Mosquito behavioural aspects of vector-human interactions in the Anopheles gambiae complex

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Abstract. The behaviour of two of the most anthropophilic malaria vectors in the world, Anopheles gambiae Giles and An. arabiensis Patton, is revisited with respect to recent studies on their host preferences and the chemical ecology of host-seeking. Issues are discussed in relation to the ways anthropophily may have arisen in the complex, and the opportunities the study of olfaction and host-seeking behaviour offers to malaria control in Africa.

Key words: Anopheles arabiensis, Anopheles gambiae, host preferences, anthropophily, host-seeking behaviour, olfaction, semiochemicals, carbon dioxide, malaria.

To be vectors of malaria, anopheline mosquitoes are expected to bite humans not sporadically, i.e. they have to be characterised by intrinsic behavioural patterns determining their association with both the human environment and the human host (Gillies, 1988). Although easier to be bitten because of their almost hairless skin, humans actually constitute a rather difficult target for blood feeding arthropods, mainly because of cultural adaptations enhancing various host defence mechanisms. For example, to reach humans when they sleep indoors requires the development of the capacity to penetrate inside a room and bite humans there, i.e. endophagy, a specific behavioural trait never evolved in some haematophagous Diptera such as Tabanidae, Glossinidae and Simuliidae, though typically present in many malaria vector mosquitoes. Their anthropophily is likely to be the product of environmental conditions involving selection pressures leading to a reorganization of the genome for the expression of the complex ethophysiological traits needed to exploit the new vertebrate host. The behaviour of two of the most anthropophilic vectors in the world Anopheles gambiae Giles and Anopheles arabiensis Patton, is here revisited with respect to recent studies on their host preferences and the chemical ecology of host seeking. Issues are discussed in relation to the ways anthropophily may have arisen in the An. gambiae complex, and the opportunities the study of olfaction and host-seeking behaviour offers to malaria control in Africa.

Feeding habits of Afrotropical malaria vectors

The study of the feeding habits of the main Afrotropical malaria vectors throughout their distribution has been traditionally based on the identification of the blood meal origin of field-collected mosquitoes, expressing the proportion of feeds off humans in the form of the Human Blood Index (HBI) (Garrett-Jones, 1964). The main shortcoming of this type of analysis lies in the difficulty of obtaining unbiased samples; nevertheless, the HBI can be of great help in the evaluation of the relative degree of contact between the available hosts and the vectors. The evaluation of the degree of anthropophily based solely on the HBI, however, is contingent upon the specific conditions in which samples are taken, i.e. extrapolation to different conditions is strictly not possible. This is because the HBI is a measure of the mosquito final host selection, which is the endpoint of a complex series of behavioural responses to internal and external stimuli, as they are modulated by chance and the environment (cf. below). Keeping in mind these limitations when gauging results from such kind of data, a relatively coherent picture of the feeding habits of An. gambiae and An. arabiensis has emerged so far.

An. gambiae is highly anthropophilic throughout its distribution, whereas the host selection of An. arabiensis is more difficult to generalise. Depending on the availability of humans relative to alternative hosts, the HBI of this species is generally high, but in villages where cattle are abundant this proportion can drop in favour of bovid feeds (Coluzzi et al., 1979; Gillies and Coetzee, 1987). Although one explanation would be that An. arabiensis is simply a more opportunistic feeder, its feeding pattern is not always proportional to host abundance. East African populations are generally less anthropophilic than West African ones, while in Madagascar mainly zoophilic populations of An. arabiensis are known to occur independently of the human-bovid ratio (Ralisoa Randrianasolo and Coluzzi, 1987).
In the other four members of the complex, zoophilic gradually increases from the mineral-water species Anopheles bwamba White to the two salt-water species Anopheles melas Theobald and Anopheles merus Dsnitz, eventually peaking in the two allopatric taxa of Anopheles quadriannulatus Theobald, both of whom are completely zoophilic, and are considered the most primitive members of the complex on ecological (White, 1974), and cyto-genetical (Coluzzi et al., 1979) grounds. One way to understand more precisely anthropophily is to address the question of what are the behavioural mechanisms leading mosquitoes specifically towards humans and what physiological and genetic determinants control them.

Mosquito host-seeking behaviour

The process by which mosquitoes arrive at a host is composed of a series of responses to internal and external stimuli, in a chain of events whose outcome is an increased probability of encountering and contacting the host. Generally, the first host cues to reach a mosquito are volatile chemicals emanating from the skin, breath and waste products of a potential host (Takken, 1991), carried by air currents. The probability that the mosquito responds to these cues and the intensity of its response depend on the strength of the host-derived stimuli, of the competing external stimuli (e.g. odours from other sources, prohibitive wind speeds, etc.), its internal state (e.g. circadian phase, gonotrophic and nutritional status, etc.), and its genotype (i.e. the genetic component of the responsiveness to given stimuli). Optomotor anemotaxis (Kennedy, 1939) in response to host odours and other odour-mediated responses (e.g. klino- and orthokinesis) can bring the mosquito nearer the host, thereby increasing the availability of additional host stimuli such as visual cues, temperature and humidity gradients, convective currents, volatile and tactile chemical cues, which may induce the mosquito to land on the host. Finally, once on the host, probing and biting are affected and initiated by the quality and quantity of stimuli such as heat and phagostimulants.

This complex behavioural sequence, however, is not a rigid chain of fixed action patterns released by key stimuli early on in the sequence and thereby expressed unmodified in a stereotyped way, but it is rather a flexible continuum of responses modulated by the interaction and integration in the CNS of internal and external components as they are experienced by the mosquito. Accordingly, it is somewhat useful as proposed by Gillies and Wilkes (1969) to classify arbitrarily the approaching flight towards the host in a long-range and a short-range phase, on the basis of the sequence of additional cues available to the flying mosquito as they cumulate on its flight path (Fig. 1). In the real world, the situation is certainly much more complex than what the representation of Figure 1 can help to visualise (Murlis and Jones, 1981; Murlis et al., 1992). For example, swinging winds cause odour plumes to snake extensively in certain habitats (Brady et al., 1990), and air turbulence breaks down the plume in small eddies that confer it structural properties exploited by flying insects to locate the odour source (Mafra-Neto and Carde, 1995). Nevertheless, this classification can be helpful if we consider the contrasting nature of the information that host cues should convey to the approaching mosquito. On one hand, semiochemicals acting during the long-range phase must set the mosquito ‘up and moving’, i.e. they must have a general positive kinetic effect, whereas once the mosquito is close enough to their source, they should decelerate it and ultimately stop it after landing on the host. It is possible, therefore, that the same semiochemicals may elicit even contrasting responses with respect to the background in which they operate, i.e. according to the presence of other cues mosquitoes exploit to gauge their position relative to that of the host. Indeed, in the natural situation it is the integration from all sensorial inputs that determines any behavioural response (Harris and Foster, 1995).

Gillies and Wilkes (1969) further distinguished between middle- and long-range phase depending on the limits of the carbon dioxide active space. This distinction bears some interest because carbon dioxide plays as role as activator of the host-seeking process, i.e. it elicits take-off or sustained flight (Gillies, 1980), and it probably has other orientating effects on the mosquito flight behaviour. From a series of field experiments (Gillies and Wilkes, 1974 and references therein), these authors inferred the existence of differences among species in the ability to initiate their flight towards the host while out of the carbon dioxide active space. The active space of
carbon dioxide is limited by the relatively high ambient background levels of this molecule (c. 300 ppm) that mosquitoes must discriminate from. Indeed mosquitoes are well adapted to detect changes in carbon dioxide concentrations as low as 50 ppm above ambient (Bowen, 1991), although the behavioural threshold for activation in *An. gambiae* has been assessed in a wind tunnel to be slightly higher (100 ppm) (Healy and Copland, 1995). *An. melas* initiated to converge towards one or two calves, or several bird species at a distance greater than when their carbon dioxide equivalent was employed (Gillies and Wilkes, 1969). Although the physical properties of the carbon dioxide plume might be different when released from breathing animals or from a gas cylinder, it is possible that activation may take place for species as *An. melas* in the long-range phase (*sensu* Gillies) by modulation of compounds other than carbon dioxide. As carbon dioxide is a universal kairomone released by vertebrates with respiration, such observation is relevant to the early expression of host preferences as further examined below.

**Measurement of host preferences**

Host preferences could be expressed at any stage of the host-seeking sequence. Given the temporal and energetic costs of host-seeking, and the risks involved in landing on the ‘wrong’ host (Randolph et al., 1992), it is likely that natural selection promotes their earliest expression. According to the specific sensorial capabilities and the use that each species makes of the available semiochemicals, the first contact with host cues is in most cases within the long-range phase. The probability of responding to such a first contact clearly affects the outcome of the mosquito’s quest for the host, and it is therefore the first possible state for the expression of host preferences.

For species as *An. gambiae* showing a ‘patrolling’ or ‘ranging’ flight strategy increasing the chances of encountering host cues, the usual response when first contacting host odours is positive anemotaxis. Activation and positive anemotaxis in response to host odours, therefore, give us the opportunity to measure host preferences at their earliest stage of expression. With this aim in mind, a trap blowing out host odours, thus exploiting anemotactic behaviour to guide mosquitoes towards its entrance has been developed: this is the odour-baited entry trap, or OBET (Costantini et al., 1993; Fig. 2). The concomitant release of odours from alternative hosts by two OBETs put side-by-side offers the possibility to have a sort of field olfactometer. This set-up may increase the experimental variance because of the varying environmental conditions of the tests, but it has the great advantage of avoiding the manipulation of laboratory mosquito strains that generally interferes with the expression of ‘normal’ behaviour and represents an imponderable and usually highly misleading source of experimental variation. Using this system, Costantini et al. (1998) found that in

![Fig. 2. The odour-baited entry trap (OBET). Above: structure of the trap, consisting of a metal frame cm 40x40x60, covered with netting and transparent plastic; entry of the trap on the left and, on the right, the plastic tube which links the trap to the source of odours via inflated ‘lay-flat’ polyethylene tubing. Centre: the OBET operated outdoors showing the inflated ‘lay-flat’ polyethylene tubing connecting the host-holding tent (on the right) to the trap. Below: measuring the speed of the air current from the entrance of the OBET.](image)

West Africa *An. gambiae* 'chose' human over calf odours with more than 20:1 odds. Such response was so precise that practically no *An. gambiae* 'chose' calf odours, almost all mosquitoes of the *An.


gambiae complex collected in the calf-baited trap being An. arabiensis. With the same set-up, the choice was in favour of human odours against an equivalent amount of carbon dioxide with 2:1 odds (Costantini et al., 1996). Interestingly, when cattle waste products were added to the human odour stream, a significant reduction in the response was apparent (Costantini et al., unpublished data).

The implications of these field experiments are at least three-fold: first of all, for species with specialised feeding habits a stream of host odours is sufficient to discriminate their preferred host, in accordance with the original hypothesis. The expression of host preferences appears already in the initial stages of host-seeking. Second, anthropophilic An. gambiae and An. arabiensis respond to bovid odours in a way as to increase their capacity to discriminate human odours against pure carbon dioxide. In fact, under the hypothesis that An. gambiae and An. arabiensis do not respond to calf odours, one would expect to find similar odds in both of the above choice experiments, and no reduction in response to a mixed human-bovid odour stream. The significance of this phenomenon is further discussed below. Third, from the practical point of view of the medical entomologist, choice tests with two OBETs placed side-by-side represent to date the most objective way we can use to measure host odour preferences in the field.

Responses to carbon dioxide

Any mosquito has the potential of being attracted to, and ultimately feeding on, a wide range of hosts by responding to carbon dioxide, a universal kairomone released by vertebrates. It is generally assumed that for species with highly specialised feeding habits, the preference for certain hosts derives instead from responses to more specific kairomones, or other host-specific cues. Differences in the relative contribution of carbon dioxide to the overall ‘attractiveness’ of a human have been related to the feeding behaviour of several species. Mosquitoes having more catholic habits, as Mansonia uniformis or Anopheles pharoensis, rely proportionally more on carbon dioxide than those species with a narrower host range as An. gambiae or An. arabiensis (Costantini et al., 1996). Such effect was apparent even at a finer level of detail, when comparing the proportional contribution of carbon dioxide in the human ‘attractiveness’ for An. gambiae and its more plastic sibling An. arabiensis (Costantini et al., 1996), or between the latter and An. quadrimaculatus (Dekker and Takken, 1998). The hypothesis that carbon dioxide does not play a role in modulating host preferences of specialist anophelines was independently supported by the comparison of the ‘attractiveness’ of a human against that of a calf by standardizing their carbon dioxide outputs to the same concentration in OBET choice-tests (Costantini et al., 1998). In this experimental situation, the host preferences of species as An. gambiae, An. arabiensis, or Anopheles rufipes were still clearly expressed. Human-specific compounds would appear, therefore, as the key factor in determining anthropophily. There is some evidence, however, that this might not be the whole truth.

Towards the identification of human kairomones

The blend of volatiles released by humans with their metabolism is extremely complex, with more than 300-400 compounds identified to date (Sastry et al., 1980). The sensorial capacities of An. gambiae are quite broad, as this species can respond at the peripheral receptor level to a wide range of compounds, many of which are not human-specific. Cork and Park (1996) have measured the electroantennogram (EAG) response of An. gambiae to a series of saturated carboxylic acids found in sweat having an increasing number of carbon atoms in the molecular chain. They found a significant response above that of the blank solvent for most acids, up to the C9 chain length. Similarly, a synthetic mixture of 12 human-aspecific aliphatic acids, by-product of the metabolism of bacteria found in Limburger cheese elicited significant responses in an olfactometer (Knols et al., 1997). Moreover, this anthropophilic species showed EAG dose-dependent responses to 4-methylphenol and 1-octen-3-ol (Cork and Park, 1996), two compounds found, among others, in ox urine and breath, respectively. These compounds are among the constituents of tsetse lures mimicking ox odour (Vale, 1993).

An. gambiae is capable to respond at the peripheral receptor and behavioural level to human-specific odours as well. Two unsaturated carboxylic acids (3-methyl-2-hexenoic acid and 7-octenoic acid) specifically present in human sweat, and related to bacterial activity at the skin secretory sites in the axillae, elicit EAG responses greater than those from the commercial repellent N,N-diethyl-3-methylbenzamidine (DEET) (Costantini et al., 1999). In OBET dual-choice field tests, the two acids combined reduced the response of a mixed An. arabiensis-An. gambiae population to whole human odour or to carbon dioxide. When presented individually, however, one of the acids had some attractive efficacy when tested against carbon dioxide, whereas the other one did not have any effect. Similar results were confirmed in wind tunnel studies for laboratory strains of An. gambiae from East and West Africa.

Again, the lessons to be learned are at least three-fold. First, interactions among semiochemicals can be complex and result in variable responses according to the way and dose they are presented, as inferred by the differences obtained when the two acids were presented individually or in combination. Second, the host odour profile may be a push-pull system of attractive and repellent components, as found in tsetses where certain human odour fractions act as repellents, especially so for Glossina palidipes females (Vale, 1974). Similarly, it is known that higher doses of 2-methoxyphenol and pentanoic acid – two compounds found in ox odour – reduce
the catch of G. pallidipes in ox baited traps. This phenomenon might modulate the contrasting dynamics that host cues need to elicit with respect to the position of the mosquito relative to the cues’ source. Third, the skin bacterial activity appears as an important component in the release of host specific and aspecific semiochemicals. This introduces another level of complexity, as temporal changes in environmental and physiological conditions inducing modifications of the skin flora may affect the ‘attractiveness’ of the same individual host.

Variation in human attractiveness

From what outlined above, it is clear that the identification of behaviourally-relevant host odours and the understanding of the responses they elicit are not simple tasks. Theoretically, we could learn a lot by studying the variability among individuals in their degree of attractiveness. It is the layman knowledge that individuals can differ in the degree they are bitten by mosquitoes and other haematophagous insects. Indeed, such belief has been confirmed under disparate experimental conditions, both in the laboratory and in the field. Individual ‘attractiveness’ has been related to sex, age (Maibach et al., 1966a; Carnevale et al., 1978), body mass (Port et al., 1980), properties of the skin (Thornton et al., 1976), and patogenic conditions (Maibach et al., 1966b; Akuamo, 1971). Given the fundamental role of olfaction in mosquito host-seeking, it is possible that the individual odour profile may contribute to such variability. By comparing the odour profile of highly attractive to that of less attractive individuals, one could pick up the molecules making up the difference, and concentrate further research efforts on these.

Such an approach, however, must take into account the experimental context in which differences in ‘attractiveness’ are evaluated and put them in relation to the behaviour of the mosquito in the natural situation (Costantini, 1996b). For example, under field conditions it can be difficult to ascertain individual differences due to kairomones other than carbon dioxide, as the latter accounts for a non-negligible proportion of the attractiveness of a human even for strongly anthropophilic species as An. gambiæ. The response of An. gambiæ to carbon dioxide is steeply dose-dependent (Costantini et al., 1996), so that humans with different carbon dioxide outputs can attract variable numbers of mosquitoes, thereby confounding differences due to other semiochemicals that individually contribute to a lower proportion of the host overall attractiveness. Moreover, one has to be careful when dealing with different phases of the host-seeking sequence, as more attractive individuals in the long-range phase may not necessarily be also more attractive during the short-range phase (Fig. 3), as found for An. gambiæ s.l. (Costantini, 1996a) and in simulids (Schofield and Sutcliffe, 1996; 1997). Possibly, the role played by semiochemicals released by the same individual may change along the host-seeking sequence.

Fig. 3. Ranking the ‘attractiveness’ of twelve adult men (identified by uppercase letters) on the basis of the mean number of An. gambiæ s.l. collected in OBETs baited with their odours (black bars on the left) or landing on their legs (grey bars on the right). Longer bars denote higher ‘attractiveness’. The men were allocated to three teams of four individuals each. The between-men variability in carbon dioxide output during the OBET collections was left unchanged (upper diagram) or standardised to the same concentration (0.15%) for each man (lower diagram).

Evolution of An. gambiæ anthropophily

The two behavioural extremes in the An. gambiæ complex range from the complete zoophily and exophillyphy of An. quadriannulatus to the marked anthropophily and endophily of An. gambiæ. The latter behavioural traits presumably emerged only during the last ten thousand years, since conditions for their selection were highly improbable in pre-Neolithic human environments without settled communities and compact villages.

The adaptation to the human environment and to biting on humans can be hypothesised following three (not mutually exclusive) main scenarios, as suggested by Coluzzi (1997): (i) progressive phyletic adjustment to human biting from ancestral primate-feeding habits; (ii) association to human dwellings as a source of ‘protective’ micro-environments allowing an increase in fitness by avoidance of unfavourable circadian and/or seasonal climatic periods; (iii) association to those humans acting as the producers of breeding sites, thus exploited by mosquitoes both as hosts and as a guide for breeding opportunities.

Of the three working hypotheses reported above, the shift from primitive primatophilic habits appears the least likely in the case of the An. gambiæ complex. The anthropophily of An. gambiæ and An. arabiensist appears to be a special adaptation from
primitive feeding on large ungulates, as few *Anopheles* and no species of the group *Pyrethrophorus* are known to take their blood meal predominantly off primates. This is also suggested by the feeding habits of the other, closely-related, siblings of the complex. Large ungulates, bovids in particular, constitute the main source of blood for all of them. Further hints come from the consideration of the behavioural plasticity of *An. arabiensis* under particular environmental conditions, and the peculiar zoophily of its malagasyan populations, both of whom are probably a legacy to such a primitive condition.

Considering that anthropophily in *An. gambiae* and *An. arabiensis* stems from their specific capacity to find humans when searching for a host, the high degree of endophily of these two species, is probably not the proximate cause but rather a consequence of their adaptation to feeding on humans. We have to suppose, therefore, that the shift from zoophily, especially for *An. gambiae*, must have been a dramatic event under the control of major selective forces which are likely to operate when the process is one of intimate adaptation to humans as ecological markers of the larval habitat (i.e. the third working hypothesis).

When both animal and human hosts are associated to the mosquito breeding sites, as it is generally observed in the savanna pastoral/agricultural communities, the expected evolutionary trend should favour a flexible genetic system responsive to selection for the prevalent host, thereby originating wide local variations. This appears to be the case in point for *An. arabiensis* but not for *An. gambiae*, as the latter taxon is uniformly characterised all over Africa by endophily combined with the highest degree of anthropophily, as if humans constituted an exclusive target during its speciation process.

Precise inferences on the evolution of the edophilic and anthropophilic behavioural traits are limited by our insufficient knowledge of their genetic determinants. However, further insight into the hypothetical evolutionary process of *An. gambiae* may be gained considering its larval bionomics and the results from polytene chromosome studies (Coluzzi et al., 1979, 1985). Permanent, swamp-like or shaded pools are avoided by *An. gambiae*, which typically breed in temporary fresh-water pools well exposed to sunlight and with bare-soil edges, mostly human-made and dependent on rainfall or on emergence by filtration of the superficial water table. Dependence from human activities is more important in humid rather than dry savannahs, peaking in the tropical rain forest where the high amount of rainfall provides to *An. gambiae* enormous breeding potential, provided that agricultural communities destroy the thick vegetation cover and the very abundant layer of humus of the forest floor. The focus on the rain forest is brought in again by the analysis of the polytene chromosome complement of the members of the *An. gambiae* complex. Among various rearrangements of the chromosome-2 of *An gambiae*, the standard is the one prevailing in all forest populations, and this arrangement is also the ancestral one, being shared with or directly related to the most primitive members of the complex (Coluzzi et al., 1985).

Speciation in the tropical rain forest actually satisfies the conditions needed for the evolution of the highly specific anthropophily typical of this taxon. The agricultural communities were the producers of the unique breeding sites available to *An. gambiae* in the tropical rain forest environment, and, consequently, the most reliable biological indicators for the new ecological niche created by deforestation. Any loss of contact with humans by the ancestral adult females of *An. gambiae* would have resulted in a high risk of extinction due to their inability to reproduce effectively in the uncleared rain forest. Thus, breeding in rare and scattered spots of cleared forest would imply continuous selection for anthropophily and probable involvement in flush and crash cycles (Powell, 1978) that may explain the origin of the major malaria vector in the world and its highly human-specific biting behaviour (Coluzzi, 1982, 1997; Coluzzi et al., 1985).

**Perspectives in malaria control**

The study of olfaction and its role in mosquito host preferences and host-seeking behaviour, has the potential for new developments in the control of vectors and vector-borne diseases. First of all, there has been some interest in developing odour-baited traps to improve the quality and quantity of samples for eco-epidemiological purposes, e.g. to monitor vector inoculation rates in control programmes without recurring to the ethically-doubtful and impractical collections on human baits (Costantini et al., 1993). Moreover, semiochemicals may be employed as lures in mass-trapping devices or decoys deviating attacking mosquitoes away from humans. The usefulness of mass-trapping devices for malaria control purposes as compared to the widespread use of insecticide-impregnated bednets is yet to demonstrate. However, if the ‘attractiveness’ of lures to the relevant *Anopheles* vector species will approach or exceed that of humans, and by careful deployment in sufficient numbers (Day and Sjögren, 1994), they might be a valuable additional tool in integrated vector management efforts as they have been in the control of tsetse flies in certain parts of Africa (Colvin and Gibson, 1992).

As its promotion will arguably carry on in the near future, malaria control will more and more rely on the community-wide use of bednets and curtains impregnated with pyrethroids whose excito-repellent and deterrent properties appear to approach in importance their insecticidal effects in the reduction of disease transmission (Curtis et al., 1991). Because of the modifications they can effect, insecticides should be evaluated by behavioural assays that are relevant to the performance of the vector under natural conditions. Simple and elegant efforts in this direction have been achieved by Hossain and Curtis.
(1991), and Miller and Gibson (1994). Their laboratory assays can assist in the identification of the most appropriate target doses and formulations under varying operational conditions, and they can help to analyse how the possible behavioural effects of resistance genes can affect in a positive or negative way vector control campaigns (Hodjati and Curtis, 1997). Validation of laboratory results under field conditions, however, remains always a necessity.

Another area that should benefit from deeper understanding of olfaction and behaviour, is that concerned with the development of insect repellents. As outlined above, the reduction of human-vector contact is playing a revamped role in malaria control, thus the use of cheap, safe, efficient, persistent, and cosmetically-acceptable repellents could contribute greatly to widen its scope. Since the discovery in the fifties of DEET has set the golden standard, however, no new molecule has overcome its main limitations so far. DEET is less efficient and has a short protection time for important malaria vectors as e.g. An. albimanus and An. gambiae, it is occasionally toxic to some individuals under prolonged and intense use, and cosmetically fastidious. Efficient and persistent plant-derived compounds, as e.g. p-menthane-3,8-diol (Trigg, 1996), can set back some of these shortcomings, especially when it is possible to grow the producing trees or plants in tropical countries without their need to import synthetic compounds paid in hard foreign currency.

One of the reasons for the difficulty in developing ‘good’ repellents is our as yet poor understanding of how repellents work, i.e. their mode of action at the mosquito physiological and behavioural level. Also, there is no clear-cut correlation between molecular structure and repellent properties. Identification of new molecules is usually carried out by preliminary screening of a large number of molecules in standardized rapid assays without a theoretical framework assisting in the selection of the compounds to test. Preliminary screening is usually performed testing individual compounds, while such procedure may well overlook molecules eliciting the proper behavioural effect only when released in combination with other compounds. This was the case in point for the two human-specific carboxylic acids we presented above. We certainly need to know more about the interactions and synergism among different compounds.

It is important to recognize, however, that repellents usually have a relative rather than absolute mode of action, i.e. they are more efficient when host-seeking mosquitoes have alternative ways of expressing their ‘normal’ behaviour on unprotected surfaces (Curtis et al., 1987). Protecting an entire community with repellents, therefore, may not achieve the scope. Intuitively, it seems more promising to intervene on behavioural mechanisms that are more rigidly encoded within the genetic makeup of a species, by e.g. exploiting the host preferences of those mosquitoes with a narrow host range, An. gambiae being the case in point. Masking the normal odour profile of humans by releasing the same compounds An. gambiae use to discriminate the ‘wrong’ hosts, we might sensibly reduce human ‘attractiveness’ to this species, in the same way as some human odour fractions are known to mask the ‘attractiveness’ of cattle to tsetse. This approach, of course, is subject to the same constraints outlined above for commercial repellents, but has the advantage of a rationale seeking to avoid the behavioural plasticity of the target species. However, its interest clearly lies in disease rather than nuisance control.

Transgenic technologies are opening up new ways and hopes for the control of malaria in its African stronghold (Collins, 1994) and, among the targets for transformation ethophysiological characters should be carefully considered, possibly extending the analysis also to behavioural patterns others than those involved in human biting. Incidentally, oviposition behaviour and the regulatory mechanisms of the gonotrophic cycle might prove an even more useful target. Whether the introduction of genes for resistance to infection into vectors will succeed in its ultimate task remains to be verified, though limitations have already been identified (Spilman, 1994). Apart from the inevitable questions about the ethical constraints and the risks involved with any permanent modification of natural vector populations, there is still the possibility for the parasite to evolve ways to escape a single resistance mechanism. Moreover, Anopheles genetically-modified for Plasmodium resistance will still be able to transmit other vector-borne diseases, and contribute to the overall mosquito nuisance, lowering the acceptability and social impact of such control programmes (Curtis, 1994). The introduction of genes for zoophilic into populations of anthropophilic species, therefore, appears in principle as a better option. Such genes could be introgressed from zoophilic species when closely-related taxa with alternative feeding habits can be crossed and back-crossed in the laboratory producing viable hybrid progeny. This is exactly the case for the anthropophilic species of the An. gambiae complex and one of their most zoophilic members, An. quadriannulatus (Pates et al., work in progress). Laboratory introgression of zoophilic by crossing experiments, however, has the possible disadvantage of ‘hitchhiking’ other genes that might lead to undesirable epistatic effects. Use in such crossing programmes of ecologically more successful or more closely-related taxa, as e.g. different populations of An. arabiensis, might prove a better path to follow. The ultimate aim is the precise identification of the genes responsible for anthropophily and the behaviour they control. Such approach could use the same genetic driving mechanisms envisaged for the spread of Plasmodium-resistant Anopheles, thereby outweighing or otherwise balancing some of the potential problems related to the fitness of transgenic vectors.
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