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The impact of host species and vector control measures on the fitness of African malaria vectors

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Summary

Many malaria vector mosquitoes in Africa have an extreme preference for feeding on humans. This specialization allows them to sustain much higher levels of transmission than elsewhere, but there is little understanding of the evolutionary forces that drive this behaviour. In Tanzania, we used a semi-field system to test whether the well-documented preferences of the vectors *An. arabiensis* and *An. gambiae* s.s. for cattle and humans respectively are predicted by the fitness they obtain from host-seeking on these species relative to other available hosts. Mosquito fitness was contrasted when humans were fully exposed, and when they were protected by a typical bednet. The fitness of both vectors varied between host species. The predicted relationship between host preference and fitness was confirmed in *An. arabiensis*, but not in *An. gambiae* s.s. whose fitness was similar on humans and other mammals. Use of typical, imperfect bednets generated only minor reductions in *An. gambiae* s.s. feeding success and fitness on humans, but was predicted to generate a significant reduction in the lifetime reproductive success of *An. arabiensis* on human relative to cows. This supports the hypothesis that such human-protective measures could additionally benefit malaria control by increasing selection for zoophily in vectors.

Key words: host specialization, selection, mosquito vectors, malaria, bednets
Introduction

Evolutionary change by pathogens and their invertebrate vectors is generally perceived as detrimental to disease control [1, 2]. However, control measures could potentially generate selection upon disease vectors that provides them a fitness reward for adopting phenotypes that reduce their transmission ability [3-5]. This approach could be amenable for vector-borne diseases where the host specificity of parasites and their vectors differ. This mismatch presents an opportunity to reduce disease transmission by generating selection on vectors to shift their host use towards non-permissive species through ecological manipulation of the fitness benefits of host selection.

A potential candidate for such an approach is malaria, a disease caused by *Plasmodium* parasites transmitted by *Anopheles* mosquitoes. The host range of *Anopheline* species varies from avian and mammalian generalists, to those specific to one host species [6]. In contrast, most human infectious *Plasmodia* can survive only in humans (*P. knowlesi* being an exception [7]). Consequently the degree to which *Anopheles* vectors specialize on humans (anthrophily) is a prime determinant of malaria transmission intensity [8], and any shift from anthrophily to feeding on other animals will reduce transmission (e.g. zooprophylaxis [9]). Current malaria control strategies are based on reducing human exposure to mosquito bites and/or mosquito density [10].

Here we investigate the potential for these approaches to generate additional benefits by creating an evolutionary incentive for mosquito vectors to switch their host species use from humans to other animals commonly available in malaria endemic settings.

Prediction of the potential impact of control measures on the evolution of mosquito host range requires an understanding of the selective forces underpinning it. The host species range of haematophagous insects has undoubtedly been shaped by natural selection, but there has been relatively little empirical investigation of how host selection influences their fitness [6, 11]. Theoretically, host specialization is predicted to
arise due to a trade-off between the performance of foragers on different host types [12, 13], with selection being generated for the development of preferences for those which provide the greatest fitness reward. By extension, environmental changes that diminish the fitness advantage associated with particular hosts could undermine selection for their preference. In the case of African malaria vectors, bednet usage is an example of an environmental change that could reduce fitness advantages associated with anthropophily. Should the expected fitness returns that mosquitoes obtain from attempting to feed on humans protected by bednets fall below those from foraging on other available animals, wide use of these interventions could generate selection on vectors to adopt more generalist feeding behaviours and/or switch their specialization to other host species. Both these phenomena could substantially reduce malaria transmission.

There has been speculation about the causes of anthropophily in African vectors [reviewed in 6], but it remains unclear which factors are most responsible for driving it. Hypotheses include innate physiological or behavioural properties of humans that influence the fitness value of bloodmeals acquired from them, their high relative abundance, and/or the environmental suitability of their habitats (houses) [6]. These can be grouped into two non-mutually exclusive routes through which selection for host specialization could arise: (1) on the basis of the relative abundance of hosts [12]; and (2) on the basis of the expected fitness obtained per host encounter. Bednet use does not directly influence human abundance, but will reduce the efficiency with which mosquitoes can extract blood on encounter. As vertebrate blood is vital for malaria vector reproduction and survival [6,14,15], interventions that interfere with the efficiency of blood extraction from a host could impair mosquito fitness and generate selection on host species use.

Although the coverage of insecticidal nets in Africa has increased dramatically over the past 10 years [16, 17], untreated or poorly treated bednets remain the most
common protective measure against mosquito biting in many locations [17]. We experimentally investigated how the fitness of the two most important African malaria vectors, *An. gambiae* s.s and *An. arabiensis*, varied on encounter with different host species, and whether the use of such bednets reduced the relative fitness expected from foraging on humans relative to commonly available animal alternatives. We also tested whether the well-established preferences of these vectors towards specific host species are positively correlated with a fitness advantage from feeding upon them. These vectors are closely related and widely distributed throughout Africa [18], but vary in their host preference with *An. gambiae* s.s being almost exclusively anthrophilic [8], and *An. arabiensis* generally preferring cows over humans when both are available [19].

**Materials and Methods**

The study was conducted at the Ifakara Health Institute (IHI) in the Kilombero valley, Tanzania, where high levels of malaria transmission are sustained year-round by *Anopheles arabiensis*, *An. gambiae* s.s, and *An. funestus*. Experiments were conducted using *An. arabiensis* and *An. gambiae* s.s from colonies at the IHI. The *An. arabiensis* colony was established a few months before the start of experiments with individuals from Sagamaganga village (~15km from IHI) and is maintained in a semi-field insectary [20]. The *An. gambiae* s.s colony was established with individuals from Njage village in 1996 (~70 km from IHI) and is maintained in an indoor insectary (26 ± 2.5°C, 80 ±10% R.H.). Both colonies are maintained on human-blood provided thrice weekly by arm feeding.

**Experimental set up**

An experimental hut (3.5 X 4 X 2.5 m) was built in a netting-enclosed chamber (9.1 X 9.6 X 3.7 m) of the IHI semi-field system (SFS, ESM1 in supplementary materials).
Mosquitoes could enter and exit the hut through its open eaves as they do in nature [21], or exit via the six windows. Mosquitoes leaving the hut were caught outside or in window exit traps. Mosquito feeding success and fitness were evaluated on humans and 4 other species commonly kept in or near houses in the Kilombero Valley: chickens, cattle, dogs and goats. Two sub-categories of cattle were tested: adult cows and calves. Within other host types, animals were roughly the same age and size. Humans were presented either exposed or sleeping under an untreated bednet. ‘Typical’ bednets were created following the World Health Organization’s standard protocol for simulating the average condition of bednets in operational use by cutting 6 moderately sized holes into the sides (4 X 4 cm) [22].

For each experiment, an individual from one of the 7 host types was placed inside the hut at dusk. Human volunteers were provided with a bed and instructed to sleep and react to mosquito biting as normal (e.g. swatting as desired). Two hundred unfed *An. arabiensis* or *An. gambiae s.s* females (4-6 days old) were then released into the chamber corners (maximum ~4.5 m from host). The next morning, the chamber and hut were intensively searched to recapture mosquitoes (by aspirator). Those recaptured were identified as being bloodfed, unfed, live or dead. Six replicates (on different host individuals) were performed for each of the 7 host types, for each mosquito species (84 trials in total). Experiments were run in one-week blocks within which seven nights of consecutive trials were performed. The order in which host species were used was randomly allocated over the week to minimize potential for carry-over effects.

**Fitness measurements**

Mosquito feeding success was measured as: (1) the proportion of mosquitoes recaptured alive and blood fed, (2) the proportion of mosquitoes dead at recapture, and (3) blood meal size. For blood meal size measurement, mosquitoes visually identified as
blood fed were moved into individual 30 ml tubes for 3 days (provided with 10% glucose solution) in the semi-field insectary. Mosquitoes were subsequently moved into individual paper cups lined with damp filter paper to stimulate oviposition, and the hematin content of excreta deposited in initial holding tubes measured to provide an index of the mass of blood ingested [23]. Oviposition cups were inspected daily and the number of eggs laid within them counted. Mosquitoes remained in holding cups and were monitored daily until death to estimate their host-species dependent survival.

**Ethical considerations**

This study was approved by the Institutional Ethical Review Board (IRB) of the IHI (IHRDC/IRB/No.A015), the Medical Research Coordination Committee of the Tanzania National Institute for Medical Research (NIMR1HQ/R.8a/Vol.IX/708) and the University of Glasgow (for details see ESM 2).

**Statistical Analyses**

Variation in the probability of blood feeding, death on recapture and oviposition (all binomial), and blood meal size and fecundity (continuous) were analysed using generalized linear mixed effect models (GLMM) with appropriate link functions in the R software package [24]. Here ‘host species’ and ‘mosquito species’ were treated as fixed effects, and ‘host individual’ as a random effect. For each response variable, a maximal model was generated and the significance of fixed effects evaluated through stepwise deletion of terms using Likelihood Ratio Tests (LRTs). For variables in which host species was identified as statistically significant, Dunnett’s post hoc test (adjusting for multiple comparisons) was used to identify statistically significant two-way differences between the unprotected human reference group and other host types. The Cox Proportional Hazards Model was used to test for differences in the post-feeding survival
of mosquitoes due to host species. In these models, a frailty function [25] was used to incorporate the random effect of host individual, and host and mosquito species were fit as main effects [24]. Reported chi-square values refer to LRTs conducted on the output of GLMMs, and z-values are for two-way comparison between a human reference group and other host species. ‘OR’ values are odds ratios from Cox proportional hazard models.

**Modeling the impact of host species on lifetime reproductive success**

A mosquito life-history model was constructed assuming that to produce eggs, a female must acquire a blood meal during one night of seeking on the $j^{th}$ host type (with probability $\beta_j$), survive the period between feeding and oviposition of $d_{ov}$ days (with a daily survival probability $s_{ov,j}$) and oviposit (with probability $\gamma_j$) a total of $F_j$ eggs. We assumed females who fed but did not obtain enough blood to trigger oviposition on one night (with probability $= 1 - \beta_j \gamma_j$) can attempt to feed again on ‘k’ successive nights until they succeed or die. After oviposition, females can initiate another feeding cycle. While the daily survival of unfed mosquitoes ($s_f$) was assumed to be independent of host type, survival of mosquitoes between blood consumption and oviposition was assumed to be dependent on host type ($s_{ov,j}$). The expected number of eggs resulting from the first feeding cycle $R(j)$, is thus:

$$R(j) = \sum_{k=0}^{\infty} \left( (1 - \beta_j \gamma_j) s_f \right)^k \beta_j \left( s_{ov,j} \gamma_j F_j \right)$$

and the lifetime reproductive success (LRS, estimated by $R_0$) expected from multiple feeding cycles $i$ is given by $R_{0j} = \sum_{i=1}^{\infty} s_f^{i-1} R(j)$, assuming age-independent survival.

Most values for host-specific mosquito fitness traits were directly estimated from experiments described above, with the exception of survival between feeding and
oviposition ($s_{ov}$). Rather than directly applying survival probabilities that were
experimentally measured under semi-field conditions here (likely an overestimate of their
value in nature), we estimated the odds of survival after feeding on different hosts
relative to having fed on an unprotected human and used this to adjust published values
of the daily survival of human-fed mosquitoes in the field (ESM 3).

Confidence intervals around the predicted mean values of $R_{0j}$ were generated by
conducting ten thousand simulations of the total lifetime reproductive success ($R_0$) of an
individual $\text{An. arabiensis}$ or $\text{An. gambiae}$ s.s. when feeding exclusively on each host
type. Further simulations were conducted to assess the total lifetime reproductive
success ($R_0$) of $\text{An. arabiensis}$ when mixing its feeding between humans using a bednet
and cows to varying degrees. Unlike $\text{An. gambiae}$ s.s. which rarely feeds on anything
other than humans in nature, $\text{An. arabiensis}$ is known capable of feeding on humans and
cattle to varying degrees depending on their local availability [6]. Uncertainty within each
simulation was introduced by selecting the value of each host-specific parameter
randomly from a Bernoulli (probability of feeding, surviving and oviposition) or normal
distribution (number of eggs laid) with a mean and standard error from the appropriate
statistical model. Observations over the first couple of trials of both vector species
indicated that more than ~90% of fecund mosquitoes laid their eggs on the first day that
an oviposition substrate was provided (4 days after feeding). It was thus assumed that
the period between feeding and oviposition was independent of host species in this
model.

To test for statistically significant differences in $R_0$ between host types,
bootstrapping analyses were performed on the 21 possible two-way host comparisons
between the 7 host types. Values of $R_0$ for host type 1 and 2 were randomly drawn from
their simulated distributions. The proportion of 10,000 such draws in which the $R_0$ of one
host type was greater than the other was used as an estimate of the probability that the
LRS of mosquitoes on these host types was significantly different (if $p < 0.05$).

Results

The foraging success and subsequent fitness of 16,517 Anopheles vectors was
tracked over 84 trials (ESM 4&5), and used to parameterize a life-history model for
prediction of mosquito LRS on different host types. For all mosquito traits analyzed,
there was a statistically significant interaction between mosquito and host species
($p<0.001$ in all cases except for fecundity where $p=0.03$). Consequently all subsequent
statistical analyses were performed for each mosquito species separately. The random
effect of ‘host individual’ was highly significant ($p<0.001$) for all response variables
examined except for the proportion of An. arabiensis found dead at recapture ($p=0.02$)
and all results are from models including this random effect. Data and model results
were used to address three questions.

a) Does host species influence mosquito vector fitness?

The proportion of mosquitoes recaptured did not vary between host species in
An. arabiensis ($\chi^2_6 = 9.76$, $P = 0.13$, Fig 1a) or An. gambiae s.s ($\chi^2_6 = 9.49$, $P = 0.15$, Fig
1b). However host species was a significant predictor of feeding probability in both An.
arabiensis ($\chi^2_6 = 52.80$, $P < 0.001$, Fig 1c) and An. gambiae s.s ($\chi^2_6 = 23.89$, $P < 0.001$,
Fig 1d). The proportion of mosquitoes dead on recapture was independent of host
species (An. arabiensis: $\chi^2_6 = 1.89$, $P = 0.93$, Fig 1e; An. gambiae s.s: $\chi^2_6 = 8.21$, $P =
0.22$, Fig 1f).

Whereas An. arabiensis obtained similarly sized blood meals from all hosts ($\chi^2_6 =
3.28$, $P = 0.77$, Fig 2a), An. gambiae s.s acquired larger meals from humans and cows
than any other hosts ($\chi^2_6 = 22.50$, $P < 0.001$, Fig 2b). Host species influenced the
probability of oviposition after blood feeding (An. arabiensis: \( \chi^2_6 = 14.85, P = 0.02 \), Fig 2c; An. gambiae s.s: \( \chi^2_6 = 21.03, P = 0.002 \), Fig 2d), but not the number of eggs laid (An. arabiensis: \( \chi^2_6 = 1.46, P > 0.05 \), An. gambiae s.s: \( \chi^2_6 = 5.73, P > 0.05 \), Fig 2e & f). The impact of host species on mosquito survival also varied between mosquito species. Whereas An. arabiensis had similar survival on all host species \( \chi^2_6 = 8.6, P = 0.2 \), Fig 3a, Table 1), in An. gambiae s.s the odds of mortality between the ‘best’ (humans and cows) and ‘worst’ host types (chickens) differed by 1.7-fold \( \chi^2_6 = 106.4, P < 0.001 \), Fig 3b, Table 1). Combining these impacts of host species on mosquito fitness, the life-history model predicted the LRS of both An. arabiensis and An. gambiae s.s to vary significantly between host species (Fig 4a & c, ESM 6).

b) Is mosquito fitness highest on naturally preferred host species?

In accordance with their natural feeding preference, An. arabiensis had greater feeding success on cows than any other host species \( P < 0.001 \) in all cases, Fig 1c). However, An. arabiensis did not obtain larger blood meals \( P > 0.05 \) in all pair wise comparisons, Fig 2a), have higher oviposition probability \( P > 0.05 \), Fig 2d), egg production \( P > 0.05 \) in all cases, Fig 2e) or survival \( P > 0.05 \), Fig 3a) on cows than other host types. As a consequence of their higher feeding success, however, the LRS of An. arabiensis was predicted to be highest on cattle hosts (Fig 4a, ESM 6).

The feeding probability of An. gambiae s.s on their naturally preferred humans was no higher than on any other host type except chickens (Fig 1d). Anopheles gambiae s.s obtained significantly larger blood meals from exposed humans than from other host types except cows \( z = -1.76, P = 0.31 \), Fig 2b), but their oviposition probability and fecundity after feeding on humans was no higher than any other host species (Fig 2d & f). The survival of An. gambiae s.s, was significantly higher after feeding on exposed humans than on other host type except cows \( \chi^2_1 = 1.13, P = 0.29 \),
Fig 3b, Table 1). When all estimates of host–dependent fitness were combined to predict the LRS of *An. gambiae* s.s., there was no evidence of an advantage associated with human feeding (Fig 4c, ESM 6).

c) Could the use of bednets alter the fitness value of humans relative to other host species?

*Anopheles arabiensis* was significantly more likely to feed on cows than on humans (*z* = -3.89, *P* = 0.002, Fig 1c). This difference was even more pronounced when humans used bednets (*z* = -6.07, *P* < 0.001, Fig 1c). The fecundity (Fig 2c & e) and survival (Fig 3a & Table 1) of *An. arabiensis* that fed despite the presence of bednets was not significantly lower than on an unprotected human or other host species.

Although the LRS of *An. arabiensis* was predicted to be highest on cows (Fig 4a), the advantage of cattle over humans only achieved statistical significance when the latter was assumed to use bednets (ESM 6). Analysis of mixed human-cattle feeding strategies indicated that *An. arabiensis* which take > 60% of their bloodmeals from cows should have a significantly higher LRS than those who attempt to feed only on bednet-protected humans (Fig 4b, ESM6).

Use of bednets was associated with a moderate, but not statistically significant reduction in *An. gambiae* s.s. feeding success on humans (*z* = 1.49, *P* = 0.47, Fig 1d). The oviposition and fecundity of *An. gambiae* s.s that fed on people using bednets was no different from those who fed on unprotected people (Fig 2d & f). However, the survival of *An. gambiae* s.s that succeeded in feeding on humans using nets was significantly reduced relative to those who fed on fully exposed humans (Table 1). The use of bednets was predicted to reduce the human-associated LRS of *An. gambiae* s.s. to below that predicted for several other host species (cattle, dogs and goats, Fig 4c),
however these differences were not statistically significant after correcting for multiple
comparisons (ESM 6).

Discussion

We show that the fitness that the malaria vectors *An. arabiensis* and *An. gambiae*
s. s derive from host encounter varies significantly between the host species most
commonly available to them. However, evidence of positive correlations between the
known natural host preferences of these vectors and their expected fitness from feeding
on them was mixed. Whereas the LRS of *An. arabiensis* was predicted to be highest on
its preferred cow hosts, that of *An. gambiae* s. s was estimated to be relatively similar on
their preferred humans and most other host species. This challenges the assumption
that innate host–specific behavioural or physiological properties are responsible for the
evolution of anthropophily in this important vector.

Evidence that untreated bednet use could reduce the relative rewards of
anthropophily was also mixed. For *An. arabiensis*, a significant fitness advantage from
foraging on cow instead of humans was only predicted if the latter use nets. Thus in
addition to the personal protection provided by such bednets [26], they may also be
capable of imposing a cost on anthropophily that could exert selection for reduced human
feeding in settings where bednet coverage is high and cattle readily available. In
contrast, protecting humans with ‘typical’, untreated bednets was predicted to have
minimal impact on the fitness of *An. gambiae* s. s and unlikely to reduce the fitness
ranking of humans relative to other animal alternatives.

While not all of the mosquito fitness traits investigated here varied between host
species, at least one did so for each vector. In *An. arabiensis*, host species primarily
determined their probability of acquiring a blood meal, but not their post-feeding fitness.
In contrast, under these experimental conditions *An. gambiae* s.s had a similar feeding
probability on all mammalian hosts, but variable reproductive success and survival afterwards. This suggests there may be trade-offs in the value of host resources for different life-history processes. Although mosquito blood meal size and egg production have been widely correlated in previous work [27-29], the smaller blood meals associated with some host species here did not consistently translate into reduced egg production. Most previous studies have examined this relationship only within one host species, and it is possible there are additional sources of haematological variation between host species that cause this relationship to breakdown when comparing blood meals taken from across them.

Evolutionary theory predicts that the fitness of specialists is highest when preferred resources are consumed [30]. Our life history model predicted this to be true for *An. arabiensis* whose LRS was estimated to be substantially higher on its naturally preferred cow hosts. However although some *An. gambiae* s.s fitness traits were highest on their preferred humans (blood meal size), there was no evidence of an overall advantage to their LRS associated with these hosts. Failure to detect correlations between host preference and performance have been documented in other insect systems [31], and attributed to ecological variation that modifies the quality of hosts in different environments. Similarly, our ability to detect host preference-performance relationships in *An. gambiae* s.s may have been limited by experimental conditions. Here we presented hosts to mosquitoes under a ‘no choice’ scenario in an indoor environment. This design was used to distinguish between fitness effects arising from innate biological properties of hosts (e.g. physiological and/or behavioural), from those arising indirectly due to variation in their use of habitats. While livestock are frequently kept inside buildings at night in our study area, in other settings livestock may be kept outside during vector activity periods. *Anopheles gambiae* s.s has a strong preference for biting indoors [32, 33], whereas *An. arabiensis* bites hosts both indoors and outside.
If *An. gambiae*’s preference for feeding on humans is an indirect consequence of an advantage arising from indoor biting (irrespective of host species), the relative advantages of anthrophily in this vector species may be underestimated here relative to other environments where animals are generally outside. Further investigation within this environmentally - realistic yet experimentally tractable system can help evaluate this hypothesis.

While this study demonstrates that untreated bednets have potential to diminish the relative fitness benefits of anthrophily in some malaria vector species, there has been relatively little evidence of such phenomena occurring in response to the use of this intervention in nature [reviewed in 6]. A potential explanation is that our results indicate that the use of this intervention has relatively minor impacts on many mosquito fitness traits (e.g. in *An. gambiae* s.s) and only led to statistically significant disadvantage of humans relative to animal hosts in a limited range of scenarios (*An. arabiensis* choosing between humans and cows). This reinforces the need to maintain good quality, intact and insecticidal-treated nets to reap the greatest epidemiological and evolutionary benefits for control.

Evaluation of the accuracy with which effects described here reflect the nature of selection acting on host species range in nature will require further investigation of several areas that at present are intractable within the semi-field conditions used here. First, the host-specific feeding probabilities estimated here may be upwardly-biased because they were measured under ‘no choice’ conditions (e.g. our observation that *An. gambiae* s.s. fed on all host species with similar probability contrasts with their known preference for humans in nature [8]). Giving *An. gambiae* s.s a choice between hosts may have significantly increased predicted feeding rates on humans at the expense of those estimated for other animals. However, this may not substantially alter our conclusions about the relative benefits of anthrophily as *An. gambiae* s.s. were shown
capable of feeding on other animals to the same degree as humans when no choice was available, with no consistent reduction in their fitness relative to those obtained from human bloodmeals. Ideally this expectation could be confirmed by simultaneous measurement of mosquito host choice and subsequent fitness. Presently this is not possible because the host choice of mosquitoes captured blood fed can only be confirmed by killing them to analyze their stomach contents, which prevents any further measurement of their fitness. Should non-invasive methods become available for bloodmeal identification, follow up investigation of mosquito fitness under choice scenarios should be pursued. Further investigation of other potential advantages of anthrophily beyond which could be measured here, including habitat-dependent foraging success (higher inside houses), or benefits from host seeking on aggregated populations, is encouraged. Finally, the requirement for large numbers of similarly aged, malaria-free mosquitoes required the use of insectary-reared mosquitoes in this study. Although both insectary colonies were initiated from mosquito populations in the local area and maintained on a natural blood source, the process of colonization can modify host discrimination behaviour [34]. Where possible, further study using F1 mosquitoes from wild populations is encouraged to identify potential biases arising from the use of colonized mosquitoes.

Our model predictions are based on several assumptions that also require validation for assessment of potential implications of these results to field. One is that the host-specific impacts on mosquito fitness measured are similar on all feeding cycles. Here mosquito fitness was measured after one blood meal, whereas in nature vectors feed every 2-4 days [35]. Repeated blood feeding could potentially cancel out or magnify the host-specific effects described here. A previous laboratory study showed that An. gambiae s.s. fed one bloodmeal using an artificial membrane feeder exhibited
similar host-specific survival as documented here [15]. However when mosquitoes were
given 2 blood meals consisting of blood from humans followed by another animal their
longevity was similar [15]. This suggests that negative fitness effects arising from
bloodmeals on poor quality hosts could be reduced by further meals from a ‘high quality’
hosts. Furthermore, mosquitoes may be able to increase their feeding frequency from
what was assumed here to compensate for lower quality bloodmeals. Had mosquitoes
been provided with an oviposition substrate earlier than the standard 4-day post-feeding
period used here, it is possible those fed on poorer quality host types could have brought
forward their oviposition to increase future feeding opportunities. This phenomenon has
not yet been documented in Anopheles gambiae s.l., but is worthy of further
investigation once reliable methods for individually marking and repeatedly sampling
mosquitoes at different time points during their feeding cycle become available.

For most of the past 20 years, untreated bednets have been the primary vector
control intervention in many malaria endemic regions including our study area. For
example, recent estimates suggest approximately 75-91% of households in the
Kilombero Valley are covered by untreated nets [36]. However in the past 5 years, these
simple interventions are being rapidly replaced by the distribution of more effective
insecticide-treated (ITN) and Long-Lasting Insecticidal (LLINs) nets in many African
countries. While increases in ITN and LLIN coverage over this period have been
massive, the median proportion of households across sub-Saharan Africa reporting
ownership of at least one ITN/LLIN is ~50% [17]. Thus there remains a significant
proportion of households that do not have access to these more effective insecticidal
interventions and continue to rely on their untreated counterparts. Understanding the
nature of selection that may have been generated by this widespread predecessor to
ITN/LLINs can provide a useful framework for anticipating the future evolutionary
changes these interventions may exert on mosquito behaviour. We hypothesize that the
addition of insecticides to nets would substantially increase the fitness costs of anthrophily and generate stronger selection for a shift away from human feeding; especially as results obtained here and our previous work [15] suggest that these vectors can reproduce and survive equally well on at least some of the commonly available alternative animal hosts.

At present the genetic basis of host species preferences in malaria vectors is poorly understood, although early work [37] illustrated that *An. gambiae* can be selected for increased zoophily within a few generations (<5). These experimental data combined with growing evidence from field settings that malaria vectors are modifying their feeding behaviour in response to insecticide-based interventions [38] suggest that their host preference is a phenotype that can evolve. Assuming such genetic variation exists, due caution would still be required before embarking on a strategy of using interventions to drive selection on mosquito host species choice. Specifically, it would need to be demonstrated that the epidemiological benefits of facilitating selection for zoophily would not be outweighed by the disadvantages of providing mosquitoes with alternative ‘refuge’ hosts that would allow their populations to be maintained even when all humans are protected by LLINs [17, 39]. However, these results highlight opportunities that interventions present for generating selection against mosquito behaviours that facilitate disease transmission. Opportunities to reduce human biting either through short-term diversion to non-permissive animal species (e.g. zooprophylaxis) or longer-term selection on anthrophily should be exploited as a means to reinforce control.

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Figure Legends

**Fig 1:** Estimated proportions (± 1 s.e.) of *An. arabiensis* (left column) and *An. gambiae* s.s. (right column) that were recaptured in trials with different host species (a,b), successfully obtained a blood meal (c,d), or died during host seeking (e,f). Host types are: CH = chicken, CA = calf, CO = cow, DG = dog, GT = goat, H = unprotected human, and H(+N) = human sleeping under an untreated net. Colours indicate the nature of statistical differences between the "human without a net" reference group and other host treatments (determined by Dunnett's post hoc test, adjusting for multiple comparisons). Dark grey indicates treatments that had a statistically higher value than the human reference group, light grey indicates treatments that had a statistically lower value than the reference group, and white refers to treatments that were not significantly different from the reference group.

**Fig 2:** Estimates (± 1 s.e.) of the mean bloodmeal size (μg of hematin, a,b), oviposition rate (c,d), and number of eggs laid (e,f) by *An. arabiensis* (left column) and *An. gambiae* s.s. (right column) after feeding on different host types. Host type abbreviations are as specified in Figure 1. Colours indicate the nature of statistical differences between the "human without a net" reference group and all other host treatments, as detailed for Figure 1.

**Fig 3:** Survival of (a) *An. arabiensis* and (b) *An. gambiae* s.s after taking a bloodmeal from different host species. Lines represent the survival function as estimated from fitting the Cox proportion hazard model. Host type abbreviations are as specified in Figure 1.
Fig 4: Predicted distributions of the lifetime egg production of *An. arabiensis*: (a) feeding exclusively on hosts of different species and (b) taking a mixture of bloodmeals from cows and humans using bednets. The dotted black line represents *An. arabiensis* fitness under a ‘human using a bednet’-only diet, and the solid blue line a cow-only host diet. Dotted lines show expected distributions for variable proportions of cow-feeding (all other meals from humans using a bednet). Blue lines indicate host diets yielding a statistically significant advantage over an exclusive human-using-a-bednet diet (black dotted line). (c) shows the predicted distribution of *An. gambiae* s.s. lifetime egg production feeding exclusively on different host species. All distributions are based on 10,000 simulations, with host type abbreviations as specified in Figure 1.
An. arabiensis

Proportion recaptured (± 1SE)

Proportion fed (± 1SE)

Proportion dead on recapture (± 1SE)

Host species

An. gambiae s.s.
Host species

An. arabiensis

An. gambiae s.s

Blood meal size (µg ± 1SE)

Proportion ovipositing (± 1SE)

Number of eggs (± 1SE)
Proportion surviving

An. arabiensis

Days

An. gambiae s.s.

Proportion surviving

Days
An. arabiensis - single host feeding (a)

An. arabiensis - mixed host feeding (b)

An. gambiae s.s. - single host feeding (c)
Table 1. Relative odds of mortality in mosquito vectors after feeding on different host species. Numbers in brackets are 95% confidence intervals.

<table>
<thead>
<tr>
<th>Host species</th>
<th>An. arabiensis</th>
<th>An. gambiae s.s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goat</td>
<td>1.25 (1.02 – 1.54)</td>
<td>1.44 (1.23 – 1.69)</td>
</tr>
<tr>
<td>Dog</td>
<td>1.19 (0.95 – 1.50)</td>
<td>1.48 (1.28 – 1.71)</td>
</tr>
<tr>
<td>Chicken</td>
<td>1.41 (0.95 – 2.09)</td>
<td>1.71 (1.27 – 2.31)</td>
</tr>
<tr>
<td>Calf</td>
<td>1.08 (0.91 – 1.29)</td>
<td>1.48 (1.26 – 1.72)</td>
</tr>
<tr>
<td>Cow</td>
<td>1.19 (1.01 – 1.40)</td>
<td>0.92 (0.78 – 1.08)</td>
</tr>
<tr>
<td>Human with untreated net</td>
<td>1.08 (0.85 – 1.38)</td>
<td>1.83 (1.56 – 2.14)</td>
</tr>
</tbody>
</table>

**b) Relative to human with a net**

<table>
<thead>
<tr>
<th>Host species</th>
<th>An. arabiensis</th>
<th>An. gambiae s.s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goat</td>
<td>1.16 (0.89 – 1.50)</td>
<td>0.79 (0.68 – 0.92)</td>
</tr>
<tr>
<td>Dog</td>
<td>1.10 (0.83 – 1.46)</td>
<td>0.81 (0.71 – 0.93)</td>
</tr>
<tr>
<td>Chicken</td>
<td>1.29 (0.85 – 1.99)</td>
<td>0.94 (0.70 – 1.26)</td>
</tr>
<tr>
<td>Calf</td>
<td>0.99 (0.79 – 1.27)</td>
<td>0.81 (0.69 – 0.94)</td>
</tr>
<tr>
<td>Cow</td>
<td>1.10 (0.87 – 1.38)</td>
<td>0.50 (0.43 – 0.59)</td>
</tr>
<tr>
<td>Human no net</td>
<td>0.92 (0.73 – 1.17)</td>
<td>0.55 (0.47 – 0.64)</td>
</tr>
</tbody>
</table>
Figure ESM1

a

b
Ethical considerations
Mosquitoes used in these experiments had not been blood fed prior to use and thus were guaranteed free of malaria and other directly transmitted blood-borne pathogens. All human hosts were adult volunteers from the research team who provided written informed consent prior to participation. Human volunteers were tested for malaria by Rapid Diagnostic Test immediately prior to participation. Anyone who tested positive was provided with treatment and did not participate. Animals used in these trials were volunteered for participation by their owners in the local community after informed consent was provided. Only animals that had no history of topical insecticide treatment within 2 – 3 months were enrolled for these experiments.
**Table ESM 3.** Description of the source of fixed parameters used to model the lifetime reproductive success of *Anopheles* mosquitoes on different host species.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Symbol</th>
<th>Value</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daily survival during host seeking</td>
<td>$s_f$</td>
<td>0.8</td>
<td>[1]</td>
</tr>
<tr>
<td>Daily survival between feeding and oviposition after feeding on an unprotected human</td>
<td>$S_{ov(h)}$</td>
<td>0.9</td>
<td>[1]</td>
</tr>
<tr>
<td>No. days between feeding and oviposition</td>
<td>$d_{ov}$</td>
<td>3</td>
<td>[2]</td>
</tr>
<tr>
<td>No. days between oviposition and seeking new host</td>
<td>$d_f$</td>
<td>1</td>
<td>[2]</td>
</tr>
</tbody>
</table>


**Table 4:** Number of female mosquitoes that participated in the live host feeding trials under semi-field conditions. Trials were replicated 42 times per vector species to estimate several indices of host–species dependent fitness (feeding probability, blood meal size, fecundity and survival).

<table>
<thead>
<tr>
<th>Sample size</th>
<th>An. arabiensis</th>
<th>An. gambiae s.s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number (N) released</td>
<td>8356</td>
<td>8117</td>
</tr>
<tr>
<td>N recaptured</td>
<td>3832</td>
<td>5595</td>
</tr>
<tr>
<td>N alive and blood fed on recapture</td>
<td>1872</td>
<td>2774</td>
</tr>
<tr>
<td>N observed dead on recapture</td>
<td>3</td>
<td>161</td>
</tr>
<tr>
<td>N blood meals measured</td>
<td>1755</td>
<td>2461</td>
</tr>
<tr>
<td>N egg batches laid</td>
<td>347</td>
<td>1222</td>
</tr>
<tr>
<td>N monitored for long-term survival</td>
<td>1835</td>
<td>2663</td>
</tr>
</tbody>
</table>
Table ESM5. Fitness indices of vectors participating in the host feeding trials, combined over all host species and individuals. Numbers in brackets are 95% confidence intervals and mean values are shown with ± 1 s.e.

<table>
<thead>
<tr>
<th>Mosquito fitness trait</th>
<th>An. arabiensis</th>
<th>An. gambiae s.s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean body size (mm)</td>
<td>3.40 ± 0.02</td>
<td>2.81 ± 0.04</td>
</tr>
<tr>
<td>Proportion feeding</td>
<td>0.28(0.23– 0.33)</td>
<td>0.71 (0.69 – 0.72)</td>
</tr>
<tr>
<td>Mean blood meal size (µg)</td>
<td>17.11 ± 0.51</td>
<td>7.33 ± 0.22</td>
</tr>
<tr>
<td>Mean fecundity</td>
<td>80.10 ± 2.03</td>
<td>54.97 ± 1.51</td>
</tr>
<tr>
<td>Median survival (days)</td>
<td>13(12-14)</td>
<td>7 (7-8)</td>
</tr>
</tbody>
</table>
Table ESM6. Proportion of times (in 10,000 runs) that a randomly selected value of the predicted lifetime egg production of mosquitoes on one host type (left hand column) was higher than on another (right hand column). The standard cut-off value for significance ($\alpha = 0.05$) was adjusted for all multiple comparisons (21 possible for host species, 15 for mixed feeding strategies in *An. arabiensis*).

Asterisks (*) indicate statistically significant differences between host diet treatments.

<table>
<thead>
<tr>
<th>Anopheles arabiensis – single host species</th>
<th>CA</th>
<th>CH</th>
<th>CO</th>
<th>DG</th>
<th>GT</th>
<th>H</th>
<th>H+N</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA</td>
<td>--</td>
<td>0.9989*</td>
<td>0.0572</td>
<td>0.4222</td>
<td>0.9417</td>
<td>0.7172</td>
<td>0.9889</td>
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<tr>
<td>CH</td>
<td>--</td>
<td>&lt;0.0001*</td>
<td>0.0010</td>
<td>0.0863</td>
<td>0.0053</td>
<td>0.2406</td>
<td></td>
</tr>
<tr>
<td>CO</td>
<td>--</td>
<td>0.9203</td>
<td>0.9990*</td>
<td>0.9886</td>
<td>&gt;0.9999*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DG</td>
<td>--</td>
<td>--</td>
<td>0.9560</td>
<td>0.7707</td>
<td>0.9919</td>
<td></td>
<td></td>
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<tr>
<td>GT</td>
<td>--</td>
<td>--</td>
<td>0.1525</td>
<td>0.7400</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>--</td>
<td>--</td>
<td>0.9576</td>
<td></td>
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<tr>
<td>H+N</td>
<td>--</td>
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<table>
<thead>
<tr>
<th>Anopheles arabiensis – mixed feeding on humans using nets and cows</th>
<th>CA</th>
<th>CH</th>
<th>CO</th>
<th>DG</th>
<th>GT</th>
<th>H</th>
<th>H+N</th>
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<tbody>
<tr>
<td>% bloodmeals on cows</td>
<td>0</td>
<td>20</td>
<td>40</td>
<td>60</td>
<td>80</td>
<td>100</td>
<td></td>
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<tr>
<td>0</td>
<td>--</td>
<td>0.1090</td>
<td>0.0.147</td>
<td>0.0011*</td>
<td>&gt;0.0001*</td>
<td>&gt;0.0001*</td>
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<tr>
<td>20</td>
<td>--</td>
<td>0.2311</td>
<td>0.0825</td>
<td>0.0227</td>
<td>0.0040</td>
<td></td>
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</tr>
<tr>
<td>40</td>
<td>--</td>
<td>0.2642</td>
<td>0.1031</td>
<td>0.0275</td>
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<td></td>
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<tr>
<td>60</td>
<td>--</td>
<td>0.2616</td>
<td>0.1030</td>
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<tr>
<td>80</td>
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<td>0.2694</td>
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</tr>
<tr>
<td>100</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Anopheles gambiae s.s. – single host species</th>
<th>CA</th>
<th>CH</th>
<th>CO</th>
<th>DG</th>
<th>GT</th>
<th>H</th>
<th>H+N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Host type</td>
<td>CA</td>
<td>CH</td>
<td>CO</td>
<td>DG</td>
<td>GT</td>
<td>H</td>
<td>H+N</td>
</tr>
<tr>
<td>CA</td>
<td>--</td>
<td>0.9999*</td>
<td>0.5428</td>
<td>0.4152</td>
<td>0.9146</td>
<td>0.6709</td>
<td>0.9863</td>
</tr>
<tr>
<td>CH</td>
<td>--</td>
<td>0.0010*</td>
<td>&lt;0.0001*</td>
<td>0.0082</td>
<td>0.0020*</td>
<td>0.0306</td>
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<tr>
<td>CO</td>
<td>--</td>
<td>0.3881</td>
<td>0.8637</td>
<td>0.6255</td>
<td>0.9633</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DG</td>
<td>--</td>
<td>0.9461</td>
<td>0.7490</td>
<td>0.9937</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>GT</td>
<td>--</td>
<td>0.2234</td>
<td>0.7495</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>--</td>
<td>--</td>
<td>0.9210</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H+N</td>
<td>--</td>
<td>--</td>
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</tr>
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</table>