

# No accounting for taste: host preference in malaria vectors

Nora J. Besansky<sup>1</sup>, Catherine A. Hill<sup>2</sup> and Carlo Costantini<sup>3</sup>

<sup>1</sup>Center for Tropical Disease Research and Training, Department of Biological Sciences, University of Notre Dame, Notre Dame, IN 46556, USA

<sup>2</sup>Department of Entomology, Purdue University, West Lafayette, IN 47907, USA

<sup>3</sup>Parasitology Unit, Department of Public Health, University of Rome, La Sapienza, 00185, Rome, Italy

**The ability of a few Afro-tropical *Anopheles* species to target humans as a source of blood and shelter, a feature generally defined as anthropophily, represents a key factor in the recent evolutionary success of these species and in their capacity to transmit malaria. Anthropophily presumably results from specific and coordinated behavioural repertoires, but these have been only superficially described and their genetic basis is unknown. A comparative approach exploiting recent molecular and ethological advances promises to shed light on the genetics of anthropophily, thereby presenting novel targets for vector control.**

Malaria is found throughout tropical regions, which share problems of poverty, lack of affordable healthcare and poor infrastructure. However, the intensity of malaria transmission is not uniform in these regions. Out of the >500 species of anopheline mosquitoes worldwide, only three are involved in the majority of human malaria transmission: *Anopheles gambiae*, *Anopheles arabiensis* and *Anopheles funestus*. The broad ranges of these three species coincide in Africa, which carries ~90% of the global malaria burden. The unique feature that helps explain the intensity of transmission in Africa is the extremely anthropophilic behaviour of the *Anopheles* vectors. Key anthropophilic behaviours include preference for humans as a bloodmeal source, oviposition in water bodies created through anthropogenic environmental modification, and endophily (the tendency to enter and rest inside houses). Such extreme specialization on a single host species, particularly humans, is rare among mosquitoes, most of which feed mainly on mammals and birds [1]. Until now, these anthropophilic repertoires have been only superficially described, and we do not understand the genes and corresponding molecular mechanisms involved in determining such behaviours. The challenge for future research is to define the relationship between genotype and complex phenotype. This information is crucial. First, it will lead to the identification of candidate genes that could serve as targets for behavioural modification of the vector, leading to reduced vector–human contact. Second, it will enable us to understand behaviours that might impact the successful deployment of future control strategies such as population replacement.

Specialization on humans at all stages of the mosquito life cycle is, by definition, a recent evolutionary innovation. Only since the rise of agriculture within the past 10 000 years have human populations burgeoned in association with permanent settlements [2]. Relatively dense and stable agricultural communities offered a reliable blood-meal source, and novel breeding sites were created as a result of deforestation, irrigation and excavation. The recent emergence of anthropophily is also suggested by the fact that *An. gambiae*, *An. arabiensis* and *An. funestus* belong to groups of species whose members are very closely related and recently diverged, yet they differ markedly in host specialization, endophily and oviposition behaviour. It is noteworthy that *Aedes aegypti* and some members of the *Culex pipiens* complex are also anthropophilic species, albeit distant mosquito relatives of *Anopheles*, that are major vectors of dengue and yellow fever, or filariasis and West Nile Virus, respectively. Forthcoming genome projects for these disease vectors will advance investigations into the genetic basis of anthropophilic behaviours. Of the behavioural repertoires that define anthropophily, host preference has received the most attention.

## Genes versus environment

Host preference has been defined as the genetically based tendency to respond to particular host cues [1]. The expression of host preference in Nature is modulated by many variables (e.g. relative host abundance and accessibility). These confounding factors have hindered substantial progress in our understanding of the contribution of the innate component in the process of host selection. Traditional approaches based on the analysis of mosquito bloodmeals or collection rates on alternative hosts [1] do not give reliable information on host preference because they measure the endpoint of the complex interaction between genetic and environmental components. As such, results from these ecological approaches are difficult to extrapolate to differing environmental conditions. Moreover, these techniques often suffer from sampling bias to an unknown extent. It must be recognized, however, that they have been instrumental to the early recognition of the wide array of blood-feeding patterns in mosquitoes. In this respect, a case deserving special mention is the Afro-tropical *An. gambiae* sibling species complex, whose isomorphic members show an impressive diversity of feeding patterns [3,4]. The Afro-tropical *An. gambiae*

Corresponding author: Nora J. Besansky (nbesansk@nd.edu).

species complex is one of the most highly anthropophilic mosquitoes known, whereas *Anopheles quadriannulatus* feeds almost exclusively on animals. *Anopheles arabiensis* is an opportunistic species that feeds preferentially on humans in many parts of Africa, but can be diverted to domestic animals as their density increases. By contrast, *An. arabiensis* feeds preferentially on domestic animals in Madagascar. The remaining species, although considered predominantly zoophilic [5,6], readily bite humans.

### Signal versus noise

Laboratory attempts to study host preference have encountered variable success [7–10]. Constraining factors in the interpretation of results from such studies are the unknown effects of laboratory colonization and maintenance of mosquito strains, and other departures from natural conditions. Field-based experimental approaches appear more promising, but they require appropriate tools and care must be taken to interpret results within the framework of each specific experimental context. Recognition of the importance of olfaction in the host-selection process of mosquitoes has led to the development of an experimental field set-up mimicking that of laboratory olfactometers: mosquito responses to odours from two hosts can be compared with paired odour-baited entry traps (OBETs). The OBET captures mosquitoes when preferences are first expressed in response to host odours. Advantages of this field-based test are elimination of perturbations due to human collectors, removal of confounding visual cues, and minimization of accidental capture of mosquitoes not responding to host cues. Standardization of this or similar kinds of tests can make results easier to interpret across different mosquito populations. Indeed, by using OBET choice tests, it has been possible to compare the host preferences of *An. gambiae* and *An. arabiensis* across a wide geographical area, from Senegal and Burkina Faso [11,12] to Ethiopia (T. Habtewold *et al.*, unpublished) and Madagascar [13]. This is a distinct advantage in light of increasing evidence from field studies that blood-feeding patterns, even within the same species, are not necessarily rigid. In *An. gambiae*, lower rates of anthropophily have been detected in parts of West Africa [14–16] and Madagascar [13]. The extent to which variation in the proportion of human bloodmeals taken by *An. gambiae* and *An. arabiensis* is a result of genetic differences, environmental factors and/or the experimental design is not yet clear. One problem is that the evidence for lower rates of anthropophily comes from disparate geographical locations and techniques that measure different steps in the behavioural sequence of host seeking. The remarkably different host preference in the *An. gambiae* complex, not only between closely related species, but also within *An. gambiae sensu stricto* or *An. arabiensis*, suggests that its genetic underpinning is evolutionarily labile.

### Nature versus nurture

The relatively loud biological ‘noise’ revealed by behavioural studies of host preference helps explain the almost complete lack of progress at understanding its genetic basis. The indications that do exist come from two lines of evidence: polymorphic chromosomal inversions and

selection experiments. First, it has been shown that in *An. arabiensis*, *An. gambiae* and *An. funestus*, carriers of certain chromosomal arrangements are preferentially associated with human habitations and therefore have an increased probability of contact with humans [17–19]. More direct evidence comes from studies with *An. arabiensis* [20] and *An. funestus* [19] that have shown significant associations between certain karyotypes and human blood. Second, rapid shifts in host choice in response to divergent selection pressure were obtained from a wild anthropophilic population of *An. gambiae* from Tanzania [21]. This indicates an underlying polymorphism for host preference and suggests that host preference in this species is controlled by a few genes of large phenotypic effect, or perhaps several genes protected from recombination by an inversion.

Evidence from field studies suggests that host preferences are mediated by differential responses to host odours other than, and in combination with, carbon dioxide, including microflora of human skin and parasite-related olfactory cues [22]. Attractive and repellent components of the host odour profile can induce positive and negative responses, resulting in a complex push–pull system [2]. Significant advances in the description of the molecular arsenal of olfactory receptors are being made (e.g. Refs [23–25]). Detailed knowledge of the behavioural, physiological and molecular aspects of mosquito olfaction are likely to uncover important targets for novel and more effective means of vector control, including new repellents, and attractants useful for mass trapping or diversion to nonhuman hosts.

### An outlook for the future

Although mosquito behaviours such as host preference are influenced by the environment, there is an underlying genetic basis. With the appropriate choice of genetic backgrounds and phenotypic assays, ultimately, it should be possible to understand at the molecular and genetic level how olfactory, visual, taste and sound cues operate to trigger behaviours that directly or indirectly impact vectorial capacity, for example, house entry, indoor resting, choice of oviposition site, host finding and host preference. Some candidate genes involved in gustatory, odorant and visual processes are already known [23–25]; further candidates remain to be discovered through exploratory approaches including quantitative trait loci (QTL) mapping, microarray studies, functional genomics (e.g. RNA interference) and comparative genomics. Comparisons between closely related species that differ strikingly in anthropophilic behaviours, such as *An. gambiae* and *An. quadriannulatus*, or between more-distantly related species that share strongly anthropophilic tendencies, such as *An. gambiae* and *An. funestus*, can provide powerful aids in this discovery process. Much needed research in this neglected area of study will help to unravel the complex genetic basis of behaviours that contribute to vectorial capacity, while revealing new targets for vector control.

### Acknowledgements

Some content of this review is adapted from a more comprehensive treatment of host use in flies, which will appear in K.E. Filchak *et al.* The

ecological genetics of host use in the Diptera. In: *The Evolutionary Biology of Flies*. (Wiegman, B.M. and Yeates, D.K., eds.), Columbia University Press (in press).

## References

- Clements, A.N. (1999) *The Biology of Mosquitoes*, CABI Publishing
- Costantini, C. *et al.* (1999) Mosquito behavioural aspects of vector-human interactions in the *Anopheles gambiae* complex. *Parassitologia* 41, 209–217
- White, G.B. (1974) *Anopheles gambiae* complex and disease transmission in Africa. *Trans. R. Soc. Trop. Med. Hyg.* 68, 278–301
- Gillies, M.T. and Coetzee, M. (1987) *A Supplement to the Anophelinae of Africa South of the Sahara*, Publication no. 55, The South African Institute for Medical Research, Johannesburg, South Africa
- Gillies, M.T. (1967) Experiments on host selection in the *Anopheles gambiae* complex. *Ann. Trop. Med. Parasitol.* 61, 68–75
- Bryan, J.H. *et al.* (1987) Adult behavior of members of the *Anopheles gambiae* complex in The Gambia with special reference to *An. melas* and its chromosomal variants. *Parassitologia* 29, 221–249
- Dekker, T. *et al.* (2002) L-lactic acid: a human-signifying host cue for the anthropophilic mosquito *Anopheles gambiae*. *Med. Vet. Entomol.* 16, 91–98
- Dekker, T. *et al.* (2001) Innate preference for host-odor blends modulates degree of anthropophagy of *Anopheles gambiae sensu lato* (Diptera: Culicidae). *J. Med. Entomol.* 38, 868–871
- Pates, H.V. *et al.* (2001) Unexpected anthropophagic behaviour in *Anopheles quadriannulatus*. *Med. Vet. Entomol.* 15, 293–298
- Pates, H.V. *et al.* (2001) Differential behaviour of *Anopheles gambiae sensu stricto* (Diptera: Culicidae) to human and cow odours in the laboratory. *Bull. Entomol. Res.* 91, 289–296
- Costantini, C. and Diallo, M. (2001) Preliminary lack of evidence for simian odour preferences of savanna populations of *Anopheles gambiae* and other malaria vectors. *Parassitologia* 43, 179–182
- Costantini, C. *et al.* (1998) Odor-mediated host preferences of West African mosquitoes, with particular reference to malaria vectors. *Am. J. Trop. Med. Hyg.* 58, 56–63
- Duchemin, J.B. *et al.* (2001) Zoophily of *Anopheles arabiensis* and *An. gambiae* in Madagascar demonstrated by odour-baited entry traps. *Med. Vet. Entomol.* 15, 50–57
- Diatta, M. *et al.* (1998) Similar feeding preferences of *Anopheles gambiae* and *A. arabiensis* in Senegal. *Trans. R. Soc. Trop. Med. Hyg.* 92, 270–272
- Lemasson, J.J. *et al.* (1997) Comparison of behavior and vector efficiency of *Anopheles gambiae* and *An. arabiensis* (Diptera: Culicidae) in Barkedji, a Sahelian area of Senegal. *J. Med. Entomol.* 34, 396–403
- Sousa, C.A. *et al.* (2001) Dogs as a favored host choice of *Anopheles gambiae sensu stricto* (Diptera: Culicidae) of Sao Tome West Africa. *J. Med. Entomol.* 38, 122–125
- Coluzzi, M. *et al.* (1977) Behavioural divergences between mosquitoes with different inversion karyotypes in polymorphic populations of the *Anopheles gambiae* complex. *Nature* 266, 832–833
- Coluzzi, M. *et al.* (1979) Chromosomal differentiation and adaptation to human environments in the *Anopheles gambiae* complex. *Trans. R. Soc. Trop. Med. Hyg.* 73, 483–497
- Costantini, C. *et al.* (1999) Chromosomal and bionomic heterogeneities suggest incipient speciation in *Anopheles funestus* from Burkina Faso. *Parassitologia* 41, 595–611
- Petrarca, V. and Beier, J.C. (1992) Intraspecific chromosomal polymorphism in the *Anopheles gambiae* complex as a factor affecting malaria transmission in the Kisumu area of Kenya. *Am. J. Trop. Med. Hyg.* 46, 229–237
- Gillies, M.T. (1964) Selection for host preference in *Anopheles gambiae*. *Nature* 203, 852–854
- Takken, W. and Knols, B.G. (1999) Odor-mediated behavior of Afrotropical malaria mosquitoes. *Annu. Rev. Entomol.* 44, 131–157
- Hill, C.A. *et al.* (2002) G protein-coupled receptors in *Anopheles gambiae*. *Science* 298, 176–178
- Merrill, C.E. *et al.* (2002) Visual arrestins in olfactory pathways of *Drosophila* and the malaria vector mosquito *Anopheles gambiae*. *Proc. Natl. Acad. Sci. U. S. A.* 99, 1633–1638
- Biessmann, H. *et al.* (2002) Isolation of cDNA clones encoding putative odourant binding proteins from the antennae of the malaria-transmitting mosquito, *Anopheles gambiae*. *Insect Mol. Biol.* 11, 123–132

1471-4922/\$ - see front matter © 2004 Elsevier Ltd. All rights reserved.  
doi:10.1016/j.pt.2004.03.007

# Problems in speciation in the genus *Blastocystis*

Hisao Yoshikawa<sup>1</sup>, Keiko Morimoto<sup>1</sup>, Zhiliang Wu<sup>2</sup>, Mulkit Singh<sup>3</sup> and Tetsuo Hashimoto<sup>4</sup>

<sup>1</sup>Department of Biological Science, Faculty of Science, Nara Women's University, Kitauoya-Nishimachi, Nara 630-8506, Japan

<sup>2</sup>Department of Parasitology, Gifu University School of Medicine, Tsukasa, Gifu 500-8705, Japan

<sup>3</sup>Department of Microbiology, Faculty of Medicine, National University of Singapore, 5 Science Drive 2, 117597, Singapore

<sup>4</sup>Institute of Biological Sciences, University of Tsukuba, Tsukuba 305-8572, Japan

**As the species *Blastocystis hominis* is thought to target only human hosts, many new species have been proposed for isolates from nonhuman hosts. However, recent molecular studies have revealed zoonotic potential of *Blastocystis* isolates from various animals. On the basis of the phylogenetic analysis of the small-subunit ribosomal RNA genes, we show here that *Blastocystis* isolates from some reptiles and amphibians are closely related to human *B. hominis*, further compounding the difficulties of speciation in the genus *Blastocystis*.**

Ever since *Blastocystis hominis* was first recognized in human stool specimens, many *Blastocystis*-like organisms have been found in a variety of animals, including mammals, birds, reptiles and, rarely, insects. Although *B. hominis* has been accepted as the species for isolates from humans, all other isolates from nonhuman hosts have been reported as either '*B. hominis*', '*Blastocystis* sp.' or given another specific name as a new species. So far, species isolated from mammals (six species), birds (five), reptiles (four) and an amphibian (one) have been proposed as new species based on the differences in host origins, morphology, *in vitro* culture characteristics and/or karyotype [1–7]. However, it is now well recognized that

Corresponding author: Hisao Yoshikawa (h.yoshikawa@cc.nara-wu.ac.jp).