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Influence of soil quality in the larval habitat on development of *Anopheles gambiae* Giles

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ABSTRACT : Larval ecology is an important aspect of the population dynamics of anopheline mosquitoes (Diptera: Culicidae), the vectors of malaria. *Anopheles* larvae live in pools of stagnant water and adult fitness may be correlated with the nutritional conditions under which larvae develop. A study was conducted in Mbita, Western Kenya, to investigate how properties of the soil substrate of *Anopheles gambiae* breeding pools can influence development of this mosquito species. *An. gambiae* eggs from an established colony were dispensed into experimental plastic troughs containing soil samples from a range of natural *Anopheles* larval habitats and filtered Lake Victoria water. The duration of larval development (8-15 days), pupation rate (0-79 %), and adult body size (20.28-26.91 mm^3) varied among different soil types. The total organic matter (3.61-21.25%), organic carbon (0.63-7.18%), and total nitrogen (0.06-0.58%) levels of the soils were positively correlated with pupation rate and negatively correlated with development time and adult body size.

Keyword Index: Anopheles gambiae, mosquito development, larval habitat.

INTRODUCTION

The larval aquatic habitat is an important part of the mosquito life cycle and may strongly influence the distribution and abundance of malaria vectors. Although an array of mosquito habitats exist, the larval stage is mainly confined to stagnant water pools and, as such, is quite vulnerable. Adult mosquitoes are difficult to control since they can fly relatively long distances and survive in a range of microhabitats, including houses, vegetation, holes in rocks and soil, among others (Gilles and Warrell 1993). Nevertheless, the adult stage has been the main target in mosquito control for decades (Fillinger et al. 2004). Larval control has only been taken into account recently, and is still underestimated in many areas (Killeen et al. 2002).

The characteristics of *Anopheles* larval habitats are variable: a shallow larval habitat with the presence of algae is a common characteristic of anophelines (Savage et al. 1990; Gimnig et al. 2001, Gimnig et al. 2002, Oo et al. 2002), although such a correlation is not systematic. Many such larval habitats consist of animal hoof or foot prints, or small ponds of still water created by irrigation projects or rainfall. Only a few anopheline species are found in artificial containers (Gilles and Warrell 1993). The characteristics of the larval habitat that are adequate for a given species are still unclear. Many environmental variables can have a direct or indirect effect on anopheline oviposition (Sumba et al. 2004, Rejmànkovà et al. 2005) as well as on larval distribution, density, and development (Gimnig et al. 2001, Oo et al. 2002) and adult fitness (Briegel 1990a, Briegel 2003). Improving our knowledge of the bioecology of mosquito aquatic stages and a detailed analysis of factors that are essential to the maintenance and dispersion of mosquitoes, particularly for species of medical importance, is therefore critical for disease control.

Physicochemical factors of the water, such as temperature, salinity, concentration of carbonates and nitrates have been shown to correlate with the presence or development quality of *Anopheles* larvae in pools (Robert et al. 1998, Gimnig et al. 2001, Oo et al. 2002). Otherwise, knowledge on this topic is very sparse, though the soil substrate is most probably of critical importance in the *Anopheles* larval habitat. Indeed, since the majority of *Anopheles* larval habitats are temporary due to seasonal rains, the only permanent part of this system is the soil in which most of the biological and chemical components of the habitat can persist during the dry season. Surprisingly, the influence of soil substrate on anopheline mosquito development and body size has not, to our knowledge, been studied. The present study aims to investigate possible relationships between the quality of different soil substrates (total organic matter, organic carbon, and nitrogen) and development and size of emerging *Anopheles* adults.

MATERIALS AND METHODS

Study area

This study was conducted in the Suba district of Nyanza Province, Western Kenya, where malaria transmission is endemic throughout the year. The main malaria vector in the area is *Anopheles gambiae*, followed by *An. arabiensis* and *An. funestus* (Shililu et al. 2003). *P. falciparum* is the most prevalent malaria agent (98 to 99%), with *P. malariae* as the only other malaria parasite in the region (Gouagna et al. 2004). Suba district lies at the northeast shores of Lake Victoria with an equatorial climate. There are two

rainy seasons - long rains extend from mid-March to June and short rains from October to December. However, intermittent rainfall may occur throughout the year in this area. Annual rainfall ranges from 700 to 1150 mm, and annual temperature from 16° C to 34° C. At the collecting sites chosen (see below), soils are well to excessively drained, on volcanic bedrock, with fertility ranging from very low to variable values (Jaetzold and Schmidt 1982, Rees et al. 1997).

Soil was collected from two zones: on the hilly island of Rusinga and on the tableland of Lwanda, 10 km distant. All experiments described below were conducted between August 2003 and March 2004 in the *MalariaSphere* screenhouse, described by Knols et al. (2002), at the Mbita-Point Field Station (MPFS, 00°25 S, 34°13 E, altitude 1,170 m) of the International Centre of Insect Physiology and Ecology (ICIPE), on the shore of Lake Victoria.

Preliminary soil sampling and analysis

A total of twenty previously known high-potential *An. gambiae* natural larval habitats (George Sonye, MPFS-ICIPE, personal communication) was sampled for preliminary analysis (ten each from the Lwanda and Rusinga zones). Sites were chosen in order to cover different mosquito breeding habitats in each zone. None of the sites were flooded at the time of the first sampling (18-20 August 2003). Two 30 ml plastic tubes were filled with soil collected within 15 cm of the surface in each habitat. Each was placed in a glass Petri dish and dried overnight at 105° C. Subsequently, 5-10 g of dry ground soil was placed in a crucible and burned for 15 min on a Bunsen flame. Weighing the soil and crucible before and after burning permitted the estimation of the total organic matter.

Of the twenty initial habitats, four were chosen in order to provide a gradient in total organic matter, i.e., 3.61% (code R9), 5.23% (R8), 20.65% (L8), and 21.25% (R6). This small sample of habitats also confined the study to the small rain and dry seasons. Three of the chosen sites were located on Rusinga Island (codes R6, R8, and R9) and one in Lwanda (code L8). Fresh soil was collected twice at each site, at ca. 8-week intervals. Experiments were conducted on a soil sample from only one site at a time.

Soil collection and larval rearing

At each collection, a plastic container (40 cm diameter, 1 m deep) was filled with surface soil from one of the larval habitats and its contents immediately divided equally (5 x 10 liters) between five plastic troughs (40 cm diameter, 20 cm deep) and flooded with filtered Victoria lake water (water depth max 5 cm). This provided a total of 10 replicate troughs for each site. After three days the troughs were checked for mosquito larvae (visible on the surface) in case wild eggs had survived in the soils. Two additional troughs containing filtered lake water only (no soil) were used as controls. The troughs were exposed to the sun through the mosquito netting roof of the screenhouse from which wild mosquitoes were excluded. Three to four days after soil flooding, 450-500 eggs from an *Anopheles gambiae* Giles *sensu stricto* (*Mbita strain*) rearing (hatching rate 90-100%) were dispensed into each of the troughs (on the assumption that the different treatments had no effect on hatching rate). Larvae in the control troughs were fed with defined quantities of Tetramin® fish food every day for optimal development, following a rearing protocol (Okech et al. 2003). The water level was checked each day and filtered lake water was added if necessary to all troughs. Pupae were counted daily and transferred to a holding cage for emergence. One of the wings of each female was measured with a micrometer eye-piece, and body size was estimated by the cubic value of the wing length (Briegel 1990b). The experiments were designed to determine indicators of larval development duration, pupation rate, and adult mosquito body size for each soil sample.

Soil analysis

A portion of the first sample taken from each of the four sites was deep-frozen and brought to the Department of Plant Ecology, Institute of Botany, University of Neuchâtel, Switzerland, for further analysis. At Neuchâtel, precise determination of the total organic matter value was made by loss on ignition (as values measured at MPFS could have been underestimated). Other soil parameters analyzed were the organic carbon by the simplified Ann method (oxidation in 8% $K_2Cr_2O_7$ with H_2SO_4 solution and back-titration with a solution of Mohr salt), and total nitrogen by the Kjeldahl method (Kjeldahl 1883). All parameters are given in % (g / 100 g) of dry soil (Table 1). The total organic matter, organic carbon, and nitrogen values obtained were used as indices for the different sites and their contribution to variations in larval development variables and adult size

Table 1. Soil characteristics of the larval habitats (codes R6, R8, R9, and L8 correspond to the different larval habitat sites).

Larval habitat code	R9	R8	L8	R6
Total Organic Matter [%]	3.61	5.23	20.65	21.25
Organic Carbon [%]	0.85	0.63	3.47	7.18
Total Nitrogen [%]	0.1	0.06	0.32	0.58

Table 2. *An. gambiae* pupation rate, development time, and body size in four larval habitats (codes R6, R8, R9, and L8 correspond to the different larval habitat sites). Absolute data are presented here, but all analyses were made on ratios of the corresponding control (see methods).

Larval habitat code		R9	R8	L8	R6
Median duration of larval development [days] (IQR*)	experimental	15 (3)	10 (3)	14 (4)	8 (4)
	control	9 (3)	10 (3)	10 (2)	8 (1)
Median no. pupae per trough (IQR)	experimental	10 (5)	1 (3)	0 (48)	279 (100)
	control	322 (59)	391 (150)	483 (70)	355 (154)
Mean body size [mm^3] (SD**)	experimental	23.81 (6.18)	20.67 (4.09)	20.28 (3.44)	26.91 (8.89)
	control	29.44 (8.54)	26.71 (6.93)	25.86 (4.35)	30.70 (6.24)

* IQR: the interquartile range is the difference between 25th and 75th percentile.
** SD: standard deviation.

was determined.

Statistical analysis

Statistical tests were conducted on experimental/control data, i.e. each value of any output variable was expressed as a fraction of the median of the corresponding control. This procedure minimized the influence of external factors, such as temperature and rainfall for larval development. The repetitions for each site were grouped for statistical analysis. The influence of total organic matter, organic carbon, and total nitrogen content of soils on development and size parameters of *An. gambiae* was analyzed using Spearman's rank correlation test (coefficient represented by ρ) for the non-parametric variables (pupation rate in 40 troughs and larval development duration providing 3,181 pupae), while Pearson's product moment correlation "r" was used for the parametric variable (body size of 248 adults). All troughs, including those with no pupae, were included in the pupation rate analysis. Statistical analyses were made using SPLUS® 6.1 for Windows and graphics were made with SPSS Sigmaplot 2001 for Windows.

RESULTS

Effect of total organic matter on larval development and adult body size

The total organic matter of soil was correlated with the number of pupae produced in each trough in that the soils with a low organic content, R9 3.61% (seven troughs) and R8 5.23% (eight troughs, Table 2) produced no more than ten pupae, whereas the soils with a higher organic content, L8 20.65% (ten troughs) and R6 21.25% (ten troughs), showed either variation in the number of pupae obtained (L8) or permitted development of more than 250 pupae (R6) (Spearman**:** $\rho = 0.5$, $P < 5x10^{-3}$, Table 2).

The number of days necessary for the larvae to develop from hatching to pupation and the total organic matter of soil was inversely correlated ($\rho = -0.33$, $P = \sim 0$), i.e. *An. gambiae* larvae grew faster when the total organic matter of the soil substrate was high (Figure 1), but the total organic matter had a negative influence on body size (Pearson's moment correlation: $r = -0.29$, $P < 5x10^{-4}$), i.e. mosquitoes emerging from a habitat with soils of high organic matter content were smaller (Figure 2).

Effect of organic carbon on larval development and adult body size

The organic carbon content of the soil was positively correlated with the number of pupae ($\rho = 0.63$, $P < 5x10^{4}$), but the development time was inversely correlated with organic carbon content of soil ($\rho = -0.33$, $P = \sim 0$), i.e. when the organic carbon level in the soil was high, larvae developed faster. In addition, the amount of organic carbon in soil had a negative influence on body size of adult female *An. gambiae* ($r = -0.21$, $P < 0.001$).

Effect of total nitrogen on larval development and adult body size

The total nitrogen content of the soil was positively correlated with the number of pupae produced ($\rho = 0.63$, $P < 5x10^{-4}$), but development time was inversely correlated with the total nitrogen level of soil ($\rho = -0.33$, $P = \sim 0$), i.e. larvae developed faster on soil rich in total nitrogen. Finally, the amount of total nitrogen in soil had a negative influence on body size of adult female *An. gambiae* ($r = -0.21$, $P < 0.001$; Figure 3).

DISCUSSION

This study shows that when the quantity of total organic matter, organic carbon, and nitrogen varies among larval habitat soils, it can significantly influence the development and body size of emerging *An. gambiae*. Larval development duration and adult body size decrease but pupation rate increases when the organic content of the soil substrate increases. This supports the relation between larval habitat quality and mosquito response in terms of development time and body size of adult mosquitoes as

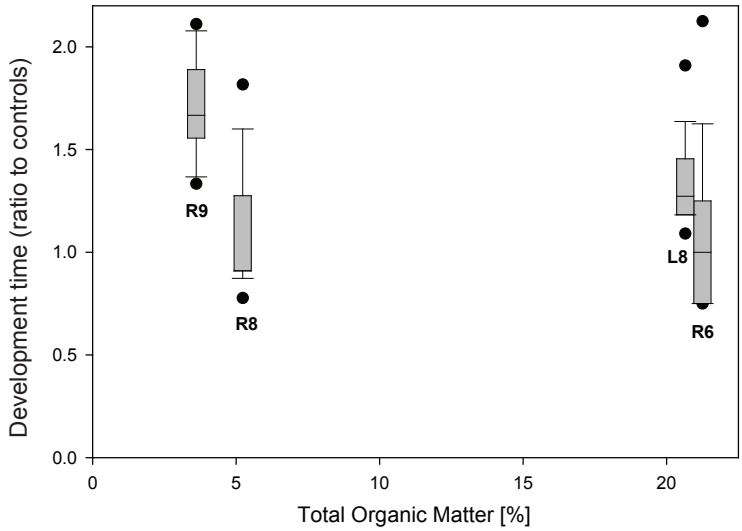

Figure 1. Influence of the total organic matter of soil in the habitat on An. gambiae larval development (n=3181). Each box represents data for one of the sites (codes R6, R8, R9, and L8). In the box plots, the line within a box marks the median, the lower and upper boundary lines of a box indicate the 25th and 75th percentiles, bars below and above indicate the 10th and 90th percentiles, respectively, and the points represent data beyond these limits. The median line for R8 is not visible as it lies on the 25th percentile value.

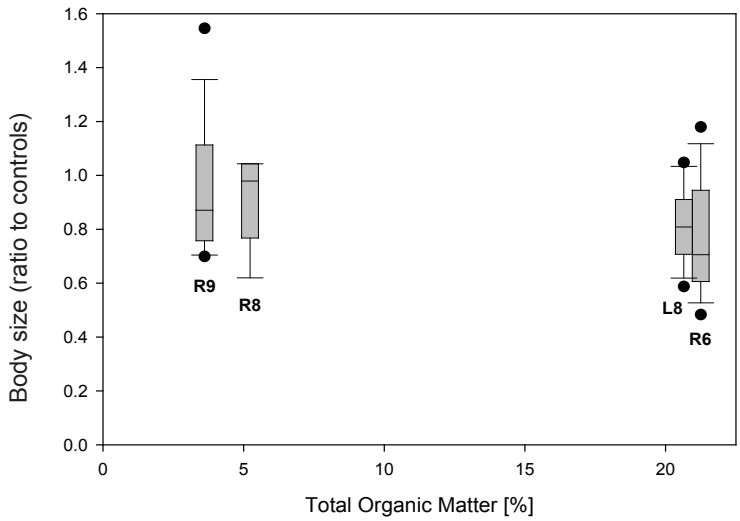

Figure 2. Influence of the total organic matter of soil in the larval habitat on *An. gambiae* adult body size (n=248). Each box represents data for one of the sites (codes R6, R8, R9, and L8). For explanation of box plots see Figure 1 legend.

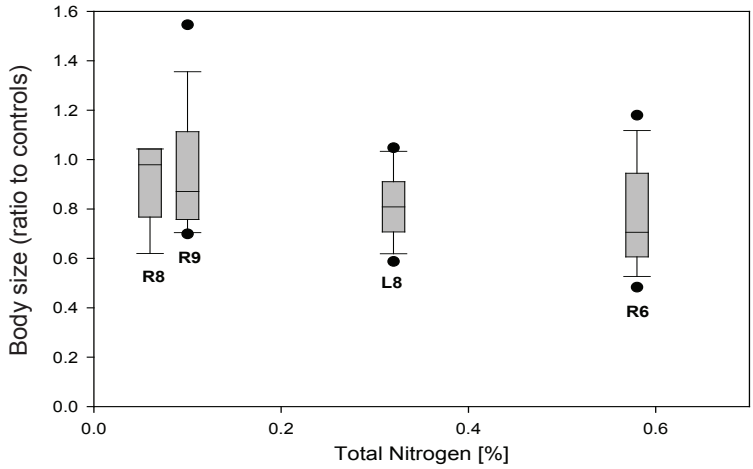

Figure 3. Influence of the total nitrogen content of soil in the larval habitat on *An. gambiae* adult body size (n=248). Each box represents data for one of the sites (codes R6, R8, R9, and L8). For explanation of box plots see Figure 1 legend.

demonstrated in previous studies. Wotton et al. (1997) observed that *An. gambiae* and *An. quadrimaculatus* grow most rapidly in habitats where a surface microlayer (the air/water interface) is enriched with microorganisms which may act as an important source of nutrition for *Anopheles* larvae. Timmermann and Briegel (1993) demonstrated that larval crowding (i.e., reduced nutrient input per individual) resulted in an extended larval developmental period, reduced pupation rate, and reduced adult body size. Our study confirms that the soil type of mosquito aquatic habitat is critical for larval development, but the underlying determinants for the development of mosquito larvae are probably quite complex.

The association between mosquito developmental variables and adult body size and the soil parameters recorded here (total organic matter, organic carbon, and total nitrogen) all follow the same trend. This is to be expected since organic carbon and organic matter are tightly linked, while nitrogen content depends on organic matter quantity and the decomposition level of the soil (Singer and Munns 1996, Sumner 2000). All the surface soils in this study have a low carbon/nitrogen (C/N) ratio (ranging from 8.6 to 12.3), which is an indication of soils with a fast organic matter turnover. As such, these soils reach a high degree of decomposition rapidly, a common characteristic of tropical soils. A high proportion of the land in sub-Saharan Africa has, however, been transformed into agricultural land to the detriment of forests. This conversion of forests to agricultural land generally results in a decline in organic carbon content (Shang and Tiessen 2000), which, according to this study, should lead to a lower pupation rate, a longer larval development time, and production of bigger *An. gambiae* adults. The land of Suba district, on the shores of Lake Victoria, is mainly exploited for agriculture and cattle pasture, with its organic content being consequently reduced. This should tend to render the soil of Suba district relatively sub-optimal for *Anopheles* larval development, though larval habitats containing a high density of *An. gambiae* larvae are present in the area. This may mean that there are more important factors than total organic matter of the larval habitat soil that are associated with mosquito population dynamics in such pools. Still, heterogeneity in soil characteristics is crucial and the role of larval habitat "quality" on vector dynamics and distribution should be considered further. Identifying specific soil-related factors underlying larval habitat productivity is a critical step towards predicting how the aquatic habitat quality and associated spatio-temporal variability affects vector population dynamics.

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