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Towards a fuller understanding of mosquito behaviour: use of electrocuting grids to compare the odour-orientated responses of *Anopheles arabiensis* and *An. quadriannulatus* in the field

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Abstract. The epidemiological role of and control options for any mosquito species depend on its degree of 'anthropophily'. However, the behavioural basis of this term is poorly understood. Accordingly, studies in Zimbabwe quantified the effects of natural odours from cattle and humans, and synthetic components of these odours, on the attraction, entry and landing responses of *Anopheles arabiensis* Giles (Diptera: Culicidae) and *Anopheles quadriannulatus* Theobald. The numbers of mosquitoes attracted to human or cattle odour were compared using electrocuting nets (E-nets), and entry responses were gauged by the catch from an odour-baited entry trap (OBET) relative to that from an odour-baited E-net. Landing responses were estimated by comparing the catches from E-nets and cloth targets covered with an electrocuting grid. For *An. arabiensis*, E-nets baited with odour from a single ox or a single man caught similar numbers, and increasing the dose of human odour from one to three men increased the catch four-fold. For *An. quadriannulatus*, catches from E-nets increased up to six-fold in the progression: man, three men, ox, and man + ox, with catch being correlated with bait mass. Entry responses of *An. arabiensis* were stronger with human odour (entry response 62%) than with ox odour (6%) or a mixture of cattle and human odours (15%). For *An. quadriannulatus*, the entry response was low ($< 2\%$) with both cattle and human odour. *Anopheles arabiensis* did not exhibit a strong entry response to carbon dioxide (CO_2) (0.2–2 L/min). The trends observed using OBETs and E-nets also applied to mosquitoes approaching and entering a hut. Catches from an electrocuting target baited with either CO_2 or a blend of acetone, 1-octen-3-ol, 4-methylphenol and 3-n-propylphenol – components of natural ox odour – showed that virtually all mosquitoes arriving there alighted on it. The propensity of *An. arabiensis* to enter human habitation seemed to be mediated by odours other than CO_2 alone. Characterizing 'anthropophily' by comparing the numbers of mosquitoes caught by traps baited with different host odours can lead to spurious conclusions; OBETs baited with human odour caught around two to four times more *An. arabiensis* than cattle-baited OBETs, whereas a human-baited E-net caught less (~ 0.7) *An. arabiensis* than a cattle-baited E-net. Similar caution is warranted for other species of mosquito vectors. A fuller understanding of how to exploit mosquito behaviour for control and surveys requires wider approaches and more use of appropriate tools.

Key words. *Anopheles arabiensis*, *Anopheles quadriannulatus*, anthropophilic, electric nets, feeding behaviour, host odours, malaria vector, zoophilic, Zimbabwe.

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Introduction

Anthropophily in mosquitoes is considered to include taking a preponderance of bloodmeals from humans (anthropophagy), and the tendency to feed indoors (endophagy) and rest indoors (endophily). Vector-competent species of mosquito exhibiting this set of characteristics are likely to have high vectorial capacities (Garrett-Jones, 1964). For instance, in Africa *Anopheles gambiae* Giles *sensu stricto*, considered strongly anthropophagic, endophagic and endophilic, is the main vector of malaria, whereas the morphologically identical sibling species *Anopheles quadriannulatus* Theobald is strongly zoophagic, exophagic and exophilic and plays no significant epidemiological role in malaria.

Characterizing the behavioural ecology of vectors not only clarifies epidemiology but also may guide control strategies. For instance, strongly anthropophilic species are likely to be susceptible to measures such as indoor spraying of insecticides and the use of insecticide-treated bednets, whereas zoophilic species are likely to be more susceptible to insecticide-treated cattle (Rowland *et al.*, 2001).

Although the concept of anthropophily affects much of our thinking about the epidemiology and control of vector-borne diseases of humans, its application can be problematic because anthropophily is not an irreducible quality but, rather, the manifestation of a complex of behavioural repertoires relating to host location, feeding and resting. Each of these, in turn, is composed of a chain of single responses to distinctive stimuli. Thus, a given mosquito feeding on a particular host species, for instance, is the result of: (a) olfactory responses such as activation, anemotaxis, kineses and enhanced alighting on hosts; (b) visual responses to host colour, shape and movement, and (c) direct insect–host interactions as the vector bites and the host defends itself (Gibson & Torr, 1999). Kennedy (1978) highlighted the problems created by designating semiochemicals by their apparent end result (e.g. 'attractant', 'arrestant') rather than analysing the multiple stimulus–response relationships that lead to this result. The confusions associated with blanket teleological labels are even greater with terms such as 'anthropophily' as it encompasses so many disparate categories of behaviour.

It is usual to characterize 'anthropophily' and 'endophily' by gross phenomena, such as the contribution of humans to diet (Garrett-Jones, 1964), the relative catches from human- and cattle-baited traps (Costantini *et al.*, 1998a; Dekker & Takken, 1998; Trung *et al.*, 2005; Tirados *et al.*, 2006) and the numbers of mosquitoes resting or biting indoors vs. outdoors (Service, 1993). This basis for identifying important vectors and appropriate control strategies may be adequate for strongly anthropophilic vectors such as *An. gambiae s.s.* However, for species, such as *Anopheles arabiensis* Patton, that feed on a wider range of hosts, the results are ambiguous. Analysis of their bloodmeals is hampered by the inability to sample with equal efficiency from a vector's various resting sites, and the responses that result in a mosquito being caught in a trap, for instance, are not all the same as those that culminate in it biting a host. Hence, host odours that increase or decrease trap catches may mean little about ultimate host preference unless we know exactly why the odour changes the catch. Experience with tsetse illustrates the problem: 2-methoxyphenol and short-chain fatty acids found in ox odour reduce the catch in traps by ~ 90% but do not alter the numbers feeding on cattle (Torr *et al.*, 1996).

Costantini *et al.* (1993) used an odour-baited entry trap (OBET) to study mosquito entry into a hut. An index of anthropophily/zoophily was then developed by comparing the catches of two OBETs placed side by side, one offering human odour from its entrance, and the other offering ox odour (Costantini *et al.*, 1998a). The results were in general accord with the expectations that anthropophilic and zoophilic populations would be biased towards the human- and cattle-baited traps, respectively (Costantini *et al.*, 1998a; Duchemin *et al.*, 2001; Tirados *et al.*, 2006). However, to interpret these results we need to know how the insects behave *before* entering the OBET or huts. For instance, how many insects travel up the plumes of host odour? How does host odour affect the trap entry response? To address these questions we need tools more appropriate than traps alone; the devices used for tsetse (Diptera: Glossinidae) could be useful.

Studies with tsetse made extensive use of video (Gibson & Brady, 1985) and electrocuting nets (E-nets) (Vale, 1974a) to measure individual responses such as kineses (Gibson & Brady, 1988), the direction (Vale, 1974b; Gibson & Brady, 1985), velocity (Gibson & Brady, 1985) and altitude (Torr, 1988) of flight, and landing responses (Green, 1986). Despite the paucity of proven tools for analysing the field behaviour of mosquitoes, the methods employed successfully for tsetse have not been widely used against mosquitoes, for several reasons. For example, unequivocally identifying a mosquito species using video alone is difficult, if not impossible, especially when a species complex such as *An. gambiae s.l.* is being observed. Moreover, E-nets were designed primarily to catch relatively large, fast, day-active tsetse, so there is concern that the devices may not be effective for mosquitoes (Knols *et al.*, 1998).

The present work began with an assessment of the efficacy of electrocuting devices and then applied them to analyse the responses that culminate in the appearance of mosquitoes in traps and huts.

Materials and methods

Study area

From December 2005 to April 2006, and in February and March 2007, work was performed in and among the buildings of Rekomitjie Tsetse Research Station (16°08′ S, 29°24′ E; altitude 500 m a.s.l.) occupying 0.3 km^2 of partially cleared woodland in the Mana Pools Game Reserve of Zimbabwe. About 50 people live on the station and a herd of some 25 cattle graze within 1 km and are kraaled overnight 180 m from the nearest human dwelling.

Odours

Natural odours were obtained from an ox, one man or three men in a tent of plastic-coated canvas, 2.0 m high × 2.0 m wide × 2.5 m long. Various individual hosts were taken from a pool of nine indigenous cattle averaging 257 kg (range 225–305 kg) and 24 men averaging 63 kg (52–75 kg). A co-axial fan (12 v, 0.38 amp, maximum airflow ~ 2000 L/min) exhausted odour-laden air from the tent down a plastic tube, 10 cm in diameter, to a net-covered outlet 20 m away where various catching devices were placed. To compare different doses of ox odour, an ox that was about 10% heavier than the average was placed in a tent from which the effluent was split two ways: one-eleventh went to an adjacent empty tent, was mixed with the standard air-flow there and then sent to the catching site to be regarded as 10% ox odour. The remaining 10-elevenths (i.e. 10 times as much) went to the catching site as 100% ox odour. Mixtures of ox and man odours were created by three methods. Firstly, the convergence method piped the odours separately to the catching site where the side-by-side outlet pipes were aimed so that the odour streams converged at ~ 30 cm downwind (i.e. 20 cm upwind of the catching device). Secondly, the junction method used a shutter to halve the flow from each tent and the separate tubes were joined before they reached the single outlet. Thirdly, a less powerful (12 v, 0.19 amp) fan sent air through a tube, 40 cm long and 10 cm in diameter, from the man tent to the ox tent; the mixed odour from the ox tent was then taken by the normal tube to the outlet.

Artificial odours consisted of: (a) carbon dioxide (CO_2) from a pressurized cylinder, and (b) the attractants, termed AOP, commonly used for tsetse in Zimbabwe (acetone at 100 mg/h and a 1: 4: 8 mixture of 3*n*-propylphenol: 1-octen-3-ol: 4-methylphenol dispensed at a combined rate of ~ 1 mg/h following the methods of Torr *et al.* [1997]). Carbon dioxide dispensed at 0.2 L/min and 2 L/min simulated the doses from man and ox, respectively.

On nights of unsettled weather the wind was variable in direction and gusted up to several metres per second, as measured by a hot-wire anemometer. However, on most nights, when the air was relatively calm, there was a catabatic drift of ~ 0.2 m/s to the east or slightly northeast (i.e. down the mainly gradual slope to the Rekomitjie River). In any event, all outlets for piped odour pointed east, ensuring that the predominant downwind direction at and near the outlet was east.

Catching devices

Odour-baited entry trap. Following Costantini *et al.* (1993), the OBET consisted of a cage 60 cm long × 40 cm wide × 40 cm high, covered in clear polythene, with odour from the tent delivered at ~ 0.5 m/s through an insect entry port 19 cm in diameter and 10 cm deep. Two adjoining OBETs were commonly used, set side by side, with the trap entrance facing downwind, and mounted 60 cm above the ground on metal struts (Fig. 1A). Usually one OBET was used for ox odour alone and the other for human odour alone, with separate but adjacent tents housing the odour sources. The positions of the ox-baited and human-baited cages were alternated between the left and right (north and south) positions from one night to the next.

Electric nets. Unless indicated otherwise, the E-nets used to catch insects in flight were 1 m high and 50 cm wide, of the type developed for tsetse (Vale, 1974a) (Fig. 1B). Each device consisted of a sheet of black polyester net (quality no. 188; Swisstulle UK plc, Nottingham, U.K.) sandwiched between two banks of vertical

Fig. 1. Examples of odour-baited entry traps (OBETs) and electric nets (E-nets) in use in the field. (A) Paired OBETs connected to tents containing different baits. (B) E-net placed downwind of an outlet from a tent with the hopper raised out of its trench to show the sump. (C) Close-up of small E-nets placed 50 cm downwind of paired OBETs to assess the flight direction of mosquitoes.

copper wires, 0.2 mm in diameter and 8 mm apart, placed 8 mm from each side of the net. The fine net is intended to be invisible to flying insects, but prevents them from flying straight through the grid. Alternate wires in each bank were earthed or charged by a transformer with a DC input of 12 v/3 amps and an output of ~ 50 kV pulsing at ~ 70 Hz. Insects killed or stunned after colliding with the grids fell to a hopper, usually extending 44 cm from each side of the net, and slid down to a sump with soapy water (Fig. 2A). A sheet-metal roof was provided to protect the grids from rain and prevent the sumps from flooding.

E-nets were usually the sole catching device at any one site, and were at ground level, held upright, with the length of the grid perpendicular to the prevailing wind; the catch in the downwind hopper comprised mosquitoes flying upwind and vice versa. When used on their own, E-nets were placed 50 cm downwind of the net-covered outlets which evacuated odour-laden air from the tents. However, an E-net was also used to cover the window of an experimental hut (Fig. 2D) and small E-nets, 22 × 30 cm, were sometimes used to sample insects near the entrance of each of the paired OBETs (Figs 1C, 2E). Insects caught by the small E-nets fell into water-filled trays.

For experiments comparing an OBET and E-net used separately, the E-net was always roofed (Fig. 2A), so a similar roof was provided for the OBET. To ensure that the OBET was well clear of the roof, it was mounted 15 cm off the ground (i.e. 45 cm lower than usual and just above the level of the grass).

Electric targets. The fine net between the electric grids of an E-net was replaced by a solid piece of cloth measuring 1 × 1 m, intended to serve as a visual target. The electric target (E-target) caught insects as they landed on the cloth. The sheet of cotton cloth consisted of two vertical panels, one black and one Phthalogen blue, each 50 cm wide. These two colours were chosen to test the effect of colour on mosquito landing rates because they are known to affect the landing behaviour of a wide range of biting Diptera (Gibson & Torr, 1999). The panels were alternated between the left and right positions from night to night. The target was protected from rain by a gently sloping roof of corrugated iron, measuring 3.5 × 5.5 m, at 2.4 m above the ground. The hopper for the E-target, and for the 1-m-wide E-net that sometimes replaced it, extended 50 cm from the grids. Electric targets were also placed upright, with the length of the target perpendicular to the prevailing wind direction, and with the outlet of the odour pipe at its base, as for E-nets.

Light trap. A miniature CDC (Centers for Disease Control, U.S.A.) light trap (model 512; J.W. Hock Inc., Gainesville, FL, U.S.A.) was baited with human or ox odour. The trap was suspended with its incandescent bulb ~ 1 m above the ground. Unless stated otherwise, the bulb was illuminated. A modified light trap (mCDC; Fig. 2C) was used to assess whether the crackles, flashes, ozone and electric field produced by E-nets and E-targets affected mosquito behaviour. This modified trap produced particularly intense sparks at points either side of the entrance; insects were not killed by these sparks because access closer than 3 cm was prevented by fine netting.

Sticky nets. Sticky nets were similar to E-nets except that rather than using banks of wires to catch insects, the net was treated with a thin layer of Temocid® (Kollant SpA, Vigonovo, Italy), an adhesive that retains insects as they make contact with it. The net was cleaned of insects daily and replaced weekly. When used on their own, sticky nets were placed 50 cm downwind of the net-covered outlets that evacuated odour-laden air from the tents.

Experimental hut. Studies were made of the numbers of mosquitoes entering a brick hut (2.4 m wide × 3.3 m long × 2.7 m high) with a metal roof and a single window, 115 cm high × 45 cm wide, placed 1 m above the outside ground. The window was the only entry and exit point because the small gaps around the door were sealed. An ox, or a man protected by a bednet, was placed in the hut and the window was fitted with either an E-net to catch mosquitoes immediately outside, or an exit trap (Fig. 2D). The E-net caught mosquitoes entering the hut through the window. The exit trap allowed mosquitoes to enter the hut through a netting entrance cone covering the top half of the window, and caught mosquitoes in a cage as they exited the hut via a slit 43 cm long × 2 cm high in the bottom half of the window. Mosquitoes that did not leave by morning were killed by non-persistent pyrethroid aerosol and were collected from a sheet on the floor. Hence, the total catches of the cage and the spraying represented the total number entering the hut.

Species identification

Mosquitoes were first identified morphologically (Gillies & De Meillon, 1968; Gillies & Coetzee, 1987). All *An. gambiae s.l.* were stored in plastic tubes (1.5 mL), containing silica-gel desiccant under cotton wool, and a sub-sample of 1267 specimens was sent to the University of Rome for species identification according to Scott *et al.* (1993). This is performed by dipping a single wing directly into the polymerase chain reaction (PCR) mix in order to avoid the DNA-extraction step. Although several anopheline and culicine species were collected during the trials, results in the following sections refer only to mosquitoes of the *An. gambiae s.l.* complex, unless specified otherwise.

Experimental design and analysis

Experiments were conducted between 21.00 hours and 05.00 hours at sites 10–25 m from a dwelling and, unless stated otherwise, about 100 m from one another. Most experiments followed a randomized Latin square design of treatments × sites × nights. However, for experiments with the experimental hut, a randomized block design was used. Groups of consecutive nights were regarded as different blocks and treatments were allocated randomly to nights within these blocks. To assess the significance of differences in catches from devices operated at separate sites, nightly catches (n) were transformed to $\log_{10}(n+1)$ for ANOVA (analysis of variance). Mean transformed catches were detransformed for reporting. For experiments where two devices were operated simultaneously at the same site (e.g. paired OBETs), the catches were subjected to logistic regression using GLIM4 (Francis *et al.*, 1993); the

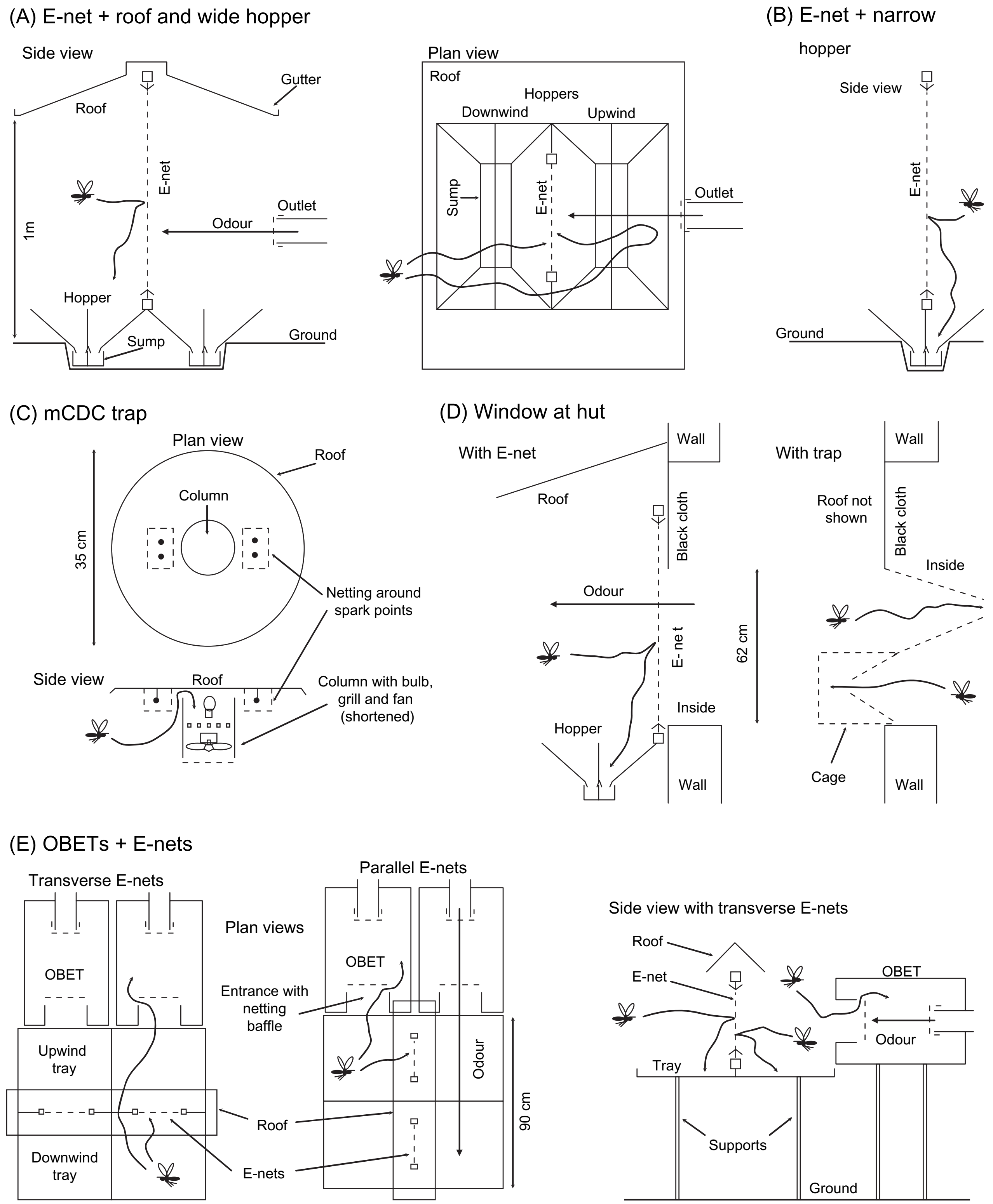

Fig. 2. Arrangements of electric nets (E-nets) used to capture mosquitoes in the vicinity of (A, B) odour sources, (C) a CDC trap, (D) the window of a hut, or (E) a pair of odour-baited entry traps (OBETs).

pooled nightly catch from both devices was the binomial denominator and the significance of changes in deviance was assessed by either a chi-square test or, if the data were overdispersed, an *F*-test following re-scaling (Crawley, 1993). Unless stated otherwise, the term 'significant' implies $P < 0.05$; means are accompanied by their standard errors.

Overview of experiments

The present study comprised three series of experiments. The first was concerned primarily with validating tools to analyse the behaviour of mosquitoes in the field. After this, and as the main body of the study, the tools were used to analyse the responses of mosquitoes to host odours, in particular, the numbers: (a) attracted to, and (b) entering either OBETs or the hut baited with odours from humans and/or cattle. The final experiments assessed the effects of odour on landing responses.

Validation of sampling devices. The numbers of mosquitoes caught by an OBET, CDC trap, sticky net or an E-net, each baited with ox odour, were compared (Experiment 1). After this, the catch from CDC traps operated outdoors with or without a light bulb and/or ox odour (Experiments 2 and 3) were compared to quantify the relative importance of visual and olfactory stimuli in the performance of the CDC trap. The effect of the physical and chemical stimuli (e.g. sparks, ozone) produced by E-nets on mosquito behaviour was assessed by comparing the catch from a modified CDC trap (mCDC) with or without a small E-net attached (Experiment 4). Finally, the effect of varying the distance between wires (4 mm, 6 mm or 8 mm) (Experiment 5) or adding a roof (Experiment 6) on the catch from an odour-baited E-net was assessed.

Flight and entry responses of mosquitoes to host odours. Experiments were conducted to compare the catches from OBETs (Experiments 7 and 8) or E-nets (Experiments 9 and 10) baited with ox and/or human odour. E-nets caught mosquitoes flying in the vicinity of a given odour source and hence provided a relative measure of the numbers of mosquitoes attracted to different odours. The OBET caught mosquitoes that were attracted to *and* entered the trap and hence direct comparisons of the catches from OBETs or E-nets baited with matching odours (Experiments 11–13) provided estimates of the relative proportions that enter an OBET. Complementary experiments, with E-nets placed downwind of the OBETs, were used to analyse, in greater detail, the flight behaviour of mosquitoes in the vicinity of OBETs baited with different odours. The series concluded with experiments designed to confirm that the trends observed with OBETs also apply to the more natural situation of hosts in huts. In one experiment (Experiment 14), the numbers of mosquitoes caught in a hut containing a human, or an ox, or CO_2 dispensed at the rates produced by these hosts, were compared. In a second experiment (Experiment 15), the relative numbers of mosquitoes approaching or entering a hut containing a human or an ox were assessed.

Alighting responses. The final alighting responses of mosquitoes were measured by comparing the catches from E-targets baited with or without AOP and/or CO_2 (Experiments 16 and 17).

Anopheles arabiensis *vs.* An. quadriannulatus

Only sub-samples of the catches of *An. gambiae s.l.* were identified to species level, and in only some experiments. Hence, the actual numbers of *An. arabiensis* and *An. quadriannulatus* caught were not known precisely and so could not be subject to separate statistical analyses. Accordingly, we report first the statistics for *An. gambiae s.l.* as a whole and then present the identifications to assess the extent to which the overall indications apply to each species in the complex.

Indices of anthropophily

Comparisons of catches from human- and animal-baited traps have been widely used to provide indices of anthropophily for various species of mosquito (Dekker & Takken, 1998; Costantini *et al.*, 1998a; Mboera & Takken, 1999; Trung *et al.*, 2005; Lardeux *et al.*, 2007). The present study provided an opportunity to assess the validity of this general approach using various capture protocols: (a) paired OBETs; (b) OBETs operated singly; (c) E-nets operated singly; (d) an E-net placed at the window of a hut to sample mosquitoes entering, and (e) collections from within a hut. The catch from the human-baited device was expressed as a proportion of the total catch from both devices. For example, if the human-baited device catches, say, 75 mosquitoes/night compared with 25 mosquitoes/night by a cattle-baited device, then the index of anthropophily is 0.75.

Results

Validation of sampling devices

The ox odour-baited CDC trap and the E-net caught > 40 times more mosquitoes than the OBET and the sticky net (Table 1, Experiment 1). As demonstrated in previous studies (Costantini *et al.*, 1998b; Mboera *et al.*, 2000), the CDC trap requires both visual (the light bulb; Table 1, Experiment 2) – and olfactory cues (Table 1, Experiment 3) to catch the numbers of mosquitoes caught by E-nets with odours alone. These preliminary results suggested that the E-net was likely to be the most effective tool for gauging the numbers of mosquitoes attracted to an odour source. Accordingly, several experiments were performed to assess further the performance of electrocuting systems.

The observed catches of the mCDC trap with sparks were about half of those without (Table 1, Experiment 4). Although this effect was not significant ($P > 0.1$), it raised the suspicion that the particularly fierce sparking at the mCDC trap interfered with the responses of some mosquitoes, and therefore that some interference might result from the more moderate sparking at standard grids. Comparison of E-nets with grid spacings of 4 mm, 6 mm or 8 mm (Table 1, Experiment 5) suggested that the 8-mm spacing was no worse than the narrower spacings. The roof, normally present to protect the E-net from rain, had no significant effect on catch during dry weather (Table 1, Experiment 6).

The hopper used for the E-net in Experiment 1 (Table 1) extended only 22 cm from the grid, whereas for all other experiments the hoppers extended 44 cm. Pooling the total catch

Table 1. Comparison of capture devices. Total and detransformed mean catches (transformed means in brackets) of *An. gambiae s.l.* in six separate experiments to compare catching devices used with various odours. For each experiment, means not associated with the same letter differ at $P < 0.05$.

Expt	Device	Odour	Total	Nightly mean	Transformed SE, & *P* by *F*-test	Replicates
1	OBET	Ox	35	1.7 (0.42)a		12 (Jan–Feb)
	CDC trap		1205	58.0 (1.77)b		
	Sticky net		31	0.9 (0.28)a		
	E-net, 8 mm grid[1]		1552	79.1 (1.90)b		
					0.135 <0.001	
2	CDC trap, + bulb	Ox	1076	101.6 (2.01)a		6 (Feb–Mar)
	CDC trap, – bulb		111	10.5 (1.06)b		
					0.133 <0.01	
3	CDC trap, + bulb	None	2	0.3 (0.10)a		6 (Feb–Mar)
	CDC trap, – bulb		3	0.3 (0.10)a		
					0.095 >0.1	
4	mCDC trap, + sparks	None	142	16.6 (1.25)a		6 (Mar)
	mCDC trap, – sparks		341	40.8 (1.62)a		
					0.137 >0.1	
5	E-net, 8 mm grid	Ox	752	39.6 (1.61)a		12 (Mar–Apr)
	E-net, 6 mm grid		568	35.6 (1.56)a		
	E-net, 4 mm grid		394	26.8 (1.45)a		
					0.063 >0.1	
6	E-net +roof	3 men	430	63.7 (1.81)a		6 (Mar–Apr)
	E-net –roof		380	57.5 (1.77)a		
					0.079 >0.1	

[1]Hopper extended 22 cm from E-net. Hoppers with all E-nets in all other experiments of all tables extended 44 cm.

of mosquitoes (Anophelines and Culicines) from the 112 replicates with wider hoppers showed that 9% (753/8688) of the upwind catch and 22% (1124/5179) of the downwind catch were caught 22–44 cm from the grid. Presumably the higher percentage on the downwind side was caused by mosquitoes being dispersed by the breeze. Judging from trajectories of mosquitoes dislodged after being stuck on the grids, it seems that hoppers that extend further than 44 cm would be needed to collect all of the catch. In any event, the catches in the narrow hoppers in Experiment 1 (Table 1) should be increased by 16% for comparison with catches in the wider hoppers. The results in Table 1 suggested that the standard E-net fitted with a roof and a wide hopper was the most effective and reliable means of catching mosquitoes in the vicinity of odour sources.

Flight and entry responses of mosquitoes to host odours

OBETs baited with host odours. When the human- and ox-baited OBETs were operated alone (Table 2, Experiment 7), the ox-baited OBET caught significantly more mosquitoes than the human-baited trap. Surprisingly, the catch from a single OBET baited with a mixture of these odours was significantly lower than the combined catch from human- and ox-baited OBETs operated side-by-side (i.e. when the odours became mixed only at some distance downwind). For the latter treatment, the total catch from the human-baited OBET was about twice that from the ox-baited OBET. The bias towards the human-baited OBET of the human-and-ox pair was even more marked when the dose of human odour was increased (Table 2, Experiment 8); the human-baited OBET gave 91.4% (± 1.4) of the total catch when three men were used, compared with 76.9% (± 2.5) with one man ($P < 0.01$ for difference between means).

E-nets baited with host odours. The above results from the OBETs (Table 2) provided conflicting indications of the relative response of mosquitoes to human and cattle odour, depending on whether the odours were separate or mixed. To explain these peculiar results, it was necessary to establish first how many mosquitoes arrived at the various odour sources, as distinct from entering an OBET. The numbers arriving were assessed by using the standard E-net with the odours.

An unbaited E-net caught few mosquitoes; one-man odour caught only a third of the catch with ox odour, and the addition of one-man odour to ox odour, by the convergence method, produced no significant increase in catches (Table 3, Experiment 9). The relatively low catch with human odour seemed to be related to the mass of the bait since increasing the human bait from one to three men tripled the catch (Table 3, Experiment 10).

In some ways the catches with the E-net (Table 3) make simple sense when compared with the OBET catches (Table 2): with both devices, the catches with three men and with the ox alone were greater than with one man alone. However, although the OBET catch with the mixture of human and ox odours was relatively poor, the E-net caught many with this mixture. Hence, it seemed that the peculiar results of the OBET experiments (Table 2, Experiment 7) might reflect the various odours having markedly distinctive effects on the entry responses (i.e. the percentage of arriving flies that enter the OBET).

Comparison of OBETs and E-nets baited with host odours. The effect of human and ox odour on the OBET-entering response was assessed by comparing the catches from OBETs and E-nets used separately and baited with the odours from humans only or humans + cattle. It is assumed that catches from the E-net and OBET represented roughly the number of insects: (a) arriving near an odour source, and (b) the proportion that subsequently entered the trap, respectively. The three experiments (Table 4) compared the response to odour from three men (Experiment 11), one man (Experiment 12) and man + ox mixed (Experiment 13). These data can be augmented by the data for the OBET and E-net catches with ox odour in Experiment 1 (Table 1), provided the catches of the E-net in that experiment are enhanced to correct for the narrow hopper then used. The detransformed mean catches from the OBET expressed as a proportion of that from the E-net represent the strength of the entry response, which was highest with one-man odour (4.6/20.8 = 0.22; Experiment 12), lower with three-man odour (6.8/64.6 = 0.11; Experiment 11), and very low with ox odour (1.7/79.1 = 0.02; Experiment 1) or a mixture of human and cattle odours (0.5/41.8 = 0.01; Experiment 13).

Trap entry responses: OBETs operated in conjunction with E-nets. A more convenient way of assessing the strength of trap entry is to place an E-net just downwind of the trap entrance and compare the numbers of mosquitoes arriving at the OBET with the numbers caught inside, as a function of host odour type (ox, 10% ox, human, CO_2 at 2 L/min or 0.2 L/min). Adjacent OBETs were each baited with a specific odour or odour combination, and a small E-net was placed 50 cm

Table 2. Comparison of catches from OBETs baited with human and ox odour dispensed alone or together. Total and detransformed mean catches (transformed means in brackets) of *An. gambiae s.l.* in two separate experiments using various odours at a single OBET or a pair of adjacent OBETs. For each experiment, means not associated with the same letter differ at $P < 0.05$.

Expt	Odour	Cage[1]	Total	Nightly mean	Transformed SE, & *P* by *F*-test	Replicates
7	Man and ox, adjacent	Man	476			12 (Dec–Mar)
		Ox	253			
		Total	729	48.2 (1.69)a		
	Man alone		197	12.5 (1.13)b		
	Ox alone		393	19.0 (1.30)c		
	Man+ox, mixed		283	14.9 (1.20)bc		
					0.086	
					<0.001	
8	Man and ox, adjacent	Man	403			6 (Jan–Mar)
		Ox	121			
		Total	524	62.2 (1.80)a		
	Three men and ox, adjacent	Men	762			
		Ox	72			
		Total	834	97.9 (2.00)a		
					0.069	
					>0.1	

[1]Specified only when two cages were adjacent. In other cases catches were at a single cage.

Table 3. Comparison of catches from E-nets baited with human and ox odour dispensed alone or together. Total and detransformed mean catches (transformed means in brackets) of *An. gambiae s.l.* in two separate experiments to compare different odours dispensed near an E-net. For each experiment, means not associated with the same letter differ at $P < 0.05$.

Expt	Odour	Total	Nightly mean	Transformed SE & *P* by *F*-test	Replicates
9	Nil[1]	13	0.6 (0.22)-		12 (Feb)
	Man alone	524	34.0 (1.54)a		
	Ox alone	1771	120.4 (2.08)b		
	Man+ox, mix	2239	147.9 (2.17)b		
				0.076	
				<0.001	
10	One man	159	15.2 (1.21)a		6 (Mar)
	Three men	586	57.0 (1.76)b		
				0.076	
				<0.01	

[1]Mean not included in ANOVA.

downwind of each OBET entrance, with their lengths perpendicular to the prevailing wind direction, such that mosquitoes flying upwind would be caught in the downwind hopper (D), those flying downwind would be caught in the upwind (U) hopper and those entering the trap (T) would be caught inside the OBET (Fig. 2E, transverse E-nets).

Various pairs of odours were compared (6 replicates in 2006, 8 replicates in 2007). As the overall abundance of mosquitoes varied between years, the catches are presented as a percentage catch distribution (Fig. 3A–G).

For each of the paired axes (OBET plus respective E-net), the distribution of catches in the trap (T) and on the upwind (U) and downwind (D) sides of the net seemed much the same for an individual odour, whatever the other odour of the pair. For example, the proportion caught in the OBET was always low on an ox axis (Fig. 3A, B, E, F; solid bars) and much higher on a one-man axis (Fig. 3A, C, D, G; open bars). The distribution of total catches between the axes was not so grossly different. Consequently, in summarizing the results it seems simplest to pool the data for each type of odour, irrespective of the adjacent odour. It is then most pertinent to focus on T/(T + U) (i.e. the number caught in the OBET expressed as a proportion of the number caught in either in the OBET [T] or flying away from it [U]). This statistic provides a sensitive index of the strength of the entering response.

The results (Fig. 3H) show that the entry response was relatively high with human odour (0.41–0.54) and low with ox odour (0.03–0.12), CO_2 (0–0.26) or a mixture of human and cattle odour (0.21). The results with natural odours accord with

Table 4. Comparison of catches from OBETs or E-nets baited with human odour, ox odour and a mixture of the two. Total and detransformed mean catches (transformed means in brackets) of *An. gambiae s.l.* in three separate experiments to compare OBET and E-net catches with various odours. For each experiment, means not associated with the same letter differ at $P < 0.05$.

Expt	Device	Odour	Total	Nightly Mean	Transformed SE & *P* by *F*-test	Replicates
11	OBET	Three men	109	6.8 (0.89)a		12 (Mar)
	E-net		921	64.6 (1.82)b		
					0.064	
					<0.001	
12	OBET	Man	72	4.6 (0.75)a		10 (Apr)
	E-net		260	20.8 (1.34)b		
					0.077	
					<0.001	
13	OBET	Man+ox, mixed	11	0.5 (0.19)a		10 (Apr)
	E-net		639	41.8 (1.63)b		
					0.101	
					<0.001	

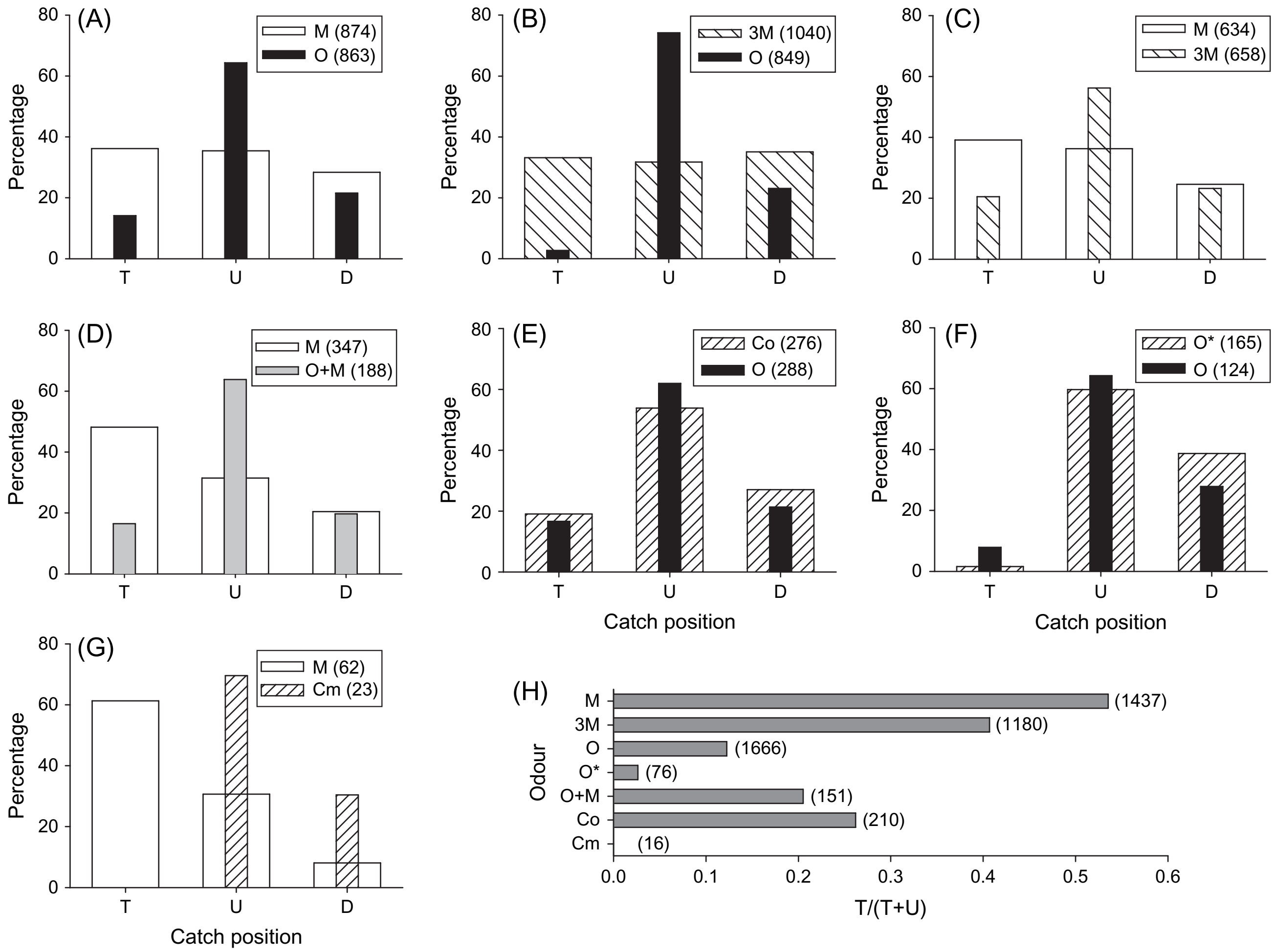

Fig. 3. Entry responses of *Anopheles gambiae s.l.* to paired odour-baited entry traps (OBETs) baited with natural doses of odour from oxen (O = 100% ox odour; O* = 10% ox odour), men (M) or CO_2 (Co = 2 L/min; Cm = 0.2 L/min). Percentage distributions of catches of *An. gambiae s.l.* caught in adjacent OBETs (T) or on the (U) upwind or (D) downwind side of E-nets placed 50 cm downwind of each OBET (see Fig. 2E) are based on total catches (shown in brackets) from the OBET + E-net over six to 10 replicates. Inset (H) shows the pooled catches OBET (T) expressed as a percentage of the pooled catches from the OBET + upwind (T + U) for each odour. Total catches from each treatment are shown in brackets.

the previous indications for the effects of such odours on entry responses. The poor entry response associated with ox odour does not seem to reflect its high concentration, because even when dispensed at a 10% dose it produced a very low entry response (0.03). The low entry response with a mixture of human and ox odour (0.21) suggests that some component(s) of the latter reduces the entry response. By contrast, as the entry response was low with CO_2 dispensed at rates simulating those in human or ox odour, it seems that human odour contains a component(s) other than CO_2 that elicits trap entry.

Mosquitoes that fly away downwind from the entrance can be expected to maintain contact with the odour and so might return. Hence, the catches on the downwind side of the E-nets (i.e. insects travelling upwind) are not necessarily composed entirely of mosquitoes making their first approach. Moreover, mosquitoes travelling downwind from one OBET might subsequently return to the other if there is a crosswind component in flight. This was investigated in April 2006 using eight replicates of the E-nets in the parallel arrangement. As expected, the catch in the man-baited OBET was again greater than in the ox-baited trap (Fig. 4). Many mosquitoes were caught on the E-nets, suggesting that there was much crosswind travel. The total numbers of *An. gambiae s.l.* caught on the ox side (i.e. flying from the ox axis to the man axis) were 136 at the net closest to the entrance and 139 at the more distant net, compared with 126 and 173, respectively, in the opposite direction. These results, and the fact that total catches on the ox axis were roughly the same as those on the man axis with the transverse nets (Fig. 3A), suggest that the swarm around the paired OBETs was largely a melée in which mosquitoes visited the entrances of each OBET about equally.

Experimental hut studies. Work at the hut was performed to confirm the main indications from the OBET studies and to explore further the role of CO_2 as a component of host odour. The number of mosquitoes caught within the hut (Table 5, Experiment 14) was greater with ox odour than with human odour. Carbon

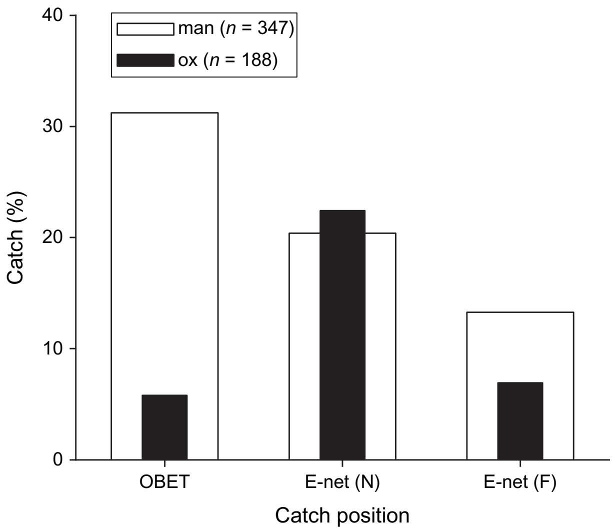

Fig. 4. Percentage distribution of *Anopheles gambiae s.l.* caught in adjacent human- and cattle-baited odour-baited entry traps (OBETs) or electric nets (E-nets) near (N) (10–32 cm) or far (F) (65–87 cm) from the OBET entrances. E-nets were arranged parallel to the wind direction. Total (OBET + E-net) catches from each treatment are shown in brackets.

dioxide was effective, especially at 2 L/min, but this gas seemed unable to account for most of the efficacy of natural odours.

The catches from the E-net outside the hut were greatest with ox odour, confirming that this odour produced the greater number of arrivals (Table 5, Experiment 15). Moreover, the number of mosquitoes caught inside the hut expressed as a proportion of the outside catch was greater with the man (4.7/3.6 ≈ 1.3; Experiment 15) than with the ox (6.1/26.3 ≈ 0.2; Experiment 15), confirming that human odour induces the stronger entry responses. Three matters must be borne in mind when assessing the strength of the entering responses at the hut. Firstly, the window of the hut was larger than the entrance to the OBET, so that entry responses might be expected to be stronger. Second, the E-net used at the hut was right against the entry point, and so measured the abundance of mosquitoes on the threshold of entering. Thirdly, the wall of the hut encouraged relatively strong breezes parallel to the wall, possibly taking some electrocuted mosquitoes well clear of the hoppers and reducing the E-net catch. For these reasons, it is not surprising that the E-net catch relative to the entry catch was not as great as in the previous OBET work.

Alighting responses

An unbaited E-target caught few *An. gambiae s.l.* and adding AOP had no significant effect on catches (Table 6, Experiment 16). In a comparison of E-targets and E-nets baited with CO_2 and/or AOP (Table 6, Experiment 17), AOP appeared to *reduce* the mean catches when added to CO_2 released adjacent to the E-target and E-net, although the effect was significant only with the E-net. The more salient points are that CO_2 increased the catches, and that the catches with the E-target were comparable with those of the E-net (i.e. virtually all the mosquitoes attracted to the odours appeared to alight on the target). Of the total number of Anophelines and Culicines found stuck on the target, 82 were on the black half and 59 on the blue. This distribution does not differ significantly from 1: 1.

Table 5. Comparison of mosquitoes caught inside or outside an experimental hut containing an ox, or a human or carbon dioxide. Total and detransformed mean catches (transformed means in brackets). Numbers of *An. gambiae s.l.* inside the hut estimated from the combined catch from an exit trap+pyrethroid spray catch; numbers outside estimated from an E-net attached to the hut's window. See text for further details. For each experiment, means not associated with the same letter differ at $P < 0.05$.

Expt	Sample	Odour	Total	Nightly mean	Transformed SE & *P* by *F*-test	Replicates
14	Inside	Nil	4	0.4 (0.15)a		6 (Jan–Feb)
		CO_2, 0.2 L/min	24	2.4 (0.53)b		
		CO_2, 2 L/min	391	56.3 (1.76)c		
		Man	251	32.9 (1.53)c		
		Ox	811	131.0 (2.12)d		
					0.118	
					<0.001	
15	Inside	Man	71	4.7 (0.76)a		10 (Feb–Apr)
		Ox	168	6.1 (0.85)a		
	Outside	Nil	1	0.1 (0.03)b		
		Man	66	3.6 (0.66)a		
		Ox	397	26.3 (1.44)c		
					0.125	
					<0.001	

Table 6. Comparison of catches from E-targets and E-nets baited with synthetic host odours. Total and detransformed mean catches (transformed means in brackets) of *An. gambiae s.l.* in two separate experiments using AOP (acetone, 1-octen-3ol and phenols) and carbon dioxide (2 L/min) with an E-target or E-net, each 1 m wide. For each experiment, means not associated with the same letter differ at $P < 0.05$.

Expt	Device	Odour	Total	Nightly mean	Transformed SE & *P* by *F*-test	Replicates
16	E-target	Nil	11	0.9 (0.271)a		6 (Mar)
		AOP	20	2.0 (0.476)a		
					0.191	
					>0.1	
17	E-target	AOP	31	2.8 (0.576)a		7 (Mar–Apr)
		CO_2	312	33.7 (1.541)bc		
		AOP+ CO_2	344	23.0 (1.381)bc		
	E-net	AOP	20	1.8 (0.454)a		
		CO_2	390	47.2 (1.683)b		
		AOP + CO_2	299	13.4 (1.158)c		
					0.148	
					<0.001	

Anopheles arabiensis *vs.* An. quadriannulatus

All samples of *An. gambiae s.l.* identified were from catches in 2006. There was no evidence that season had any marked effect on species balance. For instance, the percentage of *An. arabiensis*, as against *An. quadriannulatus*, in the human-baited OBET ranged from 100% in January ($n = 47$) to 92% in April ($n = 34$) compared with 31% ($n = 100$) and 33% ($n = 9$), respectively, for the ox-baited OBET. Hence, the data for all sampling times were pooled for each catching device. In general, the percentage of *An. quadriannulatus* was greater with E-nets and with devices baited with ox odour (Fig. 5). The compositions of the catch from the ox- and human-baited OBETs operated singly (97% vs. 42%, respectively) (Fig. 5) were very similar to the compositions when they were operated in pairs (97% [$n = 76$] vs. 38% [$n = 24$]). These data, taken with the mean catches of *An. gambiae s.l.* reported above, allow an estimate of the numbers of each species in each catch, as below.

Single OBET: arrivals. The best estimates of the number of mosquitoes arriving near the OBETs is provided by the catches from the E-nets (Table 1, Experiment 1; Table 4). It emerges (Fig. 6A) that the number of *An. quadriannulatus* arriving as a result of various odours increased six-fold in the progression: man, three men, ox, man + ox. This accords roughly with the biomasses involved. Catches of *An. arabiensis* showed a biomass effect in that the estimated catch with three men was about four times greater than that with one man. However, an E-net baited with the odour from one man caught only slightly fewer *An. arabiensis* than one baited with the odour from a single ox.

Single OBET: entry responses. The percentage of each species entering the OBET was assessed from the estimated numbers of each species arriving near the OBET, above, and the estimated number caught in the OBET. The results (Fig. 6B) show that with all odours the entry response of *An. quadriannulatus* was consistently low (< 2%). It was much greater with *An. arabiensis*, especially with the odour from one man (62%).

Hut studies: arrivals. In Experiment 15 (Table 5), it was estimated from the E-net data that the numbers of *An. arabiensis* arriving amounted to two mosquitoes/night with the man bait and one mosquito/night with the ox bait. Comparative figures for *An. quadriannulatus* were two mosquitoes/night and 25 mosquitoes/night, respectively. As with arrivals at the OBET, above, the ox odour was particularly effective for *An. quadriannulatus*, whereas ox and human odour seemed equally effective for *An. arabiensis*.

Hut studies: entry responses. The percentage of arriving *An. arabiensis* that entered was estimated as 42% for man bait and 12% for ox bait. For *An. quadriannulatus* the figures were 9% and < 1%, respectively. Again the indications are much the same as with the OBET. None of the *An. gambiae s.l.* caught with CO_2 at E-nets was identified to species level, but the species compositions of entry catches with CO_2 and ox were about the same (Fig. 5C).

Indices of anthropophily

The results (Fig. 7) show that the anthropophily index for *An. arabiensis* ranged widely, from 0.28 and 0.86, according to the method used. Hence, comparing the catches of cattle and human-baited devices to gauge ‘anthropophily’ gives equivocal results for *An. arabiensis*. For instance, the catches from the paired OBETs, single OBETs, hut collection #2 and the window E-net suggest that *An. arabiensis* is anthropophilic, whereas the catches from the free-standing E-net, and hut collection #1, suggest

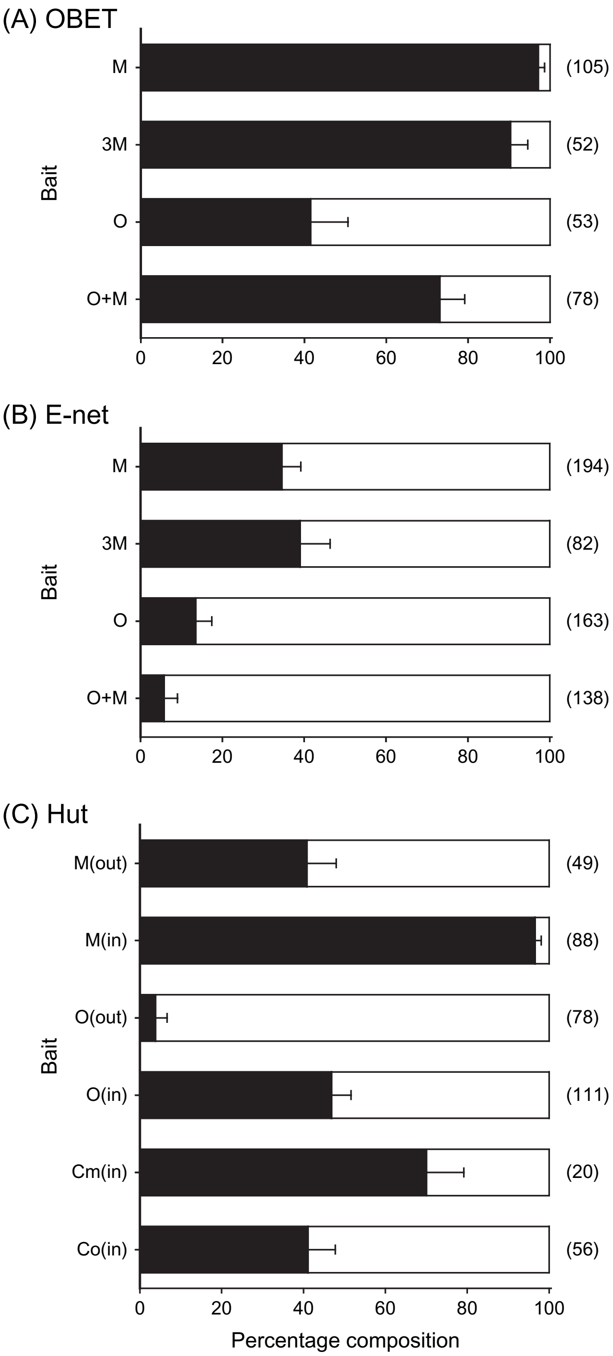

Fig. 5. Percentage composition (+ standard error [SE]) of *Anopheles arabiensis* (black portion of bar) and *Anopheles quadriannulatus* (white portion of bar) in samples from (A) an odour-baited entry trap (OBET), (B) an electric net (E-net), and (C) an experimental hut baited with odours from men (M) and/or oxen (O) or CO_2 (Cm, 2 L/min; Co, 0.2 L/min). Results from the hut show the composition of catches caught outside (out) or inside (in) the hut separately. Sample sizes are shown in brackets.

it is zoophilic. The indices for *An. quadriannulatus* were less variable (0.02–0.18) and broadly consistent with the generally accepted notion that this species is zoophilic.

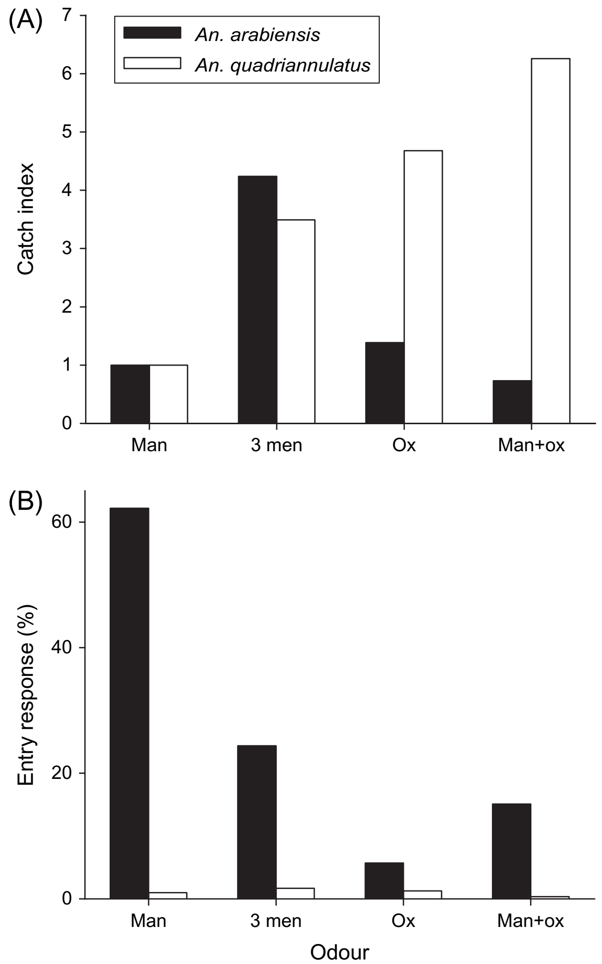

Fig. 6. Attraction and entering responses of *Anopheles arabiensis* and *Anopheles quadriannulatus* to host odours. (A) Attraction response. Catch index is the detransformed mean nightly catch from an odour-baited electric net (E-net) expressed as a proportion of the catch from an E-net baited with the odour from one man. (B) Entry response is the catch from an odour-baited entry trap expressed as a percentage of the catch from an E-net baited with the same odour.

Other species

Almost all mosquitoes other than *An. gambiae s.l.* ($n = 25\,820$) were *Anopheles pretoriensis* Theobald ($n = 2653$) and Culicines ($n = 7503$). They showed behaviour patterns more like those of *An. quadriannulatus* than *An. arabiensis*. For example, the detransformed mean catches of *An. pretoriensis* at the E-nets of Experiment 1 (Table 3) with no odour, man alone, ox alone and man + ox were 0.1, 0.7, 3.0 and 3.8, respectively. For Culicines the figures were 0.5, 10.4, 46.5 and 43.5, respectively. In each dataset, catches with ox alone and man + ox were not significantly different, but each differed significantly from the catch with man alone; as with *An. quadriannulatus*, the biomass of the host, not the host type, seemed to be the determining factor.

Midges, comprising *Culicoides schultzei* Enderlein, *Culicoides subschultzei* Cornet & Brunhes and *Culicoides fulvus*

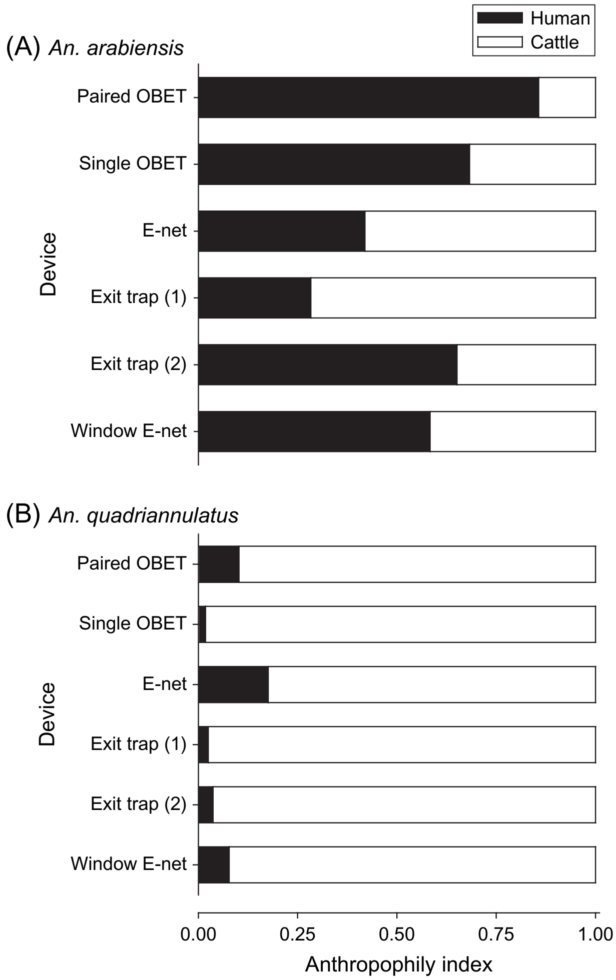

Fig. 7. Indices of anthropophily based on capture of *Anopheles arabiensis* and *Anopheles quadriannulatus* from various devices baited with human or cattle odour. Black sections in the bars greater or less than 0.5 denote anthropophilic or zoophilic behaviour, respectively. For each device the total (paired odour-baited entry trap [OBET]) or mean (all other devices) catch with human odour was expressed as a proportion of the sum of the catches from the human- and cattle-baited traps. Data for indices were derived from molecular identifications (see Fig. 4 and text) and catches of *Anopheles gambiae s.l.* presented in Tables 2 (single and paired OBETs), 3 (E-nets) and 5 (Experiment 14 for exit trap [1]; Experiment 15 for exit trap [2] and window E-net).

Sen & Das Gupta, were counted in E-net catches with ox odour for nine nights in March 2007. The total count was 11 897, against 487 *An. gambiae* s.l. The ~ 3-mm wingspan of the midges seemed no gross prejudice against capture by grids spaced at 8 mm.

Discussion

The present findings have several implications for the monitoring and control of mosquitoes and, more generally, for the analysis of vector behaviour.

Artificial baits for surveillance and control of mosquitoes

It appears that ox and human odours contain chemical(s), other than CO_2, that attract *An. quadriannulatus* and *An. arabiensis* to the vicinity of the host. For *An. arabiensis*, human odour also appears to contain chemical(s) that elicit an entry response and there is some indication that ox odour contains chemical(s) that antagonize this behaviour. Identification of these chemicals might provide the basis for the development of improved devices to sample and control *An. arabiensis* and/or strategies to reduce malaria risk. The comparison of the E-target and E-net showed that the target, which required a landing response, caught as many mosquitoes as the net, which caught mosquitoes in flight. This contrasts with the results for tsetse and other day-active vectors which tend to approach small targets but not to land on them (Vale, 1982b). By analogy with the proven vector control strategy of impregnating bednets with insecticides, these results suggest that *An. gambiae s.l.* might be readily induced to land on, say, an odour-baited target treated with insecticide. Further work to study the landing responses of *An. gambiae s.l.* is merited.

Insecticide-treated cattle

The present results also have implications for the potential use of insecticide-treated cattle to control *An. arabiensis*. Predicting the impact of this method of mosquito control relies on assessing the relative probability that the vector feeds on cattle rather than humans (Tirados *et al.*, 2006). Traditional methods of assessing this probability, based on the use of the Human Biting Index (HBI), have provided equivocal indications of the relative importance of cattle and humans in the diet of *An. arabiensis* (White *et al.*, 1972; Highton *et al.*, 1979; Molineaux & Grammiccia, 1980; Fontenille *et al.*, 1997; Tirados *et al.*, 2006) and studies using OBETs have suggested that, in mainland Africa, *An. arabiensis* is inherently anthropophilic (Costantini *et al.*, 1998a; Tirados *et al.*, 2006).

However, the present studies undertaken with E-nets indicate that outdoors, odours from single humans and cattle attract equal numbers of *An. arabiensis* and hence cattle and humans may be bitten equally outdoors. The results also imply that the probability of a mosquito entering a hut is strongly dependent on the hut structure and the odours produced from the hosts within: even with human odour, the entry response was $< 100\%$ and hence being indoors might reduce the availability of a host to mosquitoes. Accordingly, in the typical setting of an African village at night, where cattle are outdoors and humans are asleep in their huts, cattle may represent the main diet of *An. arabiensis*. However, results from some field studies suggest that the landing and/or feeding responses of *An. gambiae s.l.* to humans may be stronger indoors (Service, 1963). Further studies of the close range responses of mosquitoes to their hosts are required to assess the likely impact of insecticide-treated cattle on mosquito populations.

Anthropophily and the analysis of mosquito behaviour

Although the relative importance of humans and non-humans in the diet is a crucial determinant of vectorial capacity in mosquitoes, obtaining unbiased estimates of diet composition is difficult. Estimates based on the HBI are biased by the patchy distribution of hosts, and present results show that using host-baited devices to estimate the relative importance of potential hosts can be misleading: the catches from paired OBETs suggest that *An. arabiensis* is anthropophilic, whereas E-net catches suggest it is zoophilic. At the relatively crude level of analysis used in the present study, the number of mosquitoes in a trap can be seen as the product of: (a) the number attracted to its vicinity, and (b) the proportion that enter it. The results show that host odours have distinctive effects on these two responses, with both ox and human odour attracting *An. arabiensis* and *An. quadriannulatus* to the vicinity, but only human odour eliciting a strong entry response, and only with *An. arabiensis*. Rather than attempting to label mosquito species with gross terms such as 'anthropophilic', 'zoophilic' or 'endophilic', we need to focus on analysing the specific elements of behaviour that contribute directly to the matters of interest. For instance, if we are developing baits to catch or kill mosquitoes, we need to identify the stimuli that: (a) bring mosquitoes to the general vicinity of the bait, and (b) induce them to contact it or enter a trap. We should not confine ourselves to analysing, say, only human odours because the present data indicate that important attractants for *An. arabiensis* are also present in cattle odour. Similarly, the indications that ox odour inhibits the entry response may provide the basis of repellents to divert mosquitoes from humans to natural or artificial insecticide-treated baits.

In terms of mosquito control strategies, the finding that alighting responses on inanimate targets were much stronger than with tsetse and other day-flying Diptera (Vale, 1982a) may be important. Hence, the principle of odour-baited and insecticide-treated targets used successfully against tsetse (Vale *et al.*, 1988) might be even more applicable to mosquitoes. Indeed, insecticide-treated bednets are a sort of human-baited target. If we assume, pending further research, that cattle do not produce an alighting inhibitor for *An. arabiensis*, then the fact that this important vector of malaria arrives in large numbers at sources of ox odour reinforces the idea (Rowland *et al.*, 2001; Tirados *et al.*, 2006) that insecticide treatment of cattle could be a powerful control technique. Ideally, perhaps, the cattle should be outdoors, but indoors might do if, say, the cattle were baited with those chemical(s) present in humans which elicit a strong entry response. Alternatively, insecticide-treated netting near cowshed windows or netted kraals might be effective.

Suitable tools

The present results confirm that the entrance to an OBET simulates a hole in a window. The fact that mosquitoes were caught downwind of both entrances of paired OBETs suggests that the paired OBET technique (Costantini *et al.*, 1998a) is a quick and simple means of gauging the *relative* efficacy of various odours in inducing passage through windows. However, OBET catches alone cannot estimate the *absolute* efficiency of passage.

Moreover, the present work with the CDC trap illustrates the problems of studying behaviour by relying on traps designed primarily for surveying and monitoring populations. Catches with the CDC trap were large only when it was used with both light and odour. We do not know whether, or to what extent, this is because the odour attracted mosquitoes and the light induced entry, or because the light was attractive and the odour encouraged entry. One could argue, somewhat vaguely, that since a few mosquitoes were caught at a CDC trap with odour and no light, then the odour must have attracted at least some mosquitoes and did not completely inhibit entry. Certainly, however, one could not claim that: (a) light attracts nothing; (b) odours produce no inhibition, or (c) catches at a CDC trap baited with various odours are fair indices of the relative numbers attracted to the odours – with or without a light. Such complexities in interpretation can be addressed only by tools that quantify specific aspects of behaviour.

Electrocuting grids exemplify the type of tool required, although the standard grids for tsetse do have some problems when employed against mosquitoes. For example, trays for collecting the electrocuted mosquitoes must be wide. Unlike tsetse, mosquitoes must be studied mainly in the wet season, so the grids must be protected from rain to prevent shorting. More worryingly, the especially profuse sparking at the mCDC trap reduced the observed catches substantially. Although this effect was not significant, it does suggest that the more moderate sparking on the normal grids might have affected behaviour in some way. However, even if this were true, the sparking would have *reduced* catches (i.e. the superiority of E-net catches would indicate that the other devices were even less efficient than shown here). In any event, the sparking on the grids can be reduced by making simple adjustments to the power supply and circuitry (G. A. Vale, unpublished data, 2008).

On the positive side, almost all the mosquitoes caught were suitable for molecular identification. Concerns that the standard grid spacing of 8 mm is too wide for mosquitoes (Knols *et al.*, 1998) seem unfounded, especially as this grid caught many minute midges. A roof to protect the grid from rain had, itself, no material effect. The low catches with unbaited E-nets suggest that the sparking did not attract many mosquitoes. The grids allowed studies of the probabilities of mosquitoes: (a) travelling upwind, downwind or crosswind at various distances from baits; (b) entering a trap or hut, and (c) landing on a target. In principle the sticky nets are alternatives to E-nets, but their catches were much smaller and we lack information on a number of parameters such as the probability of capture at first contact, and the potential behaviour-modifying effects of the glue itself. None of the other sampling devices provide the required information.

Hence, electrocuting technology, largely in its present form, opens important routes to an essential topic: the fuller understanding of mosquito behaviour.

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