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

3

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27 **Summary**

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

44

45 **Key words:** host specialization, selection, mosquito vectors, malaria, bednets

## 46 **Introduction**

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

55 A potential candidate for such an approach is malaria, a disease caused by  
56 *Plasmodium* parasites transmitted by *Anopheles* mosquitoes. The host range of  
57 *Anopheline* species varies from avian and mammalian generalists, to those specific to  
58 one host species [6]. In contrast, most human infectious *Plasmodia* can survive only in  
59 humans (*P. knowlesi* being an exception [7]). Consequently the degree to which  
60 *Anopheles* vectors specialize on humans (anthrophily) is a prime determinant of malaria  
61 transmission intensity [8], and any shift from anthrophily to feeding on other animals will  
62 reduce transmission (e.g. zooprophyllaxis [9]). Current malaria control strategies are  
63 based on reducing human exposure to mosquito bites and/or mosquito density [10].  
64 Here we investigate the potential for these approaches to generate additional benefits by  
65 creating an evolutionary incentive for mosquito vectors to switch their host species use  
66 from humans to other animals commonly available in malaria endemic settings.

67 Prediction of the potential impact of control measures on the evolution of  
68 mosquito host range requires an understanding of the selective forces underpinning it.  
69 The host species range of haematophagous insects has undoubtedly been shaped by  
70 natural selection, but there has been relatively little empirical investigation of how host  
71 selection influences their fitness [6, 11]. Theoretically, host specialization is predicted to

72 arise due to a trade-off between the performance of foragers on different host types [12,  
73 13], with selection being generated for the development of preferences for those which  
74 provide the greatest fitness reward. By extension, environmental changes that diminish  
75 the fitness advantage associated with particular hosts could undermine selection for their  
76 preference. In the case of African malaria vectors, bednet usage is an example of an  
77 environmental change that could reduce fitness advantages associated with anthropily.  
78 Should the expected fitness returns that mosquitoes obtain from attempting to feed on  
79 humans protected by bednets fall below those from foraging on other available animals,  
80 wide use of these interventions could generate selection on vectors to adopt more  
81 generalist feeding behaviours and/or switch their specialization to other host species.  
82 Both these phenomena could substantially reduce malaria transmission.

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

96         Although the coverage of insecticidal nets in Africa has increased dramatically  
97 over the past 10 years [16, 17], untreated or poorly treated bednets remain the most

98 common protective measure against mosquito biting in many locations [17]. We  
99 experimentally investigated how the fitness of the two most important African malaria  
100 vectors, *An. gambiae s.s* and *An. arabiensis*, varied on encounter with different host  
101 species, and whether the use of such bednets reduced the relative fitness expected from  
102 foraging on humans relative to commonly available animal alternatives. We also tested  
103 whether the well-established preferences of these vectors towards specific host species  
104 are positively correlated with a fitness advantage from feeding upon them. These  
105 vectors are closely related and widely distributed throughout Africa [18], but vary in their  
106 host preference with *An. gambiae s.s* being almost exclusively anthrophilic [8], and *An.*  
107 *arabiensis* generally preferring cows over humans when both are available [19].

108

## 109 **Materials and Methods**

110 The study was conducted at the Ifakara Health Institute (IHI) in the Kilombero valley,  
111 Tanzania, where high levels of malaria transmission are sustained year-round by  
112 *Anopheles arabiensis*, *An. gambiae s.s*, and *An. funestus*. Experiments were conducted  
113 using *An. arabiensis* and *An. gambiae s.s* from colonies at the IHI. The *An. arabiensis*  
114 colony was established a few months before the start of experiments with individuals  
115 from Sagamaganga village (~15km from IHI) and is maintained in a semi-field insectary  
116 [20]. The *An. gambiae s.s* colony was established with individuals from Njage village in  
117 1996 (~70 km from IHI) and is maintained in an indoor insectary (26 ± 2.5°C, 80 ±10% R.  
118 H.). Both colonies are maintained on human-blood provided thrice weekly by arm  
119 feeding.

120

### 121 *Experimental set up*

122 An experimental hut (3.5 X 4 X 2.5 m) was built in a netting-enclosed chamber  
123 (9.1 X 9.6 X 3.7 m) of the IHI semi-field system (SFS, ESM1 in supplementary materials)

124 [20]. Mosquitoes could enter and exit the hut through its open eaves as they do in  
125 nature [21], or exit via the six windows. Mosquitoes leaving the hut were caught outside  
126 or in window exit traps. Mosquito feeding success and fitness were evaluated on  
127 humans and 4 other species commonly kept in or near houses in the Kilombero Valley:  
128 chickens, cattle, dogs and goats. Two sub-categories of cattle were tested: adult cows  
129 and calves. Within other host types, animals were roughly the same age and size.  
130 Humans were presented either exposed or sleeping under an untreated bednet.  
131 'Typical' bednets were created following the World Health Organization's standard  
132 protocol for simulating the average condition of bednets in operational use by cutting 6  
133 moderately sized holes into the sides (4 X 4 cm) [22].

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

145

#### 146 *Fitness measurements*

147 Mosquito feeding success was measured as: (1) the proportion of mosquitoes  
148 recaptured alive and blood fed, (2) the proportion of mosquitoes dead at recapture, and  
149 (3) blood meal size. For blood meal size measurement, mosquitoes visually identified as

150 blood fed were moved into individual 30 ml tubes for 3 days (provided with 10% glucose  
151 solution) in the semi-field insectary. Mosquitoes were subsequently moved into  
152 individual paper cups lined with damp filter paper to stimulate oviposition, and the  
153 hematin content of excreta deposited in initial holding tubes measured to provide an  
154 index of the mass of blood ingested [23]. Oviposition cups were inspected daily and the  
155 number of eggs laid within them counted. Mosquitoes remained in holding cups and  
156 were monitored daily until death to estimate their host-species dependent survival.

157

### 158 *Ethical considerations*

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

163

### 164 *Statistical Analyses*

165 Variation in the probability of blood feeding, death on recapture and oviposition  
166 (all binomial), and blood meal size and fecundity (continuous) were analysed using  
167 generalized linear mixed effect models (GLMM) with appropriate link functions in the R  
168 software package [24]. Here 'host species' and 'mosquito species' were treated as fixed  
169 effects, and 'host individual' as a random effect. For each response variable, a maximal  
170 model was generated and the significance of fixed effects evaluated through stepwise  
171 deletion of terms using Likelihood Ratio Tests (LRTs). For variables in which host  
172 species was identified as statistically significant, Dunnett's post hoc test (adjusting for  
173 multiple comparisons) was used to identify statistically significant two-way differences  
174 between the unprotected human reference group and other host types. The Cox  
175 Proportional Hazards Model was used to test for differences in the post-feeding survival



176 of mosquitoes due to host species. In these models, a frailty function [25] was used to  
 177 incorporate the random effect of host individual, and host and mosquito species were fit  
 178 as main effects [24]. Reported chi-square values refer to LRTs conducted on the output  
 179 of GLMMs, and z-values are for two-way comparison between a human reference group  
 180 and other host species. 'OR' values are odds ratios from Cox proportional hazard  
 181 models.

182

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

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

$$195 \quad R(j) = \sum_{k=0}^{\infty} [(1 - \beta_j \gamma_j) s_f]^k \beta_j (s_{ov,j}^{d_{ov}} \gamma_j F_j)$$

196 and the lifetime reproductive success (LRS, estimated by  $R_{0j}$ ) expected from multiple

197 feeding cycles  $i$  is given by  $R_{0j} = \sum_{i=1}^{\infty} s_f^{i-1} R(j)$ , assuming age-independent survival.

198 Most values for host-specific mosquito fitness traits were directly estimated from  
 199 experiments described above, with the exception of survival between feeding and

200 oviposition ( $s_{ovj}$ ). Rather than directly applying survival probabilities that were  
201 experimentally measured under semi-field conditions here (likely an overestimate of their  
202 value in nature), we estimated the odds of survival after feeding on different hosts  
203 relative to having fed on an unprotected human and used this to adjust published values  
204 of the daily survival of human-fed mosquitoes in the field (ESM 3).

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

221 To test for statistically significant differences in  $R_0$  between host types,  
222 bootstrapping analyses were performed on the 21 possible two-way host comparisons  
223 between the 7 host types. Values of  $R_0$  for host type 1 and 2 were randomly drawn from  
224 their simulated distributions. The proportion of 10,000 such draws in which the  $R_0$  of one

225 host type was greater than the other was used as an estimate of the probability that the  
226 LRS of mosquitoes on these host types was significantly different (if  $p < 0.05$ ).

227

## 228 **Results**

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

239

### 240 a) *Does host species influence mosquito vector fitness?*

241 The proportion of mosquitoes recaptured did not vary between host species in  
242 *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  
243 1b). However host species was a significant predictor of feeding probability in both *An.*  
244 *arabiensis* ( $\chi^2_6 = 52.80$ ,  $P < 0.001$ , Fig 1c) and *An. gambiae* s.s ( $\chi^2_6 = 23.89$ ,  $P < 0.001$ ,  
245 Fig 1d). The proportion of mosquitoes dead on recapture was independent of host  
246 species (*An. arabiensis*:  $\chi^2_6 = 1.89$ ,  $P = 0.93$ , Fig 1e; *An. gambiae* s.s :  $\chi^2_6 = 8.21$ ,  $P =$   
247 0.22, Fig 1f).

248 Whereas *An. arabiensis* obtained similarly sized blood meals from all hosts ( $\chi^2_6 =$   
249 3.28,  $P = 0.77$ , Fig 2a), *An. gambiae* s.s acquired larger meals from humans and cows  
250 than any other hosts ( $\chi^2_6 = 22.50$ ,  $P < 0.001$ , Fig 2b). Host species influenced the

251 probability of oviposition after blood feeding (*An. arabiensis*:  $\chi^2_6 = 14.85$ ,  $P = 0.02$ , Fig  
252 2c; *An. gambiae* s.s:  $\chi^2_6 = 21.03$ ,  $P = 0.002$ , Fig 2d), but not the number of eggs laid (*An.*  
253 *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  
254 impact of host species on mosquito survival also varied between mosquito species.  
255 Whereas *An. arabiensis* had similar survival on all host species ( $\chi^2_6 = 8.6$ ,  $P = 0.2$ , Fig  
256 3a, Table 1), in *An. gambiae* s.s the odds of mortality between the 'best' (humans and  
257 cows) and 'worst' host types (chickens) differed by 1.7-fold ( $\chi^2_6 = 106.4$ ,  $P < 0.001$ , Fig  
258 3b, Table 1). Combining these impacts of host species on mosquito fitness, the life-  
259 history model predicted the LRS of both *An. arabiensis* and *An. gambiae* s.s to vary  
260 significantly between host species (Fig 4a & c, ESM 6).

261

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

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

270 The feeding probability of *An. gambiae* s.s on their naturally preferred humans  
271 was no higher than on any other host type except chickens (Fig 1d). *Anopheles*  
272 *gambiae* s.s obtained significantly larger blood meals from exposed humans than from  
273 other host types except cows ( $z = -1.76$ ,  $P = 0.31$ , Fig 2b), but their oviposition  
274 probability and fecundity after feeding on humans was no higher than any other host  
275 species (Fig 2d & f). The survival of *An. gambiae* s.s, was significantly higher after  
276 feeding on exposed humans than on other host type except cows ( $\chi^2_1 = 1.13$ ,  $P = 0.29$ ,

277 Fig 3b, Table 1). When all estimates of host –dependent fitness were combined to  
278 predict the LRS of *An. gambiae* s.s., there was no evidence of an advantage associated  
279 with human feeding (Fig 4c, ESM 6).

280

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

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

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

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

302 however these differences were not statistically significant after correcting for multiple  
303 comparisons (ESM 6).

304

## 305 **Discussion**

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

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

324 While not all of the mosquito fitness traits investigated here varied between host  
325 species, at least one did so for each vector. In *An. arabiensis*, host species primarily  
326 determined their probability of acquiring a blood meal, but not their post-feeding fitness.  
327 In contrast, under these experimental conditions *An. gambiae* s.s had a similar feeding

328 probability on all mammalian hosts, but variable reproductive success and survival  
329 afterwards. This suggests there may be trade-offs in the value of host resources for  
330 different life-history processes. Although mosquito blood meal size and egg production  
331 have been widely correlated in previous work [27-29], the smaller blood meals  
332 associated with some host species here did not consistently translate into reduced egg  
333 production. Most previous studies have examined this relationship only within one host  
334 species, and it is possible there are additional sources of haematological variation  
335 between host species that cause this relationship to breakdown when comparing blood  
336 meals taken from across them.

337         Evolutionary theory predicts that the fitness of specialists is highest when  
338 preferred resources are consumed [30]. Our life history model predicted this to be true  
339 for *An. arabiensis* whose LRS was estimated to be substantially higher on its naturally  
340 preferred cow hosts. However although some *An. gambiae* s.s fitness traits were  
341 highest on their preferred humans (blood meal size), there was no evidence of an overall  
342 advantage to their LRS associated with these hosts. Failure to detect correlations  
343 between host preference and performance have been documented in other insect  
344 systems [31], and attributed to ecological variation that modifies the quality of hosts in  
345 different environments. Similarly, our ability to detect host preference-performance  
346 relationships in *An. gambiae* s.s may have been limited by experimental conditions.  
347 Here we presented hosts to mosquitoes under a 'no choice' scenario in an indoor  
348 environment. This design was used to distinguish between fitness effects arising from  
349 innate biological properties of hosts (e.g. physiological and/or behavioural), from those  
350 arising indirectly due to variation in their use of habitats. While livestock are frequently  
351 kept inside buildings at night in our study area, in other settings livestock may be kept  
352 outside during vector activity periods. *Anopheles gambiae* s.s has a strong preference  
353 for biting indoors [32, 33], whereas *An. arabiensis* bites hosts both indoors and outside.

354 If *An. gambiae*'s preference for feeding on humans is an indirect consequence of an  
355 advantage arising from indoor biting (irrespective of host species), the relative  
356 advantages of anthrophily in this vector species may be underestimated here relative to  
357 other environments where animals are generally outside. Further investigation within  
358 this environmentally - realistic yet experimentally tractable system can help evaluate this  
359 hypothesis.

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

370 Evaluation of the accuracy with which effects described here reflect the nature of  
371 selection acting on host species range in nature will require further investigation of  
372 several areas that at present are intractable within the semi-field conditions used here.  
373 First, the host-specific feeding probabilities estimated here may be upwardly-biased  
374 because they were measured under 'no choice' conditions (e.g. our observation that *An.*  
375 *gambiae* s.s. fed on all host species with similar probability contrasts with their known  
376 preference for humans in nature [8]). Giving *An. gambiae* s.s a choice between hosts  
377 may have significantly increased predicted feeding rates on humans at the expense of  
378 those estimated for other animals. However, this may not substantially alter our  
379 conclusions about the relative benefits of anthrophily as *An. gambiae* s.s. were shown



380 capable of feeding on other animals to the same degree as humans when no choice was  
381 available, with no consistent reduction in their fitness relative to those obtained from  
382 human bloodmeals. Ideally this expectation could be confirmed by simultaneous  
383 measurement of mosquito host choice and subsequent fitness. Presently this is not  
384 possible because the host choice of mosquitoes captured blood fed can only be  
385 confirmed by killing them to analyze their stomach contents, which prevents any further  
386 measurement of their fitness. Should non-invasive methods become available for  
387 bloodmeal identification, follow up investigation of mosquito fitness under choice  
388 scenarios should be pursued. Further investigation of other potential advantages of  
389 anthrophily beyond which could be measured here, including habitat-dependent foraging  
390 success (higher inside houses), or benefits from host seeking on aggregated  
391 populations, is encouraged. Finally, the requirement for large numbers of similarly aged,  
392 malaria-free mosquitoes required the use of insectary-reared mosquitoes in this study.  
393 Although both insectary colonies were initiated from mosquito populations in the local  
394 area and maintained on a natural blood source, the process of colonization can modify  
395 host discrimination behaviour [34]. Where possible, further study using F1 mosquitoes  
396 from wild populations is encouraged to identify potential biases arising from the use of  
397 colonized mosquitoes.

398

399 Our model predictions are based on several assumptions that also require  
400 validation for assessment of potential implications of these results to field. One is that  
401 the host-specific impacts on mosquito fitness measured are similar on all feeding cycles.  
402 Here mosquito fitness was measured after one blood meal, whereas in nature vectors  
403 feed every 2-4 days [35]. Repeated blood feeding could potentially cancel out or  
404 magnify the host-specific effects described here. A previous laboratory study showed  
405 that *An. gambiae* s.s. fed one bloodmeal using an artificial membrane feeder exhibited

406 similar host-specific survival as documented here [15]. However when mosquitoes were  
407 given 2 blood meals consisting of blood from humans followed by another animal their  
408 longevity was similar [15]. This suggests that negative fitness effects arising from  
409 bloodmeals on poor quality hosts could be reduced by further meals from a 'high quality'  
410 hosts. Furthermore, mosquitoes may be able to increase their feeding frequency from  
411 what was assumed here to compensate for lower quality bloodmeals. Had mosquitoes  
412 been provided with an oviposition substrate earlier than the standard 4-day post-feeding  
413 period used here, it is possible those fed on poorer quality host types could have brought  
414 forward their oviposition to increase future feeding opportunities. This phenomenon has  
415 not yet been documented in *Anopheles gambiae* s.l., but is worthy of further  
416 investigation once reliable methods for individually marking and repeatedly sampling  
417 mosquitoes at different time points during their feeding cycle become available.

418 For most of the past 20 years, untreated bednets have been the primary vector  
419 control intervention in many malaria endemic regions including our study area. For  
420 example, recent estimates suggest approximately 75-91% of households in the  
421 Kilombero Valley are covered by untreated nets [36]. However in the past 5 years, these  
422 simple interventions are being rapidly replaced by the distribution of more effective  
423 insecticide-treated (ITN) and Long-Lasting Insecticidal (LLINs) nets in many African  
424 countries. While increases in ITN and LLIN coverage over this period have been  
425 massive, the median proportion of households across sub-Saharan Africa reporting  
426 ownership of at least one ITN/LLIN is ~50% [17]. Thus there remains a significant  
427 proportion of households that do not have access to these more effective insecticidal  
428 interventions and continue to rely on their untreated counterparts. Understanding the  
429 nature of selection that may have been generated by this widespread predecessor to  
430 ITN/LLINs can provide a useful framework for anticipating the future evolutionary  
431 changes these interventions may exert on mosquito behaviour. We hypothesize that the

432 addition of insecticides to nets would substantially increase the fitness costs of  
433 anthrophily and generate stronger selection for a shift away from human feeding;  
434 especially as results obtained here and our previous work [15] suggest that these  
435 vectors can reproduce and survive equally well on at least some of the commonly  
436 available alternative animal hosts.

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

453

#### 454 **Acknowledgements**

455 We thank IHI insectary technicians for rearing mosquitoes, Dr Sarah Moore for logistical  
456 support, Drs Gerry Killeen and Lisa Ranford-Cartwright for discussion. We also thank all  
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595 **Figure Legends**

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

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608 **Fig 2:** Estimates ( $\pm 1$  s.e.) of the mean bloodmeal size ( $\mu\text{g}$  of hematin, a,b), oviposition  
609 rate (c,d), and number of eggs laid (e,f) by *An. arabiensis* (left column) and *An. gambiae*  
610 s.s. (right column) after feeding on different host types. Host type abbreviations are as  
611 specified in Figure 1. Colours indicate the nature of statistical differences between the  
612 “human without a net” reference group and all other host treatments, as detailed for  
613 Figure 1.

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615 **Fig 3:** Survival of (a) *An. arabiensis* and (b) *An. gambiae* s.s after taking a bloodmeal  
616 from different host species. Lines represent the survival function as estimated from  
617 fitting the Cox proportion hazard model. Host type abbreviations are as specified in  
618 Figure 1.

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621 Fig 4: Predicted distributions of the lifetime egg production of *An. arabiensis*: (a) feeding  
622 exclusively on hosts of different species and (b) taking a mixture of bloodmeals from  
623 cows and humans using bednets. The dotted black line represents *An. arabiensis* fitness  
624 under a 'human using a bednet'-only diet, and the solid blue line a cow-only host diet.  
625 Dotted lines show expected distributions for variable proportions of cow-feeding (all other  
626 meals from humans using a bednet). Blue lines indicate host diets yielding a statistically  
627 significant advantage over an exclusive human-using-a-bednet diet (black dotted line).  
628 (c) shows the predicted distribution of *An. gambiae* s.s. lifetime egg production feeding  
629 exclusively on different host species. All distributions are based on 10,000 simulations,  
630 with host type abbreviations as specified in Figure 1

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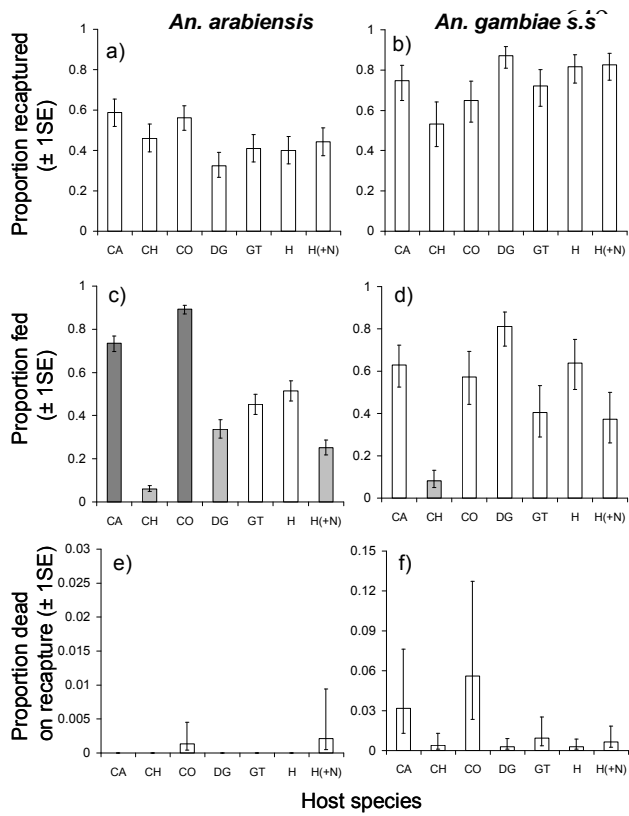
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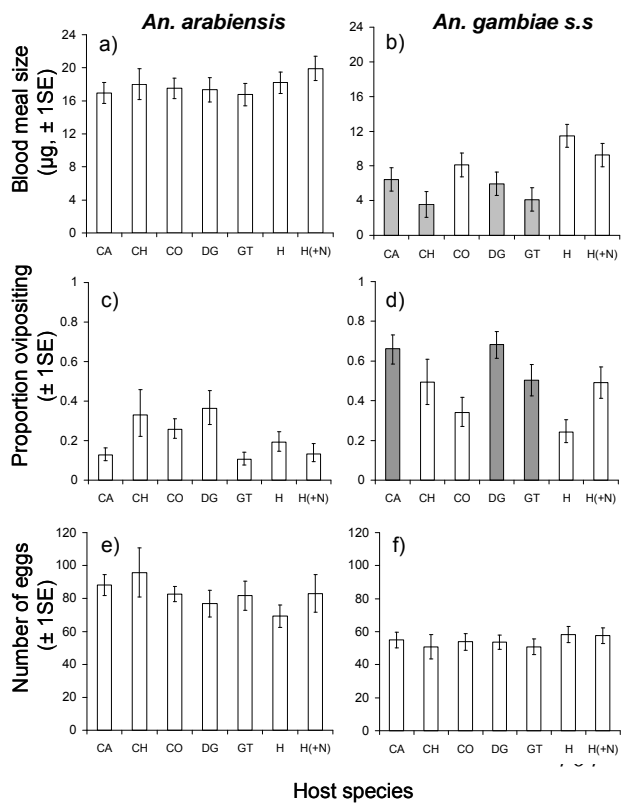
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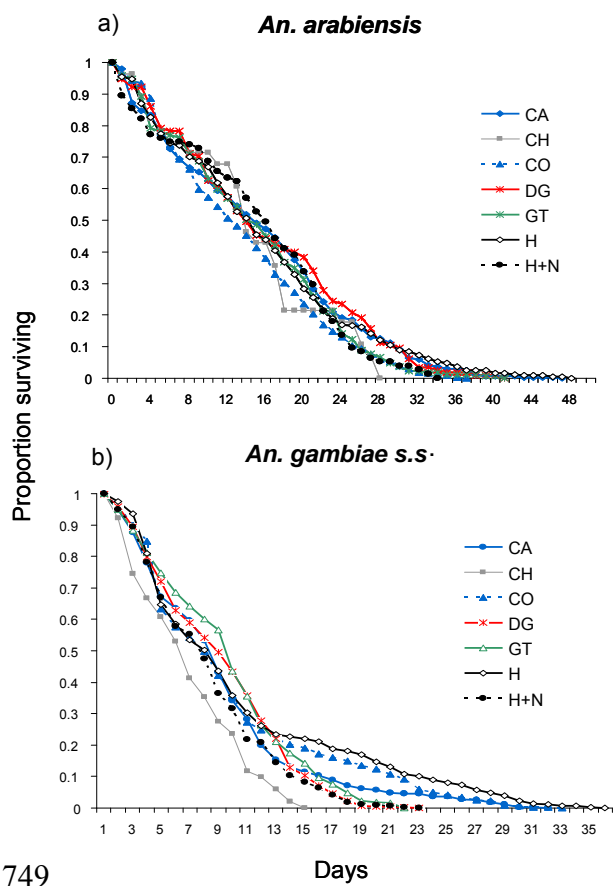
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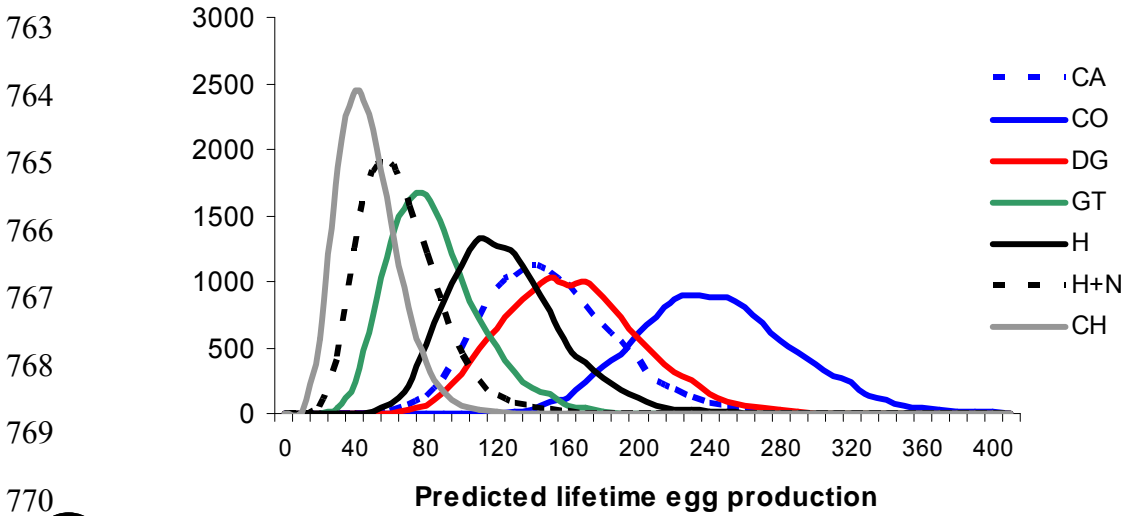
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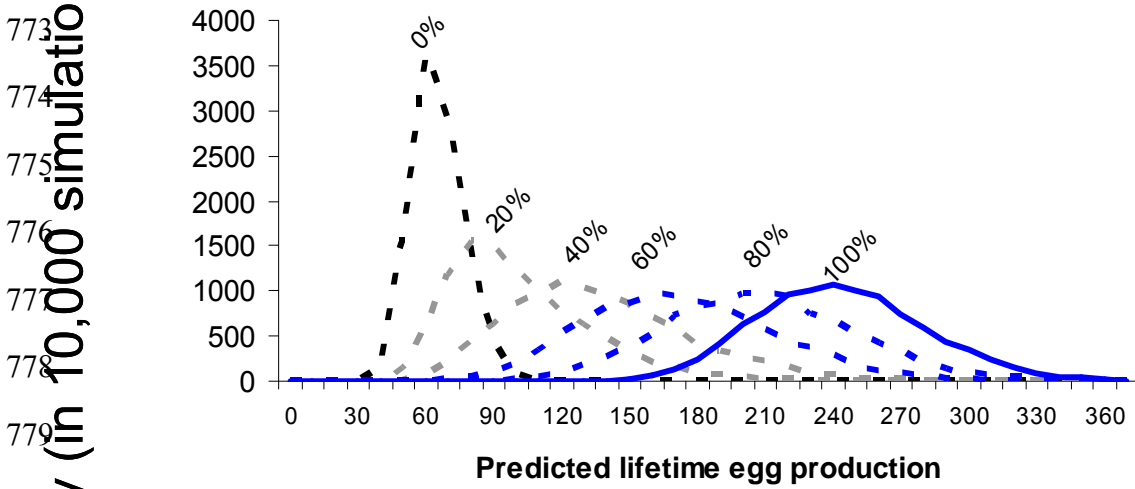
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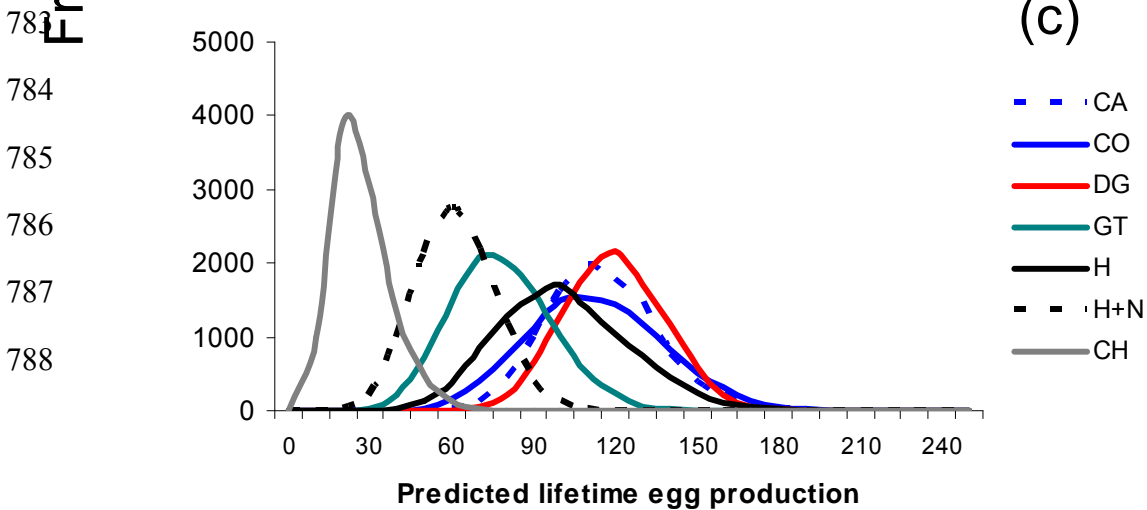
*An. arabiensis* - single host feeding (a)



*An. arabiensis* - mixed host feeding (b)



*An. gambiae s.s.* - single host feeding (c)



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790 **Table 1.** Relative odds of mortality in mosquito vectors after feeding on different  
791 host species. Numbers in brackets are 95% confidence intervals.

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<b>Odds Ratio (OR) of mortality</b>		
<b>a) Relative to human without a net</b>		
<b>Host species</b>	<b><i>An. arabiensis</i></b>	<b><i>An. gambiae s.s</i></b>
Goat	1.25(1.02 – 1.54)	1.44(1.23 – 1.69)
Dog	1.19(0.95 – 1.50)	1.48 (1.28 – 1.71)
Chicken	1.41(0.95 – 2.09)	1.71 (1.27 – 2.31)
Calf	1.08(0.91 – 1.29)	1.48(1.26 – 1.72)
Cow	1.19(1.01 – 1.40)	0.92 (0.78 – 1.08)
Human with untreated net	1.08(0.85 – 1.38)	1.83 (1.56 – 2.14)
<b>b) Relative to human with a net</b>		
Goat	1.16(0.89 – 1.50)	0.79(0.68 – 0.92)
Dog	1.10(0.83 – 1.46)	0.81 (0.71 – 0.93)
Chicken	1.29(0.85 – 1.99)	0.94(0.70 – 1.26)
Calf	0.99(0.79 – 1.27)	0.81(0.69 – 0.94)
Cow	1.10(0.87 – 1.38)	0.50 (0.43 – 0.59)
Human no net	0.92(0.73 – 1.17)	0.55 (0.47 – 0.64)

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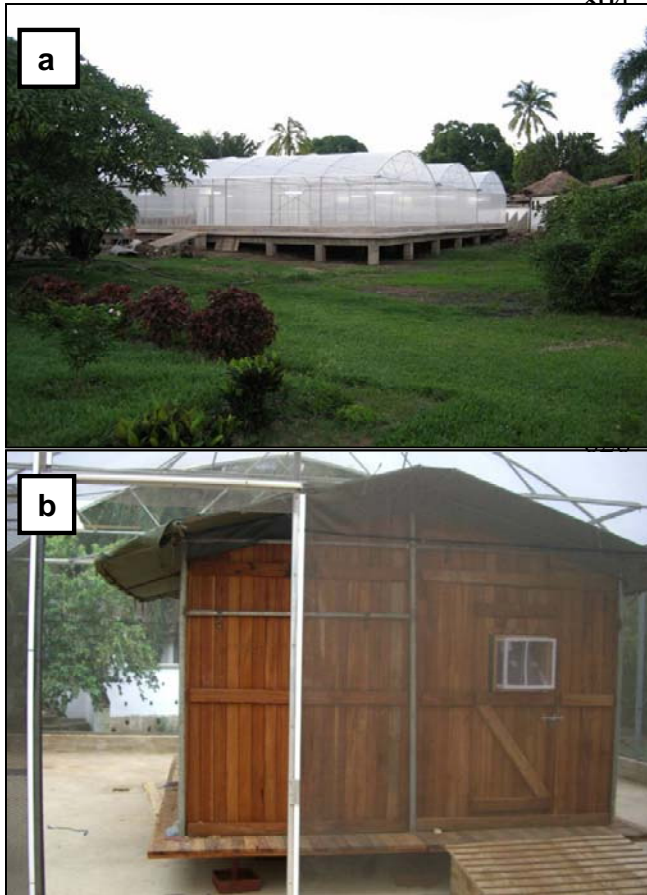


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801 Figure ESM1

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863 ***Ethical considerations***

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865 Mosquitoes used in these experiments had not been blood fed prior to use and thus

866 were guaranteed free of malaria and other directly transmitted blood-borne pathogens.

867 All human hosts were adult volunteers from the research team who provided written

868 informed consent prior to participation. Human volunteers were tested for malaria by

869 Rapid Diagnostic Test immediately prior to participation. Anyone who tested positive

870 was provided with treatment and did not participate. Animals used in these trials were

871 volunteered for participation by their owners in the local community after informed

872 consent was provided. Only animals that had no history of topical insecticide treatment

873 within 2 – 3 months were enrolled for these experiments.

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891 **Table ESM 3.** Description of the source of fixed parameters used to model the

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893 lifetime reproductive success of *Anopheles* mosquitoes on different host species.

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

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902 Med J 30: 129-135.

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912 **Table 4:** Number of female mosquitoes that participated in the live host feeding  
913 trials under semi-field conditions. Trials were replicated 42 times per vector  
914 species to estimate several indices of host –species dependent fitness (feeding  
915 probability, blood meal size, fecundity and survival).  
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<b>Sample size</b>	<b><i>An. arabiensis</i></b>	<b><i>An. gambiae s.s</i></b>
Total number (N) released	8356	8117
N recaptured	3832	5595
N alive and blood fed on recapture	1872	2774
N observed dead on recapture	3	161
N blood meals measured	1755	2461
N egg batches laid	347	1222
N monitored for long-term survival	1835	2663

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931 **Table ESM5.** Fitness indices of vectors participating in the host feeding trials,  
932 combined over all host species and individuals. Numbers in brackets are 95%  
933 confidence intervals and mean values are shown with  $\pm 1$  s.e.  
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<b>Mosquito fitness trait</b>	<b><i>An. arabiensis</i></b>	<b><i>An. gambiae s.s</i></b>
Mean body size (mm)	3.40 $\pm$ 0.02	2.81 $\pm$ 0.04
Proportion feeding	0.28(0.23– 0.33)	0.71 (0.69 – 0.72)
Mean blood meal size ( $\mu$ g)	17.11 $\pm$ 0.51	7.33 $\pm$ 0.22
Mean fecundity	80.10 $\pm$ 2.03	54.97 $\pm$ 1.51
Median survival (days)	13(12-14)	7 (7-8)

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951 **Table ESM6.** Proportion of times (in 10,000 runs) that a randomly selected  
 952 value of the predicted lifetime egg production of mosquitoes on one host type (left  
 953 hand column) was higher than on another (right hand column). The standard cut-  
 954 off value for significance ( $\alpha = 0.05$ ) was adjusted for all multiple comparisons (21  
 955 possible for host species, 15 for mixed feeding strategies in *An. arabiensis*).  
 956 Asterisks (\*) indicate statistically significant differences between host diet  
 957 treatments.  
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<b><i>Anopheles arabiensis</i> – single host species</b>							
Host type	CA	CH	CO	DG	GT	H	H+N
CA	--	0.9989*	0.0572	0.4222	0.9417	0.7172	0.9889
CH		--	<0.0001*	0.0010	0.0863	0.0053	0.2406
CO			--	0.9203	0.9990*	0.9886	>0.9999*
DG				--	0.9560	0.7707	0.9919
GT					--	0.1525	0.7400
H						--	0.9576
H+N							--
<b><i>Anopheles arabiensis</i> – mixed feeding on humans using nets and cows</b>							
% bloodmeals on cows							
	0	20	40	60	80	100	
0	--	0.1090	0.0.147	0.0011*	>0.0001*	>0.0001*	
20		--	0.2311	0.0825	0.0227	0.0040	
40			--	0.2642	0.1031	0.0275	
60				--	0.2616	0.1030	
80					--	0.2694	
100						--	
<b><i>Anopheles gambiae</i> s.s. – single host species</b>							
Host type	CA	CH	CO	DG	GT	H	H+N
CA	--	0.9999*	0.5428	0.4152	0.9146	0.6709	0.9863
CH		--	0.0010*	<0.0001*	0.0082	0.0020*	0.0306
CO			--	0.3881	0.8637	0.6255	0.9633
DG				--	0.9461	0.7490	0.9937
GT					--	0.2234	0.7495
H						--	0.9210
H+N							--

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