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Blood-feeding behaviour of the malarial mosquito *Anopheles arabiensis*: implications for vector control

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Abstract. Feeding behaviour of the malaria vector *Anopheles arabiensis* Patton (Diptera: Culicidae) was monitored for 12 months (March 2003−February 2004) in the Konso District of southern Ethiopia (5°15′N, 37°28′E). More than 45 000 *An. arabiensis* females were collected by host-baited sampling methods (light-traps, human landing catches, cattle-baited traps) and from resting sites (huts and pit shelters). In the village of Fuchucha, where the ratio of cattle : humans was 0.6 : 1, 51% of outdoor-resting mosquitoes and 66% of those collected indoors had fed on humans, human baits outdoors caught > 2.5 times more mosquitoes than those indoors and the mean catch of mosquitoes from pit shelters was about five times that from huts. Overall, the vast majority of feeding and resting occurred outdoors. In the cattle camps of Konso, where humans slept outdoors close to their cattle, ∼ 46% of resting mosquitoes collected outdoors had fed on humans despite the high cattle : human ratio (17 : 1). In both places, relatively high proportions of bloodmeals were mixed cow + human: 22–25% at Fuchucha and 37% in the cattle camps. Anthropophily was also gauged experimentally by comparing the numbers of mosquitoes caught in odour-baited entry traps baited with either human or cattle odour. The human-baited trap caught about five times as many mosquitoes as the cattle-baited one. Notwithstanding the potential pitfalls of using standard sampling devices to analyse mosquito behaviour, the results suggest that the *An. arabiensis* population is inherently anthropophagic, but this is counterbalanced by exophagic and postprandial exophilic tendencies. Consequently, the population feeds sufficiently on humans to transmit malaria (sporozoite rates: 0.3% for *Plasmodium falciparum* and 0.5% for *P. vivax*, by detection of circumsporozoite antigen) but also takes a high proportion of meals from non-human hosts, with 59–91% of resting mosquitoes containing blood from cattle. Hence, classical zooprophylaxis is unlikely to have a significant impact on the malaria vectorial capacity of *An. arabiensis* in Konso, whereas treating cattle with insecticide might do.

Key words. *Anopheles arabiensis*, anthropophilic, baited traps, endophagic, endophilic, exophagic, exophilic, host preference, malaria vector, zoophagic, zoophilic, zooprophylaxis, Ethiopia.

Introduction

In the less humid countries of tropical Africa, *Anopheles arabiensis* Patton, a member of the *An. gambiae* Giles species complex, is a major vector of malaria, particularly in southern Africa, Madagascar and along a wide east–west belt fringing the Sahel. Generally, *An. arabiensis* is regarded as being more exophilic, exophagic and zoophilic than *An. gambiae* Giles *sensu stricto,* but, although it is a highly efficient vector of malaria (White, 1974), there appears to be an east–west behavioural

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cline. For *An. arabiensis* populations in western Africa, the proportion of bloodmeals from humans (human blood index, HBI) is 80–100% indoors, and most feeding and resting occurs indoors (Molineaux & Gramiccia, 1980; Fontenille *et al.*, 1997; Bøgh *et al.*, 2001). By contrast, in eastern Africa, a greater proportion of *An. arabiensis* feed on cattle and rest outdoors. In Tanzania for instance, White *et al.* (1972) reported HBIs of 61% and 7% for samples collected indoors and outdoors, respectively, and *An. arabiensis* was 2.2 times more likely than *An. gambiae s.s.* to be found outdoors. Similar results were reported by Highton *et al.* (1979) for Kenya, whereas in Madagascar the HBI is $< 10\%$ and few samples are found resting indoors (Randrianasolo & Coluzzi, 1987; Fontenille *et al.*, 1990; Rajaonarivelo *et al.*, 2004). These behavioural differences may ultimately be linked to observed genetic variability (Coluzzi *et al.*, 1979; Mekuria *et al.*, 1982; Randrianasolo & Coluzzi, 1987; Coosemans *et al.*, 1989; Simard *et al.*, 1999; Petrarca *et al.*, 2000).

The resting and feeding behaviour of *An. arabiensis* is of great importance with respect to the efficacy of control measures. Indoor-based methods of control, such as the use of insecticide-impregnated bednets and house-spraying with residual insecticides, are highly effective against *An. gambiae s.s.*, which mainly feeds indoors on humans and rests there once fed. The degree to which *An. arabiensis* feeds on non-human hosts and rests outdoors reduces the efficacy of these control methods, but also offers opportunities for other approaches. Amongst these is the use of insecticide treatment on cattle, which has been employed successfully to control *An. stephensi*, a zoophilic vector of malaria in Pakistan (Hewitt & Rowland, 1999; Rowland *et al.*, 2001). Ironically, this technique has never been applied, let alone tested, in Africa, despite the fact that insecticide-treated cattle are used to control vector-borne diseases of livestock in areas where *An. arabiensis* transmits malaria (Eisler *et al.*, 2003). One such area is the Konso District of south-west Ethiopia, where *An. arabiensis* is the main vector of malaria (Habtewold *et al.*, 2001) and where, for the past decade, the local people have treated their cattle, albeit sporadically, with pyrethroid insecticides to control animal trypanosomiasis transmitted by tsetse flies (Diptera: Glossinidae).

The people of Konso graze many of their cattle in lower lying areas of the district > 20 km from the main villages. The herders accompanying these cattle consequently spend weeks living in makeshift camps with little shelter and in close proximity to their cattle. In the villages, by contrast, cattle are less abundant and people sleep in huts with their livestock outside. The combination of the high density of cattle, their treatment with residual pyrethroids to control tsetse and the role of *An. arabiensis* as the main vector of malaria makes Konso a particularly suitable site for assessing the use of insecticide-treated cattle to control malaria in this Afrotropical agroecosystem.

In a previous publication we reported on the behaviour and mortality rates of mosquitoes exposed to insecticide-treated cattle (Habtewold *et al.*, 2004): individuals of *An. arabiensis* were susceptible to the deltamethrin dosage used for tsetse control, although the treatment itself did not deter them from feeding on cattle. The impact of pyrethroid-treated cattle on a mosquito population depends, however, on the degrees to which the population feeds on cattle vs. humans and other hosts. In this study, therefore, we attempted to quantify the feeding behaviour of *An. arabiensis* with respect to cattle and humans in Konso.

The standard method of measuring host preference relies on the collection and analysis of blood-fed mosquitoes (Service, 1993). This approach, however, can be biased by the uneven spatial distribution of hosts. Generally, humans sleep indoors and livestock are kept outdoors. Consequently, mosquito samples collected from within huts generally show higher proportions of human bloodmeals than those collected outside, and we seldom know which estimate more closely reflects the feeding patterns of the population. Therefore, in addition to identifying the host type(s) of bloodmeals, we indirectly estimated the relative numbers of *An. arabiensis* feeding on humans (indoors and outdoors) and cattle (outdoors only) in villages and cattle camps, using various sampling methods. Unfortunately, the standard methods used to assess biting densities are not free of bias either. A second theme to emerge in the course of analysing our results therefore involves consideration of the implications of sampling bias.

Materials and methods

Study area

The study was undertaken between March 2003 and February 2004, in the district of Konso (Fig. 1), a rural, semiarid area in the Rift Valley of southern Ethiopia, located at 5°15′N, 37°28′E. The annual rainfall is ~700–800 mm, with the main wet season in April–May and secondary rains in October (1999–2001; National Meteorological Services Agency). Two sites were selected for the study, representing the main types of human settlement in the area: an established village, Fuchucha (~1300 m a.s.l.), and a lowland site at Jarso (~800 m a.s.l.), where herders periodically bring their cattle to graze.

Fuchucha. The village of Fuchucha has ~1000 people living in ~230 compounds, plus ~600 cattle, giving a cattle : human ratio of ~0.6 : 1. At night, cattle stay within the compounds, close to the huts in which people sleep. Fuchucha extends linearly away from the main river (Fig. 1); sampling sites were chosen along a transect at right angles to the river to control for the effect of distance to the river on mosquito densities. The data for sampling sites were eventually pooled into two sectors, giving mean values for those near to and those further from the river.

Jarso. The site comprises a number of temporary cattle camps that move according to the availability of suitable grazing. Each camp consists of a cooking area and a basic kraal of thornbush to keep the cattle together at night. To avoid mosquito bites, the herdsmen sleep on platforms in trees, ~5–10 m above ground. The specific camps used in the present study comprised ~30 male herdsmen and ~570 cattle, distributed between about eight camps, giving a mean cattle : human ratio of ~17 : 1. Cattle always greatly outnumbered humans, with a range of ~10–30 cattle/human for each camp, as opposed to < 1 animal/human in Fuchucha. Entomological sampling was conducted within 200 m of the main campsites of Jarso.

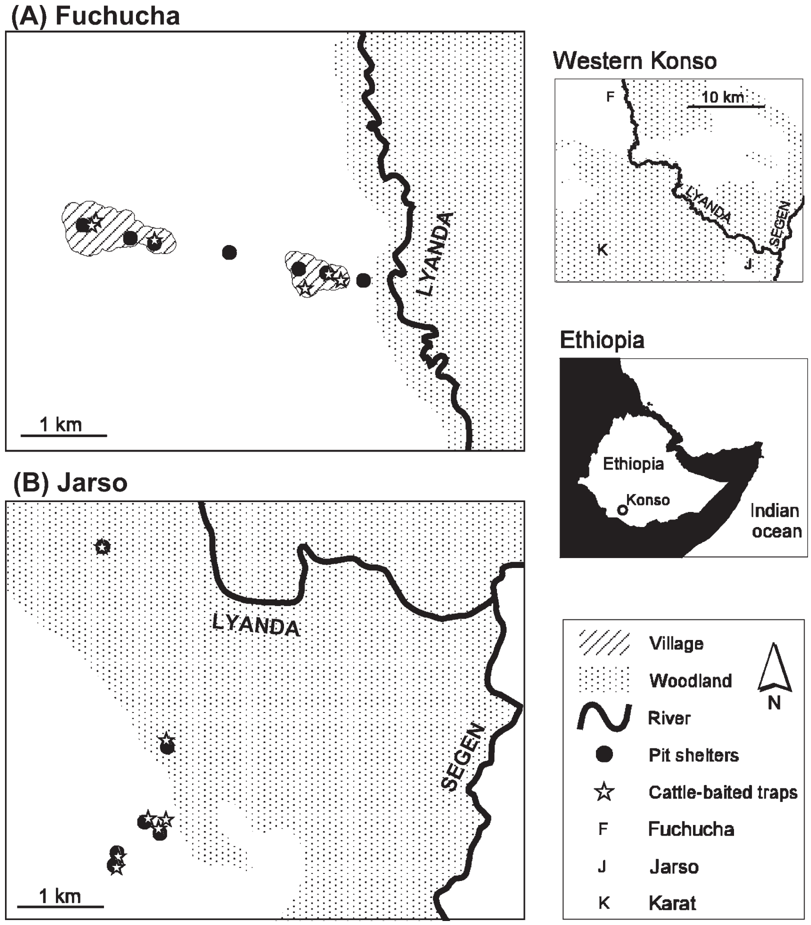

Fig. 1. Position of fixed sampling sites in (A) Fuchucha village lying perpendicular to the Lyanda river and (B) Jarso cattle camp area. The smaller maps show the geographical relationship between the two study sites (Fuchucha, F.; Jarso, J.) and the administrative centre of Konso (Karat, K), and their location within southern Ethiopia.

The positions of huts, compounds, cattle camps and all sampling points were recorded using a global positioning system (GPS) (Garmin 12XL; Garmin, Romsey, U.K.). The numbers of humans and livestock in each of the huts and compounds were recorded. These data were incorporated into a geographical information system (GIS) using Arcview (ESRI, Aylesbury, U.K.). The GIS also included the positions of rivers and roads obtained from Government of Ethiopia maps (scale 1 : 50 000), and a vegetation map (scale 1 : 50 000) derived from a survey conducted in 2001 (T. Woldeyes & J. Sandford, unpublished data). This information was used to design the stratified random sampling regime and for preparing study area maps.

Entomological sampling

Resting behaviour. Samples of outdoor-resting mosquitoes were collected by manual aspirators from pit shelters covered by a thatched roof, constructed for the purpose (Service, 1993). Mosquitoes were collected at least twice/week from seven pit shelters in Fuchucha and once weekly from six pit shelters in Jarso, totalling 6–14 samples/week.

Indoor-resting mosquitoes could only be collected from Fuchucha, as there were no equivalent human shelters in Jarso. Pyrethroid spray catches (PSCs) (Service, 1993) were conducted in 15–20 randomly selected dwellings throughout Fuchucha each month, totalling 4–5 samples/week.

Outdoor-biting catches. Mosquito biting behaviour was assessed by human landing catches (HLCs) and cattle-baited traps (CBTs). Landing catches on humans were conducted by trained assistants working in pairs (6-h shifts at 19.00–07.00 hours) to collect mosquitoes as they landed on exposed legs. Hourly collections were kept in separate cups. The collectors were provided with malaria prophylaxis under medical supervision, according to the ethical provisions of the study. Collections were made at five different sites throughout Fuchucha about twice a week, totalling ~10 samples/week. In Jarso, similar collections were performed at four sites within the vicinity of the cattle camps about once a week, totalling four samples/week. Unfortunately, it was not possible to begin using HLCs in Jarso until the third month of the study.

Cattle-baited traps were constructed to collect cattle-biting mosquitoes. These traps consisted of a wooden frame enclosure ~2.0 m^2 × 1.8 m high, covered with light cotton cloth which was raised ~30 cm above the ground along one edge. A single ox was placed in the enclosure overnight, from 19.00 hours to 06.00 hours, and at 05.30 hours (~30 min before sunrise) the cloth was lowered to retain all the mosquitoes. After sunrise, mosquitoes resting inside the enclosure were collected by manual aspirator. Cattle-baited catches were collected in Fuchucha twice a week from each of six traps, totalling ~12 samples/week, and in Jarso once a week from each of four or five traps, totalling four samples/week.

A calibration study was undertaken in Jarso to directly compare the results of the CBTs and HLCs. For 12 nights during June–July 2003, each method was randomly assigned to two sites, ~100 m apart, using a 2 × 2 Latin square experimental design.

Indoor-biting catches. The indoor-biting rate in Fuchucha was assumed to be approximated by light-trap catches (LTCs), which were conducted several times each week. A miniature CDC (Center for Disease Control, Atlanta, GA, U.S.A.) light-trap (model 512; J.W. Hock, Gainesville, FL, U.S.A.), fitted with an incandescent lightbulb, was placed next to a person sleeping under a bednet. The light-traps were operated from 19.00 hours to 07.00 hours. Twenty LTCs were conducted throughout Fuchucha about twice a week, totalling ~40 samples/week.

Gradually, light-traps are replacing human-baited catches wherever a significant correlation can be demonstrated, as in Burkina Faso (Costantini *et al.*, 1998b), Sierra Leone (Magbity *et al.*, 2002), Tanzania (Lines *et al.*, 1991; Davis *et al.*, 1995) and Kenya (Githeko *et al.*, 1994). For this study, we used the results of Habtewold (2005), who compared the results of LTCs and HLCs at villages near Arba Minch, ~40 km north of Fuchucha. Habtewold (2005) showed that there was a significant correlation ($P < 0.001$, $r = 0.77$) between indoor LTCs and indoor HLCs for *An. arabiensis*, with the LTC representing 59% (95% confidence interval [CI] 46–76%) of the HLC.

Odour-baited entry traps (OBETs) baited with cattle or human odour were used as an alternative measure of the relative importance of cattle and human hosts (Costantini *et al.*, 1993, 1998a; Duchemin *et al.*, 2001). These entry traps were originally designed to compare the propensity of mosquitoes to enter a house in response to different host odours, hence providing a combined measure of two major components of anthropophily (endophily and olfactory responses to human odours). Accordingly, two OBETs were placed adjacent to one another (Costantini *et al.*, 1998a) and each trap was connected to a separate tent (2.5 × 1.5 m, 1.5 m high) via polythene tubing (~10 m long, 20 cm in diameter). A single human (~60 kg) or a single ox (~150 kg) was placed in each tent and their odour was blown by 12-V fans (~0.5 m/s) via the polythene tubing through the traps. To obviate any site effects, the relative position of the traps was swapped randomly, following a 2 × 2 Latin square design, and different pairs of cattle and human baits were used for each block of 4 nights. The traps were operated from 19.00 hours to 07.00 hours for 12 nights and the mosquitoes were collected from the traps with aspirators the following morning.

Field-processing of mosquitoes

Mosquitoes were identified using morphological keys (Gillies & De Meillon, 1968; Gillies & Coetzee, 1987), sorted according to species, sex, abdominal condition, time and place, and counted. All specimens were then stored in plastic tubes (1.5 mL, containing silica gel desiccant beneath a layer of cotton wool) and taken to the Natural Resources Institute (Chatham, U.K.) for laboratory analyses.

Laboratory analyses

Sporozoite infection rates. A representative sample of mosquitoes from each of the baited collections was used to assess the rate of infection with sporozoites of the three *Plasmodium* types present (*P. falciparum, P. vivax*$_{210}$ and *P. vivax*$_{247}$). Sporozoite infections in the crushed head and thorax of dried mosquitoes were detected by an enzyme-linked immunosorbent assay (ELISA) using specific monoclonal antibodies to detect and differentiate the circumsporozoite (CS) protein epitopes of each *Plasmodium* type (Wirtz *et al.*, 1987, 1992; Centers for Disease Control and Prevention). Due to the low expected sporozoite rate (Habtewold *et al.*, 2001) mosquitoes were pooled in groups of 10 per ELISA well.

Bloodmeal identification. The bloodmeals from all fed mosquitoes collected from pit shelters or by PSC in huts, were identified by the precipitin test of Washino & Tempelis (1983), testing against antibovine and antihuman sera.

Statistical analyses

For the purpose of analysing and presenting the overall results, daily catch data (n) were normalized and variation homogenized by transforming to $\log_{10}(n + 1)$, according to common practice (e.g. Ijumba *et al.*, 2002), and daily means were calculated for each week. Results are presented as the back-transformed mean catch accompanied by their respective transformed mean ± standard error of the mean (SEM).

For more detailed analyses of the relationship between sampling methods, a mean daily catch/week was calculated, using the original untransformed data. The mean weekly catches were then subjected to a square-root transformation and weighted by sample size (i.e. the number of collections) and the data used to calculate monthly means. Finally, the monthly values were back-transformed to produce mean monthly catches. This procedure reduced sampling error noise, diminished pseudo-replication by using monthly rather than weekly means and normalized the data.

Standard regression analysis to assess the relationship between the mean monthly catches of two different sampling techniques ('x' and 'y') is not appropriate, as both measures are subject to sampling error. Accordingly, we plotted the mean catches against each other and estimated the elliptical region in the bivariate plane containing 95% of the observations. The overall Catch Index (Y/X) was then estimated by regularly re-sampling within the ellipse to obtain a frequency distribution of monthly catch indices (y/x) from which we calculated the median and interquartile range.

For the direct comparison of the CBT and the HLC, the data (n) were normalized by transformation to $\log_{10}(n + 1)$ and subjected to analysis of variance.

Data for OBET collections were analysed with GLIM4 (Francis *et al.*, 1993), using a binomial error structure with a logit link, with the pooled nightly catch from both OBETs as the binomial denominator. The data were overdispersed, so the significance of changes in deviance was assessed by an *F*-test after re-scaling by dividing Pearson's χ^2 by the degrees of freedom (Crawley, 1993).

The bloodmeal data were also analysed using GLIM4 with a binomial error structure. The host identity of each fed mosquito was treated as a binary variable – a mosquito fed on a human or not, or, conversely, a bovid or not. Analyses were undertaken to assess whether the distribution or abundance of cattle and humans affected the proportion of meals taken from these hosts. Using data from the GIS, each fed mosquito collected indoors was classed as being from a domestic compound comprising: humans only; mostly humans (number of cattle < number of humans) or mostly cattle (number of cattle > number of humans). Analyses were then undertaken to assess whether there was a significant difference in the proportion of human- or cattle-fed mosquitoes from these three types of compound. For samples collected outdoors, analyses were undertaken to assess whether there was a significant difference in the proportion of cattle- or human-fed mosquitoes collected from different pit shelters within Fuchucha or between Fuchucha and Jarso.

Results

Over the 12-month sampling period, 63 194 mosquitoes were collected. Three species of anopheline mosquitoes predominated: 4218 *An. pharoensis* Theobald; 13 241 *An. funestus* group and, 45 527 *An. gambiae sensu lato,* assumed to be *An. arabiensis* (Habtewold *et al.*, 2001, 2004; Habtewold, 2005). A small sub-sample of 181 *An. gambiae s.l.* collected by odour-baited entry traps was molecularly identified according to the protocol of *Scott et al.* (1993). Only one *An. quadriannulatus* was found; all the remaining mosquitoes were *An. arabiensis*, confirming that this species is largely the most abundant member of the complex present in the study area. As the first two have seldom been implicated as vectors in this part of Ethiopia, we focus on *An. arabiensis* as the principal malaria vector species in Konso.

Results obtained by the various sampling methods showed a similar seasonal pattern for *An. arabiensis* (Fig. 2). In general, catches increased rapidly with the onset of the main rains in April, peaked in late May/early June and then declined exponentially until August, after which they remained at a generally steady low level until the end of the study in February 2004. Seasonal variation in the apparent abundance of *An. arabiensis* was more marked in Jarso than Fuchucha. For instance, the mean weekly HLC range was 0.1–372 mosquitoes/night in Jarso compared with only 2.4–62 mosquitoes/night in Fuchucha. The persistence of the *An. arabiensis* population in Fuchucha during the dry season is probably due, at least in part, to the greater abundance of breeding sites associated with the Lyanda River, which flows throughout the year at Fuchucha. The rivers and pools in the vicinity of Jarso dried up within a month or so of the end of the main rains.

Although some of the sampling techniques used were originally developed primarily to compare changes in the relative density of mosquitoes resting or biting in different habitats, and were not designed to assess behaviour directly, some inferences about the resting and biting behaviour of *An. arabiensis* in Konso can be drawn by comparing the mean monthly catches from various sampling methods.

Resting behaviour

Hut vs. pit shelter catches. To assess the relationship between the density of females resting indoors and outdoors, the results of pyrethroid spray catches (PSCs) and pit shelter catches (PITs) were compared. Overall, the mean daily catch of female *An. arabiensis* from PSCs was only 0.2 (0.07 ± 0.007, *n* = 995), compared with 1.3 (0.35 ± 0.017, *n* = 670) from PITs.

Plotting the mean monthly catches from the huts and the pit shelters (Fig. 3A) shows that the median Catch Index for PSC/PIT = 0.19 (interquartile range 0.117–0.300), showing that about five times as many mosquitoes were caught resting outdoors as indoors. Pearson's correlation coefficient was only 0.76 (95% CI 0.48–0.88), which is reflected in the relatively wide ellipse in Fig. 3(A).

Comparison of pyrethroid spray catches vs. light-trap catches. The relatively low number of *An. arabiensis* resting in the huts could mean that females which fed indoors left immediately after feeding (endophagy, plus postprandial exophily), and/or that females generally fed outdoors and remained there (exophagy, plus exophily). It is therefore pertinent to compare PSCs with LTCs to assess the relationship between the number of mosquitoes resting indoors and feeding indoors.

Overall, the mean LTC was 0.4 (0.15 ± 0.012, *n* = 1164), compared with 0.2 for PSCs. The median Catch Index for PSC/LTC was 0.16 (interquartile range 0.109−0.248), which differs from an index that could be calculated using the mean catches (i.e. 0.2/0.4 = 0.5), reflecting the skewed nature of the original catch data and hence the need to use the Catch Index. Thus, of the *An. arabiensis* females that entered houses at night, only ∼16% of that number were found resting there during the day, which is consistent with a large degree of exophilic resting behaviour. Taken together, the low Catch Indices for PSC/PIT and PSC/LTC suggest that few outdoor feeding *An. arabiensis* come indoors to rest afterwards. Pearson's correlation coefficient for PSC/LTC = 0.83 (95% CI 0.58–0.92), which is higher than that for PSC/PIT.

Feeding behaviour: endophagy vs. exophagy

Light-trap vs. human landing catch. To gauge the relative degree of indoor and outdoor feeding, the indoor LTC was compared with the outdoor HLC. The mean LTC was 0.4 (0.15 ± 0.012, *n* = 1164), the mean HLC was 3.1 (0.61 ± 0.035 *n* = 337) and the median Catch Index for LTC/HLC was 0.23 (interquartile range 0.144–0.327), which suggests that more than four times as many *An. arabiensis* females fed outdoors as indoors. Even if we take into account the fact that under some circumstances in Ethiopia, an LTC can represent as little as 0.59 of an

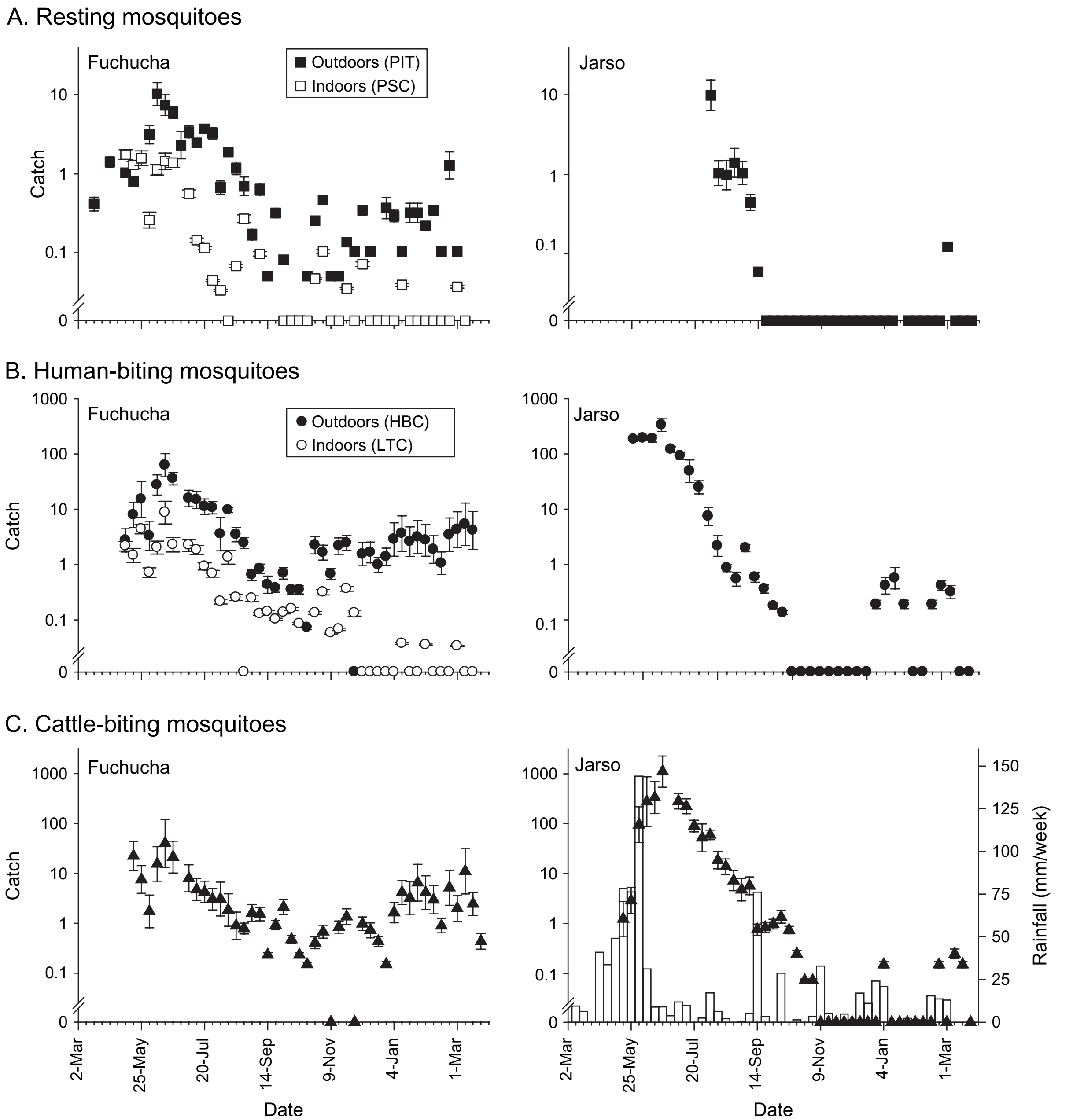

Fig. 2. Comparison of mean daily catches (± standard error of the mean) for each week of the study in Fuchucha and Jarso. (A) Resting mosquitoes caught either outdoors by pit shelter collection (PIT), or indoors by pyrethroid spray catch (PSC). (B) Human-landing mosquitoes caught outdoors by human landing catch (HLC), or indoors by light-trap catch (LTC). Note: indoor catches were not possible in Jarso cattle camps. (C) Cattle-biting mosquitoes caught by cattle-baited trap (CBT) and total rainfall data for the whole Konso area during the study period. Each datapoint represents the mean catch from the following numbers of catches/week: PIT, $n = 6–14$; PSC, $n = 4–5$; HLC, $n = 4–10$; LTC, $n = 40$; CBT, $n = 4–12$.

HLC (Habtewold, 2005), our results still indicate that ~2.5 times as many *An. arabiensis* fed outdoors as indoors. Pearson's correlation coefficient was 0.97 (95% CI 0.93–0.99), the highest value for all comparisons, as shown by the shape of the ellipse in Fig. 4(A).

Feeding behaviour: zoophily vs. anthropophily

Cattle-baited trap vs. human landing catch. Following the approach used with the previous comparisons, we compared the monthly mean catches from HLCs and CBTs operated outdoors

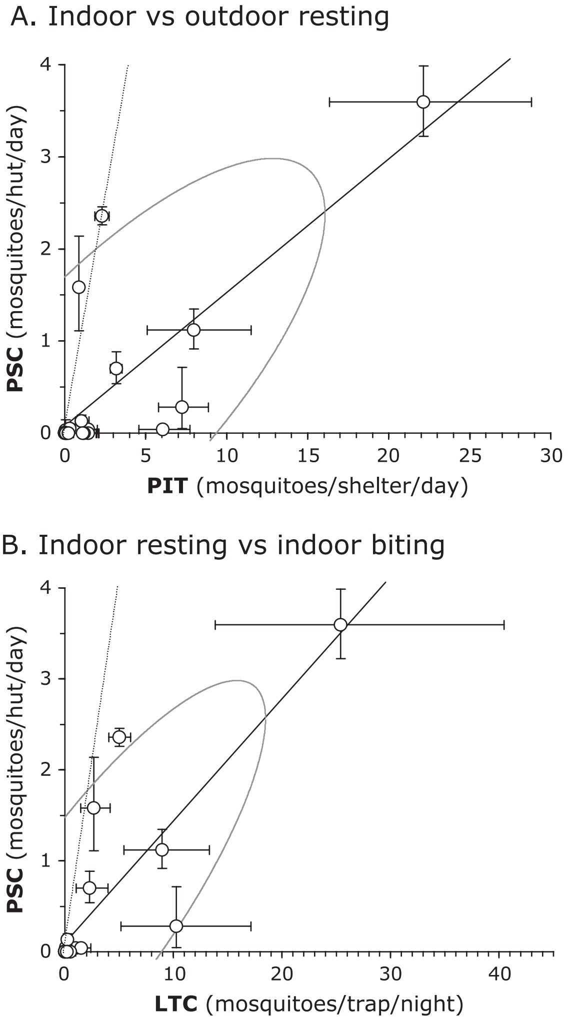

Fig. 3. Analysis of resting site preferences. (A) Scattergram of mean daily catch from pyrethroid spray catch (PSC) plotted against pit shelter catch (PIT). (B) PSC plotted against light-trap catch (LTC). Points show the mean daily catches (± 95% confidence interval) for the two techniques, paired by month and site, plotted against one another. The elliptical region is the bivariate plane containing 95% of the predicted observations and the solid and broken lines show the observed major axis regression line and the expected line if the catches from the two techniques were equivalent, respectively.

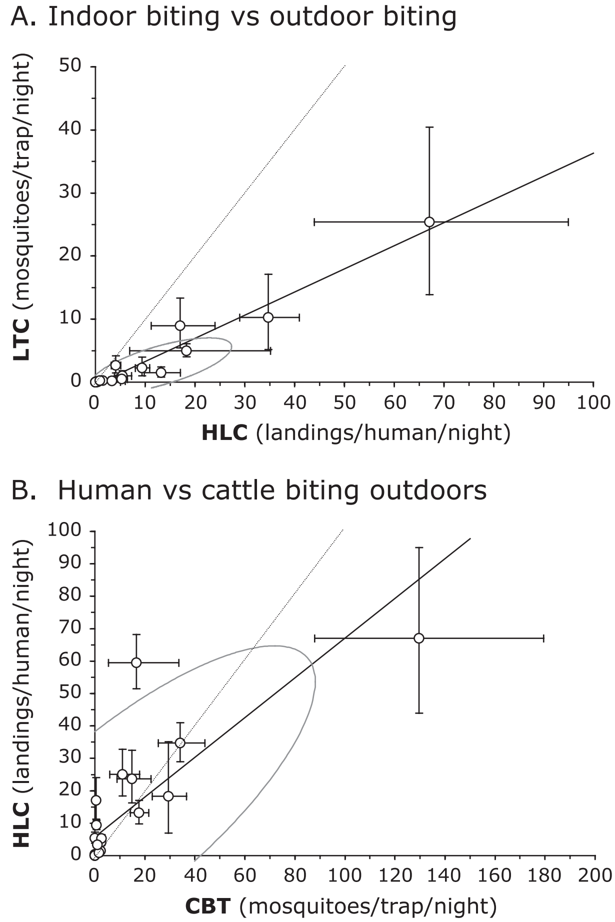

Fig. 4. Analysis of feeding behaviour. (A) Scattergram of mean daily light-trap catch (LTC) plotted against human landing catch (HLC). (B) HLC plotted against cattle-baited trap catch (CBT). Points show the mean daily catches (± 95% confidence interval) for the two techniques, paired by month and site, plotted against each other. The elliptical region is the bivariate plane containing 95% of the predicted observations and the solid and broken lines show the observed major axis regression line and the expected line if the catches from the two techniques were equivalent, respectively.

in Fuchucha (Fig. 4B). The mean CBT was 2.1 (0.49 ± 0.028, $n = 302$) compared with a mean HLC of 3.1 (0.61 ± 0.035, $n = 337$). The median Catch Index for CBT/HLC was 0.76 (interquartile range 0.500–1.220) and Pearson's correlation coefficient was 0.79 (95% CI 0.53–0.89).

Calibration of human landing and cattle-bait catches in Jarso. Direct comparison of HLCs and CBTs showed that CBTs caught, on average, 26.0 mosquitoes/trap/night (1.43 ± 0.149, $n = 12$ nights) compared with HLCs at 163.8 mosquitoes/trap/night (2.22 ± 0.107, $n = 12$) ($F_{1,10} = 35.9$; $P < 0.001$ for difference between means). There was a six-fold difference in this direct comparison, which is markedly greater than the 1.3 times difference observed with the year-long comparison. However, if we consider only the catches in Fuchucha during June and July, when the direct comparison was being undertaken in Jarso, then the mean HLC was ~2.7 times the CBT, with a mean catch/night of 10.2 (1.05 ± 0.060, $n = 54$) and 3.7 (0.67 ± 0.096, $n = 55$), respectively.

These two comparisons of cattle- and human-baited sampling systems show that the human-baited system caught more mosquitoes than the cattle-baited one, which might indicate that *An. arabiensis* is anthropophilic. However, we do not know the relative efficiency of the two techniques, and it may be that more mosquitoes were attracted to the vicinity of the cattle-bait than to the human, but the human may have been more efficient at catching mosquitoes. Thus, the greater catch from the human-baited systems does not provide unequivocal evidence for anthropophily.

Cattle- and human-baited OBETs. An alternative and arguably less biased measure of anthropophily is provided by comparing the catches of human- and cattle-baited OBETs. The total catch from the paired OBETs, over 12 nights, was 802 *An. arabiensis* females, with 14.3% (115/802) caught in the cattle-baited OBET compared with 85.7% (687/802) in the human-baited one. To assess the statistical significance of the difference, we specified the *y*-variable as the daily catch from the OBET on one side (west), with the total catch from both OBETs as the binomial denominator. Using this approach, 10.7% (± 3.8) were caught in the western OBET when it was baited with cattle odour, compared with 83.6% (± 3.2) when it was baited with human odour ($F_{1,10} = 460$, $P < 0.001$ for difference between means). The percentages do not sum to 100% because the estimates for proportion caught with cattle and human odour are independent of one another. These results suggest that *An. arabiensis* is strongly anthropophilic and are consistent with the similar, albeit equivocal, indications provided by the comparisons of HLCs and CBTs.

Human-blood index. A fourth measure of anthropophily is provided by the relative proportions of bloodmeals from humans and cattle. In Fuchucha, 66% of mosquitoes collected from indoors contained blood from humans and 59% contained bovine blood; the percentages sum to > 100% because 25% of mosquitoes contained blood from both hosts (Table 1). For samples collected outdoors, the percentages containing human or bovine blood were 51% and 71%, respectively.

Within Fuchucha, there was no marked evidence that local variation in the abundance of cattle and humans affected the proportion of meals from cattle: there were no significant differences in the proportion of cattle-fed mosquitoes collected from different pit shelters and the relative abundance of cattle and humans within compounds did not have a significant effect on the proportion of cattle-fed mosquitoes. For instance, 56% (47/84) of mosquitoes collected from human-only compounds contained bovine blood, compared with 54% (37/69) for those collected from compounds that contained more cattle than humans.

It is surprising that the proportion of human bloodmeals in the samples collected indoors was so low, given that livestock were rarely kept indoors. However, it is also surprising that the outdoor collections contained a relatively high proportion of human bloodmeals, despite the relatively high proportion of cattle outdoors at night. These results suggest that *An. arabiensis* show a strong tendency to leave the immediate vicinity of the host after feeding, with the consequence that significant numbers of cattle-fed *An. arabiensis* come into human dwellings to rest after feeding and many human-fed mosquitoes also leave dwellings and rest outdoors and/or that many humans are bitten before they go indoors at night. The HBI was lowest in Jarso (46%), although this is surprisingly high considering that the cattle : human ratio was 17 : 1.

The feeding ratio (FR, the proportion of bloodmeals from host A in relation to the proportion of host A in the host population) corrects for the proportion of hosts available (Hess *et al.*, 1968). In Fuchucha, the FR is close to 1 generally, suggesting that *An. arabiensis* did not exhibit a marked host selection whereas in Jarso the ratio was 8.3, indicating that more than eight times as many mosquitoes fed on humans than expected according to host availability (Table 1). The apparent difference may reflect the relative degree of protection afforded by sleeping indoors in Fuchucha vs. on tree platforms in Jarso.

The proportion of mixed human/cattle bloodmeals was relatively high in all contexts, ranging from 22% outdoors in Fuchucha to 37% outdoors in Jarso. It is remarkable, given the high ratio of cattle to humans in Jarso, that more than a third of the biting *An. arabiensis* fed on humans and cattle on the same night, which suggests that *An. arabiensis* does not always feed to repletion on the first host they encounter. The sample size of $n = 43$ is low, and this result must be treated with caution.

Seasonal patterns in biting cycle

At first sight, the bloodmeal results suggested that humans might have been more available to mosquitoes in Jarso than in Fuchucha, perhaps because people slept outdoors in Jarso. Even in Jarso, however, humans were to some extent more protected than cattle, as many people slept on platforms in the trees and

Table 1. Analysis of identified bloodmeals in *Anopheles arabiensis* from Fuchucha and Jarso, containing blood from human or bovid hosts and the respective feeding rations (FR). Outdoor and indoor samples were collected (*C*) from pit shelters and huts, respectively. The number of meals containing blood from humans or bovines is expressed as a percentage of the number of meals identified successfully (*Ci*). These percentages sum to > 100% because a number of meals contained blood from both hosts (i.e. 'mixed bloodmeals'). The number of simple, single-host meals from bovines and humans expressed as a percentage of all simple meals is shown in brackets.

	Host population						Bloodmeal source			
Village	Human	Cattle	Sample		*C*	*Ci*	Humans +	Bovine +	Mixed	FR*
Fuchucha	1065	603	Indoors	%			66.4 (55.2)	58.6 (44.8)	25.0	1.03 (0.9)
				n	250	232	154	136	58	
			Outdoors	%			51.2 (37.4)	70.8 (62.6)	22.0	0.8 (0.6)
				n	820	699	358	495	154	
Jarso	34	572	Outdoors	%			46.5 (14.8)	90.7 (85.2)	37.2	8.3 (2.5)
				n	43	43	20	27	16	

*Feeding ratio (FR) = proportion of bloodmeals from host A in relation to proportion of host A in total host population. The FR is estimated using either a percentage of all meals, including mixed meals, containing human blood, or simple meals only (in brackets).

there is evidence that this would reduce their availability to mosquitoes (Habtewold, 2005). Indeed, the people in Jarso specifically said that their sleeping arrangements were designed to reduce the probability of being bitten. However, people spend ~ 3 h, at ~19.00–22.00 hours, cooking and eating at ground level before retiring to bed. It is therefore pertinent to consider the temporal pattern of feeding across the night to determine whether there is a significant degree of feeding prior to ~22.00 hours.

An analysis of the pattern of human biting across the night, based on pooled data for *An. arabiensis* from HLC collections in Fuchucha (total catch = 4514) shows that, overall, ~20% of the catch came from the first few hours of the night (19.00–22.00 hours) before people generally went indoors (Fig. 5A), which is in agreement with the results of a smaller scale study carried out in nearby Sille (Taye *et al.*, 2006). This pattern varied with season, however. In the months normally associated with the lowest densities, > 50% of the catch was obtained before 22.00 hours. The most notable shift occurred between the months of May (~20% before 22.00 hours) and July (~60%), as shown in Fig. 5(B, C). The pattern of biting in Jarso was not the same as in Fuchucha, however (Fig. 5C); although there was also an early peak in biting before 22.00 hours, it was not as pronounced, and accounted for less of the night's total biting activity.

Sporozoite rates

Samples of *An arabiensis* females from HLCs, CBTs and LTCs were analysed for CS protein to identify *Plasmodium* infectivity. Samples were taken from the June and July catches (i.e. from the second half of the peak in density, when mosquitoes might be expected to be older generally and therefore more likely to be infected). The overall *Plasmodium* infection rate for the 6810 mosquitoes tested was ~0.78%, with ~0.28% *P. falciparum* and 0.50% *P. vivax* (Table 2), compared with the 0.50% and 1.76%, respectively, reported by Taye *et al.* (2006) in Sille (n = 796).

Discussion

Problems with characterizing behaviour

Several standard sampling techniques were used to assess the feeding behaviour of mosquitoes. Admittedly, most of these techniques were developed simply to measure changes in mosquito density, the overall biting rate on humans or the effects of control measures on entomological parameters of malaria transmission. Each of the techniques is likely to be affected by various sampling biases that may lead us to draw incorrect inferences, and are therefore not necessarily the ideal means of characterizing behavioural processes. For instance, it is generally assumed that the methods of collecting indoor-biting and indoor-resting mosquitoes sample the same population of mosquitoes, and therefore can be used interchangeably to make inferences regarding the numbers of mosquitoes entering and

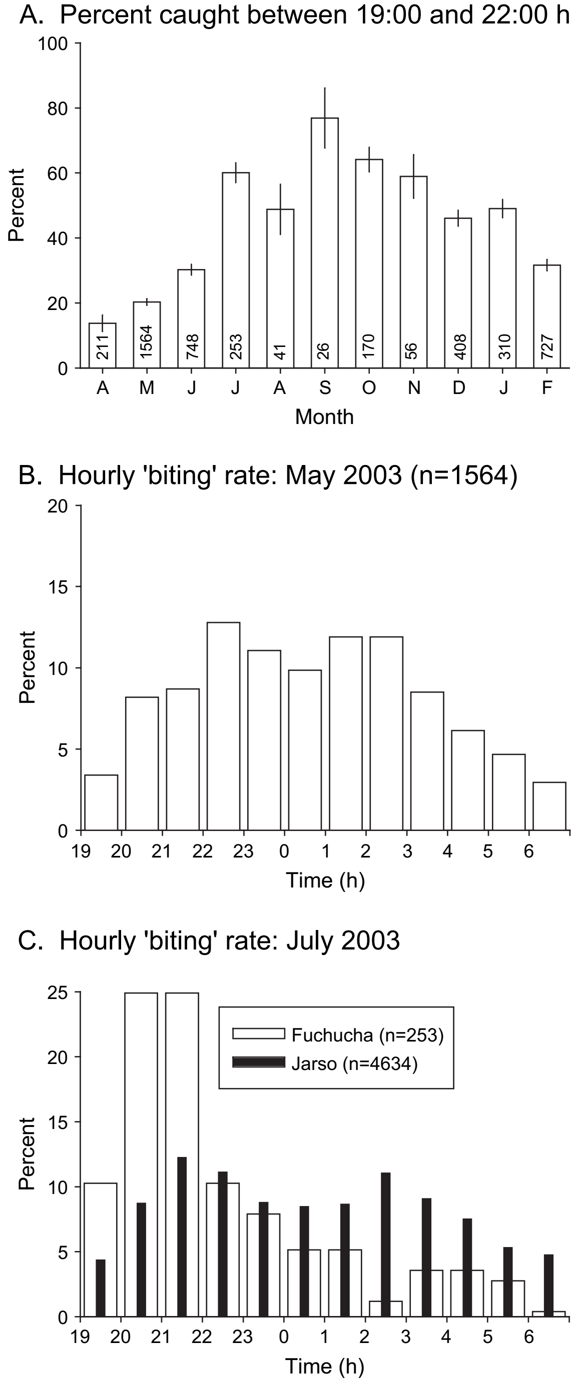

Fig. 5. Hourly biting activity throughout the night (19.00–07.00 hours), based on human landing catch (HLC). (A) Percentage of HLC collected between 19.00 and 22.00 hours, per month across the year in Fuchucha. The total number caught per month is shown at the base of the columns. (B) Percentage of pooled HLC for the month of May collected each hour of the night in Fuchucha (n = 1564). (C) Percentage of pooled HLC for the month of July collected each hour of the night in Fuchucha (open columns) and Jarso (closed columns).

Table 2. Rates of infection with *Plasmodium falciparum* and *P. vivax* in samples of *Anopheles gambiae* attracted to human or cattle hosts during June and July.

Village	Bait	Nights	*n*	*P. falciparum* # CS +	*P. falciparum* % CS +	*P. vivax* # CS +	*P. vivax* % CS +	Infected %
Fuchucha								
	Human	264	1840	7	0.38	13	0.71	1.09
	Cattle	14	550	1	0.18	5	0.91	1.09
Jarso								
	Human	32	2560	4	0.16	1	0.04	0.20
	Cattle	28	1860	7	0.38	15	0.81	1.18

CS = circumsporozoite.

resting in a dwelling, which is clearly not always valid. In addition, like many others, we have previously remarked on the tautological problem of using analyses of bloodmeals to assess the degree of anthropophily.

In the present study we attempted to overcome these problems by including behavioural assays designed to assess the strength of response to the respective host types, but these too are susceptible to bias. Although OBETs are intended to assess the trap entry response of mosquitoes in the presence of host odours, there is likely to be an interaction between these two behaviours: of the mosquitoes that approach an OBET, the proportion that enter may be affected by the type of host odour present.

Moreover, with the declining use of the HLC as a standard sampling tool, for logistical and ethical reasons, the techniques available for reliably measuring biting rates of human-biting mosquitoes are likely to diminish. If we are to obtain reliable information about mosquito behaviour to enable us to develop better means of controlling malaria and other mosquito-borne diseases, then there is a pressing need to develop and use objective methods to quantify behaviour in much the same way that electric nets and video have been used to study tsetse (Vale, 1993; Torr, 1994; Gibson & Torr, 1999).

Bearing in mind these caveats, there is still value in employing techniques that have been used widely across Africa, particularly in the case of *An. arabiensis*, the better to relate our findings to the wider body of knowledge about a species notable for its geographical variation in behavioural and ecological attributes.

Feeding behaviour

Anthropophagic or zoophagic? The present results provide apparently contradictory indications of the host preference of *An. arabiensis* in southern Ethiopia. On the one hand, there are several results that suggest that this species is strongly anthropophagic. First, HLCs caught significantly more mosquitoes than CBTs. This difference may be due to differences in the efficiency of the two sampling techniques. However, by comparing the catch of human- and cattle-baited OBETs, we were able to use the same sampling technique with different baits. The results show that the human-baited trap caught significantly more mosquitoes than the cattle-baited one, suggesting that *An. arabiensis* in Konso is inherently anthropophagic. This inference is also consistent with the results of Habtewold *et al.*, 2004) which showed that placing an ox adjacent to an HLC did not reduce the catch of *An. arabiensis,* whereas the catch of the highly zoophagic species *An. pharoensis* was reduced.

By contrast, analyses of bloodmeals did not indicate marked anthropophagy: only ~66% of mosquitoes collected from huts in Fuchucha had fed from humans, despite the fact that virtually no livestock were in the huts and across the village as a whole the ratio of cattle : humans was ~0.6 : 1. For mosquitoes collected outdoors, in either Fuchucha or Jarso, about half the mosquitoes had fed on humans, although given the preponderance of cattle in Jarso (cattle : human ratio ~17 : 1), the high percentage of human meals from there can be interpreted as evidence of anthropophily as reflected by a feeding ratio >>1.

How might relatively high levels of zoophagy (i.e. 60–90% of meals from cattle) result in a population of mosquitoes that is inherently anthropophagic? First, if mixed meals represent interrupted meals that are resumed on another host, in the case of Jarso, the chances are high that the second meal will be bovine.

Endophagic or exophagic? Second *de facto* zoophagy may also arise in Konso because the human hosts are less available as a consequence of being indoors. The HLCs, conducted *outdoors* caught about four times as many mosquitoes as the LTCs conducted *indoors*. Notwithstanding the lower efficiency of the light-trap, these results suggest that a human is at less risk of being bitten indoors than outdoors. Thus, in situations where humans are predominantly indoors and cattle outside, zoophagy might arise from an inherently anthropophagic population of *An. arabiensis*.

The high rate of biting humans in Jarso, despite the high cattle : human ratio, shows that when humans were available, they were indeed the preferred host. Although in Jarso people reduce their availability to mosquitoes by sleeping on raised platforms, they would be available to *An. arabiensis* during the first 3 h of the night before they retire to bed. The proportion of biting that occurs in the early part of the night in our study area appears to be highly variable, however, between sites and over time (Fig. 5), for reasons which remain unidentified. This variability in biting pattern was also observed for *An. arabiensis* in Zimbabwe (Crees, 1996) and in Madagascar (Marrama *et al.*, 2004).

Endophilic or exophilic? The present results provide indications that in Konso *An. arabiensis* exhibits postprandial exophily. The most compelling evidence is that the mean number of mosquitoes entering a hut, as measured by the catch of a human-baited light-trap, was about five times greater than the number collected resting in a hut, suggesting that the mosquitoes entered, fed and then left. A significant proportion of the mosquitoes caught in the pit shelters were human-fed, which is also consistent with postprandial exophily. However, the more surprising result is the large proportion of cattle-fed mosquitoes (~45%) caught indoors, where virtually no cattle were kept. Waka *et al.* (2005), working in Eritrea, also reported similar proportions of human- and animal-fed *An. arabiensis* from pit shelters and indoor spray catches, although few animals were kept indoors. Perhaps these counter-intuitive results reflect our anthropocentric definitions of 'indoors' and 'outdoors'. Is a roofed pit shelter markedly different from a hut to a mosquito, except that hosts are not present in the former? Perhaps a more parsimonious inference is that there is a general tendency for *An. arabiensis* to disperse away from a host after feeding.

Practical implications

Insecticide-treated cattle. The main aim of this study was to quantify the proportion of meals taken from cattle and hence to predict whether the treatment of cattle with pyrethroids might control malaria.

If we look at the results of the bloodmeal analyses only, they appear to be encouraging as they imply that 47–91% of meals came from cattle. This suggests that the daily mortality on the mosquito population produced by treating cattle with insecticide would be at least as great as that produced by insecticide-treated bednets, if we regard the latter as equivalent to treating human hosts with insecticide. Moreover, the observation that cattle- and human-fed mosquitoes had similar rates of infection suggests that there are not subpopulations feeding on different hosts.

The results from the OBET experiment and the comparisons of the human- and cattle-baited traps suggest that *An. arabiensis* is inherently more likely to feed from humans than cattle if both are equally available. Indeed, the results of the OBET experiment indicate a marked bias towards humans, with ~80% of mosquitoes being caught in the human-baited trap. Hence, on the basis of these observations, killing mosquitoes as they attack humans indoors is likely to be more effective than treating cattle. The results of the OBET experiment agree with those from similar studies of *An. arabiensis* elsewhere in Ethiopia (Habtewold, 2005), Burkina Faso (Costantini *et al.*, 1998a), and Zimbabwe (Costantini *et al.,* 2005). The implication emerging from these results is therefore that *An. arabiensis* across large parts of Africa is inherently anthropophagic. However, our results suggest that geographical variation in the tendency for *An. arabiensis* to feed indoors may have a profound effect on the proportion of bloodmeals taken on cattle. This interaction between host preference and biting site preference means that in areas such as Konso, an anthropophagic population of mosquitoes actually feeds largely on cattle, and hence, insecticidal cattle are likely to exert a greater mortality than insecticide-treated humans, particularly where a substantial proportion of biting occurs in the early part of the night when most people are still outdoors and therefore not under treated bednets.

The variability in the nightly biting pattern seen here and elsewhere in *An. arabiensis* (Crees, 1996; Marrama *et al.*, 2003) suggests that the behaviour is highly labile. There is also evidence that the use of insecticide-treated bednets has enhanced exophagy and/or zoophily, as evidenced by a reduction in the HBI of *An. arabiensis* in Natal, South Africa (Sharp & Le Sueur, 1991), and in *An. gambiae s.s.* populations in Gambia (Lindsay *et al.*, 1989) and Kenya (Bøgh *et al.*, 1998), with concomitant increase in cattle bloodmeals. Accordingly, the use of insecticide-treated cattle may provide a means of 'mopping up' mosquitoes that evade contact with insecticides indoors.

Killing mosquitoes does not necessarily control malaria and the precise impact of insecticide-treated cattle and/or bednets will vary according to the particular epidemiological circumstances obtaining in any given location. We explore this matter further in a subsequent paper.

Classical zooprophylaxis. The present data also have implications for zooprophylactic approaches to controlling malaria, whereby untreated livestock divert malaria mosquitoes away from humans. In Jarso, humans were greatly outnumbered by cattle but the overall sporozoite infection rate in the mosquito population was 0.6% (27/4420). During June, when *An. arabiensis* were most abundant, the mean HLC was ~187 mosquitoes/night, implying an inoculation rate of ~1 infective bite/night. Habtewold *et al.* (2001) analysed the bloodmeals of *An. arabiensis* from Jarso and found that 95% of meals were from livestock and only 5% were from humans. They argued that this suggested that 'active zooprophylaxis was effective in protecting people from bites of *An. arabiensis*'. However, the present data suggest that although the majority of meals are from livestock, the biting rate on humans is still sufficiently high that for humans living in Jarso the probability of contracting malaria approaches 1 at certain times of year.

Through reasonably intensive sampling of *An. arabiensis* (> 45 000 female mosquitoes over 12 months) in two diverse habitats, we have established several key points: (a) although the majority of bloodmeals may have come from cattle, this population of mosquitoes is inherently more strongly attracted to humans, so much so that the feeding ratio on humans is eight times higher than expected in an area where cattle outnumber humans by 17 : 1; (b) the high proportion of cattle bloodmeals probably reflects a high degree of exophagy and exophily, which will compromise the efficacy of control measures targeted at mosquitoes indoors; (c) the sporozoite rates in human- and cattle-fed mosquitoes are not significantly different, so there is no evidence of the existence of behavioural subpopulations with different host preferences; (d) the HBI of resting mosquitoes (indoors vs. outdoors) is not well-matched with the distribution of hosts and therefore we conclude that mosquitoes may move away from the feeding habitat quite soon after feeding, and (e) the nightly biting pattern shows that humans are exposed to a significant proportion of biting mosquitoes when they are outdoors and unprotected in the early evening.

On the basis of these results, we conclude that, if insecticide-treated cattle could make a significant impact on the rate of malaria transmission by *An. arabiensis* anywhere, it would be in a place like Jarso.

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