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Zoophily of *Anopheles arabiensis* and *An. gambiae* in Madagascar demonstrated by odour-baited entry traps

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Abstract. In Madagascar we used odour-baited entry traps (OBETs) for host choice tests of wild female anopheline mosquitoes (Diptera: Culicidae) at representative localities on the East and West sides of the island (villages Fenoarivo and Tsararano, respectively) and at the southern margin of the central plateau (Zazafotsy village, 800 m altitude). No insecticide house-spraying operations have been undertaken at these villages. Odours from a man and a calf of similar mass, concealed in different tents, were drawn by fans into separate OBETs set side by side. Traps were alternated to compensate for position effects, and different pairs of individual baits were employed for successive replicates. Totals of 266 *An. funestus* Giles *sensu stricto* and 362 *An. gambiae* Giles *sensu lato* were collected in 48 trap nights during March–June 1999. For each mosquito species the 'index of anthropophily' was defined as the proportion of females caught in the human-baited trap. For *An. funestus* this index was found to be consistently greater than 0.5 (value for random choice between traps/hosts), indicating that this species 'preferred' human to calf odour (index = 0.83). Conversely, the index of anthropophily for *An. gambiae s.l.* indicated they 'chose' calf in preference to human odour (index = 0.26). No significant differences of relative preference for calf or man were detected between villages; geographical variance accounted for < 8% of the total experimental variance. Molecular identifications of 181 specimens of the *An. gambiae* complex (≈ 50% of the samples) revealed only *An. arabiensis* Patton at Tsararano and Zazafotsy, but > 97% *An. gambiae* Giles *sensu stricto* at Fenoarivo, in accordance with prior knowledge of the differential distributions of these sibling species on the island. Predominant zoophily (i.e. intrinsic 'preference' for cattle odours) by both *An. arabiensis* and *An. gambiae s.s.* in Madagascar contrasts with their greater anthropophily in continental Africa.

Key words. *Anopheles arabiensis, An. funestus, An. gambiae, An. mascarensis,* host preferences, feeding behaviour, kairomones, odours, polymerase chain reaction, Madagascar.

Introduction

The main vectors of malaria in Madagascar are *Anopheles funestus* and two well known members of the *Anopheles gambiae* complex: *An. arabiensis* and *An. gambiae sensu stricto*, but their distributions and vectorial roles are not homogeneous on the island (Chauvet, 1969b; Mouchet *et al*., 1993). *Anopheles arabiensis* is generally the most widespread but least important vector. Historically in Madagascar, *An. funestus* was responsible for malaria transmission on the central plateau (altitudes 1000–1600 m a.s.l.), whereas *An. gambiae s.s.* is limited to lower altitudes. Reappearance of *An. funestus* in the highlands, after its elimination there by DDT house-spraying during

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the 1950s, produced malaria outbreaks from the mid 1980s and major epidemics in 1987–88 (Fontenille & Rakotoarivony, 1988). On the plateau, breeding sites of both *An. funestus* and *An. arabiensis* are mainly in the extensive rice fields that produce the staple crop of the islanders (Ravoniharimelina *et al*., 1992; Laventure *et al*., 1996). In coastal areas of Madagascar both *An. funestus* and *An. gambiae s.s.* are efficient malaria vectors, depending on local ecosystems, especially on the East coast where they also transmit filariasis (Brunhes, 1975) and the humid climate helps *An. gambiae s.s.* to be the predominant vector (Fontenille *et al*., 1992).

Anopheles arabiensis occurs all over Madagascar and is most prevalent in the arid southern areas. Its contribution to the overall vectorial capacity is generally lower (Fontenille *et al*., 1990), although its role as a malaria vector is not negligible. Marked exophagy and exophily of *An. arabiensis* in Madagascar hinder its control by indoor residual spraying. Moreover, *An. arabiensis* shows a high degree of zoophily in Madagascar, as demonstrated by the low human blood indices (HBI) reported from various environmental settings (Ralisoa Randrianasolo & Coluzzi, 1987; Fontenille *et al*., 1990). These peculiar behavioural characteristics set it apart from most African continental populations of *An. arabiensis*, although chromosomal analysis and crossing experiments found no evidence of intrinsic barriers to gene flow (Ralisoa Randrianasolo & Coluzzi, 1987). Microsatellite analysis of *An. arabiensis*, however, demonstrated high levels of genetic differentiation between populations from West Africa and those from Madagascar and other eastern outer islands (Simard *et al*., 1999). This differentiation also involves the host feeding habits of *An. arabiensis*: populations in West and South Africa show high levels of anthropophily (Costantini *et al*., 1998; Dekker & Takken, 1998) unless humans are greatly outnumbered by alternative hosts, particularly bovids (Coluzzi *et al*., 1979; Lindsay *et al*., 1993).

Host preferences of female mosquitoes are difficult to assess in the field because they are generally quantified by the proportion of successful feeds on each specific host, a measure that is affected by numerous environmental variables (Garrett-Jones, 1964; Garrett-Jones *et al*., 1980; Gillies, 1988). Thus, the zoophily of Malagasy *An. arabiensis* populations might result from the great abundance of cattle on the island. Recent statistics (www.fao.org, October 1999) show ≈ 10 million cattle vs. ≈ 10 million rural people inhabiting Madagascar (land area 587 000 km^2), a ratio and density considerably higher than in most countries of continental Africa. *Anopheles arabiensis* populations in Madagascar, therefore, may not be intrinsically zoophilic, but rather express zoophily in response to the prevailing environmental conditions. To test this hypothesis, we employed the experimental approach of Costantini *et al*. (1998) to assess and compare, by means of field odour choice tests, the host preferences of *An. arabiensis*, *An. gambiae* and *An. funestus* in Madagascar.

Materials and Methods

Study area

A detailed description of the climate and vegetation of Madagascar in relation to distributions of members of the *An. gambiae* complex can be found in Chauvet (1969b). Our experiments were implemented in March–June 1999 in three villages with different ratios of cattle to humans in contrasted eco-climatic settings.

Fenoarivo East (17°22′ S 49°24′ E; 5 m a.s.l.) is almost at sea level on the eastern coast; the climate is humid tropical with a mean annual rainfall of about 3000 mm and no dry season. Mean minimum and maximum temperatures are, respectively, > 20°C and 24°C during the cool season; > 24°C and 28°C during the warm season from October to May. This village is the main staging post for cattle coming from northern Madagascar to be marketed locally or in the nearby port of Toamasina.

Tsararano (16°11′ S 46°40′ E; 50 m a.s.l.) is in the region of Marovoay ~80 km from the West coast, in an area economically important for rice production. The climate is characterized by a rainy season from December to May (annual rainfall ~1400 mm) and higher temperatures than in the other two villages: maxima of 32–33°C during both the cool and warm seasons; minima of 17°C and 23°C, respectively. Bovids are considerably less abundant and generally used only for ploughing the rice fields; about 50 zebu cattle resided in Tsararano at the time of the experiment.

Zazafotsy (22°12′ S 46°21′ E; 800 m a.s.l.) is in the semiarid zone (~800 mm annual rainfall) along the southern edge of the central plateau. Thermal amplitudes are greater than in Fenoarivo East: temperature range 17–30°C during the warm season and 10–24°C during the cold season. Here there is a long dry season from April to December. The village lies close to a permanent river allowing rice cultivation determined by climate, relief and irrigation regime. Livestock breeding in this area is a principal activity for the local economy. Moreover, Zazafotsy lies on the main northward track of cattle coming plentifully from southern Madagascar.

There has been no anti-malaria house-spraying of insecticides at these three villages for decades. The villagers possess few mosquito nets and, during the study period, there was no programme for large-scale community implementation of insecticide-impregnated bednets for malaria control. Due to the relatively low economic level of these communities, consumer insecticides (aerosols, coils, etc.) are seldom used domestically; hence, the behaviour of adult mosquito populations was not directly or indirectly influenced by domestic use of insecticides.

Experimental protocol

The experimental protocol was similar to that of Costantini *et al*. (1998). Within each village, near to the residents' compounds, two odour-baited entry traps (OBETs: Costantini *et al*., 1993) were set up side by side with their entrances facing

down-wind, and a choice of odours from two alternative hosts was presented to the approaching mosquitoes. One adult man 25–40-years-old and a calf of similar mass were concealed in two separate tents and their odour drawn by 12 V fans to the OBETs via inflatable 'lay-flat' polythene tubing. The mean speed of the air current coming out of the OBETs was measured at the trap entrance with a hot-wire anemometer and adjusted to ≈ 0.6 m/s. On any trapping night, the OBETs were operated from 19.00 to 05.00 hours. On successive nights the traps were exchanged from side to side in order to compensate for any position effects; each replicate therefore consisted of two nights trapping. A fresh pair of alternative odour baits (i.e. man and calf) was employed for each replicate. In each village four replicate tests were conducted, totalling 16 trap nights (i.e. one pair of nights × 2 traps × 4 replicates) per village.

To monitor the anopheline mosquito population density we used two standard sampling methods (Service, 1993) in all three study villages. Human-bait landing catches were made both inside or just outside of three huts within the village, throughout each night of OBET use, by pairs of collectors (local residents) working alternate shifts from 19.00 to 05.00 hours. Each morning after the OBETs had been operated overnight, we implemented insecticide knock-down spray-sheet collections (SSC) using a commercial aerosol (active ingredient: dichlorvos 0.5%) in ~10 houses occupied by humans during the previous night. Also (in Fenoarivo East only) CDC light-traps were operated overnight by putting the trap close to a volunteer sleeping under an insecticide-free bednet. Houses sprayed during SSCs were not used for any other sampling purpose.

Processing of specimens

Mosquito species were identified under a stereo-microscope by reference to keys for Afrotropical and Malagasy anophelines (De Meillon, 1947; Grjebine, 1966). Specimens of *Anopheles gambiae sensu lato (s.l.)* were kept individually in wells of microtitre plates (with desiccant) for subsequent molecular identification. Polymerase chain reaction (PCR) analysis of ribosomal DNA intergenic spacers was performed on mosquito legs, without previous DNA extraction, according to the procedure of Scott *et al*. (1993). A mixture of specific primers (for *An. gambiae s.s*., *An. arabiensis* and *An. merus/melas*) was used for diagnosis according to the amplicon size from electrophoretic runs on agarose. For some of the specimens from Fenoarivo East, specific primers for *An. quadriannulatus* Theobald were also tested. Abdomens of fully engorged *Anopheles* females were subjected to direct enzyme-linked immunosorbent assay (ELISA) to identify bloodmeals of human, bovine or canine origin (Beier *et al*., 1988).

Statistical analysis

The number of mosquitoes retrieved from the human-baited OBET was expressed as the mean proportion p of the total number collected nightly from both traps, i.e. the index of anthropophily. To meet the assumptions of analysis of variance (ANOVA), p was arcsine$\sqrt{\text{p}}$-transformed (p_t) and normality checked by a goodness-of-fit test against the expected normal distribution calculated from the observed mean and variance. The variance of p_t was partitioned into its geographical and experimental components according to the guidelines of Sokal & Rohlf (1981) for a mixed-model nested ANOVA. Departures from a random choice (i.e. $P = 0.5$) were assessed by one-tailed Student t-tests against $p_t = 0.785$, which defines a 50% proportion on the angular scale. Statistical differences between species were assessed by one-way ANOVA on the mean p_t for each village.

Results

In 48 trap nights the OBETs caught no males and totals of 1930 female mosquitoes belonging to 22 species and four genera (*Anopheles*, *Mansonia*, *Aedes* and *Culex*; Table 1). *Mansonia uniformis* (Theobald) was the most frequently caught species ($n = 767$), followed by the main malaria vectors *An. gambiae s.l.* ($n = 362$) and *An. funestus* ($n = 266$). Other common species ($n > 48$) included, in decreasing order of frequency, *Culex antennatus* Becker ($n = 169$), *An. coustani* Laveran ($n = 143$) and *Cx. tritaeniorhynchus* Giles ($n = 94$). The locally relevant malaria vector *An. mascarensis* De Meillon was occasionally caught ($n = 7$ in 4 trapping nights) from all villages but only in the calf-baited OBETs (Table 1), thus showing a marked zoophilic tendency ($P < 0.01$ calculated from the expected cumulative probability of a positive binomial distribution with $P = 0.5$ and $n = 7$).

The greatest numbers of the *An. gambiae* complex were collected in Fenoarivo, whereas the highest yield of *An. funestus* was in Zazafotsy. Comparing the mean number/night caught by different sampling methods (Table 2), each OBET captured 51% as many *An. funestus* plus 57% as many *An. gambiae s.l.* as were collected on human bait (average for indoors and outdoors). The relative paucity of indoor-resting *An. gambiae s.l.* obtained by SSC (Table 2) confirmed their relative exophily in Madagascar. The frequency distribution of p_t conformed to normal expectations for *An. funestus* ($\chi^2 = 2.33$; d.f. = 1; $P > 0.12$) and *An. gambiae s.l.* ($\chi^2 = 0.92$; d.f. = 2; $P > 0.63$).

As expected, *An. funestus* females 'preferred' the OBET with human odour in all three villages. Conversely, *An. gambiae s.l.* 'preferred' the OBET baited with calf odour, with by far the lowest index of anthropophily value observed in Fenoarivo (Fig. 1). Differences between villages in the index of anthropophily accounted for < 8% of the total experimental variance and were not statistically significant for *An. funestus* or *An. gambiae s.l.* (Table 3). Most variability was due to temporal and position effects (replicates within Table 3 data), accounting for ≈ 60–75% of the total experimental variance. Heterogeneities in the index of anthropophily among different odour-bait pairs were the second most important source of experimental variability, accounting for about 20–33% of the total variance, but they were not statistically significant

Table 1. Total numbers of mosquito females collected in 48 trap nights from three Malagasy villages by entry traps set side-by-side in a choice-test arrangement and baited with odours from a man and a calf.

	Odour bait		
Species	Calf	Human	Total
Anopheles (*Anopheles*) *coustani*	70	73	143
Anopheles (*Cellia*) *flavicosta*	1	0	1
Anopheles (*Cellia*) *funestus*	44	222	266
Anopheles (*Cellia*) *gambiae s.l.*	287	75	362
Anopheles (*Cellia*) *mascarensis*	7	0	7
Anopheles (*Cellia*) *pauliani*	3	0	3
Anopheles (*Cellia*) *pretoriensis*	1	0	1
Anopheles (*Cellia*) *rufipes*	4	1	5
Anopheles (*Cellia*) *squamosus/cydippis*	32	8	40
Mansonia (*Mansonioides*) *uniformis*	386	381	767
Aedes (*Stegomyia*) *albopictus*	0	1	1
Aedes (*Stegomyia*) *tiptoni*	2	1	3
Aedes (*Aedimorphus*) *argenteopunctatus*	1	0	1
Aedes (*Aedimorphus*) *fowleri*	2	1	3
Culex (*Culex*) *antennatus*	79	90	169
Culex (*Culex*) *bitaeniorhynchus*	1	0	1
Culex (*Culex*) *decens* gr.	1	0	1
Culex (*Culex*) *giganteus*	2	1	3
Culex (*Culex*) *poicilipes*	11	5	16
Culex (*Culex*) *quinquefasciatus*	4	32	36
Culex (*Culex*) *tritaeniorhynchus*	68	26	94
Culex (*Culex*) *univittatus*	5	2	7
Grand total	1011	919	1930

(Table 3), i.e. there was insufficient evidence that the mosquito 'choice' differed according to the calf or human individual serving as odour bait (although for *An. gambiae s.l.* the F-statistic approached the threshold of statistical significance, $P = 0.1$).

Results for each mosquito taxon were therefore pooled across villages and compared between taxa, showing that their index of anthropophily differed significantly ($F_{1,4} = 18.52$; $P < 0.02$). The index was significantly greater than random for *An. funestus* ($p_t = 1.15 \pm 0.07$; index = 0.83; $t_2 = 5.23$; $P < 0.02$), confirming its anthropophilic tendency, whereas the index was significantly lower than random for *An. gambiae s.l.* ($p_t = 0.54 \pm 0.06$; index = 0.26; $t_2 = 3.90$; $P < 0.03$), indicating its zoophilic tendency.

Among samples of the *An. gambiae* complex trapped by OBETs the proportions of *An. arabiensis* and *An. gambiae s.s.* differed strongly between the three villages. All of 88 specimens from Tsararano and Zazafotsy successfully identified by PCR were *An. arabiensis*. By contrast, at Fenoarivo only one *An. arabiensis* was identified, together with 92 *An. gambiae s.s.* (Table 4) but no *An. quadriannulatus*. No *An. merus* was found in the OBET samples from any of the three villages. Sub-samples submitted to molecular analysis represented ≈53–63% (mean ≈60%) of the total trap yield from each village. Of these, ≈79–90% (mean ≈85%) were successfully identified (Table 4). Probably because of problems with DNA preservation of stocked specimens, direct DNA amplification without extraction gave often better results. The predominance of *An. gambiae s.s.* in Fenoarivo was confirmed by the PCR analysis of 44 females collected by indoor and outdoor landing catches. Of these, ≈98% (43/44) were *An. gambiae s.s.* and only one was *An. arabiensis*.

Anthropophily of *An. funestus* in Fenoarivo was confirmed by the bloodmeal analysis of 23 indoor-resting females from SSC. Of these, 19 tested positive for human blood, three for canine blood and one for mixed human/canine blood. Two additional specimens from CDC light-traps tested positive for human blood, giving an overall Human Blood Index (HBI) of 0.84. Interestingly, one fed female captured in the calf-baited OBET and morphologically identified as *An. funestus* tested positive for bovine blood, indicating repeated zoophily in foraging for a multiple bloodmeal. Because so few indoor-resting *An. gambiae s.l.* were obtained by SSC (Table 2), these specimens were all submitted to other analyses (PCR and morphometry) and no bloodmeals were identified from *An. gambiae s.s.* or *An. arabiensis.*

Discussion

Mosquito selection of feeding on different types of host, under field conditions, is usually assessed by the identification of

Table 2. Mean number of *Anopheles* females collected per night in OBETs during tests of host odour choice (mosquitoes/OBET/night); from biting catches on human bait (mosquitoes per man-night) performed simultaneously indoors (HBC in) and outdoors (HBC out); by CDC light-trap catches (LTC: mosquitoes/trap/night) and by pyrethrum knock-down spray-sheet collections in the early morning (SSC: mosquitoes/house/day), at three study villages.

	Sample				
Village	HBC in	HBC out	LTC	SSC	OBET
An. funestus					
Fenoarivo	30.7	27.1	33.2	14.9	2.0
Tsararano	2.9	1.6	–	17.3	7.1
Zazafotsy	1.7	1.9	–	1.8	7.7
An. gambiae s.l.					
Fenoarivo	19.7	29.1	27.2	1.6	12.4
Tsararano	3.4	6.8	–	3.3	2.1
Zazafotsy	4.0	11.6	–	0.1	6.8

– = sampling not performed.

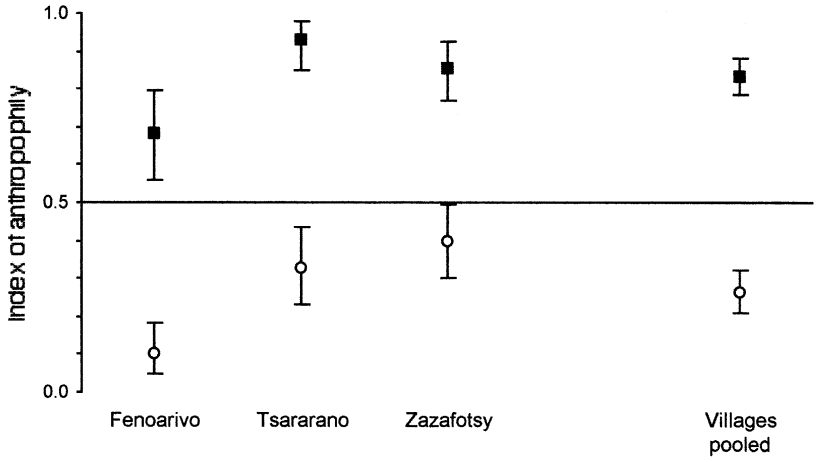

Fig. 1. Mean ± SE index of anthropophily of *Anopheles funestus* (■) and *Anopheles gambiae s.l.* (○) assessed by OBET choice tests comparing the odour of a human vs. that of a calf. The solid line (index = 0.5) represents a random choice. Values < 0.5 define a preference for calf odour, whereas values > 0.5 define a preference for human odour.

blood meals from field-collected specimens. For epidemiological purposes it is necessary to determine the HBI, i.e. proportion of bloodmeals taken from humans (Garrett-Jones *et al*., 1980). Apart from the difficulties of obtaining unbiased samples, the HBI is an unreliable indicator of the genetic and other endogenous affinities of mosquitoes for particular hosts because, in the field, many environmental variables circumstantially influence host selection (Gillies, 1972, 1988; Gibson, 1996; Costantini *et al*., 1999). The OBET choice test is designed to minimize the influence of extrinsic variables, thus highlighting the expression of intrinsic host preferences of mosquitoes during an early phase of the 'host-seeking' behavioural sequence. The genomic component of 'preference' is not completely isolated by such tests, as the 'choice' expressed by individual mosquitoes might still depend on their internal state or prior experience. Moreover, the test deliberately avoids assessment of preference for a host when cues other than its odour are available to the mosquito. Despite these limitations, the OBET choice test is an objective way of testing host preferences in the field. Moreover, even if the experimental variance is generally high (cf. Table 3), field choice tests have the advantage of avoiding artificial sources of variability due to manipulation of laboratory strains.

Our PCR identifications agree closely with other information on the distribution of members of the *An. gambiae* complex in Madagascar, *An. arabiensis* being most widespread and *An. gambiae s.s.* prevalent on the East coast but absent from the highlands (Chauvet, 1969b). OBET choice tests at Zazafotsy and Tsararano undoubtedly represent the behaviour of *An. arabiensis*.

Table 3. Partitioning the geographical and experimental variance of the OBET choice tests by nested ANOVA. Sum of squares (SS), degrees of freedom (d.f.), mean squares (MS), expected variance (s^2), and percent proportion of total variance ($\%s^2$). NS = P > 0.05.

Source of variation	SS	d.f.	MS	F	s^2	$\%s^2$
An. funestus						
Among villages	0.441	2	0.221	1.174 NS	0.013	4.1
Among baits within villages	1.136	10	0.114	0.464 NS	0.066	20.3
Among replicates within baits	3.185	13	0.245		0.245	75.6
Total	4.763	25		0.324	100.0	
An. gambiae s.l.						
Among villages	0.558	2	0.279	1.501 NS	0.012	7.8
Among baits within villages	1.859	10	0.186	2.100 NS	0.049	32.7
Among replicates within baits	1.151	13	0.089		0.089	59.5
Total	3.568	25			0.149	100.0

Table 4. Identifications of three species of anopheline females collected in OBETs at three study villages. Figures for the sibling species *Anopheles arabiensis* and *An. gambiae* represent the subsample submitted to the polymerase chain reaction (PCR) technique of Scott *et al.* (1993). Specimens that did not give readable bands are shown as 'PCR –ve' and the percentage of trapped specimens tested is given as 'Tested'. The last column shows, for each village and bait, the proportion (%) of assayed specimens that could be identified.

	Species					
Odour bait	*An. funestus*	*An. arabiensis*	*An. gambiae*	PCR –ve	Tested (%)	Identified (%)
Fenoarivo						
Calf	5	1	82	16	54.1	83.8
Human	27	0	10	4	87.5	71.4
Total	32	1	92	20	56.8	82.3
Tsararano						
Calf	12	8	0	3	50.0	72.7
Human	102	6	0	1	58.3	85.7
Total	114	14	0	4	52.9	77.8
Zazafotsy						
Calf	27	38	0	4	51.2	90.5
Human	93	36	0	4	85.1	90.0
Total	120	74	0	8	63.6	90.2

We confirmed the typical anthropophily of *An. funestus* in Madagascar and demonstrated the marked zoophily of Malagasy populations of *An. arabiensis*, something that had not been investigated experimentally before. African continental populations of *An. arabiensis* are demonstrably more anthropophilic (Costantini *et al.*, 1998; Dekker & Takken, 1998) and generally more endophagic, whereas those in Madagascar are extremely exophagic and exophilic as well as strongly zoophilic. These behavioural contrasts reflect genetic divergences between geographical populations of *An. arabiensis* (Simard *et al.*, 1999). Zoophily of the Malagasy populations raises the question of whether such feeding habits represent original behaviour or result from several years of selection pressure from indoor residual spraying with DDT (Chauvet *et al.*, 1964). OBET choice tests were undertaken in villages where houses are not sprayed at least since the spraying campaigns of the 1950s (Mouchet *et al.*, 1993). Our results suggest that, even if DDT did select for behavioural change, it was on a genetic background including zoophily in the behavioural repertoire of *An. arabiensis*, as this trait prevails after decades without house-spraying.

Unexpectedly, we found that *An. gambiae s.s.* 'preferred' calf to human odour in Fenoarivo. This town is at a crossroad of the cattle trade, many large herds converging here every year. The abundance of cattle outnumbering people locally (about 5–10 bovids per human) might have raised selective pressure against anthropophily in this otherwise highly anthropophilic species. It would be interesting to clarify whether zoophily is peculiar to the Fenoarivo population of *An. gambiae s.s.* or if this behaviour is a general characteristic of populations of this species elsewhere in the island. Circumstantial evidence of low anthropophily of Malagasy *An. gambiae s.s.* can be inferred from the mark–release–recapture experiments of Chauvet (1969a).

The zoophilic sibling species *An. quadriannulatus* has not been found in Madagascar, although it is widespread in southern Africa (Coetzee *et al*., 1993) and was reported from the island of Zanzibar (Davidson, 1966). PCR assays on females of *An. gambiae s.l.* from Fenoarivo, using primers for *An. quadriannulatus* and *An. gambiae s.s.* in parallel on the same specimens, yielded typical bands with the latter but not the former primers, confirming the specimens to be *An. gambiae s.s.* and not *An. quadriannulatus.* Further studies on the host preferences of *An. gambiae s.s.* in Madagascar, possibly using other independent genetic markers and a wide array of sampling tools might help to identify factors of potential use for genetic manipulation and vector control purposes.

Geographical variability in host preferences of members of the *An. gambiae* complex across the Afrotropical region is worth further investigations on the macro- and microspatial scales, especially in areas where this issue has not yet been addressed, for improved awareness of malaria epidemiology and vector control prospects. For example, Diatta *et al*. (1998) reported similar zoophilic tendencies in *An. gambiae* and *An. arabiensis* from Senegal, contradicting the recognized high anthropophily of the former species in West Africa. For such purposes Madagascar constitutes an ideal study area because environmental conditions often show dramatic contrasts on microspatial scale, equivalent to moving hundreds of kilometres in continental Africa. Although we did not demonstrate significant differences of the index of anthropophily between our test villages, the relatively low contribution of geographical variability to the total experimental variance implies that more replicates would be increasingly likely to reveal geographical divergences among *An. arabiensis* populations (cf. Simard *et al*., 1999; Donelly & Townson, 2000; Petrarca *et al*., 2000).

These results suggest another pathway towards identification of genes conferring zoophilic feeding habits to be exploited in transgenic vector programmes (Curtis, 1994). Introgression of *An. quadriannulatus* genes by crossing and back-crossing this species with *An. gambiae* is currently underway to isolate and identify the genetic determinants of zoophily (Curtis *et al*., 1999). This approach, however, has the possible disadvantage of 'hitch-hiking' other genes that might lead to undesirable epistatic and pleiotropic effects. Use in such crossing programmes of taxa that are ecologically more successful and more closely related, as, e.g. populations of *An. arabiensis* from Madagascar and West Africa, or the uniquely zoophilic *An. gambiae s.s.* from Fenoarivo, might provide better options (Costantini *et al*., 1999).

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