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Anopheles gambiae (Diptera: Culicidae) oviposition as influenced by type of water infused into black and red soils of western Kenya

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Abstract. The aim of this study was to document what water types were most stimulatory to oviposition by the malaria mosquito, *Anopheles gambiae* Giles *s.s.* In laboratory choice tests, dishes of black cotton and red laterite soils from around Kisumu, Kenya, equally stimulated *An. gambiae* oviposition when saturated with water. When infused into either soil type, water from Lake Victoria was significantly more stimulatory than any other type of water or infusion tested. Water from natural *An. gambiae* puddle habitats, borehole water, cow dung infusion, Bermuda grass infusion and distilled water followed in declining rank order. Relative to *An. gambiae* puddle-habitat water and distilled water, Lake Victoria water appears to provide the strongest chemical cues for *An. gambiae* oviposition recorded to date. This work suggests that this important vector of human malaria may oviposit in and around Lake Victoria, as well as in its usual puddle habitats. Lake Victoria should be explored for its possible contribution to malaria mosquito populations.

Key words: *Anopheles gambiae*, ovipositional site selection, resource preference, malaria vector, Lake Victoria

Introduction

Anopheles gambiae Giles *s.s.* (Diptera: Culicidae) is a key vector of human malaria in sub-Saharan Africa. Knowing where this mosquito deposits eggs and what factors trigger oviposition are important to understanding the basic biology of this disease vector so as to progress towards effective vector management. As is true for other mosquitoes (Bentley and Day, 1989; Clements, 1999), *An. gambiae* oviposition is thought to be influenced by visual, tactile and chemical cues from potential egg depositional sites. In laboratory choice tests, *An. gambiae* deposits many more eggs on dark- than light-coloured substrates (McCrae, 1984; Huang *et al.*, 2007), while moist substrates receive more eggs than do dry ones (Minakawa *et al.*, 2001; Huang *et al.*, 2005). Substrates comprising a wide range of differing particle sizes were equally acceptable to gravid females (Huang *et al.*, 2005). Requirements for odour and taste are not yet clear.

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Anopheles gambiae females will deposit many eggs on dark substrates wetted with distilled water and no additional chemical cues (Huang *et al.*, 2006). However, some studies have reported that females given choices deposit more eggs into water from natural larval habitats than into pure water (McCrae, 1984; Bray, 2003; Sumba *et al.*, 2004). On the other hand, Munga *et al.* (2006) found that females discriminate against water previously holding high densities of *An. gambiae* larvae. Yet-unidentified microbial odours have been suggested to play a role in *An. gambiae* oviposition (Knols *et al.*, 2004). But, it is unclear whether or not the outcomes of some such studies were confounded by variation in relative darkness of ovipositional resources. Nevertheless, the picture emerging is that these females oviposit readily and are not very discriminating. Subsets of the various ovipositional cues reported to date seem sufficient to trigger copious egg output. The full complement of optimized cues may not be necessary in nature, although it may be preferred under some circumstances where various choices happen to be available.

The objective of the current research was to revisit which types of water treatments were most stimulatory to *An. gambiae* oviposition when visual and tactile cues of the substrate were carefully standardized. High densities of *An. gambiae* larvae have been noted in zones with many cattle footprints containing water. Cow manure is also common in such areas. Thus, cow dung infusion was included in this study to test the idea that bodies of water rich in organic material might be conducive to oviposition because they might also support larval growth and development via enhanced microbial populations. Grass-infusion was included for comparison; it was not expected to stimulate *An. gambiae* oviposition.

Materials and methods

Mosquitoes

Adult *An. gambiae* s.s. Kisumu and pink-eye strains (F2) obtained from the insectary of the Kenya Medical Research Institute (KEMRI) (Kisumu, Kenya) were held in 60 × 60 × 60 cm white BugDorm-2 insect-rearing cages (MegaView Science Education Services Co., Taiwan) (Huang *et al.*, 2005). The insects were maintained at 26–29 °C, 70–85% RH and a 13:11 LD photoperiod. When 4 days old, females were fed on rabbit blood. Ovipositional tests commenced 2 days after blood feeding. At all times, mosquitoes were given access to cotton wicks soaked with 10% honey solution.

Ovipositional bioassays

The experimental design was randomized complete block; a set of one copy of each ovipositional treatment added to one BugDorm-2 cage containing 20 gravid females for one night constituted a block. Within each block, positions of treatments were randomized and (when more than two) arranged in a circle on the cage floor. Blocks were replicated through time and used different batches of females. Ovipositional treatments were inserted into the cages at *c.* 1700 h and removed *c.* 0800 h the following morning. Eggs were immediately counted under a dissecting microscope. Natural skylight provided lighting for oviposition. Data were transformed by $(x + 1)^{1/2}$ before analysis by three-way ANOVA. Means were separated by the LSD test. A paired *t*-test was used to determine the statistical significance when only one pair of treatments was included in one experiment.

Experiment 1: black and red soils infused with Lake Victoria water

Black cotton and red laterite soils are typical of the Kisumu, Kenya region where *An. gambiae* is abundant. Soil samples were obtained outside the KEMRI campus at Kisian where there were many *An. gambiae* puddle-habitats. Sun-dried soil was sieved to yield 38–45 μm particles; 70 g portions were dispensed into plastic Petri dishes of 9 cm in diameter before being saturated with water. The two soil types were compared in a two-choice test using lake water collected just offshore from the Korando B sub-location west of Kisumu. Positions of the soil types were alternated on the cage floor between each of the five replicate runs.

Experiment 2: oviposition as influenced by water and infusion type

This five-choice test included: (i) distilled water, (ii) water from a deep borehole on the KEMRI campus, (iii) Lake Victoria water, (iv) cow dung infusion, obtained by mixing 100 g fresh cow dung in 1 l of distilled water that then stood at room temperature for 6 h before decantation and (v) Bermuda grass, *Cynodon dactylon* (L) Pers. infusion resulting from soaking 200 g of fresh lawnmower clippings in 1 l of water at 85 °C for 30 min before decantation. Four blocks of this experiment were conducted using black soil and three blocks with red soil, respectively.

Experiment 3: comparison of An. gambiae *habitat water with Lake Victoria water*

This four-choice test included: (i) distilled water, (ii) borehole water, (iii) Lake Victoria water and (iv) water taken from natural *An. gambiae* puddle habitats in the Kisian area. Five replicates were conducted using each soil type, black and red.

Results

Experiment 1: black and red soils infused with Lake Victoria water

On black and red soils, 90 ± 35 (SE) and 95 ± 40 eggs were laid, respectively. This difference was not significant by paired t-test ($t = -0.1$, $\mathrm{df} = 4$, $P = 0.9$).

Experiment 2: oviposition as influenced by water and infusion type

There was no significant soil effect ($F = 1.3$, $\mathrm{df} = 1{,}22$, $P = 0.3$) or block effect ($F = 0.2$, $\mathrm{df} = 3{,}22$, $P = 0.6$) on the numbers of eggs deposited in the various water treatments; therefore, data were pooled across soil types in further analysis. The numbers of eggs were significantly higher for Lake Victoria water than any other treatment (Fig. 1). Borehole water was significantly different from distilled water, but the infusions were not.

Experiment 3: comparison of An. gambiae *habitat water with Lake Victoria water*

No significant soil ($F < 1$, $\mathrm{df} = 3{,}12$, $P = 0.1$) or block ($F < 1$, $\mathrm{df} = 4{,}12$, $P = 0.9$) effect was observed in this test. Lake Victoria water was significantly more stimulatory than the other treatments (Fig. 2). *Anopheles gambiae* habitat water and borehole water were equivalent, and both received significantly more eggs in this test than did distilled water.

Discussion

Using common dark soil substrates against a white background, the current experiments permitted comparisons of the effects of hydration medium on *An. gambiae* oviposition with no confounding effects of differing visual cues like resource darkness and contrast that can profoundly influence ovipositional outcomes (Huang *et al.*, 2006). This work was done in the laboratory using mosquitoes confined in cages. Thus, caution is advised in extrapolating to the field situation. Additionally, low numbers of eggs were produced per average female in these tests. This was likely to be due to low mating success, as males and females were never held together at high densities. Nevertheless, the blocked experimental design provided sufficient statistical power to resolve clear difference in ovipositional stimulation among various treatments.

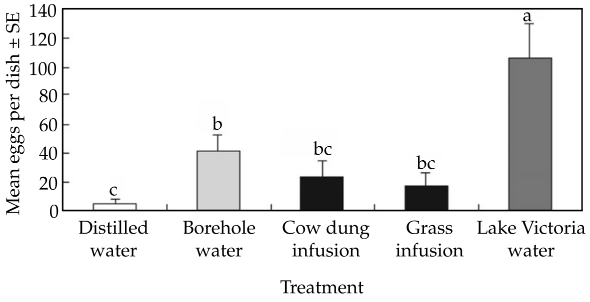

Fig. 1. Oviposition by *Anopheles gambiae* as influenced by type of water used to hydrate black and red Kenyan soil. The total number of eggs recovered in this test was 1370. Means sharing a common letter are not significantly different at $P = 0.05$

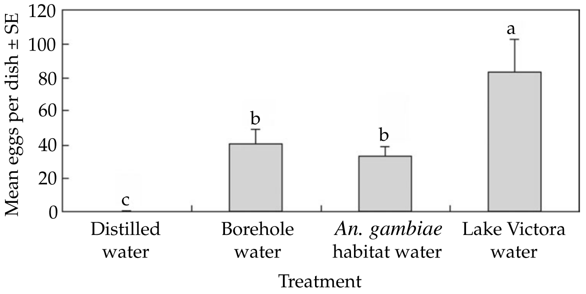

Fig. 2. Comparison of *Anopheles gambiae* oviposition in response to water from puddles inhabited by larvae versus water from the shore of Lake Victoria. The total number of eggs recovered in this test was 1574. Means sharing a common letter are not significantly different at $P = 0.05$

Lake Victoria has not been recognized as a habitat for *An. gambiae* immatures. However, hydrating either black or red soil with lake water surprisingly yielded more eggs than did any water or infusion type, including water from puddle habitats supporting larvae and thought to be ideal larval habitat. Relative to numbers of eggs laid on distilled water (McCrae, 1984; Bray, 2003; Sumba *et al.*, 2004), Lake Victoria water appears to be the most stimulatory water treatment uncovered to date. Its chemistry should be explored in the search for cues eliciting oviposition. We speculate that algal volatiles (Kambourova *et al.*, 2003; Höckelmann, 2004) might play some role in ovipositional stimulation.

Miller *et al.* (2007) have found that: (i) some *An. gambiae* can successfully develop in moving water and (ii) *An. gambiae s.s.* larvae can be found in Lake Victoria and a stream leading into it. Those findings and the current ovipositional results suggest that permanent bodies of water, including Lake Victoria, need to be examined for their ability to sustain this important vector of human malaria. Although such habitats are not likely to be optimal, they may serve as a harbourage of *An. gambiae* during dry seasons.

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References

Bentley M. D. and Day J. F. (1989) Chemical ecology and behavioural aspects of mosquito oviposition. *Annual Review of Entomology* 34, 401–421.

Bray A. M. (2003) Ovipositional behavior of *Anopheles gambiae* as influenced by variable substrates and chemicals. MSc thesis, Michigan State University, USA.

Clements A. N. (1999) *The Biology of Mosquitoes, II Sensory Reception and Behaviour*. CABI Publishing, New York.

Huang J., Walker E. D., Giroux P. Y., Vulule J. and Miller J. R. (2005) Ovipositional site selection by *Anopheles gambiae*: influences of substrate moisture and texture. *Medical and Veterinary Entomology* 19, 442–450.

Huang J., Walker E. D., Vulule J. and Miller J. R. (2007) The influence of darkness and visual contrast on oviposition by *Anopheles gambiae* in moist and dry substrates. *Physiological Entomology* 32, 34–40.

Höckelmann C. (2004) Odor compounds from cyanobacterial biofilms acting as attractants and repellents for free-living nematodes. *Limnology and Oceanography* 49, 1809–1819.

Kambourova R., Bankova V. and Petkov G. (2003) Volatile substances of the green alga *Scenedesmus incrassatulus*. *Zeitschrift für Naturforschung* 58c, 187–190.

Knols B. G. J., Sumba L. A., Guda T. O., Deng A. L., Hassanali A. and Beier J. C. (2004) Mediation of oviposition site selection in the African malaria mosquito *Anopheles gambiae* (Diptera: Culicidae) by semiochemicals of microbial origin. *International Journal of Tropical Insect Science* 24, 260–265.

McCrae A. W. R. (1984) Oviposition by African malaria vector mosquitoes II. Effects of site tone, water type and conspecific immatures on target selection by freshwater *Anopheles gambiae* Giles *sensu lato*. *Annals of Tropical Medicine and Parasitology* 78, 307–318.

Miller J. R., Giroux P. Y., Letutchaia J., Huang J., Vuluke J. and Walker E. D. (2007) Capacity of *Anopheles gambiae* to inhabit and develop in moving water. *Journal of the American Mosquito Control Association* (in press).

Minakawa J., Githure J. I., Beier J. C. and Yan G. (2001) Anopheline mosquito survival strategies during the dry period in Western Kenya. *Journal of Medical Entomology* 38, 388–392.

Munga S., Minakawa N., Zhou G., Okeyo-Owuor J. B., Githeko A. and Yan G. (2006) Effects of larval competition and predators on oviposition site selection of *Anopheles gambiae* sensu stricto. *Journal of Medical Entomology* 43, 221–224.

Sumba A. L., Okoth K., Deng A. L., Githure J. I., Knols B., Beier J. C. and Hassanali A. (2004) Daily oviposition patterns of the African malaria mosquito *Anopheles gambiae* Giles (Diptera: Culicidae) on different types of aqueous substrates. *Journal of Circadian Rhythms* 2, 6.