ANOPHELES GAMBIAE COMPLEX EGG-STAGE SURVIVAL IN DRY SOIL FROM LARVAL DEVELOPMENT SITES IN WESTERN KENYA

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ABSTRACT. The potential for *Anopheles* egg survival in dry soil from larval development sites was investigated in western Kenya. A total of 230 dry soil samples collected in 1987, 1988 and 1989 yielded 126 first-instar *Anopheles gambiae s.l.* larvae from 2 to 5 days after flooding with water. These larvae were from dried animal hoofprints along streams (57.9%), from dried edges of permanent and temporary pools (41.3%) and from dried stream beds (0.8%). Larval density was 1.2 larvae/kg of soil from positive microhabitats in 1987 and 2.4 larvae/kg in 1988. Thirteen larvae from the 1989 soil samples, reared to adults, were identified by DNA probes as *Anopheles gambiae sensu strictu* ($n = 6$) and *Anopheles arabiensis* ($n = 7$). Experimentally, eggs from field-collected females remained viable up to 12 days for *An. gambiae s.l.* and 10 days for *An. funestus*. In western Kenya, egg viability in dry soil may represent a significant, short-term survival mechanism for 2 species of the *An. gambiae* complex.

INTRODUCTION

Resistance of eggs to desiccation may represent, for some *Anopheles* species, a significant short-term population survival mechanism. The ability of anopheline eggs to withstand drying in nature has been studied infrequently for few species, despite early observations that larval development sites may contain numerous anopheline larvae almost immediately after rain (Howard et al. 1912).

Unlike eggs of aedine species which can remain dormant for months or years (Bates 1949), anopheline eggs are not adapted for long-term viability in the absence of free water. The maximum period that anopheline eggs can remain viable in drying soil may be about one month, based on observations in Panama involving *An. albimanus* Wiedemann, *An. punctimacula* Dyar and Knab and *An. tarsimaculatus* Goeldi (sic) (Stone and Reynolds 1939). Eggs have been recovered from moist soil for *An. balabacensis* Baisas in Malaysia (Rajapaksa 1971) and for *An. dirus* Peyton and Harrison in Bangladesh (Rosenberg 1982). In the laboratory, eggs of *An. balabacensis* remain viable for up to 27 days (Rajapaksa 1971). Thus, some *Anopheles* species exhibit a potential for short term egg-stage resistance to desiccation. Similarly, females of some *Anopheles* species oviposit on moist soil (Giglioli 1965) or on soil above the water surface (Rosenberg 1982). Adaptation allowing egg embryonation before larval hatching stimulated by rainfall facilitates larval survival in ephemeral habitats. Normally, larval stages cannot survive more than 2 days without water (Muirhead-Thomson 1945).

Anopheles gambiae complex populations in tropical Africa characteristically increase rapidly early in the rainy season. This is remarkable in some areas of Africa because the dry season extends from 6 to 9 months. Under such conditions, populations exist at low levels in few, size-limited larval habitats and in the adult stages (Omer and Cloudsley-Thompson 1968, 1970); there is no evidence for long term egg-stage resistance to desiccation (Ramsdale and Fontaine 1970a, 1970b). Experimentally, a small percentage of *An. gambiae* Giles *s.l.* eggs can remain viable for up to 16 (Holstein 1954) or 18 days (Deane and Causey 1943). In Sierra Leone, Muirhead-Thomson (1945) observed that *Anopheles melas* Theobald can remain viable in damp mud for at least 6 days. This appears to be the only observation that egg stages of species in the *An. gambiae* complex are adapted for short-term survival in drying larval development sites.

In areas of sub-Saharan Africa lacking extended dry seasons, egg-stage resistance to desiccation may be a significant short-term population survival mechanism for species in the *An. gambiae* complex. We investigated this possibility in western Kenya. Observations are presented on the presence of viable eggs of *An. gambiae* and *An. arabiensis* Patton in dry soil from various types of larval development sites, the timing and densities of first-instars hatched from soil samples and corresponding laboratory investigations on egg-stage survival potential.

MATERIALS AND METHODS

Study site: The study was conducted in Kisian, a rural village 10 km west of Kisumu (Kisumu District, Nyanza Province), in western Kenya. Holo-endemic malaria, primarily *Psorophora falcinarum,* is maintained in this site by *An. funestus* Giles, and by *An. gambiae* and *An.*

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arabiensis, two species in the *An. gambiae* complex (Fontaine et al. 1978).

Soil samples: Soil samples were collected in Kisian 7 times at weekly intervals from July 23 to September 3, 1987, once on July 8, 1988, and once on March 9, 1989. Sampling was during dry periods after the long rainy seasons of 1987 and 1988, and immediately before the long rainy season of 1989. Daily inspections of rain gauges at 2 houses in Kisian indicated that less than 43 mm of rain had fallen within 30 days of any collection in 1987, 31 mm in 1988 and 8 mm in 1989. Soil samples, weighing around 400 g (1987) or 1,500 g (1988), were collected from permanent pools, temporary pools, and cow hoofprints along a stream bed, microhabitats confirmed as *An. gambiae s.l.* larval development sites. Samples were also taken from stream banks and ploughed fields, unlikely sites for larval development which served as controls. Samples consisted of dry soil (moisture content not determined) removed from the top 3 cm of the surface from locations at least 1 m from standing water. Soil was placed in labeled plastic bags, and samples were transported to the Kisumu laboratory.

The following day, each 400 g soil sample (in 1987) was placed in a plastic bowl (20 cm diam × 3 cm deep) and flooded with 250 ml tap water. Similarly, fifteen 1,500 g samples in 1988 were placed in enamel bowls, flooded with 1 liter of river water, and held at room temperature (21–28°C) in Kisumu. Seven corresponding samples in 1988 were transported to Nairobi, set up as above and held at 27°C. Soil samples were dissolved by stirring and each bowl was covered by mesh screen in Kisumu or by plexiglass in Nairobi.

Each bowl was observed daily, over 6 consecutive days, for the presence of mosquito larvae. Soil was stirred daily and tap water was added to replace evaporated water. Hatched first-instar larvae were transferred to bowls of clean tap water and reared by feeding them ground fat-free dog chow. Species identifications were based on the morphology of 4th instar larvae or adults. For mosquitoes collected in 1989, adult *An. gambiae* emerging from eggs collected from soil samples were identified to species using DNA probes for the *An. gambiae* complex (Collins et al. 1988).

Experimental egg survival: The effects of 3 storage conditions on the survival of eggs obtained from field-collected *An. gambiae s.l.* and *An. funestus* were determined experimentally. Blood-fed *Anopheles,* collected by aspiration from inside houses in Kisian, were held individually in screened 50-ml plastic vials. An oviposition substrate in each vial consisted of a small piece of filter paper above cotton; this was covered by 1–2 cm of river water. Eggs were collected daily, counted in groups of 50 and stored on a 4 cm diam round filter paper, inside a covered plastic petri dish (5 cm diam). Replicates of 50 eggs were held either at room temperature wet (RTW), room temperature dry (RTD) or at 4°C in a refrigerator. All were immediately sealed in a plastic bag (15 × 15 cm) containing a wet paper towel, except RTD samples which were air dried for 2 days before sealing. From days 2 to 12, at least 3 replicates (up to 12 replicates) of 50 eggs for each species, were placed in 12 × 12 cm square plastic weighing boats (1 cm deep) containing tap water to stimulate hatching. First-instar larvae were removed and counted daily for a period of 6 days. The percent hatch per replicate was calculated as the number of first-instar larvae hatched/total eggs, times 100. Daily percent hatch for each species was obtained as the mean of all replicates tested; standard errors of the mean were computed.

RESULTS

Anopheles gambiae first-instar larvae were obtained from soil samples collected in Kisian during 6 of 7 weekly collections from July 23 to September 3, 1987 and again on July 8, 1988 (Table 1). Of the 53 larvae obtained in the 1987 collections, 24.5% were from permanent pools, 5.7% were from temporary pools and 69.8% were from cow hoofprints along streams. The 54 larvae from the 1988 collection consisted of 48.1% from temporary pools and 51.9% from cowprints along streams. Larvae were not obtained from soil samples collected from a dry stream bank or in a ploughed field. Larval density was estimated to be 1.2 larvae/kg for the positive microhabitats sampled in 1987 (53 larvae from 111–400 g samples) and 2.4 larvae/kg in 1988 (54 larvae from 15–1,500 g samples).

The 22 soil samples collected on July 8, 1988, (Table 1) were divided randomly into 15 held in Kisumu at room temperature and 7 held in Nairobi in an insectary at 27°C. In Kisumu, 8 and 17 larvae were obtained, respectively, from 4 temporary pool and 6 cow hoofprint samples. Samples held in the Nairobi insectary yielded 18 larvae from 2 temporary pool samples and 11 larvae from 3 cow hoofprint samples. The number of larvae per kg of soil was estimated to be 1.67 (25 larvae from 10 positive samples) for samples held in Kisumu vs. 3.87 (29 larvae from 5 positive samples) for samples held in Nairobi ($F = 4.30$, $df = 1,13$, $P = 0.059$). Thus, flooded soil samples held at 27°C in Nairobi yielded 2.3 times more larvae than corresponding samples held at room temperature in Kisumu.

Table 1. First-instar *Anopheles gambiae s.l.* larvae obtained from soil samples representing 5 microhabitats in Kisian, western Kenya.

Microhabitat	July 23 to September 3, 1987			July 8, 1988		
	No. soil samples	No. larvae	Larvae/kg soil	No. soil samples	No. larvae	Larvae/kg soil
Permanent pools	13	13	2.5	0	—	—
Temporary pools	20	3	0.4	6	26	2.9
Cow hoofprints along stream bed	78	37	1.2	9	28	2.1
Stream bank	9	0	0	4	0	0
Ploughed fields	31	0	0	3	0	0
Total	151	53	0.9	22	54	1.6

The temporal duration of *An. gambiae* hatching from eggs ranged from day 2 to day 5 after water was added to soil samples; no larvae were observed on days 1 or 6. For soil samples held in Kisumu at room temperature, 98.1% (52/53) of the larvae from the 1987 collections were obtained on days 4 and 5; the 1988 samples held in Kisumu differed in that 32% (8/25) of the larvae were collected on days 2 and 3 ($\chi^2 = 14.96$, df = 1, $P < 0.005$). For the 1988 samples held at 27°C in Nairobi, 86.2% (25/29) of the larvae were detected by day 3; 68.0% (17/25) of larvae from corresponding samples held at room temperature in Kisumu were not observed until days 4 and 5 ($\chi^2 = 23.65$, df = 1, $P < 0.005$).

DNA probes were used to determine *An. gambiae* complex species obtained from 57 soil samples collected on March 9, 1989, from the Kisian site. Nineteen larvae were collected from 14 flooded soil samples representing dry pools (52.6%), cow and hippopotamus hoofprints (42.1%) and a dry stream bed (5.3%). DNA analysis indicated that 7 of 13 readable preparations from adult mosquitoes were *An. arabiensis* and 6 were *An. gambiae s.l.*

Storage conditions for eggs from ovipositing field-collected *An. gambiae* and *An. funestus* affected daily hatching rates. Highest egg hatching rates were observed when eggs were held at RTW (Fig. 1); hatching rates decreased progressively over 12 days for *An. gambiae* and over 7 days for *An. funestus* (3.8% hatched on day 10). Maximum hatching rates were on day 2 for *An. gambiae* (90.7%) and *An. funestus* (74.0%) and at least 20% of the eggs hatched through day 10 for *An. gambiae* in contrast to 5 days for *An. funestus*. At RTD and 4°C storage conditions, hatching rates were less than 20% after day 5 for *An. gambiae* and after day 2 for *An. funestus*. At 4°C, *An. gambiae* and An. *funestus* eggs did not hatch after days 9 and 10, respectively. At RTD, 12.5% and 0.5% of *An. gambiae* eggs hatched on days 9 and 11, respectively, and 1.0% of *An. funestus* eggs hatched on day 10.

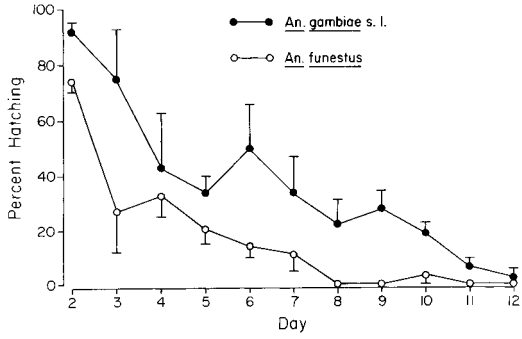

Fig. 1. Percent egg hatching (and standard errors of the mean) per day for *Anopheles gambiae s.l.* and *An. funestus* eggs collected from field-caught females and stored moist at room temperature.

DISCUSSION

Anopheles gambiae complex species in western Kenya are adapted for egg-stage survival in soil from dry larval development sites. First-instar larvae hatched from flooded soil samples in 8 of 9 collections over 3 years. Periods longer than 15 days without rainfall are rare in the Kisumu area, which averages ca. 1,500 mm of rain per year. Thus, in the absence of a true dry season, it is likely that the hatching of eggs in soil by rainfall occurs throughout the year. The demonstration of viable eggs in soil immediately prior to the long and short rainy seasons suggests that this survival mechanism could contribute to the predictable exponential increases of adult *An. gambiae s.l.* immediately after the onset of the rainy seasons.

DNA probes confirmed that viable eggs of both *An. gambiae* and *An. arabiensis* were present in soil samples. Although the number of identifications was limited ($n = 13$), the proportions of each species were nearly identical to previous cytogenetic observations on 571 larvae collected in Tiengre, a site adjacent to Kisian (Service et al. 1978).

The number of first-instar larvae per kg of flooded soil indicated a high potential for larval production in permanent and temporary pools, and in animal hoofprints. In positive habitats, from 0.4 to 2.9 larvae were hatched per kg of soil. By extrapolation, 1,000 kg of topsoil could yield from 400 to 2,900 larvae. Considering that such larvae hatch in habitats likely to be free of predators (Service 1977) and at relatively low densities, there would be a high potential for larval survival and adult production.

Eggs of field collected *An. gambiae s.l.* held in a moist environment at room temperature showed a progressive decrease in hatching from 91% on day 2 to 1% on day 12, similar to previous laboratory observations (Deane and Causey 1943, Holstein 1954). *Anopheles funestus,* a species which generally develops in permanent aquatic habitats, showed less potential for egg-stage survival under the same test conditions. Dry or cool conditions resulted in significantly lower egg survival. In this respect, the experimental conditions did not simulate the natural conditions whereby eggs on moist soil dry slowly and likely become embedded in drying soil.

These observations raise important questions regarding the survival potential of *An. gambiae* eggs in soil. For example, it is unclear whether eggs of *An. gambiae* or *An. arabiensis* in soil result from drying habitats or whether eggs are deposited onto moist soil, as in *An. melas* (Muirhead-Thomson 1945). The duration of egg-stage survival in soil is unknown; although soil samples were collected during dry periods, longitudinal observations on the same habitats were not made. Similarly, egg survival relative to soil moisture is unknown; samples appeared very dry but soil moisture was not determined. Another consideration is that larval density could be estimated more realistically in some habitats, such as animal hoofprints, by direct flooding and observations on hatching larvae; high temperatures in exposed habitats may promote corresponding high hatching rates after flooding. It is also unclear whether certain karyotypes of the *An. gambiae* complex show differences in resistance to egg-stage desiccation. Clearly, further observations are needed to define the ecological and genetic factors permitting egg-stage survival and affecting subsequent adult production.

The geographical extent of natural egg-stage resistance in soil for species in the *An. gambiae* complex is unknown. The Kisian site represents a rural area of guinea savanna vegetation in East Africa. The same phenomenon apparently does not occur for species in the drier areas of the Sudan (Omer and Cloudsley-Thompson 1970) or in Nigeria (Ramsdale and Fontaine 1970a, 1970b). Thus, the situation may differ in other areas depending upon environmental factors, soil types or the composition of karyotypes in the *An. gambiae* complex. Special attention should be given to irrigated rice fields. In Liberia, *An. gambiae s.l.* first-instar larvae appear at high densities almost immediately after flooding previously cultivated rice fields, but colonization in newly ploughed fields is slower (F. Bolay, personal communication).

Traditionally, a drying period of 4 days is considered essential to kill anopheline eggs and larvae in larval habitats (Gerbert 1937). In light of the present observations, larval vector control for the *An. gambiae* complex may be much more complicated.

In conclusion, these observations again raise the question of the potential for anopheline egg-stage survival in dry soil from larval development sites. In western Kenya, the adaptation of species in the *An. gambiae* complex for egg-stage survival in dry larval habitats represents a significant short-term survival mechanism. Indirectly, this adaptation contributes to the high malaria vectorial capacity of both *An. gambiae* and *An. arabiensis* for the transmission of malaria in this area. These observations demonstrate a need to identify environmental factors influencing natural egg-resistance to desiccation and to determine whether this physiological adaptation is common for species in the *An. gambiae* complex in different ecological zones.

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