Medical and Veterinary Entomology

Medical and Veterinary Entomology (2013) **27**, 332–338 doi: 10.1111/j.1365-2915.2012.01065.x

Landing response of *Aedes (Stegomyia) polynesiensis* mosquitoes to coloured targets

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Abstract. *Aedes polynesiensis* Marks (Diptera: Culicidae) is the primary vector of lymphatic filariasis (LF) in the island countries and territories of the South Pacific. In the development of a novel control tool, the response of *Ae. polynesiensis* to six different colours (three solid fabrics, two patterned fabrics and a plastic tarp) was measured using a digital photographic system. Adult mosquitoes were placed into an environmental chamber and allowed to choose between a white target and one of six experimental targets. Mosquito landing frequency and landing duration were calculated. Adult female *Ae. polynesiensis* preferred all of the experimental targets to the white control target. Mosquito landing frequency was highest for the solid targets (black, navy blue and red) followed in turn by the two colour pattern targets and the polyethylene target. Mosquito landing duration was greater for experimental targets when compared with white control targets. Mosquito landing frequencies did not change over time during the course of the assay. The response of male *Ae. polynesiensis* was also measured when exposed to a 100% cotton black target. Male mosquitoes preferred the black target to the white control target, although at levels lower than that observed in female mosquitoes. The results suggest that future investigations evaluating the visual responses of *Ae. polynesiensis* mosquitoes are warranted, with a special emphasis on semi-field and field-based experiments.

Key words. *Aedes polynesiensis*, filariasis control, mosquito control, targets.

Introduction

Lymphatic filariasis (LF) is a painful and disfiguring disease endemic throughout much of the tropics and sub-tropics. Global efforts to control LF are based upon the mass administration of anti-filarial compounds to at-risk populations. Although this strategy has successfully led to a reduction of LF in many areas of the world (Ramzy *et al*., 2006; Weil *et al*., 2008; Hooper *et al*., 2009), its impact in the South Pacific has been uneven in spite of, in some locales, many years of drug treatment (Esterre *et al*., 2001; Burkot *et al*., 2002; Huppatz *et al*., 2009).

Aedes polynesiensis Marks (Diptera: Culicidae) is the most important vector of LF throughout much of the South Pacific (Belkin, 1962; Ramalingam & Belkin, 1964). Mass drug administration (MDA) alone may be insufficient to control LF in the South Pacific because of the unique biology of the *Ae. polynesiensis* mosquito (Ramalingam & Belkin, 1964). This mosquito exhibits a negative density-dependent phenotype that allows it to become a more efficient vector as the microfilaremia in the human population is reduced (Pichon, 2002; Snow *et al*., 2006), a condition that arises after multiple rounds of mass drug administration.

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Vector control integrated with MDA could play a pivotal role in the effort to eliminate LF from the South Pacific. Because *Ae. polynesiensis* is a diurnal, exophilic, semi-urban mosquito, many of the conventional methods used to control other aedine mosquitoes have not been effective in the islands and atolls of the South Pacific. It is, therefore, critical that novel methods of vector control be developed. These methods must be affordable and effective while also being acceptable to the residents of the communities in which they will be deployed.

Outdoor traps and targets based upon insect colour preference have proven effective in controlling agricultural insect pests such as tephritid fruit flies (Greany *et al*., 1977; Sivinski, 1990; Vargas *et al*., 1991), biting and non-biting muscid and tabanid flies (Mihok *et al*., 2006; Mihok & Carlson, 2007; Mohamed-Ahmed & Mihok, 2009), and the tsetse vector of African trypanosomiasis in sub-Saharan Africa (Vale, 1974; Laveissiere *et al*., 1987; Green, 1988; Kappmeier & Nevill, 1999; Lindh *et al*., 2009). Visual preference in mosquitoes has also been exploited in order to develop oviposition traps that appeal to gravid female *Aedes aegypti* (L.) mosquitoes seeking an optimal location to lay eggs (Fay & Eliason, 1966).

One potential strategy for the control of *Ae. polynesiensis* could be the deployment of a colour-based target impregnated with an insecticide, similar to targets deployed for the control of tsetse flies, and similar to insecticide-treated bednets that are extensively used to protect humans from anopheline mosquitoes and have proven effective in reducing malaria transmission in multiple locales throughout the world (Curtis *et al*., 2003; Lengeler, 2004; Sochantha *et al*., 2006).

A modification of this approach may also prove to be a valid intervention in reducing the transmission of LF by *Aedes* mosquitoes. In lieu of using insecticide-treated bednets, that would not provide protection against infective bites from the outdoor, day-biting *Ae. polynesiensis* mosquito, it is proposed that visual lethal targets (LT) be placed in strategic locations outdoors. These visual targets could be deployed in shaded locations near homes, outbuildings and other structures that *Ae. polynesiensis* mosquitoes might frequent while searching for bloodmeals, resting sites and/or oviposition sites. These targets would need to be placed outdoors as *Ae. polynesiensis* is an exophilic, day-biting mosquito that only rarely ventures into homes and similar structures (Jachowski, 1954).

In order to control *Aedes* mosquito populations using this strategy, it is essential that the target colour be attractive to *Ae. polynesiensis* mosquitoes. This study describes experiments designed to test this criteria; the attractiveness of six different colours of non-insecticide-treated targets for *Ae. polynesiensis* mosquitoes. Mosquito attractiveness was measured as a function of mosquito landing frequency and resting time. Research findings are discussed in the context of further lethal target development.

Materials and methods

Mosquitoes

Aedes polynesiensis Maupiti (APM) strain mosquitoes reared in the insectary at the Medical Entomology Unit of the Louis Malardé Institute, Tahiti were used for this study. Mosquitoes were reared in 21 × 21 × 7.5 cm disposable plastic pans (Pactive, Lake Forest, IL, U.S.A.). Larvae were fed 60 g/L liver powder suspension *ad libitum*. Mosquitoes were maintained in 30 × 30 × 30 cm BioQuip no. 1450B aluminum screened cages (BioQuip, Rancho Dominguez, CA, U.S.A.). Adult mosquitoes were provided with a constant supply of 10% sucrose solution. Mosquitoes used in this study were between 4 and 12 days old post-eclosion.

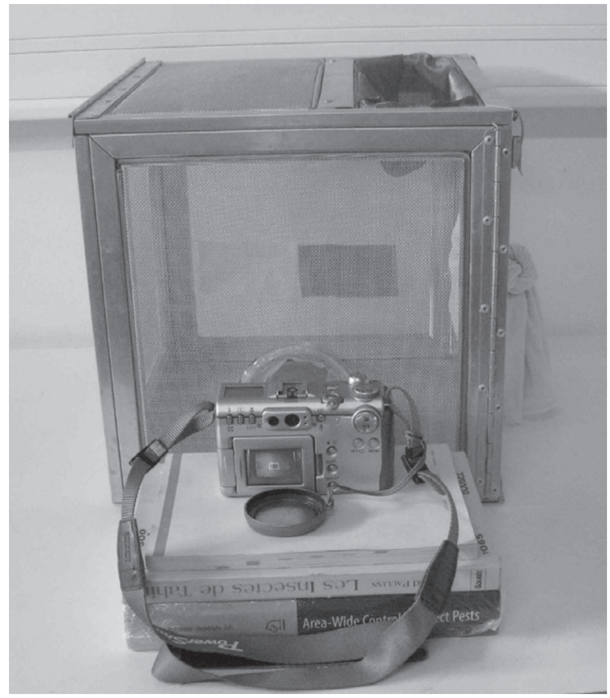

Fig. 1. Experimental assay chamber showing the camera system and the experimental and control targets side by side.

Attractiveness bioassay

Fifty host-seeking females were released into an aluminum 30 × 30 × 30 cm bioassay chamber (BioQuip), fitted with an 8-cm plastic Petri dish glued into an opening cut in the wall of the cage (Fig. 1). The Petri dish served as a window and allowed for images to be captured during the course of each trial.

Four solid colour targets representing a range of colours within the visual colour spectrum, black (320–400 nm), navy blue (445 nm), sky blue (475 nm) and red (650 nm), were evaluated in this study. These colours were selected based upon previous studies with *Aedes* spp. (Brett, 1938; Brown, 1954; Yap, 1975; Muir *et al*., 1992) which indicated that host-seeking females preferred colours in the lower range of the colour spectrum (320–500 nm) In addition, two patterned pareo cloth targets (black and white; red, blue and light blue) were also tested. The pareo patterns represented a form of Polynesian folk art and it was felt that these patterns would potentially be better accepted by the local population if

Fig. 2. Representative image of the broken pattern black and white traditional pareo design.

insecticide-treated targets were deployed in villages and towns. A 5.7 cm × 10.2 cm experimental target was compared with a solid white target of the same size in choice-test experiments to measure female mosquito target attractiveness. Only the black target was used to assess the landing behaviour of male mosquitoes. The white, black, navy blue and red targets were 100% cotton; the two patterned pareo targets were rayon (Fig. 2) and the sky blue target was a polyethylene tarpaulin.

Choice-test comparisons were made between a target of interest and a control (white) target. Targets were adhered to the wall opposite the Petri dish window to facilitate the image capture. Six replicates were performed for each target colour. A new target was used with each replicate. As a result of the complex colour pattern of the pareo targets, each replicate for an individual target varied so that different colour patterns within a given pareo were evaluated. Each replicate was performed with a new cohort of *Ae. polynesiensis* mosquitoes.

Image capture

Images were captured using a Canon PowerShot G3 digital camera (Canon, Tokyo, Japan). The intervalometer function was used with an interval time of 1 min. Photographic images were recorded for 80 min at 1-min intervals. The first 20 min after mosquito introduction into the assay chamber were considered an acclimatization period for the mosquitoes and were excluded from the final analysis.

Analysis of targets

All targets were analysed using ImageJ v. 1.43 software (Abramoff *et al*., 2004). A series of 60 images (i.e. 1 per min) was imported into the programme. The number of mosquitoes resting on the target for a given frame and the duration of the mosquito resting event were determined using the PLUGINS: MTRACKJ: Add tracks submenu. Beginning with frame one, an individual mosquito was visually identified and the mouse cursor was placed over the mosquito. The mouse was clicked at that position until the mosquito was no longer observed to be in that position. A mosquito was assumed to have moved from a given position once the body of the mosquito was no longer within the cross hairs of the mouse cursor image. Once all mosquitoes were identified and tracked, the Save tracks submenu was selected in order to save the position of each mosquito at every frame. The Measure tracks submenu was selected and the Display point measurements, Display track measurements, Display cluster measurements and Display assembly measurements were each selected.

Landing duration and frequency

For each 60-min recording period, the number of landings at each 1-min interval was determined for both the control (e.g. white) and experimental targets. The number of mosquitoes resting on each target was summed over the entire 60-min recording period and divided by 50 to determine the mean number of landings per mosquito for each target. The number of mosquitoes resting on the control target and the experimental target were compared with each other at each 1-min time point. The MTrackJ program determined the resting time of each mosquito landing on each of the targets. The contact time for each mosquito was summed for the entire 60-min test and divided by the number of landings to determine the mean resting duration per landing.

Statistical analysis

All data were tested for normal distribution using the Shapiro–Wilk test. The mean number of mosquitoes for each 1-min measurement resting on the experimental target was compared with the mean number of mosquitoes for each 1-min measurement resting on the control target. The paired Student's t-test ($P < 0.05$) was used to determine differences between the control target and the experimental target for normally distributed data. Differences between data that were not normally distributed were compared using Wilcoxon's signed rank test ($P < 0.05$). The Kruskal–Wallis test was used to determine if there were any differences between the six experimental targets. Wilcoxon's Rank sum test with Bonferroni's correction ($P < 0.008$) was used to make pairwise comparisons between each of the six experimental targets. The mean resting time for mosquitoes landing on the experimental targets was compared with those landing on the control targets using Wilcoxon's Rank sum test ($P < 0.05$). Differences between the mean resting time per mosquito landing between experimental targets were examined using the Kruskal–Wallis test. Statistical analyses were performed using JMP v. 8.0.1 (SAS Institute Inc., Cary, NC, U.S.A.).

Results

Mosquito landing frequency

The mean number [± standard error (SE)] of mosquito landings per minute for each of the six individual targets

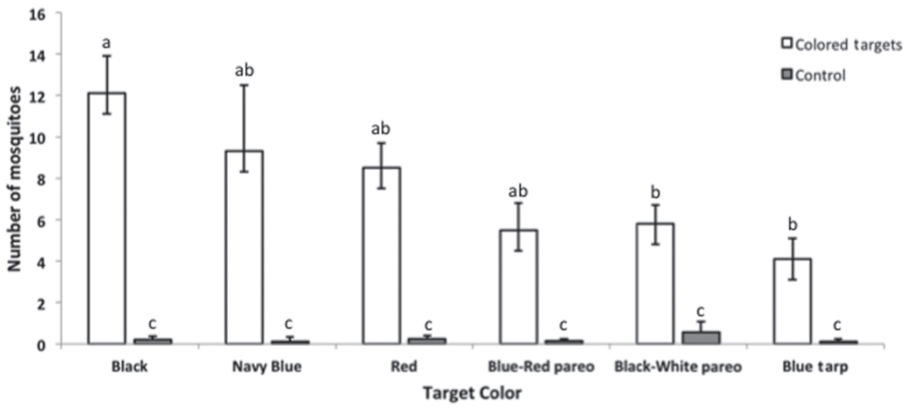

Fig. 3. The mean number of female *Aedes polynesiensis*/min resting on experimental and control targets. Mean are displayed with ± standard errors; $n = 6$ for all means. Targets with different superscripts are significantly different at the $P < 0.05$ level.

during a 60-min test period ranged from a high of 12.1 ± 1.85 mosquitoes/min for the black target to a low of 4.32 ± 0.87 mosquitoes/min for the blue polyethylene tarp target (Fig. 3). In the choice test comparisons, the mean landings/min for each experimental target was significantly greater than its corresponding white control (Wilcoxon's Rank Sum test; $P < 0.01$).

There was a significant difference in mean mosquito landings/min (Fig. 3) among the six experimental targets (Kruskal–Wallis test; $P < 0.05$). Pair-wise comparisons between each of the experimental targets found a significant difference between the black target and the black-white pareo target, and the black target and the sky blue polyethylene target (Wilcoxon's Rank Sum test; $P < 0.008$).

Table 1. Means ± S.E. of mosquito resting time per mosquito landing for female *Aedes polynesiensis* mosquitoes exposed to colored targets and white controls.

Treatment	Mean resting time/landing (min) Control	Target	N
Black	8.3 ± 5.2	13 ± 3.1	6
Navy blue	3.5 ± 1.7	10 ± 2.0*	6
Red	5.7 ± 3.3	9.7 ± 1.1	6
Black white pareo	8.1 ± 5.9	15.2 ± 4.2	6
Blue red pareo	1.7 ± 1.6	13.7 ± 4.4*	6
Blue tarp	8.0 ± 5.9	11.9 ± 3.2	6

*Experimental target is significantly different ($P < 0.05$) from control target.

Female landing duration

The mean ± SE of the resting time for mosquito landings by colour treatment ranged from a high of 15.2 ± 4.2 min/landing for mosquitoes landing on the Black-white pareo target to a low of 9.7 ± 1.1 min/landing for the red target (Table 1). The mean landing duration per mosquito landing was significantly higher for two of the experimental targets when compared with their white control. Pair-wise comparisons found a significant difference between the navy blue target and white control (Wilcoxon's Rank Sum test; $P < 0.05$), and the blue/red pareo target and white control (Wilcoxon's Rank Sum test; $P < 0.005$). There was no significant difference in the mean landing duration/mosquito landing among the six experimental targets (Kruskal–Wallis test; $P = 0.99$).

Female landing behaviour over time

The landing frequency behaviour of *Ae. polynesiensis* female mosquitoes for each of the six experimental targets was compared at six different time points (Fig. 4). There was no change in landing frequency for any of the six experimental targets ($P > 0.05$; Kruskal–Wallis test). The change in the number of mosquitoes resting on each of the six targets was also measured by comparing the number of mosquitoes resting on the target at 5 min to those resting on the target at 60 min (Fig. 4). There was no change in the number of resting mosquitoes for any of the six targets ($P > 0.05$; Wilcoxon's Rank Sum test).

Male landing behaviour

Male landing behaviour was evaluated using male *Ae. polynesiensis* mosquitoes and performing a choice comparison test with the black and white targets. The mean number (± SE) of mosquito landings per minute for male *Ae. polynesiensis* mosquitoes exposed to the black target was 3.2 ± 0.67 and there was a significant difference between the mean number of mosquito landings per minute on the black target when compared with the mean number of landings/min on the white control ($P < 0.005$; Wilcoxon's Rank Sum test). The mean duration landing time/mosquito landing of male mosquitoes landing on the black target was 8.1 ± 2.8 min/mosquito landing and the mean landing duration was significantly higher for mosquitoes landing on the black target than for those landing on the control target ($P < 0.05$; Wilcoxon's Rank Sum test).

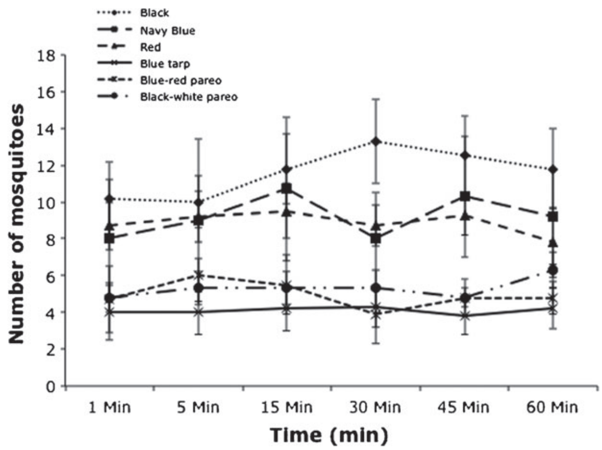

Fig. 4. Comparison of the mean number of female *Aedes polynesiensis* mosquitoes resting on each of the six experimental targets at six different time points: 1, 5, 15, 30, 45, 60 min. Means are displayed with ± standard errors; $n = 6$ for all means.

Female vs. male landings

There was a significant difference ($P < 0.005$; Student's t-test) between female (12.1 ± 1.8 landings/min) and male (3.2 ± 0.68 landings/min) mosquitoes. There was no difference ($P = 0.13$; Student's t-test) in landing duration/mosquito landing between female mosquitoes (13.0 ± 3.1 min/landing) and male mosquitoes (8.1 ± 2.8 min landing).

Discussion

This study is the first to evaluate the behavioural response of *Ae. polynesiensis* mosquitoes to visual cues using a colour choice preference assay. *Aedes polynesiensis* mosquitoes were allowed to choose between an experimental coloured target, a white control target or the remaining sides of the cage. In all the comparisons, female mosquitoes preferred each of the six experimental targets to the white control target. Furthermore, female *Ae. polynesiensis* mosquitoes exhibited a marked preference for the solid, darker, low-reflectance colours that were tested (black, navy blue and red) rather than the broken pattern pareo targets. The variations in reflectance exhibited by these broken pattern targets may be play a role in this finding. Although this study does not address the potential bias that may exist as a result of mosquitoes that are active responders (i.e. mosquitoes that land on the target, leave the target and then return to the target), it should be noted that the mean mosquito resting time per landing was greater than 10 min per landing in five of the six targets tested. This would indicate that the same individual mosquito was not being counted repeatedly during each assay replicate.

Male *Ae. polynesiensis* mosquitoes also exhibited a marked preference for the black target over the white control target but not to the degree that female mosquitoes exhibited. The landing duration of each mosquito that landed on a target was determined and no difference in landing duration between targets was observed.

The response of several species of mosquitoes to visual cues has been investigated in efforts to develop visual-based control strategies. The mosquito *Ae. aegypti* has been shown to exhibit a preference for dark, low reflectance colours (Brett, 1938; Muir *et al*., 1992) as have species of *Aedes* mosquitoes from Canada, including *Aedes punctor* (Kirby) (Brown, 1954). *Culex quinquefasciatus* Say was shown to prefer black and brown coloured targets in the laboratory setting (Wen *et al*., 1997), whereas in field settings it was demonstrated that *Aedes albopictus* (Skuse) is highly attracted to black and red oviposition traps (Yap, 1975). This phenomenon of mosquito colour preference has led to the development of oviposition traps, resting traps and other adult traps (e.g. BG-Sentinel-BGS) that are based wholly or in part on mosquito colour preference (Fay & Eliason, 1966; Edman *et al*., 1997; Williams *et al*., 2006; Ball & Ritchie, 2010).

This study describes initial efforts to characterize colour preference in the mosquito *Ae. polynesiensis* in order to identify colours that could be useful in the development of insecticide-impregnated lethal targets. Although previous work indicated that *Ae. polynesiensis* responded in a positive fashion to both sticky ovitraps and BGS adult traps, both of these trapping methods relied on olfactory signals as well as visual cues (Russell & Ritchie, 2004; Schmaedick *et al*., 2008; Chambers *et al*., 2009). In this study, visual cues were measured in the absence of olfactory signals using a novel small cage assay design.

In addition to the novel design, this study also employed a novel method for determining and tracking mosquito landings and movement on both the experimental and control targets. ImageJ is a public domain java-image processing program that is freely downloadable. It allows the user to easily edit, analyze and process digital images in a variety of formats. Because the software supports the use of 'stacks' (a series of images that share a single window), it allows the user to easily align the images and track the images through space and time. In medical entomology, it has been applied to determine the number of eggs oviposited in a container (Mains *et al*., 2008), to estimate the number of oocysts developing in a mosquito (Delves & Sinden, 2010) and to quantify the flight movement of mosquitoes in a container (Hoffmann *et al*., 2010). In this present study, the method allowed for the fast and accurate identification of multiple resting individuals on a small target.

In order to develop insecticide-impregnated lethal targets that might impact disease-transmitting mosquitoes, it is vital that the target be attractive to the arthropod vector. In this study, we demonstrated that *Ae. polynesiensis* mosquitoes, the primary vector of LF in the South Pacific, exhibit a marked preference for solid, dark colours in the lower range of the visual colour spectrum (320–450 nm). This study, although preliminary in nature, served as a model assay for detecting behavioural differences in mosquitoes and is useful in guiding the selection of colours for more laborious semi-field trials. Future studies will also need to focus on the response of *Ae. polynesiensis* mosquitoes to these targets when they have been treated with insecticide. It will be critical that laboratory-based studies focus on mosquito landing behaviour as well as on insecticide toxicity for the target organism. The implications of this work focusing on *Ae. polynesiensis* colour preference could be translated to alternate methods of colour preference-based vector control. Such an alternative strategy was successfully employed in Peru where *Aedes aegypti* (L.) mosquitoes disseminated the juvenile hormone analogue pyriproxyfen to oviposition sites after resting in buckets lined with a dark cloth that had been treated with the compound (Devine *et al*., 2009). Additional studies at the semi-field level will also need to be conducted in order to determine if mosquitoes are (a) still attracted to and (b) impacted by the targets when impregnated with insecticide.

Finally, although the lethal target strategy outlined in this study is based upon mosquito colour preferences, there exists the potential for coupling such an approach with targets/traps utilizing olfactory cues. Recent work has indicated that *Ae. aegypti* mosquitoes exhibit a marked preference for traps baited with synthetic compounds that mimic a volatile chemical emitted by human beings (Silva *et al*., 2005; Krockel *et al*., 2006; Bernier *et al*., 2007; Okumu *et al*., 2010). Furthermore, there is a potential for lethal targets to be coupled with attractive sugar baits (ASB). *Aedes albopictus* mosquitoes have shown differential attraction to sugar compounds produced by a variety of flowers and fruits (Muller *et al*., 2011) and these compounds when mixed with boric acid have proven toxic to both *A. albopictus* and *C. quinquefasciatus* (Muller *et al*., 2010; Xue *et al*., 2011).

Acknowledgements

We thank Albert Tetuanui, Tuterarii Paoaafaite and Michel Germain for their technical support. This study was supported by a Bill and Melinda Gates Foundation grant BMGF no. 44190.

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Accepted 22 August 2012
First published online 22 January 2013