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**OLFACTION IN
MOSQUITO-HOST
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Introduction III: odours for host-finding mosquitoes

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Host odours, together with visual and physical stimuli such as warm and moist convective currents, provide the necessary cues for mosquitoes to locate their hosts, although their relative importance is still debated (Kellogg & Wright 1962, Price et al 1979, Takken 1991). Cork (1996, this volume) has reviewed the relevance of several compounds as attractants (here termed kairomones, *sensu* Shorey 1977) for mosquitoes and other haematophagous Diptera. I will concentrate here on the effects of host odours on mosquito host finding and emphasize how a better knowledge of behaviour would help in the search for a synthetic bait to use in mosquito surveillance and control programmes (Kline 1994a), and in those studies aiming at the genetic manipulation of host preference of vectors of disease (Curtis 1994).

Behaviourally active compounds

One striking characteristic, when one reviews the current literature on odour-mediated mosquito host finding, is that, in spite of their theoretical interest and dramatic practical potential, very few active molecules have been discovered so far, and little is known about their actual mode of action at the physiological and behavioural level. Rather, some broad blends of volatiles obtained from sebum, sweat, blood, breath, or human arm, palm and foot emanations, plus some members of molecular families such as steroids, carboxylic acids and amino acids, have been recognized (with some conflicting conclusions) as potential kairomones (see Takken 1991, de Jong & Knols 1995). Moreover, their ability in eliciting behavioural responses when used without CO₂ is usually several times less potent than that of whole host odour, thus limiting their usefulness in field applications. CO₂, L-lactic acid and 1-octen-3-ol are the only compounds whose recognition as kairomones is at present less controversial. Their effects on behaviour, however, are still poorly known.

CO₂ is the molecule whose effects are best understood, although its specific role has been the subject of discussion (Bar-Zeev et al 1977), with some

proposing that it both activates and 'attracts' mosquitoes (Van Thiel 1947, Brown 1951, Snow 1970), and others suggesting that it merely activates them (Willis 1947, Kellogg & Wright 1962, Daykin et al 1965, Khan & Maibach 1966). Gillies (1980) reviewed the subject and concluded that CO₂ has two distinct actions: first, as an activator eliciting kinesis and optomotor anemotaxis; and second as a 'synergist' in combination with warm moist convective currents at close range, and with odours at a distance from the host. In still air anemotaxis cannot take place, and only the kinetic effect is manifested.

L-Lactic acid has been occasionally reported as 'not attractive' or even as slightly repellent (Brown et al 1951), but observations in different experimental conditions have confirmed its role in host location at the doses normally released by human skin (Smith et al 1970). Alone, it neither activates nor elicits landing and probing behaviour. With CO₂, it increases take off and, in some concentration combinations, flight activity, landing and probing (Eiras & Jepson 1991).

No reports have been published on the behavioural effects of 1-octen-3-ol in mosquitoes. Its role has been mainly inferred from its enhancing effect on trap catches of certain culicid species, but only when used in conjunction with CO₂. Its efficacy on its own is less clear (Kline 1994b).

From a historical perspective, I suggest that part of the confusion and difference of opinion that is found in mosquito odour research (as outlined by Hocking [1963]), and the difficulty with which advances have been made compared to other areas of insect chemical ecology, are due in part to: (a) the loose use of the ambiguous term 'attraction'; (b) the variety of experimental conditions in which odours have been tested without proper recognition of the precise behavioural context examined; (c) the insufficient recognition of the different role that the same chemical may have at different concentrations, and in different behavioural contexts; (d) the underestimation of interspecific differences in behaviour other than the presence of certain general mechanisms; (e) the lack of links to the ecology of each species; and (f) the weak reciprocal feedback between laboratory and field studies.

Kairomones and attractants

Traditionally, debate has arisen as to whether to consider a certain substance an 'attractant' or a 'non-attractant', often based on the comparison of results obtained with different experimental techniques. Kennedy (1977a) warned us of how the term 'attractancy', when used to embrace all locomotory responses that bring a mosquito close to the source of odour, can be a vague and even misleading word. The problem goes beyond an academic dispute on terminology. This term has been used sometimes to describe the aggregative effect of a substance, whatever the underlying mechanisms of aggregation (e.g.

sugar, a non-volatile, is 'attractive' to houseflies). Thus, even compounds that cause activated mosquitoes to stop or slow down in front of the port of an olfactometer, or to increase their rate of turning if in flight, may confusingly be called 'attractants'.

Strictly speaking, attraction should involve only 'drawing from a distance', and it should describe only those substances whose behavioural effect is an oriented response towards the source, i.e. a taxis (Dethier et al 1960). However, the term 'attractant' has sometimes been attached to behavioural responses, such as probing and feeding, which do not involve any orientation towards the host. Such an approach underlies the counterproductive view of attraction as a unitary process (Kennedy 1977a), whereas a more precise description of the behavioural effects elicited by each substance would avoid ambiguities, and clear the field of potential teleological, anthropomorphic interpretations of host location.

Methodological approaches

Several models of olfactometers have been traditionally employed in studies of kairomones. Some of them use odour-laden air currents coming out of closely spaced ports, and repeated counts are made of the mosquitoes approaching the ports within a given distance or of those standing (and in certain instances of those probing) just in front of the port (Willis 1947, Van Thiel 1947). Bos & Laarman (1975) have pointed out that in a confined space, the mere presence of dead mosquitoes in front of one of the ports can bias the distribution of other responding mosquitoes: some sort of mass effect. An improvement to this basic design, as explained by Smith et al (1970), is to add some kind of trapping device at the ports where stimuli are presented; in this way responding mosquitoes are counted only once. Apart from the benefit of avoiding statistical pseudoreplication, this means that the mean response of the population cannot be unduly influenced by the behaviour of only a few individuals responding repeatedly and consistently. Moreover, kinetic effects alone are probably less likely to be effective, although this possibility cannot be ruled out. This design has been employed to demonstrate the 'attractive' effect of L-lactic acid (Acree et al 1968, Smith et al 1970). Nowadays, wind tunnels are more frequently used in studies of these kind of responses (Mayer & James 1969, Omer 1979, Eiras & Jepson 1991, Healy & Copland 1995). Even if they usually allow for several behaviours to be expressed and independently assessed, a detailed dissection of the in-flight behavioural mechanisms is possible only with video techniques (e.g. for tsetse flies, Colvin et al 1989).

Other authors have used either large cages (Brown & Carmichael 1961), rooms (Khan & Maibach 1972) or vertical towers (Khan et al 1966, 1967), with the stimuli presented from below in still air or carried along arising convective currents generated by heat and/or moisture. In these conditions, which

simulate the final short-range approach of mosquitoes to the host, it is possible that those stimuli which intervene during previous phases of the host-finding process, either do not act at all, or may not do so in the same way. Directional cues are provided only by convective currents or, possibly, by odour concentration gradients (but see Kennedy [1977b] for a discussion on chemotaxis in flying insects).

Comparisons between different types of olfactometers are therefore meaningful only if it is kept in mind that, usually, completely different behaviours are measured, and sometimes different behavioural contexts are examined. Several key questions need to be addressed each time. What is being measured under the given experimental conditions? Which behavioural repertoires can be potentially expressed in these conditions, and which sequences are actually elicited? How are the stimuli presented, and how does this affect the specific behavioural responses?

Interspecific behavioural differences

Our knowledge of odour-mediated mosquito host location is even more incomplete than it appears because most of the laboratory studies have been on a single species, *Aedes aegypti*. The reasons for this are mainly the ease with which this species is reared in the laboratory, its diurnal activity and the traditional expertise of North American investigators (the most active in this particular field of medical entomology) on yellow fever and dengue vectors. Much less is known about the odour-mediated behaviour of other groups of Culicidae, such as the anophelines, although field studies usually show there is high interspecific variability in the response of mosquitoes to the chemicals tested (Kline et al 1990, Kline 1994b).

Given the importance of the malaria problem in the world (Curtis 1996, this volume), interest has been growing on the host-finding behaviour of the most powerful malaria vectors in the world, *Anopheles gambiae sensu stricto*, and its sibling, *Anopheles arabiensis*. In contrast to *Ae. aegypti*, these two species are typical night-biters and they actively disperse. Therefore, it would not be surprising to discover differences in the cues they use and how they use them compared to *Aedes* mosquitoes. Costantini et al (1996) have shown differences in the response to CO₂ between two anthropophilic members of the *An. gambiae* complex and more generalist sympatric species. Reeves (1953) long ago associated the CO₂ dose-response activities of three culicine species to their feeding habits. Gillies & Wilkes (1969) demonstrated interspecific differences in the active space of whole host odours. Omer (1979) highlighted differences between *An. arabiensis* and *Culex quinquefasciatus* in their upwind response to CO₂. It is likely that new insights in how mosquitoes use odour cues, and how these cues might modulate their host preferences, will come from such a comparative approach.

Feedback between laboratory and field studies

Relatively few studies have been carried out in the field on the odour-mediated host finding of mosquitoes. Even fewer studies have tried to verify laboratory results (Brown 1951), or to test laboratory-active molecules, and when this has been done, in some instances the attractiveness has not been confirmed (e.g. L-lactic acid, Stryker & Young 1970). Gillies & Wilkes (1969) concluded that *Anopheles melas* start responding to odours at greater distances than to CO₂. Unfortunately, this result did not lead to the search for non-CO₂ odours that might have an activating effect in this species.

For the most part, kairomones such as CO₂ have been employed for the practical scope of increasing the yield of trapping devices, such as light traps (see Service 1993). Unlike tsetse flies, where electric nets paved the way for field studies of kairomones, few specific trapping systems are available to collect mosquitoes mainly or exclusively by means of host odours. Unlighted light traps have been employed with the goal of studying the potential role of chemicals such as 1-octen-3-ol (see Kline 1994b). A more specific trapping device has been recently developed to catch mosquitoes using mainly odour cues, and therefore to study their odour-mediated 'host-seeking' behaviour in the field (Costantini et al 1993). This trap generates air currents against which mosquitoes must fly upwind and actively find their way into a cage, so that it catches negligible numbers without an appropriate odour bait. It is therefore sensitive even with odours that have a weak effect on the trap catch. Furthermore, it generates an odour plume that favours the entry of mosquitoes and, as the substances used to lure the trap are drawn from a tent under which there is a bait, whole odour from different kinds of hosts can be compared.

There are, of course, obvious important limitations with field studies. External, and to a certain extent even experimental, variables cannot be properly controlled and assessed by the experimenter. Responses are usually measured as the number of insects trapped, so they depend on the mode of action of the trap, and how that is perceived by the insect; video recording techniques may help in some of these interpretation difficulties (Griffiths & Brady 1994). Continuous feedback between laboratory and field studies has been certainly one of the reasons for the success of tsetse fly odour development (Colvin & Gibson 1992, Torr 1994).

Concluding remarks

A quantum leap in the discovery of attractants for tsetse flies (Hall et al 1984) and *Cochliomyia hominivorax* (Cork 1994) was the application of gas chromatography linked electrophysiology (Cork et al 1990). Even if their usefulness is still to be explored with mosquitoes (Cork 1996, this volume), it is

likely that the employment of electroantennography will contribute to the identification of active molecules. Certainly, combined physiological-behavioural studies will considerably speed up our knowledge of behaviourally active compounds.

Clearly, there are still wide gaps in our knowledge of odour-mediated mosquito-host interactions. These will be filled only if there is a close interaction between chemists, physiologists, ethologists, geneticists, molecular biologists and entomologists, working both in the laboratory and in the field. The example of how such interactions have worked so successfully in achieving the surveillance and the control of tsetse flies through odour technology (Colvin & Gibson 1992, Torr 1994) should be a challenging model.

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References

- Acree F Jr, Turner RB, Gouck HK, Beroza M, Smith N 1968 L-Lactic acid: a mosquito attractant isolated from humans. *Science* 161:1346-1347
- Bar-Zeev M, Maibach HI, Khan AA 1977 Studies on the attraction of *Aedes aegypti* (Diptera: Culicidae) to man. *J Med Entomol* 14:113-120
- Bos HJ, Laarman JJ 1975 Guinea pig, lysine, cadaverine and estradiol as attractants for the malaria mosquito *Anopheles stephensi*. *Entomol Exp Appl* 18:161-172
- Brown AWA 1951 Studies of the responses of the female *Aedes* mosquito. IV. Field experiments on Canadian species. *Bull Entomol Res* 42:575-582
- Brown AWA, Carmichael AG 1961 Lysine and alanine as mosquito attractants. *J Econ Entomol* 54:317-324
- Brown AWA, Sarkaria DS, Thompson RP 1951 Studies on the responses of the female *Aedes* mosquito. I. The search for attractant vapours. *Bull Entomol Res* 42:105-114
- Colvin J, Gibson G 1992 Host-seeking behavior and management of tsetse. *Annu Rev Entomol* 37:21-40
- Colvin J, Brady J, Gibson G 1989 Visually-guided, upwind turning behaviour of free-flying tsetse flies in odour-laden wind: a wind-tunnel study. *Physiol Entomol* 14:31-39
- Cork A 1994 Identification of electrophysiologically-active compounds for New World screwworm, *Cochliomyia hominivorax*, in larval wound fluid. *Med Vet Entomol* 8:151-159
- Cork A 1996 Olfactory basis of host location by mosquitoes and other haematophagous Diptera. In: *Olfaction and mosquito-host interactions*. Wiley, Chichester (Ciba Found Symp 200) p 71-88

- Cork A, Beevor PS, Gough JE, Hall DR 1990 Gas chromatography linked to electroantennography: a versatile technique for identifying insect semiochemicals. In: McCaffery AR, Wilson ID (eds) Chromatography and isolation of insect hormones and pheromones. Plenum, New York, p 271–280
- Costantini C, Gibson G, Brady J, Merzagora L, Coluzzi M 1993 A new odour-baited trap to collect host-seeking mosquitoes. *Parassitologia* 35:5–9
- Costantini C, Gibson G, Sagnon N'F, della Torre A, Brady J, Coluzzi M 1996 Mosquito responses to carbon dioxide in a West African Sudan savanna village. *Med Vet Entomol* 10, in press
- Curtis CF 1994 The case for malaria control by genetic manipulation of its vectors. *Parasitol Today* 10:371–374
- Curtis CF 1996 Introduction I: an overview of mosquito biology, behaviour and importance. In: *Olfaction in mosquito–host interactions*. Wiley, Chichester (Ciba Found Symp 200) p 3–7
- de Jong R, Knols BGJ 1995 Selection of biting sites on man by two malaria mosquito species. *Experientia* 51:80–84
- Daykin PN, Kellogg FE, Wright RH 1965 Host-finding and repulsion of *Aedes aegypti*. *Can Entomol* 97:239–263
- Dethier VG, Barton Browne L, Smith CN 1960 The designation of chemicals in terms of the responses they elicit from insects. *J Econ Entomol* 53:134–136
- Eiras AE, Jepson PC 1991 Host location by *Aedes aegypti* (Diptera: Culicidae): a wind tunnel study of chemical cues. *Bull Entomol Res* 81:151–160
- Gillies MT 1980 The role of carbon dioxide in host-finding by mosquitoes (Diptera: Culicidae): a review. *Bull Entomol Res* 70:525–532
- Gillies MT, Wilkes TJ 1969 A comparison of the range of attraction of animal baits and of carbon dioxide for some West African mosquitoes. *Bull Entomol Res* 59:441–456
- Griffiths N, Brady J 1994 Analysis of the components of 'electric nets' that affect their sampling efficiency for tsetse flies (Diptera: Glossinidae). *Bull Entomol Res* 84:325–330
- Hall DR, Beevor PS, Cork A, Nesbitt B, Vale GA 1984 1-Octen-3-ol: a potent olfactory stimulant and attractant for tsetse isolated from cattle odours. *Insect Sci Appl* 5:335–339
- Healy TP, Copland MJW 1995 Activation of *Anopheles gambiae* mosquitoes by carbon dioxide and human breath. *Med Vet Entomol* 9:331–336
- Hocking B 1963 The use of attractants and repellents in vector control. *Bull WHO (suppl)* 29:121–126
- Kellogg FE, Wright RH 1962 The guidance of flying insects. V. Mosquito attraction. *Can Entomol* 94:1009–1016
- Kennedy JS 1977a Behaviorally discriminating assays of attractants and repellents. In: Shorey HH, McKelvey JJ Jr (eds) *Chemical control of insect behavior. Theory and application*. Wiley, New York, p 215–229
- Kennedy JS 1977b Olfactory responses to distant plants and other odor sources. In: Shorey HH, McKelvey JJ Jr (eds) *Chemical control of insect behavior. Theory and application*. Wiley, New York, p 67–91
- Khan AA, Maibach HI 1966 Quantitation of effect of several stimuli on landing and probing by *Aedes aegypti*. *J Econ Entomol* 59:902–905
- Khan AA, Maibach HI 1972 Effect of human breath on mosquito attraction to man. *Mosq News* 32:11–15
- Khan AA, Maibach HI, Strauss WG, Fenley WR 1966 Quantitation of several stimuli on the approach of *Aedes aegypti*. *J Econ Entomol* 59:690–694

- Khan AA, Strauss WG, Maibach HI, Fenley WR 1967 Comparison of the attractiveness of the human palm and other stimuli to the yellow-fever mosquito. *J Econ Entomol* 60:318–320
- Kline DL 1994a Introduction to symposium on attractants for mosquito surveillance and control. *J Am Mosq Control Assoc* 10:253–257
- Kline DL 1994b Olfactory attractants for mosquito surveillance and control: 1-octen-3-ol. *J Am Mosq Control Assoc* 10:280–287
- Kline DL, Takken W, Wood JR, Carlson DA 1990 Field studies on the potential of butanone, carbon dioxide, honey extract, 1-octen-3-ol, L-lactic acid and phenols as attractants for mosquitoes. *Med Vet Entomol* 4:383–391
- Mayer MS, James JD 1969 Attraction of *Aedes aegypti* (L.): responses to human arms, carbon dioxide and air currents in a new type of olfactometer. *Bull Entomol Res* 58:629–642
- Omer SM 1979 Responses of females of *Anopheles arabiensis* and *Culex pipiens fatigans* to air currents, carbon dioxide and human hands in a flight-tunnel. *Entomol Exp Appl* 26:142–151
- Price GD, Smith N, Carlson DA 1979 The attraction of female mosquitoes (*Anopheles quadrimaculatus* Say) to stored human emanations in conjunction with adjusted levels of relative humidity, temperature, and carbon dioxide. *J Chem Ecol* 5:383–395
- Reeves WC 1953 Quantitative field studies on a carbon dioxide chemotropism of mosquitoes. *Am J Trop Med Hyg* 2:325–331
- Service MW 1993 *Mosquito ecology: field sampling methods*, 2nd edn. Elsevier Science, New York
- Shorey HH 1977 Interaction of insects with their chemical environment. In: Shorey HH, McKelvey JJ Jr (eds) *Chemical control of insect behavior. Theory and application*. Wiley, New York, p 1–5
- Smith CN, Smith N, Gouck HK et al 1970 L-Lactic acid as a factor in the attraction of *Aedes aegypti* (Diptera: Culicidae) to human hosts. *Ann Entomol Soc Am* 63:760–770
- Snow WF 1970 The effect of a reduction in expired carbon dioxide on the attractiveness of human subjects to mosquitoes. *Bull Entomol Res* 60:43–48
- Stryker RG, Young WW 1970 Effectiveness of carbon dioxide and L(+) lactic acid in mosquito light traps with and without light. *Mosq News* 30:388–393
- Takken W 1991 The role of olfaction in host-seeking of mosquitoes: a review. *Insect Sci Appl* 12:287–295
- Torr SJ 1994 The tsetse (Diptera: Glossinidae) story: implications for mosquitoes. *J Am Mosq Control Assoc* 10:258–265
- Van Thiel PH 1947 Attraction exercée sur *Anopheles maculipennis atroparvus* par l'acide carbonique dans un olfactomètre. *Acta Trop* 4:10–20
- Willis ER 1947 The olfactory responses of female mosquitoes. *J Econ Entomol* 40:769–778

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