Medical and Veterinary Entomology (1996) **10**, 220–227

Mosquito responses to carbon dioxide in a West African Sudan savanna village

CARLO COSTANTINI,[1,2] GABRIELLA GIBSON,[1] N'FALÉ SAGNON,[3] ALESSANDRA DELLA TORRE,[2] JOHN BRADY[1] and MARIO COLUZZI[2]
[1]Department of Biology, Imperial College, University of London, U.K., [2]Istituto di Parassitologia* and Fondazione Pasteur–Cenci Bolognetti, Università 'La Sapienza', Rome, Italy, and [3]Centre National de Lutte contre le Paludisme, Ouagadougou, Burkina Faso

Abstract. Mosquito responses to carbon dioxide were investigated in Noungou village, 30 km northeast of Ouagadougou in the Sudan savanna belt of Burkina Faso, West Africa. Species of primary interest were the main malaria vectors *Anopheles gambiae s.s.* and *An.arabiensis*, sibling species belonging to the *An.gambiae* complex. Data for *An.funestus, An.pharoensis, Culex quinquefasciatus* and *Mansonia uniformis* were also analysed.

Carbon dioxide was used at concentrations of 0.04–0.6% (cf. 0.03% ambient concentration) for attracting mosquitoes to odour-baited entry traps (OBETs). The 'attractiveness' of whole human odour was also compared with CO_2 emitted at a rate equivalent to that released by the human bait. In a direct choice test with two OBETs placed side-by-side, the number of *An.gambiae s.l.* entering the trap with human odour was double the number trapped with CO_2 alone (at the human equivalent rate), but there was no significant difference between OBETs for the other species of mosquitoes. When OBETs were positioned 20 m apart, again CO_2 alone attracted half as many *An.gambiae s.l.* and only 40% *An.funestus, 65% Ma.uniformis* but twice as many *An.pharoensis* compared to the number trapped with human odour.

The dose–response for all mosquito species was essentially similar: a linear increase in catch with increasing dose on a log–log scale. The slopes of the dose-response curves were not significantly different between species, although there were significant differences in the relative numbers caught. If the dose–response data are considered in relation to a standard human bait collection (HBC), however, the behaviour of each species was quite different. At one extreme, even the highest dose of CO_2 did not catch more *An.gambiae s.l.* than one HBC. At the other extreme, the three highest doses of CO_2 caught significantly more *Ma.uniformis* than did one HBC. *An.pharoensis* and *Cx quinquefasciatus* showed a threshold response to CO_2, responding only at doses above that normally released by one man. *An.funestus* did not respond to CO_2 alone at any dose in sufficient numbers to assess the dose response. Within the *An.gambiae* complex, *An.arabiensis* 'chose' the CO_2-baited trap with a higher probability than *An.gambiae s.s.* Also *An.arabiensis*, the less anthropophilic of the two species, was more abundant in CO_2-baited OBETs than in human bait collections.

Key words. *Anopheles arabiensis, An.funestus, An.gambiae, An.pharoensis, Culex quinquefasciatus, Mansonia uniformis*, carbon dioxide, odour-baited traps, malaria vectors, host-seeking behaviour, Sudan savanna, Burkina Faso, West Africa.

*W.H.O. Collaborating Centre for Malaria Epidemiology.

Correspondence: Dott. C. Costantini, Department of Biology, Imperial College at Silwood Park, Ascot, Berkshire SL5 7PY, U.K.

Introduction

A wealth of evidence shows that carbon dioxide (CO_2) is probably the single most important cue used by mosquitoes (Diptera: Culicidae) and other haematophagous arthropods for locating a source of blood. This role of CO_2, compared with other chemical and physical variables, has usually been investigated under controlled laboratory conditions with the culicine mosquito *Aedes aegypti* (L.), as reviewed by Gillies (1980). Fewer studies of CO_2 effects have involved malaria vector anopheline mosquitoes: *Anopheles atroparvus* Van Thiel (Van Thiel, 1947; Van Thiel & Weurman, 1947; Laarman, 1955), *An.stephensi* Liston (Brouwer, 1960; Bos & Laarman, 1975), *An.albimanus* Wiedemann (Knols *et al.*, 1994b), and members of the *An.gambiae* Giles complex: *An.arabiensis* Patton (Omer, 1979) and *An.gambiae* Giles *sensu stricto* (Knols *et al.*, 1994a; Knols, 1996).

Reeves (1953) was among the first to demonstrate the 'attractiveness' of CO_2 for mosquitoes in the field, and how this varies according to the dose and the species considered. Since then, CO_2 has been used as a means of increasing both the number of mosquitoes caught and the species diversity in various trapping devices (Service, 1993). Reduction in the amount of CO_2 released by a natural host decreases the number of mosquitoes approaching it, but not the proportion biting (Snow, 1970). In West Africa, Gillies & Wilkes (1969, 1970, 1972, 1974) investigated the range of attraction of host-equivalent amounts of 'synthetic' carbon dioxide, compared to one calf (1970), two calves (1969, 1972) and several bird species (1974). Similar studies were undertaken by Schreck *et al.* (1972) with North American mosquitoes. Gillies (1980) later plotted a curve of the range of attractiveness of CO_2 at different emission rates. An important conclusion was that this range varies between mosquito species. Moreover, Gillies & Wilkes (1969) proposed CO_2 as a middle-range factor so that for species such as *An.melas* Theobald, for example, approach towards the host is initiated by the presence of other host odours at longer ranges.

The role of CO_2 in the 'host-seeking' behaviour of mosquitoes is a matter of debate. Some authors regard it as only an 'activator', activation being defined as the induction of flight activity (Laarman, 1955; Kellogg & Wright, 1962; Daykin *et al.*, 1965; Khan & Maibach, 1966). Gillies (1980), on the other hand, concluded that "it acts as an 'attractant', orientation towards the host being mediated by kinesis and optomotor anemotaxis", but "in the absence of moving air currents" (as for some cage experiments) "only the kinetic or 'activating' effect is manifested".

With respect to the *An.gambiae* complex, it has been shown that host odours play an important role in host location (Gillies, 1988; Takken, 1991; Costantini *et al.*, 1993), but the relative importance of individual odours is unknown. Among the six sibling species in this complex there are well-documented variations in host preference (White, 1974), ranging from strong anthropophily in *An.gambiae s.s.* to almos complete zoophily in *An.quadriannulatus* (Theobald). Of the species we studied, *An.gambiae s.s.* is highly anthropophilic throughout its distribution, whereas the host preference of *An.arabiensis* is more difficult to generalize. In areas where humans are the main host available, the proportion of bloodmeals that come from humans (the human blood index, HBI) is high for both *An.gambiae* and *An.arabiensis*. In villages where cattle are abundant, however, the HBI for *An.arabiensis* drops in favour of bovid feeds (Coz, 1973; White, 1974; Coluzzi *et al.*, 1975; Gillies & Coetzee, 1987). Although one explanation would be that *An.arabiensis* is simply a more opportunistic feeder, the pattern of feeding on different types of host mammals by *An.arabiensis* is not always proportional to host abundance. For example, in Madagascar independently of frequency of humans, *An.arabiensis* is mainly zoophilic (Ralisoa Randrianasolo & Coluzzi, 1987).

We report here a preliminary field study to investigate the influence of CO_2 relative to other host odours in the 'host-seeking' behaviour of *An.gambiae s.s.* and *An.arabiensis*, in comparison to other sympatric, human-biting, but more opportunistic mosquito species.

Materials and Methods

Study site and trapping methods. The experiments were conducted at Noungou village (12° 30' N, 1° 20' W) in the Sudanese savanna belt *c.* 30 km north-east of Ouagadougou, Burkina Faso, West Africa, during the 1993 rainy season. This village has *c.* 300 huts occupied by 500 people and covers *c.* 4 km^2 on the edge of a large permanent lake. The rainy season lasts from June to September, with a mean annual rainfall of *c.* 800 mm. Mosquito bionomics are well known for this area (Hamon, 1963; Hamon *et al.*, 1966). Millet and sorghum are the main crops, cultivated around and within the village. Domestic animals in the village are mainly goats, sheep and donkeys, with very few cattle and no pigs. Two types of hut usually made of mud-brick walls, with one room, an open window and open eaves, are grouped in family compounds: circular huts (*c.* 3 m diameter) with a thatched roof, and rectangular huts (*c.* 3 × 5 m) with a corrugated-iron roof. These types of hut are known in the entomological literature as 'Mossi huts' and 'Bobo huts', respectively (Darriet *et al.*, 1985; Majori *et al.*, 1987).

At Noungou in the wet season the main malaria vectors are *An.gambiae s.s.* and *An.arabiensis*. 'Mopti' genotypes (Coluzzi *et al.*, 1985) represent the prevailing chromosomal form of *An.gambiae s.s.* At the end of the wet season *An.funestus* Giles densities rise to a peak in November and then decline until the beginning of January (Hamon *et al.*, 1966; Merzagora, 1993).

The main experiments were done with odour-baited entry traps (OBETs) designed to catch mosquitoes attracted by host odours alone, with no visual host cues present (Costantini *et al.*, 1993). The standard bait was a man, sleeping in a tent. The tent was positioned inside an experimental hut of similar construction to local 'Mossi' dwellings. The man's host odour was brought to the trap via *c.* 9 m of polythene 'lay-flat' tubing, leading from a 12 V fan at one end of the tent. As much of the tubing as possible was wound around the outside of the hut to enable the odour-conditioned air to approach ambient temperature. The speed of the air-stream released from the opening of the OBET was *c.* 50 cm s^{-1}.

The OBET was placed outdoors on a table at window level (*c.* 1.5 m above ground), and covered as much of the window as possible, so that other cues experienced by the mosquito were similar to those which would normally attract them to enter by flying through the windows of human dwellings in response to host odours. This standard arrangement was modified for each experiment, as described below. Experiments began at 21.00 hours and ended at 05.00 hours local time, when the traps were closed and the mosquitoes were collected.

Carbon dioxide was released from cylinders, its flow controlled with two-stage pressure regulators, measured with CO_2-rotameters and adjusted with needle valves. CO_2 concentrations were monitored with a portable infra-red gas analyser (ADC2000, range of measurable concentrations 0–2%, resolution ±0.01%). The cylinders were hidden behind the experimental huts to remove any visual stimuli.

To facilitate comparisons between experiments and to obtain background information about mosquito densities, a human biting catch (HBC) was conducted each night either indoors or outdoors at four different randomized positions within the village. Collections were made of the mosquitoes landing on the exposed lower legs of one sitting catcher from 21.00 to 05.00 hours. Collectors changed every 2 h and were allocated to different time sets on subsequent nights, according to randomized 4 × 4 latin squares (Cochran & Cox, 1957).

Species identification. Mosquitoes were identified using the key of Gillies & Coetzee (1987) for anophelines and the key of Edwards (1941) for culicines. Among the *An.gambiae* complex, representative subsamples were tested for species identification by the PCR molecular technique of Scott *et al.* (1993). Generally the mosquito species were identified morphologically as the catches were counted, except for *Mansonia (Mansonioides)* spp. which were dried and kept for later examination. Only *c.* 5% of these specimens were *Mansonia africana* (Theobald); the rest were *Ma.uniformis* (Theobald). It was decided, therefore, to consider the *Mansonia* samples to be mostly representative of *Ma.uniformis*.

Experiment 1. Whole human odour v. carbon dioxide: choice test

Two OBETs were placed side-by-side near the window of one of the experimental huts. One OBET was set up as the standard 'man-baited' treatment (as above). The other OBET was baited with CO_2 via a rubber tube inserted into the lay-flat tubing near the fan. The fan for the CO_2 was placed in the hut eaves so that it would draw air mainly from outdoors to avoid contamination with residual human odours from inside the hut.

The concentration of CO_2 emitted by the men used as baits varied with person and with time, although it was relatively stable on any given night. Each night, therefore, the CO_2 concentration of the air-stream coming out of the man-baited trap was measured with the infra-red CO_2 gas monitor and the same concentration was released through the CO_2 -baited trap. This was to ensure that mosquito 'choice' was not affected by different levels of CO_2 released from the two traps, but by the presence or absence of other human odours. Each trap stayed in the same place, but the relative positions of the two odours were alternated each night (to control for possible side-effects by swapping positions of the 'lay-flat' tubing connections). Ten replicates were performed, using a different human bait every night to reduce the effect of differences in attractiveness between baits.

To allow for spatial and temporal variability, a CO_2/human *catch index* was calculated, defined as the log-transformed ratio of the CO_2-baited trap catch + 1, over the human-baited trap catch + 1. The antilogarithm of the catch index mean, weighted by the nightly sample size, gave an estimate of the mean proportion of mosquitoes caught with CO_2 alone relative to whole human odour. Departures of the index from unity were tested using the Mann-Whitney U test.

Experiment 2: Whole human odour v. carbon dioxide: distance comparison

The arrangement for this experiment was similar to the choice test except that the 'man-baited' and 'CO_2-baited' traps were set up at different huts separated by at least 20 m. Four OBET experimental hut units were used in a random rotation, although only two were used each night. One OBET was baited with a sleeping man (as above), and another OBET was baited with the same concentration of CO_2 as was released by the 'man-baited' trap that night. Four human baits were used, each of which was tested twice, so the overall number of replicates was eight.

Experiment 3. Dose response to carbon dioxide

The standard arrangement of experimental hut and OBET was used, with the human bait odours replaced by CO_2 released into the 'lay-flat' tubing at the fan level. The concentration was measured with the CO_2 gas monitor at the trap entrance and adjusted with a flowmeter at the start of the experiment. Five test concentrations of CO_2 were chosen: 0.04%, 0.06%, 0.15%, 0.30% and 0.60%. The ambient concentration was 0.03%. The amount of CO_2 typically released at the trap entrance by a man sleeping in the tent ranged between 0.05% and 0.12%. Higher concentrations of CO_2 were achievable, but required more than one cylinder per night and caused the valves to freeze.

Due to limitations on availability of CO_2 cylinders, only three or four concentrations could be tested on any one night. The protocol was therefore set up as a partially balanced incomplete block design with two associate classes (Cochran & Cox, 1957). Over fifteen nights each concentration was tested nine times. Even within the rainy season the density of mosquitoes can suddenly change dramatically. To overcome this source of variability in the data the results were analysed in two ways: (1) as a log–log dose–response experiment; and (2) as a dose response normalized to a standard human biting catch (HBC). The aims of normalizing the dose response were (a) to reduce the effects of density variation, by relating the OBET catches on a given night to another independent measure of mosquito density; and (b) to investigate further the relative importance of CO_2 and whole human odour for each species of mosquito.

For the statistical analysis a generalized linear modelling package, GLIM® (Payne, 1987), was used to obtain the best fit to the data. For the log–log model, raw count data were log $(x + 1)$-transformed and differences in the slope of their respective dose–response curves were assessed by the change in deviance caused by the removal of the interaction term DOSE × SPECIES from the full model.

For analysis of the HBC-normalized model, a CO_2/HBC *catch index* was calculated for each CO_2 treatment, expressed as the ratio of the OBET catch over the simultaneous HBC. The sequence of HBCs was two nights indoors, two nights outdoors consecutively. The mean number of *Ma.uniformis* caught outdoors was marginally greater than caught indoors. For *An.gambiae s.l.*, however, significantly more were caught indoors than outdoors by HBC. Therefore, for both species, the dose response was investigated separately for each HBC position. For *An.pharoensis* Theobald and *Culex quinquefasciatus* Say, significantly more mosquitoes were caught outdoors than indoors by HBC, most of the collections indoors giving very low or zero counts. For nights when there was no outdoor HBC, therefore, the *An.pharoensis* and *Cx quinquefasciatus* data were discarded. For those few occasions when the HBC caught no mosquitoes, the data were also discarded.

The square-root of the catch index was plotted against CO_2 log-concentration, expressed as parts per million (ppm) above ambient. The statistical analysis followed procedures analogous to the log–log model.

Results

Experiment 1. Choice test: whole human odour v. carbon dioxide

Table 1 shows the geometric mean catches of five species of mosquitoes in paired, differentially baited OBETs. When the distribution of mosquitoes in the two traps was tested against the binomial distribution with an expectation of random 'choice' (probability 50%), only *An.gambiae s.l.* showed a significant difference: 33% were caught in the CO_2-baited trap v. 67% in the human-odour trap. *An.pharoensis* was the only species for which the proportion caught in the CO_2-baited trap (60%) exceeded that caught in the human-baited trap (40%) but this difference was not significant. To facilitate comparisons between experiments 1 and 2, the difference in catches between the two paired traps are also expressed in Table 1 as the CO_2/human catch index. Analysis of the data for *An.gambiae s.s.* and *An.arabiensis* shows that both sibling species preferred the human bait, but *An.arabiensis* did so with a lower probability: 60% *v* 70% for *An.gambiae s.s.* ($n = 442$; G = 4.8; d.f. = 1; $P < 0.03$).

An ANOVA of log (x + 1)-transformed counts showed that there were no significant trap position effects. For *An.gambiae s.l.* there was significant day-to-day heterogeneity (G = 18.4; d.f. = 4; $P < 0.01$), but the 'choice' was always in the same direction: the man-baited trap always caught as many or more *An.gambiae s.l.* than the CO_2 -baited trap. The pooled G-test was also significant (G = 46.9; d.f. = 1; $P < 0.01$), showing that, overall, this species made a significant 'choice'. For *An.pharoensis* and *Ma.uniformis* there was significant heterogeneity ($P < 0.05$), indicating that they did not choose the same bait every day. The pooled data show that there was no significant difference in the catch between the two traps, so these species did not make a significant 'choice' overall.

Experiment 2. Distance comparison: whole human odour v. carbon dioxide

Table 2 shows that, for *An.gambiae s.s.* and *An.arabiensis*, when human odours and CO_2 were presented separately, in OBETs ≥20 m apart, CO_2 attracted less than half as many mosquitoes as did the whole human odour. Numbers caught were too few to allow comparison between these two sibling species.

An.funestus was attracted even less than *An.gambiae s.l.* to CO_2 alone. Conversely, twice as many *An.pharoensis* were attracted

Table 1. Choice test (experiment 1). Mean numbers (geometric mean) of mosquitoes caught per night (21.00–05.00 hours) using a man-baited trap versus a CO_2-baited trap placed side-by-side outside a hut window: *n*, total number of individuals caught; catch index, mean proportion of mosquitoes caught in a CO_2-baited trap relative to the numbers caught in a trap with whole human odour. Significance levels: $^{**}P < 0.01$, $^{***}P < 0.001$, ns = not significant.

	Mean catch (95% c.l.)				
Species	Man	CO_2	$F_{(1,16)}$	*n*	Catch index
An. gambiae s.l.	27.3 (19.8–37.5)	13.5 (8.9–20.2)	9.48**	450	0.51***
An.funestus	2.0 (0.5–5.2)	2.2 (0.8–4.9)	0.01^{ns}	75	0.85^{ns}
An.pharoensis	2.1 (0.8–4.2)	3.0 (1.2–6.4)	0.68^{ns}	75	1.61^{ns}
Ma.uniformis	11.7 (4.8–26.4)	11.6 (5.6–23.3)	0.01^{ns}	311	0.87^{ns}
Cx quinquefasciatus	2.0 (0.7–4.3)	2.0 (0.9–3.8)	0.01^{ns}	63	1.12^{ns}

Table 2. Whole human odour *v.* CO_2 (experiment 2). Overall sample size (*n*) and catch index (mean proportion of mosquitoes caught in a CO_2-baited trap relative to the numbers caught in a trap with whole human odour) in paired OBETs separated by at least 20 m.

		Catch index		Mann-Whitney U	
Species	*n*	Mean	95% c.l.	Z	*P*
An. gambaie s.l.	137	0.48	(0.25–0.91)	–2.69	0.01
An.funestus	235	0.39	(0.25–0.60)	–2.69	0.01
An.pharoensis	30	2.09	(1.36–3.21)	–1.94	0.05
Ma.uniformis	142	0.66	(0.45–0.98)	–1.39	0.16
Kruskal-Wallis *H*				13.35	0.004

to CO_2 on its own as compared to whole human odour. There was no significant difference in the numbers of *Ma.uniformis* attracted to the CO_2-baited and human-baited traps when they were separated. Heterogeneities between these four species were significant (Kruskal-Wallis test).

Experiment 3. Dose response to carbon dioxide

Dose–response relationships were clearest for *An.gambiae s.l.* and *Ma.uniformis* (Fig. 1a). Regression lines fitted through the log–log plots have similar slopes and their intercept values reflect the different numbers of each species caught. Whereas the dose response to CO_2 of these species is similar, Fig. 1(a) also shows that their response to CO_2 in relation to the HBC is quite different. The highest dose of CO_2 did not attract as many *An.gambiae s.l.* as one HBC, whereas for *Ma.uniformis* the CO_2 dose closest to that released by a single man attracted as many mosquitoes as one HBC. This relationship is discussed in greater detail below. An even better fit to the data for *An.gambiae s.l.* was achieved by fitting an asymptotic exponential curve of the form:

$$\ln(\text{catch}+1) = 5.102\,(1 - 2.632e^{-0.28\ln([CO_2])}) \quad (1)$$

whose parameters were estimated by maximum likelihood.

The same relative proportions of *An.gambiae s.s.* (62.9% and 62.5%) and *An.arabiensis* (37.1% and 37.5%) were caught at the lowest and highest doses of CO_2 ($n = 466$; $G = 0.005$; d.f. = 1; $P > 0.95$), indicating that these two members of the *An.gambiae* complex do not differ in their dose response to CO_2. Among the HBC samples, however, 71.5% of the catch was *An.gambiae s.s.* and only 28.5% *An.arabiensis*, showing a significant increase ($n = 1065$; $G = 9.2$; d.f. = 1; $P < 0.05$) in the excess of *An.gambiae s.s.* over *An.arabiensis* when human bait was employed.

For *An.pharoensis* and *Cx quinquefasciatus* there was evidence of a threshold effect (Fig. 1b), with little response at the two lowest doses, at or below the concentration released by a single man, whereas the catch increased by at least an order of magnitude at the highest doses tested. Fig. 1(b) also shows that the numbers caught at the man-equivalent dose of CO_2 are comparable to one HBC for both species.

Fig. 2 compares mosquito responses to a whole human bait (i.e. CO_2 plus the other human odours and short-range factors)

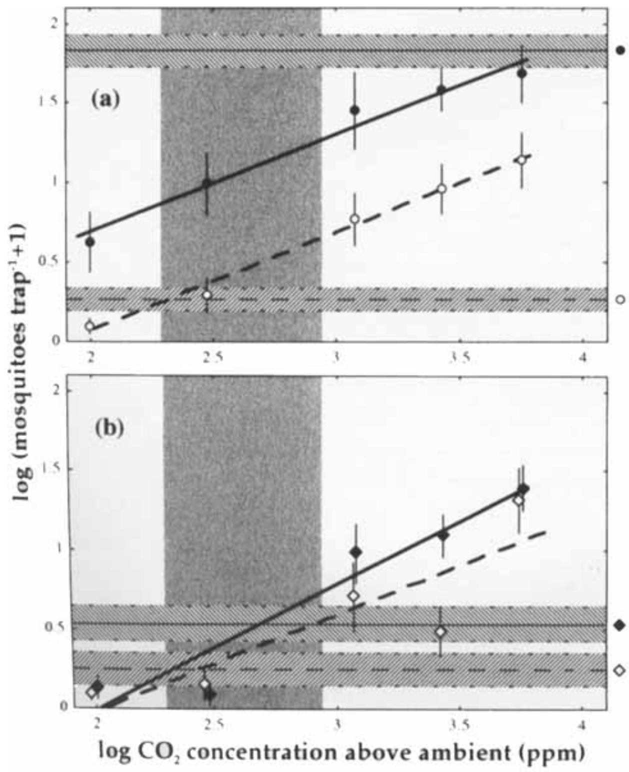

Fig. 1. Dose response of mosquitoes to CO_2 (log–log). (a) *An.gambiae s.l.* (closed circles and solid line), and *Ma.uniformis* (open circles and dashed line), (b) *An.pharoensis* (closed diamonds and solid line), and *Cx quinquefasciatus* (open diamonds and dashed line). Slopes show best linear fit. Horizontal shaded bands depict mean human-biting catch per night (±SE) for the species symbol shown to the right of the mean line. Shaded vertical areas define the range of CO_2 concentrations (measured at the OBET entrance) arising from a man sleeping in the tent indoors (see Materials and Methods for details).

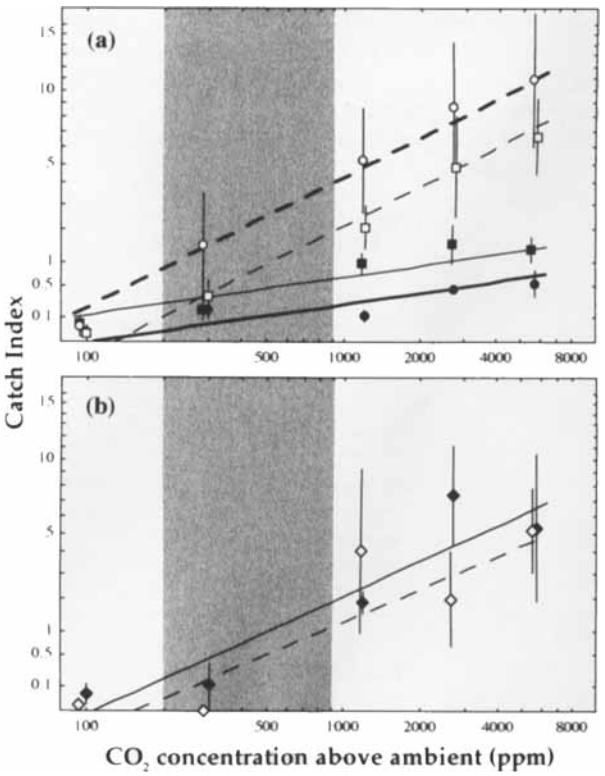

Fig. 2. Dose response of mosquitoes to CO_2 in relation to a standard human-biting catch (square root-log). (a) *An.gambiae s.l.* (closed circles/squares and solid lines), and *Ma.uniformis* (open circles/squares and dashed lines); (b) *An.pharoensis* (closed diamonds and solid line), and *Cx quinquefasciatus* (open diamonds and dashed line). In (a) squares depict the outdoor catch index (thin regression lines), circles the indoor catch index (thick regression lines). Shaded vertical areas define the range of CO_2 concentrations (measured at the OBET entrance) arising from a man sleeping in the tent indoors (see Materials and Methods for details).

versus an OBET baited with CO_2 alone. For *An.gambiae s.l.* the increased catch with rising CO_2 concentration never went above the equivalent of one HBC (catch index = 1), whereas for *Ma.uniformis* there was a much steeper increase in catch with CO_2 alone (solid lines *v.* dashed lines in Fig. 2a). Slopes of the regression lines for these two species differ significantly ($F = 24.5$; d.f. = 1,83; $P < 0.001$), reinforcing the indications from previous experiments that, compared with *Ma.uniformis, An.gambiae s.l.* is more responsive to whole human odour than to CO_2. The catch index for both species differs between HBCs undertaken indoors or outdoors (thick lines *v.* thin lines, Fig. 2a) but, for each species, the slopes of the lines relating both HBCs (indoors and outdoors) to CO_2 concentration were parallel. Thus, for *An.gambiae s.l.*, with higher concentrations, the index surpassed a value of one for HBCs outdoors, but reached only 0.6–0.7 for HBCs indoors. Conversely, for *Ma.uniformis*, the catch index for HBCs outdoors was consistently lower than for indoors (Fig. 2a). With *An.pharoensis* and *Cx quinquefasciatus*, the HBC was equal to or less than the OBET catch at CO_2 concentrations equal to or greater than the man-equivalent (Fig. 2b), but this relationship was not straightforward for either species. Although some *An.funestus* were caught in the CO_2-baited traps, the numbers were too few to assess the dose–response for this species.

Discussion

Host odours influence mosquitoes in their response to CO_2 and mosquito species differ in their response to human odours. For *An.gambiae s.s.* and *An.arabiensis*, whether CO_2 is presented (i) near human odour, (ii) on its own, or (iii) at high concentrations, it is not as attractive as a human host indoors or his odour equivalent. These results are also consistent with the finding by Snow (1970) that, for *An.gambiae s.s.*, CO_2 accounted for *c.* 40% of the attractiveness of a human bait wearing CO_2-absorption apparatus. Carbon dioxide is only one, albeit important, component of the attractiveness of humans.

For *Ma.uniformis*, however, CO_2 is apparently one of the more important cues for locating hosts. The response to whole human odour was never significantly greater than to the man-equivalent dose of CO_2 and there was a steep dose-response curve for CO_2. *Cx quinquefasciatus* and *An.pharoensis* were also attracted to CO_2 alone, but appreciably so only when there was at least a man-equivalent dose.

Because more *An.pharoensis* were attracted to the CO_2-baited trap than to the man-baited trap (experiment 2), it is tempting to suggest that the human odours or other physical gradients generated by the live host inhibited or repelled mosquitoes from the man-baited trap, since the CO_2 concentration was the same in both. However, the dose of CO_2 used was just at the threshold of change in *An.pharoensis* responsiveness (Fig. 2b), where even small differences in CO_2 dose might be expected to give large differences in the catch between two CO_2-baited traps.

The response of *An.funestus* was less clear, in that it showed no preference in the choice test (experiment 1, small samples), and yet only about 40% of the man-baited OBET catch was accounted for by CO_2 (experiment 2, adequate samples). *An.funestus* was not attracted to CO_2 on its own in sufficient numbers to analyse the dose response (partly due to the relatively low population densities prevailing during the first half of our investigation).

Overall, these results reveal aspects of the response to host cues leading *An.gambiae s.l.* (and *An.funestus*) to be anthropophilic and the other species to be more generalist feeders. Only *An.gambiae s.l.* 'chose' man over CO_2 in a direct choice test and no amount of CO_2 attracted more *An.gambiae s.l.* than a man. *An.gambiae s.s.* were attracted more than *An.arabiensis* by the man in the choice test whereas, for the other three species caught, the presence of man had no effect on the choice test and higher doses of CO_2 caught more than a man in an HBC. One might speculate that, for such species, the number approaching a human host is limited mainly by the amount of CO_2 produced by the person. Moreover, the dose–response relationship suggests that the amount of CO_2 released by a host may be one of the chief sources of variability in the number of mosquitoes approaching it; Schofield & Sutcliffe (1996) found similar effects for *Simulium* blackflies. *An.arabiensis* 'chose' the CO_2-baited trap with a higher probability than did *An.gambiae s.s.*, and the proportion of *An.arabiensis* was greater in CO_2-baited OBETs than in HBCs, supporting the view that *An.gambiae* is the more anthropophilic of these two sibling species.

It is not possible to determine from these experiments the basis for differences in the response to CO_2 between the species. The response variable was simply the number of individuals trapped in OBETs (and not escaping before dawn), without any indication of which behavioural repertoires led them to being trapped. In the choice test experiment, for example, we cannot tell whether a significant 'choice' was due to differences in trap-entry behaviour or to specific orientation factors. Dose responses in the field reflect both changes in the number responding, and in the strength of response of individual mosquitoes. Increases in catch with dose may be due, *inter alia*, to increases in the size of the CO_2 plume (i.e. the active space), or to an increase in the strength of the stimulus allowing insects to orient toward the source more effectively, or a combination of these factors.

With the exception of *An.gambiae s.l.*, there was no indication (within the range of doses tested) of a plateau in the response to carbon dioxide alone on the log–log scale, as shown for tsetse flies by Torr (1990). Such an effect might be expected at higher doses for several reasons: there may be limits to the efficiency with which mosquitoes can navigate odour plumes, or CO_2 may become repellent and even anaesthetic at very high concentrations. The asymptotic pattern of the *An.gambiae s.l.* response (Fig. 1a and equation 1) may be due to extraneous factors, such as a density-dependence in the escape rate from the OBET, or to a more significant specific difference in its response to CO_2.

Acknowledgments

Field work of this study was based at the Centre National de Lutte contre le Paludisme (CNLP), Ouagadougou, sponsored by the Ministry of Health of Burkina Faso and the Programma di Assistenza Tecnica of the Direzione Generale per la Cooperazione allo Sviluppo of the Italian Ministry of Foreign Affairs. We are deeply indebted to the personnel of these organizations, especially to the CNLP director, Dr L. Lamizana, and to those who participated in the labour-intensive mosquito collections. We are particularly grateful to Dr S. Young for his constant support and advice with electronics, and for designing the fan speed regulator circuit. Drs D. R. Hall, A. Cork and S. J. Torr kindly provided us with the carbon dioxide gas monitor and gave very useful suggestions. The skilful technical collaboration of Mrs S. Bagalino and G. Croce for the PCR analysis is highly acknowledged. This work is part of the project 'Behavioural studies on malaria vectors' funded by the European Community under contract Nos. TS3-CT92-0101 and TS3-CT91-0032.

References

Bos, H.J. & Laarman, J.J. (1975) Guinea pig, lysine, cadaverine and estradiol as attractants for the malaria mosquito *Anopheles stephensi*. *Entomologia Experimentalis et Applicata*, **18**, 161–172.

Brouwer, R. (1960) Variations in human body odour as a cause of individual differences of attraction for malaria mosquitoes. *Tropical and Geographical Medicine*, **2**, 186–192.

Cochran, W.G. & Cox, G.M. (1957) *Experimental Designs*, 2nd edn. Wiley, New York.

Coluzzi, M., Petrarca, V. & Di Deco, M.A. (1985) Chromosomal inversion intergradation and incipient speciation in *Anopheles gambiae*. *Bollettino di Zoologia*, **52**, 45–63.

Coluzzi, M., Sabatini, A & Petrarca, V. (1975) Chromosomal investigations on the species A and B of the *Anopheles gambiae* complex in the Garki Project (Kano State, Nigeria): results of species identification from 1971–1974. Unpublished document MPD/TN/75.1, World Health Organization, Geneva.

Costantini, C., Gibson, G., Brady, J., Merzagora, L. & Coluzzi, M. (1993) A new odour-baited trap to collect host-seeking mosquitoes. *Parassitologia*, **35**, 5–9.

Coz, J. (1973) Contribution à la biologie du complexe *Anopheles gambiae* Giles en Afrique Occidentale. *Cahiers O.R.S.T.O.M., Série Entomologie Médicale et Parasitologie*, **11**, 33–40.

Darriet, F., Robert, V. & Carnevale, P. (1985) Evaluation en phase II, dans la station expérimentale de Soumousso (Burkina Faso) de l'efficacité sur les vecteurs du paludisme de deux insecticides: l'OMS-3002 à 0.1 g/m^2 et l'OMS-3004 à 0.1 g/m^2 en aspersions intradomiciliares de cases Bobo et Mossi. *Document Technicale de l'OCCGE*, **No. 8679**, 28pp.

Daykin, P.N., Kellogg, F.E. & Wright, R.H. (1965) Host finding and repulsion of *Aedes aegypti*. *Canadian Entomologist*, **97**, 239–263.

Edwards, F.W. (1941) *Mosquitoes of the Ethiopian Region. III. Culicine adults and pupae*. British Museum (Natural History), London.

Gillies, M.T. (1980) The role of carbon dioxide in host-finding by mosquitoes (Diptera: Culicidae): a review. *Bulletin of Entomological Research*, **70**, 525–532.

Gillies, M.T. (1988) Anopheline mosquitos: vector behaviour and bionomics. *Malaria Principles and Practice of Malariology* (ed. by W. H. Wernsdorfer and I. McGregor), pp. 453–485. Churchill Livingstone, New York.

Gillies, M.T. & Coetzee, M. (1987) *A Supplement to the Anophelinae of Africa South of the Sahara (Afrotropical Region)*. Publications of the South African Institute for Medical Research, **No. 55**, 143pp.

Gillies, M.T. & Wilkes, T.J. (1969) A comparison of the range of attraction of animal baits and of carbon dioxide for some West African mosquitoes. *Bulletin of Entomological Research*, **59**, 441–456.

Gillies, M.T. & Wilkes, T.J. (1970) The range of attraction of single baits for some West African mosquitoes. *Bulletin of Entomological Research*, **60**, 225–235.

Gillies, M.T. & Wilkes, T.J. (1972) The range of attraction of animal baits and carbon dioxide for mosquitoes: studies in a freshwater area of West Africa. *Bulletin of Entomological Research*, **61**, 389–404.

Gillies, M.T. & Wilkes, T.J. (1974) The range of attraction of birds as baits for some West African mosquitoes (Diptera: Culicidae). *Bulletin of Entomological Research*, **63**, 573–581.

Hamon, J. (1963) Les moustiques anthropophiles de la région de Bobo-Dioulasso (République de Haute-Volta): cycles d'agressivité et variations saisonnières. *Annales de la Société Entomologique de France*, **132**, 85–144.

Hamon, J., Coz, J., Adam, J.P., Holstein, M., Rickenbach, A., Brengues, J., Subra, R., Sales, S. & Eyraud, M. (1966) Contribution à l'étude de la répartition des *Anopheles* en Afrique Occidentale. *Cahiers O.R.S.T.O.M., Série Entomologie Médicale*, **4**, 13–70.

Kellogg, F.E. & Wright, R.H. (1962) The guidance of flying insects. V. Mosquito attraction. *Canadian Entomologist*, **94**, 1009–1016.

Khan, A.A. & Maibach, H.I. (1966) Quantitation of effect of several stimuli on landing and probing by *Aedes aegypti*. *Journal of Economic Entomology*, **64**, 442–443.

Knols, B.G.J. (1996) Odour-mediated host-seeking behaviour of the Afro-tropical malaria vector *Anopheles gambiae* Giles. Doctoral thesis, University of Wageningen.

Knols, B.G.J., De Jong, R. & Takken, W. (1994a) Trapping system for testing olfactory responses of the malaria mosquito *Anopheles gambiae* in a wind tunnel. *Medical and Veterinary Entomology*, **8**, 386–388.

Knols, B.G.J., Takken, W. & De Jong, R. (1994b) Influence of human breath on selection of biting sites by *Anopheles albimanus*. *Journal of the American Mosquito Control Association*, **10**, 423–426.

Laarman, J.J. (1955) The host-seeking behaviour of the malaria mosquito *Anopheles maculipennis atroparvus*. *Acta Leidensia*, **25**, 1–144.

Majori, G., Sabatinelli, G. & Coluzzi, M. (1987) Efficacy of permethrin-impregnated curtains for malaria vector control. *Medical and Veterinary Entomology*, **1**, 185–192.

Merzagora, L. (1993) Variazioni ecologiche ed epidemiologia della malaria in una zona di savana sudanese presso Ouagadougou, Burkina Faso. Ph.D. thesis. Università di Roma 'La Sapienza'.

Omer, S.M. (1979) Responses of females of *Anopheles arabiensis* and *Culex pipiens fatigans* to air currents, carbon dioxide and human hands in a flight-tunnel. *Entomologia Experimentalis et Applicata*, **26**, 142–151.

Payne, C.D. (ed.) (1987) *The GLIM® System Release 3.77 Manual*, 2nd edn. Numerical Algorithms Group Ltd, Oxford.

Ralisoa Randrianasolo, B.O. & Coluzzi, M. (1987) Genetical investigations on zoophilic and exophilic *Anopheles arabiensis* from Antananarivo area (Madagascar). *Parassitologia*, **29**, 93–97.

Reeves, W.C. (1953) Quantitative field studies on a carbon dioxide chemotropism of mosquitoes. *American Journal of Tropical Medicine and Hygiene*, **2**, 325–331.

Schofield, S.W. & Sutcliffe, J.F. (1996) Human individuals vary in attractiveness for host-seeking black flies (Diptera: Simuliidae) based on exhaled carbon dioxide. *Journal of Medical Entomology*, **33**, 102–108.

Schreck, C.E., Gouck, H.K. & Posey, K.H. (1972) The range of effectiveness and trapping efficiency of a plexiglass mosquito trap baited with carbon dioxide. *Mosquito News*, **32**, 496–501.

Scott, J.A., Brogdon, W.G. & Collins, F.H. (1993) Identification of single specimens of the *Anopheles gambiae* complex by the polymerase chain reaction. *American Journal of Tropical Medicine and Hygiene*, **49**, 520–529.

Service, M.W. (1993) *Mosquito Ecology: Field Sampling Methods*, 2nd edn. Elsevier Applied Science Publishers, London.

Snow, W.F. (1970) The effect of a reduction in expired carbon dioxide on the attractiveness of human subjects to mosquitoes. *Bulletin of Entomological Research*, **60**, 43–48.

Takken, W. (1991) The role of olfaction in host-seeking of mosquitoes: a review. *Insect Science and its Application*, **12**, 287–295.

Torr, S.J. (1990) Dose responses of tsetse flies (*Glossina*) to carbon dioxide, acetone and octenol in the field. *Physiological Entomology*, **15**, 93–103.

Van Thiel, P.H. (1947) Attraction excercée sur *Anopheles maculipennis atroparvus* par l'acide carbonique dans un olfactomètre. *Acta Tropica*, **4**, 10–20.

Van Thiel, P.H. & Weurman, C. (1947) L'attraction excercée sur *Anopheles maculipennis atroparvus* par l'acide carbonique dans l'appareil de choix II. *Acta Tropica*, **4**, 1–9.

White, G.B. (1974) *Anopheles gambiae* complex and disease transmission in Africa. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **68**, 278–301.

Accepted 31 January 1996