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1 **The impact of uniform and mixed species blood meals on the fitness of the mosquito**

2 **vector *An. gambiae s.s:* does a specialist pay for diversifying its host species diet?**

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4 **Running title:** Host diet diversity in *An. gambiae s.s.*

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26  **Abstract**

27  We investigated the fitness consequences of specialization in an organism whose host

28  choice has an immense impact on human health: the African malaria vector *Anopheles*

29  *gambiae* s.s.. We tested whether this mosquito's specialism on humans can be attributed to

30  the relative fitness benefits of specialist versus generalist feeding strategies by contrasting

31  their fecundity and survival on human-only and mixed host diets consisting of bloodmeals

32  from humans and animals. When given only one blood meal, *An. gambiae* s.s. survived

33  significantly longer on human and bovine blood, than on canine or avian blood. However,

34  when blood fed repeatedly, there was no evidence that the fitness of *An. gambiae* s.l. fed a

35  human –only diet was greater than those fed generalist diets. This suggests that the

36  adoption of generalist host feeding strategies in *An. gambiae* s.s. is not constrained by

37  intraspecific variation in the resource quality of blood from other available host species.

38  **Introduction**

39      Evolutionary theory predicts that resource specialization evolves only when there is a

40  greater fitness advantage from concentrating feeding on one dietary resource rather than a

41  mixture (Levins, 1962; MacArthur & Pianka, 1966b; Pyke *et al.*, 1977). In contrast,

42  generalism is predicted to evolve when there are only moderate fitness differences resulting

43  from feeding on different resources such that there is no net advantage to being selective

44  (Egas *et al.*, 2004; Abrams, 2006a; b). Numerous studies have attempted to test for the

45  existence of dietary fitness trade-offs associated with specialist and generalist feeding, with

46  the majority focusing on the experimental study of phytophagous insects (Price *et al.*, 1980;

47  Futuyma & Moreno, 1988; Jaenike, 1990; Via, 1990). These studies provide some support

48  for the prediction that generalists maximize their fitness by feeding on a variety of resources

49  rather than selecting only one (Bernays *et al.*, 1994; Allard & Yeargan, 2005; Michaud &

50  Jyoti, 2008), and conversely that specialists experience a reduction in fitness when they

51  switch to an atypical host and/or mix their diet between host species (Thomas et al., 2010).

52        Although phytophagous insects serve as excellent models for study of host

53    specialization, their broader applicability to other organisms that rely on living hosts such as

54    ectoparasites and insect disease vectors of humans and animals is largely untested.   Failure

55    to test theoretical predictions more widely on these and other parasitic organisms limits their

56    application to identify and diminish the factors that drive selection for insect vector

57    specialization on disease-susceptible hosts.  Like phytophagous insects, the host range of

58    insect disease vectors varies extensively between extreme specialism and generalism

59    (Lyimo & Ferguson, 2009).  Amongst many of the most important insect vectors of human

60    disease, specialization on humans (anthrophily) is common (Lyimo & Ferguson, 2009).

61    Furthermore, variation in the degree of anthrophily within and between vector populations is

62    a strong predictor of spatial variation in disease transmission intensity (Kiszewski *et al.*,

63    2004; Kilpatrick *et al.*, 2006; Kilpatrick *et al.*, 2007).  Consequently identifying the factors that

64    generate selection for extreme anthrophily is a vital first step for investigation of if and how

65    this behaviour could be manipulated to reduce disease transmission.

66        Mosquitoes in the genus *Anopheles* are responsible for malaria transmission to

67    humans (Kelly-Hope et al., 2009).  There is substantial variation in the host range of

68    Anophelin*e* species ranging from those that feed on a wide range of mammals and birds, to

69    those that feed on just one species (Lyimo & Ferguson, 2009).  The most extreme specialist

70    within this genera is probably the African malaria vector *An. gambiae s.s,* which feeds almost

71    exclusively on humans throughout its range (Kiszewski et al., 2004).  According to

72    evolutionary theory, such specialism should only arise if the fitness that these mosquitoes

73    derive from exclusive anthrophagy is substantially higher than from mixing between other

74    available host species.  Similar to most haematophagous insects, the key resource that *An.*

75    *gambiae* s.s. require from their hosts is protein (required for reproduction) that they acquire

76    from haemoglobin in vertebrate red blood cells (Hurd *et al.*, 1995).  Specialization could arise

77    if there is underlying variation in the protein content or nutritional value of host blood, and/or

78    associated behavioural, physiological or ecological differences between hosts that influence

79    the energetic value that mosquitoes derive from them (Lyimo & Ferguson, 2009).  Here we

80    explicitly test the impact of only potential determinant of selection on host species choice,

81    the resource quality of vertebrate blood, and investigate whether its influence on *An.*

82    *gambiae* s.s. fitness is sufficient to explain its extreme specialism on humans.

83        Several haematological properties including biochemical composition and red cell

84    density vary between vertebrate species  (Wintrobe, 1933; Harrington *et al.*, 2001), and could

85    influence the nutritive value and subsequent fitness of mosquitoes that imbibe it (Harrington

86    *et al.*, 2001; Lyimo & Ferguson, 2009).  Here we investigated the fitness consequences of

87    consuming a specialist or generalist host diet in *An. gambiae s.s.* with the aim of testing the

88    prediction that specialists pay a fitness cost for diversifying their host species diet.  The first

89    hypothesis tested was whether the fitness of *An. gambiae s.s* after feeding on the blood of

90    their preferred human hosts is greater than after consuming blood from other host types

91    commonly available in the same environment (avian, bovine and canine).  Secondly we

92    tested whether over the course of the average reproductive lifespan of *An. gambiae s.s.* (in

93    which a median of two bloodmeals are taken, Gillies & Wilkes, 1965) their fitness is higher

94    after feeding exclusively on human blood than after consuming a generalist diet consisting of

95    a mixture of bloodmeals from humans and common animal alternatives.  Lastly, we tested

96    whether the relative fitness of mosquitoes was similar across a range of generalist host diets

97    (human plus one other host species), or depends primarily on the specific composition of

98    host types consumed.  Our aim was to evaluate whether variation in the fitness value that

99    *An. gambiae* s.s. obtains from the blood of vertebrate species commonly available to it is

100    sufficient to account for its extreme host specialization on humans.  The experimental system

101    used allowed us to control for all sources of variation in mosquito-host interactions other than

102    vertebrate blood resource quality, thus enabling the first comprehensive test of its role in

103    generating selection on the host species range of this important vector.

104    **Material and Methods**
105

106   *Mosquito rearing:* The *An. gambiae s.s* Keele strain (Hurd, 2005) was used in this study.

107   This is an outbred line created from the balanced interbreeding of individuals from four

108   laboratory colonies derived different African populations (Kenya, north Tanzania, south

109   Tanzania and the Gambia, Hurd *et al.*, 2005). This colony was established at the University

110   of Glasgow four years prior to these experiments, where it is maintained on human blood that

111   is provided to mosquitoes by membrane feeding thrice weekly. Mosquitoes are maintained

112   under standard insectary conditions of $26 \pm 1^{\circ}C$, 80% relative humidity, and a 12:12 hour

113   light/dark cycle. Adult mosquitoes are provided with a solution of 5% glucose/0.05 % para-

114   amino-benzoic acid (PABA).

115

116   *Mosquito blood feeding:* Two days before experiments, previously unfed adult females of a

117   similar age (3-5 days post emergence) were randomly selected and transferred into

118   cardboard holding cartons (9.5 cm diameter × 9 cm depth) sealed with mesh at the top.

119   Approximately 100-200 females were placed in each carton and held for 2 days under

120   insectary conditions before being offered their first blood meal. On the day of the feed,

121   mosquitoes were offered a blood meal from a membrane feeder as described previously

122   (Carter et al., 1993). All the vertebrate bloods (human and animal) used in these

123   experiments were collected, stored and processed in a similar manner prior to

124   experimentation. Whole human and animal blood was collected aseptically from healthy

125   adult individuals. All blood was stored in sterile vacutainers containing sodium citrate

126   preservative and held in a fridge at $4^{\circ}C$ prior to use. Blood was stored for no more than 7

127   days after being withdrawn from a host before being fed to mosquitoes.

128

129   *Host species impacts from one blood meal:* In nature *An. gambiae* s.s. will blood feed and

130   produce an egg batch once every 2-4 days, with the median number produced before death

131   being two (Gillies & Wilkes, 1965) However, an initial set of experiments was performed in

132   which mosquitoes were provided with only one blood meal to test intrinsic variation in the

133   resource quality of blood from different host species. Mosquitoes were randomly allocated

134  to one of 4 host treatment groups: chicken, cow, dog or human blood (Lorne Laboratories,

135  Reading, UK).  Within each treatment group, mosquitoes were further randomly subdivided

136  into 4 feeding cartons (holding 40-50 mosquitoes each) which served as independent

137  replicates of each diet treatment (4 host treatments x 4 replicates/treatment = 16 cartons).

138  Each of these 16 replicates was fed on the blood of a different host individual (4 per species)

139  provided from a different membrane feeder).  Two hours after the feed, cartons were

140  inspected and all unfed mosquitoes were removed and killed.  Blood fed mosquitoes were

141  kept in these original holding cartons for 3 days and provided access to a solution of 5%

142  glucose/0.05% para-amino-benzoic acid.  On day 3, survivors were transferred into individual

143  30 ml universal tubes filled with approximately 1cm of distilled water to allow them to oviposit.

144  Mosquito oviposition rate (proportion laying eggs) and fecundity (the number of eggs laid)

145  were evaluated the following day by examining each tube and counting any eggs laid with

146  the aid of a dissecting microscope.  The survival of mosquitoes was checked on a daily basis

147  from the first day after the blood feed.  Mosquitoes were provided with the standard glucose

148  solution on cotton wool pads during this period.

149

150  *Uniform and mixed host species diets:*  For their first blood meal, all *An. gambiae s.s* were

151  provided with human blood (Patricell UK, Nottingham, UK).  Mosquitoes were split into three

152  groups, with each group being fed from the blood of a different human donor.  Three days

153  after the feed, all survivors were transferred into individual 30 ml universal tubes filled for

154  oviposition as described above.   After tubes had been inspected for oviposition the next day,

155  all surviving mosquitoes were pooled in a holding cage and then randomly allocated into one

156  of four different treatments for their second blood meal: human (Patricell UK, Nottingham,

157  UK), cow, dog or chicken blood (Harlan Laboratories Ltd, Belton, UK).  With each of these 4

158  treatments, mosquitoes were further randomly subdivided into three feeding cartons (holding

159  10-25 mosquitoes each) which served as independent replicates of each diet treatment (4

160  host treatments x 3 replicates/treatment = 12 replicates overall).  Each of these twelve

161  replicate groups was fed on the blood of a different host individual, provided from a different

162    membrane feeder.  The 4-day gap between the first and second blood meal was selected to

163    mimic the blood feeding cycle of *An. gambiae* s.s. in nature (e.g. 2 -4 days between blood

164    feeds, Gillies, 1953).  After the second blood feed, all mosquitoes that were observed to have

165    fed (by visual inspection 2 hours after the feed) were held in cartons for 3 days and then

166    transferred into individual universal tubes containing 1 cm of distilled water for oviposition.

167    The following day, tubes were inspected to measure oviposition and fecundity as described

168    above.  All mosquitoes were then transferred into dry universal tubes and maintained there

169    until the end of the experiment (provided with a solution of 5% glucose/0.5% para-amino-

170    benzoic acid (PABA) as described above).  All mosquitoes were checked on a daily basis for

171    further 18 days after their second blood feed (23 -25 days from emergence) to monitor

172    survival.  This entire experimental procedure was repeated twice to make two blocks.

173

174    *Statistical analyses:*  Statistical analysis was conducted to assess the impact of host diet

175    diversity on 3 key measures of mosquito fitness: oviposition, fecundity and survival.

176    First, we investigated whether the proportion of mosquitoes that laid eggs after feeding was

177    influenced by host treatment using generalized linear mixed effect models with binomial

178    errors (glmer) in the R statistical software package (Crawley, 2007).  Here 'host treatment'

179    was taken as the main effect and the different host individuals within a host species were fit

180    as a random effect.  A base statistical model including only the random effect of host

181    individual was constructed, to which the main effect of 'host treatment' was added to form the

182    full model.  The significance of host treatment was evaluated using likelihood ratio tests

183    (Burnham & Anderson, 2002).  Variation in mosquito fecundity (the number of eggs laid) was

184    similarly analysed using generalized linear mixed effect models (lmer) in the R statistical

185    software package (Crawley, 2007).

186        As the median number of egg batches produced by *An. gambiae* s.s. before death is

187    two (Gillies & Wilkes, 1965), analysis of mosquito cumulative egg production over their first

188    two blood meals can provide an approximation of the lifetime reproductive success of an

189    average individual.  Here we estimated the cumulative number of eggs produced over 2

190    feeding cycles in order to approximate the impact of specialist and generalist blood diets on

191    lifetime reproductive success.  In these experiments it was not possible to measure the

192    lifetime egg production of individual mosquitoes, because individuals had to be pooled into

193    groups for blood feeding within which they could not be individually tracked.  However,

194    cumulative egg production over two blood meals was estimated by summing the distribution

195    of egg numbers produced after the first and second blood meals on different host individuals.

196    As all mosquitoes initially fed on humans, it was assumed that their fecundity after the first

197    blood meal was similarly distributed.  The observed range of eggs produced from the first

198    blood meal (block 1: 0 – 103 eggs, block 2: 0 – 159 eggs) was split into 11 intervals to obtain

199    a distribution describing fecundity in units of 15 eggs.  Distributions of eggs laid after the

200    second blood meal were computed on a similar basis for each of the 4 host treatments, and

201    an estimate of cumulative egg production obtained by summing distributions from the first

202    and second bloodmeals.  A Kruskal-Wallis test was used to evaluate if there were statistically

203    significant differences in cumulative egg production between host blood treatments.

204          The impact of host treatment on mosquito survival was analysed using the Cox

205    Proportional Hazard Model (COXPH) in the R statistical software package (Crawley, 2007).

206    Differences in survival between treatment groups were assessed from the day after the first

207    blood meal in single host experiments, and from the second blood meal onwards in mixed

208    host diet experiments.  In these analyses, host treatment was considered as a main effect,

209    and a frailty function was used to incorporate the random effect of host individual (within

210    each host species treatment) into the Cox model.

211

212    **Results**

213    *Host species impacts from one blood meal:*  The oviposition rate of mosquitoes after one

214    blood meal varied between host species ($\chi^2_3$ = 26.02, P < 0.001), with *An. gambiae s.s.*

215    having a significantly higher probability of laying eggs after feeding on human and cow blood,

216    than chicken or dog (Figure 1a).  However, the number of eggs laid by ovipositing

217    mosquitoes was similar across host species ($\chi^2_3$ = 1.60, P = 0.66, Figure 1b).  Mosquito

218      survival after taking one blood meal varied between host species ($\chi^2_3$ = 56.5, P < 0.001,

219      Figure 2).  The longevity of *An. gambiae* s.s. was similarly high after taking a human and cow

220      blood meal, but was significantly lower on chicken and dog blood than on humans (Table 1).

221      *Uniform and mixed host species diets*: Over the two experimental blocks, the reproduction of

222      a total of 140 and 200 *An. gambiae* s.s. were assayed respectively.  The oviposition rate of

223      *An. gambiae s.s* after feeding on two human blood meals did not differ from those fed on  a

224      meal of human followed by cow, chicken or dog blood (Block 1: $\chi^2_3$ = 5.37, P = 0.15, Figure

225      3a; Block 2: $\chi^2_3$ = 2.18, P = 0.53, Figure 3b).  Furthermore, the number of eggs laid by

226      ovipositing *An. gambiae s.s* following their second blood meal did not vary between

227      mosquitoes in the human-only and mixed host species diets (Block 1: $\chi^2_3$ = 1.78, P = 0.62,

228      Figure 3c: Block 2: $\chi^2_3$ = 3.84, P = 0.28, Figure 3d).  Finally the estimated cumulative egg

229      production of mosquitoes over two blood meals did not vary between those fed a diet of only

230      human blood, or a combination of human and animal blood (Block 1: $\chi^2_3$ = 0.12, P = 0.99,

231      Figure 4a, Block 2: $\chi^2_3$ = 0.10, P = 0.99, Figure 4b).  Post hoc comparison also indicated

232      that there was no significant differences between the three different generalist host blood

233      diets (human-cow, human-chicken and human-dog) in terms of mosquito oviposition (Block

234      1: $\chi^2_2$ = 4.42, P =0.11, Figure 3a,  Block 2: $\chi^2_2$ = 1.31, P =0.52, Figure 3b), fecundity (Block

235      1: $\chi^2_2$ = 2.09, P = 0.35, Figure 3c  Block 2: $\chi^2_2$ = 0.49, P = 0.78, Figure 3d), or cumulative

236      egg production over two blood meals (Block 1: $\chi^2_2$ = 0.09, P = 0.95, Figure 4a, Block 2: $\chi^2_2$

237      = 0.10, P = 0.95, Figure 4b).

238          The survival of *An. gambiae s.s* after feeding on two blood meals did not vary

239      between those fed only human blood, and those given human and cow, chicken or dog blood

240      (Figure 5a & b, Table 2).  Similarly, no significant differences in mortality were observed

241      between mosquitoes in the three different generalist host diet groups (Block 1: $\chi^2_2$ = 0.92, P

242      = 0.63, Block 2: $\chi^2_2$ = 0.86, P = 0.65).

**Discussion**

243

244      Here we experimentally investigated the consequences of host species diversity for

245    the malaria mosquito *An. gambiae* s.s. to test the evolutionary prediction that organisms

246    evolved for specialism have reduced fitness on generalist diets.  Comparisons of the fitness

247    that mosquitoes derived from single bloodmeals indicated the existence of some underlying

248    variation in the resource quality of host blood, with *An. gambiae* s.s. having greater

249    oviposition success and survival after feeding on human and bovine blood, than on dog or

250    chicken.  However when evaluated within the context of the multiple feeding events that *An.*

251    *gambiae* s.s. is expected to take during its lifetime (median of 2), neither their reproductive

252    success nor long term survival was influenced by the diversity of their host species diet

253    (human-only or a mixture of human followed by cow, ,chicken or dog).  Furthermore, the

254    number of eggs laid by *An. gambiae* s.s. after one or two bloodmeals was unrelated to host

255    species, and there was no evidence from either single or multiple feeding experiments that

256    human blood gave rise to a distinct advantage over all other host types that could account for

257    its extreme specialization in nature (e.g. human blood was associated with some advantages

258    over avian and canine blood, but not bovine).  Thus we hypothesize that selection for

259    specialization in this mosquito is unlikely to be generated by underlying variation in quality

260    between their preferred human hosts and other alternatives readily available in the same

261    environment.

262      Haematological properties such as red blood cell size, density and amino acid

263    composition are known to vary between the host species investigated here (Wintrobe, 1933;

264    Nemi, 1986; Hawkey, 1991; Hawkey *et al.*, 1991).  Such variation could be responsible for

265    between-host differences in mosquito survival and oviposition rate we observed after one

266    blood meal here.  However there was negligible impact of interspecific haematological

267    variation after the second blood meal, possibly because mosquitoes acquired enough

268    nutrients from their first (human) blood meal to offset any modest deficits in the nutrient

269    quality of later meals.   As human blood was identified as one of the better resources  in the

270    single-feed experiments, it is possible that by providing this first before meals from other host

271      species could have masked the costs of a generalist host diet in which non-human hosts

272      were consumed first or repeatedly.  However putting together the two 'best' host types as

273      identified in single feed experiments (human and cow) did not give rise to greater fitness than

274      a combination of one of the best and the worst (human and chicken). This suggests that the

275      fitness effects of individual blood meals may not be additive, and that any deficits arising

276      from interspecific host haematological variation can be offset provided mosquitoes obtain

277      more than one blood meal.  Further experiments in which the order and frequency of human

278      and animal blood meals given to *An. gambiae s.s.* is varied are required to confirm this.

279           We found no impact of host species on the number of egg laid by ovipositing

280      mosquitoes in either single of multiple feeding experiments.  This result contrasts with some

281      previous studies indicating that mosquito fecundity depends on host species (Woke, 1937a;

282      Woke, 1937b; Bennett, 1970; Downe & Archer, 1975; Mather & DeFoliart, 1983).

283      Discrepancies between these and the current study may reflect genuine biological

284      differences in the impact of host species on different mosquito species.  Alternatively the

285      impact of host species may have been underestimated in these experiments due to the

286      manner in which blood was presented.  Here, a fixed volume of blood was presented to

287      mosquitoes in standardized membrane feeding devices, for a fixed time period (longer than

288      the average duration of a feeding event in the wild).  This design allowed us to isolate the

289      specific impact of blood on mosquito fitness, while controlling for variation in all other host

290      physiological and behavioural traits that could influence blood intake.  However the lack of

291      time limitation may have allowed mosquitoes to adapt their feeding effort (e.g. by adjusting

292      the volume of blood taken, or the time spent feeding) to maximize their resource intake

293      regardless of the type of blood consumed.  If such compensations are less possible when

294      mosquitoes are feeding of live hosts, the impact of inherent variation in blood quality could

295      have a substantially greater impact on mosquito fitness in the wild than estimated here.

296           In this study, the long-term survival of *An. gambiae s.s* was influenced by host

297      species only when blood intake was restricted to one meal, but not when multiple meals were

298      taken.  These results are consistent with the majority of previous laboratory studies which

299    indicate mosquito survival is unrelated to host blood meal diversity (reviewed in (Lyimo &

300    Ferguson, 2009).  However, Harrington et al 2001 did find that the survival of *Ae. aegypti*

301    was significantly longer on its naturally preferred human hosts than that  of laboratory

302    rodents.  This was postulated as being a consequence of variation in the isoleucine content

303    of blood between these species, which was correlated with the acquisition of energetic

304    reserves rom blood feeding (Harrington *et al.*, 2001).  It is unknown whether variation in

305    blood isoleucine content could account for the enhanced survival of *An. gambiae* s.s. after

306    taking one blood meal from humans or cows here.  However if these host species do exhibit

307    significant biological variation in blood isoleucine content, it had little impact on the fitness

308    that *An. gambiae* s.s. acquired from their second blood meal.  If the impact of host-specific

309    variation in haematological parameters such as isoleucine concentration is highly context

310    dependent (e.g. dependent on the number and type of previous bloodmeals taken), it may be

311    unlikely to provide a sufficiently reliable signal to drive selection for host choice.

312          Although our results indicate blood meal diversity does not have a large impact on

313    *An. gambiae* s.s. survival, it is possible it does have a moderate effects that would be unlikely

314    to detect under standard laboratory conditions.  For example as is standard practice for

315    laboratory studies, all blood fed mosquitoes were given unlimited access to glucose and

316    water throughout their lifetime.  Sugar feeding is known to extend mosquito longevity (Gary &

317    Foster, 2001; 2004), and although *Anopheles* are known to do so in nature (Beier, 1996;

318    Gary & Foster, 2006; Gary *et al.*, 2009)  it is unlikely do so as readily in the wild as in these

319    laboratory conditions.  Consequently this provision of sugar could have masked differences

320    in mosquito survival arising from their blood diet.  Previous studies have also shown that

321    sugar provision in addition to blood can reduce (Gary & Foster, 2001; Bracks *et al.*, 2006) or

322    enhance *An. gambiae* s.s. reproductive success (Manda *et al.*, 2007).  Further experiments

323    including contrasts of blood diets in the presence and absence of sugar meals are required

324    to confirm the predicted impacts of host species on mosquito fitness described here.

325          Although the existence of fitness trade-offs in resource exploitation have been widely

326    predicted for dietary specialists (Levins, 1962; MacArthur & Pianka, 1966a; Pyke *et al.*,

327    1977), their existence has failed to be confirmed in several insect model systems (Agrawal et

328    al., 2002).  In these studies, it has been hypothesized that the lack of fitness differences

329    between single and mixed host diets is because the plant resources incorporated in both

330    meal types had similar nutritional value (Rapport, 1980; Hauge *et al.*, 1998), which may also

331    be true for the blood sources investigated here.  Alternatively, failure to detect trade-offs

332    associated with host dietary diversity in this and other studies may be a consequence of the

333    homogeneous background of insect lines used in laboratory studies.  Although these

334    experiments used a relatively outbred line of *An. gambiae* s.s. (Hurd et al., 2005), the

335    adaptations that favour specialization on humans could have been partially eroded during the

336    colonization process and limited our ability to detect an advantage of human blood.

337    Although we found no evidence of an overall fitness advantage from feeding only on

338    human blood here, it is premature to dismiss the possibility that trade-offs in host exploitation

339    under more natural conditions that may explain why *An. gambiae s.s* has evolved a specialist

340    feeding strategy.  For example, defensive behaviour in response to mosquito biting can vary

341    between host species (Edman & Scott, 1987), and may generate selection for specialization

342    on weakly protective species.  Other host factors such as the ease with which their skin can

343    be penetrated, range over which their odour cues can be detected (Gillies & Wilkes, 1972),

344    suitability of microhabitats and relative availability will influence their encounter and

345    exploitation rates by mosquitoes, and could exert stronger selection on host choice than

346    variation in blood quality.  Further examination of these host-specific factors under natural

347    conditions are needed to evaluate their role in structuring *An. gambiae* s.s. host range.

348    The intense specialism of *An. gambiae* s.s. on humans is largely responsible for its

349    ability to maintain malaria transmission levels within its sub-Saharan African range that are

350    well beyond those achieved in other parts of the globe.  In addition to the immediate need to

351    protect humans from their bites using Insecticide Treated Nets and other measures, it is

352    possible that longer-term reductions in exposure could be achieved through environmental

353    manipulation of the selective forces that promote anthrophily.  These results suggest that

354    transition from a human-specialist to more generalist strategy need not be impeded by the

355  relative fitness value of uniform and mixed species blood diets, and that any potential costs

356  of generalism are more like to arise through ecological or behavioural factors.

357  **Authors' contributions**

358  INL designed and executed experiments, performed data analyses and prepared the

359  manuscript draft.  SPK executed single bloodmeal experiments. HMF supervised the

360  execution of experiments, data analysis and reviewed the manuscript.  LCR advised on

361  methodology, reviewed the manuscript and provided comments.

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372 **References**

373 Abrams, P.A. 2006a. The effects of switching behavior on the evolutionary diversification of

374          generalist consumers. *Am Nat* **168:** 645-659.

375 Abrams, P.A. 2006b. The prerequisites for and likelihood of generalist-specialist coexistence.

376          *Am Nat* **167:** 329-342.

377 Agrawal, A.A., Vala, F. & Sabelis, M.W. 2002. Induction of preference and performance after

378          acclimation to novel hosts in a phytophagous spider mite: Adaptive plasticity? *Am Nat*

379          **159:** 553-565.

380 Allard, C.M. & Yeargan, K.V. 2005. Effect of diet on development and reproduction of the

381          harvestman *Phalangium opilio* (Opiliones : Phalangiidae). *Env Entomol* **34:** 6-13.

382 Beier, J.C. 1996. Frequent blood-feeding and restrictive sugar-feeding behaviour enhance

383          the malaria vector potential of *Anopheles gambiae* s.l. and *An. funestus* (Diptera:

384          Culicidae) in Western Kenya. *J Med Entomol* **33:** 613 - 618.

385 Bennett, G.F. 1970. The influence of the blood meal type on the fecundity of *Aedes aegypti*

386          (Stegomyia)L. (Diptera:Culicidae). *Canad J Zool* **48:** 539-543.

387 Bernays, E.A., Bright, K.L., Gonzalez, N. & Angel, J. 1994. Dietary mixing in a generalist

388          herbivore: tests of two hypotheses. *Ecology* **75:** 1997-2006.

389 Bracks, M.A.H., Juliano, S.A. & Lounibos, L.P. 2006. Superior reproductive success on

390          human blood without sugar is not limited to highly anthropophilic mosquito species.

391          *Med Vet Entomol* **20:** 53 - 59.

392 Burnham, K.P. & Anderson, D.R. 2002. *Model selection and multimodel inference a practical*

393          *information-theoretic approach*. Springer, New York.

394     Carter, R., Ranford-Cartwright, L.C. & Alano, P. 1993. The culture and preparation of

395         gametocytes of *Plasmodium falciparum* for immunochemical, molecular and mosquito

396         infectivity studies. *In "Methods in molecular biology, vol 21; protocols in molecular*

397         *parasitology".* Humana Press Inc, Totowa, New Jersey. **21:** 67-89.

398     Crawley, M.J. 2007. *The R book.* John Wiley & Sons Inc, Chichester, England.

399     Downe, A.E.R. & Archer, J.A. 1975. Effects of different bloodmeal sources on digestion and

400         egg production in   *Culex tarsalis* Coq (Diptera:Culicidae). *J Med Entomol* **12:** 431-

401         437.

402     Edman, J.D. & Scott, T.W. 1987. Host defensive behaviour and the feeding success of

403         mosquitoes. *Insect Sci Applic* **8:** 617-622.

404     Egas, M., Dieckmann, U. & Sabelis, M.W. 2004. Evolution restricts the coexistence of

405         specialists and generalists: The role of trade-off structure. *Am Nat* **163:** 518-531.

406     Futuyma, D.J. & Moreno, G. 1988. The evolution of ecological specialization. *Ann Rev Ecol*

407         *Syst* **19:** 207-233.

408     Gary, R.E. & Foster, W.A. 2001. Effects of availability of sugar on the reproductive fitness

409         and vectorial capacity of the malaria vector *Anopheles gambiae* (Diptera: Culicidae).

410         *J Med Entomol* **38:** 22 - 28.

411     Gary, R.E. & Foster, W.A. 2004. *Anopheles gambiae* feeding and survival on honeydew and

412         extra-floral nectar of peridomestic plants. *Med Vet Entomol* **18:** 102 - 107.

413     Gary, R.E. & Foster, W.A. 2006. Diel timing and frequency of sugar feeding in the

414         mosquitoes *Anopheles gambiae*, depending on sex, gonotrophic state and resource

415         availability. *Med Vet Entomol* **20:** 308-316.

416   Gary, R.E., Cannon, J.W. & Foster, W.A. 2009. Effect of sugar on male *Anopheles gambiae*

417       mating performance, as modified by temperature, space and body size. *Parasites &*

418       *Vectors* **2:** 1 - 13.

419   Gillies, M.T. 1953. The duration of the gonotrophic cycle in *Anopheles gambiae* and

420       *Anopheles funestus*, with a note on the efficiency of hand catching. *East Afr Med J*

421       **30:** 129-135.

422   Gillies, M.T. & Wilkes, T.J. 1965. A study of the age-composition of populations of *Anopheles*

423       *gambiae* Giles and *An. funestus* Giles in North - Eastern Tanzania. *Bull Entomol Res*

424       **56:** 237 - 262.

425   Gillies, M.T. & Wilkes, T.J. 1972. The range of attraction of animal baits and carbon dioxide

426       for mosquitoes. Studies in a freshwater area of West Africa. *Bull Entomol Res* **61:**

427       389 - 404.

428   Harrington, L.C., Edman, J.D. & Scott, T.W. 2001. Why do female *Aedes aegypti* (Diptera:

429       Culicidae) feed preferentially and frequently on human blood? *J Med Entomol* **38:**

430       411-422.

431   Hauge, M.S., Nielsen, F.H. & Toft, S. 1998. The influence of three cereal aphid species and

432       mixed diet on larval survival, development and adult weight of *Coccinella*

433       *septempunctata. Entomol Expt Applic* **89:** 319-322.

434   Hawkey, C.M. 1991. The value of comparative haematological studies. *Comp Haem Intl* **1:** 1-

435       9.

436   Hawkey, C.M., Bennett, P.M., Gascoyne, S.C., Hart, M.G. & Kirkwood, J.K. 1991.

437       Erythrocyte size, number and haemoglobin content in vertebrates. *Brit J Haem* **77:**

438       392-397.

439    Hurd 2005. Evaluating the costs of mosquito resistance to malaria parasites. *International*

440           *Evolution* **59:** 2560-2572.

441    Hurd, H., Hogg, J.C. & Renshaw, M. 1995. Interractions between bloodfeeding, fecundity and

442           infection in mosquitoes. *Parasitol Today* **11:** 411-416.

443    Hurd, H., Taylor, P.J., Adams, D., Underhill, A. & Eggleston, P. 2005. Evaluating the costs of

444           mosquito resistance to malaria parasites. *Evolution* **59:** 2560-2572.

445    Jaenike, J. 1990. Host Specialization in Phytophagous Insects. *Ann Re Ecol Syst* **21:** 243-

446           273.

447    Kelly-Hope, L.A., Hemingway, J. & McKenzie, F.E. 2009. Environmental factors associated

448           with the malaria vectors *Anopheles gambiae* and *Anopheles funestus* in Kenya.

449           *Malaria J* **8**. 268.

450    Kilpatrick, A.M., Kramer, L.D., Jones, M.J., Marra, P.P. & Daszak, P. 2006. West Nile virus

451           epidemics in North America are driven by shifts in mosquito feeding behavior. *PLoS*

452           *Biol* **4:** e82.

453    Kilpatrick, A.M., Kramer, L.D., Jones, M.J., Marra, P.P., Daszak, P. & Fonseca, D.M. 2007.

454           Genetic influences on mosquito feeding behavior and the emergence of zoonotic

455           pathogens. *Am J Trop Med Hyg* **77:** 667.

456    Kiszewski, A., Mellinger, A., Spielman, A., Malaney, P., Sachs, S.E. & Sachs, J. 2004. A

457           global index representing the stability of malaria transmission. *Am J Trop Med Hyg*

458           **70:** 486 - 498.

459    Levins, R. 1962. Theory of fitness in a heterogeneous environment. i. The fitness set and

460           adaptive function. *Am Nat* **96:** 361-373.

461 Lyimo, I.N. & Ferguson, H.M. 2009. Ecological and evolutionary determinants of host species
462     choice in mosquito vectors. *Trends Parasitol* **25:** 189-196.

463 MacArthur, R.H. & Pianka, E.R. 1966a. On optimal use of a patchy environment. *Am Nat*
464     **100:** 603-609.

465 Manda, H., Gouagna, L.C., Nyandat, E., Kabiru, E.W., Jackson, R.R., Foster, W.A., Githure,
466     J.I., Beier, J.C. & Hassanali, A. 2007. Discriminative feeding behaviour of *Anopheles*
467     *gambiae* s.s. on endemic plants in western Kenya. *Med Vet Entomol* **21:** 103 - 111.

468 Mather, T.N. & DeFoliart, G.R. 1983. Effect of host blood source on the gonotrophic cycle of
469     *Aedes triseriatus. Am J Trop Med Hyg* **32:** 189.

470 Michaud, J.P. & Jyoti, J.L. 2008. Dietary complementation across life stages in the
471     polyphagous lady beetle *Coleomegilla maculata. Entomol Expt Appl* **126:** 40-45.

472 Nemi, J.C. 1986. Essentials of Veterinary Hematology, Lea & Febiger, Philadelphia.

473 Price, P.W., Bouton, C.E., Gross, P., McPheron, B.A., Thompson, J.N. & Weis, A.E. 1980.
474     Interactions among three trophic levels: Influence of plants on interactions between
475     insect herbivores and natural enemies. *Ann Rev Ecol Syst* **11:** 41-65.

476 Pyke, G.H., Pulliam, H.R. & Charnov, E.L. 1977. Optimal foraging: A selective review of
477     theory and tests. *Q Rev Biol* **52:** 137-154.

478 Rapport, D.J. 1980. Optimal Foraging for Complementary Resources. *Am Nat* **116:** 324-346.

479 Thomas, H.Q., Zalom, F.G. & Roush, R.T. 2010. Laboratory and field evidence of post-
480     release changes to the ecological host range of *Diorhabda elongata*: Has this
481     improved biological control efficacy? *Biol Cont* **53:** 353-359.

482    Via, S. 1990. Ecological genetics and host adaptation in herbivorous insects - the

483        experimental study of evolution in natural and agricultural systems. *Ann Rev Entomol*

484        **35:** 421-446.

485    Wintrobe, M.M. 1933. Variations in the size and hemoglobin content of erythrocytes in the

486        blood of various vertebrates. *Folia Haem* **51:** 32-49.

487    Woke, P.A. 1937a. Comparative effects of the blood of different species of vertebrates on

488        egg production of *Aedes aegypti*. *Am J Trop Med Hyg* **17:** 729-745.

489    Woke, P.A. 1937b. Comparative effects of the blood of man and of canary on egg-production

490        of *Culex pipiens* Linn. *J Parasitol* **23:** 311-313.

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**Figure legends**

**Figure 1 - Reproductive success of *An. gambiae s.s* after one blood meal on different host species.** Figure 1a indicates the oviposition rate (proportion of mosquitoes that laid eggs) after taking one blood meal from different host species, and figure 1b the average number of eggs laid by mosquitoes who oviposited after one blood meal.

**Figure 2 - The survival of *An. gambiae s.s* after taking one blood meal from different host species.**

**Figure 3 - Reproductive success of *An. gambiae s.s* after their second blood meal on different host species.** Host species treatments are represented by abbreviations, with the first letter referring to the host species whose blood was consumed on the first feed (always human, 'H'), and the second letter to the host species whose blood was consumed on the second feed: CH - chicken, CO – cow, H + DG – dog, and uniform H – two successive human blood meals. Figure 1a and b indicate the oviposition rate of mosquitoes after taking a second blood meal from different host species, and figure 1c and d indicate the average number of eggs laid by mosquitoes who oviposited after their second blood meal.

**Figure 4 - Estimated cumulative distribution of eggs laid by *An. gambiae s.s* over two gonotrophic cycles in experimental blocks 1(a) and 2(b).** Host species treatments are represented by abbreviations, with the first letter referring to the host species whose blood was consumed on the first feed (always human, 'H'), and the second letter to the host species whose blood was consumed on the second feed: CH - chicken, CO – cow, H + DG – dog, and uniform H – two successive human blood meals

**Figure 5 - The survival of *An. gambiae s.s* after feeding after two blood meals from either human-only (specialist) or human and animal (generalist) host sources.** Host species treatments are represented by abbreviations, with the first letter referring to the host species whose blood was consumed on the first feed (always human, 'H'), and the second letter to the host species whose blood was consumed on the second feed: H + CH: human +

531  chicken, H + CO – human and cow, H + DG –human and dog, and uniform H – two

532  successive human blood meals

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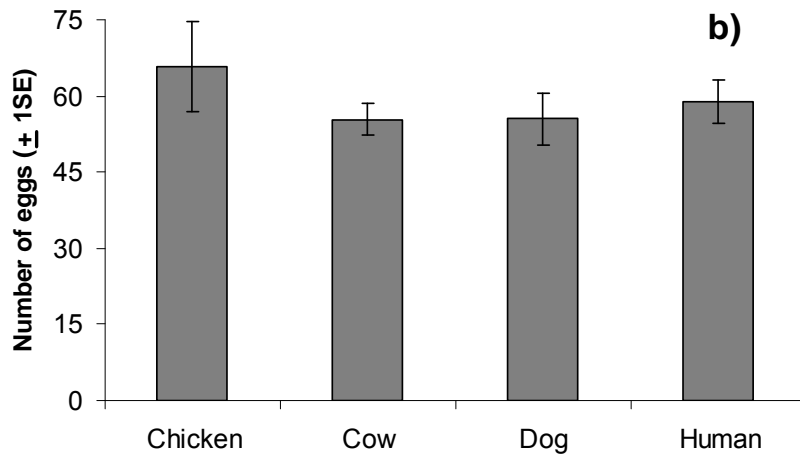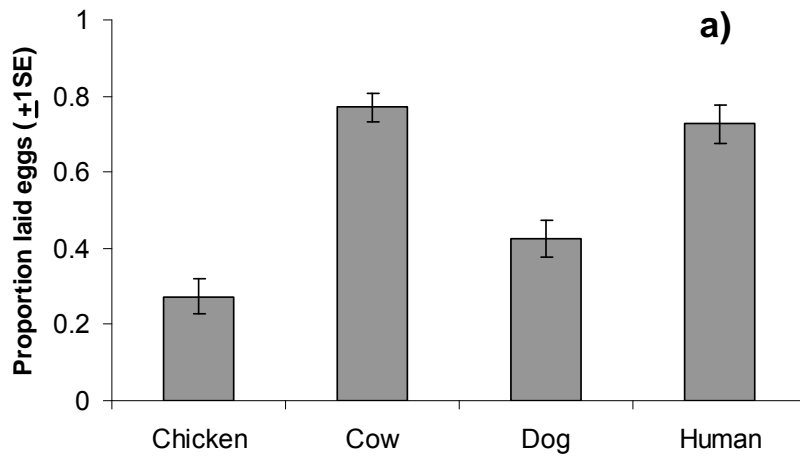
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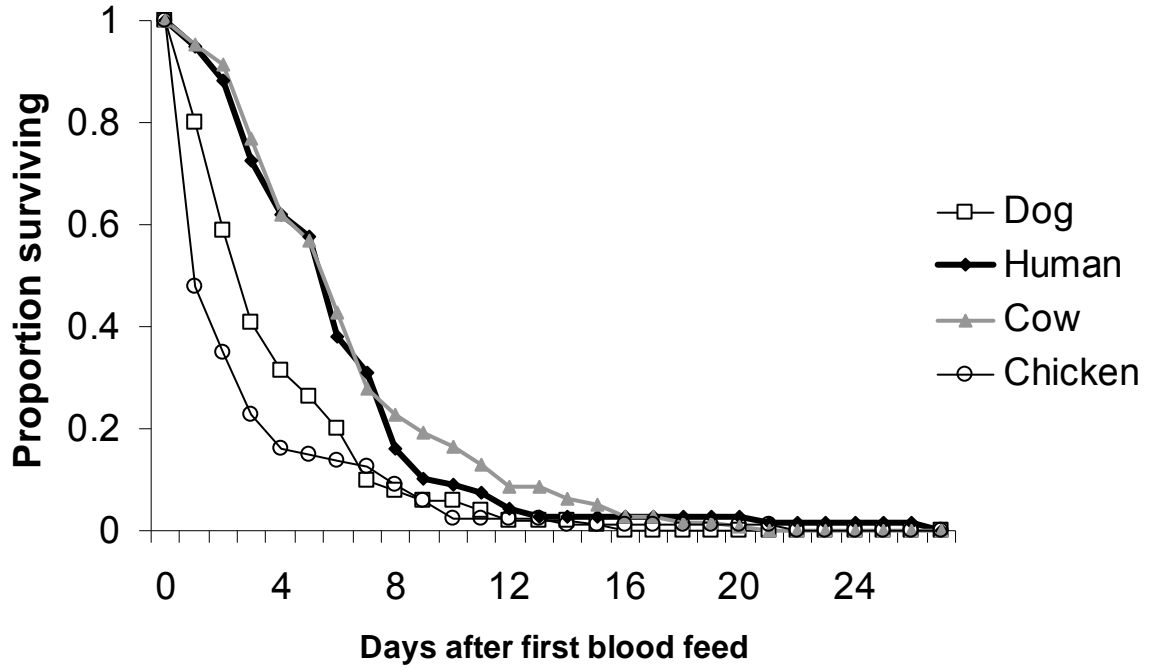
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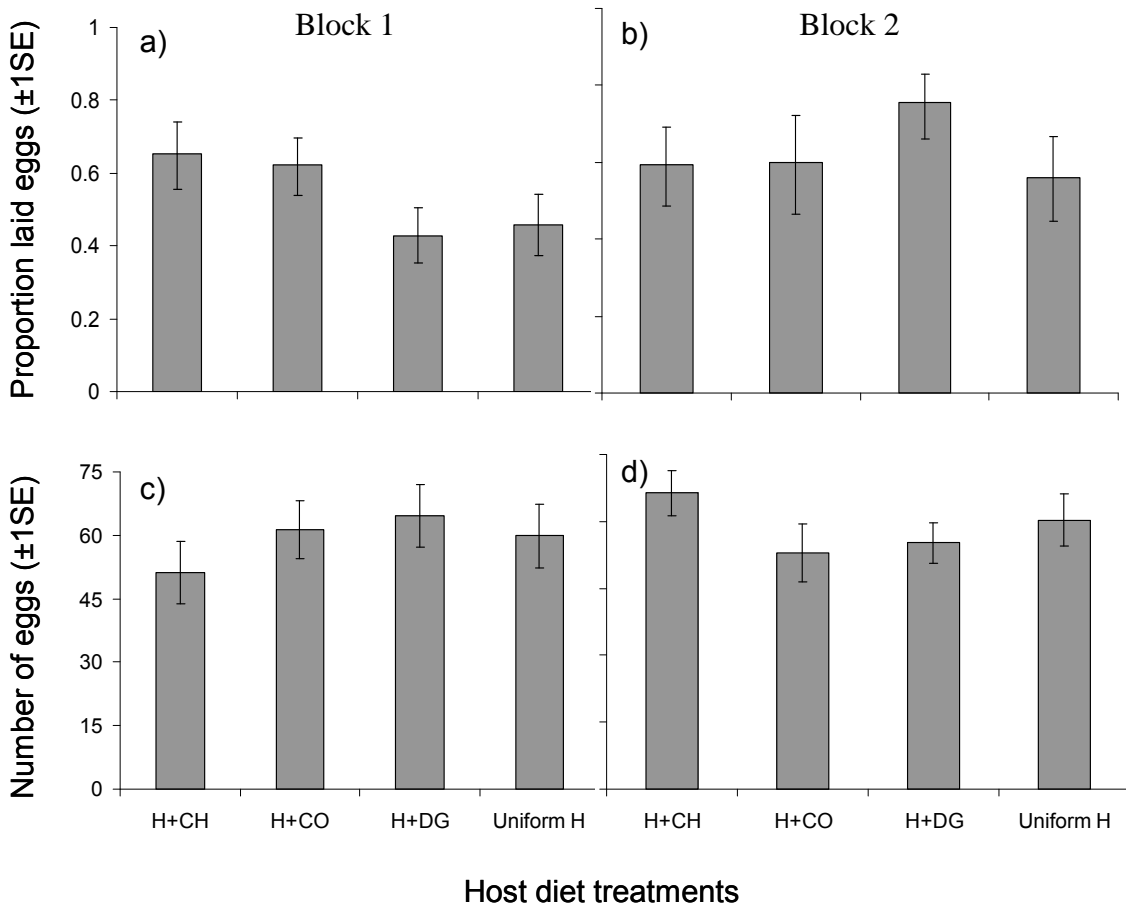

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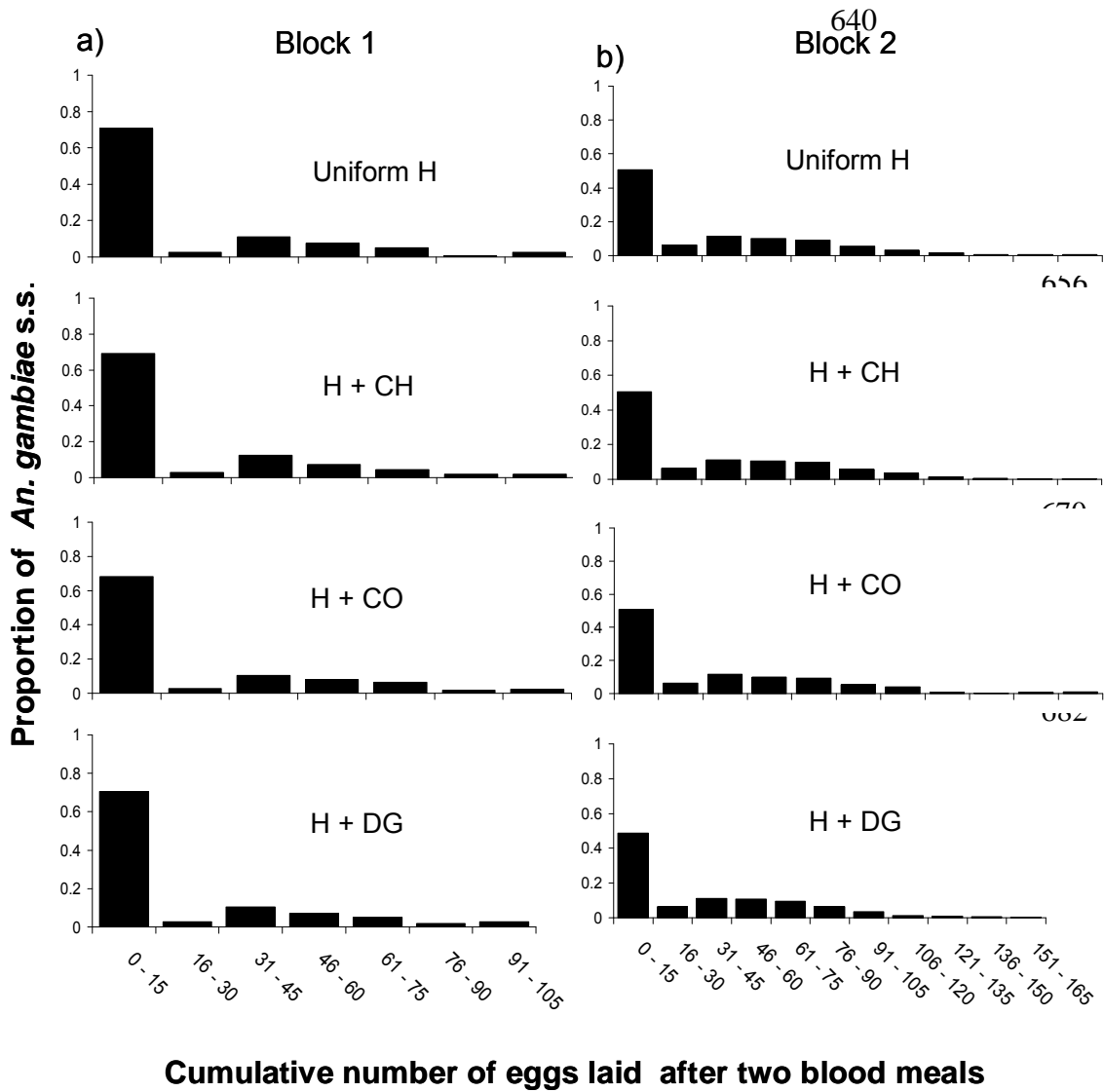
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Figure 4

a) **Block 1**    b) **Block 2**



**Proportion of *An. gambiae s.s.***

Uniform H

H + CH

H + CO

H + DG

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**Cumulative number of eggs laid  after two blood meals**

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**Block 1**
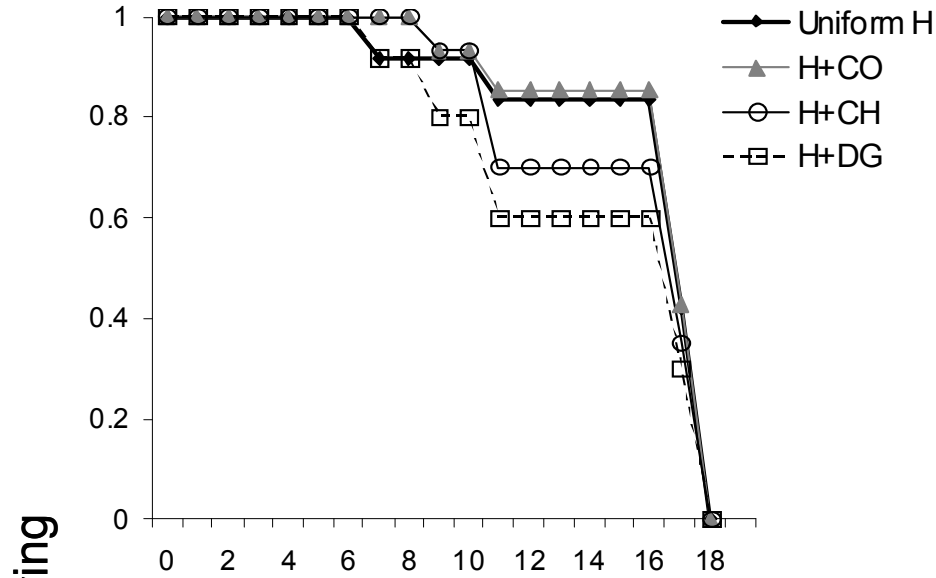


**Proportion surviving**

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**Block 2**
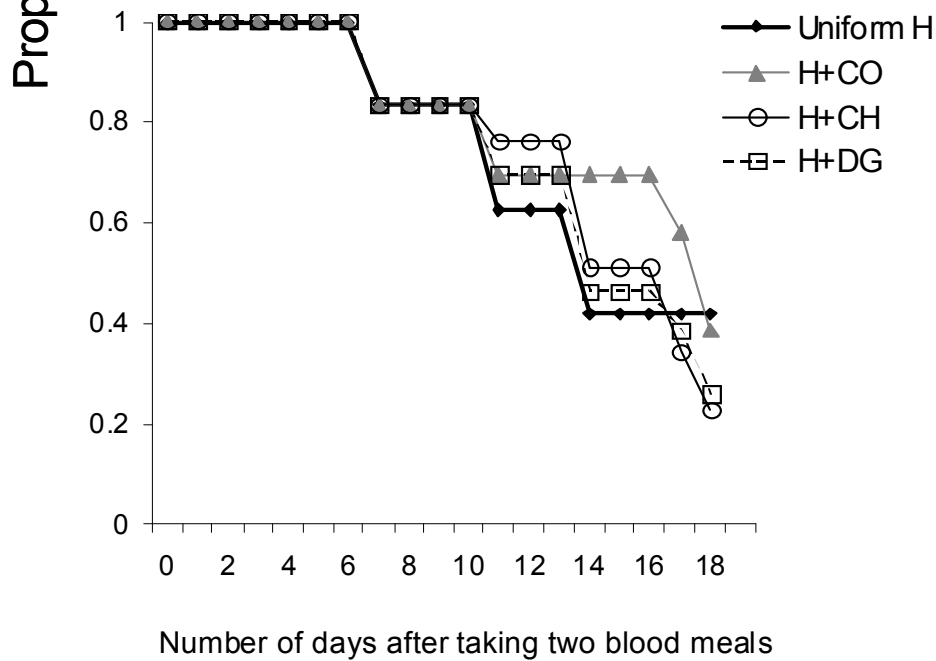


Number of days after taking two blood meals

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**Tables**

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821  **Table 1. Survival of *An. gambiae s.s* after one blood meal:** The estimated odds of

822  mortality of *An. gambiae s.s* after taking one blood meal from different host species relative

823  to human blood.  Numbers in brackets are the 95% confidence intervals of the odds ratio.

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| Host species | Odds Ratio |
|---|---|
| Chicken | 2.47 (1.80-3.39) |
| Cow | 0.94 (0.70-1.27) |
| Dog | 1.78 (1.31-2.41) |

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843 **Table 2. Survival of *An. gambiae s.s* after two blood meals:** The estimated odds of

844 mortality of *An. gambiae s.s* after taking two blood meals from different combinations of host

845 species relative to human-only blood meals.  The numbers in brackets are the 95%

846 confidence intervals of the odds ratio (OR).

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| Host diet treatment | Block 1 | Block 2 |
| --- | --- | --- |
| Human + Chicken | 1.27 (0.50 – 3.21) | 1.12 (0.45 – 2.76) |
| Human + Cow | 0.99 (0.37 – 2.65) | 0.75 (0.28 – 2.02) |
| Human + Dog | 1.53 (0.63 – 3.75) | 1.12 (0.46 – 2.76) |

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