Malaria Journal

Research

Open Access

Larval habitats of *Anopheles gambiae* s.s. (Diptera: Culicidae) influences vector competence to *Plasmodium falciparum* parasites

B a A O c *[1,2,6], L - C G - a a[2,3], G- - Ya [5], J I G - [2] a J C B [4]

A : [1]C B c , R a c a D (CBRD), K a M ca R a c I - , P. O. B 54840, Na b , K a, [2]H- a H a D , I a a C I c P a Ec (ICIPE), P. O. B 30772, Na b , K a, [3]D a S c Sa UR 016, I - R c c P - D (IRD), P.O. B 64501, 34394 M C 5, F a c , [4]D a E a P- b c H a , U M a Sc M c , 12500 SW, 152 S , B- B M a , FL 33177, USA, [5]P a P- b c H a , C H a Sc c , U Ca a, I , H Ha R 3038, I , CA 92697-4050, USA a [6]W Lab a Ma B c c , U F a, 9505 Oc a S B - a , S A- , 32080-8610, FL, USA

E a B a A O c * - c @ . - - L - C G - a a - L - -C .G - a a@ .b ; G- - Ya - - - @- c. - J I G - - - @ c . ; J C B - b @ . a . -

* C a-

Published: 30 April 2007

Malaria Journal 2007, **6**:50 doi:10.1186/1475-2875-6-50

Received: 17 January 2007
Accepted: 30 April 2007

This article is available from: http://www.malariajournal.com/content/6/1/50

Abstract

Background: The origin of highly competent malaria vectors has been linked to productive larval habitats in the field, but there isn't solid quantitative or qualitative data to support it. To test this, the effect of larval habitat soil substrates on larval development time, pupation rates and vector competence of *Anopheles gambiae* to *Plasmodium falciparum* were examined.

Methods: Soils were collected from active larval habitats with sandy and clay substrates from field sites and their total organic matter estimated. *An. gambiae* larvae were reared on these soil substrates and the larval development time and pupation rates monitored. The emerging adult mosquitoes were then artificially fed blood with infectious *P. falciparum* gametocytes from human volunteers and their midguts examined for oocyst infection after seven days. The wing sizes of the mosquitoes were also measured. The effect of autoclaving the soil substrates was also evaluated.

Results: The total organic matter was significantly different between clay and sandy soils after autoclaving (P = 0.022). A generalized liner model (GLM) analysis identified habitat type (clay soil, sandy soil, or lake water) and autoclaving (that reduces presence of microbes) as significant factors affecting larval development time and oocyst infection intensities in adults. Autoclaving the soils resulted in the production of significantly smaller sized mosquitoes (P = 0.008). Autoclaving clay soils resulted in a significant reduction in *Plasmodium falciparum* oocyst intensities (P = 0.041) in clay soils (unautoclaved clay soils (4.28 ± 0.18 oocysts/midgut; autoclaved clay soils = 1.17 ± 0.55 oocysts/midgut) although no difference (P = 0.480) in infection rates was observed between clay soils (10.4%), sandy soils (5.3%) or lake water (7.9%).

Conclusion: This study suggests an important nutritional role for organic matter and microbial fauna on mosquito fitness and vector competence. It shows that the quality of natural aquatic habitats of mosquito larvae may influence malaria parasite transmission potential by *An. gambiae*. This information can be important in targeting larval habitats for malaria control.

Background

Ma a a c *Anopheles gambiae* c a a a b a a a ab a [1]. ab a ca a a b ca c a ac c, c c c b- a ab- a c a a - a- [2]. W ab a - a a a - - a c c c c [3,4], ac a a - *An. gambiae* a a a a a a - c a, a c a c - a a a ab- a - [4].

An. gambiae a a a b a b a c a b c c a ac c - a a, - a c b- b a a a [5,6]. I a b a - a - a a ab- a a - - [3], a a a a - b a a a c [4], - a b c - a- - - a a ab a - b a a a c - - *Plasmodium falciparum* a a c a - - . - c a a c a c a a , a - - - a c a a a a a a *An. gambiae* a b - a .

T a - c - b- a a a a a - c c - c *An. gambiae* *P falciparum* a a a a . S - b a a a a ab a b a ca a K a. B - *P. falciparum* a - c - a a *An. gambiae* a a a - a a a - b a , a - c- *P. falciparum* - a - c a - - a a a b a ac a - . - a c - a - a a a a - a - c ab a a a a c c c c . S- a a - - a a ac a a a ab a .

Materials and methods

Study area

- a c - a I a a C I c P a Ec (ICIPE) a O a- b Ca - ca - K a La V c a, S- a D c. a a - - b - a - a a a - - ba ac a . W a a a ; a R- a, c a a b a ca- a . S a a a b a a a - b a - a . - b ab a - a a a . c a , a , c , , , a , a - a , a - c c a c a [7]. a a - a a a a a a 1200 1600 a, b- a a a b a a a (ICIPE a O a b Ca - ca a a a). a - - Ma c J- a a - Oc b D c b . Ma a a a a c, *An. gambiae* . ., *An. arabiensis*, a *An. funestus* c b- a - a - a a a a a b 0 a 1.55 c - b [8].

a ca c - - ba ab- a c a a, c , a a c . (La : 0 , 28.26 S; L : 34 , 17.311 E) a L a a V a a - a a a a a b ac c - (ca). a b a b (c , , , a , a a) a a a - a . c (La : 0 24 S; L : 34 10 E) a Wa a a V R- a I a a a a a a a a a a a a a a . a a a ca a c a b a (c , a a) a - a . I a a a - a , b ab- a - a - c ab a a a.

Selection and sampling of habitat soils for experiments

T c a a ab a - , c b ab - - ab- a . A - c a b a a b a a a c . a a a b a . I a ab a a a a b ac- a - a Pa - c a a a c - . T a a ab a c ac - (L a a a R- a) ba a . S c c ab a a ac a a b - 10 c a a - ICIPE a O a b ca - - a a a ab a . - a a a ab a - - - c - - [9] c a - c ab ac a - c , - a a a a c , c- , c - ab a a a a c c - - , a ac a a a .

Manipulation of soils collected from field sites

Rearing mosquitoes on soil substrates

Recruitment of **P. falciparum** *gametocyte carriers*

Experimental infection and assessment of oocyst stages in mosquitoes

Statistical analysis

T a T- (a - u - a a a c) a - u c a a c a b - ua- u c a a a- u c a c a a a A a a (GLM) - u- a a a a a - u a c ab- a a a- u c a a a , - u b - ua , a - u b a - u - . GLM - u a a a a a - u a c ac (, a- u c a) c - - u - u. T c a a a c a b L a S ca D c (LSD) GLM - u a a a - u a a a a , c , a a - u . P a c - a a a a a - ua a , - c a , a . T c a c b - u a a a - u ANOVA a a T- u .

Results

The organic content in unautoclaved and autoclaved soils

A a 6 a a c c a c a c . T ca - c a a c a b - ua- u c a c a a - ua- u c a a (a T- : P = 0.853). I a- u c a a ca - c a a a c a c b c a a a (T- : P = 0.022), a c a b c a (2.12% 0.09) a a (1.07% 0.39). T c a- u c a c b a a a c a , a c a a a c a c a b a a- u c a .

Effect of larval habitat soils on larval development

T a a a - ua c a (5.0 a 0.57) a a (5.7 a 0.88) a a 0.33). T a a a- u c a (Tab 1), - u a a- u c a (a - c b a ac) a c a a . H , a a a - ca b (P = 0.606) a a- u c a (P = 0.661) a a - a a a (GLM) - u a a a a a ab a a a ca ac a c a a a - ua , a ca a a ca c a a (Tab 2). T ac- b ab a a ca - u a - a c a a ab a c a c a a a a . T - u b - ua a a - u ca a c b ab a a- u c a (Tab 2). B - u-a- u c a , - u (c a = 3.134 0.26; a a = 3.131 0.26) ca (ANOVA: = 1, F = 0.010, P = 0.920). H , a a a- u c a , a - u ca (ANOVA: = 1, F = 7.161, P = 0.008) b c a (3.012 0.205) a a (2.946 0.28). O a a a , a , a- u c a ca a c a - u - c a (ANOVA: = 2, F = 0.233, P = 0.796) a (ANOVA: = 2, F = 0.120, P = 0.888).

Effect of larval habitat soils on oocyst infections in mosquitoes

T c c a - u a c a a (10.4%) a a a a - u a a (5.3%) c (7.9%) (Tab 3). N ca c c c a b *An. gambiae* - u a - u-a- u c a b (C a - : χ^2 = 27.71, = 28, P = 0.480). A GLM - u a a a a a a ab a a a- u c a ca a c c - u - u (Tab 4). A c ,

Table 1: The influence of autoclaving larval habitat soils on fitness parameters of *An. gambiae* mosquitoes.

Mosquito parameter measured.	Rearing substrates for larvae			
	Insectary	Clay soils	Sandy soils	Prob.
Un-autoclaved soils				
Larval development time (d) ± SD	6.33 ± 0.33	5.0 ± 0.57	5.7 ± 0.88	0.606
Pupation rates	94.1%	43.3%	55.3%	0.260
Wing sizes ± SE	3.12 ± 0.22	3.08 ± 0.10	3.16 ± 0.07	0.523
Autoclaved soils				
Larval development time (d) ± SD	6.3 ± 0.33	5.7 ± 0.88	5.3 ± 0.88	0.661
Pupation rates	82.7%	55.3%	65.7%	0.451
Wing size ± SE	2.85 ± 0.12	2.94 ± 0.11	2.86 ± 0.13	0.641

#Values are means ± SD of readings from 12 experiments for all the 3 habitat types. In the control group, mosquitoes were reared in lake water using standard protocols.

c a - u a a a a a (6.33 a a c a - u ca

Discussion

Table 3: The infectivity of *An. gambiae* s.l. mosquitoes reared on unautoclaved clay and sandy soil substrates obtained from mosquito larval habitats in western Kenya and then co-infected with different gametocyte carriers (mean gametocyte density = 173.8 ± 121.4).

Gametocyte		Oocyst infection rates			Oocyst intensity		
Carrier #	Density	Lab	Clay	Sandy	Lab	Clay	Sandy
1	48	15.4 (2/13)	21.6 (8/37)	0 (0/56)	2.0	2.1	0
2	16	2.2 (1/45)	9.5 (8/84)	0 (0/38)	2.0	1.6	0
3	32	9.4 (5/53)	27.3 (12/44)	0 (0/51)	1.4	2.0	0
4	32	ND	8.7 (2/23)	0 (0/34)	ND	2.5	0
5	128	40 (2/5)	0 (0/20)	13.9 (16/115)	2.0	0	2.0
6	16	9.7 (3/31)	0 (0/17)	2.8 (3/108)	2.33	0	1.7
7	16	6.9 (2/29)	0 (0/40)	16.1 (5/31)	1.50	0	2.8
8	16	0 (0/14)	0 (0/23)	0 (0/24)	0.0	0	0.0
		7.9% (15/190)	10.4% (30/288)	5.3% (24/457)	1.60 ± 0.29	2.06 ± 0.18	1.62 ± 0.59

Only 8 out of 22 experiments successfully yielded infected mosquitoes. The analyse

Table 4: A GLM univariate test on the effect of habitat type and treatment of habitat soils by autoclaving on midgut oocyst intensities in *An. gambiae* mosquitoes.

Source of variation	DF	MS	F	Prob.
Intercept	1	1.275	6.423	0.022
†Habitat type	1	0.978	4.928	0.041*
$Treatment	1	1.214	6.120	0.025*
Habitat type × Treatment	1	0.664	3.347	0.086
Total organic matter	1	0.401	2.018	0.175
Gametocyte density	1	1.580	7.965	0.012*
Mosquito size	1	1.193	6.011	0.026*
Error	16	0.198		

†Habitat refers to clay or sandy soils mixed with water. $Treatment refers to autoclaving or not autoclaving the soils; *Probability, P < 0.05.

[illegible]

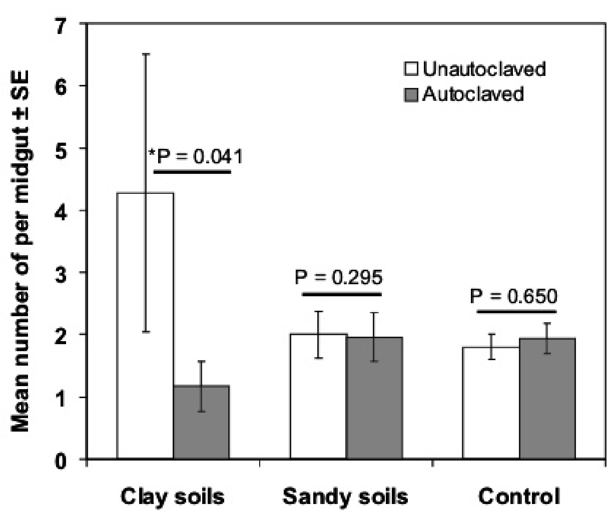

Figure 1
The effect of autoclaving soils from clay and sandy larval habitats on the oocyst infection intensities in the midguts of *An. gambiae* mosquitoes.

[illegible]

(Pa- uM , . c .) a c c c .
A a ∂ - u , c∂ a ∂ ab a - au
- u c b a a a ∂ a c a
c a c ∂ b- u *Anopheles*
- u a ∂ a a a a
c a a . I ∂ , a u - c∂
- c a a ∂ ab a a - u a ∂ a
a c b- u a a a c .

Authors' contributions

BAO D , c c a a a
a a, a - uc ; LCG Da a c c a
a - uc ; JCB R a a a a - uc ; GY
R a - uc ; JIG A a - u a
a - uc . A a- u∂ ∂ a a a a
∂ ∂ c ∂ a .

Acknowledgements

All the ICIPE Thomas Odhiambo Campus staff members, particularly the vector competence project team members, for their input in this study. Also appreciation to the local chiefs, clan elders, village communities, schools, and head teachers for the cooperation received from them in the recruitment of gametocyte carriers. Many thanks to the personnel of the Mbita Health Centre for their support. This study was supported by NIH grants U19AI45511, D43TWO1143, D43TW00920 and D43TW01505. BAO received an African Regional Postgraduate Programme in Insect Science (ARPPIS) training fellowship from ICIPE. This paper has been published with the permission of the Director, Kenya Medical Research Institute (KEMRI).

References

1. Service MW: **Mosquito Ecology: Field Sampling Methods.** 2nd Edition edition. London, Elsevier Applied Sciences; 1993.
2. Minakawa N, Mutero CM, Githure JI, Beier JC, Yan G: **Spatial distribution and habitat characterization of anopheline mosquito larvae in Western Kenya.** *Am J Trop Med Hyg* 1999, **61:**1010-1016.
3. Grimstad PR, Walker ED: **Aedes triseriatus (Diptera: Culicidae) and La Crosse virus. IV. Nutritional deprivation of larvae affects the adult barriers to infection and transmission.** *J Med Entomol* 1991, **28:**378-386.
4. Lyimo EO, Koella JC: **Relationship between body size of adult Anopheles gambiae s.l. and infection with the malaria parasite Plasmodium falciparum.** *Parasitology* 1992, **104 (Pt 2):**233-237.
5. Gimnig JE, Ombok M, Kamau L, Hawley WA: **Characteristics of larval anopheline (Diptera: Culicidae) habitats in Western Kenya.** *J Med Entomol* 2001, **38:**282-288.
6. Gimnig JE, Ombok M, Otieno S, Kaufman MG, Vulule JM, Walker ED: **Density-dependent development of Anopheles gambiae (Diptera: Culicidae) larvae in artificial habitats.** *J Med Entomol* 2002, **39:**162-172.
7. Fillinger U, Sonye G, Killeen GF, Knols BG, Becker N: **The practical importance of permanent and semipermanent habitats for controlling aquatic stages of Anopheles gambiae sensu lato mosquitoes: operational observations from a rural town in western Kenya.** *Trop Med Int Health* 2004, **9:**1274-1289.
8. Shililu J Mbogo C, Mutero C, Gunter J, Swalm C, Regens J, Keating J, Yan G, Githure J, Beier, J: **Spatial distribution of Anopheles gambiae and Anopheles funestus and malaria transmission in Suba District, western Kenya.** *Insect Science and Its Application* 2003, **23:**187-196.
9. Knols BG, Njiru BN, Mathenge EM, Mukabana WR, Beier JC, Killeen GF: **MalariaSphere: a greenhouse-enclosed simulation of a natural Anopheles gambiae (Diptera: Culicidae) ecosystem in western Kenya.** *Malar J* 2002, **1:**19.
10. Beier JC CR Oyaro C, Masinya A, Odago WO, Oduor S, Koech DK, Roberts CR: **Anopheles gambiae complex egg-stage survival in dry soil from larval development sites in western Kenya.** *J Am Mosq Control Assoc* 1990, **6:**105-109.
11. Shililu JI, Grueber WB, Mbogo CM, Githure JI, Riddiford LM, Beier JC: **Development and survival of Anopheles gambiae eggs in drying soil: influence of the rate of drying, egg age, and soil type.** *J Am Mosq Control Assoc* 2004, **20:**243-247.
12. Gouagna LC, Okech BA, Kabiru EW, Killeen GF, Obare P, Ombonya S, Bier JC, Knols BG, Githure JI, Yan G: **Infectivity of Plasmodium falciparum gametocytes in patients attending rural health centres in western Kenya.** *East Afr Med J* 2003, **80:**627-634.
13. Takken W, Klowden MJ, Chambers GM: **Effect of body size on host seeking and blood meal utilization in Anopheles gambiae sensu stricto (Diptera: Culicidae): the disadvantage of being small.** *J Med Entomol* 1998, **35:**639-645.
14. Wotton RS, Chaloner DT, Yardley CA, Merritt RW: **Growth of Anopheles mosquito larvae on dietary microbiota in aquatic surface microlayers.** *Med Vet Entomol* 1997, **11:**65-70.
15. Walker ED, Olds EJ, Merritt RW: **Gut content analysis of mosquito larvae (Diptera: Culicidae) using DAPI stain and epifluorescence microscopy.** *J Med Entomol* 1988, **25:**551-554.
16. Merritt RW, Dadd RH, Walker ED: **Feeding behavior, natural food, and nutritional relationships of larval mosquitoes.** *Annu Rev Entomol* 1992, **37:**349-376.
17. Clements AN: **Development, Nutrition and Reproduction.** In *The Biology of Mosquitoes Volume 1*. London, Chapman & Hall; 1992.