensis*
1	Masai camp	2, a	6	15
2	Kivukoni	2, a	6	20
3	Limaumau a	2, a, c	23	10
4	Limaumau b	1, a	4	0
5	Limaumau c	3, b, c	6	6
6	Town by church	1, a	12	2
7	Town by hospital	1, a	15	2
8	Town by market	1, a	10	11
9	Town by laboratory	1, a	6	1
10	Airstrip	4, b, c	12	2

1, open puddle; 2, large pool; 3, rice field; 4, flowing; a, turbid; b, clean; c, with emergent vegetation.

Total DNA was extracted from larvae using the phenol/chloroform/polymerase method (Paskewitz and Collins 1990). A thinly cut session of dried filter paper containing the whole larvae was placed in a 1.5-ml microfuge tube containing 100 μl of lysis buffer and crushed with a micropestle. Proteinase K (20 μg) was added and the tubes incubated at 55°C for 1.5 h. Carrier 1 μg (salmon sperm) DNA was added to the lysate and the aqueous fraction phenol/chloroform extracted twice. Mosquito DNA was precipitated from the aqueous layer with isopropanol, washed in 70% ethanol, dried and dissolved in 10 μl sterile distilled water, and stored at −20°C. Two microliters mosquito DNA was used for PCR. DNA templates were amplified with primers specific for *An. gambiae* and *An. arabiensis* as described by Scott et al. (1993). PCR amplified products were visualized in ethidium bromide stained agarose gels.

Results and Discussion

Every pool examined contained mosquito larvae. Those not containing *Anopheles* larvae contained the predatory larvae of *Culex tigrepes*. Anophelines were collected from 20 sites and were identified to species from 10. Habitats varied from small puddles by the side of footpaths to large, shallow pools with emergent vegetation. Water was either clear or turbid, still to gently flowing (Table 1). Puddles of turbid water contained most larvae.

The DNA extracted from single larvae dried on filter paper was amplified successfully. Bands corresponding to 390 for *An. gambiae* and 315 bp for *An. arabiensis* were observed. One mosquito with a band size equivalent to 290 bp was not classified. All but 1 site contained a mixed population of species (Table 1). The only site that contained larvae

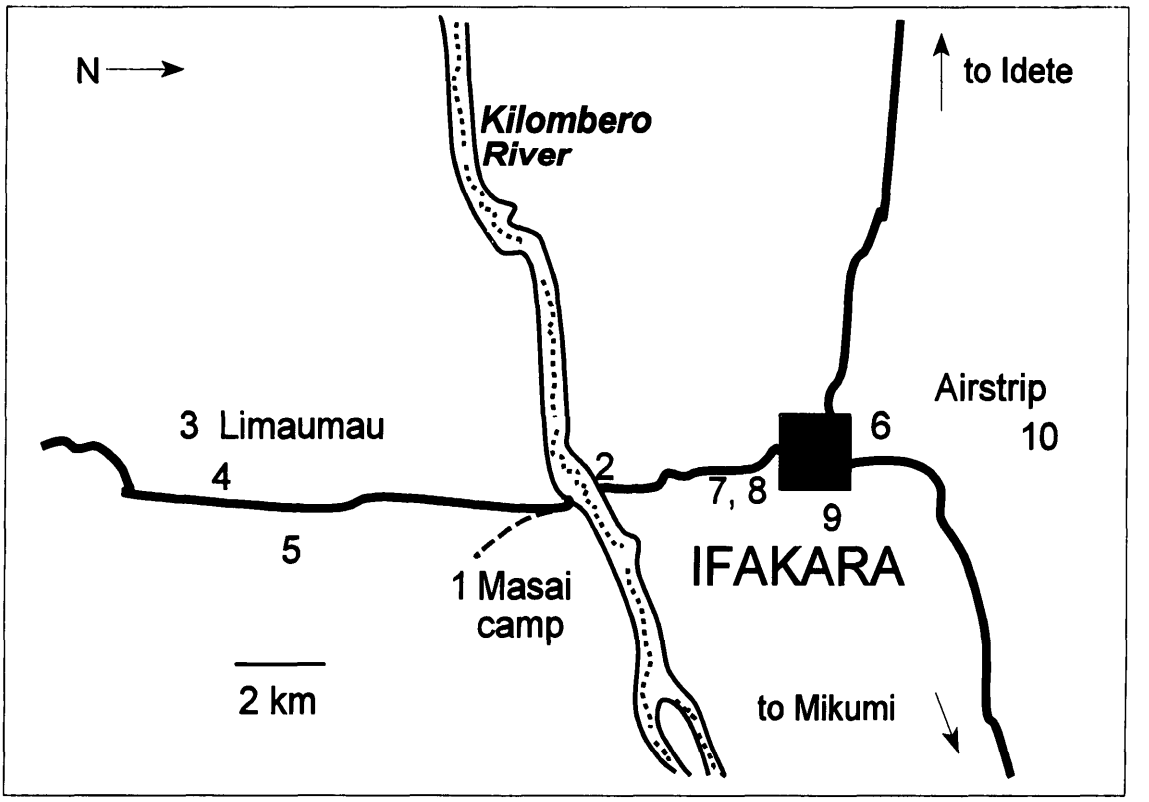

Fig. 1. Distribution of collection sites of larvae from around the town of Ifakara, southern Tanzania, May 1994. Numbers correspond to locations given in Table 1.

Table 2. Number of *An. gambiae* complex larvae identified to species according to instar, Ifakara, May 1994

Species	Instar			
	1st	2nd	3rd	4th
An. gambiae	15	20	24	40
An. arabiensis	13	20	24	12

of just *An. gambiae* was a turbid, 0.5 m deep puddle that people constantly walked through.

The relative proportions of the 2 species varied among locations rather than types of water. As in Segera (White et al. 1972), significantly more *An. arabiensis* than *An. gambiae* were collected in the area close to where cattle were kept by Masai (χ^2 = 44.2, df = 9, $P > 0.0005$). The latter species predominated at all other sites (Fig. 1). Although the species composition among instars 1st–3rd was similar, most 4th instars were identified to be *An. gambiae* (Table 2).

Anopheles gambiae may be able to survive heavy rainstorms better than *An. arabiensis*. A few days before the samples were taken heavy rain eliminated a large *An. arabiensis* population from an area close to Ifakara. Differential survival may, in part, explain the relative preponderance of 4th instar *An. gambiae* in our samples and, perhaps, the annual population cycles of the 2 species observed in this and other areas.

Our preliminary study demonstrated that the PCR technique was a useful method to obtain detailed information about the ecology of larvae of the *An. gambiae* complex. Our results indicated that the larval requirements of *An gambiae* and *An. arabiensis* were similar and that distribution may be largely explained by host rather by breeding site availability. Nevertheless even the smallest pool has an edge and a middle (Morrison and Andreadis 1992), and it is possible that a more careful evaluation of location of the larvae within breeding sites may reveal unsuspected differences in microhabitat use between these species. Because this technique also can be used to examine intraspecific differences a longitudinal study monitoring population composition in a number of sites would seem apposite.

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