Chemical ecology of the behaviour of the filariasis mosquito *Culex quinquefasciatus* Say

Promotor: dr. J.C. van Lenteren
Hoogleraar in de Entomologie

Co-promotor: dr.ir. W. Takken,
Universitair Docent
Laboratorium voor Entomologie
Departement Plantenwetenschappen

Chemical ecology of the behaviour of the filariasis mosquito *Culex quinquefasciatus* Say

Leonard Ernest Gustavin Mboera

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PROPOSITIONS (STELLINGEN)

1. Contrary to previous assumptions, host seeking of *Culex quinquefasciatus* and *Anopheles gambiae*, at close range, is not random and this affects mosquito catch size, parity rate and hence the estimation of entomological inoculation rates.

This thesis.
Lines, J.D. *et al.*, 1991. *Bull Entomol. Res.* **81**, 77-84.
Mbogo, C.N.M. *et al.*, 1993. *J. Am. Mosq. Control Assoc.* 9, 260-263.
Davis, J.S. *et al.*, 1995. *Med. Vet. Entomol.* 9, 249-255.

2. The species *Culex quinquefasciatus* has the fascinating characteristic that it is ornithophilic in North America and anthropophilic in East Africa.

This thesis.
Tempelis, C.H. 1974. *J. Med. Entomol.* 11, 635-653.
White, G.B. 1971. *Trans. R. Soc.Trop. Med.* **65**, 819-829.
Beier, J.C. *et al.*, 1990. *J. Am. Mosq. Control Assoc.* **6**, 207-212.

3. The observation that *Culex quinquefasciatus* responds synergistically to oviposition pheromone and waterborne stimuli suggests that the pheromone enhances the reliability of the identification of a suitable breeding site.

This thesis.
Millar, J.G. *et al.*, 1994. *J. Am. Mosq. Control Assoc.* **10,** 374-379.

4. Assessing the transmission risk of malaria and bancroftian filariasis can be made more accurate by the use of odour-baited traps.

This thesis.
Macdonald, G. 1957. *The epidemiology and control of malaria.*

5. Urbanisation and industrialisation have brought *Culex quinquefasciatus* and man close together, thereby increasing disease problems.

6. Vectors and vector-borne diseases are keeping Africa green and are responsible for the natural maintenance of the continent's eco-systems.

7. Man is a victim of his own odour in malaria and bancroftian filariasis transmission in Africa.

Propositions with the thesis '**Chemical ecology of the behaviour of the filariasis mosquito *Culex quinquefasciatus* Say**'.

Leonard E.G. Mboera Wageningen, April 27, 1999.

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Preface

The time spent on preparation of this work constituted time taken from routine research activities and departmental administration, and I am extremely grateful for the extraordinary tolerance extended to me by my employer and colleagues. But of course, I have more than mere tolerance to applaud on Amani Medical Research Centre's part: several research scientists have been extremely encouraging and directly supportive throughout this effort. I would like to thank Yohana Matola and Kato Njunwa, former Centre's Directors, and Fred Salum, Williams Makunde, Martha Lemnge, Mathias Kamugisha, Robert Malima, William Kisinza and Julius Massaga for their word of encouragement and insight. Prof. Wen Kilama, former Director General, National Institute for Medical Research (NIMR) is thanked for his initiatives, support and encouragement in securing the PhD fellowship. Dr. Andrew Kitua, Director General, NIMR, is thanked for supporting the final part of this work. I am greatly indebted to them.

Some of the earliest work in this thesis was carried out while a collaborating scientist in the European Community (EC) funded project titled: *Behavioural studies on malaria vectors,* and I thank the EC for their support. Crucial to this work was the Ifakara Health Research and Development Centre (IHRDC) and Ubwari Field Station. For two years, I had the distinct honour of being a visiting research scientist to the IHRDC. I received thoughtful assistance from Dr. Andrew Kitua, the former Centre's Director, Edith Lyimo and Ikupa Akim, and the entire staff of the Centre. Japheth Kihonda, Aniseth Kihonda, Godwin Ulungi, Raphael Kapendamweni provided me with the excellent technical and field assistance that produced parts of this thesis. My stay in Namawala, Kikulukutu, Njage and Mchombe was a memorable one. Many colleagues at Ubwari Field Station assisted along the way, providing field and technical assistance. I am extremely grateful to Kasembe Mdira, Grace Chuwa, Mayunga Maega, Edward Sambu, Azael Kimambo, Abdallah Telaki, Stephen Mkongewa, Benjamin Chambika, Josephine Nyongole, Jane Kayamba, Joseph Myamba and the entire staff of the station. In addition, I would like to thank the villagers of Kikulukutu, Njage and Ubwari for their co-operation and provision of free access to their house premises and pit latrines.

I am especially indebted to Prof. Joop van Lenteren and Dr.ir. Willem Takken, first for accepting to be my promotor and co-promotor respectively, secondly for providing me with advice during my research work and writing of the thesis, and lastly for leaving me the freedom to conduct experiments as I found them appropriate. The two provided me with valuable comments on all the chapters. Willem, thank you very much for your continuous support in the laboratory, field and for your most useful advice at various stages of this work. I would also like to thank Profs. Chris Curtis and John Pickett, Dr. Philip McCall, Paul Lyakurwa, Said Mgenzi, Marieta Braks, G. Mmbuji and Fikirini Msuya for their substantial contributions toward the successful execution of this work. Dr. Dan Kline is thanked for the permission to adopt figure 1 in chapter 6.

Special gratitude are due to my colleague and friend, Dr.ir. Bart Knols, who assisted me throughout the study period, providing reading critiques, research guidance and crucial insight. Bart and Ingeborg, you were my hosts while in Holland

and Ifakara, and always ready to give a first hand assistance when I was stuck materially or morally. Bart, you have played a great role in refining some of the designs and ideas. You have reviewed all the chapters and publications resulting from this thesis despite your tight research schedule. Thank you very much. Indeed, I hope the collaboration we have had since 1993 shall continue, and the "Pwagu na Pwaguzi" company shall flourish throughout our research career.

Various people provided invaluable assistance during my laboratory work and stay in Wageningen. Leo Koopman, Frans van Angelen, André Gidding and Piet Huisman are thanked for making sure my mosquito colony was healthy and the olfactometer was well maintained. I am grateful to Piet Kostense for drawing most of the figures in this thesis. I thank Henk Snellen and Menno de Lind van Wijngaarden for taking me to various interesting parts of the Netherlands. Françoise, Nicolas and Daniel Takken, for making me feel welcome at their home. Kido Mtunda, Hawa Msham, Salim Al-Ismaily, Joseph Chata, Nelly Numbi, Jeremy Mowo, Joyce Shoo, Ephraim Senkondo and Richard Mukabana for turning miserable moments into joyful scenarios.

Anyone who has ever been involved in this kind of research work knows what a toll its execution and writing takes on friends and relatives. Thanks to all of you, for putting with me throughout this process, especially my parents, sisters and brothers. I particularly thank my wife, Haika Kafui, for working at an unusually frenetic pace in order to support me both psychologically and morally and for offering much appreciated encouragement. I wouldn't forget my sons, Jeremy and Noel who assisted me in some of the field experiments. Thank you very much for making sure that my experimental animals (calf and goat) were well fed throughout the study period.

This work was supported generously by the European Community, Wageningen Agricultural University, The Netherlands, The Tanzanian Health Research Training Fund and the Tanzanian National Institute for Medical Research. I am very grateful to these institutions.

Dedicated to Mringi, Mam and Riki.

Summary

Behavioural and chemical ecology of *Culex quinquefasciatus*

There are many similarities between host-location and oviposition behaviours of mosquitoes. Both require complex integration of physical and chemical cues by the searching mosquitoes. Long-range cues, probably involving vision, allow mosquitoes to identify different habitats, oviposition sites and specific host characteristics. Once an oviposition site or a host has been identified, short-range cues become increasingly important. Oviposition semiochemicals for some mosquito species have been identified and have shown considerable potential for both increasing the sensitivity for monitoring populations of culicine mosquitoes and for potential control programmes. Since most anthropophilic mosquitoes in tropical Africa seek their blood meals indoors at night, their host-location behaviour is thought to be regulated to a large extent by olfaction. Chemical orientation is, therefore, assumed to play an important role in resource-location behaviour of mosquitoes.

Culex quinquefasciatus is an important vector of urban bancroftian filariasis in the tropical world. Despite its public health importance, much of its olfactory mediated behaviour is poorly understood. Studies on resource-location behaviour, in particular the role of semiochemicals in its behaviour, are required to understand the relationship between the mosquito, its host and the surrounding environment to effectively control bancroftian filariasis. In this thesis the role of semiochemicals in the oviposition site-selection and host-location behaviour of *Cx quinquefasciatus* has been examined. The major findings can be summarised as follows:

Carbon dioxide chemotropism in mosquitoes

Carbon dioxide chemotropism in mosquitoes and its potential role in mosquito surveillance and management programmes is reviewed in Chapter 2. The responses of various mosquito species to carbon dioxide differ. However, the compound plays a role in activation, upwind anemotaxis, and selection of biting sites on hosts. Carbon dioxide has been used as a kairomone over the last four decades and its usefulness in mosquito surveillance, notably for North American and Australian nuisance mosquitoes and for arbovirus and filariasis vectors in endemic countries, is recognised. This has prompted the development of carbon dioxide-baited trapping systems for large-scale removal of nuisance mosquitoes. In spite of these successes, carbon dioxide does not appear to be a useful kairomone for the surveillance of Afrotropical anthropophilic vectors of malaria and bancroftian filariasis and little is known about its mode of action in this important group of mosquitoes.

Odour-mediated host-location behaviour of *Cx quinquefasciatus*

The olfactory responses of the host-seeking female *Cx quinquefasciatus* to various odour stimuli were studied in a dual-choice olfactometer (Chapter 3). The numbers of mosquitoes entering each olfactometer port were studied towards clean conditioned air (control), foot skin emanations (collected on polyamide stockings by wearing them), carbon dioxide (4.5%), moistened air and various combinations thereof.

Foot skin emanations were significantly more attractive than clean stockings. The mosquitoes were also significantly more attracted to foot skin emanations than to a clean stocking to which water was added. A moistened clean stocking, however, was slightly more attractive than a dry stocking. Carbon dioxide did not elicit higher responses than clean air, and no synergistic effect was observed in combination with foot skin emanations. These results present evidence that kairomones used during the host-seeking process by *Cx quinquefasciatus* are present in human emanations. Next, the preference of *Cx quinquefasciatus* between humans, and domestic animals, was investigated (Chapter 4). The response of the mosquito to the humans, cattle and goats was also compared to its response to carbon dioxide. The mosquito responded to man-baited tents in significantly larger numbers than to the calf- or goat-baited tents. The response of the mosquito to either a calf-baited tent or goat-baited tent was similar. The number of mosquitoes caught in a man-baited tent was significantly larger than that caught in a carbon dioxide baited tent. It was further found that the number of *Cx quinquefasciatus* responding to a tent baited with a calf or a goat was not significantly different from the number of mosquitoes responding to a carbon dioxide-baited tent. It can be concluded that with equal availability of the three vertebrates, *Cx quinquefasciatus* would respond significantly more to cues from human hosts than from either a calf or a goat. It is also likely that carbon dioxide is one of the major cues from a goat or a calf, to which the mosquito responds.

Furthermore, the response of *Cx quinquefasciatus, An. gambiae s.l.* and *An. funestus* to human odour was compared with that to carbon dioxide in the field (Chapter 5). Human odour, pumped from an underground pit into a bed net, attracted a similar number of *Anopheles* mosquitoes as a bed net occupied by a man. Significantly fewer mosquitoes were caught in a tent into which carbon dioxide at human equivalent release rates was pumped, than in a human odour-baited tent. Similarly, human odour attracted a larger number of host-seeking *Cx quinquefasciatus* than carbon dioxide. The parity rate of *Cx quinquefasciatus* responding to human odour was similar to that of mosquitoes responding to carbon dioxide. It can be concluded that in the indoor situation, human body odour other than carbon dioxide is the principal cue to which these mosquito species respond and that the physical cues from a host and carbon dioxide, when used as a kairomone on its own, account for a minor part of the overall attractiveness of man.

Finally, skin emanations and various specific organochemical compounds known to attract other haematophagous Diptera were tested for *Cx quinquefasciatus* in the field (Chapter 6). It was found that *Cx quinquefasciatus* responded significantly more to a Counterflow Geometry (CFG) trap baited with human skin emanations than to the unbaited trap. Furthermore a larger number of *Cx quinquefasciatus* were caught in traps baited with carbon dioxide than in traps baited with either acetone, octenol or butyric acid. The combination of carbon dioxide and skin emanations resulted in an additive effect. However, the combination of carbon dioxide and octenol did not produce a synergistic response. These results indicate that under field conditions *Cx quinquefasciatus* can be sampled by traps baited with skin emanations and/or carbon dioxide.

Odour-mediated oviposition behaviour

The response of *Culex* mosquitoes to acetoxyhexadecanolide (a synthetic oviposition pheromone), emanations from soakage pit water and grass infusions was investigated in pit latrines (Chapter 7). Oviposition by *Cx quinquefasciatus* and *Cx cinereus* occurred more frequently in water treated with the synthetic oviposition pheromone than in untreated tap water. The biological activity of the synthetic oviposition pheromone was found to last for over 9 days. The pheromone, however, caused little oviposition away from known breeding sites. It was further found that the combination of synthetic oviposition pheromone and soakage pit water or grass infusions resulted in a synergistic response in the oviposition by *Cx quinquefasciatus, Cx cinereus* and *Cx tigripes.*

In Chapter 8, the oviposition and behavioural responses of *Cx quinquefasciatus* to 3-methylindole (skatole) were investigated under field conditions of Tanzania. The daily oviposition rhythm of *Cx quinquefasciatus* showed two peaks, the higher at twilight and the lower peak at dawn. *Cx quinquefasciatus* was found to oviposit more egg rafts at 10^{-5} μg/l skatole than at lower or higher concentrations. The oviposition response of gravid *Cx quinquefasciatus* to skatole was poor when compared to that to the synthetic oviposition pheromone. A blend of synthetic oviposition pheromone and skatole resulted into an additive oviposition response by *Cx quinquefasciatus.* The duration of activity of skatole lasted for about 7 days. The conclusion derived from this part of the thesis is that oviposition semiochemicals can be used as ovitraps or gravid mosquito traps to monitor *Cx quinquefasciatus* populations or to attract mosquitoes to sites treated with a biopesticide for vector control.

Odour-baited sampling systems

It was found that the efficiency of the widely used Centers for Disease Control (CDC) light-trap in sampling an indoor population of *Cx quinquefasciatus* and *An. gambiae* is affected by the position of the trap in relation to the human-baited bed net. Significantly higher catches were recorded for both species when the trap was positioned at the foot-end of the bed, near the top of the bed net. Parity rates were significantly higher near the top of the net than at the level of the host. In addition, infective *Cx quinquefasciatus* were caught in the trap positioned above the foot-end of the bed net (Chapter 9).

The efficiency of various trapping systems baited with carbon dioxide were compared in sampling outdoor populations of *An. gambiae* and *Cx quinquefasciatus* (Chapter 10). The efficiency of the CFG trap was similar to that of electric nets (ENT). Both CDC traps with light or without light were less efficient in collecting *An. gambiae* or *Cx quinquefasciatus* outdoors. It is possible, therefore, to collect the two mosquito species outdoors with CFG traps or ENT baited with carbon dioxide. Finally an assessment of traps baited with oviposition semiochemicals in sampling of gravid *Cx quinquefasciatus* is reported in Chapter 11. A CFG trap baited with either a synthetic oviposition pheromone, grass infusions or the combination of the two was found to be a

useful tool for collecting gravid *Cx quinquefasciatus* both indoors and outdoors. However, the proportion of gravid mosquitoes in the catches increased when the traps were placed away from mosquito emergence sites. In conclusion, the study shows that chemical ecology plays a significant role in the life cycle of *Cx quinquefasciatus* and this principle can be used to develop new control strategies.

Samenvatting

Chemische ecologie van het gedrag van *Culex quinquefasciatus*

Er bestaan veel overeenkomsten tussen gastheerzoekgedrag en ovipositiegedrag van muggen. Beiden vereisen een complexe integratie van fysische en chemische signalen bij de muggen. Muggen kunnen verschillende habitats, ovipositieplaatsen en gastheereigenschappen herkennen met behulp van signalen welke over grote afstand werkzaam zijn. Hierbij zijn waarschijnlijk ook visuele stimuli betrokken. Na herkenning van de gastheer of ovipositieplaats worden 'korte afstand' signalen in toenemende mate belangrijk. Voor enkele muggensoorten zijn ovipositie-specificieke signaalstoffen geïdentificeerd en deze kunnen worden ingezet bij de bemonstering van muggen populaties alsmede voor bestrijdingsprogramma's. Omdat de meeste antropofiele muggensoorten in Afrika haar bloedmaaltijden 's nachts zoeken in woonhuizen, wordt aangenomen dat het gastheerzoekgedrag voor een belangrijk deel gereguleerd wordt door olfactorische stimuli. Om bovengenoemde redenen speelt chemische oriëntatie dus een belangrijke rol bij het foerageergedrag van muggen.

Culex quinquefasciatus is een belangrijke vector van filariasis bancrofti in steden in de tropen. Ondanks het belang van deze mug voor de volksgezondheid is er weinig bekend over het door geurstoffen beïnvloedde gedrag. Onderzoek naar het foerageergedrag, in het bijzonder het gedrag beïnvloed door geurstoffen, van deze mug is nodig om de relatie te begrijpen tussen de mug, haar gastheer en haar omgeving voor een effectieve bestrijding van filariasis bancrofti. In dit proefschrift zijn de rol van signaalstoffen bij de selectie van ovipositieplaatsen en bij het gastheerzoekgedrag van *Cx quinquefasciatus* onderzocht. De belangrijkste resultaten worden hieronder samengevat.

Koolzuur chemotropie bij muggen

Een overzicht van de chemotropie bij muggen onder invloed van koolzuur en de rol van koolzuur bij de bemonstering en bestrijdingsprogramma's wordt gegeven in Hoofdstuk 2. Tussen muggensoorten kan de reactie op koolzuur verschillen. Dit gas speelt een rol bij de activatie, windopwaartse anemotaxis en de keuze van een steekplaats op de gastheer. Gedurende de afgelopen 40 jaar is koolzuur veelvuldig toegepast als een kairomoon, en het nut van dit gas wordt algemeen erkend voor de bemonstering van muggen, met name van soorten die overlast veroorzaken in de Verenigde Staten van Amerika en Australië, alsmede van de vectoren van arbovirussen en filariasis in endemische gebieden. Vangtechnieken, gebaseerd op lokvallen voorzien van koolzuur, zijn ontwikkeld voor het op grote schaal wegvangen van steekmuggen. Ondanks deze successen blijkt koolzuur geen geschikt kairomoon voor de bemonstering van antropofiele vectoren van malaria en filariasis bancrofti in Afrika. Er is weinig bekend over het werkingsmechanisme van koolzuur bij deze belangrijke groep van muggen.

Olfactorisch gastheerzoekgedrag van *Cx quinquefasciatus*

De olfactorische gedragingen van gastheerzoekende vrouwtjes van *Cx quinquefasciatus*, welke waren blootgesteld aan verschillende chemische stimuli, werden onderzocht in een windtunnel (Hoofdstuk 3). Het aantal muggen dat een poort van de windtunnel binnenvloog werd bestudeerd bij schone lucht (contrôle), vluchtige componenten afkomstig van de voet (verzameld op polyamide sokjes), koolzuur (4,5%), bevochtigde lucht en hun kombinaties. Vluchtige componenten van de voet waren aantrekkelijker dan schone sokjes. De muggen werden ook signifikant meer aangetrokken door voetcomponenten dan door een schoon sokje waaraan water was toegevoegd. Een vochtig sokje was echter aantrekkelijker dan een droog sokje. Koolzuur was niet aantrekkelijker dan schone lucht, en er was geen synergistisch effect van koolzuur en vluchtige componenten van de voet. De resultaten tonen aan dat vluchtige verbindingen van de mens kairomonen voor *Cx quinquefasciatus* bevatten welke gebruikt worden bij het gastheerzoekgedrag.

Vervolgens is de gastheervoorkeur van *Cx quinquefasciatus* voor de mens en landbouwhuisdieren onderzocht (Hoofdstuk 4). De aantrekking van de mug voor een mens, koe (kalf) en geit en voor koolzuur werd vergeleken. Signifikant grotere aantallen muggen werden gevangen in tenten waarin zich een mens bevond in vergelijking tot tenten met een kalf of geit. Het aantal muggen gevangen in een tent met een kalf of geit was gelijk. De tent waarin zich een mens bevond trok signifikant meer muggen aan dan een tent waarin zich een koolzuurbron bevond. Het aantal muggen dat een tent binnenvloog waarin zich een kalf of geit bevond was gelijk aan dat wat gevangen werd bij koolzuur alleen. Geconcludeerd wordt dat, bij gelijke beschikbaarheid van de drie vertebraten, *Cx quinquefasciatus* sterker zal worden aangetrokken door de signaalstoffen van de mens dan die van een kalf of geit. De data suggereren ook dat koolzuur de belangrijkste signaalstof van een kalf of geit is waarop de mug reageert.

Ten derde is de aantrekking van *Cx quinquefasciatus*, *Anopheles gambiae s.l.* en *An. funestus* voor menselijke geuren vergeleken met die voor koolzuur in een veldsituatie (Hoofstuk 5). Menselijke geuren, welke vanuit een ondergronds hol gepompt werden in een bovengrondse klamboe in een tent, trokken een gelijk aantal Anopheles muggen aan als een mens onder een klamboe. Koolzuur, vrijgelaten vanuit een tent in een concentratie en volume gelijk aan die van een mens, trok signifikant minder muggen aan dan de geuren van een mens. Eveneens was het aantal *Cx quinquefasciatus* aangetrokken door koolzuur minder dan bij de geuren van een mens. Het percentage *Cx quinquefasciatus* dat eerder eitjes had afgezet en op menselijke geuren reageerde was gelijk aan het percentage dat reageerde op koolzuur. Uit deze experimenten wordt geconcludeerd dat, binnenshuis, menselijke geuren het voornaamste signaal zijn waarop deze muggen reageren en dat de fysische gastheer stimuli alsmede koolzuur slechts een beperkte rol spelen onder de stimuli welke de aantrekkelijkheid van een mens voor deze muggen bepalen.

Als laatste zijn menselijke huidstoffen en een aantal organische verbindingen welke aantrekkelijk zijn voor andere haematofage Diptera onderzocht op hun aantrekkelijkheid voor *Cx quinquefasciatus* in een veldsituatie (Hoofdstuk 6). *Cx* *quinquefasciatus* werd meer aangetrokken door een 'Counterflow Geometry' val (CFG) van waaruit vluchtige humane huidverbindingen werden afgegeven dan van een geurvrije CFG val. Ook werd een groter aantal *Cx quinquefasciatus* gevangen in vallen met koolzuur als lokstof dan in vallen met aceton, 1-octen-3-ol of boterzuur. De kombinatie van koolzuur en vluchtige huidverbindingen als lokstof resulteerde in een additief effect. Er was geen extra effect bij een kombinatie van koolzuur en 1-octen-3-ol. De resultaten tonen aan dat, in een veldsituatie, *Cx quinquefasciatus* gevangen kan worden in lokvallen voorzien van humane huidverbindingen en/of koolzuur.

Olfactorisch ovipositiegedrag

De aantrekking van *Culex* soorten tot acetoxyhexadecanolide (een synthetisch ovipositieferomoon), en vluchtige verbindingen uit organisch verrijkt water of water met rottend gras, werd bestudeerd in latrines (Hoofdstuk 7). *Cx quinquefasciatus* en *Cx cinereus* legden vaker eitjes in water waarin het feromoon was opgelost dan in schoon kraanwater. De biologische aktiviteit van het feromoon strekte zich uit over meer dan 9 dagen. In waterreservoirs buiten de reeds in gebruik zijnde broedplaatsen van *Cx quinquefasciatus* veroorzaakte het feromoon weinig ovipositie. De combinatie van het feromoon en organisch verrijkt water of water met rottend gras veroorzaakte een synergistische ovipositie respons in *Cx quinquefasciatus*, *Cx cinereus* en *Cx tigripes*.

Hoofdstuk 8 beschrijft het onderzoek naar de rol van 3-methylindol (skatol) bij de ovipositie en het gedrag van *Cx quinquefasciatus* onder veldomstandigheden in Tanzania. Het dagelijkse ovipositie ritme van *Cx quinquefasciatus* heeft twee maxima, de hoogste bij zonsondergang, en de tweede bij zonsopgang. Een optimum eileg werd gevonden bij 10^{-5} µl/l skatol. De eileg van eidragende *Cx quinquefasciatus* bij skatol was laag vergeleken met die bij het synthetisch ovipositieferomoon. Een mengsel van skatol en het feromoon resulteerde in een additief effect. De overblijvende aktiviteit van skatol bedroeg ongeveer 7 dagen. Uit deze onderzoekingen wordt geconcludeerd dat signaalstoffen welke een rol spelen bij de eileg van *Cx quinquefasciatus* gebruikt kunnen worden in eilegvallen of vallen voor eidragende muggen voor het meten van populatiedichtheden of om muggen te lokken naar plaatsen behandeld met een biopesticide voor vector bestrijding.

Lokvallen voor het meten van populatiedichtheden

Uit het onderzoek (Hoofdstuk 9) bleek dat de efficiëntie van de algemeen gebruikte CDC lichtval voor het bemonsteren van populaties van *Cx quinquefasciatus* en *An. gambiae* wordt beinvloedt door de plaats van de muggenval in relatie tot de klamboe (waaronder zich een mens als geurbron bevindt) welke naast de val is opgehangen. Een val opgehangen aan het voeteneinde van het bed en aan de bovenrand van de klamboe, ving significant meer muggen van beide soorten. Het percentage muggen dat reeds eitjes gelegd had was ook significant hoger in de hoger opgehangen vallen dan in vallen op dezelfde hoogte als de gastheer. De val welke] opgehangen was boven het voeteneinde van het bed ving ook *Cx quinquefasciatus* welke met *Wuchereria bancrofti* besmet was.

De efficiëntie van verschillende vangsystemen, met koolzuur als lokstof, en buitenshuis, werd vergeleken met vangsten van *An. gambiae* en *Cx quinquefasciatus* (Hoofdstuk 10). De efficiëntie van de CFG val was vergelijkbaar met die van geëlectrificeerde vallen (ENTs). Twee CDC vallen, met en zonder licht, waren minder efficient voor het bemonsteren van *An. gambiae* en *Cx quinquefasciatus* dan de CFG en ENT vallen. Buitenshuis kunnen beide muggensoorten dus gevangen worden met de CFG val of met de ENT val met koolzuur als lokstof.

Als laatste is een vergelijkende studie gedaan naar eilegvallen voorzien van ovipositiesignaalstoffen (Hoofdstuk 11). Het bleek dat de CFG val, met het synthetisch eilegferomoon, of met water voorzien van rottend gras of met een combinatie van beide als lokstof, zeer bruikbaar was voor het vangen van eidragende *Cx quinquefasciatus*, zowel binnen als buiten. Het aandeel van de eilegdragende vrouwtjes steeg sterk naarmate de val op grotere afstand van een broedplaats geplaatst was.

Uit het onderzoek wordt geconcludeerd dat de chemische ecologie een zeer belangrijke rol speelt in de levenscyclus van *Cx quinquefasciatus* en dat deze kennis gebruikt kan worden voor de ontwikkeling van nieuwe strategiëen voor de bestrijding van deze mug.

1

General introduction

1. 1. Distribution of *Culex quinquefasciatus*

Culex quinquefasciatus Say 1823 (Diptera: Culicidae) is one of the major vectors of bancroftian filariasis in the world (White, 1971a; Subra, 1981). In addition, it frequently causes considerable annoyance to people exposed to its constant biting. The mosquito is widely distributed in tropical and subtropical areas, and is the third most commonly distributed mosquito in the world. In most tropical areas *Cx quinquefasciatus* has become established in towns and areas where urban life has led to the creation of its favourite larval breeding places. Rapid urbanisation in Africa, for example, has led to public health problems related to water supply, drainage and inadequate disposal of sewage and refuse. The resulting increase of stagnant polluted water in urban areas has multiplied the number of breeding sites suitable for *Cx quinquefasciatus* (Cairncross *et al.*, 1988). Thus *Cx quinquefasciatus* is the dominant if not the sole species of mosquito in most of the urban areas of West and Central Africa. In East Africa, in addition to urban areas, *Cx quinquefasciatus* is also very abundant in a number of rural localities (Nelson *et al.*, 1962; White, 1969; Subra, 1981; Beier *et al.*, 1990; Mosha *et al.*, 1992; Njau *et al.*, 1993; Mboera *et al.*, 1997a; Wakibara *et al.*, 1997).

1. 2. Taxonomy of *Culex quinquefasciatus*

Cx quinquefasciatus is a member of the *Culex pipiens* complex, which includes among others *Cx pipiens* Linnaeus *sensu stricto (s.s.).* The mosquitoes *Cx quinquefasciatus* and *Cx pipiens* are generally regarded as a pair of sister species or subspecies, the main members of the cosmopolitan *Culex (Culex) pipiens* group or species complex, collectively known as *Cx pipiens sensu lato (s.l.).* Until the 1980s *Cx quinquefasciatus* was often called *Cx fatigans* Wiedemann 1828, a junior synonym (Belkin, 1977). *Cx quinquefasciatus* (redescribed by Sirivanakarn & White, 1978) is an urban pest in the tropics, breeding perennially in polluted water. *Cx pipiens* (redescribed by Harbach *et al.*, 1985) is essentially a temperate Holarctic mosquito, although it occurs in highland areas of eastern Africa, at lower altitudes in southern Africa, and in southern Argentina, Australia and Asia (Service, 1993a). Over much of *Cx pipiens s.s.* geographical range, it feeds mainly on birds (ornithophagic) and mates in nuptial flight (eurygamous), although man-biting stenogamous populations are present in other areas (Service, 1986). *Cx pipiens* is the type-species of the genus *Culex* L., from which the family name Culicidae is derived (Edwards, 1932). Typical *Cx pipiens s.s.* is not anthropophilic, but biotype *Cx pipiens* form *molestus* Forskal 1775 (redescribed by Harbach *et al.*, 1984) is a widespread man-biting nuisance mosquito characterised by an autogeny (production of the first batch of egg without a blood meal) and stenogamy without diapause. Intergradation between *pipiens* and *quinquefasciatus* occurs in some hybrid zones, e.g. around the Madagascan plateau (Urbanelli *et al.*, 1997) and in North America between latitude 36^0 and 39^0 North (Barr, 1957), but these two taxa remain generally distinct world-wide. Adults of *Cx quinquefasciatus* and *Cx pipiens* can usually be separated by the morphology of the male external terminalia. In *Cx quinquefasciatus* the dorsal arms of the phallosome are closely approximated, whereas in *Cx pipiens s.s.* they are widely divergent. Intermediate forms, however, occur. *Cx molestus* differs biologically from both species in being autogenous, but the male terminalia resemble those of *Cx quinquefasciatus* (Service, 1986).

1. 3. Bancroftian filariasis as a disease transmitted by *Culex quinquefasciatus*

In much of the urban areas of the tropics *Cx quinquefasciatus* is the principal vector of *Wuchereria bancrofti*, Cobbold (Nematoda: Onchocercidae) (White, 1971a; Subra, 1981). Studies in Tanzania have, in addition, shown that *Anopheles gambiae*, *An. arabiensis* and *An. funestus* are important vectors of the parasite in rural areas (White & Magayuka, 1969; White, 1971b; Mboera *et al.*, 1997a). Since most of the anopheline vectors are also the major vectors of malaria, it is not surprising that malaria and bancroftian filariasis control often go hand in hand (Mboera *et al.*, 1997a).

W. bancrofti is the causative agent of a chronic infection affecting mainly the lymphatic system of man. About 73 million people are infected with *W. bancrofti* globally and the disease occurs in most tropical regions of Latin America, Africa, Asia and the Pacific, extending into some subtropical regions (Service, 1993b). Despite some efforts to control the infection, the disease is considered to be the fastest spreading insect-borne disease of man in the tropical world (W.H.O., 1992). In some African countries such as Tanzania, bancroftian filariasis is of both medical and public health importance causing serious morbidity in the extensively highly endemic areas (Minjas & Kihamia, 1991). The disease causes a considerable economic drain of scarce health resources and it is therefore a major impediment to economic development in the developing countries.

The life cycle of the parasite involves man as the definitive host and mosquito as an intermediate host. *W. bancrofti* microfilariae, circulating in the peripheral blood of man, are ingested with a blood meal by a mosquito vector. In the mosquito midgut the parasites shed their sheaths. A proportion manage to penetrate the gut epithelium before the formation of the periotrophic membrane to reach the haemocoel through which they migrate to the thoracic flight muscles (O'Connor & Beatty, 1936). In the muscle, they undergo metamorphosis and change into short 'sausage' forms which then undergo two moults before developing into elongate third stage infective larvae in about two weeks. The mature third stage larvae leave the thoracic musculature and enter the haemocoel in which they move around, the majority accumulating in the head. They escape when the mosquito feeds by entering the labium and rupturing the labella. They are usually deposited in a drop of haemolymph and enter the skin through the puncture made by the feeding mosquito (Sasa, 1976). The larvae pass through the lymphatics where they mature to thread-like, 4-8 cm long adults and pass to regional lymphatic glands. After mating they remain in the glands where the females develop eggs and larvae which are released as microfilariae into the peripheral circulation (Peters, 1992).

It is estimated that 15,000-30,000 accumulated microfilariae are needed to develop a chronic and clinical case of bancroftian filariasis. An infective mosquito rarely contains more than four or five developing larvae, since an overburden would impair the ability of the mosquito to fly and hence to further the life of the parasite (Burgess & Cowan, 1993). In Africa, the microfilariae of *W. bancrofti* are nocturnally periodic, their densities in peripheral blood reaching a peak during the four hour period around midnight coinciding with the peak biting activity of the vectors. They are scanty or absent during the day light hours (Minjas & Kihamia, 1991).

Bancroftian filariasis is characterised by acute and chronic inflammation of the lymphatics. The disease processes result from the host's immune response to the presence of filarial worms in the lymphatics, which cause lymphatic blockage and chronic oedema of the affected parts (elephantiasis). Clinically it is manifested by recurrent fever and inflamed lymphatic vessels and glands, orchitis followed by hydrocele, or abscesses in limb lymphatics. Involvement of abdominal lymphatics may result in the passage of milky white urine (chyluria) or chylous ascites (Burgess & Cowan, 1993). In some populations a tropical pulmonary eosinophilia syndrome may be a feature. This is characterised by an abnormal immune response in the lungs which leads to cough and shortness of breath with eosinophilic lung infiltrates and peripheral blood eosinophilia (Burgess & Cowan, 1993).

Although morbidity due to filariasis is both acute and chronic, lesions in the latter are more overt. For example, in heavily infected villages of Tanzania, hydrocele was found to be the most important lesion affecting about 30-50% of adult males (McMahon *et al.*, 1979; Wegesa *et al.*, 1979). Elephantiasis of the legs and scrotum is the second commonest chronic lesion of the disease. Death due to bancroftian filariasis is rare, however, there is no reason to suppose that it does not occur. Most often death may probably follow the chronic sequel of the disease (Minjas & Kihamia, 1991). Moreover, suicide sometimes occurs because of severely deformed legs especially among young people.

In addition to bancroftian filariasis, *Cx quinquefasciatus* has been reported to play some role in the transmission of viral diseases of man and animals. In the United States of America, it contributes to the transmission of St. Louis encephalitis virus and other arboviruses (Reisen *et al.*, 1992). In southern Tanzania, the mosquito was found to be infected with Chikungunya virus during an epidemic that occurred in 1953 (Robinson, 1955). Twelve years later, hepatitis B virus was demonstrated in the mouth parts of these mosquitoes in Senegal (Wills *et al.*, 1976). *Cx quinquefasciatus* has also been incriminated as a vector of fowl pox virus (Lee *et al.*, 1958), West Nile virus and Rift Valley fever virus (W.H.O., 1978).

1. 4. Bionomics of *Culex quinquefasciatus*

Eggs. Cx quinquefasciatus eggs are laid in the form of a pear-shaped egg-raft which can float on the surface of water. At the time of laying they are whitish but they darken within a few hours. An average of 155 eggs per raft was observed by Subra (1981) in Kenya. In Dubai, Service (1986) observed an average of about 228 eggs per raft in egg-rafts collected from natural larval habitats. Recently in Northeast Tanzania, L.E.G. Mboera (unpublished data) has observed a mean range of 221-289 eggs per raft in different breeding habitats. Interestingly, significantly larger egg rafts (more eggs per raft) were collected in cattle water troughs contaminated with plant debris, than in soakage pits, builders' or septic tanks. In tropical areas hatching of eggs usually occurs one day after egg-laying.

Larvae and pupae. Usually the female and male larvae hatch simultaneously. However, the length of the larval stage in the males is shorter than in the females, while the reverse is true for the pupal stage. The larval stage lasts between six and eight days and pupal stage about 40 hours. The two main factors that regulate mosquito larval growth are nutrition and the temperature of the water in the breeding places (Clements, 1963).

Cx quinquefasciatus develops mainly in habitats containing highly polluted water rich in organic matter that the larvae can use for nourishment (Subra, 1981). The habitats can generally be divided into enclosed on-site sanitation structures and open breeding sites. The most common types of sanitation structures include pit latrines, which receive faeces and sometimes sullage water; soakage pits, which receive sullage water only; septic tanks which receive water and solids from flush toilets as well as sullage water and cesspits, serving as soakaway for water from septic tanks. The open breeding sites of *Cx quinquefasciatus* include: flooded grasslands, ditches or drains, water from bathrooms, leaking sanitation structures and rubbish pits (Chavasse *et al.*, 1995a). Although waste-water represents the main breeding sources, the larvae can develop in virtually any type of breeding place found in the human environment. Most breeding sites are of medium size, or small and the species can develop in either sunny or shady places.

In Rangoon, Myanmar (Burma), Self & Tunn (1970) found that concrete drains were the most important breeding site of *Cx quinquefasciatus*. Similar observations have been reported by Coene (1993) in Kinshasa. In the coastal areas of East Africa the same type of breeding places are found both in the towns and big villages. In Mombasa (Teesdale, 1959) and in smaller towns in Kenya (Wijers & Kiilu, 1978; Subra & Dransfield, 1984), pit latrines are the most common breeding places. A rather similar observation has been made in both Zanzibar (Mansfield-Aders, 1927; Maxwell *et al.*, 1990) and continental Tanzania (White, 1971a; Menu & Kilama, 1972; Bang *et al.*, 1975; Mosha *et al.,* 1992; Chavasse *et al.*, 1995a). Soakage pits also provide breeding places for *Cx quinquefasciatus* (Aders, 1917; Teesdale, 1959; Wijers *et al.*, 1978; Chavasse *et al.*, 1995a). In a recent assessment of *Cx quinquefasciatus* breeding sites in Dar es Salaam, Chavasse *et al.* (1995a) found that the mosquito breeds prolifically in septic tanks and cesspits. The greater productivity of septic tanks and cesspits was attributed to the fact that they are continuously wet, the water surface is less disturbed and scum is encountered less often. Wastewater from sisal factories is also suitable for the development of *Cx quinquefasciatus* larvae (Subra, 1981). Various containers and peridomestic breeding places can be colonised by *Cx quinquefasciatus* (Harris, 1942; Van Someren *et al.*, 1955; Beehler & Mulla, 1995). Otieno *et al.* (1988a) observed that in western Kenya the main breeding sites of *Cx quinquefasciatus* were concrete builders' pits, which are made to hold water during the construction of houses. These are left behind once building has been completed. Recently in Muheza and Tanga, Northeast Tanzania, the mosquito was observed to breed in cattle water troughs (Mboera *et al.*, 1997b; R.A. Shabani & L.E.G. Mboera, unpublished data). The species even develops in rice fields in some areas (Surtees, 1970; Chandler & Highton, 1975) and more in natural habitats such as banana leaf axil (Teesdale, 1941), or tree-holes (Van Someren *et al.*, 1955).

Adults. Adults emerge from the breeding sites in two peaks, one before sunset and the other, which is very marked, between 20.00 and 21.00 h (De Meillon & Sebastian, 1967). Before adults leave the breeding place, a resting phase of varying length occurs. This is followed by a mass departure at nightfall (Yasuno & Harinasuta, 1967). Mating takes place 26 to 48 hours after emergence or even later, mainly at dusk (Sebastian & De Meillon, 1967; Yasuno & Harinasuta, 1967). Usually females are fertilised before their first blood meal, but sometimes fertilisation occurs when the females have already taken blood (Yasuno & Harinasuta, 1967). In nature the period between emergence and the first blood meal is believed to be only about 48 hours (Subra, 1981).

1. 5. Adult behaviour

1. 5. 1. Flight behaviour

Lindquist *et al.* (1967) reported that some *Cx quinquefasciatus* travelled up to 1 km and a 'great number' up to 600 m during dispersion studies in Rangoon. In the same city, Self *et al.* (1971) later made a more or less identical estimate, with most mosquitoes flying less than 500 to 600 m from the point of release. However, evidence from mark-release studies in India showed that *Cx quinquefasciatus* was capable of flying up to 7 km in a densely populated rural area (Yasuno *et al.*, 1978). In Reunion, Hamon (1953, as cited by Subra, 1981) estimated that *Cx quinquefasciatus* could fly a distance of 8 km. Interestingly, Brooks *et al.* (1976) recorded a distance of 11 km, much greater than any known distance to-date. Generally it can be concluded that, in its classical environment, an inhabited region, *Cx quinquefasciatus* rarely flies more than a few hundred metres. Nonetheless, this distance does not represent potentialities in the species which, in an uninhabited area, can travel much further. The distance covered may be influenced by climatic conditions, and the vicinity of the potential hosts and breeding places. *Cx quinquefasciatus* may move faster and cover greater distances in the hot rather than cold season. The distance travelled by females seems to be related to their state of nutrition, unfed females covering greater distances (Subra, 1981).

1. 5. 2. Host-seeking behaviour

Host preference

Mosquitoes feed from a range of different host animals. Some species have developed a characteristic host preference, which may or may not be fixed. For most blood-sucking insects the spectrum of host choice differs between geographical regions and seasons and the proportions of available hosts (Lehane, 1991). Host preference is a poorly understood phenomenon mainly because much about the complex mixture of odours, emanating from animals that must underlie the ability of a blood-sucking insect to recognise and locate its preferred host, is still unknown (Davis & Friend, 1995). In most mosquito species, for instance, a definite genetically determined host preference is present. However, the preference shown by a particular species of mosquito for one vertebrate host or another is likely to be influenced by environmental conditions

(Takken, 1991). In cases of "selective" host preference, the insect may still feed on a non-preferred host if this is the only one present.

Cx quinquefasciatus has been described by many workers in Africa to be anthropophilic in its feeding behaviour (Thomas, 1956; Heisch *et al.*, 1959; White, 1971b; Chandler *et al.*, 1975; Beier *et al.*, 1990; Bϕgh *et al.*, 1998). The degree of anthropophily varies according to the place where female mosquitoes are collected. Catches made indoors provide a larger proportion of anthropophilic females than those out of doors (Lee *et al.*, 1954; Beier *et al.*, 1990). However, Smith (1961) and De Meillon & Sebastian (1967) reported that anthropophily predominated both in indoor and outdoor catches. The probability that a mosquito will feed on humans depends both on a function of host numbers and easy access to them (Laarman, 1955; Burkot, 1988; Gillies, 1988). If a mosquito prefers a human host a variety of factors will determine whether or not a particular individual is bitten (Burkot, 1988).

Host location

Location of a host is an essential part of the life of any insect. Phytophagous insects, for example, show specialised feeding habits. Host location in these insects consists of a sequence of behavioural responses to an array of stimuli associated with host and non-host plants. The phytophagous insects are equipped with sensory receptors enabling them to perceive these stimuli. Plant stimuli involved include visual, mechanical, gustatory, and olfactory characteristics (Ahmad, 1983; Visser, 1986). Likewise, host-seeking haematophagous insects recognise a variety of stimuli from prospective hosts which in turn activates their host-finding behaviour.

Host location is an integrated, but flexible, behavioural package which gathers momentum as the host is tracked down. Although a variety of host signals are used in host finding, information on what signals are used and the processes involved is, however, still fragmentary. The various behaviour patterns involved in host location have been conveniently divided into three phases by Sutcliffe (1987): (a) Appetitive searching - appetence initiates a series of behavioural responses that are likely to bring an insect into contact with stimuli derived from a potential host. Appetence is preceded by hunger, which in turn is influenced by the insect's physiological condition. (b) Activation and orientation - upon receipt of host stimuli the insect switches from behaviour patterns driven from within to oriented host location behaviour. This is driven by host stimuli which are of increasing variety and strength as the insect and host come closer together. (c) Attraction - the final phase, in which host stimuli are used to bring the insect into host's immediate vicinity, and in which the decision of whether or not to contact the potential host is made.

In insects, receptivity to the host varies over time and is mainly controlled by endogenous rhythms and physiological condition (Sutcliffe, 1987). Insects exhibit internally programmed circadian activity occurring over the 24 hour cycle (Saunders, 1982). In mosquitoes daily activity cycles have been demonstrated by Taylor & Jones (1969), Nayar & Sauerman (1971) and Jones (1976). The insects' spontaneous activity corresponds with normal periods of feeding activity in nature. While circadian rhythms modulate receptivity over the 24 hour cycle, hunger as determined by physiological state, operates over days or weeks (Jones & Gubbins, 1978).

Visual cues. Vision is an important stimulus in the activation and orientation of many blood-sucking insects (Allan *et al.*, 1987; Sutcliffe, 1987). It is most widely used by diurnal insects which live in open habitats (Lehane, 1991). There are two different types of visual information available to blood-sucking insects. The first set of information comes from the intensity contrast between the target and the background. The ease with which the target-background combination is seen by the insect also depends on the intensity (brightness) of the objects. The second set of information comes from colour. The role of vision in host-location behaviour is well documented in Simuliidae (Fredeen, 1961; Peschken & Thorsteinson, 1965; Bradbury & Bennett, 1974; Thompson, 1976; Browne & Bennett, 1980), Tabanidae (Allan & Stoffolano, 1986), and Muscidae (Pospisil & Zdarek, 1965; La Breque *et al.*, 1972). Vision, as well as being important in activating and orientating insects to the host, is also vital in the attraction of haematophagous insects and in their decision of whether and where to land. Generally, host-seeking insects prefer to land on dark, low intensity colours similar to those of many host animals.

Diurnal and possibly crepuscular mosquitoes may orient visually to their hosts (Allan *et al.*, 1987), but the role of vision in host seeking by night-biting mosquitoes is unclear and needs further studies. Vision may also be important in plant location (Davis & Friend, 1995). The response to coloured visual stimuli by mosquitoes has been studied by many workers. Some of the earlier observations showed that *Culex* and *Aedes* mosquitoes preferred dark, rather than light colours (Howlett, 1910; Ko, 1925; Brett, 1938; O'Gower, 1963; Browne & Bennett, 1981). Brett (1938) suggested that this preference for darker colours might be a response to surfaces with a low reflectance factor. Brown's (1951, 1954) observations on some mosquito species showed that the attractiveness of coloured cloths varied inversely with reflectivity or brightness between 475 and 625 nm wavelengths. The lower the reflectivity (i.e. the darker the colour), the more attractive the cloth. O'Gower (1963) found that the dominant stimuli affecting *Ae. aegypti* during the host-seeking phase were visual reflectance and appearance. Gilbert & Gouck (1957) observed that the darker shades attracted the most *Ae. aegypti* while the lighter shades attracted the most *Ae. taeniorhynchus*. The darker shades of the brighter colours (yellow, orange) and the lighter shades of the darker colours (blue) were the most attractive to *Ae. sollicitans*. Studies by Browne & Bennett (1981) showed that *Mansonia pertubans* preferred black, red and blue over white and yellow. This mosquito species showed substantial colour differentiation during both day and night. In a recent study, Mutinga *et al.* (1995) found that *An. gambiae* prefer to rest onto white surfaces, followed by yellow, black and red respectively in that order.

Insects have been observed to show preference for various shapes of targets. For example, different tabanid species select markedly different landing sites on cattle (Mullens & Gerhardt, 1979). This is probably visual recognition of the preferred site because flying insects show definite preferences for particular parts of complex inanimate targets and also for targets of particular shapes. Many Simuliidae and Culicidae show a preference for the extremities of a target (Fallis *et al.*, 1967; Browne & Bennett, 1981). A good example of the effect of shape on the alighting response is given in the five-fold increase in tsetse flies landing on horizontally elongated targets over the same target presented vertically (Vale, 1974). Given a target of the right shape, blood-sucking insects will often choose to alight at a colour or intensity border, or edge caused by the confluence of two angled planes (Fallis *et al.*, 1967; Turner & Invest, 1973; Bradbury & Bennett, 1974; Browne & Bennett, 1980; Allan & Stoffolano, 1986; Brady & Shereni, 1988). The size of the target may also be important in determining landing rates for tsetse flies (Hargrove, 1980).

The influence of the shape of an object on its role in the attraction of mosquitoes has not been extensively studied. Studies by Brown (1952) demonstrated the importance of contour in mosquito attraction. *Ae. cantator* and *Ma. pertubans* responded in greater numbers to cuboid rather than pyramidal targets, while *Ae. punctor* was taken in larger numbers from pyramids. All culicines differentially selected the end or projecting parts of the 3-dimensional rectangular targets. Other workers have reported mosquito preference for targets with protruding rather than flat surfaces indicating geometry to be of some importance (Kellogg & Wright, 1962). For many species of blood-sucking insect, the evidence on shape preference is conflicting. This is because in most cases orientation and attraction are often confused in these studies. Flies may well show a preference for a particular shape to orientate towards but, on close approach to the target, they are not attracted to it and sheer off (Lehane, 1991).

How widespread the use of movement detection is in the finding of hosts is unclear. Tsetse flies, males in particular, are drawn by large, dark, moving objects (Brady, 1972; Gatehouse, 1972; Vale, 1974). However, under field conditions, it seems that most tsetse drawn to moving objects mate rather than feed, while those drawn to stationary objects are almost exclusively interested in feeding, not mating (Vale, 1974; Owaga & Challier, 1985). Movement is also important for host finding in some mosquito species (Sippel & Brown, 1953).

Chemical cues. Chemical communication, a process by which information is exchanged between organisms by use of chemical stimuli, plays a very important role in the lives of many insects. Olfaction is considered to be the most universally employed mode of communication in insects (Lewis, 1984). The variety of chemical interactions that occur between organisms and their environment has prompted efforts to classify chemical stimuli according to the type of interaction. A semiochemical is a volatile compound that carries information from one organism to another. Semiochemicals are divided into two groups: a pheromone is a semiochemical that acts between individuals of the same species, i.e. the interaction is intraspecific, and an allelochemical is a semiochemical that acts between species, for interspecific interactions (Dusenbery, 1992; Lovett *et al.*, 1989). Allelochemicals are subdivided according to the advantage of the interaction, into allomones, kairomones, synomones and apneumones (Nordlund & Lewis, 1976). However, semiochemical terminology according to Nordlund & Lewis (1976) is based to a large extent on the origin of the compounds in specific interactions. It was Dicke & Sabelis (1988) who described infochemical terminology based on cost-benefit analysis. An infochemical is therefore a chemical that, in the natural context, conveys information in an interaction between two individuals, evoking in the receiver a behavioural or physiological response that is adaptive to either one of the interactants or to both. A pheromone is an infochemical that mediates an interaction between organisms of the same species in which the benefit is to the origin-related organism, to the receiver or to both. An allelochemical is defined as an infochemical that mediates an interaction between two individuals that belong to different species.

Studies on the chemical ecology of arthropods, particularly the phytophagous insects, have increased dramatically in recent years (Whittaker & Feeny, 1971; Barbier, 1979; Bell & Cardè, 1984; Cardè & Bell, 1995; Jackson & Morgan, 1993). In haematophagous Diptera, much published information on chemical ecology is available for *Glossina* species (see Vale, 1993; Willemse & Takken, 1994). Several types of semiochemicals have been described in this group of insects, particularly those involved in host-location behaviour.

It has been established that blood seeking mosquitoes use airborne olfactory cues produced by their host to orientate themselves to that host (Takken, 1991). According to Gillett (1979) odours may be the long distance signals guiding female mosquitoes to their vertebrate hosts. The mosquitoes then respond to other signals such as heat, moist air, and movement Laarman, 1955; Gillies, 1980, 1988; Takken, 1991; Eiras & Jepson, 1991, 1994).

Olfactory stimuli implicated in host location by haematophagous insects to date include carbon dioxide, lactic acid, acetone, butanone, 1-octen-3-ol (hereinafter octenol) and phenolic components of urine (Lehane, 1991). The role of various chemical compounds in host-seeking of mosquitoes has been comprehensively reviewed by Takken (1991) who concluded that the effectiveness of mosquito kairomones is dependent on species, release rate and age of the mosquito population. It is not clear, however, which precise behavioural processes these compounds exert on mosquitoes. Because of its significance in odour-mediated behaviour of mosquitoes, the role of carbon dioxide in mosquito behaviour is reviewed comprehensively in Chapter 2.

Physical cues. Heat is also an important host-seeking signal in most haematophagous insects, with high sensitivity to small gradients of temperature. As early as 1910, Howlett reported that a test-tube filled with hot water had a very strong attraction for females of *Aedes* mosquitoes. He concluded that heat is the dominating factor that guides mosquitoes to their hosts. However, Rudolfs (1922) suggested that it is heat in combination with other factors which is of special importance in the attractive action. The factor heat was further investigated thoroughly by Peterson & Brown (1951) who showed that the attraction of mosquitoes by a warm body depends upon stimulation by convection heat. Studies by Khan & Maibach (1966) and Khan *et al.* (1966) have shown that heat in the presence of carbon dioxide increases the landing responses of female *Ae. aegypti*. Furthermore, in a recent field study, Kline & Lemire (1995) demonstrated that heat significantly increases the collection of *Ae. taeniorhynchus, An. atropos*, and *Cx nigripalpus*. Little information, however, is available on the distance over which heat is effective. There are marked convection currents, with local thermal differences of 1^0C or more, at up to and beyond 40 cm from a human arm (Wright, 1968). However, moisture in a host-odour filament can carry thermal information over several metres, and the small temperature fluctuations associated with air compression waves resulting from movement of the animal host can be detected by warm and cold thermal receptor pairs on the mosquito's antennae over a distance of more than 3 m(Davis & Sokolove, 1975). Thermal convection currents arising from a host also carry host odours while creating a sufficient air flow for anemotaxis (Davis & Friend, 1995).

Moisture is an important factor in mosquito attraction (Brown, 1951; Laarman, 1955). It is thought to be necessary for alighting of mosquitoes on the host's body. Nonetheless, most of the studies on water vapour perception by blood-sucking insects have been physiological or structural (Altner & Loftus, 1985) rather than behavioural, and therefore the degree of involvement of water vapour in short-range orientation/attraction is unclear. When moisture has been reported as important it is usually as a synergistic agent with another stimulus. Recently, Takken *et al.* (1997a) showed that the behavioural responses of *An. gambiae s.s.* were modulated by small gradients in relative humidity. A significantly greater attraction to an odour source was found when relative humidity was greater than the background compared to equal or lower background relative humidity. Thermal convection, humidity gradient and olfactory cues are thus important for guiding mosquitoes to a human host and for eliciting landing behaviour (Clements, 1963; Gillies, 1980; Takken, 1991).

In general, visual and olfactory stimuli are the most important signals when the insect is still at some distance from the host and result in anemotactic and optomotor responses. Nearer the host different stimuli such as heat and moisture, become important (Lehane, 1991).

Biting behaviour

Night feeding man-biting mosquitoes bite man either inside dwellings (endophagy) or outside (exophagy). Sometimes certain individuals of the same species facultatively feed indoors and others outdoors. In Africa *Cx quinquefasciatus* are markedly endophagous (Van Someren *et al.*, 1958; Smith, 1961). The large majority of female *Cx quinquefasciatus* feeds at night and the peak of biting activity has been observed to occur around midnight (Smith, 1961; Subra, 1981). The proportion of endophagous females could however, vary according to locality. In Bobo Dioulasso, Subra (1972, as cited in Subra, 1981) observed that endophagy was more marked during the cold season, when the difference between lower and upper extreme temperatures was greater.

According to Self *et al.* (1969) *Cx quinquefasciatus* bites man mainly below the knee. However, recent findings by De Jong & Knols (1996) show that *Cx quinquefasciatus* bites randomly over the body, exhibiting a preference for body parts with low densities of eccrine sweat glands. In addition, it seems that the biting pattern of this mosquito is not influenced by exhaled breath (De Jong & Knols, 1996) which supports findings by Service (1993b) that carbon dioxide exerts limited attraction of this species. Different mosquito species exhibit different biting site preferences even on one and the same individual host (De Jong & Knols, 1996; Dekker *et al.*, 1998). The biting site preference is thought to be governed by mainly four factors viz., skin temperature, skin humidity, vision and body odours. Smith (1961) observed significant differences in catches of *Cx quinquefasciatus* between two individuals of the same race and between different races. Similarly, Knols *et al.* (1995) observed significant differences in attractiveness for *Cx quinquefasciatus* between individuals occupying tent-traps.

Most biting by *Cx quinquefasciatus* occurs after midnight (Brunhes, 1975; Van Someren *et al.*, 1958; Smith, 1961), the peak being between 01.00 h and 02.00 h (Brunhes, 1975). However, bites during the first part of the night have also been reported (Subra, 1981). This could be a result of a marked fall in temperature during the night, which lead to a decrease in number of females seeking a blood meal. Of endophagous females and only in the rainy season, a majority of parous individuals has been observed biting at the beginning and end of the night. This special behaviour of parous females during the rainy season might be connected with the oviposition rhythm (Subra, 1981).

1. 5. 3. ***Resting behaviour***

Some *Cx quinquefasciatus* females choose resting places inside dwellings, while others find shelters outdoors (Subra, 1981). Mosha *et al.* (1992) observed that *Cx quinquefasciatus* was one of the most abundant species of endophilic mosquitoes in some villages of northern Tanzania. The density of *Cx quinquefasciatus* resting inside houses has been observed to correlate with the number of sleepers. White (1969) reported that in the Muheza area of Northeast Tanzania, the average density of *Cx quinquefasciatus* in houses (with corrugated iron roof and smooth wall) was higher than in huts (with thatched roof and mud walls) and the increasing size of the dwellings was correlated with the rise in density.

Cx quinquefasciatus tends to fly out of houses more than out of huts before reaching the gravid condition. Similar observations have been reported in Phnom Pehn, Kampuchea by Kohn (1991). This author further found that in the localities having the character of a town *Cx quinquefasciatus* was more abundant in houses constructed from corrugated plate and in those of which the floor was situated 2-3.5 m above the ground. By contrast, in the locality of a village character, the mosquito was most numerous in brick houses with the floor at ground level. *Cx quinquefasciatus* is said to be especially common in damp houses (Edwards, 1942).

1. 5. 4. ***Oviposition behaviour***

Mosquito egg-laying has been defined as two distinct behaviours: preoviposition, which includes all of the behaviours involved in orientation of the gravid mosquito to an oviposition site, and oviposition, the actual deposition of eggs on the substrate. With few exceptions, oviposition flights of many species occur during twilight (Nielsen & Nielsen, 1953; Haddow & Ssenkubuge, 1962), thus most mosquito species lay eggs at dusk or during the night. Preoviposition and oviposition behaviours in mosquitoes are controlled by endogenous factors associated with mating and egg maturation, by exogenous cues associated with oviposition sites, and by inherent circadian rhythms (Isoe *et al*, 1995). Mosquito preoviposition and oviposition behaviours consist of stimulation to take flight, orientated upwind flight in response to attractants, assessment and sampling of a site, and finally, oviposition stimulation (Klowden & Blackmer, 1987; Bentley & Day, 1989). Oviposition site selection by mosquitoes is a critical factor in both survival and population dynamics and is therefore an essential part of the life history of all species.

The location and selection of an oviposition site involves visual, olfactory, and tactile response (Bentley & Day, 1989). Sometimes volatile chemicals produced by decomposition of organic debris are the principal attractants. For example, log-ponds are particularly attractive oviposition sites for *Cx tarsalis* and *Cx quinquefasciatus*, and water contaminated with chicken manure or rice-straw infusions are very attractive to *Cx pallens* (Service, 1993a). *Culex* mosquitoes deposit egg rafts directly on the water surface and use contact stimuli to evaluate water chemistry and other factors prior to oviposition (Bentley & Day, 1989).

Oviposition in *Cx quinquefasciatus* is mediated mainly by semiochemicals. Infusions of organic matter have long been reported as oviposition attractants for culicine mosquitoes (see Bentley & Day, 1989; Takken & Knols, 1999). *Ae. albopictus* (Hein, 1976; Holck *et al.*, 1988; Allan & Kline, 1995), *Ae. aegypti* (Gjullin *et al.*, 1965; Chadee, 1993), *Ae. triseriatus* (Holch *et al.*, 1988), *Cx nigripalpus* (Ritchie, 1984), *Cx pipiens* (Madder *et al.*, 1980; Reiter, 1986), *Cx quinquefasciatus* (Gjullin *et al.*, 1965; Reisen & Meyer, 1990; Millar *et al.*, 1992), *Cx restuans* (Madder *et al.*, 1980; Reiter, 1986), and *Cx tarsalis* (Reisen & Meyer, 1990) have been observed to be attracted to hay and grass infusions for oviposition. Millar *et al.* (1992) isolated from *Cynodon dactylon* L. infusion a mixture of five compounds which proved to be attractive to ovipositing *Cx quinquefasciatus*. The compounds included phenol, 4-methylindole, 4-ethylphenol, indole and 3-methylindole. However, 3-methylindole (skatole) accounted for most of the attraction.

Numerous other chemical attractants have been identified, and many are associated with high organic or bacterial content (Beehler *et al.*, 1994a,b). Substances that stimulated oviposition by *Cx quinquefasciatus* were produced in cultures of *Pseudomonas reptilivora* isolated from natural oviposition sites of this mosquito species (Ikeshoji *et al.*, 1967). Organically enriched temporary water sources produce large numbers of *Culex* mosquitoes (Beehler & Mulla, 1995). Recently in a laboratory experiment Beehler *et al.* (1994a) were able to identify proteinaceous hydrolysates which are attractive to ovipositing female *Cx quinquefasciatus*. These workers observed that lactalbumin hydrolysate and bacterial contaminants were attractive to gravid *Cx quinquefasciatus*.

Apart from semiochemicals produced in plant infusions and by micro-organisms, early behavioural observations showed that egg rafts attracted gravid females of *Cx quinquefasciatus*, *Cx tarsalis* and *Cx pipiens molestus* to lay at the same oviposition sites. There is increasing evidence that in many instances pheromones emanating from eggs, provide cues for oviposition site location of many culicine mosquitoes. The response is elicited by apical droplet material, *erythro*-6-acetoxy-5-hexadecanolide (acetoxyhexadecanolide). Acetoxyhexadecanolide, first isolated from egg rafts, has been found to be a true oviposition pheromone (Laurence & Pickett, 1985). A synthetic oviposition pheromone has been used to concentrate egg-laying of *Cx quinquefasciatus* within specific areas in oviposition sites in Kenya (Otieno *et al.*, 1988a). Following their laboratory studies Pile *et al.* (1991, 1993) suggested that at long range acetoxyhexadecanolide stimulates upwind flight of *Cx quinquefasciatus* towards the pheromone source. At close range it produces an orthokinetic reduction in flight speed. Moreover, at an oviposition site, the pheromone induces a higher proportion of visiting females to oviposit.

In the field, it is likely that there is an interaction between pheromones and other infochemicals in guiding gravid mosquitoes to a suitable oviposition site (McCall & Cameron, 1995). However, it is difficult to determine the relative importance of oviposition pheromones versus other stimuli (such as olfactory, physical and/or visual stimuli) to the overall behavioural response i.e. aggregated oviposition (Bentley & Day, 1989). Indicators of site suitability are probably more important in the initial selection, with specific aggregation pheromones operating subsequently (McCall & Cameron, 1995).

According to McCall & Cameron (1995) oviposition site selection by insects is crucial for maximising survival of their progeny. Aggregated oviposition may have several benefits for the aggregating species. An increase in numbers of eggs and larvae may provide physical protection. For *Culex* species, a large number of larvae may prevent the formation of scum on top of organically rich oviposition site. Aggregation may also reduce the effects of predation; fewer of the offspring of one individual female may be lost when diluted within a large population of eggs or immatures (Pulliam & Caraco, 1984). Pickett & Woodcock (1996) also suggest that, since *Cx quinquefasciatus* has evolved into an urban insect, to be near people it would run in the risk of trying to oviposit in sites that are mechanically disturbed. Thus, it employs the oviposition pheromone to establish whether an oviposition site is mechanically safe or not. The oviposition pheromone therefore, denotes the safety of the site as it is present only after the eggs mature.

Physiological factors such as nutritional status and insemination are known to influence mosquito oviposition behaviour. Prolonged exposure to sugar inhibits ovipositional behaviour in the laboratory (De Meillon *et al.*, 1967; Hudson, 1970). Insemination has also been observed to affect oviposition in the laboratory. In culicines matrone, a substance produced by the male accessory gland, is transferred to the female during copulation, rendering her refractory to further insemination (Craig, 1967). This substance is known to be an ovipositional stimulant in several mosquito species (Leahy & Craig, 1965; Hiss & Fuchs, 1972). The initiation of an ovipositional flight is also linked with environmental factors, especially rainfall, relative humidity, temperature, and wind speed.

The chemical cues discussed above are not the only factors that determine the selection of oviposition sites by gravid *Cx quinquefasciatus* females. The structure of the breeding places also plays an essential part. Other factors include: volume of water, distance between the surface of water in the breeding places and ground level, and the degree to which the opening into the breeding places was obstructed (see Subra, 1981). This author observed in Burkina Faso that for an equal surface area, breeding places with the most water attracted the largest number of ovipositions. The number of ovipositions was greater, the further below ground level and surface of the water. Female oviposited more readily in breeding places with an unobstructed entrance than in those in which it was obstructed.

Oviposition attractants have been effectively utilised in traps for monitoring a number of culicine species, particularly as they attract predominantly gravid females, the cohort of the population most likely to be infected and infectious (Millar *et al.*, 1992). Grass infusions, for example, have been used to enhance collections in CDC light traps (Reiter *et al.*, 1991), gravid female traps (Reiter, 1983; Reisen & Meyer, 1990) and ovitraps (Hock *et al.*, 1988; Kitron *et al.*, 1989).

1. 6. Population dynamics

In Burkina Faso, peak female densities of *Cx quinquefasciatus* in urban areas have been recorded in the rainy season and at the beginning of the dry season (Subra, 1981). In northern Nigeria, near Kaduna, peak densities occur at the beginning of the rainy season (Service, 1965). In Kenya, two periods of peak densities, during and after the rainy seasons, have been reported (Van Someren *et al.,* 1958; Teesdale, 1959; Wijers & Kiilu, 1978). In Northeast Tanzania *Cx quinquefasciatus* densities had two peaks corresponding to the two rainy seasons that occur in the area (White, 1971b), the peak densities during the lesser rainy season are usually from October to December. In northern Tanzania, Mosha *et al.* (1992) found that *Cx quinquefasciatus* was present throughout the year with the densities increasing with the rising water level in pit latrines following heavy rains.

Based on this information, it can be concluded that in Africa, there is a generally marked relationship between rainfall and the peak breeding periods. A single peak corresponds to a single rainy season as seen in West Africa while two peaks corresponds to the two rainy seasons observed in East Africa. One possible explanation for seasonal changes in the *Cx quinquefasciatus* population is that the climate may differentially affect other species competing with *Cx quinquefasciatus* for breeding resources. Competitor species such as *Cx cinereus* and *Cx decens* may have greater reproductive capacity than *Cx quinquefasciatus* during the times of the year more favourable for their own survival in the adult stage (Irving-Bell *et al.*, 1987).

The population density of *Cx quinquefasciatus* in many breeding places is affected by many physical, chemical and ecological factors. Competition in preferred breeding sites may have density-dependent effects leading to higher proportion of wet sites being infested as the less favourable sites become colonised. It appears that competition from other *Culex* species such as *Cx cinereus* and *Cx nebulosus* may lead to the replacement of *Cx quinquefasciatus* in on-site sanitation structures (Subra & Dransfield, 1984; Irving-Bell *et al.*, 1987). According to the former, given the regularity of the displacement of *Cx quinquefasciatus* by *Cx cinereus* each year, it would appear that this is a case of competition-mediated succession. *Cx quinquefasciatus* has the characteristics of an *r*-selected species (MacArthur & Wilson, 1967) being able to colonise sites immediately after flooding but unable to withstand competition from *Cx cinereus* when sites become suitable for that species. However, Irving-Bell *et al.*'s (1987) observations indicate that *Cx quinquefasciatus* displaced both *Cx cinereus* and *Cx decens,* the latter remaining as a co-breeder for longer than the former. It has been observed by many workers that larvae of *Cx quinquefasciatus* cohabit with larvae of other culicine mosquitoes such as *Cx cinereus*, *Cx nebulosus*, *Cx decens* and *Ae. aegypti*

(White, 1967; Subra & Dransfield, 1984; Irving-Bell *et al.*, 1987; Lyimo & Irving-Bell, 1988; Mboera *et al.*, 1997b; R.A. Shabani & L.E.G. Mboera unpublished data).

1. 7. Control of *Culex quinquefasciatus*

Chemical control

The preimaginal stages of *Cx quinquefasciatus* develop mainly in habitats containing highly polluted water, and the most appropriate and classical means of control are larviciding and/or environmental management. Until recently, control of *Culex* mosquitoes in some African countries depended primarily on larvicidal treatments with organophosphates, including diazinon, chlorpyrifos and chlorfenvinphos in pit latrines, septic tanks, drains and other polluted water sources (White, 1971b; Kolstrup *et al.*, 1981). In Tanzania, polluted water suitable for breeding of *Culex* species was treated with oil containing DDT or gamma-HCH until 1958 when malathion was recommended (Menu & Kilama, 1972; Bang *et al.*, 1973). However, in pit latrines, the presence of detergents emulsifies oil layers, and malathion was found to deteriorate rapidly in polluted water (White, 1971b). Recently, pyriproxyfen, a juvenile hormone mimic, was shown to be effective in controlling *Cx quinquefasciatus* when applied in open breeding sites in Dar es Salaam (Chavasse *et al.*, 1995b).

Extensive use of insecticides against *Cx quinquefasciatus*, applied to breeding sites contiguous to human habitations, has resulted in the development of resistance to chlorinated hydrocarbons, organophosphates and carbamates as well as to pyrethroids (Tang & Wood, 1986; W.H.O., 1986). Resistance to various organophosphates and carbamate (Georghiou *et al.*, 1966; Curtis & Pasteur, 1981; Amin & White, 1984, 1985; Yebakima *et al.*, 1995; Chandre *et al.*, 1997) as well as pyrethroid (Curtis *et al.*, 1984; Halliday & Georghiou, 1985; Magnin *et al.*, 1988; Amin & Hemingway, 1989; Chandre *et al.*, 1998) in this species is well documented. Detailed studies by Khayrandish & Wood (1993a, 1993b) have revealed that strains of *Cx quinquefasciatus* from Northeast Tanzania, show a moderate to very high level of resistance to organophosphorus compounds, carbamates and pyrethroids. As a consequence, insect growth regulators are now in use.

Residual house spraying against malaria mosquitoes has shown little impact in the control of *Cx quinquefasciatus.* Chavasse *et al.* (1995b) reported that the large number of *Cx quinquefasciatus* was a major obstacle to public acceptance of the initiative to control malaria vectors in Dar es Salaam, through house spraying and larviciding using fenitrothion. Although *Cx quinquefasciatus* was not the primary target of the control project, the persistence of nuisance-biting has made residents sceptical and dissatisfied with insecticide spraying (Stephens *et al.*, 1995).

One of the most commonly used methods of personal protection against mosquitoes is the use of bed nets. Bed nets provide passive protection from mosquitoes. Treatments of bed nets with a pyrethroid insecticide significantly improves this protection strategy. Insecticide impregnated bed nets act like traps that are baited with body odour emitted by the sleeper. Mosquitoes attracted to the bed net are killed when they land on the treated fabric. Nonetheless, there is a lack of reduction of *Cx quinquefasciatus* population observed in communities with widespread use of insecticide-treated nets. In a Tanzanian village, for instance, insecticide impregnated bed nets gave little reduction of indoor resting *Cx quinquefasciatus* (Magesa *et al.*, 1991). In addition, Curtis *et al.* (1996) observed that the mortality of *Cx quinquefasciatus* was strikingly less than that of *An. funestus* and *An. gambiae* with fabrics impregnated with cyfluthrin, deltamethrin, etofenprox, lambda-cyhalothrin and permethrin in the same district. Thus, although currently, impregnated bed nets are an important new tool recommended by the W.H.O. for malaria prevention, to be efficient, active community participation is required. In urban areas, without a significant reduction mosquito nuisance caused essentially by *Cx quinquefasciatus*, there is a risk that bed net programmes will be disregarded by the local community. The ineffectiveness of most insecticides against *Cx quinquefasciatus* presumably, is mainly associated with the general tolerance of the mosquitoes species to contact with insecticides (Brown & Pal, 1971; Hossain *et al.*, 1989) possibly due to the special empodium beneath *Culex* tarsi (Chandre *et al.*, 1998). In addition, it is possible that *Culex* mosquitoes are also more readily irritated than *Anopheles* and incline to leave insecticide-treated surfaces (Curtis *et al.*, 1996).

Protection from adult mosquitoes for limited periods can be obtained through the use of insect repellents applied to the skin of the head, neck, legs and arms. Mosquito coils are also often employed for personal protection and to reduce human-vector contact in many parts of Africa. Burning coils vaporise an insecticide that permeates closed rooms and provides primarily repellent effect. Esbiothrin mosquito coils have shown some promising efficacy against *Cx quinquefasciatus* in northern Tanzania (Mosha *et al.*, 1992).

Environmental management

Environmental management is probably the most effective way of controlling *Cx quinquefasciatus*. The mosquito may be controlled by prevention of stagnation in drains, by frequent cleaning and flushing together with mosquito proofing of septic tanks and pit latrines by various methods (Bang *et al.*, 1975; Curtis & Minjas, 1985). Among the major source of *Culex* species breeding in affluent urban areas are septic tanks and soakaways into which mosquitoes gain access through cracks, crevices, and faulty manholes covers. Timely repairs are essential, and constitute a more cost effective and more sustainable intervention than chemical larviciding.

Physical control

Culex and many other mosquito larvae breathe air through the water surface, hence oil and monomolecular films are used to coat the surface and thereby suffocate the larvae. However, these materials are biodegradable and require repeated applications in pit-latrines, cesspits, disused wells, flooded cellars etc.

Floating layers of expanded polystyrene beads provide permanent methods to prevent breeding of *Cx quinquefasciatus* in stagnant water confined within walls (Curtis, 1990). Polystyrene beads physically prevent adult mosquitoes from laying eggs there, suffocate the larvae and pupae, and prevent the emergence of adult mosquitoes. Polystyrene beads are non-biodegradable, and three years after application to cesspits the layers of beads have been observed to be still in place, indicating that the method lasts for a considerable period (Charlwood, 1994). Killing of larvae and/or prevention of emergence of adults by polystyrene beads has been demonstrated in individual pits in Kenya, Tanzania and Zimbabwe (W.H.O., 1992). In Tanzania, they have effectively been used in Zanzibar (Maxwell *et al*., 1990), Dar es Salaam (Curtis & Minjas, 1985; Chavasse *et al*., 1995b), Ifakara (Charlwood, 1994) and Tanga and Muheza (T.J. Wilkes, *personal communication*).

Reduction of human-mosquito contact may also be achieved by house-screening. Screening of house windows and eaves with plasticized mesh is very efficient as are mosquito nets, provided they are correctly used (Minjas & Kihamia, 1991). Window screening has for quite a long time been considered useful as a means of self-protection against man-biting mosquitoes. The major draw-backs in using these methods include high costs, poor management and frequent tear especially with low quality materials (Njau *et al*., 1993).

Biological control

Biological control using the bacterium *Bacillus sphaericus* has given good results against *Cx quinquefasciatus* in pit latrines and septic tanks in Dar es Salaam (Minjas, 1989). In this trial *Cx quinquefasciatus* breeding was prevented for six to ten weeks after the introduction of *B. sphaericus*. The bacteria tolerate pollution well and are effective against larvae resistant to conventional larvicides.

The use of larvivorous fish is one of the oldest and best known methods of controlling mosquitoes. The use of mosquito fish, *Gambusia affinis,* in subterranean urban storm drains in California to control *Cx quinquefasciatus* has been reported by Farley & Caton (1982). Another fish, *Poecilia reticulata*, has been widely used to control *Cx quinquefasciatus* in Asia (Legner, 1995). In East Africa, larvivorous fish were introduced several centuries ago by the Arabs who had founded towns and villages along the coast. The fish were placed in tanks where the water was kept for ritual ablutions and where *Ae. aegypti* and *Cx quinquefasciatus* bred. During the period after the Second World War, the use of fish was resumed to supplement control of the mosquitoes by insecticides (Subra, 1981).

Integrated control

Integrated control aims to reduce a pest organism to levels where injury is tolerated or a pest is no longer harmful. This is achieved by combining methods, which if applied separately, would not achieve the desired result but together are mutually complementary. In Tanzania combined environmental improvement by reducing the number of breeding places and the spraying of latrines and polluted water with chlorpyrifos led to a reduction of 92% in adult densities of *Cx quinquefasciatus* (Bang *et al.,* 1975). Integrated control of *Cx quinquefasciatus* has also been successful in Rangoon, Myanmar (Mathis, 1972). To be successful mosquito management programmes, however, must include seasonal monitoring to determine density and location, and year round monitoring and inspection to identify breeding sites.

2.0. Problem definition and research goals

2.1. Host-seeking behaviour

Mosquitoes and other haematophagous Diptera locate vertebrate hosts by means of various chemical, visual, and physical cues. For most of these insects the spectrum of host choice differs with changing place and season and given different proportions of available hosts. The degree of host specificity is generally defined in terms of the proportion of available host species used and therefore, lies on a continuum with ranges from the use of one host species to the use of all available host species. However, a blood-sucking insect may still use only a subset of all available hosts. Whilst searching for a blood meal, a mosquito may encounter a number of unsuitable host species on which it is unable to feed before a suitable one is met. As the location, identification of and preference for a host is an integral part of its life strategy, an insect must be able to discriminate a suitable from an unsuitable host. Depending upon the likelihood of encountering a suitable host, there may be a selective pressure to increase the efficiency by which a host species is identified. In an anthropophilic mosquito, for instance, selection should favour a host identification system in which movement towards a human host is induced only by stimuli from man. On the other hand, a mosquito with a more opportunistic feeding behaviour, which uses a broad range of host species, may rely initially on less specific host stimuli and identify a specific host only after contact. The question that arises from these arguments is: what are the cues or stimuli which are used by mosquitoes, particularly anthropophilic ones, to select and locate their hosts?.

Most anthropophilic mosquitoes in tropical Africa seek their blood meals indoors at night, and the long distance orientation behaviour is thought to be governed to a large extent by olfaction (Takken, 1991). Chemical orientation is, therefore, assumed to play an important role in their host-location behaviour and the information value of odour stimuli from the vertebrate host is considered vital to aid in host selection. To date, carbon dioxide, lactic acid and octenol have been described as mosquito kairomones (Acree *et al.*, 1968; Gillies, 1980; Takken, 1991; Kline, 1994a), the latter two mainly for a number of *Aedes* species. Recently, it was found that anthropophilic species such as *An. gambiae* poorly respond to carbon dioxide, and that human breath is not attractive at all (De Jong & Knols, 1995; Healy & Copland, 1995; Knols *et al.*, 1998). Volatile substances produced by mammalian skin have been incriminated as mosquito kairomones (Takken, 1991), however, only few compounds are currently known in the attraction of highly anthropophilic mosquitoes. Recent analysis and identification of volatile components in human sweat and Limburger cheese (which produces a human foot odour-like smell) has provided blends of chemicals shown to be attractive to *An. gambiae s.s.* in a laboratory bioassay in the absence of carbon dioxide (De Jong & Knols, 1995; Knols & De Jong, 1996; Knols *et al.*, 1997; Braks *et al.*, 1999). Studies on host-seeking behaviour, in particular the role of odours, are required to understand the role of olfactory cues in the host-seeking process of *Cx quinquefasciatus,* especially since this mosquito is also considered to be anthropophilic. Identification of attractive compounds is of major interest, considering the possibility that such compounds may be useful for mosquito sampling in epidemiological studies of mosquito-borne diseases.

The need to accurately assess mosquito population levels has long been a major component of control efforts. Hitherto, the man biting/landing catch technique is the most widely used method in attempting to measure mosquito populations and other relevant disease transmission parameters. Although this technique provides a useful animals in time and space, the results are often inherently variable due to differences overview of the mosquitoes causing nuisance and transmitting diseases to man and among the collectors. Thus man landing catches may not truly reflect the mosquito biting rates. Likewise, the Centers for Disease Control (CDC) light trap, which is often employed as a standard mosquito sampling device, is not an efficient tool as the collections may contain mosquitoes of different physiological status and the trapping technique requires the presence of man as a bait (Garrett-Jones & Magayuka, 1975). A device that samples the host-seeking section of the vector community is highly desirable.

Despite the widespread exploitation of odours from breath and urine to attract tsetse to traps and targets, a work which has significantly contributed to the development of economically cheap and environmentally safe baits (Vale, 1993) the effect of odour on the behaviour of mosquitoes has not been extensively investigated. Most of the studies on the role of kairomones in mosquito host-seeking behaviour used *Ae. aegypti* as a target species. Much remains to be done on host-oriented behaviour of other medically important mosquitoes such as *Cx quinquefasciatus*.

2.2. Oviposition site selection

Semiochemicals, including aggregation pheromones, have been used as baits to monitor and control agricultural pests (Jutsum & Gordon, 1989). Moreover, unlike most kairomones, such as octenol and carbon dioxide, which are attractant cues to other mosquitoes, aggregation pheromone baits are species-specific. Semiochemical baited traps could allow regular sampling to estimate vector population size and structure. Moreover, since oviposition pheromones specifically attract gravid females, which were exposed to infection during their previous blood meal, traps baited with oviposition attractants would more effectively aid the determination of population infection rates (McCall & Cameron, 1995) than traps that attract host-seeking mosquitoes. Oviposition attractants have considerable potential for both increasing the sensitivity for monitoring populations of culicine mosquitoes and for the potential delivery of pathogens or pesticides to larval populations (Otieno *et al.*, 1988a; Schlein & Pener, 1990; Itoh *et al.*, 1994). Ovitraps are often used for population monitoring and surveillance (Chadee *et al.*, 1988; McHugh & Hanny, 1990), and the use of oviposition attractants or stimulants to increase the sensitivity of these traps as well as gravid female traps (Reiter, 1983; Freier & Francy, 1991) has considerable potential.

There are indications that *Culex* aggregation pheromones may play an important role in mosquito management programmes in the future. When combined with an insect-growth regulator in Kenya, the pheromone directed egg laying and inhibited adult emergence (Otieno *et al.*, 1988a). Despite showing promising results in Kenya, the olfactory role of oviposition semiochemicals has not been studied in other parts of

Africa. Pheromone-baited traps could allow regular sampling to estimate vector population size and structure. It has been established that responses of gravid *Cx quinquefasciatus* to acetoxyhexadecanolide are increased when combined with 3-methylindole, found in polluted water in natural oviposition sites (Blackwell *et al.*, 1993; Millar *et al.*, 1994), but the specific behavioural role of each stimulus is not known.

Several factors acting as external stimuli affect mosquito orientation and oviposition. Both behaviours require complex integration of visual, physical and chemical cues by the searching mosquito. Based on the description of the lack of information on the role of chemical cues in the behaviour of *Cx quinquefasciatus*, the present study was undertaken to investigate its odour-mediated host-seeking and oviposition site-selection behaviour. Where relevant, studies included the behaviour of the equally anthropophilic *An. gambiae* and *An. funestus*, which often co-exist in rural areas of East Africa.

In summary, the specific objectives of the work presented in this thesis were:

1. To study host-seeking behaviour of *Cx quinquefasciatus* in relation to host preference.
2. To determine the role of specific host odours in the host-seeking behaviour of *Cx quinquefasciatus*, using: carbon dioxide, octenol, acetone and skin emanations.
3. To investigate the role of the oviposition pheromone of *Cx quinquefasciatus* in relation to the selection of oviposition sites and the interaction with breeding site associated chemicals in attracting gravid mosquitoes.
4. To evaluate the effects of host-odour and oviposition stimuli on trap entry behaviour in order to develop an odour-baited monitoring system to be used in mosquito surveillance and control programmes.

The information resulting from this study is expected to be useful (i) in understanding the biology and behavioural ecology of *Cx quinquefasciatus*, (ii) for the development of odour-baited traps to monitor the epidemiology of bancroftian filariasis and other mosquito-borne disease and (iii) for the improvement of vector control programmes.

Part I:
Chemical Ecology of Host-seeking Behaviour of *Culex quinquefasciatus*

2

Carbon dioxide chemotropism in mosquitoes (Diptera: Culicidae) and its potential in vector surveillance and management programmes: a review

ABSTRACT - Carbon dioxide chemotropism in mosquitoes and its potential role in mosquito surveillance and management programmes is reviewed. Although the responses of various species to carbon dioxide differ, the role of this compound in activation, upwind anemotaxis, and selection of biting sites on hosts is documented for a number of species of medical and veterinary importance. The mechanism of carbon dioxide perception and sensitivity are discussed as well as dose response levels and interaction with other stimuli. The use of carbon dioxide as a kairomone over the last four decades has revealed its usefulness in mosquito surveillance notably for North American and Australian nuisance mosquitoes and for arbovirus and filariasis vectors in endemic countries. This has prompted the development of carbon dioxide-baited trapping systems for large-scale removal of nuisance mosquitoes. In spite of these successes, carbon dioxide does not appear to be a useful compound for the surveillance of Afrotropical anthropophilic malaria and bancroftian filariasis vectors and little is known about its mode of action in this important group of mosquitoes.

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Introduction

The life of the female mosquito is governed to a large part, by its behavioural responses to stimuli from important resources such as resting sites, oviposition sites, nectar sources, mates, and blood sources (Sutcliffe, 1994). Although mosquitoes use a combination of physical, visual and olfactory cues to orient toward a host, the olfactory system plays a prominent role in modulating the response. Odours emanating from the host are known to be important orienting stimuli for the host-seeking behaviour of the female mosquito. It is interesting to note that the chemicals available in the greatest quantity which may carry information from vertebrate hosts to haematophagous insects are the waste products excreted by the former (Dusenbery, 1992). These are usually simple molecules, such as carbon dioxide, 1-octen-3-ol (octenol), butanone, acetone and phenols which are common constituents of vertebrate breath and urine.

The history of the study of the role of carbon dioxide in mosquito host-location behaviour goes back to the observations by Rudolfs (1922) that carbon dioxide produced by breathing was an important factor in attracting mosquitoes to their hosts. Rudolfs findings provided a basis for subsequent work on carbon dioxide chemotropism in mosquitoes. For example, Van Thiel (1947) studied the importance of carbon dioxide in the attraction of mosquito in an air current olfactometer and found that carbon dioxide added to air current had a relative attraction of 52-76% more mosquitoes than the control. Further studies of Van Thiel & Weurman (1947) indicated that carbon dioxide strongly enhanced the attractiveness of other stimuli to mosquitoes such as heat and moisture, as well as the combinations of heat and blood. Reeves (1951) succeeded in catching more *Culex* and *Anopheles* in a stationary trap baited with carbon dioxide and confirmed that various mosquito species respond to the compound. Willis & Roth (1952) were able to demonstrate quite constantly and reproducibly the attraction of *Aedes aegypti* to carbon dioxide in a large mosquito cage. A year later, Reeves (1953) reported that carbon dioxide emitted from a cylinder exerts a specific attraction on mosquitoes according to its rate of flow. Following these, and his own observations, Laarman (1955) confidently concluded that carbon dioxide plays a role in attracting mosquitoes.

Subsequent field studies by Gillies & Wilkes (1969, 1970, 1972, 1982) and Snow (1970) have shown that carbon dioxide is a mosquito kairomone. The latter found in field experiments that human breath, from which carbon dioxide was removed, attracted significantly lower numbers of mosquitoes than the normal control. It is now generally accepted that carbon dioxide is, in concert with other olfactory stimuli, a kairomone for virtually all haematophagous insects (Service, 1993). The aim of this paper is to review the current status of our knowledge concerning the responses of mosquitoes to carbon dioxide, the potential use of this compound in mosquito surveillance and management programmes, and to make recommendations for additional research.

Carbon dioxide in the environment

Carbon dioxide is normally present in the atmosphere at between about 0.03 and 0.05%, occasionally rising to 0.1% in dense vegetation at night. Its production in soil is typically in the order of 1 gram per hour per square metre of surface area (Wessenling, 1962) and its concentration is 0.2 to 1% (Black 1968), i.e. higher than atmospheric carbon dioxide. Carbon dioxide in the soil is a result of the metabolic activity of micro-organisms and plant roots (Stange, 1996). As well as being a natural component of human and animal breath, carbon dioxide is also secreted by the skin of vertebrate hosts, though at a much lower concentration. Mammals and birds expire air that contains about 5% carbon dioxide (Altman *et al.*, 1958). Human breath contains about 4.5% carbon dioxide, whereas excretion from his total skin surface is about 0.3 to 1.5% of that expired from the lungs (Gillies, 1980; Lehane, 1991). Recent measurements with an infrared analyser have shown that carbon dioxide is given off by the human hand continuously at the rate of 1.0 to 1.8 ml/h (Carlson *et al.*, 1992). Moreover, different types of animals release different amounts of carbon dioxide and its concentration in a vertebrate animal is determined by its metabolic rate.

The carbon dioxide gradients that can possibly occur in the vicinity of plant materials are much smaller than those encountered in the vicinity of warm blooded vertebrates, because the metabolic rate of a plant is smaller per unit mass and the total surface area is much larger (Stange, 1996). Carbon dioxide gradients are also generated by human activity through industrial by-product, deforestation and fuel combustion. As a result of these activities the carbon dioxide concentration in the atmosphere is gradually increasing. While the concentration was about 0.026-0.027% during the preindustrial era (Nicolas & Sillans, 1989), it is now estimated at 0.0353% with a rate of increase of 0.00015% per year (Stange, 1996). Although an increase in global temperatures is expected to alter the distribution of an arthropod-borne pathogen either by affecting the distribution of the vector, or by accelerating the development of the pathogen within the vector (Lines, 1995), the increase in the atmospheric carbon dioxide is not expected to affect the concentration in the breath of a vertebrate host. At a given distance, for a raised background, a plume of breath released by a host will therefore continue to produce a given absolute change in concentration and hence a smaller fraction of background. As a result the range over which blood sucking insects could detect carbon dioxide released by their hosts will decrease (Stange, 1996).

Vertebrates release carbon dioxide at different rates. For example, domestic chickens are estimated to produce carbon dioxide at the rate of 12-24 ml per kg body weight per minute (Barott & Pringle, 1941; Sturkie, 1965). The table of Gaddum (1961) may be a useful guide for estimation of the amount of carbon dioxide released by mammals. However, there are considerable differences in the amounts of carbon dioxide produced by fasting and lactating animals (see P.W. Moe & H.F. Tyrell unpublished data *in* Roberts, 1972). These workers found that whereas fasting dairy cows produced an average of 1123 ml/min of carbon dioxide, lactating dairy cows produced 3476 ml/min of carbon dioxide. The amount of carbon dioxide released by a human is approximately 275 ml/min (Schmidt & Nielsen, 1975). Although such large volumes of carbon dioxide are emitted by various vertebrate species, the compound is sensed by mosquitoes at a considerably lower rate. In nature considerable dilution of the carbon dioxide plume usually occurs but filaments of relatively undiluted carbon dioxide are likely to travel for quite some distance downwind and the distance from the host at which activation and orientation are still likely to occur will vary according to local meteorological conditions (Murlis & Jones, 1981).

Role of carbon dioxide in mosquito host-location behaviour

The life cycle of most arthropod-borne infectious pathogens require at least two successful contacts between vectors and their hosts. These contacts are established whenever the vector locates the host by responding to various stimuli provided by the hosts. Odours from host vertebrates give information on the availability of a blood meal and on the characteristics of the host (Takken, 1996).

The role of carbon dioxide in host-location behaviour in haematophagous insects is documented for Ceratopogonidae (Nelson, 1965), Tabanidae (Thornhill & Hays, 1972), Culicidae (Gillies, 1980), Glossinidae (Vale, 1980), Muscidae (Warnes & Finlayson, 1985a, b) and Simuliidae (Sutcliffe, 1986). Carbon dioxide as an important stimulus in mosquito host-seeking behaviour has been demonstrated both in the laboratory (Laarman, 1955; Eiras & Jepson, 1991; Knols *et al.*, 1994a; Healy & Copland, 1995; Takken *et al.*, 1997a) and in the field (Gillies & Wilkes, 1972; Costantini *et al.*, 1996; Dekker & Takken, 1998; Knols *et al.*, 1998; Chapter 5). As early as 1955, Laarman suggested that the essential role of carbon dioxide in mosquito host-seeking behaviour is that of an activating stimulus. Several workers have so far clearly demonstrated that fewer host-seeking mosquitoes arrive at the source minus carbon dioxide, indicating that carbon dioxide is functioning both as a stimulus and activating agent, orienting mosquitoes to hosts (Laarman, 1958; Snow, 1970; Omer, 1979).

It has been proposed by Gillies (1980) and later by Takken (1991) that carbon dioxide both induces take-off and sustained flight and orients mosquitoes. According to Gillies (1980), carbon dioxide acts as an activator only in still air; in moving air, the compound may be used for orientation to its source. In still air, however, any airborne attractant can at best only be an activator, because there is no directional component to the odour signal toward which the mosquito can orient (Davis & Friend, 1995). Grant *et al.* (1995) argue that increments in carbon dioxide concentration may act simply to stimulate the mosquito to initiate flight. Once airborne, other sensory cues may allow it to orient upwind where it will eventually encounter the source of the elevated carbon dioxide (Gillies, 1980). Alternatively, the flying mosquito may actually follow klinotactically the incremental changes in carbon dioxide concentration, which must occur in the natural environment, to locate the stimulus source (Gillies, 1980). A study by Eiras & Jepson (1991) has demonstrated that carbon dioxide elicits the complete sequence of behaviours from activation response to landing in *Ae. aegypti.* Further, Takken & Knols (1990) demonstrated that carbon dioxide acts as a synergist when offered in combination with skin emanations because it increases the chance that mosquitoes will arrive at the host. In a wind-tunnel study, these authors found that carbon dioxide and human skin emanations cause distinctly different flight behaviour and thus they are used for different aspects of host-seeking behaviour. The authors suggested that the role of carbon dioxide is to stimulate the mosquito to engage in upwind anemotaxis while skin emanations inform the mosquitoes about the quality and location of the host. It was recently demonstrated that carbon dioxide induces an upwind anemotaxis in mosquitoes in the field (Knols *et al.*, 1998). Since both activation and orientation are part of the process of attraction, the attractive effects of other odours on host-seeking behaviour cannot always be elicited unless carbon dioxide and the host odour are presented simultaneously (Bowen, 1991).

Following flight initiation and orientation, female mosquitoes respond to the host's stimuli by landing and probing. Grant *et al.* (1995) assume that there is a steep gradient in the concentration of transpired carbon dioxide at the host's skin surface. The location of the sensilla basiconica on the distal, ventral surface of the maxillary palps place them in close proximity to the skin during this landing and probing sequence, where transpired carbon dioxide from the host's skin may be detected. Thus, these receptor neurons may also play a role in guiding the landing and probing behaviour of mosquitoes. According to Davis & Friend (1995), probing results in contact by the sensilla at the tip of the proboscis or on the labella lobes with the substrate surface and is influenced by thermal gradients, carbon dioxide, and host airborne and surface chemical signals. Recent observations by Moskalyk & Friend (1994) that female *Ae. aegypti* feed more readily on a warmed artificial membrane containing bicarbonate further suggests that carbon dioxide released through the skin might be involved in the stimulation of feeding behaviour after landing, together with other sensory inputs. In addition, Knols *et al.* (1994b) have demonstrated that human breath and possibly its carbon dioxide content, influences the selection of biting sites of *An. albimanus*. Since the morphology of the palp and the distribution of sensilla along the palp varies considerably between mosquito species, their role in the landing and probing response may differ (Grant *et al.*, 1995).

The recent demonstration that male *Ae. aegypti* also responds with comparable sensitivity to stimulation with carbon dioxide (Grant *et al.*, 1995) has raised more questions as to the actual role of this compound in mosquito behaviour. Nelson *et al.* (1978) were able to recapture a number of marked and released male *Cx tarsalis* using carbon dioxide baited light traps in California. Male *Ae. sierrensis* was also found to respond to carbon dioxide in the same area by Garcia *et al.* (1989). Male mosquitoes do not blood feed, but they do feed on plant nectars. Thus, their ability to detect carbon dioxide is perhaps required for locating food sources. An alternative explanation of attraction by carbon dioxide is suggested by the fact that male *Ae. aegypti* have been observed to orient to human hosts where mating occurs as the females seek a blood meal (Hartberg, 1971). This behaviour has also been reported in *Ma. africana* whose mating takes place at the host and with males being known to respond to host odour (McIver *et al.*, 1980). Recently, McCall *et al.* (1996) have demonstrated in a laboratory study that male *Ae. aegypti* also show some response to host odour. The response observed by McCall *et al.* (1996) may, like their attraction by carbon dioxide, be part of mate patrolling behaviour.

Although numerous behavioural studies have explored the role of carbon dioxide in the orientation and host-seeking capabilities of female mosquitoes, either alone or as a synergist of the responses elicited by other compounds, the exact role of carbon dioxide in modulating each of these behaviours is not clear. It is apparent that there is probably an interspecific variation in the role of carbon dioxide in location behaviour. It is likely that in most species carbon dioxide activates the female mosquito, while in others, additionally,, it causes anemotactic flight and orientation towards a vertebrate host.

Moreover, work by Knols *et al.* (1994b) and Grant *et al.* (1995) present evidence that carbon dioxide is responsible for selection of biting sites in some mosquito species. Carbon dioxide chemotropism can thus be divided into a number of stages: initial activation, orientation and selection of a biting site. It is as yet unknown whether host-seeking mosquitoes use carbon dioxide plumes only for orientation or also for an estimation of distance from the source.

Chemoreception of carbon dioxide in mosquitoes

Ultrastructural studies have played a major role in the identification of specific sensory organs (sensilla) associated with host-seeking behaviour in mosquitoes. Olfactory sensilla in adult mosquitoes are located in the antennae and the maxillary palps. The antennal sensilla respond to various synthetic and natural olfactory stimuli. The maxillary palps, a pair of appendages extending from the proboscis, contain receptor neurons which respond to carbon dioxide. Palpal ablation studies (Bassler, 1958 as cited by Sutcliffe *et al.*, 1987) provided early indications of a carbon dioxide detection role for these sensilla in *Ae. aegypti*. Carbon dioxide responsiveness within a behaviourally significant range for sensilla basiconica (capitate pegs) of *Ae. aegypti* was confirmed electrophysiologically by Kellogg (1970). Kellogg reported finding a class of neurons in the sensilla basiconica, on the ventral-medial aspect of the maxillary palps, that are sensitive in a dose-dependent manner to levels of carbon dioxide found in human breath. Each of these club-shaped pegs houses three neurons, one of which detects changes in carbon dioxide (Kellogg, 1970). The receptor neurons produce phasic-tonic patterns of action potential responses to low concentrations of carbon dioxide.

Insect odour-sensitive sensilla have been described in detail by McIver (1982) and Zacharuk (1985). Sensilla basiconica are distributed along the terminal three sub-segments of the maxillary palps in female anophelines (McIver & Siemicki, 1975; Grant *et al.*, 1995), and are restricted to the fourth segment in male and female culicines (McIver & Charlton, 1970; McIver, 1971; Grant *et al.*, 1995). The spatial distribution of sensilla basiconica on the male *Ae. aegypti* palp is however, not restricted to the fourth sub-segment from the head, as it is in the female. Instead, the distribution seems to be restricted to the distal half of the third sub-segment from the head (Grant *et al.*, 1995). Numbers present range from less than 20 per palp in female *Uranotaenia* species (Omer & Gillies, 1971) to more than 200 in various *Culex* species (McIver, 1970). Males generally have fewer sensilla basiconica than females. Although generally most female mosquitoes possess sensilla with receptor neurons that respond to carbon dioxide stimulation there is much interspecific variation in both the external morphology of maxillary palp and the distribution of the sensilla along the palp (Braverman & Hulley, 1979). The palps of *An. stephensi* are significantly longer (*ca.* 1450 μm) than the palps of either *Ae. aegypti* (*ca.* 350 μm) or *Cx quinquefasciatus* (*ca.* 300 μm).

Recent studies by Grant *et al.* (1995) have shown that male *Ae. aegypti* have morphologically similar sensilla to those seen in females which are innervated by a receptor neuron responsive to carbon dioxide. The apparent sensitivity of male receptor neurons to carbon dioxide equals that of the female at lower concentrations and may exceed it at the higher levels.

Morphological structure has been used to build a strong circumstantial evidence for which dendrite is the probable carbon dioxide detector. According to Sutcliffe *et al.* (1987) the lamellate dendrite is the probable carbon dioxide detector in mosquitoes. The lamellate dendrite is the only dendrite common to the sensilla basiconica of other blood sucking Diptera such as Ceratopogonidae and Simuliidae. Previously, Bogner *et al.* (1986) have demonstrated electrophysiologically that these palpal sensilla (and therefore the lamellate dendrite within) are carbon dioxide sensitive. These data provide further circumstantial support for the lamellate dendrite of mosquitoes and other biting flies being the carbon dioxide-sensitive unit.

In mosquitoes, it is the change in concentration of carbon dioxide rather than the level of carbon dioxide encountered which is the important factor eliciting behavioural responses (Wright & Kellogg, 1962). To facilitate this, mosquitoes' sensilla basiconica receptor neurons are sensitive to very small changes in carbon dioxide levels, changes as small as 0.05% will elicit behavioural responses in a wind tunnel (Mayer & James, 1969; Grant *et al.*, 1995). Kellogg (1970) and Healy & Copland (1995) showed a range of activation threshold concentration of carbon dioxide in *An. gambiae* to be in the region of 0.01% above background. This figure corresponds closely with other wind tunnel studies on responses of mosquitoes to carbon dioxide (Eiras & Jepson, 1991). The receptors are apparently saturated at 4.0% carbon dioxide, the level present in human breath (Kellogg, 1970). The response to a single pulse of a given concentration of carbon dioxide appears to be independent of the background level of carbon dioxide (Grant *et al.*, 1995). It has been suggested that this change in sensitivity is hormonally controlled as has been shown for mosquito lactic acid receptors (Klowden & Lea, 1979; Davis, 1984; Klowden *et al.*, 1987).

In mosquitoes, carbon dioxide causes continued upwind flight when received at continuously varying concentrations. In a wind tunnel study, female *An. arabiensis* and *Cx quinquefasciatus* have been observed to respond to changing concentrations of carbon dioxide, but not to a constant concentration of 0.5% (Omer, 1979; Mayer *et al.*, 1979). Recently, *An. gambiae* have been observed to respond rapidly by taking-off and flying upwind to small increases in carbon dioxide concentration presented as a series of intermittent pulses (Healy & Copland, 1995). Under natural conditions, odours are likely to be received in the form of pulses due to the irregular and filamentous structure of the plume. Mammals release breath in pulses, turbulence will ensure the odour plume is continually mixed and diluted in an irregular fashion creating further heterogeneity (Gillies, 1980; Murlis *et al.*, 1992). Recently Mafra-Neto & Cardé (1994, 1995) showed in laboratory experiments that the structure of an odour plume greatly influence the flight tract of a resource-seeking insect. They observed that a male moth, following either turbulent or mechanically pulsed plumes, fly faster and straighter upwind and locate sources more frequently and rapidly than those following continuous narrow plumes. High pulse frequency, therefore, induces straighter upwind flights and more rapid transitions of behaviour (Mafra-Neto & Cardé, 1995).

Differential responses to carbon dioxide

Interspecific differential responses to concentrations of carbon dioxide occurs among mosquito species. There is a tendency for the number of attracted mosquito species to either increase or decrease when the discharge level of carbon dioxide is increased. For instance, raised levels of carbon dioxide increased the catches of *Ae. nigromaculis, Ae. dorsalis, Ae. taeniorhynchus, Ae. vexans, Ae. vigilax, Coquillettidia perturbans, Cx erraticus, Cx salinarius, Cx tarsalis, Psorophora ciliata, Ps. columbiae, An. funestus, An. quadrimaculatus, An. quadriannulatus, An. mcintoshi* and *An. pharoensis* (Reeves, 1953; McIver & McElligott, 1989; Takken & Kline, 1989; Kline *et al.*, 1991a; Van Essen *et al.*, 1994; Dekker & Takken, 1998; Chapter 5). However, increased rates of release had no significant effect on catches of *An. gambiae s.l., An. crucians, Culiseta inornata, Cs. morsitans, Cx restuans, Cx pipiens, Cx quinquefasciatus* and *Ps. mathesoni* (Reeves, 1953; Harden *et al.*, 1970; Kline *et al.*, 1991b; Dekker & Takken, 1998). Reeves (1953) attributed these observations on the mosquito's host preferences. That is, higher discharge rates being preferred by mosquitoes with large animal hosts while lower rates by those with small animal hosts such as birds. Since the carbon dioxide release rate of a mammal depends largely on its weight, concentrationwise carbon dioxide might be a host specific cue to a certain extent, leading zoophilic species to large mammals like cows (Dekker & Takken, 1997). It is interesting however, to observe that anthropophilic mosquitoes species (*An. gambiae, An. arabiensis* and *Cx quinquefasciatus*) respond relatively poorly to any increase in carbon dioxide release rates. Although *An. funestus,* another highly anthropophilic species, was caught in large numbers with increased rates of carbon dioxide in South-east Tanzania (Chapter 5), the mosquito in their area of study consists of a species complex of which the different species vary highly in host preference. Since the precise identity of the *An. funestus* caught was not known, these data should be considered with caution.

It is likely, however, that the host preference of the mosquito also plays a role in its response to different concentration levels of carbon dioxide. Thus mosquitoes with a broad host range will be expected to respond commonly to carbon dioxide and their response will increase as the level of the compound is raised. There is also a possibility that a threshold carbon dioxide concentration for each species exists beyond which a response can no longer be induced. For example, when the response of *An. gambiae s.l.* and *An. funestus* to tents baited with a human or carbon dioxide was compared in south-east Tanzania (Chapter 5), it was found that carbon dioxide at 300 ml/min pumped into a tent caught significantly fewer mosquitoes than a human baited tent. Raising the level of carbon dioxide five-fold did not increase the catch of *An. gambiae s.l.*, whereas it increased that of *An. funestus*. This could mean that the low dose of carbon dioxide already induced the maximum response in *An. gambiae* and *An. arabiensis* which therefore could not be increased by a higher dose. This is in agreement with results obtained by Costantini *et al.* (1996) who showed that *An. gambiae s.l.* had reached its maximum response level near 4000 ppm of carbon dioxide, which is equivalent to 300 ml/min. Although the increase in mosquito catches in some species is dose dependent in some it flatten out above a threshold (as was found in *An. gambiae s.l.*), and in yet others it probably causes a repellent effect and thus decreases the catch. In a recent study using electric nets baited with breath or carbon dioxide, Knols *et al.* (1998) found that the compound attracted mainly zoophilic mosquitoes and very small numbers of *An. gambiae s.l.* and *An. funestus* (3 and 8.5% of the total catch for human breath and carbon dioxide, respectively) were caught in an outdoor situation. Previously De Jong & Knols (1995) and Healy & Copland (1995) suggested that because of the similarity of the responses of *An. gambiae* to carbon dioxide and human breath, this mosquito species does not respond to breath-related chemicals other than carbon dioxide.

Similarly, using odour-baited traps in Burkina Faso, Costantini *et al.* (1996) observed that in a direct-choice test between carbon dioxide and human odour when two traps were placed side by side, only *An. gambiae s.l.* made a choice significantly different from random, half as many choosing the carbon dioxide bait. When the two traps were placed at least 20 m apart (with equal carbon dioxide concentrations), traps baited with carbon dioxide alone attracted half as many *An. gambiae s.l., ca* 40% as many *An. funestus, ca* 65% as many *Ma. uniformis* and twice as many *An. pharoensis* as the human odour baited traps. An increase in the dose of carbon dioxide did not increase the number of *An gambiae s.l.* over that of human bait catch, while higher doses of the compound caught significantly more *Ma. uniformis* than did human bait catches. In the same study, *An. pharoensis* and *Cx quinquefasciatus* showed a threshold response to carbon dioxide, responding only at doses above that normally released by one man. More recently, Dekker & Takken (1998), in South Africa, observed that higher release rates of carbon dioxide increased the relative proportion of *An. arabiensis* and *An. quadriannulatus* but more significantly for *An. quadriannulatus* whereas no effect of the release rate on the catches of *Cx quinquefasciatus* was observed. All these studies provide more evidence for a variation in the response to carbon dioxide among mosquito species.

Field observations (Costantini *et al.*, 1996; Knols *et al.*, 1998; Chapter 5) that *An. gambiae s.s.* and *An. arabiensis,* both anthropophilic mosquitoes (White, 1974), are only moderately to poorly attracted to carbon dioxide, confirm the laboratory findings reported by De Jong & Knols (1995) and Healy & Copland (1995). It is likely that the highly anthropophilic mosquitoes show less dependency on carbon dioxide in their host-seeking behaviour and that the compound, when used as a kairomone on its own, accounts for only a part of the overall attractiveness of man.

Although mosquito attraction to carbon dioxide has been reported from a number of field studies and the compound alone is associated with large increases in trap catches, the efficiency of carbon dioxide baited traps with respect to the proportion of the mosquito population responding or the effectiveness as related to the physiological state of the vector (Davis & Bowen, 1994) is not known. Whatever the discharge rate of carbon dioxide, the concentration of the gas in the air at various distances from the release site, depends on local environmental conditions, which can be highly variable in both time and space, and usually remain largely unknown in trapping experiments (Service, 1993b). According to Kline *et al.* (1990a), the response of mosquitoes to a source of carbon dioxide is a function of the level of carbon dioxide emitted. That is, mosquitoes respond to an increase in carbon dioxide emission rate rather than to its concentration. However, it is not clear whether the behavioural data reflect a dose-dependent effect, differences in the cross-sectional size of the carbon dioxide-containing plumes, because the sizes of the emitting sources differ, or perhaps both these factors are important (Davis & Friend, 1995).

Mosquito physiological status and response to carbon dioxide

Carbon dioxide baited traps have been shown to collect mosquitoes of different physiological status. Some workers have observed that more nulliparous mosquitoes respond to carbon dioxide than do parous and gravid mosquitoes (Morris & DeFoliart, 1969; Feldlaufer & Crans, 1979; Magnarelli, 1975; Reiter, 1983; Emmons *et al.*, 1986; Reisen & Pfuntner, 1987). Examination of the physiological age of mosquitoes by Polovodova & Magnarelli (1977) showed that females of several species caught by carbon dioxide-baited traps had completed two ovarian cycles and had begun the third. Nulliparous females with terminal follicles at the 1st or 2nd stages were frequently encountered. Russell (1985) found that female *Cx annulirostris* collected by carbon dioxide baited traps exhibited marginally lower parity rates than females collected by animal baited traps.

By contrast, a significantly higher proportion of parous females sampled by carbon dioxide-baited traps has been reported by others (Morris *et al.*, 1976; Slaff & Crans, 1981; Milby *et al.* 1983; Meyer *et al.*, 1984). Parous rates were higher among female *Cx tarsalis* sampled by carbon dioxide-baited light traps than among females sampled by other trapping techniques in California (Meyer *et al.*, 1984). Morris *et al.* (1976) found that over half of *Cs. melanura* caught in carbon dioxide-baited light traps were parous. High parity rates have also been reported for *Cx salinarius* sampled by carbon dioxide baited traps (Slaff & Crans, 1981). In field studies in the United States, Magnarelli (1975) found significant differences in the percentages of parous females between mosquitoes captured in a marsh and forest habitats. However, the carbon dioxide baited traps proved most consistent in the collection of parous females. Reisen *et al.* (1990) found that parity rates of *Cx stigmatosoma, Cx quinquefasciatus* and *Cx tarsalis* caught at the same place and time showed a similar trend in parity rates between different trapping systems, and that the rates were significantly variable among communities and seasons for *Cx stigmatosoma* but not for *Cx quinquefasciatus* and *Cx tarsalis*. Although epidemiologically, the host-seeking and parous component of a vector population is of most concern and individuals taken at carbon dioxide-baited traps are considered to represent the host-seeking population (Gillies, 1980; Reisen *et al.*, 1983), it must be emphasised that a trapping system that collects a representative sample of the mosquito population of all age groups is desirable.

Blood-fed mosquitoes have also been caught in carbon dioxide baited traps. In arbovirus studies in northern Thailand, Leake *et al.* (1986) found that blood-fed mosquitoes formed only 1-8% of the catches whereas in Argentina, Mitchell *et al.* (1987) found a considerable larger number of blood-fed mosquitoes caught in carbon dioxide-baited traps. Morris *et al.* (1980) found blood-fed and gravid mosquitoes forming 0.3% of the females caught in a carbon dioxide-baited trap. Gordon *et al.* (1991) found that of the mosquitoes collected by a carbon dioxide baited light trap set outdoors, 2.2% representing 25 species, had evidence of blood in the abdomen. Generally, blood-fed and gravid females caught with carbon dioxide traps are small in number but it is not clear as to why they should be caught among host seeking populations. Laboratory experiments by Klowden & Briegel (1994) have further indicated that *An. gambiae, An. freeborni* and *An. albimanus* show varying degrees of host-seeking behaviour while their eggs matured. These authors concluded that for some mosquito species, attempts to take multiple blood meals during a single gonotrophic cycle may be a common phenomenon.

It is known that after a mosquito obtains a blood meal, it usually will not attempt to locate another source of blood until it has at least partially processed the meal or has laid its complement of eggs. In the field, blood-fed mosquitoes have been collected attempting to feed on humans (Gillies & De Meillon, 1968; Magnarelli, 1977). The frequency of multiple blood feeding in field-collected female anophelines has been found to vary from 5 to 50% (Senior White, 1952; Davidson, 1954; Smith & Weitz 1959; Edman & Downe, 1964; Boreham & Garrett-Jones, 1973; Burkot *et al.*, 1988). Briegel (1990) and Lyimo & Takken (1993) found that smaller females of *An. albimanus* and *An. gambiae* respectively, usually require two or three blood meals to allow completion of the first gonotrophic cycle.

Culicine mosquitoes also frequently take multiple blood meals. Forty-three percent of a *Cx tarsalis* population was found to have taken more than one blood meal (Bang & Reeves, 1942). More recently, Trpis & Häusermann (1986) detected double blood meals in *Ae. aegypti.* The presence of multiple blood meals in an individual mosquito (Edman & Downe, 1964; Scott *et al.*, 1983) is an additional important indication of multiple meals taken by an individual mosquito. Although multiple feeding has been attributed to interrupted feeding as a consequence of host defensive behaviour, there is substantial evidence that the nutritive state of the mosquito also plays an important role in repeated blood feeding (Briegel & Hörler, 1993). The need for adult female anophelines to accumulate protein reserves in excess of that necessary for vitellogenesis may be the primary factor responsible for frequent blood feeding. In such situations mosquitoes, responding to carbon dioxide-baited traps are likely to be host-seeking. Nevertheless, the possibility that carbon dioxide is playing a role in location of resting sites for the blood fed and gravid mosquitoes, can not be ignored.

Carbon dioxide synergism

Carbon dioxide has a continued usefulness for blood-sucking insects because under selective pressure the host cannot avoid producing it (Dusenbery, 1992). The great disadvantage of carbon dioxide is that it has little specificity; there are many sources of it, both plant and animal. The high level of carbon dioxide in the environment makes it easy to develop receptors that are sufficiently sensitive so that the natural concentration is always above threshold (Pline & Dusenbery, 1987). Insects that make use of carbon dioxide in chemical orientation, therefore, must be equipped to detect small changes in concentrations of this compound, which may be why mosquitoes and other haematophagous insects appear to respond to other host chemicals at a longer range than they do to carbon dioxide (Gillies, 1980; Vale & Hall, 1985a).

In the natural situation, carbon dioxide is only one of several host stimuli which the insect receives. Carbon dioxide can act in concert with other stimuli giving a response which is different to that of either stimulus given alone (Gillies, 1980; Takken & Knols, 1990). There is a spectrum of responses to these dual stimuli, ranging from synergism (where the two stimuli give an overall reaction which is greater than the sum of the two stimuli given separately) to an interaction where one stimulus primes the insect to respond to the second which, if given alone, has no effect. For example, Vickery *et al.* (1966) demonstrated synergism of carbon dioxide and chick by showing that the combination of the two could increase the catch of *Cx nigripalpus* about seven and three times those collected by chick and carbon dioxide-baited traps respectively.

Lactic acid, a compound present on human skin, has been shown to activate and elicit oriented flight behaviour in mosquitoes only when carbon dioxide was also present in the air stream (Acree *et al.*, 1967; Smith *et al.*, 1970; Price *et al.*, 1979). Other components of host breath can also act in concert with carbon dioxide. For instance, octenol enhances the catches of tsetse flies in traps, especially when carbon dioxide is also released (Hall *et al.*, 1984; Vale & Hall 1985a, b). Acetone further enhances the effectiveness of these traps for sampling tsetse flies. The combination of carbon dioxide and octenol causes a synergistic increase in the collections of many mosquito species, especially species of *Aedes, Anopheles, Coquillettidia, Mansonia* and *Psorophora.* This response appears to vary geographically, seasonally, and according to the physiological state of the mosquito, especially for *Anopheles* species (Kline, 1994a). Major contributions to the knowledge of synergistic effects of kairomones in mosquito attraction have been made by Takken & Kline (1989) and Kline *et al.* (1990a, 1991b). Studies by these workers in the United States indicate that addition of octenol to carbon dioxide synergistically increases collections of aedine mosquitoes, most notably, *Ae. taeniorhynchus*. A synergistic effect of the octenol supplemented carbon dioxide treatment has also been demonstrated in *Cq. perturbans*, *Ma. titillans* and *Cx salinarius* (Kline *et al.*, 1990a; Kline, 1994a). In a field study in Queensland, Australia, Kemme *et al.* (1993) reported that using carbon dioxide-baited traps, *Ae. vigilans, Ae. funereus, Cx annulirostris* and *Cx sitiens* were collected in significantly increased numbers compared to traps baited with octenol alone. Largest collections of *Ae. vigilax* and *Ae. funereus* were obtained with traps baited with carbon dioxide and octenol simultaneously, demonstrating a synergistic effect of these chemicals. Furthermore, Ritchie & Kline (1995) have demonstrated that the addition of octenol to carbon dioxide-baited traps significantly increased the collection of *Ae. vigilax, Ae. procax* and *Cx annulirostris.* Recent studies by Becker *et al.* (1995), however, could not confirm the synergistic effect of carbon dioxide and octenol in the Upper Rhine Valley, Germany. They observed that octenol-carbon dioxide baited traps did not lead to any increase in the number of mosquito collected compared with carbon dioxide alone. The lack of a synergistic effect was accompanied by an apparent lack of any selectivity of octenol for any of the species collected in their study. Recently, Takken *et al.* (1997a) showed that carbon dioxide alone did not cause a behavioural response in *An. gambiae s.s.*, but in combination with acetone a strong response was elicited. By contrast, in this study *An. stephensi* was attracted to carbon dioxide alone, but even more so to the combination of carbon dioxide and acetone or carbon dioxide and octenol.

Why have insects developed distinctive responses to mixtures of different host-derived stimuli? Multiple stimuli are likely to be a more reliable guide to the presence of a host than one stimulus received alone. This is because while one stimulus alone may be of non-host origin, this is very unlikely for combinations of stimuli, especially when they are received in particular proportions. Responding to particular combinations of host signals may permit a degree of selection for a particular host which is still some distance away (Lehane, 1991) and thus increases host-seeking efficiency. In other words, responding to stimuli received in combination is likely to maximise the chances of host encounter while minimising energy consumption. Also, many insects show inherent preferences for particular hosts (specialists), a character which may be the result of co-evolution. Carbon dioxide is released by all potential mosquito hosts and, although it may be good at alerting the mosquitoes to the possibility of a blood meal, it does not allow them to discriminate between hosts. This is supported by studies on the response of anthropophilic mosquitoes such as *An. funestus, An. gambiae* and *Cx quinquefasciatus,* to carbon dioxide or breath (Healy & Copland, 1995; De Jong & Knols, 1995; Knols *et al.*, 1995; Knols *et al.*, 1998; Chapters 4 and 5). Body odours, however, may be characteristic for particular groups of host animal or even for particular species (Lehane, 1991). For instance, the recently confirmed response of *An. gambiae s.s.* to human skin odours has been ascribed to the characteristic microflora on the human skin (Knols, 1996).

The mechanism of putative synergistic responses to host odours is not precisely known. There are two possible explanations. First, each odour may be treated separately by the peripheral nervous system with integration and amplification of the signals occurring in the central nervous system (Eiras & Jepson, 1991). Or secondly, the response of peripheral receptors to a substance may be significantly higher in the presence of the second chemical (Mayer & James, 1969; Davis & Sokolove, 1975). Electrophysiological recordings from mosquito receptors strongly suggest that the former theory is correct (Bowen, 1991; Lehane, 1991; Anton, 1996).

Range of attraction to carbon dioxide

In some species of Culicidae and Simuliidae, hosts' body odours guide the insects towards the host over a greater distance than an equivalent artificial source of carbon dioxide (Bradbury & Bennett, 1974; Gillies & Wilkes, 1972). In these species, the upwind flight behaviour of host-seeking involves separate mechanisms for long-, middle-, and close-range orientation. Long-range attraction involves host-specific odours, while a combination of carbon dioxide and odours is probably the main guide in the insect's host-directed orientation at middle range (Bursell, 1987). In close-range orientation, where most directed visual responses occur, behaviour is affected by the physical make-up of the host: body heat, moisture, colour, size, shape, and movement (Wright & Kellogg, 1962; Bradbury & Bennett, 1974; Gillies & Wilkes, 1982). Carbon dioxide could also have a combined action with warm, moist convection current (Gillies, 1980).

The range of attraction of carbon dioxide in mosquitoes has been demonstrated in West Africa by Gillies (1969) and Gillies & Wilkes (1969, 1970, 1972) and in

Canada by McIver (1982) and McIver & McElligott (1989). The experiments reported by Gillies & Wilkes (1969) indicated that *An. melas*, *Cx thalassius*, *Cx tritaeniorhynchus* and *Aedes* species, could be attracted in significant numbers up to a distance of 18 to 37 m from the source of carbon dioxide, which was released at an equivalent of two calves. However, they concluded that, for *An. melas*, host odours were acting as a long-range attractant eliciting responses beyond the range of carbon dioxide. When the experiment was repeated using carbon dioxide at an equivalent of one calf, they found that in *An. melas*, *Cx thalassius* and *Cx tritaeniorhynchus* the range of attraction of carbon dioxide was less than 14 m (Gillies & Wilkes, 1970). In later studies Gillies & Wilkes (1974) showed that carbon dioxide released at 50 ml/min could attract ornithophagic mosquitoes from up to only 4 m. McIver & McElligott (1989) on the other hand, observed that increased levels of carbon dioxide gas attracted more mosquitoes and from longer distances than low levels. The authors found that a four-fold increase of carbon dioxide extended the range of attraction from between 3-7 m to 7-11 m for several *Aedes* species and *An. walkeri*. According to Edman (1979), for many species of mosquitoes, the orientation to chemical signals associated with hosts seems to take place over relatively short distances of for example 0-15 m. However, in his review Gillies (1980) concludes that, carbon dioxide is usually a medium- or long-range kairomone for mosquitoes. It remains to be investigated to what extent carbon dioxide interacts with other kairomones to cause distance-related behavioural responses.

Use of carbon dioxide in sampling systems

The need to accurately assess mosquito population levels has long been a major component of control efforts. Early, but still much used attempts at measuring mosquito populations, consist of capturing, counting, and identifying the species attracted to human or animal baits. The technique provides a useful overview of the mosquitoes causing nuisance and transmitting human and animal diseases in space and time. However, the results obtained are inherently variable due to various factors. Differential attractiveness of people to mosquitoes have been reported by various workers. Smith (1961), Curtis *et al.* (1987) and recently Knols *et al.* (1995) found significant differences in catches of mosquitoes between individuals in Tanzania. Knols *et al.* (1995) observed a significant difference in attractiveness for *An. gambiae s.l.*, *An. funestus* and *Cx quinquefasciatus* between isolated men sleeping in tents. In their study there was no active involvement of the test person in catching the mosquitoes, and each person occupied his own tent. The differential response of host-seeking mosquitoes towards the test person was, therefore, attributed to his body odours. Human body odours are known to be distinctive and specific (Stoddart, 1990) and varying compositions of this odour complex might determine whether or not a mosquito initiates a search for a host upon detecting host odour.

Thus the findings of Smith (1961), Curtis *et al.* (1987) and Knols *et al.* (1995) provide evidence that a mosquito tend to select a particular host while given a choice and man landing catches may not truly reflect the average mosquito biting rates in time and space. Moreover, in human bait catches, the collector is usually trying to collect as many mosquitoes as possible and therefore may catch more mosquitoes than would have bitten him under natural circumstances. Collections by a few individuals in a few selected sites within a locality are unlikely to be representative of the average number of bites received by an individual from a heterogeneous village population (Service, 1977). Similarly, in animal-bait catches the numbers of mosquitoes caught will depend not only on the host size and host density, but also on the abundance of hungry mosquitoes and the availability of natural alternative hosts. All these factors will have an influence both on effective epidemiological modelling, in which it is generally assumed that the number of bites received by humans is equal and constant, and on ecological studies such as those comparing the efficacy of light traps with human biting catches (Lines *et al.*, 1991; Githeko *et al.*, 1994).

Many scientists feel that almost any trap that actively catches mosquitoes will give a biased measure of the true mosquito fauna in a particular region (Bidlingmayer, 1974; Service, 1993b). Although the light trap is currently employed as a standard mosquito sampling device, critics of the trap indicate that the collections do not necessarily reflect the true biting fraction of the mosquito population when used outdoors because the insects are probably attracted to a light source, which is a non-host stimulus (Service, 1993b). Traps based on kairomones, sources of natural host odours, provide a more objective monitoring tool for the host-seeking fraction of disease carrying mosquitoes, provided the kairomones used consist of an odour mixture to which the insects respond naturally. Such traps can be used to study the vector biology as well as epidemiology of mosquito-borne diseases, a knowledge which is vital for the planning and evaluation of intervention strategies. For indoor collections this may be different, because the mosquitoes were first attracted by other stimuli than light, presumably host odours, and once inside consisted already of the biting fraction of the population.

As early as 1941, Headlee had reported that delivering carbon dioxide gas over a light trap increased the mosquito catch. However, the advantages of using carbon dioxide with mosquito traps were first described in detail by Reeves (1951) who demonstrated that carbon dioxide might be a major factor in attracting mosquitoes to mechanical traps in proportion to those sampled by man landing and animal-baited traps. Since then carbon dioxide has been commonly used in various trapping systems. Further studies have shown that in addition to increasing the total numbers of mosquitoes captured, the species composition of carbon dioxide-baited traps more accurately reflects the true mosquito nuisance populations in an area (Newhouse *et al.*, 1966; Morris & DeFoliart, 1969; Parsons *et al.*, 1974; Magnarelli, 1975; Slaff *et al.*, 1983; Knols *et al.*, 1998).

The combination of carbon dioxide with active trapping devices has been used to catch mosquitoes world-wide (Service, 1993b). To date carbon dioxide-baited Centers for Disease Control (CDC) portable light traps (Sudia & Chamberlain, 1962) are routinely employed in surveillance programmes in many parts of the United States. Sometimes these traps have been used with the light source removed (Takken & Kline, 1989). Observations by Herbert *et al.* (1972) have shown that the catches of some mosquito species increase when the light is removed and the traps baited with only carbon dioxide. Indoor catches of *An. gambiae s.s.* and *An. funestus* are reduced when the light is removed (Costantini *et al.*, 1998b).

Examples of where carbon dioxide has been shown to increase the collections of mosquitoes include the work of Russell (1985) in Australia which showed that a carbon dioxide-baited trap was most efficient and collected the greatest numbers of *Cx annulirostris*. Work by Kemme *et al.* (1993) in the same country, indicated that of the odour-baited traps, carbon dioxide was the only chemical compound among those tested that caused attraction of *Ae. vigilax, Ae. funereus, Cx sitiens*, and *Cx annulirostris*. Recently, Kline (1994a) reported that 34 species of mosquitoes were attracted to carbon dioxide in a trap set outdoors in the United States.

In East Africa, light traps baited with carbon dioxide have been used to sample outdoor mosquito populations at known Rift Valley Fever epizootic sites in Kenya (Linthicum *et al.*, 1985). The most abundant mosquitoes obtained in these studies were *Aedes* species, including: *Ae. lineatopennis, Ae. circumluteolus, Ae. cumminsii, Ae. sudanensis, Ae. dentatus*, and *Ae. quasiunivittatus*. In another study in western Kenya (Logan *et al.*, 1991), light traps baited with carbon dioxide and set outdoors collected mosquitoes representing 8 genera and 43 species. Species collected in abundance included: *Cx pipiens, Cx zombaensis, An. coustani, An. squamosus, Ma. uniformis, Cq. microannulatus, Uranotaenia mashonaensis, Ma. africana* and *Cq. aurites*. However, in this study *An. gambiae*, the most important malaria mosquito in Africa, formed less than 0.1% of the total collection. A similar observation has been reported from Uganda (Henderson *et al.*, 1972) and South-east Tanzania (L.E.G. Mboera & B.G.J. Knols, unpublished data). The former collected various species of *Coquillettidia, Culex, Ficalbia* and *Uranotaenia*. The latter authors observed that an unlighted CDC-trap baited with carbon dioxide and set outdoors in a village setting, caught four mosquito genera, *Aedes* species accounting for the largest proportion (58.8%) followed by *Mansonia* (37.8%) and *Culex* (2.4%). *Anopheles* mosquitoes accounted for only 1% of the total catch with only few *An. funestus* and *An. gambiae* being caught in the traps.

In West Africa, Gordon *et al.* (1991) used carbon dioxide-baited traps to study the ecology of Rift Valley fever. In their study, mosquitoes representing 25 species in 4 genera were collected, with *Anopheles* species forming a very small proportion of the total catch. Jupp & McIntosh (1990) observed that of the mosquitoes collected by carbon dioxide-baited light traps in South Africa, anophelines accounted for 69.6% of the total collection but the species collected were not named. To establish the level of response of anthropophilic mosquitoes in Africa, the collections of mosquitoes should be both outdoors and indoors. Because all the above mentioned studies were done outdoors, this may have introduced a strong bias toward exophilic species. Indoor sampling would undoubtedly have yielded a larger number of anthropophilic species.

The efficiency of carbon dioxide-baited traps as compared to other trapping methods has been described and employed by many workers in the field. Landry & DeFoliart (1986) found that traps baited with carbon dioxide caught significantly more *Ae. triseriatus* than an unbaited trap or traps baited with a mouse. The added presence of a mouse did not increase the attractiveness of carbon dioxide. They concluded that carbon dioxide is a suitable bait for monitoring the biting activity of *Ae. triseriatus*, which is a low-density, diurnal species that does not readily enter animal-baited traps and does not respond to light traps (Craig, 1983). Lloyd & Pennington (1976) compared a CDC miniature light trap baited with carbon dioxide and bovine-baited trap. Although the same number of mosquito species were collected by both methods but the total caught in the carbon dioxide baited trap was more than 5.5 times that collected in the bovine-baited trap. When collections of a carbon-dioxide baited trap were compared with those of a trap baited with a pig, Yajima (1974) found that the mean age of mosquitoes collected in the former was lower than of those collected in the latter.

In Thailand, Miller *et al.* (1969) made a direct comparison between the responses of mosquitoes to light and carbon dioxide and found that carbon dioxide-baited traps caught about 30 times as many mosquitoes as unbaited light traps. In North-western New Jersey, Slaff *et al.* (1983) found that a carbon dioxide-baited trap and the man landing catch had a similarity index of 0.92. Carbon dioxide however, proved to be a far better indicator of mosquito biting intensity than the standard light trap. Similarly, Bangs *et al.* (1986) found that of the four mosquito collecting systems, 89.9% were caught in carbon dioxide baited traps while only 8.7% were collected by human baits. The mosquito species collected included *An. occidentalis, Cx erythrothorax, Cx tarsalis,* and *Cx quinquefasciatus,* only *Cx quinquefasciatus* being collected in large numbers on the human bait. These results indicate that before drawing a conclusion about the efficiency of carbon dioxide baited-traps compared to another sampling device, the response of the particular mosquito species to carbon dioxide must be known.

Pinkovsky & Sutton (1977) compared carbon dioxide and light as attractants for CDC mosquito traps in the Philippine and found that of over 10,000 female mosquitoes, 97.3% were taken using carbon dioxide and only 2.7% with light. They also found that 22 species of mosquitoes were collected in the carbon dioxide-baited trap while only four were taken in light traps. In an attempt to sample adult *Culex* mosquitoes for arbovirus surveillance in California, Reisen & Pfuntner (1987) showed that female *Cx quinquefasciatus*, *Cx peus* and *Cx tarsalis* were most abundant in carbon dioxide-baited traps in both urban and rural environments. Since carbon dioxide-baited traps collected far more *Cx quinquefasciatus* and *Cx peus* than unbaited light traps, this suggests that females responded more sensitively to an olfactory cue (carbon dioxide) than to a visual cue (light) (Reisen & Pfuntner, 1987). Although the ratio of standard light trap to carbon dioxide-baited trap catch size has been shown above to vary among mosquito species over time and space (Milby *et al.*, 1978; Meyer *et al.*, 1984), carbon dioxide-baited traps invariably collected more female mosquitoes than did standard light traps. Processing standard light trap catches is complicated by the sorting of the mosquitoes from the other phototactic insects (Reisen & Pfuntner, 1987). Unlike light traps, carbon dioxide-baited traps attract mosquitoes during the day as well as at night, but the catch size and species composition may differ (Service, 1969).

Most of these findings therefore, suggest that the addition of carbon dioxide to light traps on a regular basis improves the consistency of mechanical collection devices in an outdoor situation, and reflects the true biting fraction of the mosquito population without the inherent errors of man landing or biting counts. Nonetheless, it should be noted that the number and species of mosquitoes collected by any trapping system is correlated spatially and is mostly influenced by various environmental factors and host behaviour. Meteorological factors, for instance, are known to have a major influence on the mosquito sample size obtained. Daily ambient temperature is one of the strongest meteorological factor that modify the daily activity of adult mosquitoes (Petric *et al.*, 1995). Reisen & Aslamkhan (1978) observed that *Anopheles* species in Pakistan tend to bite mainly in the evening during the cool season and later in the night during the warm season. A similar pattern was found for *An. merus* in South Africa (Sharp, 1983). In Florida, Bidlingmayer (1985) also found that the upper temperature threshold for *Ae. vexans* is between 16 and 18^0C, and that catches of individuals involved in host-seeking flight declined by 10% for every degree of decrease in temperature. Sudden day-to-day temperature changes can therefore generate dramatic fluctuations in mosquito sample size (Petric *et al.*, 1995). The effect of wind speed on suction trap catches of some mosquitoes has also been described by Bidlingmayer & Day (1995). These workers observed that trap catches were inversely related to wind speed. For example, although the normal mosquito flight speed is about 1 m/sec, Bidlingmayer & Day (1995) found that trap catches were reduced to about 50% and 75% by winds of 0.5 m/sec and 1 m/sec respectively.

The type of vegetation cover at the collection site may also affect the size and species of mosquitoes collected by a trapping system. For instance, Meyer *et al.* (1991) demonstrated an association between carbon dioxide trap catch size and vegetation. They observed that most host-seeking *Cx tarsalis* and *Cx quinquefasciatus* females were collected in the understorey of peripheral vegetation and under shady trees while *Ae. nigromaculis* females were collected predominantly in an open pasture and peripheral vegetation, avoiding the tree canopy. The selective trapping of the three species by carbon dioxide baited traps placed in different vegetative microhabitats demonstrates that vegetation has a significant influence on carbon dioxide trap effectiveness for sampling various mosquito species. Furthermore, Meyer (1991) found carbon dioxide baited traps were more efficient in collecting *Cx quinquefasciatus* in rural mixed agricultural areas than in urban areas. Housing density in the urban zones was associated with the reduced efficiency of the carbon dioxide traps for sampling host-seeking female *Cx quinquefasciatus*. On the other hand, the increased effectiveness of the carbon dioxide trap in the rural areas was attributed to presence of vertebrate hosts and interspersed areas of vegetative cover. This suggests the presence of a relationship between the host-feeding patterns and associated foraging strategies of the mosquito species. Interesting observations have also been reported in California by Reisen *et al.* (1990). These workers found that whereas *Cx tarsalis* and *Cx stigmatosoma* were more abundant in carbon dioxide-baited CDC traps hung in the tree canopy than in traps at ground level, *Cx quinquefasciatus* was abundant in both ground level and tree canopy traps and *Cs. incidens* was collected more frequently by ground level traps. The level at which each species was collected correlated with the habitat of its preferred host. They found that *Cx tarsalis* and *Cx stigmatosoma* fed most frequently on birds, *Cx quinquefasciatus* on both mammals and birds and *Cs. incidens* primarily on dogs.

Carbon dioxide-baited traps for monitoring mosquito populations are widely used in many mosquito surveillance programmes (Petric *et al.*, 1995) and epidemiological studies of mosquito-borne viral and filarial diseases of man and animals. Light traps supplemented with carbon dioxide, have frequently been used to monitor adult mosquito populations during surveillance of vectors of encephalitis virus (Rohe & Fall, 1979), Japanese encephalitis virus (Vythilingam *et al.*, 1995) and Tembusu and Umbre viruses of man in Malaysia (Wallace *et al.*, 1977), eastern equine encephalomyelitis (Morris *et al.,* 1980), St. Louis encephalitis and other arboviruses in the United States (Tsai *et al.*, 1989; Kline *et al.*, 1990b; Meyer *et al.*, 1991; Gilliland *et al.*, 1995), Rift Valley fever in Kenya (Linthicum *et al.,* 1985; Logan *et al.,* 1991) and Senegal (Gordon *et al.*, 1991) and Chikungunya virus in South Africa (Jupp & McIntosh, 1990). Carbon dioxide-baited traps have also been employed to study the potential vectors of *Dirofilaria immitis* in the United States (Tolbert & Johnson, 1982; Walters & Lavoipierre, 1982; Pinger, 1982). In the United States, carbon dioxide is commonly used in the routine surveillance of *Cx nigripalpus* and *Cx salinarius* to increase adult collections (Kline *et al.*, 1990b).

Currently, there is no simple and cheap method for dispensing carbon dioxide in the field. Generally, either dry ice or carbon dioxide gas from a cylinder are used as baits in mosquito traps. An obvious advantage of using cylinders for releasing carbon dioxide into traps is that its discharge can be regulated, an important consideration if it is suspected that different species respond to different emission rates. Uniform release of gas, however, necessitates a sensitive regulatory valve system and meters to control and measure flow rates, and these together with the cylinders, are more costly and bulky than dry ice (Service, 1993b). Moreover, carbon dioxide gas at doses of several litres per hour is inconvenient and expensive to use on a large scale. The alternative carbon dioxide source, dry ice, is relatively cheap and light, although in certain areas it may be more difficult to obtain than cylinders. Dry ice-baited traps need, in addition, to be examined daily to replenish the dry ice bait. Furthermore, host-seeking mosquitoes generally respond to warmth, whereas dry ice will usually lower temperature in the vicinity of the trap, which is not conducive to mosquito attraction (Service, 1977). A major disadvantage of the use of dry ice is that the release rate of carbon dioxide is highly variable throughout the collection period, and this may cause either attraction or repellency of certain mosquito species.

The limitations in the dispensing of carbon dioxide provide a challenge in its routine use as a bait in mosquito trapping systems. Other sources of carbon dioxide have been considered by some workers. Hoy (1970) proposed the use of combustion as a source of carbon dioxide that has the advantages of low cost and ease of regulation. Hoy (1970) used a 4-cylinder engine adapted to operate on liquid propane gas. This system produces both carbon dioxide and carbon monoxide. Trials with pure carbon monoxide showed that this compound does not negatively affect the catch of mosquitoes (Hoy, 1970). This author also considered a gas burner as another possible source of carbon dioxide. Another cheap alternative source of carbon dioxide gas is a 1.5 kg generator described by Shipp (1985). This carbon dioxide generator, which is operated from a 500 g propane tank, emits carbon dioxide, moisture and heat.

The use of a small gas cylinder of about 11 kg and several truck or tractor inner tubes which can be inflated with gas at the trapping sites has been suggested by others. The inner tubes can be fitted with a valve mechanism to regulate the flow of gas. This arrangement has been used successfully in Scotland for trapping Simuliids (Coupland, 1991 cited by Service, 1993b). Alternatively, simple and small pieces of commercially available laboratory apparatus can be fitted to a gas cylinder to produce small blocks of dry ice of about 500 g. This apparatus enables the rapid production of a number of uniform-sized blocks which can conveniently be made in the field for immediate use, and thus saves the necessity to transport large blocks of dry ice to the field for breaking up into irregular lumps. This would appear to have considerable potential in areas where supplies of dry ice are limited (Service, 1993b).

Conclusion

Much of the work on host-seeking behaviour of mosquitoes suggests that olfaction is the principle modality used, with carbon dioxide being employed by nearly all blood-feeding species (Takken, 1996). Indeed, carbon dioxide is widely used in mosquito surveillance programmes, even though the efficiency of the sampling systems used is often not known. Although the precise mechanism of carbon dioxide chemotropism in mosquitoes is unknown, the compound can be considered a general vertebrate kairomone for female mosquitoes. It plays an important role in activation, upwind anemotaxis and selection of biting sites. The response of engorged and gravid females of some mosquito species indicates that carbon dioxide may also play a role in the search for resting sites. Its role in mate searching by males of some mosquito species is also apparent. Recent observations have indicated that mosquito species show differential responses to carbon dioxide (Reeves, 1953; Kline *et al.*, 1991a; Van Essen *et al.*, 1994; Takken *et al.*, 1997a; Dekker & Takken, 1998; Chapter 5), and that the compound accounts for only a minor part of the overall attractiveness of humans particularly in highly anthropophilic *An. gambiae, An. arabiensis, An. funestus* and *Cx quinquefasciatus* (Table 1).

Table 1. The response (in percentage) of Anopheles gambiae s.l., An. arabiensis, An. melas, An. funestus *and* Culex quinquefasciatus *to human odour as compared to carbon dioxide*

Species	Human Odour	Carbon dioxide	Reference
An. gambiae s.l.	60	40	Snow (1970)
	67	33	Costantini *et al.* (1996)
	70	30	Costantini *et al.* (1996)
	91	9	Chapter 5
An. arabiensis	60	40	Costantini *et al.* (1996)
	80	20	Dekker & Takken (1998)
An. melas	19	81	Snow (1970)
An. funestus	60	40	Snow (1970)
	73	27	Chapter 5
Cx quinquefasciatus	40	60	Costantini *et al.* (1996)
	75	25	Chapter 4
	72	28	Chapter 5

Although a differential response of mosquitoes to carbon dioxide has been reported, no efforts have been made to determine the optimal levels of carbon dioxide for a maximum response of individual mosquito species of medical and veterinary importance. The demonstration that mosquito species are strongly attracted by carbon dioxide has already led to routine trapping systems based on carbon dioxide-baited traps for monitoring some mosquito species, particularly in the United States and Australia. For the anthropophilic *An. gambiae*, which are the most important vectors of malaria and bancroftian filariasis in tropical Africa, it appears that carbon dioxide alone cannot replace the human bait although it may play a role in host-seeking behaviour in combination with other chemicals (Takken, 1996).

Despite the fact that studies on carbon dioxide chemotropism and its synergism have made no direct contribution to mosquito control to date, they have contributed significantly to our knowledge of mosquito host-seeking behaviour (Knols *et al.*, 1998). Data currently available on the response of mosquito species to carbon dioxide provides optimism that a control strategy based on semiochemicals can be devised. Indeed, recent studies in South-west Florida indicate that a carbon dioxide-octenol barrier can protect an area from being invaded with nuisance mosquitoes (Kline & Lemire, 1998). Carbon dioxide is already used for mosquito surveillance on a routine basis.

Much remains to be learned about the mode of action of carbon dioxide and its efficiency for mosquito surveillance. Determination of why it attracts some species of mosquitoes, or some populations of the same species and not others, requires further investigation. It is yet unknown whether different mosquito species, which all respond to the same host species, use the same semiochemical(s) as a source of information. Furthermore, we know little about the effect of physiological status (mating, chronological age, nutritional and gonotrophic state, blood feeding experience) on the response to host stimuli. In particular, the possible effect of the physiological state of mosquito on the response to carbon dioxide needs to be determined. For any vector-borne disease surveillance programme, the attraction of the fraction of the mosquito population most likely to be infected and infectious is of utmost importance. It needs to be determined if over the course of a mosquito season the response rate of a particular mosquito species to a specific release rate of carbon dioxide is constant. Studies emphasising the importance of interaction of various stimuli during host finding and host selection by different mosquito species are urgently required. These questions need to be addressed to determine the potential use of carbon dioxide and other kairomones in the surveillance and management of any mosquito species. Moreover, the development of carbon dioxide bait systems suitable for objective sampling of mosquito vectors of veterinary and medical importance is urgently required. Studies on the development and improvement of the dispensing systems for carbon dioxide in mosquito trapping systems must also be emphasised. There is therefore, much that needs to be learned about mosquito semiochemistry before olfactory based sampling techniques become practical.

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3

Olfactory responses of female *Culex quinquefasciatus* (Diptera: Culicidae) in a dual-choice olfactometer

ABSTRACT - Olfactory responses of individual female (n=1010) *Culex quinquefasciatus* Say (Diptera: Culicidae) to various odour stimuli were studied in a dual-choice olfactometer. Responses (i.e. the number of mosquitoes entering either of both olfactometer ports) were studied towards clean conditioned air (control), foot skin emanations (collected on worn stockings), carbon dioxide (4.5% in clean air), moistened air and various combinations thereof. Skin emanations were significantly more attractive ($\chi^2 = 23.0$, $P<0.001$) than clean stockings (control). The mosquito was also significantly more attracted ($\chi^2 = 7.7$, $P<0.01$) to skin emanations than to a clean stocking to which water (an equivalent of that absorbed by a worn stocking) was added. A moistened (1 g H_2O) clean stocking, however, was slightly more attractive than a dry stocking ($\chi^2 = 6$, $P<0.025$). Carbon dioxide (4.5%) did not elicit higher responses than clean air, and no synergistic effect was observed in combination with skin emanations. With the aim of developing an odour-baited trap, the results indicate that *Cx quinquefasciatus* responds well to body odours which can be collected on polyamide materials.

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Introduction

Culex quinquefasciatus Say (Diptera: Culicidae) is the most important vector of bancroftian filariasis in the world. The mosquito is widely distributed in tropical and subtropical regions of the world where it has established itself in towns and areas where human habitation has led to the creation of favourable breeding sites, mainly habitats containing highly polluted water rich in organic matter that larvae use for nourishment (Subra, 1981).

Host-seeking and biting behaviour of *Cx quinquefasciatus* constitute important aspects of bancroftian filariasis epidemiology. The mosquito is markedly endophagic, anthropophilic and nocturnal (White, 1971b; Beier *et al.*, 1990), which are behavioural traits favouring the transmission of *Wuchereria bancrofti* microfilariae from the peripheral blood system to the vector. Self *et al.* (1969) reported a preference for biting the lower limbs of humans but recent experiments by De Jong & Knols (1996) showed a random distribution of bites on a naked motionless volunteer.

Host-seeking mosquitoes use visual, physical and olfactory cues produced by their host to orient themselves to that host (Takken, 1991). Of the available stimuli, host odour is considered the most important one, especially for nocturnally active species that seek their host when visual cues are at a minimum or even non-existent. Several studies have examined organochemical compounds for their role in mosquito host location. Of these, carbon dioxide is generally considered an important mosquito kairomone (reviews by Clements, 1963; Gillies, 1980; Chapter 2), although its specific role in the host-seeking behaviour of different mosquito species is poorly understood. In addition to carbon dioxide, other compounds such as L(+)-lactic acid (Acree *et al.*, 1968), 1-octen-3-ol (Takken & Kline, 1989), butanone, phenols (Kline *et al.*, 1990a), and carboxylic fatty acids (Knols *et al.*, 1997) have been shown to attract mosquitoes. The majority of these studies, however, focused on the yellow fever mosquito *Aedes aegypti* (Acree *et al.*, 1968; Carlson *et al.*, 1973) and very little is known for other species of medical and veterinary importance. The odour-mediated host-seeking behaviour of *Cx quinquefasciatus* has received remarkably little attention, despite its medical importance. In this preliminary study we sought to investigate the response of *Cx quinquefasciatus* towards foot skin emanations, carbon dioxide and moisture as a first step in the development of an effective odour-bait for this filariasis vector.

Materials and Methods

Dual-choice olfactometer. The dual-choice olfactometer (Fig. 1) was made of Perspex materials and was placed in a climate-controlled room at 27±1^{0}C and 80±5% RH with a light intensity of 110 lux. Pumped air (1400 ml/min) was cleaned by activated charcoal, humidified, and regulated by volume before entering the olfactometer. Wind speed, temperature, and relative humidity of the air passing the tunnel ports were measured before and after each test, and averaged 20±4 cm/sec, 26.9±0.3^{0}C, and 93.9±0.4% RH, respectively.

Mosquitoes. The *Cx quinquefasciatus* strain used originated from Colombo, Sri Lanka (courtesy Prof. C.F. Curtis) and has been maintained under laboratory conditions for more than ten years. Mosquitoes were kept in a climate-controlled room at 27 ± 1^{0}C, 80±5% RH and a light regime of LD 12:12. Adult mosquitoes were kept in 30 cm^3 cages and offered a 6% glucose solution. Females were offered blood from a human arm twice weekly for egg production. Wet filter paper on glass petri dishes was provided for oviposition. Larvae were reared in plastic rays containing tap water and fed Tetramin® fish food. Females used in the experiments were four to eight days old and had not received a blood meal. They were deprived of sugar water 18.00 h before testing.

Experimental procedures. Experiments were carried out during the last six hours of the dark period. Test mosquitoes were randomly selected from a colony cage and transferred individually into glass vials (5x1.5cm) using a suction tube, which were then sealed with a water-moistened plug of cotton wool. Individuals were released at the downwind end of the olfactometer and the response (i.e., entering of either of the two olfactometer ports within the 3 min. experimental period) recorded. Surgical gloves were worn at all times in order to avoid contamination. All parts of the olfactometer were cleaned with hexane and ethanol (96%) between test series.

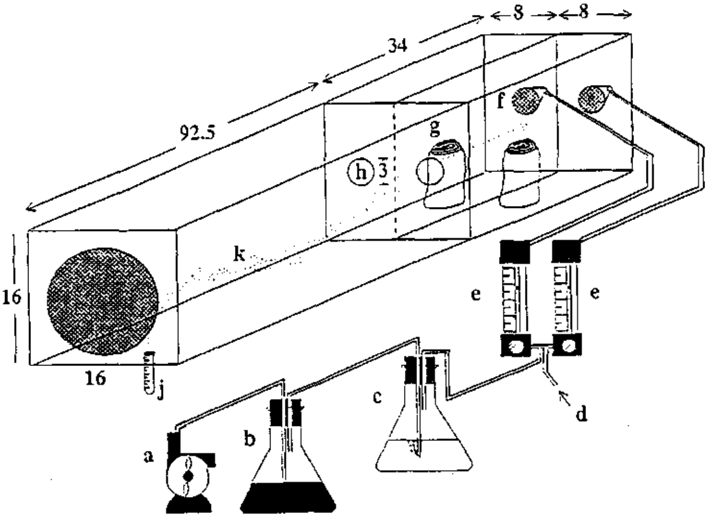

Figure 1. The dual-choice olfactometer set-up (dimensions in cm). Room air was pumped (a) at 1400 ml/min, cleaned by passing activated charcoal (b), moistened by passing distilled water (c), passed a flow meter (e) for each port, and entered the olfactometer (f). Baits (worn/clean stockings) (g), were positioned in compartments and air that passed over them entered the tunnel through the ports (h). Mosquitoes were released from glass vials (j) at the downwind end of tunnel and, an imaginary flight path is shown (k). When needed, carbon dioxide was pumped into the circuit from a pressurised gas cylinder at (d).

Before running tests in which odour stimuli were added to a clean air stream, a series of routine blank tests (clean conditioned air from both tunnel ports) was run. Similar tests were also conducted with clean polyamide stockings or carbon dioxide released from both ports in order to test the symmetry of the system. Thereafter, to counteract the effect of any biased directional response of unknown origin, the positions of the treatment and control were alternated between ports after every 10 mosquitoes.

Skin emanations were collected from a human foot on polyamide stockings by wearing them for 4-5 days between 06.00 h and 18.00 h at which time they were kept in a tightly stoppered bottle until being used for an experiment the following morning. Throughout the experiments the same volunteer provided the skin emanations. The rolled up stocking was placed on a glass petri dish 17 cm from the air entrance in the tunnel ports, and clean air was pumped over it. The control odour consisted of a clean stocking. The number of mosquitoes that responded to and probed the polyamide stocking in each treatment was recorded.

A clean polyamide stocking onto which water equal to an increase in weight (*ca.* 1.0 g) of a stocking worn for four days was pipetted, was tested against a worn (4 days) polyamide stocking in order to examine the effect of water on mosquito responses. In another series of experiments, clean stockings moistened with 1 g of water were tested against clean dry polyamide stockings.

Carbon dioxide (4.5%, the concentration in human breath (Gillies, 1980)) discharged from a cylinder at 300 ml/min was initially pumped into both ports of the tunnel. In the second experiment, carbon dioxide was compared to clean air. The control was conditioned air alone at a flow rate equal to the combined rates of flow of air and carbon dioxide in the treatment port. When running tests with carbon dioxide, the gas was allowed to flow freely through the conduit for five minutes to displace all air in the test port before the experiment started.

In a last series of experiments the possibility of synergism between foot odours and carbon dioxide was examined. Carbon dioxide (4.5% at 300 ml/min) passed over foot skin emanations on polyamide stocking was tested against either foot skin emanations on polyamide stocking or carbon dioxide (at a similar concentration).

Results

Figure 2A shows that when clean conditioned air was pumped into both ports of the tunnel, no significant preference ($\chi^2 = 0.9$, $P>0.05$) for either port was observed. Similar results were obtained when both ports were baited with clean stockings ($\chi^2 = 0.8$, $P>0.05$). Mosquitoes responded significantly more to skin emanations on polyamide stockings than to clean stockings ($\chi^2 = 23.0$, $P<0.001$). Of those mosquitoes entering the port with the worn stocking, 87.7 % landed and probed on it as compared to 12.3% of those entering the port with the clean stocking. Worn stockings were also more attractive than moistened clean stockings ($\chi^2 = 7.7$, $P<0.01$), confirming the attractiveness of body odours other than water. Nevertheless, moistened stockings were significantly more attractive than dry ones ($\chi^2 = 6$, $P<0.025$). The total percentage of mosquitoes responding to either of both test ports was significantly higher ($P<0.01$) in those treatments in which foot odours were pumped in the tunnel than in the treatment pumping clean air from both test ports. However, no difference was observed with the treatments using clean stockings in both ports or those that had water added to clean stockings.

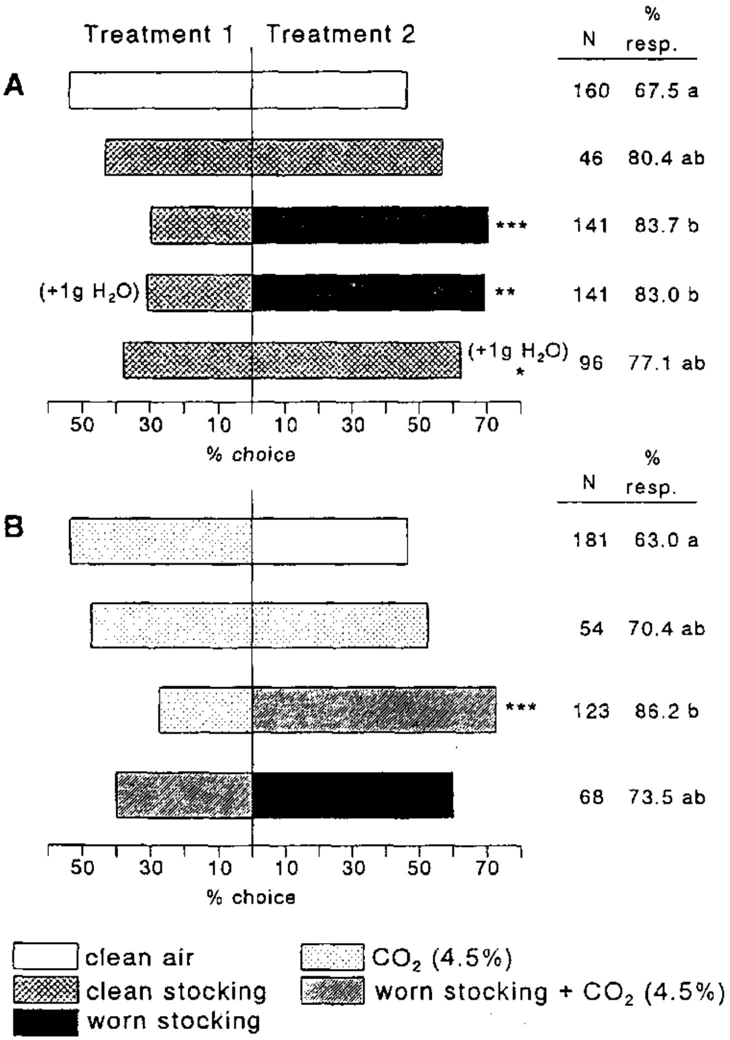

*Figure 2. Results of olfactometer tests with various odour stimuli. Treatments 1 and 2 were tested against each other, and bars indicate the percentage of responding mosquitoes flying into either test port (% choice). *:$P<0.025$; **:$P<0.01$, ***:$P<0.001$. N: total number tested. % resp.: mosquitoes that entered either of both test ports. Percentages with no letters in common are significantly different at $P<0.05$.*

When the response of *Cx quinquefasciatus* to air with added carbon dioxide (4.5%) was compared to that of atmospheric air (*ca.* 0.03%, Fig. 2B), the percentages of mosquitoes entering the ports did not differ between the treatments (χ^2= 0.9, *P*>0.05). These results indicate that carbon dioxide at human breath concentration does not attract this strain of *Cx quinquefasciatus*. When both ports of the olfactometer were baited with carbon dioxide, the mosquitoes did not show any preference (χ^2= 0.1, *P*>0.05) for either of the ports showing maintenance of symmetry of the olfactometer.

When carbon dioxide plus a worn polyamide stocking was tested versus carbon dioxide only, significantly more (χ^2= 24.6, *P*<0.001) mosquitoes were attracted to the carbon dioxide/worn stocking combination than to carbon dioxide alone (Fig 2B). On comparing the response to carbon dioxide+worn stocking versus worn stocking alone, it was found that there was no significant difference between the treatments (χ^2= 2.9, *P*>0.05), indicating that the response was more to the worn stocking than to carbon dioxide, and that no synergistic effect of skin emanations with carbon dioxide was present. The overall percentage of mosquitoes responding to any of the test ports in this second series of experiments was highest for those treatments in which foot odour and/or carbon dioxide was present in the tunnel.

Discussion

The results show that *Cx quinquefasciatus* is attracted to body odours collected on polyamide stockings, that this effect is partially caused by the uptake of water by the stocking, that carbon dioxide at a human breath concentration is not more attractive than clean air and, that the combination of body odours with carbon dioxide does not result in increased responses compared with body odour alone. Responses of *Cx quinquefasciatus* to skin emanations have also been observed in windtunnel studies by Omer (1979), who found that air passing over a hand was attractive to the mosquitoes. However, these studies did not compensate for the presence of water in the emanations that may well have influenced the results. As for human feet, hands contain high densities (>300/cm^2) of eccrine sweat glands, excreting large amounts of water (Marples, 1969). Other workers have also shown that skin emanations are attractive to a variety of mosquito species (Thompson & Brown, 1955; Khan & Maibach, 1966; Price *et al.*, 1979), and it has been found that odours collected on worn clothing attract more female *Anopheles gambiae* and *An. funestus* to an unoccupied hut than clean clothing (Haddow, 1942). Recently Geier *et al.* (1996) collected human skin odours on an ethanol-soaked pad, and demonstrated that this extract was as attractive to *Ae. aegypti* as a human hand and that the extract maintained its activity for months after storage at low temperatures. Other haematophagous insects have also been shown to be attracted to worn materials and even exhibited differential attractiveness to clothing worn on specific parts of the body. Thompson (1976) for instance, in Cameroon, attracted more blackflies belonging to the *Simulium damnosum* complex with worn trousers. From these and other studies (e.g. De Jong & Knols, 1996) it can be concluded that mosquitoes may respond to kairomones originating from some body regions, these odours can be transferred onto adsorbent cloth (cotton wool pads, polyamide material, etc.) and still retain their attractiveness. And due to a lasting effect of the attractiveness, these specific kairomones are not very volatile.

It is interesting to note that the addition of small amounts of water to clean stockings provides a sufficient stimulus to attract *Cx quinquefasciatus*. It was observed that during all experiments the stockings increased in weight by approximately 0.4 g, presumably as a result of water uptake from the humid airstream. This may have resulted in intermittent variations of the relative humidity of the air passing the tunnel ports, which may have been responsible for the overall increase of responsiveness of the mosquitoes. Like *An. gambiae* (Takken *et al.,* 1997b), it thus appears that *Cx quinquefasciatus* is also extremely sensitive to small changes in the relative humidity gradient. Earlier work showed that *Cx quinquefasciatus* bit significantly more on the drier areas of the body than on areas with a high eccrine sweat gland density (De Jong & Knols, 1996), suggesting that drier skin is preferred above a moister surface. These results are not in contrast with the present finding that moisture is a confounding factor in attraction to skin odour. Moisture is naturally present in the total emanations of the human body and it seems that *Cx quinquefasciatus* uses water vapour as an addition cue to respond to (human) hosts from a distance. As humans have a far greater eccrine sweat gland density than other mammals, moisture may be a behavioural stimulus for anthropophilic mosquitoes such as *Cx quinquefasciatus* and *An. gambiae sensu stricto* (Takken *et al.*, 1997b). Nevertheless, our studies have indicated that skin emanations elicit stronger attraction to *Cx quinquefasciatus* than variations in the moisture gradient, and that these skin emanations, left as residues in the stockings are probably a source of human odour that plays an important role in host-seeking behaviour of this species. It should be borne in mind, however, that due to the size of the olfactometer, responses were only observed over a small distance near the source. It may well be that over longer distances other kairomones also influence this behaviour. Recently it has been found that *Cx quinquefasciatus* are readily attracted to human emanations released *in-vivo* from a tent or a bed net (Costantini *et al.*, 1996; Dekker & Takken, 1998; Chapters 4 and 5) and that a Counterflow Geometry trap baited with foot skin emanations collected more *Cx quinquefasciatus* than unbaited traps (Chapter 6).

Carbon dioxide is considered to be a universal attractant for a number of haematophagous Diptera, but these results show that *Cx quinquefasciatus* is poorly attracted to this compound at a concentration equivalent to that in human breath. However, the response of the mosquito to different concentrations of carbon dioxide was not tested to determine optimum response levels for this species. Although Costantini *et al.* (1996) found that *Cx quinquefasciatus* in Burkina Faso to responded at doses above that normally released by one human, it is likely that at short range carbon dioxide plays a minor role in the attractiveness of a host. Other workers have made similar observations on different mosquito species (Crumb, 1922; Healy & Copland, 1995; De Jong & Knols, 1995). Recent work in Tanzania showed that tents baited with carbon dioxide (pure, at 300 ml/min) attracted 9% as many *An. gambiae s.l.* and 25-28% as many *Cx quinquefasciatus* as a tent baited with a human (Chapters 4 and 5). As carbon dioxide is not signifying the identity of an upwind host, it is likely that specialised mosquitoes like *Cx quinquefasciatus* show less dependency on this compound in their host-seeking behaviour than generalist species; and that, when used as a kairomone on its own, it accounts for only a minor part of the overall attractiveness of a human (Chapter 2).

No interaction between carbon dioxide and skin emanations was observed. This is contrary to earlier observations by other workers who reported the presence of a synergistic effect of carbon dioxide and other compounds for various species of mosquitoes. For example, Vickery *et al.* (1966) demonstrated synergism of carbon dioxide and chicks by showing that the combination of the two could increase the catch of mosquitoes. Lactic acid has been shown to activate and elicit oriented flight behaviour in mosquitoes only in the presence of carbon dioxide (Smith *et al.*, 1970; Price *et al.*, 1979). Takken & Kline (1989) and Kline *et al.* (1990a, 1991b) have shown that the presence of carbon dioxide in conjunction with other odours (namely 1-octen-3-ol) resulted in synergism for several mosquito species, in particular *Ae. taeniorhynchus*. Since in these experiments the combination of carbon dioxide and skin emanations could not significantly increase the number of mosquitoes responding compared to carbon dioxide only, it is likely that there is no synergistic effect for *Cx quinquefasciatus* by the combination of the two. The overall conclusion of this work is that human skin produces compounds to which *Cx quinquefasciatus* is attracted over a short distance, and that this effect may be enhanced by slight variations in relative humidity.

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4

Odour-mediated host preference of *Culex quinquefasciatus* (Diptera: Culicidae) in Tanzania

ABSTRACT - Field experiments to determine the host preference of *Culex quinquefasciatus* Say (Diptera: Culicidae), between a human host, calf and goat, were conducted in Muheza, North-east Tanzania. The responses of the mosquito to the three vertebrate hosts were also compared with responses to carbon dioxide. A total of 2565 unfed female mosquitoes were collected, of which *Cx quinquefasciatus* accounted for 96.6 % of the catch. A human-baited tent caught a larger number of host-seeking *Cx quinquefasciatus* than a calf-baited tent ($P<0.05$). The man:calf ratio was observed to be 7.8:1. Similarly, a human-baited tent caught a larger number of female *Cx quinquefasciatus* than a goat-baited tent ($P<0.05$), with a man:goat ratio of 10:1. The response of the mosquito to either a calf-baited tent or goat-baited tent was not significantly different ($P>0.05$). The number of mosquitoes attracted to a human-baited tent was significantly larger than that attracted to carbon dioxide released at 300 ml/min. Only 25.3 % of the human host response was attributed to carbon dioxide. The number of *Cx quinquefasciatus* responding to a tent baited with a calf or goat was not statistically different from the number responding to a tent baited with carbon dioxide released at 300 ml/min. It can be concluded that with equal availability of the three vertebrate hosts, *Cx quinquefasciatus* would respond more to volatile cues from a human host than from either a calf or goat, thereby supporting earlier data about its high degree of anthropophily. The major olfactory cue from a goat or a calf to which this mosquito responds is probably carbon dioxide.

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Introduction

Mosquitoes feed from a range of different host vertebrates. Some species have developed a characteristic host preference, feeding preferentially on humans (anthropophagic), mammals other than man (zoophagic) or birds (ornithophagic). For the majority of blood-sucking Diptera the spectrum of host choice differs with changing place and season and given different proportions of available hosts (Lehane, 1991). The mechanisms that determine host preference in mosquitoes are poorly understood. In many mosquito species, for instance, there is an explicit genetically determined host preference. However, the preference shown by a particular species of mosquito for one vertebrate host or another is likely to be influenced by environmental conditions (Takken, 1991), by the ecology and the behaviour of both the host and the vector, especially with regard to concurrence in space and time (Gillies, 1988). Host selection by vectors is thus a result of a combination of intrinsic preferences modulated by extrinsic factors. The probability that a mosquito will feed on a particular host depends both on a function of host numbers and easy access to them (Laarman, 1955; Burkot *et al.*, 1988; Gillies, 1988). This means that even in cases of species-specific host preference, the mosquito may still feed on a non-preferred host if this is the only one present because the opportunity to feed is more productively crucial than is the source of the blood meal.

In Africa, *Culex quinquefasciatus* has been described to be anthropophilic in its feeding behaviour (Thomas, 1956; Heisch *et al.*, 1959; White, 1971b; Mwaiko *et al.*, 1975; Beier *et al.*, 1990; Bϕgh *et al.*, 1998). This degree of anthropophily however, varies according to the place where female mosquitoes are collected. Although Smith (1961) and De Meillon & Sebastian (1967) reported that anthropophily predominated both in indoor and outdoor catches, recent observations by Beier *et al.* (1990) in Kenya have indicated that catches made indoors provide a larger proportion of anthropophilic females than those made outside. It is obvious that many of the mosquitoes that are collected indoors are likely to have taken a blood meal from a human host, most probably occupying the same house resulting into a biased higher human blood index than for mosquitoes collected outdoors.

Traditionally, mosquito host preferences have been assessed by determining the blood meal origin of freshly fed specimens (Tempelis, 1974; Garrett-Jones *et al.*, 1980). This has led to the estimation of the degree of anthropophily via the human blood index (HBI) i.e., the proportion positive for human blood in a sample of blood meals. The limitation of this approach lies in the difficulty of obtaining a sample representative for the population as a whole. Moreover, it does not measure the inherent host preference of a mosquito, but rather the final outcome of all the above mentioned factors (Costantini *et al.*, 1998a). Other problems include biased sampling of engorged endophilic mosquitoes, multiple feeds, difficulties of identifying closely related species and analysis of data (Boreham, 1975; Tempelis, 1975; Rubio-Palis *et al.*, 1994; Diatta *et al.*, 1998). Few data on mosquito host selection based on direct preference of mosquitoes to vertebrate hosts is available. For instance, in tropical Africa, although some information on host-selection preference using direct host choice is available for the important malaria mosquito species *Anopheles gambiae s.l.* (Gillies, 1964; Costantini *et al.*, 1998a; Diatta *et al.*, 1998), little is known about the preference of *Cx quinquefasciatus* to the commonest mammalian hosts. *Cx quinquefasciatus*, the tropical house mosquito, being the commonest urban mosquito in tropical Africa is more likely to encounter, in addition to humans, domesticated animals in its host-seeking process. The most common animals in the urban areas of East Africa include cattle, goats, sheep, and poultry. The aim of the present study was to investigate the host-selection preference of *Cx quinquefasciatus* to human, calf and goat hosts and to examine the role of carbon dioxide in attracting the mosquito to these hosts.

Materials and Methods

Study area. The studies were carried out at Muheza in the coastal lowlands of North-east Tanzania. Muheza ($5^0$10'S, $38^0$46'E), is 40 km inland from the coastal town of Tanga and lies at an altitude of 200 m above sea level. The rainfall pattern in the area is characterised by two seasonal peaks of rain, the main one in March-May and another less pronounced peak in November-December. The annual rainfall averages 1000 mm with a perennial high humidity. The mean annual temperature is 26^0C, with cooler months between June and September and warmer months between October and May. The experiments were conducted from mid May to mid June 1998, covering the last part of the rainy season. During the experiments, the ambient temperature was between 26.0^0C and 28.7^0C and the RH ranged from 78 - 93%. These variables were measured in *loco* by means of electronic probes. A total of 393.9 mm of rain fell during the study period.

Cattle husbandry, introduced during the early 1980s, is practised by many people in the township. There are about 500 dairy farmers keeping over 1500 heads of cattle. Most of the cattle are zero grazed (Mboera *et al.*, 1997a). Goats have been traditionally kept by the indigenous people for many years. Other animals found in the area include a few sheep, dogs, cats, poultry, pigs and donkeys.

Experimental protocol. Response of mosquitoes to a tent baited with man or calf. The response of host-seeking mosquitoes to a man inside a bed net in a tent was compared with a calf baited bed net in a tent. The male volunteer was 40 years old, weighed 70 kg, while the male calf was 6 months old and weighed 54 kg. The tents used (Fig. 1) in the experiments were floorless, had a sloping roof 2 m high at the apex, with a ground floor area of 1.9 x 1.9 m, and were made of light-grey polyvinylchloride. The tents had on both sides, slits (60x4 cm) just beneath the roof through which mosquitoes could enter. Two exit traps (Muirhead-Thomson, 1948) were fitted to the walls of the tents. Inside, an unimpregnated rectangular bed net was hung, next to which a standard miniature CDC light trap (Sudia & Chamberlain, 1962) was operated, the shield of the trap being suspended 1 m above ground level close to the net (Lines *et al.*, 1991). Each night the position of the tents and treatments were moved between the two sites. The experiment was run between 22.00 and 04.00 h for four nights.

Response of mosquitoes to a tent baited with man or goat. In the second experiment, the response of mosquitoes to a man-baited tent was compared to goat-

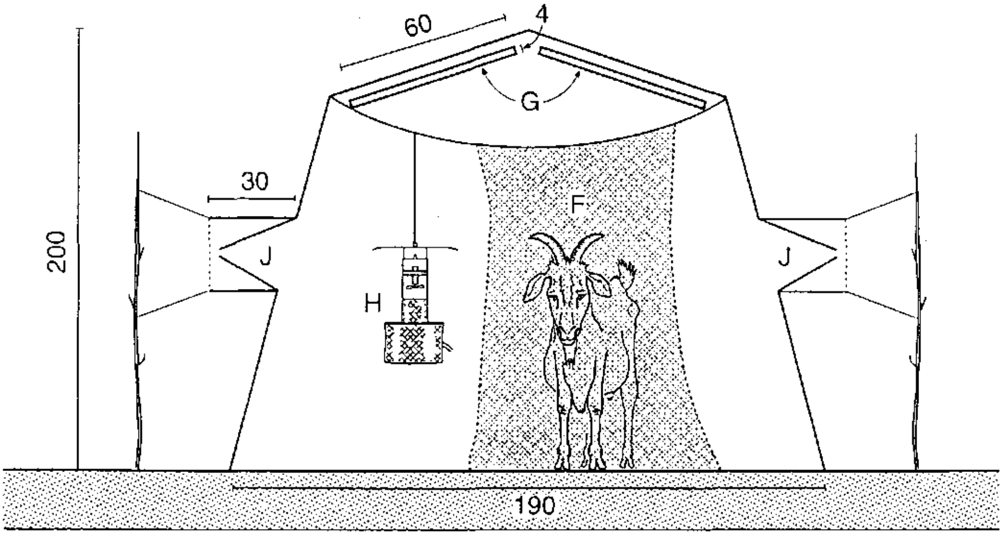

Figure 1. The experimental tent (dimensions in cm) modified for sampling mosquitoes. The test animal was placed under a bed net (F), mosquitoes could enter through entry-slits (G) and were caught by a CDC light trap (H) or Muirhead-Thomson exit traps (J).

baited tent. A six year old male goat, weighing 42 kg was placed inside a bed net in a tent in a similar experimental protocol as described above in experiment 1. The second tent was baited with a male volunteer mentioned above. The experiment was run between 22.00 and 04.00 h for four nights.

Responses of mosquitoes to tents baited with calf or goat. In the third experiment, the responses of mosquitoes to the calf-baited tent versus a goat-baited tent were assessed. The two animals are the ones used in the above experiments. The experiment was run between 20.00 and 05.00 h for four nights.

Responses of mosquitoes to a tent baited with either a man, calf or goat versus a tent baited with carbon dioxide. In the first experiment the response of mosquitoes to a tent baited with a man (described above) was compared to that baited with carbon dioxide released at 300 ml/min. In another two experiments, a calf and later a goat was compared with carbon dioxide at 300 ml/min. According to the table given by Gaddum (1961 as cited by Gillies & Wilkes, 1969), a 51-kg animal should produce 200 ml/min of carbon dioxide, or rather more in a young animal. Values between 200 and 300 ml/min would therefore have been appropriate for a body mass of 40 to 60 kg. A carbon dioxide output of 300 ml/min was aimed at as the equivalent of the animals' carbon dioxide output in these experiments.

Into one tent, carbon dioxide (300 ml/min) was pumped from a pressurised cylinder, through a 5 mm (diameter) silicon tubing and was led into the tent under the bed net. The outlet of the tubing was fixed onto a wooden pole, 0.5 m above the ground. In another experiment the attractiveness of a goat placed inside a bed net in a tent was compared with a bed net baited with carbon dioxide. Each night the flow rate was checked at the beginning and at the end of the experiment. The three experiments were each run between 21.30 and 05.30 h for four nights.

In these experiments the distance between the tents was 25 m and they were 30 m from the nearest house occupied by 5 people and an animal house containing 3 cows and 3 goats. The tents, bed nets and traps were assigned to the respective treatments and rotated daily between sites. The exit traps were emptied, and CDC light trap catches retrieved from the tents at 06.00 h every morning. The mosquitoes were taken to the laboratory where they were anaesthetised with chloroform; sorted to species, sex, and counted.

Data analysis. Catches of unfed female mosquitoes were transformed to log (n+1) and subjected to ANOVA after a satisfactory check for normality of the distribution (Snedecor & Cochran, 1989). An F-test significant at $P<0.05$ was followed by a Least Significant Difference test for comparison of treatment means. Back-transformed means are reported.

Results

A total of 2565 mosquitoes were collected during the study. The most abundant mosquito species in the area was *Cx quinquefasciatus* which accounted for 96.6 % of the total catch. No attempt was made to analyse data for the other species of mosquitoes (which included *Anopheles gambiae*, *An. funestus*, *An. coustani* and *Mansonia africana*). In the experiment to determine the response of mosquitoes to man-baited tent versus calf-baited tent, a significantly larger number of unfed host-seeking *Cx quinquefasciatus* were caught in the man-baited tent than in the calf-baited tent (Table 1A). Similarly, the man-baited tent caught significantly larger numbers of unfed female *Cx quinquefasciatus* than the goat-baited tent (Table 1B).

On comparing the response of mosquitoes to a tent baited with a calf versus a goat, it was found that *Cx quinquefasciatus* did not differ in host preference between a calf and goat ($P>0.05$) (Table 1C). When the number of mosquitoes caught in the man-baited tent was compared to that caught in the carbon dioxide-baited tent, of all mosquitoes, only 25.3 % were caught in the latter tent (Table 2A). However, the number of host-seeking unfed female mosquitoes attracted to the calf-baited tent was not significantly different from that attracted to the carbon dioxide-baited tent ($P>0.05$) (Table 2B). Likewise, the number of mosquitoes responding to the goat-baited tent was not significantly different from that responding to the carbon dioxide-baited tent ($P>0.05$) (Table 2C).

Table 1. Total (n) and geometric mean (±SD) number of catches of Culex quinquefasciatus *per day for a human-baited tent versus a calf- (A), and goat-baited tent (**B**) and a calf-baited tent versus goat-baited tent (**C**)*

	Bait	n	Mean±SD
A	Man	585	144.7±0.2a
	Calf	86	18.6±0.9b
B	Man	278	63.0±0.6a
	Goat	33	6.2±1.5b
C	Calf	200	47.5±0.4a
	Goat	153	22.9±2.0a

SD = standard deviation. Means in the same sub-table followed by a different letter are significantly different at $P<0.05$.

Table 2. Total (n) and geometric mean (±SD) number of catches of Culex quinquefasciatus *per day for a tent baited with carbon dioxide versus a man-baited tent (A), calf-baited tent (**B**), and goat-baited tent (**C**)*

	Bait	n	Mean±SD
A	Man	372	81.7±0.7a
	CO_2	94	20.7±0.8b
B	Calf	141	40.0±0.4a
	CO_2	193	45.6±0.5a
C	Goat	163	35.5±0.8a
	CO_2	179	39.0±0.9a

SD = standard deviation. Means in the same sub-table followed by a different letter are significantly different at $P<0.05$.

Discussion

Results of this study indicate that given equal access to a human host, calf and goat, *Cx quinquefasciatus* will select a human host in preference to the other two vertebrates. Recently, Dekker & Takken (1998) have shown that *Cx quinquefasciatus* entered human-baited traps significantly more than carbon dioxide or calf-baited traps. These findings support earlier reports from blood precipitin tests that *Cx quinquefasciatus* in tropical Africa is strongly anthropophagic (Beier *et al.*, 1990). The human blood index (HBI) for this mosquito has been observed to be as high as 95.5 % of mosquitoes collected indoors on the Kenyan coast (Bϕgh *et al.*, 1998). Similarly, indoor collections of *Cx quinquefasciatus* made in western Kenya also had a high HBI, but bloodfed mosquitoes collected outdoors was significantly lower (Beier *et al.*, 1990). There is little information on the response of *Cx quinquefasciatus* to goats in Africa. Beier *et al.* (1990) reported that in Kenya, goat/sheep blood meals accounted for only 0.5 % of the blood meals taken by *Cx quinquefasciatus* collected indoors. It appears that goat is one of the least preferred vertebrate hosts while it is among the most common domestic animals in East Africa. In Malaysia, Reid (1961) compared the mosquitoes attracted to two men, a calf and two goats. The man:calf ratio calculated for *Cx quinquefasciatus* caught in baited nets was 1.7:1 whereas that of man:goat was 2.8:1. In the study in Malaysia, *Cx quinquefasciatus* preferred man. Similarly, Dekker & Takken (1998) observed 1.7:1 man:calf ratio in their study in South Africa. The man:calf ratio reported by Reid (1961) and Dekker & Takken (1998), therefore, were lower than that observed in this study. This could be attributed to the different methods of mosquito collection between these studies. In Malaysia and South Africa, bed nets were used to collect mosquitoes in an outdoor setting, while in our study exit and CDC light traps were used to collect mosquito, in an indoor setting.

The analysis of blood meals of a wide range of mosquito species has demonstrated that feeding patterns, i.e., the range of host species fed on and the extent of host specificity, are highly species-specific. It is likely that differences in feeding patterns reflect differences in responsiveness to stimuli present throughout the host-location process (Costantini *et al.*, 1998a). It should be borne in mind that feeding patterns of mosquitoes obtained by analysis of blood meals might not reflect the actual mosquito preference because defensive behaviour by hosts can influence the successful feeding by mosquitoes. For instance, goats are known to be restless baits compared with sheep or calves (Gillies, 1988), reflected by having lower blood indices (Beier *et al.*, 1990) despite the fact that our study showed a similar preference of *Cx quinquefasciatus* to both calf and goat.

It was interesting to observe that the number of mosquitoes attracted to the tent-baited with a calf did not differ from that attracted to a goat. A similar trend was observed when the two mammalian species were compared with carbon dioxide released at 300 ml/min. This suggests that cues used by *Cx quinquefasciatus* to locate the two mammals may be similar and that carbon dioxide accounts for most of this attractiveness of *Cx quinquefasciatus* to these vertebrate species. The present study confirms the opinions of Costantini *et al.* (1998a) that it is possible to demonstrate a difference in the responsiveness of various mosquito species to air-borne odours from the commonly available hosts. Furthermore, these results show that the very high degree of anthropophily of *Cx quinquefasciatus* in this area can at least partly be explained as a preference for human odours other than carbon dioxide since only 25.3% of the catch could be directly ascribed to carbon dioxide.

In the natural situation, carbon dioxide is only one of several host stimuli that mosquitoes receive. Carbon dioxide in exhaled breath influences the behaviour of virtually all haematophagous insects and it probably played a significant role in the evolution of haematophagy (Knols & Meijerink, 1997). A major disadvantage of carbon dioxide is that a mosquito flying upwind in a plume of carbon dioxide cannot ascertain the species of its source, as it is a metabolic product of all living organisms. For opportunistic mosquitoes this would not pose a problem, but it turns out that anthropophilic mosquitoes have a limited choice of host species on which they feed. Because of this, other, more host-specific chemicals are also required by anthropophilic mosquitoes to facilitate host recognition. Generally, an opportunistic mosquito will respond to common mammalian kairomones such as carbon dioxide, whereas *An. gambiae, An. funestus* and *Cx quinquefasciatus*, which feed almost exclusively on human blood, would be expected to show limited response to carbon dioxide and instead select human-specific kairomones. The latter may be present in human foot emanations or human sweat, as it was recently found that anthropophilic *An. gambiae s.s.* and *Cx quinquefasciatus* are attracted to these stimuli (Braks *et al.*, 1999; Chapter 3). Variation in host-preference between mosquito species is likely to be reflected in their response to different host odours offered (Dekker & Takken, 1998). According to Gibson (1996), recent evidence from behavioural studies suggests that differences in host preference may reflect differences in the relative responsiveness to carbon dioxide and other, more specific, host odours. Although the attraction of many mosquito species towards carbon dioxide has been widely demonstrated (see Chapter 2), this common chemical does not inform the mosquito about the quality of the host nor explain the large differences in host-preference that exist between the various mosquito species (Dekker & Takken, 1998). It is likely, therefore, that the importance of carbon dioxide in the host-seeking behaviour of mosquitoes differs from species to species, and is limited in species exhibiting a strong anthropophilic behaviour (Chapter 5).

It is probably that human-specific semiochemicals act as host-specific cues for *Cx quinquefasciatus*. Carbon dioxide is likely to act in concert with other human cues in the host-location behaviour of *Cx quinquefasciatus* giving a response which is different from that of either stimulus given alone. Multiple stimuli are likely to be a more reliable guide to the presence of a specific host such as man, than one stimulus received alone. This is because, while carbon dioxide alone may be of non-host origin, this is very unlikely for combinations of stimuli, especially when they are received in particular proportions. Responding to particular combinations of host signals may permit a degree of selection for a particular host which is still some distance away, thus increasing host-seeking efficiency. With the long-term goal to make odour-baited traps for *Cx quinquefasciatus* surveillance and/or control, carbon dioxide will unlikely to be an efficient bait. Thus more research to identify human specific compound is strongly advocated.

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5

The response of *Culex quinquefasciatus, Anopheles gambiae s.l.* and *An. funestus* (Diptera: Culicidae) to tents baited with human odour or carbon dioxide in Tanzania

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ABSTRACT - The response of *Culex quinquefasciatus* Say, *Anopheles gambiae s.l.* Giles, and *An. funestus* Giles to tents baited with carbon dioxide or human odour was studied in the field in Tanzania. Two exit traps and a CDC miniature light trap set beside a bed net were used to sample mosquitoes that entered the tent. The number of host-seeking *Cx quinquefasciatus* caught in carbon dioxide (300 ml/min)-baited tent was significantly larger than that collected in unbaited tent ($P<0.05$). Human baited-tent attracted a larger number of *Cx quinquefasciatus* than carbon dioxide baited-tent. Carbon dioxide accounted for 28% of the number of *Cx quinquefasciatus* attracted to human odour. Human odour, pumped from an underground pit into a bed net attracted a similar number of *Anopheles gambiae s.l* and *An. funestus* as a bed net occupied by a man. Significantly fewer *Anopheles* mosquitoes were caught in a tent into which carbon dioxide (300 ml/min) was pumped than in a human odour baited tent (9 for *An. gambiae s.l.* and 27% for *An. funestus*). A five-fold increase of the carbon dioxide concentration (to 1500 ml/min) did not increase the catches of *An. gambiae s.l.* whereas those of *An. funestus* were increased to 69% of the catches by human odour. It is concluded that in the indoor situation described, human odour other than carbon dioxide is the principal cue to which these mosquito species are attracted and that the physical presence of a host and carbon dioxide, when used as a kairomone on its own, accounts for only a minor part of the overall attractiveness of man.

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Introduction

Culex quinquefasciatus, Anopheles gambiae s.l., and *An. funestus* are major vectors of bancroftian filariasis in East Africa. The last two species are also important vectors of malaria in the region. Effective transmission of these diseases requires at least two successful contacts between female mosquitoes and their human hosts. These contacts become established whenever a mosquito, by means of a complex behaviour, locates the host by responding to visual, physical and chemical cues produced by it (Clements, 1963; Takken 1991). The relative importance of these factors varies between mosquito species and depends mainly on the degree of host specificity. The three mosquito species are highly anthropophilic and nocturnal, and their long distance orientation behaviour is thought to be mediated to a large extent by olfaction (Takken, 1991). According to Gillett (1979) odours may be the long distance attractants guiding female mosquitoes to the vicinity of the host. Subsequently they respond to other signals such as warmth, moist air, and movement (Laarman 1955; Gillies, 1988; Takken, 1991). The extent to which each of these factors, or a combination thereof, influence the host-seeking behaviour of nocturnal mosquito species remains largely unknown. However, it has been shown recently, that host odours play an important role in host-location in *An. gambiae s.l.* (Costantini *et al.* 1993; 1996). Using odour-baited entry traps, Costantini *et al.* (1996) observed that in a direct choice test between carbon dioxide and human odour, about half as many *An. gambiae s.l.*, 40% as many *An. funestus* and about 60% as many as *Cx quinquefasciatus* were attracted to the carbon dioxide bait as compared to human odour. Previously, Snow (1970) found in field experiments that human breath from which carbon dioxide was removed attracted significantly lower numbers of mosquitoes than the untreated control.

By placing a cow in an underground pit, Vale (1974) separated visual and olfactory cues and demonstrated the importance of host odours in the host-seeking behaviour of tsetse flies (Diptera: Glossinidae). Similarly, in the forest region of Cameroon, Thompson (1976) found that a man hidden inside a trap and his exhaled breath removed through a long hose, attracted four times as many *Simulium damnosum s.l.* Theobald (Diptera: Simuliidae) as did an unbaited trap. He also found that carbon dioxide, emitted from inside the trap attracted some two-thirds as many as a man exhaling normally inside the trap. The purpose of this study was therefore, to determine the response of *Cx quinquefasciatus, An. gambiae s.l.* and *An. funestus* to human odour or carbon dioxide baited tents which were modified for sampling mosquitoes.

Materials and methods

Study area. The experiments were carried out at Ubwari ($5^0$10'S, $38^0$46'E), in Muheza district, North-east Tanzania and Kikulukutu ($8^0$09'S, $36^0$24'E), in Kilombero district, South-east Tanzania, during the long rainy season (March-June), when mosquito densities are highest. Ubwari, a village in Muheza township, is situated 40 km inland of Tanga at an altitude of 214 m above sea level on the foothills of the East Usambara mountains. The area is known for its high density of *Cx quinquefasciatus.*

Kikulukutu borders an extensive rice growing area which provides ideal breeding sites for *Anopheles* mosquitoes. The ecological setting and demography of Kikulukutu village has been described recently by Takken *et al.* (1998).

Experimental protocols. The tents used in the experiments have been described in Chapter 4. In Ubwari, the first experiment compared the response of mosquitoes to a carbon dioxide-baited tent versus unbaited tent. Into one tent, carbon dioxide at 300 ml/min was pumped from a pressurised cylinder, through a 5 mm silicon tubing and was led into the tent under the untreated rectangular bed net. The outlet of the tubing was fixed over a CDC trap fan at ground level to increase the dispersal of the gas. An unbaited tent was used as a control. The tents were 30 m apart and 50 m from the nearest human habitation. In the second experiment the attractiveness of a human seated inside a bed net in a tent was compared with a tent-baited with carbon dioxide gas. In one tent a male volunteer, 38 years old, was seated on a chair under untreated bed net. A CDC trap fan was fixed at ground level to increase the dispersal of odour. Carbon dioxide at 300 ml/min, dispensed from a pressurised gas cylinder through a silicon tubing, as described above, was led into the second tent under untreated rectangular bed net. The outlet of the tubing was fixed over a CDC trap fan at ground level to increase the dispersal of the gas. Each night the tents and treatments were moved between the two sites. The two experiments were run between 23.00 and 05.00 h for four nights and eight nights respectively. The exit traps were emptied, and CDC light traps were retrieved from the tents at 06.00 h every morning.

In Kikulukutu, the first experiment compared the attractiveness of a man-baited tent versus a tent-baited with carbon dioxide. The gas dispensed from a pressurised gas cylinder through 5 mm silicon tubing, passed through a flow-meter, and was then led into the tent under the bed net. The outlet of the tubing was fixed over a CDC trap fan at ground level to increase the dispersal of the gas. A similar fan was installed under the test person (inside a bed net) and in the control tent. Two doses of carbon dioxide were tested, 300 ml/min and 1500 ml/min, being the equivalent expired in breath by one or five adults respectively. The first dose was tested for three nights between 23.00 and 03.00 h, the second dose for six nights between 24.00 and 02.00 h. A third tent with neither a human bait nor carbon dioxide was used as a control. The tents were 30 m apart and were 50 m from the nearest house occupied by three people.

The second experiment investigated the feasibility of catching mosquitoes with only human odour being present in the tent. Underneath each tent a pit was dug, large enough to contain a test person. A 1 m long polyvinylchloride (PVC) pipe (diameter 11 cm), to which the fan of a CDC light trap was fixed, protruded 15 cm above ground level in the tent and was used to pump odours (at *ca.* 1.5 m/sec) from the pit into the bed net. The pits could be closed with a polythene sheet, pinned onto a wooden door frame. Odours from a 29 year old male volunteer was pumped from the pit between 23.00 and 03.00 h during four nights. 'Odour' from an unoccupied pit was pumped into a second tent which served as a control. Each night the test person would alternate between the pits. This would not only exclude day or site effects but would also show whether pits could become contaminated by odour residues which would temporarily make them unsuitable for use as a control treatment. The fans in the pits were left running from

03.00 h until 06.00 h as a precautionary measure against this. The third experiment compared the attractiveness of a man seated inside a bed net versus a bed net baited with human odour pumped from a pit. Two male test persons, 29 and 35 years of age, alternated between the two positions (bed net or pit) and the two sites for a total of 16 nights (between 23.00 and 06.00 h). In all experiments a tent, bed net and traps were assigned to the respective treatments to avoid contamination with odours. Exit traps were emptied, and CDC light trap catches retrieved from the tents every morning. To prevent day-time resting mosquitoes from being caught the following night the interior of the tents was thoroughly searched for mosquitoes which were then removed from it. Female *Cx quinquefasciatus* collected were dissected to determine the parity and infectivity of the host-seeking females. The proportions in which *An. arabiensis* Patton and *An gambiae s.s.* Giles occurred in catches with human odour and carbon dioxide were assessed by identifying specimens with the polymerase chain reaction (Scott *et al.*, 1993).

Data analysis. Catches from light traps and exit traps were summed, transformed to log(n+1) and after a satisfactory check for normality of the distribution subjected to Latin square analyses of variance (Snedecor & Cochran, 1989). An F-test significant at $P<0.05$ was followed by a Least Significant Difference test to sort out differences between treatment means.

Results

Throughout the study period in Ubwari, *Cx quinquefasciatus* dominated the entire mosquito collections. Few *Anopheles* species were collected in the area, and no attempt was made to analyse the data. When carbon dioxide-baited tent was compared to unbaited tent, it was observed that the former attracted significantly larger numbers of mosquitoes than the later (Table 1A). Table 1B shows that human baited-tent collected significantly more *Cx quinquefasciatus* than carbon dioxide baited tents ($P<0.05$). However, carbon dioxide could attract 28% of the mosquitoes attracted by the human bait. The parity rate of *Cx quinquefasciatus* attracted to the human bait (61.2%) was not significantly different from that of mosquitoes attracted to carbon dioxide (48.3%) (χ^2 test).

Table 2 shows that human odour, pumped from a pit, attracted significantly more *An. gambiae s.l.* and *An. funestus* than 'odour' from a control pit. Within the four day experimental period no effect of odour residues on the catch in the control treatment could be noticed, these being mean catch of 2.3 for *An. gambiae s.l.* and 1.3 for *An. funestus*.

When the catches of *Anopheles* mosquitoes collected from man-baited tent were compared to those from human odour-baited tent (Table 3) it was found that human odour was the single most important factor determining the size of catches since the physical presence of man in addition to his odour did not increase catches significantly. None of the other main effects was significant, and the two volunteers were equally attractive to both mosquito species.

Table 1. ANOVA tables, geometric mean catches of Culex quinquefasciatus *per day and indices of comparison for carbon dioxide baited tent versus unbaited tent (control) (A) or man-baited tent (**B**)*

	Source	df	MS	F-value	Treatment	n	Mean	Index
A	Day	3	0.0023	1.825ns	CO_2	82	20.12	1a
	Site	1	0.000005	0.007ns	Control	1	0.189	0.001b
	Treatments	1	3.1221	245.38***				
	Error	2	0.00127					
B	Days	7	0.2421	7.312*	Man	411	43.21	1a
	Sites	1	0.0443	1.339ns	CO_2	144	12.06	27.9b
	Treatment	1	1.1211	33.862***				
	Error	6	0.0331					

df, degrees of freedom; MS, mean squares; *: $P<0.05$; ***: $P<0.001$; ns, not significant; n, number of mosquitoes caught; indices not followed by the same letter are significantly different at $P<0.05$.

Table 2. ANOVA tables, geometric mean catches of An. funestus *and* Anopheles gambiae *per day and indices of comparison for human odours pumped into a tent from an underground pit versus odours pumped from unoccupied pit (control)*

Species	Source	df	MS	F-value	Odour	n	Mean	Index	Aa/Ag
An. funestus	Day	3	0.074	3.61ns	human	34	7.32	1a	
	Site	1	0.004	0.17ns	control	5	1.38	0.188b	
	Odour	1	0.592	24.43*					
	Error	2	0.023						
An. gambiae s.l.	Day	3	0.020	0.63ns	human	106	21.90	1a	0.84
	Site	1	0.760	24.39*	control	9	1.34	0.061b	0.89
	Odour	1	1.963	62.97**					
	Error	2	0.031						

df, degrees of freedom; MS, mean squares; *: $P<0.05$; **: $P<0.025$; ns, not significant; n, number of mosquitoes caught; indices not followed by the same letter are significantly different at $P<0.05$. Aa/Ag: proportion of *An. arabiensis*/*An. gambiae s.s.*

When tents were baited with the equivalent of carbon dioxide produced by an adult human, catches were only 9 and 27% (for *An. gambiae s.l.* and *An. funestus* respectively) of the catch obtained from a tent occupied by a man, in both cases a significant reduction was observed (Table 4). However, carbon dioxide caught significantly more mosquitoes than unbaited tents. A five-fold increase in the amount of carbon dioxide released in the tent did not affect *An. gambiae s.l.* catches, but increased the *An. funestus* catch to 69% of that by man, which made both treatments equally attractive for this species. Species identification showed that the majority of *An. gambiae s.l.* were *An. arabiensis.* The relative proportions of *An. arabiensis*/*An. gambiae s.s.* in human odour and carbon dioxide samples were not significantly different from each other (χ^2 test).

Discussion

A human-baited tent consistently collected higher numbers of *Cx quinquefasciatus* than a carbon dioxide-baited tent, indicating that human odour other than carbon dioxide plays a major role in the attraction of this mosquito species, and that carbon dioxide accounts for 28% of the attractiveness of whole human odour in the host-finding process of the mosquito. *Cx quinquefasciatus* in the area of study is highly anthropophilic (Mwaiko *et al.*, 1975; Chapter 4), it is thus not surprising that it responds more to human odour than to carbon dioxide. In another experiment in the same area, carbon dioxide-baited tent caught 25% as many *Cx quinquefasciatus* as those caught by human-baited tent (Chapter 4). However, in West Africa, Costantini *et al.* (1996) showed that 60% of the response of *Cx quinquefasciatus* to man was due to carbon dioxide and that the species shows a threshold response to carbon dioxide responding only at doses above that normally released by one man.

The parity rate of the female *Cx quinquefasciatus* attracted to the human bait was not significantly different from that of mosquitoes attracted to the carbon dioxide-baited tent. Carbon dioxide-baited traps have been shown to attract mosquitoes of different physiological status in space and time. For instance, although some workers have observed that more nulliparous mosquitoes respond to carbon dioxide than were parous and gravid mosquitoes (see Chapter 2), a significantly higher proportion of parous females sampled by carbon dioxide-baited traps has been reported by others. It is likely therefore that the parity rates of mosquito caught by carbon dioxide-baited traps vary between species, place and time.

When the attractiveness of carbon dioxide was compared with that of man, the equivalent of carbon dioxide produced by an adult human attracted significantly fewer mosquitoes of both *An. gambiae s.l.* and *An. funestus* than a test person, though for both species the catch was significantly higher than that of the control tent. The large reduction in catch compared to a human baited-tent could have been caused by a smaller range of attraction of carbon dioxide. However, a five-fold increase in carbon dioxide concentration should then have extended this range and attract more mosquitoes, which for *An. gambiae s.l.* was clearly not the case. In contrast, *An. funestus* catches rose to the

Table 3. ANOVA tables, geometric mean catches of An. funestus *and* An. gambiae *per day and indices of comparison for human odours pumped into a tent from an underground pit versus a man occupied tent.*

Species	Source	df	MS	F-value	Human inside	n	Mean	Index
An. funestus	Block	3	0.047	0.59ns	Tent	204	10.94	1a
	Site	1	0.014	0.18ns	Pit	161	8.35	0.76a
	Position	1	0.089	1.13ns				
	Person	1	0.013	0.17ns				
	Error	25	0.079					
An. gambiae s.l	Block	3	0.044	0.037ns	Tent	45	2.14	1a
	Site	1	0.002	0.01ns	Pit	68	3.01	1.41a
	Position	1	0.089	0.75ns				
	Person	1	0.007	0.06ns				
	Error	25	0.119					

df, degrees of freedom; MS, mean squares; ns, not significant; n, number of mosquitoes caught; indices not followed by the same letter are significantly different at $P<0.05$.

Table 4. ANOVA tables, geometric mean catches of An. funestus *and* Anopheles gambiae *per day and indices of comparison for human odours pumped into a tent from the underground pit versus a 300 or 1500 ml/min carbon dioxide (CO_2) baited tents*

Species	Source	df	MS	F-value	Odour	n	Mean	Index	
An. funestus	Day	2	0.001	1.52ns	Human	56	17.70	1a	
	Site	2	0.098	108.19*	CO_2 (300ml/min)	16	4.80	0.274b	
	Odour	2	1.035	1139.46**	Control	1	0.26	0.015c	
	Error	2	0.000						Aa/Ag
An. gambiae s.l.	Day	2	0.043	2.16ns	human	300	99.20	1a	0.78
	Site	2	0.039	1.95ns	CO_2 (300ml/min)	32	8.80	0.088b	0.87
	Odour	2	21.92	108.90*	Control	3	1.00	0.010c	0.50
	Error	2	0.020						
An. funestus	Day	5	0.082	1.15ns	Human	67	9.51	1a	
	Site	2	0.070	0.97ns	CO_2 (1500 ml/min)	51	6.59	0.693a	
	Odour	2	1.476	20.67**	Control	1	0.26	0.027b	
	Error	8	0.071						Aa/Ag
An. gambiae s.l.	Day	5	0.106	1.836ns	Human	126	18.37	1a	0.64
	Site	2	0.040	0.69ns	CO_2 (1500ml/min)	13	1.46	0.08b	0.55
	Odour	2	2.295	39.47**	Control	2	0.26	0.014c	1.00
	Error	8	0.058						

df, degrees of freedom; MS, mean squares; *: $P<0.01$; **: $P<0.001$; ns, not significant; n, number of mosquitoes caught; indices not followed by the same letter are significantly different at $P<0.05$. Aa/Ag: proportion of *An. arabiensis*/*An. gambiae s.s.*

extent that they were no longer different to those from the test person. For *An. gambiae s.l.* this could have meant that the low dose of carbon dioxide already induced the maximum response, which therefore could not be increased by a higher dose. For *An. funestus* the responses were clearly enhanced by the higher dose. However, several additional factors could have caused the increase of catches for this species. Firstly, in East Africa, *An. funestus* belongs to a species complex (Gillies & Coetzee, 1987), and it is possible that zoophilic members of this complex, such as *An. rivulorum* Leeson represented a larger proportion of the catch when higher doses were used. Both species occur sympatrically in this region, but their relative contributions to the catches could not be ascertained due to difficulties of identifying *An. rivulorum.* Secondly, even if similar numbers were attracted to the tents by both doses, the higher dose could have enhanced the slit-entry response, resulting in higher catches. Furthermore, even if the number that entered the tents was similar, it is not known what proportion escaped through the entry slits. The higher dose of carbon dioxide might have stimulated mosquitoes to remain around the bed net longer which therefore increased their chance of being caught. Whether differential responses towards varying doses of carbon dioxide took place at physiological or behavioural level therefore remains unclear.

Although carbon dioxide is considered to be a universal attractant for blood-feeding Diptera, our observations have indicated that the degree of attraction of these anthropophilic mosquito species to this compound is rather limited. In a separate field study by Knols *et al.* (1998) it was shown that both *Anopheles* species were not attracted to human breath or the equivalent of carbon dioxide therein in an outdoor situation. Moreover, in Burkina Faso, Costantini *et al.* (1996) found that carbon dioxide, whether presented near human odour, on its own or even at high doses, was not as attractive as a human host indoors or his odour equivalent. Similarly, using human and carbon dioxide baited bed nets in South Africa, Dekker & Takken (1998) observed that *An. arabiensis* caught with human equivalents of carbon dioxide was significantly lower than with whole human odour. It is likely that these mosquitoes show less dependency on carbon dioxide in their host-seeking behaviour because the compound provides no information on the identity of the host upwind of them. It is somewhat surprising that the more zoophilic *An. arabiensis*, which dominated in the catches, did not show a higher response to carbon dioxide but apparently relied more heavily on other odours to locate the source. However, Costantini *et al.* (1996) observed that the West African *An. arabiensis* chose the carbon dioxide-baited trap with higher probability than *An. gambiae s.s.*. The latter species is activated by (Healy & Copland, 1995) and attracted to carbon dioxide in the laboratory (Knols *et al.*, 1994b), and its role as a kairomone has clearly been established. Similar results have been reported for *An. arabiensis* (Omer, 1979). In the field, however, the biological significance of carbon dioxide might only be expressed in the interaction with other odours, perhaps in a synergistic manner, in the proximity of the host.

By pumping human odour from an underground pit into a tent we excluded the influence of visual cues, convection currents and radiant body heat, the remaining stimuli being moist air, body odours and carbon dioxide. Since the active range of attraction of carbon dioxide when leaving the tent must have been limited, probably to less than a metre (Gillies, 1980), the attraction of the two *Anopheles* species can then only be explained as orientation from a distance to body odours other than carbon dioxide. It is concluded that body odour guided the mosquitoes to the immediate vicinity of the tent, where prior to entering it, they experienced increased carbon dioxide and moisture levels near the entry slits. This is similar to what was found for *An. melas* Theobald in an outdoor situation in The Gambia, where the active range of attraction was much larger than what could be expected on the basis of carbon dioxide expired by the calves used in that study (Gillies & Wilkes, 1969; 1970).

After the mosquitoes entered the tent, the physical presence or absence of a human did not affect the total number caught. This indicates that even if visual and physical properties of the host are presented to the mosquito, chemical cues still dominate the host-seeking process at this stage of final approach, *ca.* 2 m from the host. Although it has long been thought that body odours only play a role at distances from the host where physical and visual cues are not detectable (Laarman, 1955; Clements, 1963; Gillies & Wilkes, 1972) the present results show that they continue to play a role even in the immediate vicinity of the host.

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6

The response of *Culex quinquefasciatus* Say (Diptera: Culicidae) to traps baited with carbon dioxide, 1-octen-3-ol, acetone, butyric acid and human skin emanations in Tanzania

ABSTRACT - The responses of *Culex quinquefasciatus* to traps baited with carbon dioxide, 1-octen-3-ol, acetone, butyric acid and human skin emanations were studied in the field in Muheza, North-east Tanzania. Using Counterflow Geometry (CFG) traps, it was found that significantly more *Cx quinquefasciatus* responded to skin emanations collected on polyamide (nylon) stockings than to clean stockings ($P<0.05$). Significantly more mosquitoes were caught in a CFG trap baited with carbon dioxide than in traps with either human skin emanations, acetone or butyric acid. It was also found that in an outdoor situation a carbon dioxide baited CDC light-off trap collected over 12 times more *Cx quinquefasciatus* than an unbaited CDC light-off trap and 9 times more mosquitoes than CDC traps baited with 1-octen-3-ol alone ($P<0.05$). Although a carbon dioxide baited CDC trap collected more mosquitoes than when the trap was baited with a combination of carbon dioxide and 1-octen-3-ol, the difference was not statistically significant. Moreover, the number of mosquitoes caught in a CDC trap baited with 1-octen-3-ol did not differ significantly from that of the unbaited CDC trap ($P>0.05$). These results indicate that the Afrotropical *Cx quinquefasciatus* responds significantly better to traps baited with carbon dioxide than to either octenol, acetone or butyric acid, and that human skin emanations contain stimuli to which *Cx quinquefasciatus* is attracted under field conditions.

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Introduction

Blood-seeking mosquitoes use airborne olfactory cues produced by their hosts to orient to that host. For nocturnal anthropophilic mosquitoes human body odour is probably the most important cue in their host-seeking behaviour. Odours may be the long distance cues employed by mosquitoes, while in the vicinity of the host, the insect may respond to other signals such as temperature, moist air and movement (Gillies, 1988). In recent years, several workers have demonstrated the behavioural significance of organochemical compounds as a source of attractants for anthropophilic mosquitoes (Cork 1996; Cork & Park 1996; Geier *et al.*, 1996; Knols *et al.,* 1997; Takken *et al.*, 1997a). The olfactory stimuli implicated in host location by haematophagous insects to date include carbon dioxide, lactic acid, acetone, butanone, 1-octen-3-ol (hereinafter referred to as octenol), phenolic components of urine (Hassanali *et al.*, 1986; Bursell *et al.*, 1988) and short-chain carboxylic acids (Knols *et al.*, 1997). The use of carbon dioxide as a stimulus for mosquitoes has been known for some time and its role in host-finding by mosquitoes has been reviewed comprehensively in Chapter 2.

Octenol and acetone, which were first identified as host kairomones for tsetse flies (Hall *et al.*, 1984; Hassanali *et al.*, 1986), have been shown to attract certain mosquito species in the field. The established role of octenol as a kairomone for many mosquito species has been well documented (Kline, 1994a). Most of these studies have demonstrated that octenol, in combination with carbon dioxide, significantly increased collections of *Aedes* mosquitoes. However, the response of many mosquito species to octenol is poor in the absence of carbon dioxide. Recently, it was shown that the anthropophilic *Culex quinquefasciatus* Say (Diptera: Culicidae) is attracted to human skin emanation in the laboratory (Chapter 3), while Kline (1998) showed that several other culicine mosquitoes were attracted to it in the field in the USA. These chemicals, and others, have not been previously tested as mosquito attractants under field conditions of East Africa. The studies reported herein were, therefore, conducted to further evaluate carbon dioxide, octenol, acetone, butyric acid and skin emanations as attractants for *Cx quinquefasciatus*, a serious nuisance mosquito and vector of urban bancroftian filariasis, under field conditions of North-eastern Tanzania.

Materials and methods

Study area. Field studies were conducted in Muheza ($5^0$10'S, 38^0'46'E) in North-east Tanzania. The area has been described in Chapter 4.

Traps. In this study Counterflow Geometry (CFG) and miniature Centers for Disease Control (CDC) traps were used to sample mosquitoes. The CFG trap (fig. 1, courtesy Dr. Dan Kline), which utilises a novel counterflow concept, has been described recently by Kline (1999). It is constructed from a clear polyvinylchloride (PVC) container (*ca.*11.4 l), modified by removing the bottom, adding a mounting flange and a 10.16 cm diameter x 17.78 cm length of PVC thin wall pipe. Inside the 10.16 cm pipe, a 5.08 cm diameter x 30.48 cm length of PVC pipe is mounted concentric with, and extended 7.62 cm beyond the end of 10.16 cm pipe. The lid of the jar is modified by attaching an 8 cm fan above and a suitable filter structure below the lid. The 8 cm fan is positioned to blow air out of the container and thus induce a supply draft between the 10.16 cm pipe and the 5.08 cm pipe. Inside the filter structure, a 4 cm fan is mounted so as to seal the 5.08 cm tube from the container and provide an airflow out of the container in a direction opposite to flow created by the 8 cm fan. In the side of the 5.08 cm pipe, a small tube (0.32 cm, internal diameter) is attached to provide an entrance port for carbon dioxide to enrich the exit plume from the 4 cm fan. Above the 8 cm fan, a small lid is attached to prevent rain damage to the fan and to the two 6 V batteries connected in series. The CDC traps (Model 512, John W. Hock Company, Gainesville, FL) are as described by Sudia & Chamberlain (1962).

Experimental protocol. The response of Cx quinquefasciatus *to a Counterflow Geometry (CFG) trap baited with skin emanations versus an unbaited trap.* Two CFG traps were used in this experiment. One of the traps was baited with a polyamide (nylon) stocking, worn for 4 to 7 days on a male volunteer (40 years old, 70 kg body weight) while the other trap was baited with a clean polyamide stocking. The stockings were put into a polythene bag, fixed to the trap at its carbon dioxide supply point, through a 20 cm long (5 mm inside diameter) silicon tubing. The traps were hung from a wooden pole at 25 m apart and 30 m from the nearest house. The treatments were alternated between the two sites every trapping day and the traps were operated between 19.00 and 06.00 h for four days.

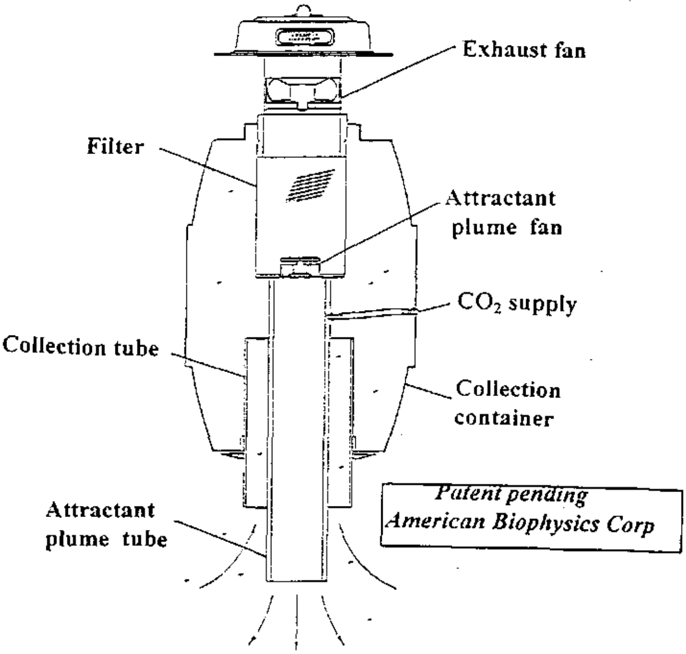

Figure 1. Counterflow Geometry (CFG) trap (after Kline, 1999)

The response of Cx quinquefasciatus *to a CFG trap baited with carbon dioxide versus a trap baited with skin emanations, acetone or butyric acid.* Into one trap, carbon dioxide released at 300 ml/min was pumped from a pressurised cylinder, through a 5 mm silicon tubing. The second trap was baited with a polyamide stocking worn for four to seven days. The stocking was put in a polythene bag fixed to the trap through a similar tubing, 20 cm long. The traps were hung from a wooden pole with the lowest part at 20 cm from the ground. In another experiment, the response of *Cx quinquefasciatus* to a CFG trap baited with carbon dioxide versus a CFG trap baited with acetone was assessed. In one trap carbon dioxide was pumped from a pressurised cylinder at a rate of 300 ml/min. In another trap, a glass bottle containing 4 ml of acetone was fixed to a silicon tube connected to the trap at its carbon dioxide supply point. The acetone was levelled to a 4 ml mark before the start of the daily experiment. In another series of experiments, the response of *Cx quinquefasciatus* to a carbon dioxide baited CFG trap versus a trap baited with butyric acid was assessed. Butyric acid was supplied from a 4-ml glass vial fitted to a silicon tubing fixed to the trap. In another trap, carbon dioxide released at 300 ml/min was pumped. During these experiments the treatments, 25 m apart and 30 m from the nearest house, were alternated between the two sites and the traps were operated between 19.00 and 06.00 h for four days.

The response of Cx quinquefasciatus *to a CFG trap baited with carbon dioxide or skin emanations versus a trap baited with carbon dioxide+skin emanations.* In this experiment carbon dioxide released at 300 ml/min was pumped into one CFG trap while in the other trap carbon dioxide at the same release rate was pumped through a polythene bag containing a polyamide stocking worn for four to seven days. Carbon dioxide was released into the polythene bag through a 5 mm silicon tubing. In the third treatment, a polythene bag containing a polyamide stocking worn for 4-7 days was fixed to a CFG trap through its carbon dioxide supply point. The traps were 25 m apart in a straight line. At each site the trap was hung from a wooden pole so that the bottom tube, through which the odour is released, was 20 cm above the ground level. In this experiment, traps were assigned to the respective treatments and rotated daily between sites for three days between 18.00 and 06.00 h.

The response of Cx quinquefasciatus *to carbon dioxide, octenol and carbon dioxide + octenol combination.* Because of an insufficient number of CFG traps, CDC traps from which the light had been removed (CDC light-off) were used in this experiment. Miniature CDC light-off traps were baited with the following odours: (1) carbon dioxide alone; (2) octenol alone; (3) carbon dioxide + octenol and (4) an unbaited CDC trap as a control. Carbon dioxide released at 300 ml/min was dispensed from a pressurised gas cylinder as described in experiment 2. The outlet of the tubing was fixed at the entrance of a CDC trap. 4 ml of octenol was dispensed from glass vials with a pipe-cleaner extending 2 cm above the septum (see Kline *et al.*, 1990a). Each trap day the octenol level was adjusted to 4 ml and the vial inverted for several seconds to ensure complete wick saturation. Vials were affixed near the trap entrance and when used in combination with carbon dioxide, they were affixed adjacent to the carbon dioxide release point. The traps were hung outdoors from wooden poles, with the trap shield at 1 m from the ground. The treatments were 25 m apart in a straight line and 30 m from the nearest house. Each trap position was provided with a different bait daily in a randomised design, from 19.00 to 06.00 h.

Data analysis. All mosquito catches were transformed to log(n+1) and were subjected to a Latin square analysis of variance (Snedecor & Cochran, 1989). An F-test significant at $P<0.05$ was followed by a Least Significant Difference test to sort out differences between treatment means.

Results

A total of 1508 mosquitoes were collected during the study period. The collections consisted of 6 species of mosquitoes, which in descending order of abundance were *Cx quinquefasciatus* (87.7%), *Anopheles gambiae* (2.9 %), *Cx cinereus* (2.1 %), *An. coustani* (1.8 %), *An. funestus* (1.0 %) and *Mansonia africana* (0.9%). Only *Cx quinquefasciatus* numbers were considered adequate to include in the statistical analyses.

Table 1. Total (n) and geometric mean catches of Culex quinquefasciatus *per day for a CFG trap baited with skin emanations versus an unbaited trap (control)* ***A****; carbon dioxide versus a trap baited with:* ***B****, skin emanations:* ***C****, acetone; and* ***D****, butyric acid outdoors.*

	Bait	n	Mean±SE
A	Skin emanations	85	21.1±0.8a
	Control	10	2.3±0.2b
B	Carbon dioxide	143	35.3±0.1a
	Skin emanations	49	11.9±0.2b
C	Carbon dioxide	176	43.4±0.1a
	Acetone	22	3.4±0.7b
D	Carbon dioxide	118	29.4±0.1a
	Butyric acid	16	3.5±0.3b

SE = standard error. Means in the same column, within a sub-table, followed by a different letter are significantly different at $P<0.05$.

In all experiments there was no significant difference in catches between days or sites. Table 1A shows that significantly more mosquitoes responded to a trap baited with skin emanations than to an unbaited trap under field conditions. However, significantly more mosquitoes were caught in a trap baited with carbon dioxide than in a trap baited with skin emanations ($P<0.05$) (Table 1B). Also significantly more mosquitoes responded to traps baited with carbon dioxide than with acetone ($P<0.05$). Similarly, a significantly larger number of mosquitoes was caught in traps baited with carbon dioxide than in traps baited with butyric acid ($P<0.05$) (Table 1C). More mosquitoes were caught in a CFG trap baited with a combination of carbon dioxide and skin emanations than to a trap baited with each stimulus separately, but the difference was

Table 2. Total (n) and geometric mean catches of Culex quinquefasciatus *per day for a CFG trap baited with carbon dioxide versus a trap baited with skin emanations or carbon dioxide + skin emanations combination outdoors.*

Bait	n	Mean±SE
Carbon dioxide	50	13.0±0.1a
Skin emanations	25	8.3±0.1a
Carbon dioxide + Skin emanations	86	27.6±0.2a

SE = standard error. Means followed by the same letter are not significantly different at $P<0.05$.

Table 3. Total (n) and geometric mean catches of Culex quinquefasciatus *per day for CDC light-off trap baited with carbon dioxide (CO_2), octenol or their combination and an unbaited CDC light-off trap (control) outdoors.*

Bait	n	Mean±SE
Control	29	5.4±0.6a
Carbon dioxide	297	65.3±0.3b
Octenol	33	7.0±0.4a
Carbon dioxide+Octenol	184	40.5±0.4b

SE = standard error. Means not followed by the same letter are significantly different at $P<0.05$.

not statistically significant ($P>0.05$) (Table 2). In this experiment, there was also no significant difference between a trap baited with carbon dioxide and one baited with skin emanations.

Cx quinquefasciatus responded more to a carbon dioxide-baited CDC trap than to traps baited with either octenol or carbon dioxide+octenol combination. A carbon dioxide-baited CDC trap collected over 12 times more *Cx quinquefasciatus* than an unbaited trap and 9 times more mosquitoes than a trap baited with octenol alone ($P<0.05$). Although fewer mosquitoes were caught in a trap baited with a combination of carbon dioxide+octenol than to carbon dioxide alone, the difference was not significant. It was further found that the number of mosquitoes caught in the octenol-baited trap did not differ significantly from that caught in the unbaited trap ($P>0.05$).

Discussion

These experiments have shown that in the field, the trap baited with worn stockings caught a significantly larger number of *Cx quinquefasciatus* than a trap baited with clean stockings. Kline (1998) also found in his field studies that a CFG trap baited with a worn sock collected significantly more mosquitoes of various species than an unbaited trap.

Although the data suggest that carbon dioxide is more attractive to *Cx quinquefasciatus* than the emanations present on worn stockings, closer analysis of the data reveal that, under field conditions both stimuli are attractive to *Cx quinquefasciatus*, and that their combination is more attractive than either stimulus alone. In this experimental design, human skin emanations accounted for 25-30 % of the attraction. This is encouraging for studies elucidating the nature of the chemical causing attraction. Recently, Kline (1998) found a synergistic interaction between worn socks and carbon dioxide in attracting most species of mosquitoes in six genera (*Aedes, Anopheles, Coquillettidia*, *Culex, Culliseta,* and *Psorophora*) during his recent field studies in the United States. It has been previously shown in laboratory studies that *Cx quinquefasciatus* is highly attracted to skin emanations collected on polyamide stockings which had been worn on the foot of a human volunteer, and significantly more than to carbon dioxide (Chapter 3). Also female *Aedes aegypti* are attracted to human skin emanations collected on a sock which had been worn on the foot of a human volunteer (Kline, 1998). Previous field studies in Kenya (Haddow, 1942) showed that other anthropophilic mosquitoes, *An. gambiae* and *An. funestus* females, preferred a hut containing worn clothing to a completely empty hut. In the same study females of *Ma. africana* and *Ma. uniformis*, a group of different zoophilic mosquitoes, showed no such preference. From previous studies (Carlson *et al.*, 1973; Knols *et al.*, 1997) it is known that the anthropophilic *Ae. aegypti* and *An. gambiae s.s.* both are attracted to carboxylic acids (fatty acids) in the laboratory. Free fatty acids constitute a quarter of the skin surface lipid of humans and are breakdown products of triglycerides to free glycerol by the action of *Corynebacterium* and *Pityrosporum,* micro-organisms residing in the sebaceous glands (Nicolaides, 1974). Therefore, carboxylic acids are likely to constitute one of the attractive stimuli present on a previously worn stocking. As there was evidence from one of the present trials that *Cx quinquefasciatus* is relatively poorly attracted to human skin emanations when compared with carbon dioxide, we have no explanation for the difference observed, other than the possibility that under the experimental field conditions other competitive odours may have been present or the skin emanations may have lost essential components while being exposed to the ambient air. Also, no attempts were made to quantify the amount of human skin emanations, whereas the concentration and dose of these stimuli may be very important in affecting host-seeking *Cx quinquefasciatus.*

The results of this study are different from what has been observed when using live human baits in both indoor and outdoor situations in the field. *Cx quinquefasciatus* was observed to be significantly more attracted to human odour-baited tents than to carbon dioxide-baited tents in the same location in Tanzania (Chapter 4). This has also been observed for the other Afrotropical anthropophilic mosquitoes *An. gambiae* and *An. funestus* (Chapter 5). The difference may be caused by the fact that live humans emit a wider range of cues than the skin emanations collected on stockings.

In this study octenol was a poor mosquito attractant. Although Ritchie & Kline (1995) showed that octenol supplemented with carbon dioxide, significantly increased collections of *Cx annulirostris* in Australia, other *Culex* species have shown a weak response to octenol in similar studies elsewhere. For instance, in Germany, *Cx pipiens* did not respond to octenol (Becker *et al.*, 1995), while from studies in the United States and Australia it is clear that few mosquito species respond to octenol alone, and many species, including several anophelines, responded only to a combination of octenol and carbon dioxide (Kline, 1994a; Kline & Lemire, 1995). Interestingly, Kline & Lemire (1995) found a significant increase in trap collections when CDC traps were baited with carbon dioxide and octenol and heat was added as an additional stimulus. This suggests an interaction between heat and olfactory stimuli as had been proposed by Laarman (1958). Octenol is a common volatile in the emanations of herbivorous mammals (Hall *et al.*, 1984) and therefore it is perhaps not surprising that it is an attractant for mosquitoes that feed predominantly on these animals. Many *Culex* species are ornithophilic or anthropophilic, and therefore, may not respond to octenol. Although the compound has also been found in human sweat (Cork, 1996), there is no information about its role on host-finding behaviour of African anthropophilic mosquitoes in the field.

Cx quinquefasciatus did not show a positive response to acetone, a chemical present in the breath of vertebrates, but in *An. gambiae* and *An. stephensi* it has been observed to cause strong behavioural responses (Takken *et al.*, 1997a). In addition, the mosquito responded poorly to butyric acid in our experiments. Butyric acid has been implicated in host attraction for mosquitoes (Ikeshoji, 1993) and the frequency with which butyric acid-sensitive cells are found in *Ae. epactius* suggests that this volatile is an important olfactory cue for this species (Bowen, 1995). Butyric acid is present in low to moderate concentrations in human dermal, faecal and urinary secretions as a by-product of bacterial metabolism (Orlowski, 1966 as cited by Bowen, 1995).

In a separate study (Chapters 4 and 5) it was found that a human host was significantly more attractive to *Cx quinquefasciatus* than carbon dioxide, which accounted for 25-28 % of the attraction. Indeed, Dekker & Takken (1998) reported a similar effect of carbon dioxide. Therefore, it can be concluded that carbon dioxide is one of the stimuli to which *Cx quinquefasciatus* is attracted and the addition of human skin emanation enhanced the response to the odour-baited trap. Further studies are likely to reveal the active compounds present in the skin emanation.

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Part II:
Chemical Ecology of Oviposition Behaviour

7

The influence of synthetic oviposition pheromone and volatiles from soakage pits and grass infusions upon oviposition site-selection of *Culex* mosquitoes in Tanzania

ABSTRACT - The response of *Culex* mosquitoes to (5*R*,6*S*)-6-acetoxy-5-hexadecanolide (the synthetic oviposition pheromone, SOP), emanations from soakage pit water (SPW) and grass infusions (GI) was studied in pit latrines in Muheza, Tanzania. Water treated with the synthetic oviposition pheromone received significantly more egg rafts of *Culex quinquefasciatus* Say and *Cx cinereus* Theobald than did the untreated water ($P<0.001$). The residual activity of SOP did not decrease over a 9-day period, with the geometric mean number of egg rafts laid in the SOP-treated water varying daily between 2.5 and 8.9 as compared to 0.1 to 1.6 egg rafts laid in tap water. However, SOP did not attract ovipositing mosquitoes to non-breeding sites and both treatment and control received few eggs. SPW and GI attracted ovipositing *Cx quinquefasciatus*, *Cx cinereus* and *Cx tigripes* Grandpré and de Charmoy with the number of eggs rafts deposited in bowls containing SPW or GI being significantly larger than that deposited in bowls with tap water ($P<0.05$). It was further found that significantly more egg rafts of *Cx quinquefasciatus* were deposited in SOP-treated water than in SPW ($P<0.05$). When SOP was compared with SOP+SPW, significantly more egg rafts of *Cx quinquefasciatus* were deposited in bowls with the latter combination than in bowls containing SOP or SPW only ($P<0.05$), indicating a synergistic effect between SOP and SPW. Similarly, when SOP-treated water and GI were compared with SOP+GI, significantly more egg rafts of *Cx quinquefasciatus* were laid in the latter bowl, indicating a synergistic effect between SOP and GI. The results indicate that the combined use of SOP and organically-enriched water can be employed in monitoring of *Culex quinquefasciatus* for control programmes. This study provides the first record of the attraction of *Cx cinereus* and *Cx tigripes* to oviposition stimuli.

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Introduction

Gravid females of culicine mosquito species show a high degree of preference in selecting specific oviposition sites. This preference may be due to the presence of oviposition pheromones or oviposition attractants and repellents in natural habitats (Bentley & Day, 1989). Oviposition pheromones may occur in nature as intraspecific messengers to inform conspecifics of suitable oviposition sites. Volatiles resulting from the microbial fermentation of organic matter in the aquatic habitat, may also function as semiochemicals for gravid mosquitoes to detect suitable or unsuitable oviposition sites (Kline, 1994b; McCall & Cameron, 1995).

Oviposition site-selection behaviour has been widely studied in *Culex quinquefasciatus* Say (Laurence & Pickett, 1985; Millar *et al.*, 1992). For this species oviposition is mediated by semiochemicals. In addition to 3-methylindole, which accounts for most of the attraction of female *Cx quinquefasciatus* to oviposition sites (Millar *et al.*, 1992), numerous other chemical attractants have been identified, and many are associated with high organic or bacterial content (Ikeshoji *et al.*, 1975; Beehler *et al.*, 1994a,b).

Apart from these semiochemicals produced from microbial degradation of organic matter, behavioural observations showed that egg rafts attracted gravid females of *Cx quinquefasciatus* to oviposit at the same sites. This response is elicited by (5*R*, 6*S*)-6-acetoxy-5-hexadecanolide (abbreviated acetoxyhexadecanolide), a pheromone released from the apical droplets of 1-d old eggs (Bruno & Laurence, 1979; Laurence & Pickett, 1982; Laurence *et al.*, 1985). There is increasing evidence that other culicine species also use pheromones, released from eggs or larvae as cues for oviposition site location (Takken & Knols, 1999). In a few instances conspecific *Culex* species have been found to respond to the oviposition pheromone of *Cx quinquefasciatus* (Osgood, 1971).

A synthetic acetoxyhexadecanolide is available and has been used to concentrate egg-laying of *Cx quinquefasciatus* within specific areas in oviposition sites in Kenya (Otieno *et al.*, 1988a). The acetoxyhexadecanolide was formulated in tablets which on contact with water release the pheromone to the surface of the water where slow evaporation, ensured by the low vapour pressure of the compound, gave a continuos rate of emission of the pheromone. However, further field trials of the synthetic oviposition pheromone (SOP) to determine the response of gravid *Cx quinquefasciatus* in various breeding sites in Africa have not been reported. Moreover, the importance of the oviposition pheromone relative to chemical cues derived from the oviposition site itself or from infusions mimicking oviposition sites has not been elucidated. The aims of this study were therefore: (i) to determine the response of gravid *Cx quinquefasciatus* to a SOP in relation to its influence in the selection of oviposition sites in pit latrines; (ii) to determine the interaction between SOP and water from natural breeding sites in resource-location behaviour of the mosquito (iii) to determine the interaction between SOP and grass infusion (GI) and, (iv) to assess the period of activity of SOP in the field.

Materials and Methods

Study site. All the experiments were conducted at Ubwari, $5^0 10'$S, $38^0 46'$E, near Muheza, north-east Tanzania. The area lies at an altitude of 200 m above sea level and is about 40 km to the west of the coastal town of Tanga. The mean annual temperature is 26^oC and the average rainfall 1000 mm. The major breeding sites of *Cx quinquefasciatus* are pit latrines and soakage pits. Most of the pit latrines are filled with water during the rainy season thus providing optimal conditions for breeding *Culex* mosquitoes. Six pit latrines were selected for the experiments. Each consisted of about 1 m^2 slab of concrete provided with a drop hole (12x25 cm) in the centre, suspended over a soakage pit. A small hut of corrugated iron was built surrounding the pit. Access was obtained by a wooden door. The presence of culicine mosquito breeding was confirmed by trapping emerging adult mosquitoes from pit latrines.

Chemicals. The synthetic oviposition pheromone (acetoxyhexadecanolide) was prepared as described previously (Dawson *et al.*, 1990). Blank effervescent tablets were laid out individually on a clean piece of paper. Using a precision syringe, 0.1 ml of SOP solution in hexane (200 mg/ml) was placed on each of the tablets. To prepare the control, 0.1 ml hexane was placed on another effervescent tablet spread out individually on a clean piece of paper. The tablets were left to dry for a few minutes at room temperature before use. For further details see Otieno *et al.* (1988a).

Experimental protocol. Each experiment was run in six latrines simultaneously, thus producing six replicates for treatment and control.

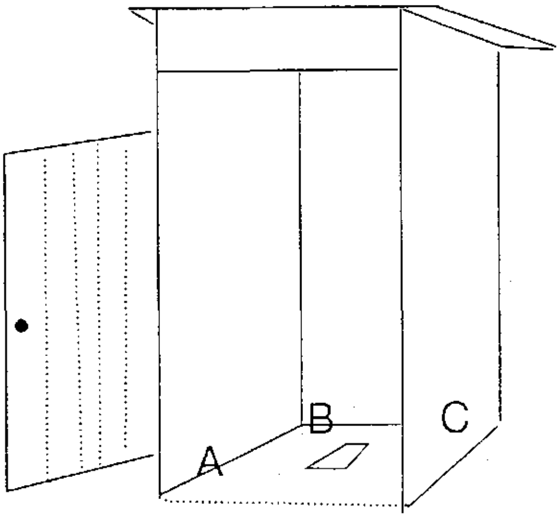

Figure 1. Schematic drawing of a latrine in which the study was conducted, showing the position of the bowls containing the oviposition pheromone, organically rich water or tap water (A, B and C).

The response of Culex *mosquitoes to SOP.* The first experiment was to assess the response of *Cx quinquefasciatus* to SOP. Two black plastic bowls (30 cm top surface diameter) were filled with 800 ml of non-chlorinated tap water. Into one of the bowls, an effervescent tablet treated with SOP solution (20 mg/tablet, viz., Otieno *et al.*, 1988a) was placed while a hexane treated tablet was placed in the other bowl as a control. The bowls were placed 1 m apart on the floor of the latrine building at 18.00 h and left overnight (Fig. 1). Each morning, at 08.00 h, the egg rafts deposited in each bowl were counted and removed. The water was discarded and the bowls rinsed with tap water each day before resetting the experiment. The position of the treatment and control bowls were alternated each day. The experiment was run for 6 days.

Residual activity of the oviposition pheromone. In order to investigate the duration of activity of the SOP, pheromone-treated bowls were not emptied daily, but only the egg rafts within the bowls were removed and the bowls set for the day's experiment. There were no further additions of SOP or control tablets. The experiment was conducted between 18.00 and 08.00 h and run for 9 days.

The response of Culex *mosquitoes to SOP, soakage pit water (SPW) and SOP+SPW.* In this experiment the effect of SPW was at first compared with unchlorinated tap water. In another experiment SPW, SOP and their combination were compared in a 3-choice arrangement. Water from a soakage pit where mosquitoes were breeding, was collected and strained through fine netting to remove all solid debris before used for the experiment. The bowls containing the treatment and control were placed on the floor of a latrine at 1 m apart. The water in both bowls was discarded before setting another day's experiment. Both experiments were conducted between 18.00 and 08.00 h, the first one ran for 8 days, while the second experiment was run for 6 days. In the latter treatment bowls were alternated daily between the three positions.

The response of Culex *mosquitoes to SOP, grass infusions (GI) and SOP+GI.* GI was prepared by 2 kg of *Digitaria* sp. cut into small pieces and soaked in 10 l of unchlorinated tap water in a plastic bucket and left to ferment for 5 days. The infusion was filtered through fine netting and frozen until needed. In one experiment six bowls of GI were compared with unchlorinated tap water. 800 ml of GI in 6 bowls and the same volume of unchlorinated tap water were then placed on the floor of a pit latrine building and the number of mosquito egg rafts deposited were recorded each morning for six days. In another experiment, GI, SOP and their combination were compared in a 3-choice arrangement. This experiment was run for 6 days. Bowls were treated as in the previous experiment.

The response of Cx quinquefasciatus *to SOP in a site which does not normally support breeding.* Six bowls of tap water were treated daily with the SOP and placed on the veranda of six houses. Six bowls with untreated tap water were used as controls, each bowl placed at 1 m from the SOP-treated bowl. The experiment was conducted between 18.00 and 08.00 h and run for 6 days.

Data analysis. In all experiments the collected egg rafts were sorted by shape and taken to the laboratory and reared separately to the adult stage when they were identified to species (Edwards, 1942; Gillett, 1972). All data were log (x+1) transformed and means of treatments and controls were compared using Student *t*-tests. Means in factorial experiments were subjected to ANOVA and an F-test significant at $P<0.05$ was followed by a Least Significant Difference test to compare treatment means.

Results

Apart from the expected presence of *Cx quinquefasciatus*, *Cx cinereus* Theobald and *Cx tigripes* Grandpré and de Charmoy were also found. *Cx quinquefasciatus* were produced from pear-shaped egg rafts, *Cx cinereus* from round egg rafts and *Cx tigripes* from rod-shaped egg rafts.

When the response of mosquitoes to SOP was tested, the bowls containing SOP received significantly more egg rafts of *Cx quinquefasciatus* and *Cx cinereus* than did those containing tap water treated with a control tablet ($P<0.001$, Table 1A). When the duration of activity of the SOP was assessed, it was found that, except for day 5, significantly more egg rafts of *Cx quinquefasciatus* were laid in water treated with SOP than in untreated water ($P<0.05$). The mean number of egg rafts deposited in the pheromone-treated bowls was 4.9 (2.5 to 8.9) with two sites being consistently more attractive than the others. In the control the mean number of egg per day was 0.5 (0.1 to 1.6) (Fig. 2).

When SPW was compared with unchlorinated tap water (Table 1B), egg rafts of *Cx quinquefasciatus*, *Cx cinereus* and *Cx tigripes* were collected and for each species the numbers in the treated bowls were significantly different from the control ($P<0.05$). In a subsequent experiment the number of egg rafts of *Cx quinquefasciatus* oviposited in SOP+SPW was significantly different from those deposited in SOP or SPW only, with more egg rafts deposited in the combination treatment ($P<0.05$). *Cx cinereus* egg rafts deposited on the SOP-treated water did not differ from those deposited on SPW. However, the number of egg rafts deposited on the SOP+SPW combination was significantly larger than that deposited in either SPW or SOP alone. Although few egg rafts of *Cx tigripes* were collected in all three treatments, the number of egg rafts deposited in SPW was not statistically different from that deposited in SOP ($P>0.05$), whereas significantly more egg rafts were deposited on SOP+SPW combination than on SOP or SPW alone ($P<0.05$) (Table 2A).

In the comparison of GI with tap water, significantly more egg rafts of *Cx quinquefasciatus*, *Cx cinereus* and *Cx tigripes* were deposited on GI than on tap water (Table 1C). When GI was compared with SOP or SOP+GI combination, the number of egg rafts of *Cx quinquefasciatus* deposited on SOP was similar to that laid on the GI. However, significantly more egg rafts were deposited on the SOP+GI combination ($P<0.05$), and this number was 2.7 times more than the sum of either treatment alone (Table 2B). The number of egg rafts of *Cx cinereus* laid on SOP was larger than that laid on GI ($P>0.05$), but not significantly different from that deposited on the SOP+GI combination. The mean number of egg rafts of *Cx tigripes* laid on the three treatments were not different from one another ($P>0.05$) (Table 2B).

When the attraction of the mosquitoes to SOP in a non-breeding area was assessed, no *Culex* egg rafts were found in the SOP treated bowl at one of the locations and only few were found in other locations. The overall oviposition of *Cx quinquefasciatus* in the SOP-treated water was not significantly different from egg-rafts laid in the control bowls ($P>0.05$), with geometric mean value (±SD) of 0.6 ±1.0 egg rafts for the former and 0.3 ± 0.5 for the latter.

Table 1. Geometric mean (±SD) number of egg rafts of Culex quinquefasciatus, Cx cinereus *and* Cx tigripes *oviposited in synthetic oviposition pheromone treated water (SOP) (A), soakage pit water (SPW) (**B**) and grass infusions (GI) (C) versus untreated water (control); (n = 6 days)*

		Cx quinquefasciatus	*Cx cinereus*	*Cx tigripes*
	Treatment	Mean± SD	Mean±SD	Mean±SD
A	SOP	5.0 ± 0.78a	3.36±0.22a	0
	Control	0.18±0.38b	0.43±0.50b	0
B	SPW	4.48±1.58a	0.17±0.47a	0.53±1.29a
	Control	0.28±0.48b	0b	0.12±0.55b
C	GI	5.64±1.58a	0.31±0.65a	0.28±0.59a
	Control	0.24±0.67b	0.02±0.13b	0.04±0.22b

SD = standard deviation. For each sub-table, means in the same column, followed by a different letter, are significantly different at $P<0.05$.

Table 2. Geometric mean (±SD) number of egg rafts of Culex quinquefasciatus, Cx cinereus *and* Cx tigripes *oviposited in synthetic oviposition pheromone treated water (SOP), soakage pit water (SPW) and their combination (A), and SOP-treated water, grass infusions (GI) and their combination (**B**) (n = 6 days)*

		Cx quinquefasciatus	*Cx cinereus*	*Cx tigripes*
	Treatment	Mean± SD	Mean±SD	Mean±SD
A	SOP	8.6 ± 3.50a	0.36±0.61a	0.06±0.15a
	SPW	3.0 ± 1.10b	0.14±0.45a	0.06±0.20a
	SOP+SPW	31.5 ±1.21c	1.74±1.13b	0.32±0.60b
B	SOP	11.02±2.28a	2.51±1.69a	0.95±1.21a
	GI	7.55±1.59a	0.56±0.74b	1.38±1.31a
	SOP+GI	49.71±1.87b	1.90±1.36a	1.21± 1.0a

SD = standard deviation. For each subtable, means in the same column, followed by a different letter, are significantly different at $P<0.05$.

Discussion

In these experiments it was observed that the water treated with SOP received significantly more egg rafts of *Cx quinquefasciatus* and *Cx cinereus* than did the control. Also, this is the first time that *Cx cinereus* is reported to respond to SOP. In nature, *Cx quinquefasciatus* and *Cx cinereus* have been observed to breed in similar habitats in Kenya, Nigeria and Tanzania (Subra & Dransfield, 1984; Irving-Bell *et al.*, 1987; Lyimo & Irving-Bell, 1988; Mboera *et al.*, 1997). It is likely that the two species respond to similar cues whilst selecting their oviposition site. Previously, it has been reported that other *Culex* species such as *Cx tarsalis* Coquillet (Hwang *et al.*, 1987) and *Cx pipiens molestus* Forskål also respond to *Cx quinquefasciatus* pheromone, and it has been suggested that the pheromone is active in conspecific of *Cx quinquefasciatus* (Millar *et al.*, 1994).

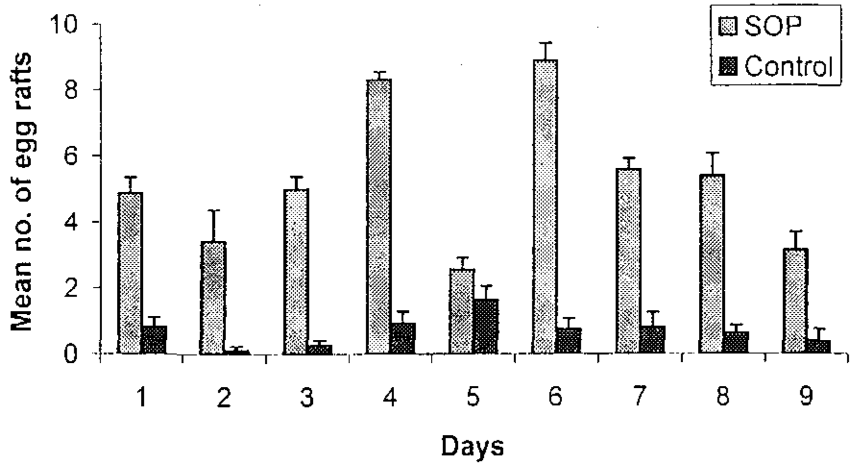

Figure 2. The residual activity of the SOP (25 mg/l) expressed as the geometric mean of egg rafts deposited in treatment and control bowls over time. Error bars represent standard errors.

Cx tigripes appeared in breeding sites of *Cx quinquefasciatus* and *Cx cinereus* towards the end of the rainy season. Larvae of *Cx tigripes* are known to prey voraciously on larvae of other mosquito species such as *Anopheles gambiae* Giles (Lyimo, 1993) and *Cx quinquefasciatus* and *Cx cinereus* (L.E.G. Mboera, unpublished data). To our knowledge, this is the first time that *Cx tigripes* has been observed to respond to olfactory cues. Thus oviposition cues, similar to those attracting *Cx quinquefasciatus* and *Cx cinereus*, will lead *Cx tigripes* to breeding sites of its prey. Several predatory mosquito species, e.g. *Toxorhynchites* spp., have been attracted to oviposition site water-borne semiochemicals to which *Aedes* spp. are also responsive (Linley, 1989). It therefore is possible that the observed phenomena is a common strategy for predacious mosquitoes to locate their prey.

It was initially surprising to find that the residual activity of the synthetic oviposition pheromone did not decrease over a 9-day period. As the total number of egg rafts laid varied greatly in between days, with a constant trend in between latrines (data not shown), the activity of the pheromone must have been above the optimum threshold (Millar *et al.*, 1994) during the experimental period. It appears worthwhile to extend the duration of this experiment in order to study the actual duration of the oviposition pheromone under natural conditions. As we did not change the pheromone-treated water in the bowls, it is possible that the two treatments were daily complemented by natural pheromone originating from the freshly deposited egg rafts. However, the relatively large dose of SOP together with the inherently low vapour pressure of this compound is known under other, but related conditions to give a level dose/response relationship once the dose is high enough to achieve the saturated vapour pressure (Laurence & Pickett, 1985), as could be happening here.

The three *Culex* species showed a stronger preference to oviposit on SPW than on unchlorinated tap water. It is likely that this polluted water from a natural mosquito breeding site contains volatile cues used by gravid *Culex* mosquitoes. When SOP-treated water was compared with SPW and SOP+SPW, more egg rafts of *Cx quinquefasciatus* were laid in the latter treatment indicating a synergistic effect. A similar phenomenon was observed when GI and SOP were compared with their combination. In two other independent laboratory studies on the effect of SOP plus polluted water (Blackwell *et al.*, 1993) and SOP plus a synthetic mixture of oviposition attractants (Millar *et al.*, 1994), only an additive effect of the combination was found and no synergism. In the field, it is unlikely that SOP will act independently, i.e. without other indicators of oviposition site suitability. Such indicators of site suitability are probably more important in the initial selection, with the oviposition pheromone operating subsequently in an augmentative role as the former are unlikely to be species-specific. Factors other than the oviposition pheromone are therefore, involved. These factors were not determined in this study, but numerous oviposition attractants have been identified and many are associated with high organic or bacterial content (Millar *et al.*, 1992; Beehler *et al.*, 1994a). Among other substances found to be attractive to ovipositing *Culex* mosquitoes are fatty acids as well as compounds produced by *Pseudomonas* and *Aerobacter* bacteria (Ikeshoji *et al.*, 1967, 1975).

The finding that, in a non-breeding area, SOP alone did not stimulate oviposition of *Cx quinquefasciatus* was unexpected, as in another study (Chapter 11) it was found that this mosquito is attracted to SOP from a distance. Initially attraction over a distance of 5.5 cm (Laurence & Pickett, 1982) and then up to 10 m (Otieno *et al.*, 1988a) were evidenced and more recently upwind anemotaxis mediated by the SOP has been demonstrated (Pile *et al.*, 1991; 1993). The combined results with significantly increased oviposition in SOP-treated bowls inside latrine buildings, suggest a strong interaction in oviposition-inducing behaviour between water-derived semiochemicals, present in natural breeding sites, and the oviposition pheromone. However, it is difficult to determine the relative importance of the pheromone versus other stimuli (such as synergistic semiochemicals, physical and/or visual stimuli) to the overall oviposition behavioural response (Bentley & Day, 1989). To date, little information is available as to the modes of action of substances that mediate oviposition. Appropriate experimental design for differentiating attractants and stimulants as well as for exploring the interplay between these factors is important.

SOP, GI and SPW clearly elicited strong oviposition responses in filed populations of gravid *Cx quinquefasciatus*, whereas oviposition by *Cx cinereus* was moderate to the SOP and GI, and low in response to SPW. Our results are in agreement with previous observation that gravid *Cx quinquefasciatus* oviposits readily in response to SOP in the laboratory and field (Laurence & Pickett, 1982; Otieno *et al.*, 1988a, 1988b). SPW and GI, such as those in used our study, consist of complex mixtures of compounds that vary over time. It has been established previously that some of the oviposition attractants from such organic infusions are products of bacterial degradation and it is therefore not surprising that *Cx quinquefasciatus* breeds more profusely in polluted water than in clean water. In Tanzania these stimuli are present in natural breeding sites. In addition, the presence of both oviposition pheromone and GI or SPW provides a stronger oviposition stimulus than the individual components alone. Oviposition semiochemicals can therefore be employed in odour-baited traps for the surveillance and control of *Cx quinquefasciatus*, an important vector of urban bancroftian filariasis.

Acknowledgements

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8

Oviposition and behavioural responses of *Culex quinquefasciatus* (Diptera: Culicidae) to skatole and synthetic oviposition pheromone in Tanzania.

ABSTRACT - Studies on the behavioural and oviposition responses of *Culex quinquefasciatus* to 3-methylindole or skatole (an oviposition site cue) and (*5R,6S*)-6-acetoxy-5-hexadecanolide (the synthetic oviposition pheromone, SOP) were conducted in Muheza, North-east Tanzania. *Cx quinquefasciatus* was found to display a bimodal pattern of oviposition. The highest peak (64.8%) was between 18.00 and 20.00 h, while the second peak (18.9%) was between 06.00 and 08.00 h. The mean number of egg rafts oviposited per day between the two peaks was statistically different ($P<0.05$). Oviposition responses of gravid *Cx quinquefasciatus* differed across the range of concentrations of skatole. Significantly larger numbers of egg rafts were deposited in bowls treated with 10^{-6} µg or 10^{-5} µg skatole than in the bowls with tap water ($P<0.05$). The number of egg rafts deposited in bowls treated with 10^{-4} or 10^{-1} µg skatole were not significantly different from that laid in tap water ($P>0.05$). Under field conditions skatole was found to be effective for a period of seven days, after which its biological activity decreased significantly. It was further found that significantly more egg-rafts of *Cx quinquefasciatus* were deposited in water treated with SOP than in water treated with skatole ($P=0.0002$). An additive effect was observed when a SOP+skatole combination was used to attract gravid *Cx quinquefasciatus* to oviposition sites. It is concluded that SOP and skatole are major components mediating oviposition site selection of *Cx quinquefasciatus* under field conditions, and may have considerable potential as odour baits for the surveillance of *Cx quinquefasciatus* populations. Combined with biopesticides, odour-baited ovitraps might provide effective control of this mosquito species.

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Introduction

Various cues are involved in mediating a wide range of mosquito behaviours, from host-seeking to the recognition of specific features of the oviposition sites. The correct choice of the most valuable oviposition site for a mosquito species is crucial to the survival of its progeny. Several studies report that oviposition pheromones and various kairomones are responsible for the attractiveness of oviposition sites of culicine mosquitoes (for review: Takken & Knols, 1999). Ikeshoji *et al.* (1975) showed that phenols were attractive to *Culex quinquefasciatus* Say whereas Millar *et al.* (1992) isolated a five-component mixture (phenol, 4-methylphenol, 4-ethylphenol, indole, and 3-methylindole or skatole) from grass infusions, reported by Reiter (1983) to be attractive or stimulatory to *Cx quinquefasciatus*. Of the five compounds, skatole has been shown to be the most stimulatory component both in the laboratory (Millar *et al.*, 1992; Mordue *et al.*, 1992) and in the field (Beehler *et al.*, 1994b).

In Tanzania it was recently shown that volatiles from soakage pit water and grass infusions attract gravid *Cx quinquefasciatus* to an oviposition site (Chapter 7), though the specific compounds responsible for the attraction have not been identified. Most of the field studies on the responses of gravid mosquitoes to skatole have been limited to the USA. As this compound has not been tested in the field as a semiochemical for *Cx quinquefasciatus* in Africa, our objective of the work reported here was to assess its biological activity and to determine its interactive effect when combined with a synthetic oviposition pheromone (SOP) under field conditions of North-east Tanzania.

Materials and Methods

Study site. All the experiments were conducted in Muheza ($5^0$10'S, $38^0$46'E), North-east Tanzania. The area has been described in Chapter 4.

Chemicals. Skatole (99% 3-Methylindole) was obtained from Sigma Chemical Co. (St. Louis, USA). A solution of skatole was made by dissolving it in 96% ethanol. The synthetic oviposition pheromone was prepared as described previously by Dawson *et al.* (1990). Blank effervescent tablets were spread out individually on a clean piece of paper. Then using a precision syringe, 0.1 ml of the mosquito oviposition pheromone solution in hexane (200 mg/ml) was placed on each of the tablets. The tablets were left to dry for a few minutes at room temperature before use.

Experimental protocol. Circadian oviposition pattern of Culex quinquefasciatus. Two pit latrines were selected for the experiments. Each pit latrine consisted of 1 m^2 slab of concrete provided with a drop hole (12x25 cm) in the centre, suspended over a soakage pit. A small hut of corrugated iron was built surrounding the pit. Access was obtained by a wooden door (see Chapter 7). Four plastic bowls (30 cm top surface diameter) were filled with 1000 ml of non-chlorinated tap water. Into each bowl, a pheromone-impregnated tablet was dissolved. Two bowls were placed 1 m apart on the floor of two latrine buildings at 16.00 h local time and left overnight. The bowls were retrieved at two hour intervals, and replaced by the same number of other bowls containing freshly made SOP. The number of egg-rafts of *Cx quinquefasciatus* laid on the water in the bowls was counted and recorded. The water was discarded and the bowls rinsed with tap water each day before resetting the experiment. The experiment was run for four days. During the experimental period, sunrise was at 06.32 h while sunset was at 18.19 h local time (Department of Meteorology, Dar es Salaam).

Dose-response of Cx quinquefasciatus *to skatole.* To determine dose-responses of *Cx quinquefasciatus* to skatole, 1 g of skatole was dissolved into 100 ml of 96 % ethanol. Taking 0.1 ml of skatole, the following concentrations were made: 10^{-6} µg/l, 10^{-5} µg/l, 10^{-4} µg/l or 10^{-1} µg/l. Four plastic bowls with 1000 ml of non-chlorinated tap water containing either of the four concentrations of skatole were compared with a bowl of water treated with 0.1 ml of ethanol, as a control. The treatments were placed randomly at 1 m apart on the floor of a pit latrine building of 6 m^2. The treatments were alternated between the five sites for a duration of 10 days. The skatole solutions were discarded and the bowls rinsed with clean tap water each day before resetting the experiment at 18.00 h local time. In the following morning, at 08.00 h, the number of egg rafts of *Culex* mosquitoes deposited in each bowl was recorded.

Oviposition response of Cx quinquefasciatus *to skatole versus tap water.* Five black plastic bowls with a litre of water containing 10^{-5} µg skatole and another 5 bowls with the same amount of tap water were treated with 0.1 ml of ethanol (as control). Two bowls, one treated with skatole and one with ethanol only, were placed at 1 m apart on the floor of five pit latrine buildings and left overnight between 18.00 and 08.00 h. Each morning, the bowls were rinsed with tap water and the experiment was set again using a freshly made skatole preparation for each experimental day.

The residual effect of skatole. In this experiment the duration of activity of skatole was determined by comparing deposition of egg rafts in 1000 ml of tap water containing 10^{-5} µg skatole in a black plastic bowl with an equal amount of water treated with 0.1 ml ethanol (as a control). This experiment was conducted in six pit latrine buildings and the treatments were placed on the floor of the buildings at 1 m apart . The experiment was run between 18.00 and 08.00 h for 10 days. Skatole-treated bowls were not emptied daily, but only egg rafts within the bowls were removed and the bowls set for the day's experiment. There were no further additions of skatole or ethanol. Egg-rafts deposited in each bowl were counted and removed at 08.00 h the following morning.

Oviposition response of Cx quinquefasciatus *to skatole versus SOP.* Two black plastic bowls were filled with 1000 ml of non-chlorinated tap water. One of the bowls was treated with 10^{-5} µg skatole while the other bowl with SOP impregnated tablet (20 mg/tablet). The bowls were placed 1 m apart on the floor of the latrine building and left overnight. Five pit latrine buildings were used for the experiment to make five replicates. The experiment was run for six days between 18.00 and 08.00 h.

In another series of experiments, the oviposition response of *Cx quinquefasciatus* to skatole, synthetic oviposition pheromone or their combination was determined. Three plastic bowls were filled with 1000 ml of non-chlorinated tap water. One of the bowls was treated with

0.1 ml (10^{-5} μg/l) skatole, the second bowl with a SOP-impregnated tablet while the third bowl contained both skatole and SOP. The three treatments (with three replicates) were placed 1 m apart on the floor of a pit latrine building away from the entrance between 18.00 and 08.00 h for 6 days. The treatments were alternated between the three sites every experimental day.

Data analysis. In all experiments the egg rafts collected were sorted by shape and taken to the laboratory and reared separately to the adult stage when they were identified to species morphologically (Edwards, 1942; Gillett, 1972). All data were log (x+1) transformed and means of treatments were compared using Student *t*-tests. Means of factorial experiments were subjected to ANOVA. An F-test significant at $P<0.05$ was followed by a Least Significant Difference test to sort out differences between treatment means.

Results

Egg rafts of two *Culex* species were collected during the experiment to determine the circadian pattern of the oviposition of *Cx quinquefasciatus*. Of the 162 egg rafts deposited, 98% were identified as those of *Cx quinquefasciatus* while the remainder were those of *Cx cinereus*. *Cx quinquefasciatus* was found to display a bimodal pattern of oviposition. The highest peak, with 64.8% of the total egg rafts, was observed between 18.00 and 20.00 h while the second peak (accounting for 18.9% of the total egg rafts collected) was between 06.00 and 08.00 h East African Standard Time (Fig. 1). During the evening peak a mean number of 20.9 egg rafts/day was recorded whereas the mean for the morning peak was 6.4 egg rafts/day. The mean daily number of egg rafts between the two peaks was significantly different ($P<0.05$).

In the experiment to determine the effect of skatole concentration on attractancy (Fig. 2), a total of 536 egg-rafts of *Cx quinquefasciatus* was collected. Oviposition responses of *Cx quinquefasciatus* females differed across the range of concentrations of skatole. Strongest oviposition response occurred at concentrations from 10^{-6} to 10^{-5} μg/l skatole, with the latter concentration receiving the largest number of egg rafts of *Cx quinquefasciatus* compared to the other concentrations ($P<0.05$). The number of egg rafts deposited in bowls with 10^{-4} or 10^{-1} μg/l skatole was not significantly different from that deposited in the control.

Table 1A shows that a significantly larger number of egg rafts of *Cx quinquefasciatus* was deposited in bowls containing skatole than in untreated tap water ($P=0.0003$). It was further found that significantly more egg-rafts of *Cx quinquefasciatus* were deposited in water treated with SOP than in water treated with skatole ($P=0.0002$) (Table 1B). When skatole, SOP and combinations thereof were compared, it was found that significantly more egg rafts were deposited on water treated with SOP than skatole. The number of eggs deposited in the SOP+skatole combination was not significantly different from that deposited in a bowl containing SOP alone. However, an additive effect was observed when skatole and SOP were combined (Table 2).

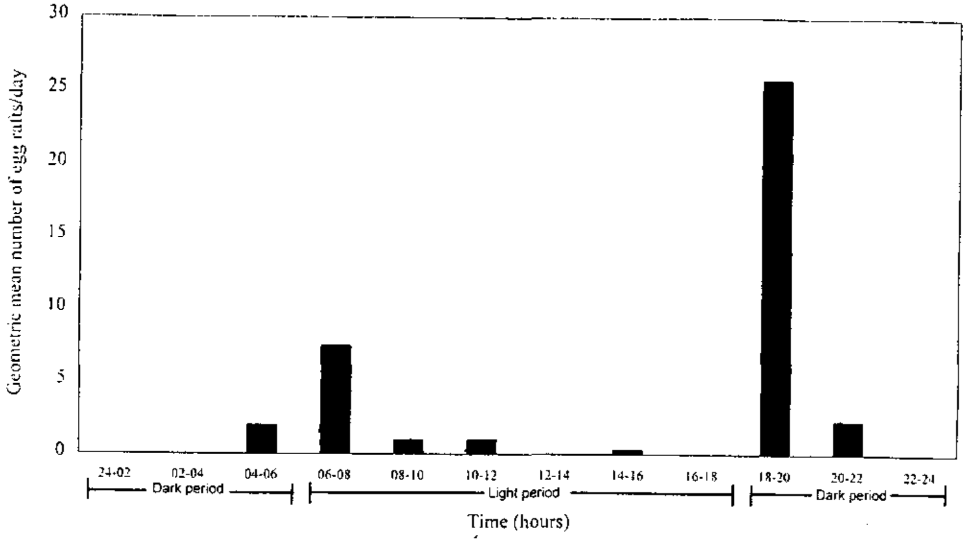

Figure 1. Circadian oviposition pattern of Culex quinquefasciatus *in the study area*

It was found that the number of egg rafts of *Cx quinquefasciatus* deposited in bowls containing skatole remained significantly higher than in bowls with water from day 1 to day 7 (Fig. 3). Thereafter, the number of egg rafts deposited in bowls with skatole was not significantly different from that deposited in bowls with tap water.

Table 1. Total (n) and geometric means(±SD) of egg rafts of Culex quinquefasciatus *deposited in water treated with skatole versus tap water (control) (A) and skatole versus synthetic oviposition pheromone (SOP) (**B**)*

	Treatment	n	Mean±SD
A	Skatole	271	3.6±2.3a
	Control	46	0.9±1.9b
B	SOP	588	9.4±2.5a
	Skatole	124	2.5±1.5b

SD = standard deviation. Means in the same column followed by a different letter are significantly different at $P<0.05$.

Table 2. Total (n) and geometric means(±SD) of egg rafts of Culex quinquefasciatus *deposited in skatole (SKT), synthetic oviposition pheromone (SOP) and their combinations (SKT+SOP)*

Treatment	n	Mean±SD
SOP	360	14.3±1.4a
SKT	152	6.6±1.1b
SKT+SOP	503	23.6±0.7a

SD = standard deviation. Means in the same column followed by a different letter are significantly different at $P<0.05$.

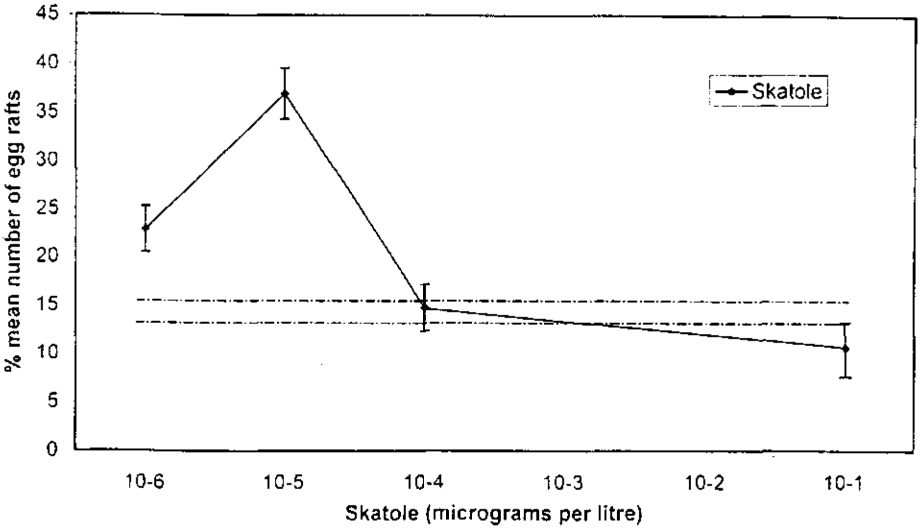

Figure 2. The dose-response of Culex quinquefasciatus *to skatole (3-Methylindole). Dotted lines indicate upper and lower limits of control response. Vertical bars represent standard errors.*

Discussion

Two ovipositional peaks for *Cx quinquefasciatus* were observed in this study, with early evening oviposition activity producing the highest peak while a lower peak was displayed at dawn. It has been reported before that in the absence of atmospheric disturbances, sunrise and sunset are the preferred times for oviposition in *Cx quinquefasciatus* females as observed in south-east Asia (De Meillon *et al.*, 1967) and in West Africa (see Subra, 1981). In the latter case, the most important peak was at sunset. Morning ovipositional activity in other species of mosquitoes have been reported for *Anopheles gambiae* Giles and *An. funestus* Giles (Gillies &

Coetzee, 1987). These evening and morning peaks also occur in other *Culex* species including *Cx pipiens* Linnaeus, and *Cx restuans* Theobald (Haddow & Gillett, 1958). According to Gillett (1956) the time of oviposition in mosquitoes is determined by an interplay of intrinsic and extrinsic factors. The intrinsic factors include an ovarian development hormone which is associated with the time of the blood meal and an ovulation hormone, which is usually dependent on mating. The extrinsic factors include the change in light intensity. However, findings by Suleman & Shirin (1981) indicate that oviposition in *Cx quinquefasciatus* is primarily an endogenous activity regulated by photoperiod. Many mosquito species exhibit characteristic oviposition patterns in response to environmental cues such as light intensity. Oviposition patterns are further modified by feeding status (Isoe & Millar, 1995) but this was not studied here.

The dose-response experiment showed that the highest proportion of egg-rafts of *Cx quinquefasciatus* was observed at 10^{-5} µg/l skatole. Skatole has also been shown to stimulate oviposition in gravid females of *Cx quinquefasciatus* at concentrations as low as 10 parts per trillion in a laboratory study (Millar *et al.*, 1992). However, studies by Blackwell *et al.* (1993) showed skatole to increase oviposition by *Cx quinquefasciatus* at concentrations as high as 10^{-5} to 10^{-2} µg/l. Like in these findings, deposition of egg rafts was significantly less at 10^{-1} µg/l skatole (Blackwell *et al.*, 1993). The decreased responses at higher concentrations (>10^{-4} µg/l) of skatole in this study may reflect repellent or deterrent effects for this species at these concentrations.

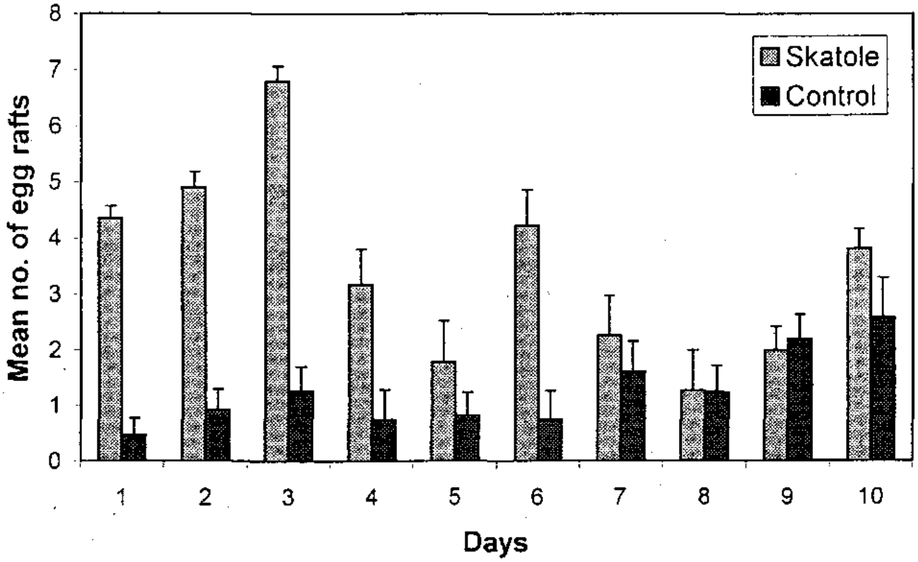

Figure 3. The residual activity of skatole as oviposition stimulus for Cx quinquefasciatus. *Error bars represent standard errors.*

In this study it was clearly shown that skatole mediates oviposition of *Cx quinquefasciatus* under field conditions. Skatole has also been shown to mediate oviposition of *Ae. albopictus* (Allan & Kline, 1995) and *Cx tarsalis* and *Cx stigmatosoma* under field conditions (Beehler *et al.*, 1994b). Although skatole was observed to influence oviposition site selection of *Cx quinquefasciatus*, the effect was significantly lower as compared to that of SOP. It is likely that, after gravid mosquitoes have been attracted to the oviposition site, they respond more to the pheromone which is a cue indicating the presence of conspecifics. Skatole on the other hand, is produced by many sites and does not necessarily provide species-specific cues. In a separate study, soakage pit water and grass infusions, of which skatole is presumed to be one of the major components, were found to attract gravid *Cx quinquefasciatus* in equal numbers to an oviposition site as SOP (Chapters 7 and 11). It is noted here, however, that SOP was superior to skatole in influencing deposition of egg-rafts by this mosquito species. Therefore, it seems likely that grass infusions or soakage pit water contain additional oviposition attractants other than skatole.

Although in these studies only an additive effect between skatole and SOP was observed, it was shown in other experiments (Chapter 7) that the oviposition pheromone acts synergistically with grass infusions or soakage pit water in influencing the oviposition site selection of gravid *Cx quinquefasciatus*. There is thus an interaction between kairomones and pheromones in attracting gravid mosquitoes to suitable natural oviposition site. Similar, but additive effects have been reported in previous studies with the pheromone and polluted water (Blackwell *et al.*, 1993) and the pheromone and a synthetic mixture of oviposition attractants (Millar *et al.*, 1994).

The duration of activity of skatole in this study was found to be seven days post-treatment. When evaluating organic enrichment of ponds, Beehler & Mulla (1995) observed that peak production of *Culex* mosquitoes was maintained for three weeks post enrichment, declining markedly thereafter. The period of activity of skatole observed in this study was, however, short as compared to that of the synthetic oviposition pheromone in the same study area (Chapter 7). Skatole is indeed more volatile and may be lost by sublimation or by biodegradation in the experiment environment thereby lowering its effectiveness in influencing oviposition site selection under field conditions. In a natural site, the production of skatole (and other attractants) is probably continuous from the activity of micro-organisms.

Ovitraps are often used for mosquito population monitoring and surveillance (Chadee *et al.*, 1988; McHugh & Hanny, 1990), and the use of oviposition attractant/ stimulants to increase the sensitivity of these traps as well as gravid mosquito traps has been demonstrated in the United States (Reiter, 1983, 1986; Reiter *et al.*, 1991) and in Tanzania (Chapter 11). Skatole and synthetic oviposition pheromone which were clearly shown to mediate attraction of *Cx quinquefasciatus* to oviposition sites, may have considerable potential as odour baits for the surveillance of *Cx quinquefasciatus* populations. Combined with biopesticides like *Bacillus sphaericus*, odour-baited ovitraps can be considered as effective control methods. Accurate and reliable information regarding *Cx quinquefasciatus* breeding habits and distribution is an essential prerequisite for the planning and execution of management strategy for the filarial mosquito, *Cx quinquefasciatus*.

Acknowledgements

I would like to thank Mayunga Maega and Aza Kimambo for their excellent field assistance. I am also grateful to Frank Mbua, Jane Kayamba, Judith Mahundi, Mohammed Alli and Hatibu Mohammed who allowed access to their latrines. Prof. John Pickett, IACR-Rothamsted, UK. IACR-Rothamsted, U.K, is thanked for providing the synthetic oviposition pheromone. This work was supported financially by the Tanzania Health Research Training Fund.

Part III:
Semiochemicals in sampling of mosquitoes

9

The influence of Centers for Disease Control light trap position, relative to a human-baited bed net, on catches of *Culex quinquefasciatus* and *Anopheles gambiae* in Tanzania

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ABSTRACT - The best position for Centers for Disease Control (Antlanta, GA) light traps, in relation to human-occupied bed nets for trapping of host-seeking *Culex quinquefasciatus* Say and *Anopheles gambiae* Giles mosquitoes, was determined in Tanzania. Significantly higher catches were recorded for both species when the trap was positioned at the foot end of the bed, near the top of the net. Parity rates were significantly higher near the top of the net than at the level of the host. Since trap position affects the catch size and the proportion of infectious mosquitoes therein, standardised use of this technique for estimating entomological inoculation rates (i.e., the number of potentially infectious bites received over a certain period of time) is recommended.

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Introduction

Various designs and applications of light-traps have been proposed and evaluated for sampling anthropophilic mosquito populations in order to indirectly estimate human biting rates (Service, 1993b). Of all light-traps, the standard Centers for Disease Control (CDC) (Antlanta, GA) miniature light trap (Sudia & Chamberlain, 1962) has been found to be a very efficient device for sampling endophagic malaria and filariasis vectors in Africa (Odetoyinbo, 1969). The optimum location of the light-trap for sampling house-visiting mosquitoes has been reported to be as close to the host(s) as possible, and its catching efficiency is greatly improved when the human bait is protected by a bed net (Garrett-Jones & Magayuka, 1975). The correlation between light-trap and human biting catches for *Anopheles gambiae* Giles, *An. funestus* Giles and *Culex quinquefasciatus* Say has been described (Lines *et al.*, 1991), and this trap-bed net system is now widely used to monitor vector populations and evaluate vector control interventions (Curtis, 1991; Shiff *et al.*, 1995; Mboera *et al.*, 1997a). However, it has been reported that light traps are biased towards sampling 2-2.3 times as many *Plasmodium falciparum*-infected females than human-biting catches (Davis *et al.*, 1995; Mbogo *et al.*, 1993). Therefore, they may not provide a reliable cross-section of the host-seeking population. Since mosquitoes prefer to bite certain parts of the human body (De Jong & Knols, 1996), we tested the hypothesis that the sensitivity of the trapping system would differ according to the location of the trap in relation to the position of the human host under the bed net.

Materials and Methods

Study area. The studies were conducted in Muheza, a township in Northeast Tanzania, 40 km west of the coastal town of Tanga and Njage, a village about 80 km from Ifakara town, South-east Tanzania. The sites were chosen because in Njage *An. gambiae s.s.* is the dominant species, while in Muheza, *Cx quinquefasciatus* is the most abundant species present. The ecological setting of Muheza has been described in Chapter 4. Njage is situated at an altitude of 300 m above sea level, 3-4 km south of the middle escarpment of the Udzungwa mountains at the edge of the Kilombero valley, a flood plain some 20 km wide. In the village, the houses are generally made of mud bricks and thatched grass. The area has two rainy seasons. The main rains start in March and extend through to May and the short rains occur in December and January. A cool, dry season follows the long rains in June and July. There are relatively few domestic animals in this part of the district and only a small number of goats, chicken and dogs may be seen in the village.

Experimental protocol. Experiments with *Cx quinquefasciatus* were done at Muheza whereas trapping of *An. gambiae* was done in Njage village. Previous investigations showed that Njage harbours a population of *An. gambiae sensu stricto* only (B.G.J. Knols unpublished data; Chapter 10). At both field sites four traditional-style houses (mud walls with thatched roofs), occupied by one male adult only, were selected. The average age of the occupants was 29 yr. (range 27-30 yr.) in Muheza and 34 yr. (range 19-48 yr.) in Njage. Each occupant was provided with an unimpregnated rectangular (110x185x178 cm) bed net. The average height of the bed above floor level was 46 cm (range 42-55) in Muheza and 42 cm (range 34-52) in

Njage. A standard miniature CDC light trap with incandescent light bulb (Model 512; John W. Hock Company, Gainesville, FL) was hung beside the bed net with the shield of the trap touching the side of the net and the trap entrance in one of four positions: the head end or foot end of the bed, at either 70 cm or 150 cm from the floor (approximately 25 cm or 105 cm above the bed respectively).

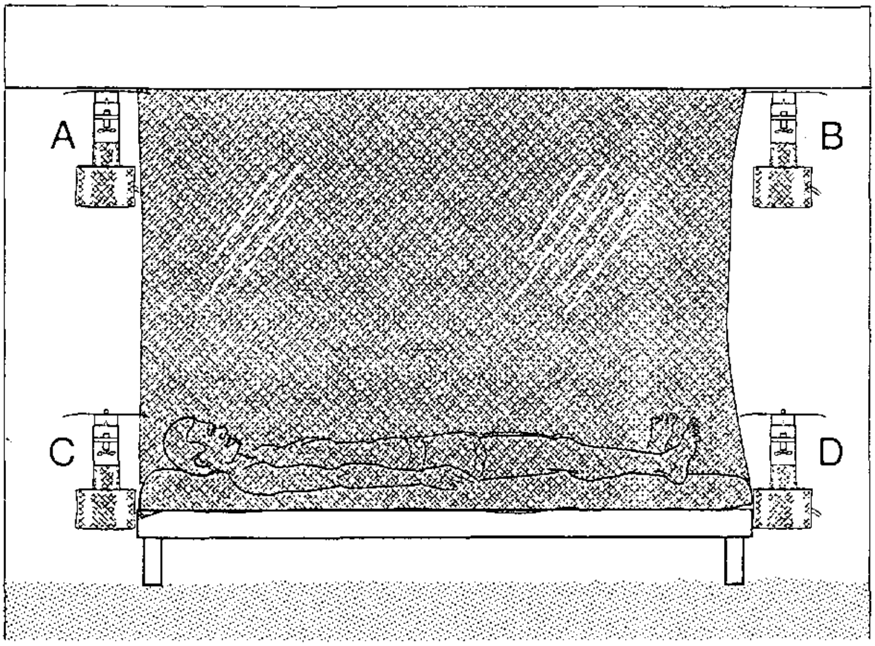

Figure 1. The experimental set-up showing the different CDC light trap positions in relation to a human-baited bed net. A: Head-high; B: Feet-high; C: Head-low and D: Feet-low.

Using a two-way ANOVA incomplete factorial design trap positions were randomised and alternated each night for every house for 8 or 12 nights (for *An. gambiae* and *Cx. quinquefasciatus* respectively). Traps were operated between 20.00 and 0600 h for *An. gambiae* and 21.00 and 06.00 h for *Cx. quinquefasciatus*. It was ascertained that all volunteers had retired at the beginning of the night and that their traps were operating properly, and we checked their presence periodically during the experimental period. All mosquitoes caught were identified, counted and classified according to sex and blood feeding stages (i.e. unfed, blood fed, half gravid, gravid). Subsamples of the catches were dissected in 0.75% phosphate buffer solution and their parity determined by coiling or uncoiling of their ovarian tracheoles (Detinova, 1962). A sample of *Cx quinquefasciatus* was also dissected for *Wuchereria bancrofti* infection status.

For a sample of female *An. gambiae*, the wing length was determined to assess the size of the host-seeking mosquitoes. Each wing length was measured under a dissecting microscope fitted with a camera lucida, connected to a computer. A wing was fitted between two object glasses that were put on a black box under the objective of the microscope at x 20 magnification. The wing length was measured from the distal end of the aula to the tip, excluding the fringe scales and the length recorded by the computer.

Data analysis. Female catches were subjected to log(x+1) transformation and subjected to Latin square ANOVA (Williams, 1993) after a satisfactory check for normality of the distribution. An F-test significant at $P<0.05$ was followed by a Least Significant Difference (LSD) for comparison of treatment means. Parity rates and wing size for the different trap positions were compared using chi-square tests for independence.

Results

A total of 4,051 *Cx quinquefasciatus* and 13,402 *An. gambiae* were caught during the experiments, of which the vast majority (>90%) were unfed specimens, presumably host-seeking while caught. Results of the ANOVA are shown in Table 1A. For both species significant house and trap position effects were observed, with the former probably reflecting variations in spatial mosquito densities, house design or host-attractiveness, and the later indicating differences in catch-size among the four trap positions.

Further analysis of trap position effects (Table 1B) showed that the feet-high position resulted in 1.3-2.1 times higher catches ($P<0.05$) for *An. gambiae* and 2.3-3.1 times higher catches ($P<0.001$) for *Cx quinquefasciatus* when compared with the other positions. Pooled data analysis (top versus bottom, head versus feet side of the bed) showed significantly ($P<0.05$) higher catches for traps near the top of the net but no difference between head/feet side of the bed. The feet-high position sampled a higher proportion of older mosquitoes than the other trap positions, and this effect was significant for *Cx quinquefasciatus*. However, pooled results showed that for both species traps near the top of the net caught significantly more parous mosquitoes than traps near the bottom ($\chi^2 = 4.36$, df = 1, $P = 0.017$ for *An. gambiae*; $\chi^2 = 15.50$, df = 1, $P = 0.0001$ for *Cx. quinquefasciatus*). Of the dissected female *Cx quinquefasciatus*, two harboured an infective larval stage (L3) of *Wuchereria bancrofti* and all were collected in traps located at the level above the feet-side of the bed net. A total of 2074 wings of *An. gambiae* were measured to determine the size of the caught mosquitoes. The mean wing length of *An. gambiae* did not significantly differ between the trap positions (Table 2).

Discussion

These results clearly demonstrate that catch-size and parity rates of host-seeking females for both species differ according to the trap position in relation to the host occupying the bed net, but the factors causing this phenomenon are not yet understood. The host-seeking behaviour of both species has been shown to be influenced by human foot odour (Knols & Meijerink, 1997) which may in part explain higher catches in the feet-high position. Nevertheless, the results indicate that mosquito behaviour around bed nets is not a random

*Table 1. A, ANOVA tables for catches of Culex quinquefasciatus and Anopheles gambiae; **B**, Catches for different CDC light trap positions in relation to a human-baited bed net*

A	*Cx quinquefasciatus*			*An. gambiae*		
Source	df	MS	F-value	df	MS	F-value
Day	11	0.245	1.261 ns	7	0.115	2.906 ns
House	3	0.251	12.94 P <0.001	3	0.673	17.02 P<0.001
Trap position	3	0.565	29.09 P<0.001	3	0.152	3.844 P<0.05
Error	30	0.019		18	0.040	

B	*Cx quinquefasciatus*			*An. gambiae*		
Trap position†	n	Mean	Parity rate‡	n	Mean	Parity rate§
Head-low	653	47.4 a	0.27 (112) ab	3060	353.1 ab	0.52 (218) a
Head-high	787	63.2 a	0.37 (183) ac	3602	287.6 ab	0.57 (188) a
Feet-low	770	56.0 a	0.24 (170) b	2700	209.8 a	0.49 (107) a
Feet-high	1841	147.4 b	0.42 (210) c	4040	444.2 b	0.60 (203) a

CDC, Centers for Disease Control; df, degrees of freedom; MS, mean sums of squares; ns, not significant; n, total number caught. Numbers in parentheses are the total number dissected. Means and parity rates not followed by the same letter are significantly different at $P<0.05$. † Head, feet: head end or foot end of bed; low= 25 cm above bed level; high = 105 cm above bed level. ‡χ^2 = 16.71, df = 3, P = 0.0008; §χ^2 = 6.46, df =3, P= 0.091.

process and suggests that increased knowledge on their behaviour may result in improved trap and bed net designs and their use for sampling mosquito populations.

It was interesting to find that the size of the host-seeking *An. gambiae* did not vary between the four trap positions. Moreover, the mosquitoes caught were of equal size and comprised of a relatively larger size (mean range: 3.06-3.08) as compared to 2.70-2.98 mm reported by Lyimo (1993) in nearby villages.

Table 2. Mean±SE wing length of Anopheles gambiae *collected from different CDC light trap positions in relation to a human-baited bed net*

Position	No. of wings	Mean±SE
Head-low	577	3.08±0.008a
Head-high	445	3.06±0.010a
Feet-low	444	3.07±0.009a
Feet-high	608	3.08±0.008a

SE= standard error. Means are followed by the same letter indicating they are not significantly different at $P<0.05$.

It can be concluded that the position of the CDC light trap next to a bed net influences the catch size and parity rate of *An. gambiae* and *Cx quinquefasciatus*. Furthermore, it follows that estimates of entomological inoculation rates (EIR) will be influenced by trap position since in turn this affects the number caught and parasite (sporozoite or filarial) rates (which will increase with higher parity rates). The estimations of the EIR will be affected as follows:

EIR = map, where:
m = relative density
a = human biting rate
p = parasite rate

Following the results obtained in this study: (i) m will be higher in the feet-high position and will raise the EIR; (ii) p will be higher in the feet-high position, due to a higher parity rate (older mosquitoes) and will thus raise the EIR. Substantial variations in EIR will be obtained if CDC light traps are not used in a standardised manner. This will greatly affect our current understanding of the relationship between disease mortality and EIR. Thus multiplying light trap catches by 1.5 to give true human biting rates (Maxwell *et al.*, 1990; Lines *et al.*, 1991; Mboera *et al.*, 1997a) may further affect good assessment of the EIR. Standardised use of sampling methods (i.e. light traps), is a prerequisite for comparing EIRs from areas with different epidemiological settings. Standardised use of light traps near bed nets (i.e. in the feet-high position) may thus contribute to a better understanding of the still poorly understood relationship between transmission intensity and disease, through more accurate measurements of the EIR. Development of new, easy to use, cheap and effective sampling tools such as tent traps or modified bed nets is required to overcome the above mentioned problems with CDC light traps.

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10

Comparison of carbon dioxide baited trapping systems for sampling outdoor mosquito populations in Tanzania

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ABSTRACT - The outdoor catching efficiency of various mosquito trapping devices baited with carbon dioxide (CO_2, 300 ml/min) was evaluated and compared in two areas of Tanzania. Four types of sampling tools viz., CDC miniature light traps (with the light bulb switched on or off), electric nets (ENT), and Counterflow Geometry (CFG) traps were used. In Njage, South-east Tanzania, *Anopheles gambiae s.s.* was the most abundant of the 7 mosquito species present comprising 74.3% of the total number caught (n = 2171). In Muheza, North-east Tanzania, *Culex quinquefasciatus* Say was the most common species (90.9% of the total caught (n = 1080)). In both areas the CFG trap was superior to both the CDC light-on and CDC light-off traps for sampling *An. gambiae* and *Cx quinquefasciatus*. The efficiency of CFG trap and ENT for sampling these species was similar ($P>0.05$). However, ENT was superior to the CDC light-off trap in sampling both species. The CDC light-off trap collected significantly more ($P<0.05$) *Cx quinquefasciatus* than the CDC light-on trap. It is concluded that the CFG trap and ENT are effective tools for sampling outdoor populations of *An. gambiae* and *Cx quinquefasciatus*. The addition of light to a CDC trap for sampling *Cx quinquefasciatus* outdoors may affect the collections negatively.

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Introduction

A number of sampling techniques have been used to measure the number of mosquitoes that are in search of a blood meal. Methods commonly used include the human-biting catch (HBC), pyrethrum spray collections and light traps (for review see: Service, 1993b). Most of these sampling techniques are aimed at collecting indoor-biting mosquitoes. Whereas the HBC attracts the fraction of mosquitoes biting humans, it is a rather subjective method, depending on the skills and motivation of the collector. Moreover, rapidly spreading drug resistance renders the HBC ethically unacceptable. Light traps do not pose these problems, but are not necessarily sampling the biting fraction of the population. Many workers have used light traps with varying success (Odetoyinbo, 1969; Service, 1970; Chandler *et al.*, 1975; Garrett-Jones & Magayuka, 1975). Recently Lines *et al.*, (1991), in Tanzania, and Costantini *et al.* (1998b), in Burkina Faso, showed that CDC light traps used inside houses, in combination with human-occupied bed nets yielded results comparable to standard human-biting collections and may be used to estimate mosquito abundance. However, several studies have shown that light traps underestimate the density of host-seeking man biting mosquitoes (Service, 1993b). The traps are biased towards sampling a greater proportion of parous and sporozoite infected mosquitoes (Mbogo *et al.*, 1993; Davis *et al.*, 1995). In addition, the position of the trap, when used next to an occupied bed net, affects catches (size and parity rates) of both *Anopheles gambiae* Giles and *Culex quinquefasciatus* Say (Chapter 9). Therefore the CDC light trap may not be a very suitable device for estimating the entomological inoculation rate.

The tools available for studying the outdoor host-seeking behaviour of anthropophilic mosquitoes in Africa are few, and most of these operate on the principle of an active air current sucking mosquitoes into collecting bags (see Service, 1993b), or have an active odour-laden air current pumped out of traps guiding mosquitoes towards them (Costantini *et al.*, 1993). These methods have two major disadvantages: Firstly, it is not known what proportion of approaching mosquitoes enter the trap upon arrival at its entrance and secondly, being non-directional sampling devices, they can not be used to study mosquito flight behaviour. Odour-baited entry traps (OBETs), similar in design to 'lobster-pot' exit traps, have recently been used to collect host-seeking mosquitoes in West Africa (Costantini *et al.*, 1993, 1996).

Electric nets, initially developed as sampling tools for tsetse flies by Vale (1974), have played an important role in studying the host-seeking behaviour of these insects. Gillies *et al.* (1978) and later Gillies & Wilkes (1981) adopted Vale's technology to study flight behaviour of mosquitoes in relation to prevailing wind directions and the influence of wind on the flight speed of West African mosquito species. Later in France, Killick-Kendrick *et al.* (1986) successfully sampled sandflies, which are considerably smaller than mosquitoes, with human odour-baited electric nets. Recently, Knols *et al.* (1998) have demonstrated that electric nets are useful for sampling outdoor mosquito populations and for studying mosquito flight behaviour.

A Counterflow Geometry (CFG) trap was recently developed by the American Biophysics Corporation and has successfully been used to sample various mosquito species outdoors in the United States (Kline, 1998, 1999) and *An. arabiensis* in South Africa (B.G.J. Knols & L.E.O. Braack, unpublished data). This is the first time the trap has been employed for sampling *An. gambiae* and *Cx quinquefasciatus* in East Africa.

Traps based on host attractants are likely to provide an objective monitoring tool for the host-seeking fraction of malaria and bancroftian filariasis vectors. Such traps can be used to study the vector biology as well as epidemiology of mosquito-borne diseases, knowledge which is vital for the planning and outcome of intervention strategies. In addition, simple odour-baited trapping devices could supplement other methods such as bed nets etc. in reducing challenge, particularly in areas without resort to insecticide. Traps could eventually become an integral part of primary health care systems. Therefore, in an effort to look for an effective sampling tool for outdoor host-seeking mosquitoes, the relative capacity of various trapping devices to sample the host-seeking population of mosquitoes was compared and evaluated in two different sites in Tanzania where *An. gambiae* and *Cx quinquefasciatus*, are abundant.

Materials and Methods

Study site. The studies were conducted in the village of Njage in South-east Tanzania and in Muheza, North-east Tanzania. These sites were chosen because in Njage *An. gambiae s.s.* is the dominant species, while in Muheza, *Cx quinquefasciatus* is the most abundant species present. Njage, 80 km to the west of the town of Ifakara, is situated at an altitude of 300 m above sea level, 3-4 km south of the middle escarpment of the Udzungwa mountains at the edge of the Kilombero river valley. The houses in this village are generally made of mud bricks and thatched grass. The area has two rainy seasons. The main rains start in March and extend through to May and the short rains occur in December and January. A cool, dry season follows the long rains in June and July. There are relatively few domestic animals in this part of the district and only a small number of goats, chicken and dogs may be seen in the village. Muheza on the other hand, is a small town, 40 km inland from the coast of the Indian Ocean. At 200 m above sea level, the town lies at the foot of the East Usambara mountains, which rise up to 1600 m above sea level. Unlike in Njage, houses in Muheza consist of cement blocks covered with a roof of corrugated iron. Many households keep some livestock in the form of cattle, goats, sheep and chickens. Like in Njage, there are two rainy seasons in Muheza: the long rains from March to May and the short rains from October to November.

Sampling tools. In this study four types of sampling tools were used. These included: Centres for Disease Control (CDC) light-on trap, CDC light-off trap, Counterflow Geometry (CFG) trap and electric nets (ENT) (Fig. 1). The miniature CDC traps (Model 512, John W. Hock Company, Gainesville, FL) are as described by Sudia & Chamberlain (1962).

CFG trap, which utilises a novel counterflow concept, has been described recently by Kline (1999). It is constructed from a clear PVC container (*ca.* 11.4 l), modified by removing the bottom, adding a mounting flange and a 10.16 cm diameter x 17.78 cm length of PVC thin wall pipe. Inside the 10.16 cm pipe, a 5.08 cm diameter x

30.48 cm length of PVC pipe is mounted concentric with, and extended 7.62 cm beyond the end of the 10.16 cm pipe. The lid of the jar is modified by attaching an 8 cm fan above and a suitable filter structure below the lid. The 8 cm fan is positioned to blow air out of the container and thus induce a supply draft between the 10.16 cm pipe and the 5.08 cm pipe. Inside the filter structure, a 4 cm fan is mounted in a manner to seal the 5.08 cm tube from the container and provide an airflow out of the container in a direction opposite the flow created by the 8 cm fan. In the side of the 5.08 cm pipe, a small tube (0.32 cm internal diameter) is attached to provide an entrance port for carbon dioxide to enrich the exit plume from the 4 cm fan. Above the 8 cm fan, a small lid is attached to prevent rain damage to the fan and to the mosquito collection. The power source to the fans was provided by two 6 V rechargeable batteries.

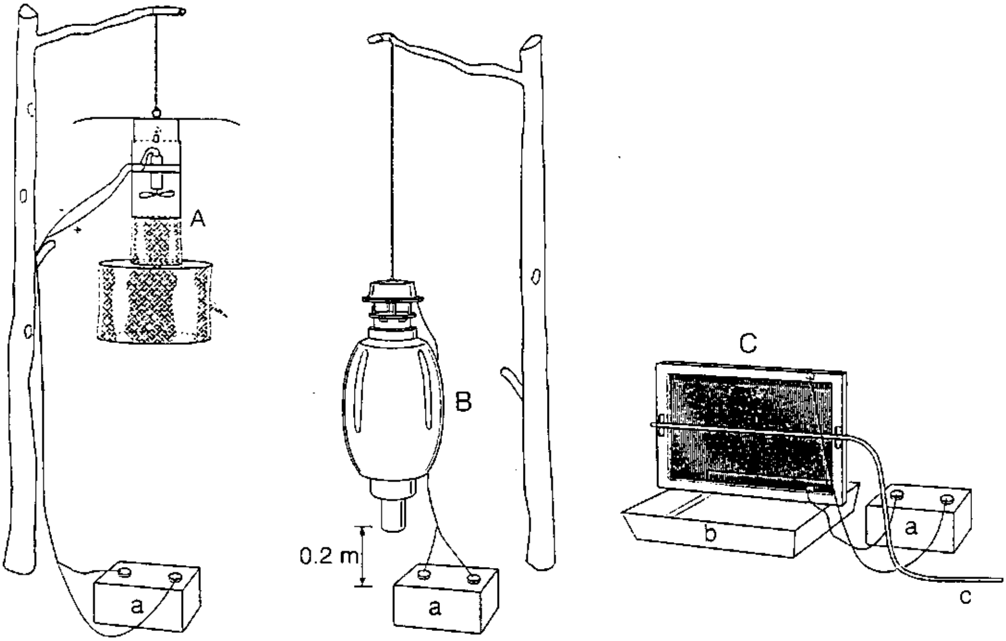

Figure 1. CO_2-baited traps used to sample outdoor mosquito populations. A: CDC light trap, B: CFG trap and C: Electric net; a: Power supply, b: water tray, and c: carbon dioxide supply tube.

Modified electric nets, 66x45 cm in size, with 150 electrocuting wires 4 mm apart were used in this study (Knols *et al.*, 1998). The wires were made of stainless steel (diameter 0.2 mm), attached to zinc plated draw springs (0.5x3.5x20 mm) on one side (conductors) and nylon loops (fishing line) on the opposite side (insulators). Alternating wires were charged with 6 kV AC or earthed respectively. The power source was an inverter transformer oscillator driven by a 12 V car battery. Electrocuted mosquitoes were collected in a plastic transparent tray (45x45 cm) underneath the net, filled with water to which a detergent powder was added. A plastic polythene sheet was suspended over the nets so that they could be operated during light rains.

Experimental protocol. Njage: In the experiment at Njage, the CFG trap was compared with the CDC light-off trap. The traps were suspended from a wooden pole with the lowest part of the traps at 20 cm above ground level. CO_2 was discharged from a pressurised gas cylinder at the rate of 300 ml/min and was dispensed through 5 mm silicon tubing and passed through a flow-meter. The outlet of the tubing was fixed at the entrance of a CDC trap, 1 m above the ground level. For the CFG trap, CO_2 was dispersed from the trap at its lowest part, 20 cm above the ground level. The traps were set 46 m apart and the nearest house, occupied by a single person, was 10 m away. Two sites, at a distance of 500 m, were chosen for the experiments. At each site the two traps were alternated between the two locations for a duration of two nights. The experiments were conducted between 20.00 and 06.00 h. In another series of experiments, a CO_2-baited CDC light-on trap was compared with a CO_2-baited CFG trap. The methodology is as described above.

In the second series of experiments, the ENT were compared with other trapping systems. In one of the experiments, two electric nets were compared, one baited with CO_2, and another unbaited. The nets were set 25 m from one another and each 10 m from a nearby house occupied by one adult man. CO_2 was dispensed as described above and the outlet of the silicon tubing was connected to a 25 cm-long perforated plastic tube fixed horizontally across the centre of the electric grid (Knols *et al.*, 1998). This created a wide front of carbon dioxide, to be dispersed with the air passing through the net. Mosquitoes caught were collected in two plastic trays filled with water, and placed under the electric nets. A mild detergent was added to the water to prevent the mosquitoes from escaping. A 2x2 Latin square design was used and the experiments were run between 20.00 and 06.00 h for four days. In another experiment, the efficiency of an electric net baited with CO_2 (as described in the previous experiment) was compared with an CFG trap baited with an equal amount of CO_2. The traps were set at the same site as described above. The CFG trap was hung from a wooden pole with the lowest part 20 cm above the ground. Finally, the ENT was compared with the CDC light-on trap. Both traps were baited with CO_2 released at 300 ml/min.

Muheza: In one experiment, the response of *Cx quinquefasciatus* to CDC light-on and CDC light-off traps baited with CO_2, was compared with an unbaited CDC light-on trap. An unbaited CDC light-off trap was used as a control. The traps with the treatments were 30 m apart in a straight line and 50 m from the nearest house. Each trap position was changed daily in a randomised design, and traps were operated from 19.00 to 06.00 h. In the second experiment, a CFG trap baited with CO_2 was compared with an unbaited CFG trap. The traps were set 30 m apart, with their lowest part at 20 cm above ground level. The experiment was run between 19.00 and 06.00 h for four nights with the treatments alternated between sites. In another experiment, a CO_2-baited CFG trap was compared with CO_2-baited CDC light-off trap. In the last series of experiments, a CO_2-baited CFG trap was compared with CO_2-baited ENT. The ENT was deployed as described above. The treatments were set 30 m apart and 50 m from the nearest house. The traps were operated between 19.00 and 06.00 h and alternated between the two sites after each experimental night. In all experiments, the CDC traps and CFG traps were retrieved at 06.00 h in the morning. For electric nets, mosquitoes caught in the wires, and those fallen in the water trays were collected in a glass tube for further processing in a field laboratory. In the laboratory, mosquitoes were sorted to species and sex. Of the *An. gambiae* collected further species identification was done using polymerase chain reaction technique (Scott *et al.*, 1993).

Data analysis. Catches of unfed female mosquitoes (presumably host-seeking) were log (n+1) transformed and means of treatments were compared using Student *t*-tests for experiment 1 in Njage. Data of the other experiments were subjected to Latin square analyses of variance (Snedecor & Cochran, 1989). An F-test significant at $P<0.05$ was followed by a Least Significant Difference test to sort out differences between treatment means.

Results

A total of 2171 unfed female mosquitoes were recovered from 20 collections made at Njage. *An. gambiae s.s.* was the most abundant of the 7 species present comprising of 74.3% of the mosquitoes collected, followed by *Mansonia africana* (9.8%), *An. coustani* (7.4%), *Aedes circumluteolus* (3.6%) *Cx quinquefasciatus* (3.3%), *An. funestus* (1.5%) and *An. wellcomei* (0.1%). A small proportion (<5%) of the *An. gambiae* collected consisted of *An. arabiensis*. In Muheza, 1080 unfed female mosquitoes were recovered from 16 night collections. Of these, 90.9% were *Cx quinquefasciatus*. Other species included *Ma. africana* (2.7%), *Cx cinereus* (2.1%), *Ae. aegypti* (1.6%), *An. funestus* (1.1%), *An. gambiae s.l.* (1.0%), *Ae. circumluteolus* (0.3%), *Ma. uniformis* (0.2%) and *An. coustani* (0.1%).

When the CFG trap was compared with the CDC light-on trap, it was observed that the former trap collected significantly more *An. gambiae* than the latter ($P<0.05$) (Table 1A). Similarly, in comparison with the CDC light-off trap (Table 1B), the CFG trap collected significantly more mosquitoes than the latter; with the number of *An. gambiae* collected in the CFG trap being significantly larger than those caught in the CDC light-on trap ($P<0.05$). For the other mosquito species, the CFG trap collected significantly larger numbers of *An. coustani* and *Ae. circumluteolus* than the CDC light-off trap (Table 1B).

The number of mosquitoes collected by ENT baited with CO_2 was significantly larger than the number of mosquitoes collected in unbaited nets ($P<0.05$). However, a small number of mosquitoes (n=14) was still collected from unbaited nets, and were probably intercepted during flight (Table 2A). When a CO_2-baited ENT was compared with a CO_2-baited CFG trap, the total number of mosquitoes collected by the CFG trap was larger than that by the ENT, but not significantly different (Table 2B). When a CO_2-baited ENT was compared with a CO_2-baited CDC light-off trap, it was found that the ENT collected significantly larger numbers of *An. gambiae* than the CDC light-off traps ($P<0.05$) (Table 2C).

The number of *Cx quinquefasciatus* collected by an unbaited CDC light-on trap was not significantly different from that collected by an unbaited CDC light-off trap (control). The CO_2-baited CDC light-off trap collected significantly more *Cx quinquefasciatus* than the other CDC traps. By adding light to a CO_2-baited CDC trap, the mosquito catch was reduced by over 50% (Table 3). The trap with light collected substantially larger numbers of other flying insects, mostly moths. These were not seen in CDC light-off traps. It was further observed that the CFG trap baited with CO_2 caught significantly more *Cx quinquefasciatus* than an unbaited CFG trap (Table 4A). In addition, a CFG trap baited with CO_2 collected more *Cx quinquefasciatus* than a CDC light-off trap baited with CO_2 ($P<0.05$) (Table 4B). The number of female *Cx quinquefasciatus* collected in the CFG trap and the ENT, when both baited with CO_2, was not statistically different ($P>0.05$) (Table 4C).

*Table 1. Total (n) and geometric mean number (±SD) of mosquitoes collected with a CO_2-baited CFG trap versus a CO_2 baited CDC trap with the light on (A) or light off (**B**)*

(**A**) Species	CFG trap		CDC light-on trap	
	n	Mean±SD	n	Mean±SD
An. gambiae	203	40.2±1.2a	21	5.0±0.4b
An. funestus	4	0.7±0.9a	2	0.4±0.5a
An. coustani	20	3.5±1.8a	5	1.0±0.8a
Cx quinquefasciatus	9	2.0±0.6a	12	1.2±2.2a
Ma. africana	11	2.0±1.2a	7	2.3±1.0a
Ae. circumluteolus	10	2.4±0.4a	15	3.1±0.6a

(**B**) Species	CFG trap		CDC light-off trap	
	n	Mean±SD	n	Mean±SD
An. gambiae	176	36.5±1.0a	33	3.8±3.3b
An. funestus	12	2.7±0.7a	4	0.9±0.8a
An. coustani	22	7.7±1.1a	0	0b
Cx quinquefasciatus	5	1.5±1.1a	3	0.2±0.4a
Ma. africana	12	2.8±0.4a	6	1.1±0.9a
Ae. circumluteolus	21	4.2±1.1a	2	0.4±0.5b

SD = standard deviation. Means in the same row followed by a different letter are significantly different ($P<0.05$).

Table 2. ANOVA tables, geometric mean catches/day of Anopheles gambiae *and indices of comparison for electric nets (ENT) baited with CO_2 versus unbaited ENT (control) (A), CFG trap (B) and CDC light-off trap (C)*

	Source	df	MS	F-value	Treatments	n	Mean	Index
(A)	Days	3	0.0163	0.576ns	ENT+CO_2	129	27.83	1a
	Sites	1	0.4361	15.445ns	Control	14	2.56	0.009b
	Treatments	1	1.6518	58.5**				
	Error	2	0.0282					
(B)	Days	3	0.0099	10.86ns	ENT+CO_2	133	29.99	1a
	Sites	1	0.0047	5.1ns	CFG+CO_2	202	44.03	1.47a
	Treatments	1	0.0527	5.76ns				
	Error	2	0.3036					
(C)	Days	3	0.0209	1.547ns	ENT+CO_2	93	22.96	1a
	Sites	1	0.0014	0.105ns	CDC+CO_2	36	8.55	0.37b
	Treatments	1	0.3192	23.56*				
	Error	2	0.0135					

df, degree of freedom; MS, mean square; *$P<0.05$; **$P<0.025$; ns, not significant; n, number of mosquitoes; indices in the same sub-table followed by a different letter are significantly different at $P<0.05$.

*Table 3. ANOVA tables (**A**) and log-transformed mean catches of Culex quinquefasciatus per day and indices of comparison for CDC light-on, CDC light-off traps baited with CO_2, and unbaited CDC light-off traps (control) (**B**)*

A	Source	df	MS	F-value
	Days	3	0.0351	0.851ns
	Sites	3	0.1173	2.981ns
	Treats	3	1.6532	42.011***
	Error	6	0.0393	
B	Treatments	n	Mean	Index
	Light-off	7	1.6	1a
	Light-on	12	3.7	2.2a
	Light-on+CO_2	104	25.0	15.4b
	Light-off+CO_2	252	57.4	35.3c

df= degrees of freedom; MS= mean squares; ***: $P<0.001$; ns= not significant; n: number of mosquitoes caught; indices not followed by the same letter are significantly different at $P<0.05$.

Discussion

The results show that CO_2-baited CFG traps and the ENT were superior to both CDC light-off and CDC light-on traps in collecting host-seeking *An. gambiae* and *Cx quinquefasciatus* when set in an outdoor environment. Several other studies in South-east Tanzania have shown that CDC light traps baited with CO_2 caught only small numbers of *An. gambiae* when set outdoors (L.E.G. Mboera & B.G.J. Knols, unpublished data). Like in our study, *An. gambiae* in Njage has previously been shown to comprise mostly of *An. gambiae s.s.* (B.G.J. Knols *et al.*, unpublished data). The CFG trap has been previously shown to effectively collect various species of mosquitoes in the United States and South Africa. For instance, in field studies against natural populations of woodland species in the United States, the CFG trap captured 7.8 times more mosquitoes than a standard professional (ABC-PRO) trap (Kline, 1999). The former trap efficiently collected *An. crucians, An. quadrimaculatus* and *Cx erraticus* than the latter.

Most studies on the anthropophilic *An. gambiae s.s.* and *Cx quinquefasciatus* are based on indoor collections, as it is the habit of those species to enter houses for blood feeding. However, for studies on odour-mediated behaviour of anthropophilic mosquitoes, it is imperative that the insects can be collected outdoors, to reduce the effect of "competitive" natural human odours. With both the CFG trap and ENT this appears possible, as the number of mosquitoes collected was very much higher than that collected in the CDC trap. Outdoor collections also have the advantage of being able to intercept the mosquitoes during their upwind flight inside an odour plume, which is

Table 4. ANOVA tables, geometric mean catches/day of Culex quinquefasciatus *and indices of comparison for a CFG trap baited with CO_2 versus an unbaited CFG trap (control) (A), CO_2-baited CDC light-off trap (**B**) and CO_2-baited ENT (C)*

	Source	df	MS	F-value	Treatments	n	Mean	Index
(A)	Days	3	0.0050	0.648	CFG+CO_2	315	70.26	1a
	Sites	1	0.0025	0.032	CFG	12	2.66	0.038b
	Treatments	1	3.3232	43.416**				
	Error	2	0.0765					
(B)	Days	3	0.0281	0.489	CFG+CO_2	274	70.62	1a
	Sites	1	0.0048	0.083	CDC+CO_2	48	10.14	0.144b
	Treatments	1	1.3062	22.79**				
	Error	2	0.0573					
(C)	Days	3	0.0088	1.379	CFG+CO_2	304	75.22	1a
	Sites	1	0.0030	0.466	ENT+CO_2	227	55.69	0.74b
	Treatments	1	0.0331	5.206ns				
	Error	2	0.0064					

df, degree of freedom; MS, mean square; **:$P<0.025$; ns, not significant; n, number of mosquitoes; indices in the same sub-table followed by a different letter are significantly different at $P<0.05$.

different from indoor collections where additional cues such as convection heat and relative humidity from the host may influence the mosquito's behaviour as well.

Previously, it was shown by Knols *et al.* (1998) that electric nets baited with either human breath or CO_2 collect mainly zoophilic mosquitoes, and only few *An. gambiae*. The large number of *An. gambiae* collected by electric nets in this study indicates that the trap efficiency in catching different species of mosquitoes varies with time and space and may depend on the density of the local mosquito populations. However, during the experimental period CDC light traps set beside a sleeping adult person under a bed net in nearby houses caught on average 419 *An. gambiae s.l.* per night (data not shown). This supports findings that the trap is an efficient tool for sampling anthropophilic mosquitoes indoors (Costantini *et al.*,1998b). The few mosquitoes caught in unbaited electric nets are probably those mosquitoes that were flying upwind in search of a host and intercepted before detecting host cues.

The role of the light in the CDC trap is unclear. Takken & Kline (1989) showed in their odour-baited studies of mosquito behaviour that light causes a distortion of the catch, attracting large number of insects other than Culicidae. When the CDC trap is used indoors for the collection of anophelines, the presence of light significantly increases the catch (Costantini *et al.*, 1998b), possibly because the mosquitoes flying around the human-occupied bed net gradually accumulate near the light. The light is not considered an 'attractant' from a distance, and it remains unclear why it was added to the trap, as the trap was developed to be used with an odour bait (mainly carbon dioxide). The results with the CO_2-baited CDC traps clearly show a significant reduction in the number of *Cx quinquefasciatus* collected. It may be assumed that the mosquitoes are attracted to the CO_2 source, and perhaps become disoriented by the presence of the light, leading to a reduced catch. It is recommended, therefore, that in outdoor studies with CDC traps, the light should be dispensed with. Moreover, light often attracts non-target insects which can make the sorting of the catch time consuming.

In an outdoor situation traps based on kairomones, sources of natural host odours, provide a more objective monitoring tool for the host-seeking fraction of pathogen carrying mosquitoes, provided the kairomones used consist of an odour mixture to which the vectors respond naturally. For indoor collections this may be different, because the mosquitoes are first attracted by other stimuli than light, presumably host odours, and once inside consist already of the biting fraction of the population (Chapter 3).

All trap collections increased when CO_2 was added as a bait. In previous studies on *An. gambiae*, it was shown that CO_2 contributes to the overall attractiveness of humans by 9% (Chapter 5) to 40% (Costantini *et al.*, 1996). From these studies it was concluded that CO_2 is only one of the kairomones responsible for the attraction of mosquitoes to humans. In recent indoor studies (Chapters 4 and 5) it was found that CO_2 was responsible for 25-28% of the *Cx quinquefasciatus* catch compared to complete human odour. Although CO_2 only attracts a fraction of the mosquito population compared to human odour, it nevertheless seems that this compound can used as an odour bait in sampling tools for anthropophilic mosquitoes, provided it is realised that the compound attracts fewer mosquitoes than a live human host. Moreover,

CO_2 has the advantage above a human host, that it can be dispensed at a constant rate and that the need for a human collector as bait is not required. Studies are required to compare the attractiveness of outdoor traps baited with human odour with that of CO_2-baited traps.

It was interesting to observe that the number of *An.gambiae* and *Cx quinquefasciatus* collected by the CFG trap did not significantly differ from that collected by ENT when baited with CO_2, although the mode of operation of the two traps are different. ENT were also superior to CDC light-off traps baited with CO_2 in collecting *An. gambiae.*

In conclusion, CO_2-baited ENT and CFG traps were found to be effective tools for sampling *An. gambiae* and *Cx quinquefasciatus.* It should be noted that while the CFG trap is easier to operate, uses small batteries, and collects live specimens, the ENT is difficult to operate, uses car batteries and kills the specimens. Electric nets though, are the instrument of choice when studying upwind odour-mediated flight behaviour of mosquitoes and other Diptera (Vale, 1974, 1993; Knols *et al.*, 1998). Both the CFG trap and ENT provide prospects of using odour-baited traps to sample an outdoor population of host-seeking *An. gambiae* and *Cx quinquefasciatus.*

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11

Sampling gravid *Culex quinquefasciatus* (Diptera: Culicidae) using traps baited with synthetic oviposition pheromone and grass infusions in Tanzania.

ABSTRACT - The assessment of traps baited with (*5R,6S*)-6-acetoxy-5-hexadecanolide (the synthetic oviposition pheromone, SOP) and grass infusions (GI) in sampling a population of gravid *Culex quinquefasciatus* was conducted in Muheza, Northeast Tanzania. A Counterflow Geometry (CFG) trap baited with SOP and set outdoors, adjacent to a pit latrine building, collected a significantly larger number of gravid *Cx quinquefasciatus* than a unlighted CDC miniature trap baited with SOP ($P<0.0001$). Inside pit latrine buildings, significantly more gravid *Cx quinquefasciatus* were collected in a CFG trap-baited with SOP than in the trap baited with tap water ($P<0.01$). Under similar conditions, significantly more gravid *Cx quinquefasciatus* were collected in a CFG trap baited with GI than in a trap baited with tap water ($P<0.0001$). CFG traps baited with either GI or SOP and set outdoors, away from known breeding sites, caught significantly more gravid *Cx quinquefasciatus* than traps baited with tap water ($P<0.05$). It was further observed that a CFG trap baited with a SOP+GI combination caught significantly more gravid *Cx quinquefasciatus* than a CFG trap-baited with either GI or SOP alone. In both cases, the proportion of gravid mosquitoes increased as traps were placed away from a natural emergence site. A SOP-baited CFG trap caught a larger number of gravid *Cx quinquefasciatus* than the corresponding number of egg rafts deposited in a jar with water treated with SOP. It is concluded that CFG traps baited with oviposition attractants can effectively be used to sample gravid *Cx quinquefasciatus*.

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Introduction

Culex quinquefasciatus Say, the principal vector of bancroftian filariasis in the urban areas of the East African coast, breeds in polluted water, mainly in wet pit latrines, septic tanks and soakage pits. The synthetic oviposition pheromone, (SOP) (*5R,6S*)-6-acetoxy-5-hexadecanolide)) has been shown to influence significantly the oviposition site selection of this mosquito in East Africa (Otieno *et al.*, 1988a; Chapter 7). Recent studies in Muheza, Tanzania, have also indicated that volatiles from soakage pits and grass infusions attract *Cx quinquefasciatus, Cx cinereus* Theobald and *Cx tigripes* Grandprè and de Charmoy to oviposition sites, particularly when a blend of synthetic oviposition pheromone was tested jointly with either soakage pit water or grass infusions (Chapter 7).

Various studies have shown that oviposition attractants show great promise for surveillance (Reiter, 1983, 1986; Millar *et al.*, 1994) and, possibly, control (Otieno *et al.*, 1988a) of *Cx quinquefasciatus*. With the specific oviposition modifying compounds such as (*5R,6S*)-6-acetoxy-5-hexadecanolide (Laurence & Pickett, 1985) and skatole (Millar *et al.*, 1992), mosquito populations can perhaps be sampled and manipulated by regulating their oviposition behaviour. Gravid females of *Cx quinquefasciatus* are able to distinguish between sites with and without an oviposition pheromone at distances of up to 10 m, as the experiments by Otieno *et al.* (1988a) have shown. However, gravid females did not oviposit immediately in water carrying pheromone alone, in an outdoor situation, away from known natural breeding sites in both Kenya (Otieno *et al.*, 1988a) and Tanzania (Chapter 7). Although GI-baited traps have proven their usefulness in sampling gravid *Culex* mosquitoes in the United States (Reiter, 1983; Ritchie, 1984), reports of sampling gravid mosquitoes in Africa are not available. It is expected that gravid mosquito traps based on semiochemicals will provide an objective monitoring tool for mosquitoes. Our objectives in the work reported here were (i) to study the effect of GI and SOP, and their combination in the vicinity of and away from known breeding sites to avoid the effect of competitive odours, (ii) to assess the efficiency of traps baited with the two oviposition attractants in sampling gravid mosquitoes, and (iii) to compare the efficiency of ovitraps versus gravid mosquito traps when baited with SOP.

Materials and method

Study site. All the experiments were conducted in Muheza, $5^0$10'S, $38^0$46'E, in North-east Tanzania. The area has been described in Chapter 4

Chemicals. The SOP was prepared as described previously by Dawson *et al.* (1990). Blank effervescent tablets were spread out individually on a clean piece of paper, and using a precision syringe, 0.1 ml of the mosquito oviposition pheromone solution in hexane (200 mg/ml) was placed on each of the tablets. The tablets were left to dry for a few minutes at room temperature before use. Grass infusion was prepared by fermenting 2 kg of *Digitaria sp.* grass cuttings in 10 l of tap water for 5 days at room temperature. The brew was filtered through fine netting and was frozen until needed. For more details see Chapter 7.

Experimental protocol. The relative efficiency of a SOP-baited CFG trap and a CDC trap for sampling of Cx quinquefasciatus. In order to know whether the CFG trap (Kline, 1999) performed as well as the widely used CDC trap (Sudia & Chamberlain, 1962), the two types of traps, baited with SOP, were at first compared. Since it had previously been shown that a CDC trap with a light collected significantly fewer *Cx quinquefasciatus* than traps without light (Chapter 10), the lamp from the CDC trap used in these experiments was removed. The pheromone-treated tablet was dissolved in 800 ml of tap water. A glass vial containing 4 ml of SOP was attached near the entrance of the CDC trap or it was fixed in a small plastic tube that was attached at the carbon dioxide entrance port on the lateral wall of the CFG trap. The two traps were hung outdoors at either side of a latrine building 50 cm from the wall, at 2 m apart. Traps were left overnight. The CFG trap was hung 20 cm from the ground level, whereas the CDC trap was hung with the shield 1 m from the ground level. The traps were operated between 18.00 and 08.00 h for eight days and alternated between the sites every experimental day.

Response of Cx quinquefasciatus *to a CFG trap baited with SOP or GI versus tap water.* 800 ml of tap water treated with either a tablet impregnated with SOP or hexane (control) were put in white plastic jars. The jars were covered with a black netting material to avoid mosquitoes ovipositing on the solution. CFG traps were hung above the jars with their lowest tip just above the netting material covering the jar (Fig 1). Two pairs of the traps were set inside two pit latrine buildings, with traps at 1 m apart in each pair. The traps were operated between 18.00 and 08.00 h for 8 days and were alternated between the two sites every experimental day.

In another experiment the response of *Cx quinquefasciatus* to a CFG trap baited with GI was compared with the response to tap water indoors. In a similar setting as described in the experiment above, two CFG traps were either baited with 800 ml of GI or tap water in a white plastic jar covered with a black netting material and were hung 1 m apart inside the latrine building. The GI and water used in the jars of each trap was replaced each night of trap operation. The traps were operated between 18.00 and 08.00 h for 8 days with the treatments alternated between the two sites.

Response of Cx quinquefasciatus *to a CFG trap baited with SOP versus GI outdoors, adjacent to a breeding site.* In this experiment 800 ml of the treatments in white plastic jars covered with a black netting material were placed under the CFG traps. The traps, with the lowest tip touching the netting material, were hung outside the latrine building, 50 cm from the latrine wall, and were placed 2 m apart. The experiment was conducted between 18.00 and 08.00 h for eight days with the treatments alternated between the two sites.

Response of Cx quinquefasciatus *to a CFG trap baited with either SOP, GI or water, away from known breeding sites.* Three CFG traps were set outdoors in an area away from known breeding sites of *Cx quinquefasciatus*. The nearest human habitation with potential breeding sites was 200 m away. The treatments, SOP, GI, and tap water (control) were put in white plastic jars covered with a black netting material to avoid mosquitoes laying their egg in it. The traps were hung from wooden poles 10 m apart, with their lowest tips touching the netting material. The traps were operated between

18.00 and 08.00 h. In a 3-choice arrangement, the three treatments were alternated between the three sites everyday for six days.

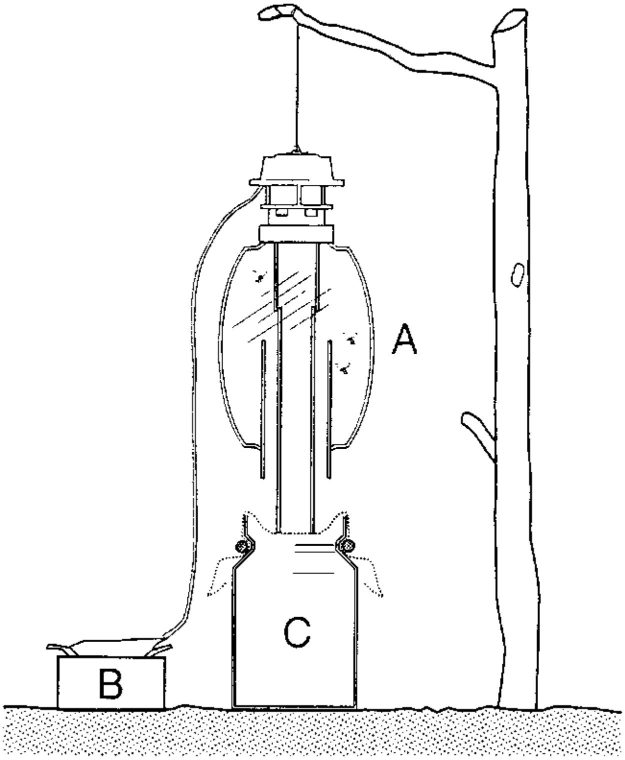

Figure 1. Counterflow Geometry (CFG) trap when used as gravid mosquito trap; A: CFG trap, B: power source (12V battery); C: jar containing the bait.

In another series of experiments, CFG traps baited with either GI, SOP or SOP+GI combination were compared. Two CFG traps, one baited with 800 ml of GI and another with 800 ml of tap water treated with SOP, were compared with 800 ml of GI treated with SOP. The traps with the lower most end over jars containing the treatments were placed in an open compound, 200 m from the nearest known breeding site of *Cx quinquefasciatus*. In a 3-choice arrangement, the traps were alternated between the three positions every trapping day and operated between 18.00 and 08.00 h for six days.

The efficiency of a SOP-baited ovitrap versus SOP-baited mosquito gravid trap. In this experiment, SOP-baited CFG traps were used as gravid mosquito traps while SOP-baited white plastic jars (4 litre capacity) were used as ovitraps. The treatments were (i) an operating CFG trap (trap-on) baited with SOP, (ii) a CFG trap not operating (trap-off) but baited with SOP, (iii) an unbaited operating CFG trap, and (iv) a jar containing SOP. The jar in treatment (i) was covered with black mosquito netting to avoid mosquitoes depositing their egg rafts in it, whereas the jars in treatment (ii) and (iv) were left uncovered. The treatments were placed at 10 m apart in a straight line (10 m from the nearest pit latrine) and were alternated between the four sites every day. The experiment was run between 18.00 and 08.00 h for four days.

Data analysis. The mosquitoes collected were sorted to species, sex and physiological status i.e. unfed, blood-fed, or gravid. The egg rafts collected were sorted by shape and taken to the laboratory and reared separately to the adult stage when they were identified to species morphologically (Edwards, 1942; Gillett, 1972). All data were log (x+1) transformed and means of treatments and controls were compared using Student *t*-tests (Snedecor & Cochran, 1989). Means in factorial experiments were subjected to ANOVA. An F-test significant at $P<0.05$ was followed by a Least Significant Difference test to sort out differences between treatment means.

Results

A CFG trap baited with SOP and set outdoors adjacent to a pit latrine building, collected significantly more gravid *Cx quinquefasciatus* than a CDC trap ($P<0.05$), with the mean value of 20.4 and 2.2 gravid mosquitoes per trap day respectively. Therefore, all other experiments were done with CFG traps only.

Inside a latrine building, significantly more gravid *Cx quinquefasciatus* were collected in a CFG trap baited with SOP than in the control ($P<0.01$) (Table 1A). The proportion of gravid females attracted to the SOP baited trap was 63.8% of the total female mosquitoes collected (n = 2059). The rest were nongravid females. Similarly, significantly more gravid *Cx quinquefasciatus* were collected in the CFG trap baited with GI than in the trap containing tap water ($P<0.0001$) (Table 1B). In this experiment the proportion of gravid *Cx quinquefasciatus* was 65.8% of the total females (n = 2480) collected. Outdoors near a pit latrine building the number of gravid *Cx quinquefasciatus* caught in the CFG trap-baited with SOP was not significantly different from that collected in the trap baited with GI ($P>0.05$) (Table 1C). The gravid females caught by the CFG trap baited with SOP and GI were 77.4% and 70.5% of the total female mosquitoes (n = 444 and 346), respectively.

When CFG traps were set outdoors away from known breeding sites, the mean number of gravid *Cx quinquefasciatus* caught in a trap baited with SOP, GI or tap water were 71.1, 94.2 and 6.32 respectively. The numbers of gravid mosquitoes caught in traps baited with SOP and GI were not significantly different ($P>0.05$). Significantly fewer gravid mosquitoes were collected in a trap containing tap water ($P<0.05$). Of the mosquitoes caught in a SOP or GI baited trap, 94.0% and 93.9% were gravid respectively. When traps baited with SOP or GI were compared with a trap baited with

SOP+GI combination, significantly more gravid *Cx quinquefasciatus* were caught in a trap baited with the SOP+GI combination than in the other two traps (Table 2). Of the female *Cx quinquefasciatus* caught in the traps, 96.9%, 94.7% and 97.7% in a trap baited with SOP, GI and the combination of the two, respectively, were gravid mosquitoes.

The number of gravid *Cx quinquefasciatus* collected in a SOP-baited CFG trap-on was greater than the number of egg rafts deposited in a jar under the non-operating trap or number of egg rafts in the jar containing SOP in the absence of the trap. Significantly fewer gravid mosquitoes were collected by the unbaited operating trap than in the SOP-baited CFG trap-on (*P*<0.05) (Table 3).

Table 1. Total (n) and geometric mean (±SD) of gravid Cx quinquefasciatus *per trap day as collected by a CFG trap baited with: **A**, synthetic oviposition pheromone (SOP), **B**, grass infusions (GI) versus a CFG trap baited with tap water (control) set indoors; and **C**, a CFG trap baited with SOP versus GI set outdoors adjacent to pit latrine buildings*

		Treatment	n	Mean±SD
Indoors	**A**	SOP	1314	140.3±0.9a*
		Control	527	57.2±0.7b
	B	GI	1632	194.0±0.4a**
		Control	370	43.1±0.5b
Outdoors	**C**	SOP	344	40.4±0.4a
		GI	244	26.5±0.7a

SD = standard deviation. Means in the same sub-table followed by a different letter are significantly different at *: *P*<0.01; **: *P*<0.001.

Table 2. Total (n) and geometric mean (±SD) catches of gravid Cx quinquefasciatus *per day for a CFG trap baited with either grass infusions (GI), a synthetic oviposition pheromone (SOP) or SOP+GI combination in an area away from a known breeding site*

Treatment	n	Mean±SD
SOP	157	24.7±0.4a
GI	195	30.7±0.4a
SOP+GI	475	77.7±0.2b

SD = standard deviation. Means not followed by the same letter are significantly different at *P*<0.05.

Discussion

In this study it was found that a CFG trap baited with SOP and set outdoors was more efficient in collecting gravid *Cx quinquefasciatus* than a CDC trap baited with SOP. The CFG trap, which utilises a counterflow concept, has two fans, one dispersing the odour (by pumping it outside the trap) while the other, a stronger fan, sucks the mosquitoes into the trap as they are flying up the odour trail. This is not the case of the CDC trap that operates with a single fan. In the CDC trap, the fan disperses the odour away from the trap entrance, thus fewer mosquitoes of those that are attracted to the trap are caught and hence its efficiency is reduced.

Table 3. Total (n) and geometric mean (±SD)of gravid Culex quinquefasciatus *caught in either CFG trap baited with SOP (SOP+trap-on) or number of egg rafts deposited in SOP treated water in the presence (SOP+trap-off) or absence (SOP no trap) of the CFG trap*

Treatment	n	Mean±SD gravid mosquitoes	Mean±SD egg rafts
SOP+trap-on	182	45.0 ±0.1a	
Unbaited trap-on	12	2.7±0.7c	
SOP+trap-off	46		11.3±0.4b
SOP no trap	56		14.2±0.1b

SD = standard deviation. Means in the same column followed by a different letter are significantly different at $P<0.05$.

Significantly more gravid *Cx quinquefasciatus* were caught in traps baited with either a SOP or GI than in traps baited with tap water. Because the trap collects responding mosquitoes by suction, before they are able to contact and sample the water in the plastic jar, the compounds must be perceived by olfaction for the mosquitoes to respond. These experiments clearly demonstrated that the compounds used are mosquito oviposition stimuli. In a separate study (Chapter 7), it was observed that *Cx quinquefasciatus* deposit more egg rafts in GI or SOP than in tap water. Similar observations on the effect of SOP have been reported by Otieno *et al.* (1988a). Strong attraction of *Cx quinquefasciatus* to hay infusions, similar to our grass infusions, has also been reported by Beehler *et al.* (1994b), and it appears that in the field the oviposition behaviour of *Cx quinquefasciatus* is mediated by both the pheromone and semiochemicals from the organically enriched water. Other mosquito species also use water-borne chemicals for oviposition (Allan & Kline, 1995) but the combined use of a pheromone and the water-borne chemicals appear to be limited to some *Culex* species.

Proportionally fewer gravid females were collected inside pit latrine buildings compared to gravid mosquitoes collected outdoors adjacent to a pit latrine building and away from known breeding areas. However, in both cases the mean number of gravid mosquitoes responding to either a GI or SOP-baited traps was larger than that responding to traps baited with water. The collection of many nongravid *Culex* in the vicinity of latrines is probably due to the fact that traps were placed near mosquito emergence sites and thus also collecting newly emerged mosquitoes. The traps, being near emergence sites for mosquitoes, are likely to suck in any mosquito flying near them and hence increase the chance of mosquitoes of different physiological status and sex to be caught in it.

In both the indoor and outdoor situations, the number of gravid *Cx quinquefasciatus* collected in the CFG trap baited with SOP did not significantly differ from that collected in the trap baited with GI. In the two situations, both near or away from known breeding sites, a trap baited with GI or SOP caught significantly more gravid *Cx quinquefasciatus* than a trap baited with tap water, showing that gravid mosquitoes respond to semiochemicals in search and selection of a suitable breeding site.

Since gravid mosquito traps baited with SOP collected more gravid mosquitoes than the corresponding number of egg rafts deposited in ovitraps, the pheromone must be perceived by olfaction and the number of egg rafts deposited does not reflect the number of gravid mosquitoes visiting an oviposition site. The gravid mosquito trap caught a larger proportion of gravid *Cx quinquefasciatus* than the corresponding number of egg rafts deposited in the ovitrap, because as the mosquitoes approached the bait, they were sucked into the trap by the fan before they either deposited their egg rafts or left the site. It may also be true that the trap fan actively dispersed a far larger plume of SOP than that dispersed passively by diffusion from the jars. Consequently, many more mosquitoes may have intercepted volatile stimuli from this larger plume and be attracted, making for a more efficient trapping method than the oviposition jar. Interestingly, results of this study have shown that SOP, when used in gravid mosquito traps, was a better measure of oviposition response than when used in an ovitrap.

It can be concluded that CFG traps baited with oviposition attractants can effectively be used to sample gravid *Cx quinquefasciatus* populations and that a combination of synthetic oviposition pheromone and GI is better than either of the two. Moreover, mosquito gravid traps are better indicators of gravid female mosquitoes than ovitraps when baited with the synthetic oviposition pheromone. Survey and monitoring with pheromones and other oviposition attractants are practised world-wide against a broad array of insect pests, and with the availability of the SOP and GI, these techniques may provide integral parts of the available intervention measures in the control programmes of bancroftian filariasis in Africa.

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General discussion and conclusions

Much of the behaviour of adult mosquitoes is mediated by olfactory cues, including such important features as mating, blood feeding, oviposition and nectar feeding (Takken & Knols, 1999). However, our understanding of the behavioural ecology of the bancroftian filariasis vector, *Culex quinquefasciatus* is still marginal. It is envisaged that, with better understanding, these resource-locating behaviours may be exploited or modified to provide new or improved methods of mosquito monitoring and control in the future. The exploitation of mosquito behaviour and general ecology to reduce contact with human hosts, similar to the development of control strategies for tsetse flies (Diptera: Glossinidae) based on simple odour-baited targets and traps (Vale, 1993), may be an alternative to chemical control of *Cx quinquefasciatus* in the future. Novel methods based on the interruption of odour-mediated behaviours such as host-seeking and oviposition of *Cx quinquefasciatus*, though widely practised for control of this and other mosquitoes in the United States, are yet to be developed in the Afrotropical region, because their basic biology is poorly known. This lack of understanding in biology has been the major basis for studying the chemical ecology of *Cx quinquefasciatus*.

Chemical ecology of host-seeking behaviour

Insects have evolved and function within a multitrophic context. They base their foraging decisions on information from different trophic levels, and chemical information plays an important role. Although the importance of semiochemicals in foraging by parasitoids and predators has been well documented (see Vet & Dicke, 1992), little is known to date of the chemical ecology of man-biting mosquitoes. Information from vertebrate hosts is important in the mosquito host-finding process. A sequence of responses to host derived cues brings the searching mosquito close to its potential host.

In this part the host-seeking and selection behaviours of *Cx quinquefasciatus* are considered to form important aspects of bancroftian filariasis epidemiology. The vector efficiency of *Cx quinquefasciatus* is associated with its high anthropophilic character, accompanied by a preference for feeding indoors at night. These factors, and its adaptation to unique habitats and conditions created by humans around their dwellings, makes the species an important mosquito in urban areas of the tropics. Indeed, *Cx quinquefasciatus* is the only important anthropophilic *Culex* mosquito in Africa. Evolution of biting at night of this mosquito has the effect of minimising host avoidance mechanism. In addition to breeding in the peridomestic man-made structures, the mosquito spends a very great proportion of its adult life resting inside houses, factors that ensure a closer association between the mosquito and its human host.

During host-location, mosquitoes use visual, physical and olfactory cues produced by their vertebrate host to orientate themselves to that host. Since most anthropophilic mosquitoes are nocturnal biters, olfactory cues are considered to be the most important stimuli. One of the objectives of this study was to determine important olfactory stimuli involved in the host-seeking and selection processes of *Cx* *quinquefasciatus*. As far as the host-seeking behaviour is concerned, results (Chapter 3) have shown that *Cx quinquefasciatus* responds readily to human skin emanations. The response of the mosquito to carbon dioxide at a human breath concentration is less as compared to skin emanations. Moreover, it appears that *Cx quinquefasciatus* is extremely sensitive to small changes in the relative humidity gradient. Thus the human body produces compounds to which *Cx quinquefasciatus* is attracted over a short distance, and this effect may be enhanced by slight variations in relative humidity. Hocking (1963) proposed that carbon dioxide and host odours are long-range kairomones, with elevated temperature and moisture contributing to close-range orientation. In addition to my observations that *Cx quinquefasciatus* responds positively to humidity gradients (Chapter 3), Takken *et al.* (1997a) showed that the behavioural responses of *An. gambiae* were modulated by small gradients in relative humidity. Humidity gradients are thought to influence the close-range orientation and landing behaviour of mosquitoes onto hosts (Brown, 1951; Laarman, 1955; Gillies 1980). This is supported by electrophysiological studies, which confirmed the presence of hygroreceptors (Kellogg 1970) on the antennae of *Ae. aegypti*. However, when Eiras & Jepson (1994) tested the effects of humidity and temperature on the behaviour of female *Ae. aegypti* in a bioassay, they found that there was no corresponding effect when the humidity is increased by up to 5%. Nonetheless, the response shown by *Cx quinquefasciatus* to a humidity gradient in my laboratory experiments was significant though lower than that to skin emanations.

Mosquitoes are categorised as being anthropophagic or zoophagic, but these terms describe only the outcome of a complex behaviour pattern, and are mostly based on the identification of blood meals. Little is known about the mechanisms by which mosquitoes arrive at a particular host. The ultimate choice of a host to feed on is related to the evolution of optimal feeding strategies, but the host-seeking process is determined by the stimuli available and the nature of the mosquitoes' responses to these stimuli. Knowledge of the feeding behaviour of the vectors of human diseases is essential in understanding the vector-host interactions, and their respective roles in a disease transmission cycle. However, host-selection behaviour of mosquitoes, and the cues that stimulate one species to be a 'generalist' feeder, and another a 'specialist' feeder are poorly understood.

Information on vector-host interactions can be obtained by studying the natural feeding habits of the mosquito vector. The analysis of blood meals of a wide range of mosquito species has demonstrated that feeding patterns, i.e., the range of host species fed on and the extent of host specificity, are highly species-specific. It is likely that differences in feeding patterns reflect differences in responsiveness to stimuli present throughout the host location process. It should be borne in mind that feeding patterns of mosquitoes obtained by analysis of blood meals might not reflect the actual mosquito preference because defensive behaviour by hosts can influence the successful feeding by mosquitoes. For instance, lower blood indices of a particular animal might only reflect defensive behaviour of the host and not its attractiveness. By studying host preference using a response choice test, the present study (Chapter 4) has indicated that, given equal opportunity of a human host, and the most common mammalian animals in the urban areas of East Africa, *Cx quinquefasciatus* will select a human host in preference to other mammals such as cattle and goats. These findings support earlier reports from blood precipitin tests that *Cx quinquefasciatus* in tropical Africa is strongly anthropophagic (Beier *et al.*, 1990; Bϕgh *et al.*, 1998). The very high degree of anthropophily of *Cx quinquefasciatus* in this area may be partly explained as a preference for human odours other than carbon dioxide. Generally, an anthropophagic mosquito would be expected to show limited attraction to carbon dioxide and instead select human-specific kairomones (Chapters 4 and 5). The present data suggest convincingly that human volatiles other than carbon dioxide play an important role in the host-seeking behaviour of *Cx quinquefasciatus.*

Field studies in Tanzania have shown that the role of carbon dioxide in attracting *Cx quinquefasciatus* to man was minor. However, the compound's contribution of 25-28% to the total attractiveness of the human host is significantly important in guiding the mosquito to its host. Moreover, carbon dioxide is likely to act in concert with other human odours giving a response which is different from that of either stimulus given alone. Multiple stimuli are likely to be a more reliable guide to the presence of a specific host such as man, than one stimulus received alone. The mosquito's response to specific blends of host cues may allow a degree of selection for a particular host, in this case a human. Since the response of *Cx quinquefasciatus* to a human host appears not to rely on carbon dioxide but rather on other, human specific olfactory cues, this behaviour might be linked to its capacity to act as an important vector of bancroftian filariasis. For a mosquito to be an efficient vector of a human disease, it must preferentially feed on human hosts.

Using the experimental design described in Chapter 5, it was possible to separate the cues that may be responsible for attracting *An. gambiae* and *An. funestus* similar to designs used by Vale (1974) to study tsetse flies in Zimbabwe. Hence it was possible to compare the proportion of mosquitoes that were attracted to the physical presence of a human bait or the body smell of the human bait or its carbon dioxide equivalence. The results showed that some 91% of *An. gambiae* and *An. arabiensis* and 73% of *An. funestus* attracted to a human bait did so in response to the host's odours other than carbon dioxide. Body odours and carbon dioxide are the main olfactory cues that lead man-biting mosquitoes to a human host.

All vertebrate hosts produce carbon dioxide and this compound therefore serves as a potential semiochemical for haematophagous arthropods. The appropriateness and usability of any kairomone, however, depends on its reliability in indicating the presence of a potential vertebrate host. Carbon dioxide will signal the presence of both human and non-human hosts. Stimuli derived from the human itself are generally the most reliable sources of information for an anthropophilic mosquito. Ideally, the cues should tell mosquitoes whether a vertebrate host is present and to which species it belongs. It may be assumed that the use of a cue that is reliable and easy to detect, enhances host-seeking efficiency and consequently fitness. According to Vet & Dicke (1992) specialist insects, being more selective, use relatively more time for searching than generalists. Thus, specialists benefit more from a reduction in search time through an effective use of infochemicals than generalists.

Mosquitoes respond to odour baits according to their known host preference. For instance, in South Africa, Dekker & Takken (1998) showed in a choice test between a calf and a human, that zoophilic mosquito species responded significantly more to calf odours and carbon dioxide whereas anthropophilic species such as *Cx quinquefasciatus* responded more to human odours. Unlike the present studies in Tanzania, where *Cx quinquefasciatus* showed a similar response to both carbon dioxide and a calf, in South Africa the mosquito preferred carbon dioxide to a calf (Dekker & Takken, 1998). In both East and West Africa, Gillies (1964, 1967) observed that *An. gambiae*, another highly anthropophilic mosquito, was attracted more to a human than to a calf. It is clear from these results and others, that the Afrotropical anthropophilic mosquitoes, particularly, *An. gambiae* and *Cx quinquefasciatus*, respond to human volatiles, of which carbon dioxide is not a very reliable constituent.

These findings are in agreement with those reported by Costantini *et al.* (1996) and Dekker & Takken (1998). In Burkina Faso, host odours were found to influence mosquitoes in the response to carbon dioxide, and *Cx quinquefasciatus* and *An. gambiae* responded less to carbon dioxide than to human body odours. In the same study, *An. arabiensis* was found to be less anthropophilic of the two members of the *An. gambiae* complex. In South Africa (Dekker & Takken, 1998) and Tanzania (Chapter 5) *An. arabiensis* responded significantly more to human body odours than to carbon dioxide. Unlike in the present experiments, in Burkina Faso (Costantini *et al.*, 1996) and South Africa (Dekker & Takken, 1998) a higher release of carbon dioxide resulted in a proportional increase in the number of *An. arabiensis*. Whereas the *An. arabiensis* at the study area in Tanzania feeds predominantly on man (Charlwood *et al.*, 1995), the South African population feeds entirely on wild animals. Dekker & Takken (1998) attribute this preference in South Africa to the higher carbon dioxide release of many wild host animals because of their larger body mass as compared to man. In both Burkina Faso (Costantini *et al.*, 1998a) and the present observations (Chapter 5) *An. funestus* responded more readily to human odours than to an equivalent concentration of carbon dioxide.

Cx quinquefasciatus was found to readily respond to traps baited with skin emanations under field conditions. This observation support earlier laboratory findings reported in Chapter 3. The response to skin emanations was slightly higher when carbon dioxide was added. Besides carbon dioxide, the field experiments have shown that *Cx quinquefasciatus* responds poorly to other organochemical compounds such as acetone, 1-octen-3-ol (octenol) and butyric acid (Chapter 6). Acetone is a chemical present in the breath of vertebrates, including humans. Takken *et al.* (1997a) showed in a laboratory bioassay that in the presence of carbon dioxide, acetone caused strong behavioural responses in *An. gambiae* and *An. stephensi* but at different concentrations. Octenol has also been reported as a semiochemical to which mosquitoes respond from a distance in the field (Kline *et al.*, 1990a,b; Takken & Kline, 1989). The response to octenol is not species-specific, as many mosquito species are known to respond to it (Kline, 1994a). The findings in this thesis show that *Cx quinquefasciatus* responds poorly to octenol or to its combination with carbon dioxide under field conditions in Tanzania. Interestingly, Kline & Lemire (1995) found a significant increase in trap collections of various mosquito species when CDC traps were baited with carbon dioxide and octenol and when heat was added as an additional stimulus. This suggests an interaction between heat and olfactory stimuli (Laarman, 1958).

Like acetone, octenol is a common volatile in the emanations of herbivores (Cork & Park, 1996; Hall *et al.*, 1984), and therefore it is perhaps not surprising that it is a kairomone for zoophilic mosquitoes. In field experiments in the United States, (Kline *et al.*, 1990b) *Culex* species did not respond to octenol alone or in combination with carbon dioxide. The lack of response from *Culex* was thought to relate to their host preferences because octenol is a mammalian components of host odour and *Culex* species are mainly ornithophilic in the United States. Further work conducted by Kline (1994a) in a range of ecological niches showed that 35 species of mosquitoes could be caught in odour-baited traps, of which, traps baited with octenol in combination with carbon dioxide caught species of *Aedes, Anopheles, Psorophora, Coquillettidia* and *Mansonia*. In conclusion, there is strong evidence that *Cx quinquefasciatus* responds highly to human volatiles and the role of carbon dioxide in its host-location behaviour is limited. Moreover, acetone and octenol are unlikely to play a role in the orientation of *Cx quinquefasciatus* to its human host.

The combined results from laboratory and field studies indicate that human skin odours constitute the major components of host cues used by *Cx quinquefasciatus* in its host-seeking behaviour. It is, however, disappointing that the response to skin residues was not significantly higher than that to carbon dioxide as previously shown in the laboratory (Chapter 3). It is probable that quick evaporation of essential chemicals, and/or competition with natural host odours in a village setting may be attributed to the field study failures. More work is therefore required, particularly on the characterisation of the possible kairomones in human skin emanations. So far, a large number of studies in Africa have shown that baiting traps with whole natural host odour or some component of odour increases the catch of mosquitoes (Costantini *et al.*, 1993, 1996, 1998a; Knols *et al.*, 1998; Dekker & Takken, 1998 and Chapters 4 & 5). These findings are important in providing a stimulus to developing a bait system for mosquitoes, similar to that used in tsetse monitoring and control. The development of odour baits to control tsetse flies (Vale, 1993) is probably the best example of the use of semiochemicals to control an arthropod vector of medical and veterinary importance in Africa. The development of this control technology has been based on an understanding of the responses of tsetse to their hosts, using research tools that quantify single and specific responses. It is suggested that the same approach to analysing the responses of mosquitoes to their hosts will produce improved baits for mosquitoes.

Chemical ecology of oviposition behaviour

The activity of *Culex quinquefasciatus* is limited to the period in between dusk and dawn, typically early in the morning and late in the evening, during the oviposition process and during the night when host-seeking. During the day, the mosquitoes rest, preferably inside houses. The gravid mosquito will leave the resting site for oviposition in the apparent absence of any oviposition site stimulus, presumably only in response to an endogenous activity rhythm modulated by the gravid state, and exogenous environmental stimuli such as falling light intensity. Outside the resting site, the mosquito is then stimulated by various chemical oviposition cues from a nearby oviposition site. In many culicine mosquitoes the selection of oviposition site has been observed to be mainly olfactory-mediated. Semiochemicals originating from natural water bodies as breakdown products of bacterial origin or from the mosquito eggs (as oviposition pheromone) have been shown to influence oviposition site selection of some *Culex* species. As with host-location, olfactory signals have clearly been shown to play an essential role in other behavioural modalities, such as the location of the oviposition site. Results obtained from this work have shown that *Cx quinquefasciatus* and *Cx cinereus* oviposit their egg rafts more readily in the presence of an oviposition pheromone (acetoxyhexadecanolide). It was further observed that the response of the gravid mosquitoes, of these two species and that of *Cx tigripes*, to volatiles from either soakage pit water or grass infusion is higher than to water. The cues used by these gravid mosquito species, therefore, include acetoxyhexadecanolide, an oviposition pheromone and/or volatiles from grass infusions and other organic matter (Chapter 7). Most of the behaviourally relevant compounds present at natural oviposition sites are phenols. Ikeshoji (1975) isolated phenols and a series of monomethylphenols, dimethylphenols and trimethylphenols from extracts of wood creosote and demonstrated that several of these compounds elicited oviposition behaviour from culicine mosquitoes. Millar *et al.* (1992), who screened systematically the environmental cues responsible for eliciting oviposition in *Cx quinquefasciatus*, identified phenol, 4-methylphenol, 4-ethylphenol, indole, and 3-methylindole in fermented *Cynodon dactylon* grass infusions. Indole and 3-methylindole (skatole) are also found in mammalian waste products and they have been found to influence oviposition site selection of a number of Muscidae and Calliphoridae that oviposit on mammals (Cragg & Ramage, 1945; Mackley & Brown, 1984; Mulla & Ridsdill-Smith, 1986).

It is clear from these studies, that volatiles from polluted water such as water from soakage pit or grass infusions in combination with oviposition pheromone provide a synergistic effect in the selection of breeding sites of *Cx quinquefasciatus, Cx cinereus* and *Cx tigripes* (Chapter 7). It is likely that the absence of a synergistic effect obtained when synthetic oviposition pheromone was combined with skatole (Chapter 8), contrary to that observed when combined with grass infusions or soakage pit water, was due to the presence of other compounds in the latter blend, or the ratio of the oviposition pheromone to skatole used in these experiments was not proportional to that found in natural breeding sites. These field studies have shown that *Cx quinquefasciatus* is quite discriminative in selecting sites for oviposition. Considerable evidence associates this site discrimination by ovipositing females as a fundamental factor in determining larval distribution. Selection of a suitable oviposition site by a gravid mosquito is of utmost importance for the survival of her progeny (McCall & Cameron, 1995). One way in which she locates an appropriate site is by orienting towards oviposition semiochemicals. This allows the female to provide an optimal food resource for her progeny at an energetically low maternal cost (Dicke & Sabelis, 1988). Since the production of oviposition pheromone occurs at the maturation of the egg rafts, this is adaptively advantageous in that it signals safety of the site, as the pheromone is only released by the eggs that have survived for at least 24 h, and may have other benefits for the development of the offspring (McCall & Cameron, 1995). Environmental cues such as volatiles from polluted water, on the other hand, give only indirect information on suitability, information that is often less predictable and reliable.

The response of *Cx quinquefasciatus* to skatole was observed at doses as low as 10^{-5} and 10^{-6} μg/l which were similar to that shown by Blackwell *et al.* (1993) but lower than those reported by Beehler *et al.* (1994b). The repellent effect seen at higher concentrations suggests that, although mosquitoes may respond positively to low levels of metabolic products from degradation of organic matter, higher concentrations of the same stimulus deter mosquitoes from ovipositing in waters that may be too polluted or otherwise detrimental to development of their offspring (Millar *et al.*, 1994). The production of skatole from human and animal wastes and plant infusions in the vicinity of human habitations, in structures such as a pit latrine, soakaways and cesspit, is an important epidemiological factor that assures *Cx quinquefasciatus* of its close association with man, for the oviposition site and source of blood meal and hence an easy transmission of bancroftian filariasis.

A better understanding of the factors influencing oviposition site selection may be utilised in mosquito control strategies. Oviposition attractants, for example, may be employed to lure mosquitoes to already treated sites and trap them directly or indirectly through their immature stages by using juvenile hormone mimics or biological control agents such as *Bacillus sphaericus*. In addition, oviposition attractants may also be used to monitor mosquito populations where polystyrene beads have been employed to control breeding of *Cx quinquefasciatus* in on-site sanitation structures. The observations that *Cx tigripes* is likely to have used the oviposition pheromone of *Cx quinquefasciatus* to locate a concentration of mosquito eggs (Chapter 7), should be studied further to see the possibility of employing this predator in the control of *Cx quinquefasciatus*.

Semiochemicals in sampling systems

Accurate means of assessing mosquito infective bites per person is essential, both for predicting outbreaks of mosquito-borne diseases and for monitoring the progress of control programmes. At present, human-biting catches are used as the most reliable measure of human-vector contact for calculating the entomological inoculation rate, i.e. the number of infective bites received on average by a person over a given time unit. This is probably not the most ideal method in terms of ethics because of the exposure of humans to the risk of contracting vector-transmitted diseases and the increasing number of drug-resistant parasites. Human biting collections are also time intensive, inconvenient, costly and difficult to standardise. Other sampling methods are available. Light-traps set beside a bed net occupied by a human bait, for example, have been widely employed in the collection of vectors of malaria and bancroftian filariasis in Africa (Garrett-Jones & Magayuka, 1975; Maxwell *et al.*, 1990; Mbogo *et al.*, 1993; Mboera *et al.*, 1997a). Although it was thought that CDC light-trap collections can be compared with human biting collections to evaluate how accurately they reflect actual human biting risk, the reliability of this technique is doubtful. For instance, Lines *et al.* (1991) working in Tanzania found that three indoor CDC light-traps collected as many *An. gambiae* and *Cx quinquefasciatus* as two human collectors and Davis *et al.* (1995) in the same country, collected 1.23 times the number of *An. gambiae* with indoor CDC light-traps versus human bait collections. In Kenya, Mbogo *et al.* (1993) observed that CDC light-traps underestimated actual biting risk when *An. gambiae* abundance was either high or low. Recently, Costantini *et al.* (1998b) showed that the catch of *An. gambiae* from one indoor CDC light-trap was 1.08 times that from a human landing collection. Moreover, the trapping of *An. gambiae s.s.* and *Cx quinquefasciatus* in an indoor situation using the standard CDC light-trap, has shown variations depending on the position where the trap was placed (Chapter 9). Catch-size and parity rates of host-seeking females for both species differed according to the trap position in relation to the host occupying the bed net, but the factors causing this phenomenon remain to be established. The biting behaviour of *An. gambiae* (De Jong & Knols, 1996) and *Cx quinquefasciatus* (Self *et al.*, 1969; Chapter 3) has been shown to be influenced by human foot which may in part explain higher catches from the feet. Higher mosquito catches in traps at a higher position may be due to convection currents at this position carrying body odours upwards as compared to catches at the level of the body. It can be deduced from these findings that the mosquito behaviour around bed nets is not a random process and the data suggest that increased knowledge on mosquito behaviour may result in improved trap and bed net designs and their use for sampling mosquito populations indoors. Furthermore, it follows that estimates of entomological inoculation rates will be influenced by trap position since in turn this affects the number and age of mosquitoes caught, and parasite rates (which will increase with higher parity rates). The current sampling techniques for anthropophilic mosquitoes in Africa, therefore, vary in efficacy. A standardised trapping device that specifically attracts the human-biting proportion of vector populations is now highly desirable. Such a device would have to be based on the main sensory cues used by vectors to locate human hosts specifically, and it would have to elicit the appropriate differential responses of the main vector species.

The principle of odour-mediated, resource-locating behaviours in mosquitoes has been widely accepted (Takken, 1996). Although many individual aspects of these behaviours are not yet understood, there are good prospects to believe that studies towards some of these processes will open the way for manipulation of the behaviours in mosquito monitoring and control in the future. For example, despite a minor role of carbon dioxide in attracting anthropophilic mosquitoes to their hosts, carbon dioxide baited CDC traps may collect larger numbers of *Cx quinquefasciatus* than most of already identified mosquito kairomones such as octenol and acetone (Chapter 6). Efforts to sample anthropophilic mosquitoes in an outdoor situation have been found to be difficult when using odour baited CDC traps. Costantini *et al.* (1993) have shown that it is possible to attract mosquitoes into a device baited with cues associated with the first stage of host location, i.e. orientation to host odours carried by wind outdoors using an odour-baited entry trap (OBET). The OBET successfully caught similar numbers of *An. gambiae s.l.* as a CDC light-trap next to a human bait under a bed net, and it caught about 33% of an all-night, human-biting catch nearby. To date, there are large numbers of studies showing that baiting traps with whole human odours increases the catch of mosquitoes (Snow, 1970; Costantini *et al.*, 1993, 1996; Knols *et al.*, 1998; Chapters 4 & 5). Further, the addition of carbon dioxide and octenol (see Chapter 2) increased the catches of mosquitoes to light traps in various parts of the world. However, it is not clear what precise behavioural effects the traps exert on an approaching host-seeking mosquito. Thus the development of improved sampling system based on an improved understanding of host-oriented behaviour is urgently required.

The screening of human odours for behavioural activity in the laboratory (Cork & Park, 1995; Braks *et al.*, 1997) in order to develop odour-baited traps for the Afrotropical anthropophilic vectors of malaria and bancroftian filariasis will require an efficient sampling device for field evaluation of the possible odour candidates. The type of a sampling tool markedly affects the efficiency of a mosquito sampling technique. For instance, carbon dioxide-baited CDC light traps have shown to be inefficient in sampling outdoor population of *An. gambiae* and *An. funestus* under field conditions of Tanzania (L.E.G. Mboera & B.G.J. Knols, unpublished data) and Kenya (Linthicum *et al.*, 1985). In this thesis it is shown that the recently developed electric nets (Knols *et al.*, 1998) and CFG traps (Kline, 1999), when baited with carbon dioxide and set outdoors, were remarkably efficient in collecting *An. gambiae s.s.* and *Cx quinquefasciatus* as compared to CDC light traps (Chapter 10). CFG traps baited with worn socks have shown for the first time that skin residues can be used to attract mosquitoes to a sampling device in both Tanzania (Chapter 6) and the USA (Kline, 1999). Carbon dioxide-baited electric nets and CFG traps can thus be used sample host-seeking *An. gambiae* and *Cx quinquefasciatus* in an outdoor situation. There are, therefore, prospects that odour-baited electric nets and CFG may provide efficient tools for sampling outdoor populations of these two Afrotropical anthropophilic mosquito species in the future. Previous studies have also indicated that the sampling efficiency of electric nets averaged 41.8% (Knols *et al.*, 1998), similar to that reported for tsetse flies by Parker & Brady (1990).

Mosquito traps that incorporate human body odour are environmentally friendly. Moreover, they may provide an alternative approach to mosquito sampling and control. Besides the unavailability of an efficient sampling device, efforts to produce a host odour attractant for the highly anthropophilic species such as the Afrotropical *An. gambiae, An. funestus* and *Cx quinquefasciatus*, may have been hindered by the chemical nature of the compounds associated with human hosts, although a number of short-chain fatty acids identified in sweat samples have been shown to be electrophysiologically active (Bowen, 1995; Knols *et al.*, 1997; J. Meijerink *et al.*, unpublished data). So, future studies on mosquito olfaction might result in the development of new low-tech traps for community use but, equally, could form the basis for new molecular approaches to the control of mosquito-borne diseases.

The identification of oviposition semiochemicals for *Cx quinquefasciatus*, and the consequent development of standardised baits containing a known blend of semiochemicals would also be of considerable benefit to mosquito surveillance programmes. In Chapter 11, oviposition semiochemicals were shown to be of great promise for surveillance and possibly control of *Cx quinquefasciatus*. With the already identified oviposition-modifying substances, perhaps mosquito populations can be sampled and manipulated through regulation of mosquito oviposition. Thus, the synthetic oviposition pheromone and skatole offer an excellent potential for developing population management techniques that could supplement currently available chemical and biological strategies developed for the control of *Cx quinquefasciatus*.

Chapter 12

Future prospects of the use of semiochemicals in surveillance and control of mosquito-borne diseases

The work presented in this thesis has demonstrated clearly that the resource finding process of *Cx quinquefasciatus* is mediated mainly by semiochemicals. Chemicals are involved in mediating a wide range of insect behaviours, from communication between conspecific individuals to the selection of oviposition sites and vertebrate and plant hosts (Cardé & Bell, 1995). Despite the apparently powerful behavioural effects of chemicals, it is generally accepted that the behaviour of mosquitoes is not governed by any one external chemical stimulus, but mediated by a large number of external and internal stimuli. It can be concluded therefore, that *Cx quinquefasciatus* exhibits a high degree of specialisation in both host and oviposition site selection. *Cx quinquefasciatus* shows a strong preference for a human host, and its oviposition site is usually restricted to organically rich water particularly in man-made structures. It is interesting to note that the Afrotropical *Cx quinquefasciatus* is an ovipositional and blood-feeding specialist. It is not surprising then to find that the species is closely associated with man in both its host-seeking and oviposition behaviours.

It is expected that in the near future, semiochemicals will result into the development of a powerful, species-specific synthetic bait. Results presented in this thesis provide additional demonstration of an oviposition pheromone and a habitat-derived semiochemical, skatole, that may be useful in mosquito monitoring programmes. Such compounds could be valuable tools in bancroftian filariasis surveillance programmes, because synthetic oviposition pheromone and skatole could be formulated and deployed in standardised and reproducible doses. A standardised synthetic bait for *Cx quinquefasciatus* would allow the reliability and repeatability of experimental manipulations to be increased and allow more rigorous comparisons between data collected on targets at different sites and between different years. A standardised bait may also help to improve species-specificity and may be capable of being presented in a concentrated form, allowing targets to be baited with the equivalent of several standard host baits. This would increase the efficiency of the sampling system and would be particularly valuable if traps or targets were to be used in any control intervention. Moreover, the development of improved monitoring and sampling systems, based on an improved understanding of host-resource seeking behaviour, will enhance greatly the rational management of disease vectors of man and livestock.

Conclusions

With respect to the specific research objectives (in bold face) the following conclusions can be drawn:

1. To study host-seeking behaviour of *Cx quinquefasciatus* in relation to host preference

- *Cx quinquefasciatus* responds to moisture gradients during its host-locating process (*Chapter 3*).
- *Cx quinquefasciatus* responds to human skin emanations both in the laboratory and field (*Chapters 3 and 6*).
- In the laboratory there was no response of *Cx quinquefasciatus* to carbon dioxide (*Chapter 3*). However, in the field this compound contributed for 25-28% of the number of *Cx quinquefasciatus* that responded to a human host (*Chapters 4 and 5*).
- *Cx quinquefasciatus* responds more to olfactory cues from a human than to those emitted by a calf or goat (*Chapter 4*).
- Host-seeking *Cx quinquefasciatus* respond equally to volatile cues of a calf, a goat and carbon dioxide (*Chapter 4*).

2. To determine the role of specific host odours in the host-seeking behaviour of *Cx quinquefasciatus*, using carbon dioxide, octenol, acetone, butyric acid and human skin emanations.

- *Cx quinquefasciatus* responds more to traps baited with a combination of skin emanations and carbon dioxide than to either of the stimuli alone under field conditions (*Chapter 6*).
- *Cx quinquefasciatus* responds poorly to traps baited with acetone, octenol or butyric acid (*Chapter 6*).

3. To investigate the role of the oviposition pheromone of *Cx quinquefasciatus* in relation to the selection of oviposition sites and the interaction with breeding site associated chemicals in attracting gravid mosquitoes.

- Synthetic oviposition pheromone (acetoxyhexadecanolide) attracts gravid *Cx quinquefasciatus* and *Cx cinereus* to an oviposition site (*Chapter 7*).
- Soakage pit water and grass infusions attract and induce oviposition in *Cx quinquefasciatus*, *Cx cinereus* and *Cx tigripes* (*Chapter 7*).
- Synthetic oviposition pheromone, in the presence of volatiles from soakage pits or grass infusions, acts synergistically in guiding gravid *Cx quinquefasciatus, Cx cinereus* and *Cx tigripes* to an oviposition site (*Chapter 7*).
- Gravid *Cx quinquefasciatus* respond to 3-methylindole (skatole) during the oviposition process (*Chapter 8*).
- The combination of synthetic oviposition pheromone and skatole elicits an increased oviposition response of gravid *Cx quinquefasciatus* (in an additive manner) compared with the effect of pheromone or skatole alone (*Chapter 8*).

4. To evaluate the effects of host-odour and oviposition stimuli on trap entry behaviour in order to develop an odour-baited monitoring system to be used in mosquito surveillance and control programmes.

- Catch-size and parity rate of host-seeking *An. gambiae* and *Cx quinquefasciatus* differ according to the CDC light-trap position in relation to the host occupying the bed net (*Chapter 9*).
- Electric nets and the Counterflow Geometry trap baited with carbon dioxide can be used successfully in sampling outdoor populations of *An. gambiae* and *Cx quinquefasciatus* (*Chapter 10*).
- The Counterflow Geometry trap baited with a combination of synthetic oviposition pheromone and grass infusions can be employed successfully in sampling outdoor populations of gravid *Cx quinquefasciatus* (*Chapter 11*).

List of publications

The chapters presented in this thesis have been or will be published as:

Mboera, L.E.G. & Takken, W. (1997) Carbon dioxide chemotropism in mosquitoes (Diptera: Culicidae) and its potential in vector surveillance and management programmes. *Review of Medical and Veterinary Entomology* **85**, 355-368.

Mboera, L.E.G., Knols, B.G.J., Takken, W. & Huisman, P.W.T. (1998) Olfactory responses of female *Culex quinquefasciatus* Say (Diptera: Culicidae) in a dual-choice olfactometer. *Journal of Vector Ecology* **23**, 107-113.

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Mboera, L.E.G., Knols, B.G.J., Takken, W. & della Torre, A. (1997) The response of *Anopheles gambiae* s.l. and *A. funestus* (Diptera: Culicidae) to tents baited with human odour or carbon dioxide in Tanzania. *Bulletin of Entomological Research* **87**, 173-178.

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Mboera, L.E.G., Mdira, K.Y., Salum, F.M., Takken, W. & Pickett, J.A. (1999) The influence of synthetic oviposition pheromone and volatiles from soakage pits and grass infusions upon oviposition site-selection of *Culex* mosquitoes in Tanzania. *Journal of Chemical Ecology* (in press).

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Curriculum vitae

The author was born in November 21, 1957 in Tanzania. He grew up in Morogoro and Moshi, where he had all his primary school education between 1965 and 1972. He joined Kibaha in 1973 and Mazengo in 1977 for his secondary school and high school education, respectively. Between 1979 and 1980 he worked as a serviceman in the Ministry of Defence and National Service. Thereafter he was employed as a field assistant with the Ministry of Agriculture and stationed in Arusha and later Monduli district. In December 1981 he joined the University of Dar es Salaam and later the Sokoine University of Agriculture for a bachelor degree course in veterinary medicine (BVM). He graduated in 1985 as the best student of the year in veterinary medicine. From December 1985 to September 1992 he worked with the Research and Training Centre of the Ministry of Agriculture, Mpwapwa as a veterinary surgeon and tutor. He obtained his MSc degree and DIC in Applied Entomology at the University of London, Imperial College of Science, Technology and Medicine, UK, in 1990. He left the Ministry of Agriculture to join the National Institute for Medical Research as a Research Scientist in October 1992. He has participated as a facilitator in various national and international courses in malaria and bancroftian filariasis in Africa. He has published 15 papers on disease vectors of human and livestock.