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ECOLOGICAL AND EVOLUTIONARY DETERMINANTS OF ANOPHELINE HOST SPECIES CHOICE AND ITS IMPLICATIONS FOR MALARIA TRANSMISSION

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Abstract

Despite the importance of host species choice of mosquito vectors to the epidemiology and control of malaria, our understanding of the ecological and evolutionary factors that drive the host species preference in these vectors is very limited. My PhD thesis aimed to experimentally investigate the potential ecological and evolutionary determinants of the host species choice of the African malaria vectors *Anopheles arabiensis* and *An. gambiae s.s*, which are amongst the most highly specialized and efficient malaria vectors in the world, and identify a control strategy that reduces their anthrophily.

I used a unique semi-field system where these vectors were able to interact naturally with hosts of different species to establish whether their fitness depends on type of host species, they encounter and feed upon. My initial prediction was that highly host-specific feeding behaviour of these vectors is a product of natural selection whereby mosquito fitness is highest on their naturally preferred host types. This prediction was met in *An. arabiensis,* whose feeding success and lifetime egg production was predicted to be higher on their naturally preferred bovid hosts. However, I did not detect any association between the preference of *An. gambiae s.s* for humans and their lifetime reproductive success, although they obtain larger blood meals and survived longer on these naturally preferred human hosts. These findings suggest the role of host species on mosquito fitness varies between vector species.

I then evaluated whether the host species-specific fitness of malaria vectors may be attributed to intrinsic defensive behaviours and haematological properties that make some host species being more beneficial than others. My initial prediction was that mosquito feeding success and fitness would be the highest in the absence of host defensive behaviours and, more specifically, that the least defensive host species would be the most highly preferred in nature. I have found that the feeding success (probability of obtaining a blood meal) of *An. arabiensis* is greater on host species with least effective defenses (e.g. bovids). However, this association was not apparent for anthrophilic *An. gambiae s.s*. Surprisingly, I found that the subsequent fitness (blood meal size and survival) of both vector species was generally greater on hosts who were free to exhibit defensive behaviours than those whose behaviours were restricted. These findings suggest that natural physical defensive behaviours made by hosts including humans may not impose strong fitness costs to malaria vectors. Therefore, I conclude that if natural host defensive behaviours shape the host species preference of malaria vectors they do so by influencing the probability of acquiring a blood meal but not the value of the blood meal if obtained.

I also assessed whether the nutritive value of host blood, as determined by haematological properties of packed cell volume (PCV) and haemoglobin concentration (Hb), could explain variation in fitness of malaria vectors on different host species. I found that the PCV and Hb of host species that are commonly encountered by malaria vectors in their natural environments vary significantly. I further found that the variation in these haematological properties influence the feeding success (e.g. blood intake rate) of the anthrophilic *An. gambiae s.s* but not the *An. arabiensis*. *Anopheles gambiae* s.s obtain full blood meal faster on hosts with low and medium levels of PCV. Surprisingly, these haematological traits were predicted to have opposite effects on the survival of both vector species. The survival of *An. gambiae s.s* was positively correlated with host PCV, but negatively correlated with their Hb. In contrast, the survival of *An. arabiensis* was predicted to be positively correlated with host Hb, but negatively related with PCV. Overall, there was no clear evidence that haematological properties of the host species preferred by these mosquito vectors are optimal for their fitness.

I then extended my investigations to a laboratory investigation to measure the impact of host species diversity on the fitness of *An. gambiae s.s* throughout their life. Under these conditions, I found that *An. gambiae s.s* had similar fitness after either feeding on a uniform (human-only) or mixed host species. These findings indicate that the blood composition of different species may be unlikely to reduce the fitness of *An. gambiae s.s*

My PhD thesis also experimentally measured the impact of using simple intervention (e.g. an untreated bed net) on reducing the fitness of malaria vectors that acquire from human hosts. I found that the lifetime reproductive output of *An. arabiensis* on protected human was significantly lower than on bovid hosts. In contrast, the use of untreated nets by humans reduced survival of anthrophilic *An. gambiae s.s*, but the reduction was not predicted to be sufficient to significantly reduce the total lifetime reproductive output of these mosquitoes on human hosts than on animal alternatives. These findings suggest that the widespread use of simple untreated net may generate selection pressures for *An. arabiensis* to maintain their feeding on bovid hosts and to a lesser extent for *An. gambiae s.s* to reduce their anthrophily.

The findings of my PhD research have implications for the epidemiology and control of malaria. I found that host species and their intrinsic properties may influence aspects of the feeding success, blood meal size and survival of malaria vectors which are the key determinants of malaria transmission intensity. I further demonstrate that selectively protecting humans with untreated nets may generate selection pressures for malaria vectors to reduce their anthrophily and consequently the transmission intensity of malaria. These findings suggest integrating existing interventions (e.g., use of untreated and insecticide treated bed nets) with environmental management that increases availability of an alternate host species (e.g. zooprophylaxis) may generate selection pressures for *An. gambiae s.s* to reduce their anthrophily, and *An. arabiensis* to maintain their feeding on alternative animal hosts (zoophily).

Overall, I discuss the impacts of host species choice and intrinsic host factors on the fitness of African malaria vectors, the impacts of intervention on their fitness and their potential to select for a host shift, and the implications to epidemiology and control of malaria. I finally highlight gaps in the knowledge of the evolution of host species choice in malaria vectors where more research is required.

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Author's declaration

I declare that the research work reported in this thesis is entirely my own, and of my own composition, except where otherwise stated. Most of the chapters in this thesis have been produced as independent papers for publication in co-aurthorship with my supervisor, and my personal contribution to each chapter as follows.

Chapter 1. Published in *Trends in Parasitology* as: Issa N. Lyimo and Heather M Ferguson: Ecological and Evolutionary determinants of host species choice in mosquito vectors. Initial concepts developed by myself during reviewing of literature and HF enhanced the first draft for publication.

Chapter 2. In preparation for submission as a short communication to *Parasites & Vectors* as: Issa N. Lyimo, Ally Daraja, Kasian Mbina, Edgar Mbehela, Dorothy Armstrong and Heather Ferguson. Establishment of a semi-field and laboratory colony of *Anopheles arabiensis* from a wild population in Tanzania. IL collected wild mosquitoes, and prepared rearing protocol. AD, KM, EM and DA implemented rearing protocol under supervision of IL and HF. Drafts were prepared by IL and reviewed by HF.

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Chapter 4. In preparation for submission to *BMC Evolutionary Biology* as: Issa N. Lyimo, Tanya Russell, Kasian Mbina, Ally Daraja and Heather M Ferguson: The influence of host defensive behaviours on the fitness of African malaria vectors: implications for the evolution of host choice. Data collection was conducted by IL with technical assistance from KM and AD. The statistical analyses were done by IL under supersion of HF. Drafts were developed by IL and HF provided comments.

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I further declare that no part of this research work has been submitted for the requirement of any other degree.

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1 General introduction

1.1 Introduction

Insects exhibit diverse resource exploitation strategies, including predation, herbivory, and parasitism. The ecological and evolutionary factors that influence the resource selection of some insects (e.g. herbivores) have been extensively investigated due to their agricultural importance. By contrast, there has been relatively little investigation of the selective forces that mediate host choice in haematophagous insects, despite their importance as vectors of disease. Therefore, it is important to understand the potential determinants of host species choice in mosquitoes, the most important insect vectors of human disease, and whether this phenotype could be manipulated to yield new disease control strategies based on vector behavioural change.

1.2 Epidemiological relevance of mosquito host species choice

Insects are the most abundant group of animals (Lehane, 2005). Due to the massive economic impact of herbivorous insects on agriculture and forestry, the determinants of their feeding preferences have been extensively investigated (Jaenike, 1990). By contrast, little is known about the determinants of host choice in insects that feed on vertebrate blood. These organisms transmit numerous human and animal diseases, with mosquitoes having the greatest impact of public health due to their role in malaria, arboviral and filariasis transmission (Lehane, 2005). Although not all mosquitoes require blood for reproduction (anautogenous), the host species choice of those that do is a critical determinant of the transmission intensity of pathogens they transmit (Kiszewski et al., 2004) (e.g. Figure 1.1). The ecological and evolutionary factors that may determine host choice in haematophagous mosquitoes are reviewed here. The central aim is to discuss the ultimate selective forces responsible for driving the evolution of host species choice and how they may be manipulated to reduce vector-borne disease, but not the proximate mechanisms used to detect and distinguish between host species or individuals (reviewed by Knols et al., 1995).

Malaria transmission is a function of the three-way interaction between humans, *Plasmodium* parasites, and the Anopheline mosquito vectors that transmit them between hosts. Transmission depends on several aspects of the outcome of their interactions and life history including human–vector contact rate, vector and parasite survival, parasite development rate inside vectors, and human and vector population size. The manner in which these parameters combine to determine malaria transmission was first proposed by Ronald Ross (Ross, 1911) , and later modified by MacDonald (MacDonald, 1957) who developed the now standard Ross-MacDonald mathematical model of malaria transmission. This model predicts malaria transmission intensity in terms of the reproductive rate, $\boldsymbol{R_0}$, defined as the number of new cases generated by one infected person in a population of susceptibles:

$$\boldsymbol{R_0} = \frac{ma^2 bce^{-dn}}{rd} \qquad \textbf{(Eqn 1)}$$

where m is the number of vectors per person, a the number of bites vectors take from humans each day, b and c the infectiousness of vectors to humans and vice versa, n the incubation period of the parasite, and $1/r$ and $1/d$ the duration of human infectiousness and mosquito longevity respectively. The parameter **'**a**'** best captures the impact of host choice on malaria transmission rates. As the propensity of mosquitoes to feed on humans increases, so does the value of **'**a**'**. As this variable is exponentially related to malaria transmission, even small changes in mosquito preference for humans have a sizeable impact on malaria transmission. No biological parameter other than mosquito survival has a greater impact on malaria transmission than the feeding rate on humans. As summarized in table 1, the vertebrate species from which mosquitoes feed can also have a direct impact on their survival, thus host species choice could make at least two independent and sizeable contributions to malaria transmission intensity.

Figure 1.1. Epidemiological significance of mosquito host choice to malaria transmission

1.3 Evolution of host choice

A substantial body of theory and empirical research has accrued to predict how organisms should select dietary resources to maximize their fitness (Begon et al., 2006). The widespread existence of dietary specialization presents a paradox: why choose a limited variety of resources when many are available? Specialization is predicted to evolve when there are trade-offs between the fitness obtained from feeding on different resources, such that net energy gain is higher when only a limited subset is consumed (Egas et al., 2004). By contrast, generalism should evolve when there are only moderate differences in energetic gains between resources (Egas et al., 2004).

By extension to mosquitoes, generalism should evolve in environments where host encounter rates are low and the advantage of waiting for an optimal host is traded-off against the risk of death before feeding. Conversely, specialism should arise when the frequency of encounter with favorable host species increases. The host breadth of haematophagous insects is likely the product of both optimal foraging on currently available hosts (Kelly and Thompson, 2000) and historical patterns of host availability as reflected by phylogenetic congruence with their vertebrate hosts (Page, 2003).

In order for mosquitoes to evolve generalist or specialist host feeding strategies as predicted, there must be genetic variation for host choice. Although rarely investigated, this phenomenon has been documented in *Anopheles gambiae* where divergent feeding preferences for humans or cows were generated within 5-6 generations of selection (Gillies, 1964), and in crossmating experiments with zoophilic *Aedes simpsoni* and anthropophilic *Ae aegypti* that generated offspring of intermediate preference (Mukwaya, 1977). Thus selection has the potential to act on mosquito feeding behaviour and has likely generated observed patterns of host species choice.

1.4 Patterns of host species choice in mosquitoes

General patterns of mosquito host species range may be apparent from large-scale entomological surveys such as those conducted on *Anopheline* malaria vectors by the World Health Organization (Bruce-Chwatt et al., 1966). From 1955-1964, over 100,000 individual anophelines were analysed to estimate the human blood index (HBI) of different species. Detailed analyses were performed on a sub-sample of 52 species to further identify the source of non-human blood. Eighty two percent of these populations exhibited dietary specialism (>50% bloodmeals taken from one host type, Figure 1.2a), indicating that selective host use is common. A critical limitation of this data, however, is that it comes from studies where mosquitoes were sampled from only a few habitat types (e.g houses or animal sheds) in which a limited range of hosts were available. Consequently the true diversity of host species on which mosquitoes feed is likely under-represented and estimates of specialization may be upwardly biased. Although many mosquito species exhibit characteristic trends in host species choice, this behaviour can vary spatially and temporally (Figure 1.3). The selective pressures that may be responsible for driving both intra and inter-specific variation in host species choice are now discussed.

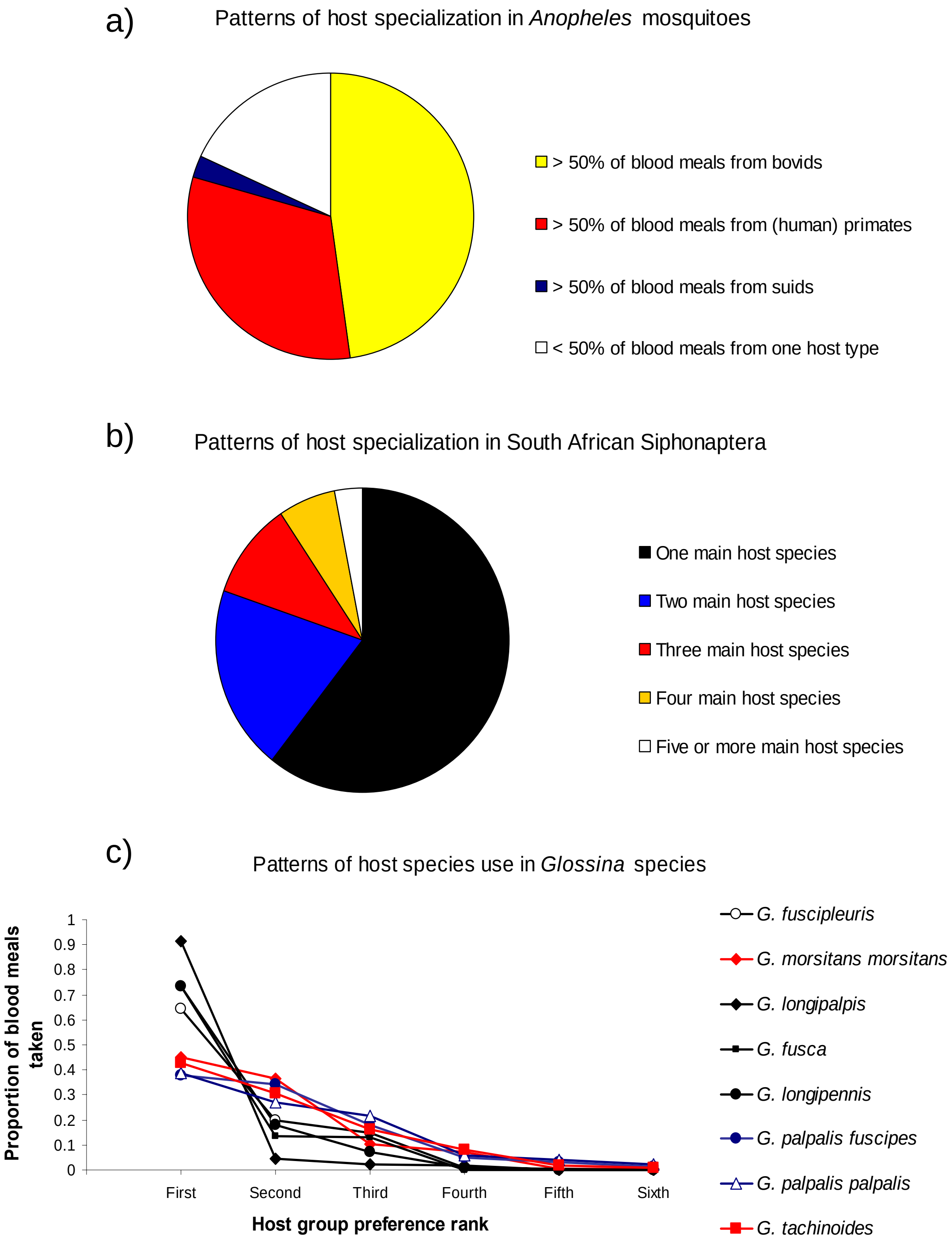

Figure 1.2. Patterns of vertebrate host specialization in various groups of haematophagous insects. (a) Proportion of Anopheline mosquito populations (n = 117, drawn from 52 different species) exhibiting preferential use of bovids, humans, suids or displaying no clear preference (e.g. no host group fed upon > 50%). Data from ref. (Bruce-Chwatt et al., 1966). (b) Proportion of South African Siphonapteran flea species (n = 96) with one or more main host species. Data from ref. (Segerman, 1995). (c) Proportion of blood meals taken from first, second, third, fourth, fifth and sixth more preferred host groups used by eight species of tsetse flies. Host groups include primates, suids, bovids, birds, reptiles and 'other mammals' (elephant, rhinoceros, hippopatamas, dog, cat, hyaena, other carnivores, porcupines and aardvark). Data from ref. (Weitz, 1963).

Geographical

In the northern hemisphere, the mosquito *Culex pipiens* in Mediterranean regions feed on mammals or both mammals and birds, while those far than north are entirely ornithophilic. The malaria vector *An. gambiae s.s.* is highly anthropophilic in mainland Africa, but prefers to feed on dogs on the island of Sao Tome (Sousa et al., 2001). Although the mosquito *Anopheles arabiensis* is known to prefer cows, it can be highly anthropophilic when livestock are absent (Mwangangi et al., 2003). Similarly, in Uganda, the population of *Aedes bromeliae* (reported as *Ae.simpsoni*) specialize by feeding on rodents at Bwayise, but preferentially feed on humans at Bwamba (Clements, 1999). Thus, geographic variation between regions could influence host availability that in turn may affect host choice in mosquitoes

Seasonal

In North America, culicine mosquitoes including *Culex tarsalis* and *Cx. nigripalpus* switch their host species choice from birds in spring and early summer, to mammals in late summer and autumn (Kilpatrick et al., 2006). Also, in Kenya, feeding pattern of *Cx.univittatus* switches from mammals to avians during long rains (Chandler et al., 1977). In some cases, it has been hypothesised that these shifts are due to seasonal changes in host availability (Chandler et al., 1977) and climatic conditions (Chandler et al., 1977, Clements, 1999), and in other cases the potential cause remains unclear (Clements, 1999).

Microhabitat

In western Kenya, the HBI of several culicine species sampled inside human dwellings was found to vary with their site of capture; human blood was more prevalent in mosquitoes caught indoors, and bovid blood was more common in those resting outdoors (Beier et al., 1990). Similarly, the HBI of *An. arabiensis* caught in houses was three times higher than in those caught outdoors (Petrarca et al., 1991). Thus, feeding preference of mosquitoes may be influenced by relative availability of the potential host in the particular microhabitat in which they are found

Foraging experience

Mosquito host choice may also be influenced by prior foraging experience that causes them to learn which hosts are most successfully fed upon. Several studies have attempted to test this possibility by marking and releasing mosquitoes to assess their tendency to return to their original location (as reviewed by Alonso and Schuck-Paim, 2006). While several studies indicated that mosquitoes return to their initial point of capture at higher than expected rates, it is unclear whether this is evidence of learning (Alonso and Schuck-Paim, 2006), or genetic and environmental factors that influence attraction to particular sites. To avoid these confounding factors, an experimental study was conducted in which mosquitoes known to have fed on either pigs or cows during their first meal were allowed to choose between these hosts on their second feed (Mwandawiro et al., 2000). While the *Aedes* species showed no host fidelity, *Cx.tritaeniorhynchus*, *Cx.gelidus* and *Cx.vishnui* were substantially more likely to choose the host species that was first fed upon for their second feed (Mwandawiro et al., 2000). As the offspring of these mosquitoes did not display similar host choice to their parents, it was concluded that the observed host fidelity was more likely a product of learning than genetic predisposition. Further investigations with similarly robust experimental designs are required to confirm the role of learning as a determinant of mosquito host species choice.

Figure 1.3. Source and examples of within-species variation in mosquito host species choice

1.4.1 Host availability

Spatial and temporal heterogeneity in host distribution influences the duration of mosquito host seeking, which, in turn, may influence energetic expenditure, survival and risk of predation while foraging. At its most extreme, host availability is known to impact mosquito feeding behaviour by influencing whether they blood feed at all. Specifically, autogeneous egg production in mosquitoes has been associated with environments where vertebrate host availability is severely limited (Corbet, 1967).

In haematophagous species, two approaches have been used to assess the dependence of host species choice on host availability. The first (and most common) is to qualitatively compare whether the proportion of blood meals taken from particular host species varies with their relative abundance, which almost always finds a positive relationship (Hess et al., 1968). While useful for evaluation of the sensitivity of host choice to availability, a more quantitative approach is required to test whether patterns of host species choice can be fully attributed to availability. One such measure is the forage ratio (FR), calculated as the proportion of bloodmeals taken from a particular host species divided by its relative abundance within the host community (Hess et al., 1968). Values of the FR >1 imply a preference for that host species, < 1 avoidance, and ~ 1 of random foraging (Kay et al., 1979). Probably due to the difficulty of conducting accurate host censuses, this technique is rarely used in practice and has been criticized for its assumption that the presence of a host implies it is ‘available’ (Kay et al., 1979). Kay *et al.*, in 1979 proposed the ‘Feeding Index’ (FI) as an alternative, which requires only an approximation of the relative abundance of two host species on which feeding is compared. As with the FR, values of the FI that are substantially different from one indicate non-random host choice. In the few studies that have calculated either a FI (Kay et al., 1979, Loyola et al., 1993) or FR (Hess et al., 1968, Lardeux et al., 2007a), values are almost always considerably lower or higher than one. Taken together with results from qualitative studies, this indicates that mosquito host species choice is sensitive to host availability but that true preferences are evident that cannot be attributed to random foraging on available host species.

1.4.2 Nutritional value of blood and energetic costs of digestion

Non-random feeding of mosquitoes may be explained by variation in nutritional rewards and corresponding fitness accruing from different host types. Experimental studies consistently indicate that the reproduction and survival of mosquitoes after blood feeding varies with host species (Table 1.1). Several haematological properties vary between vertebrate species which could influence the nutritive value of their blood (Wintrobe, 1933, Harrington et al., 2001). During feeding, mosquitoes pass ingested blood through the pyloric armature situated at the posterior of the gut (Trembley, 1951). These sclerotized teeth-like structures allow the passage of serum but trap red blood cells (Trembley, 1951), which are the primary source of protein for egg production. The number and complexity of these teeth vary between mosquito species (Vaughan et al., 1991), and red blood cell size varies between vertebrate species (Wintrobe, 1933, Hawkey et al., 1991). It has been hypothesized that mosquitoes may specialize on host species whose blood can be most efficiently filtered by their particular pyloric armature(Harrington et al., 2001, Vaughan et al., 1991), although this remains to be demonstrated. The isoleucine content of vertebrate blood has been associated with mosquito egg production, and its variation between vertebrate species was hypothesized to influence host selection (Harrington et al., 2001). However, artificial manipulation of isoleucine in blood did not influence the fecundity of *Ae. aegypti*, suggesting this amino acid may be a correlate rather than cause of fecundity variation in Nature (Harrington et al., 2001).

Even if the base nutritional value of blood from different host sources is similar, the energetic costs of digesting it may not be. Blood digestion requires a significant expenditure of energy, with, for example, the mosquito *Culex tarsalis* doubling its metabolic rate when consuming blood in comparison to sugar (Gray and Bradley, 2003). The time required to digest blood varies between host species, with *Cx. tarsalis* digesting the blood of its preferred chicken hosts at a faster rate than blood from rodents (Downe and Archer, 1975). For blood proteins to be uptaken by mosquitoes, red cells must be hemolyzed. Hemolysis can occur either mechanically or enzymatically in the mosquito gut. The cibarial armature situated at the anterior of the mosquito foregut is the primary site of mechanical hemolysis. The number and morphology of teeth rows in the cibarial armature varies between mosquito species (Coluzzi et al., 1982), and is associated with the relative magnitude of mechanical hemolysis (Coluzzi et al., 1982). As mechanical hemolysis is less energetically demanding than the production of enzymes, mosquitoes may be selected to specialize on host species whose red blood cells are most efficiently broken down by their particular cibarial armature.

Variation in physical and chemical properties of blood between vertebrate species may influence the reproductive success of mosquitoes and generate selection for preferential feeding on optimal host species. If this variation drives host choice evolution, there should be a correlation between the fitness mosquitoes gain from a host species and their preference for it in Nature. Results of laboratory studies in which mosquitoes were experimentally fed on blood from different species indicates that their reproduction and survival is often, but not consistently, the highest after feeding on host types commonly used in Nature (Table 1.1). However, these laboratory studies generally did not include all host species that the target mosquito fed upon in Nature, and/or only looked at one component of mosquito fitness which may not be indicative of their total lifetime reproductive success. Consequently, it is not yet possible to conclude how extensively mosquito host species preferences can be explained by the fitness benefits arising from selective feeding under natural conditions.

Mosquito species	Common hosts in nature	FDH	Hosts surveyed in laboratory	Fitness trait examined					Most productive hosts	Least productive hosts	ES
				FS	FD	GC	FT	SV			
Ae. aegypti	Humans‡ (Ponlanwat and Harrington, 2005)	✓	Humans and rodents	–	✓	–	–	✓	Human	Rodents	(Harrington et al., 2001)
Ae. aegypti	Humans‡(Ponlanwat and Harrington, 2005)	✓	Birds, frogs, guinea pig, humans, monkeys, rabbits, rats and turtles	–	✓	–	–	–	Rabbits, guinea pigs, frogs and turtles	Humans and monkeys	(Woke, 1937a)
Ae. aegypti	Humans‡(Ponlanwat and Harrington, 2005)	✓	Birds, humans, mice and rabbits	–	✓	–	–	–	Birds, rabbits and mice	Humans	(Nayar and Sauerman Jr, 1977)
Ae. solicitans	Deers‡(Crans et al., 1990)	✓	Birds, humans, mice and rabbits	–	✓	–	–	–	Humans	Birds, mice and rabbits	(Nayar and Sauerman Jr, 1977)
Ae. triseriatus	Deers, Chipmunks (Nasci, 1982)	✓	Chipmunks, deer, humans, mice and squirrels	–	✓	–	–	–	Chipmunks and Squirrels	Deer, humans and mice	(Mather and DeFoliart, 1983)
Ae. triseriatus	Deers, Chipmunks (Robertson et al., 1993)	✓	Chipmunks, deer, humans, mice and squirrels	–	–	✓	–	–	Deers	Chipmunks, Humans, mice and squirrels	(Mather and DeFoliart, 1983)
An. quadrimaculatus	Deers‡, Horses‡(Nasci, 1982)	✓	Birds, humans, mice and rabbits	–	✓	–	–	–	Birds, rabbits and mice	Humans	(Nayar and Sauerman Jr, 1977)
An. farauti	Humans, cattle, pigs and dogs(Foley et al., 1991)	✓	Humans, dogs and rodents	–	✓	–	✓	–	Rodents	Human and dogs	(Okazawa, 2001)
C .pipiens pipiens	Birds‡(Hamer et al., 2008)	✓	Birds, guinea pigs and humans	–	✓	–	–	–	Birds	Guinea pigs and humans	(Shroyer and Siverly, 1972)
C. nigripalpus	Birds, Humans Cattles(Gomes et al., 2003)	✓	Birds, humans, mice and rabbits	–	✓	–	–	–	Birds, rabbits and mice	Humans	(Nayar and Sauerman Jr, 1977)
C. salinarius	Birds(Cupp and Stokes, 1976)	✓	Chickens, guinea pigs, humans and turtles	✓	–	–	–	–	Humans	Chickens, guinea pigs, and turtles	(Shelton, 1972)
C. salinarius	Birds(Cupp and Stokes, 1976)	✓	Chickens, guinea pigs, humans and turtles	–	✓	–	–	–	Chickens, turtles and guinea pigs	Humans	(Shelton, 1972)

Table 1.1. Comparison of fitness traits of mosquitoes measured under controlled laboratory conditions on different host species and their feeding preference in nature. Common hosts are defined as those from whom at least 20% of blood meals are taken in at least one natural population. Common hosts distinguished by the symbol '‡ are those from which 80% or more of mosquito blood meals were taken in at least one natural population (indicating specialism). Fitness traits refer to measurements derived under controlled experimental conditions when cohorts of mosquitoes were simultaneously fed on different host species (generally restrained during feeding) and their subsequent survival and reproduction monitored. The column abbreviated by FDH indicate whether fitness vary with host types. These traits are defined as follows: FS = 'feeding success', the proportion of the mosquitoes that fed on a particular host type, FD = 'fecundity', the number of mature oocytes/or eggs laid, GC = 'gonotrophic cycle', the number of days between blood feeding and ovipositon or rate of blood digestion, FT = 'fertility', the proportion of eggs that hatched into larvae; and SV = 'survival' the longevity of the mosquito after bloodfeeding. The last column indicate the experimental studies (ES) investigating fitness traits. In the fitness columns, '√' indicates host species did affect fitness trait, and '–' that the listed fitness trait was not investigated in that study.

1.4.3 Host defensive behaviour

Whereas the defensive behaviour of plants (expressed by production of toxic metabolites) is the most important predictor of the host species choice of phytophagous insects (Jaenike, 1990), the relative importance of host defensive behaviour to mosquito host species choice is unclear. Animals defend themselves from insect bites by a variety of means including protective tissues (e.g. skin, feathers), physical movements and behavioural avoidance (as reviewed by Lehane, 2005). Additional artificial defensive meaures developed by humans such as house screening (Lindsay et al., 2002, Kirby et al., 2009) and insecticide treated nets (ITNs) are also highly effective means of reducing the feeding success of mosquitoes (Takken, 2002).

Host defensive behaviour can influence the feeding success of mosquitoes by physically blocking and/or killing them while attempting to feed (Waage and Nondo, 1982), and by causing them to abandon feeding attempts (Walker and Edman, 1985). Numerous studies have demonstrated that defensive reactions to mosquito biting varies between host species (Edman and Scott, 1987, Lehane, 2005). However, the defensiveness of particular host species usually varies as a function of mosquito density (Waage and Davies, 1986, Kelly et al., 1996), thus there may be no consistent host-specific defense phenotype on which natural selection can act. Further investigation of the relative magnitude of variation in defensiveness between and within host species is required to test whether this behaviour can explain patterns of mosquito host choice.

1.4.4 Other potential determinants of host species choice

Other physiological, behavioural and ecological factors may also play a role in mosquito host choice evolution. Vertebrate hosts mount immune responses to arthropod saliva, which can impede the feeding success, reproduction and survival of insects that subsequently bite them (Billingsley et al., 2008, Tschirren et al., 2007). Variation in immunocompetence between host species could generate selection for specialization on less responsive host types. Biochemical and physiological properties of mosquito saliva could also influence their ability to exploit different hosts. Mosquito saliva consists of a complex mixture of vasodilators, anti-platelet compounds and immune-modulating compounds (Ribeiro and Francischetti, 2003). The efficiency with which the saliva from a particular mosquito species can anaesthetize and extract blood may vary between host species, and again generate selection for preferential feeding on those whose blood can be most efficiently exploited. To our knowledge, these possibilities have not been investigated.

Finally, mosquito host species choice may in part be a by-product of favourable environmental conditions that draw mosquitoes to habitats where only a particular subset of hosts are available. Manipulative experiments in which the relative fitness obtained from feeding on hosts in common and novel environments are required to test this possibility.

1.5 Contrasting mosquitoes with other haematophagous insects

As in mosquitoes, specialization on a limited number of host species is common in other haematophagous insects such as fleas, where many taxa feed principally on only one host species (e.g. Figure 1.2b). The host choice of tsetse flies appears more evenly distributed across host types, but some host types are still fed upon substantially more often than others (Figure 1.2c).

Most information on the relationship between host availability and host species choice comes from studies of mosquitoes (see above). However, there is some evidence indicating that the host species choice of triatomids and tsetse flies varies with host availability and proximity (Clausen et al., 1998, Gurtler et al., 1997). Like mosquitoes, however, these insects still exhibit preferences that cannot simply be explained by host availability. Blood nutritive value and the cost of digestions appears to be a clear predictor of the host species choice of some haematophagous insects. The flea *Parapulex chephrenis* requires less energy and time to digest the blood of its preferred mouse host (Sarfati et al., 2005), and also has higher fecundity and survival upon it than on non-preferred gerbils (Krasnov et al., 2003). The flea *Ctenocephalides felis* also has higher reproductive success on common rather than atypical host species (Williams, 1993). Finally, host defensive behaviour also influences the feeding success of other haematophagous insects including tsetse flies and horse flies (Torr et al., 2001, Waage and Davies, 1986). As is the case with mosquitoes, it is unclear whether intrinsic variation in the defensiveness of host species is linked to host choice in these systems.

1.6 Mosquito host choice evolution in a dynamic world

Current patterns of host species choice by mosquitoes are the product of millions of generations of natural selection. Change in the abundance and diversity of host species relative to insect generation length may have occurred relatively slowly through much of this period, but has escalated recently due to rapid human population growth and associated changes in vertebrate species diversity. Recent changes in vertebrate species communities that increase mosquito preference for humans could dramatically increase the transmission of diseases such as malaria, dengue, and filariasis and undermine currently effective control strategies. Unfortunately, preliminary evidence suggests changes in host choice may already be occurring in response to some land-use activities. For example, the proportion of bloodmeals taken from humans by the malaria vector *An. fluviatilis* in Uttar Pradesh increased from 1.4% in 1938-1939, to 41.2% in 1949-1952, which was attributed to forest clearing and the intensification of rural agriculture (Bruce-Chwatt et al., 1966). Similar changes are being observed around the Amazon basin, where the human biting rate of *An. darlingi* is 278 times greater in deforested in comparison to forested areas (Vittor et al., 2006). However, it remains unclear whether host species diversity may influence fitness of highly anthrophilic mosquitoes.

The tendency for increased mosquito feeding on humans as a consequence of landscape and demographic changes, however, may be counteracted by the increased uptake of vector control tools that selectively protect people. The widespread usage of untreated and/or insecticide-treated bed nets has been associated with substantial shifts in mosquito blood-feeding from humans to animals in some (Kaburi et al., 2009, Bogh et al., 1998, Lefevre et al., 2009) but not all areas (Quinones et al., 1997). Similar reductions in the human blood index (HBI) of malaria vectors has been observed in areas undergoing Indoor Residual Spraying (Gillies and Furlong, 1964), although observance of this phenomenon varied between geographical areas, mosquito species and with the type of insecticides used (Garrett-Jones et al., 1980). It remains unclear whether observed reductions in anthropophily in response to ITNs or IRS were the result of behavioural plasticity, or an underlying genetic change in the vector population.

1.7 Can shifts in host species use be manipulated?

The idea of reducing pathogen transmission by altering the host species choice of mosquito vectors is not new. This concept, known as zooprophylaxis, has been advocated as a malaria control strategy by the WHO since 1982 (WHO, 1982); with the guiding principle being that transmission will be lowered by diverting mosquitoes from humans onto livestock hosts that cannot transmit *Plasmodium*. There have been few rigorous large-scale trials of zooprophylaxis as a malaria control tool, but available evidence is mixed suggesting that the presence of livestock may reduce (Chelbi et al., 2008, Service, 1991), enhance (Saul, 2003), or have no effect on transmission (Bogh et al., 2001). While there has been some discussion of how livestock could actually increase malaria risk (indirectly, by attracting more mosquitoes and/or creating more larval habitats, (Saul, 2003), what has so far been missed from the zooprophylaxis debate is the potential evolutionary and ecological consequences of enforcing a mass host species shift on vector populations.

As reviewed, many mosquitoes (including important vectors of human disease) are highly selective in their host species choice. Accepting the evolutionary principle that such specificity arises only when there are fitness advantages from being selective, it follows that diverting mosquitoes onto less preferred hosts will change their net reproductive output. Evidence reviewed here (Table 1.1), confirms that mosquito host species choice routinely influences their subsequent survival and reproduction. It is thus unlikely that mosquito fitness will remain constant if their host species is switched. From a disease control perspective, this has two consequences. First, it suggests zooprophylactic strategies could bring added value by lowering mosquito reproduction and survival, which in turn could reduce the growth rate and stability of their populations. Secondly and less beneficially, it suggests the inherent fitness advantages from feeding on humans will always provide an incentive for anthropophilic mosquitoes to evade control measures that reduce human contact.

A more effective means of conducting zooprophylaxis may be not only to divert mosquitoes from humans, but also decrease the relative fitness benefits gained from them. To achieve this, it is first necessary to prove anthropophilic species gain an advantage from feeding on humans, and identify the mechanism through which it arises: greater availability, higher blood nutritional value, and/or lower defensiveness as reviewed here.

Once identified, the advantage of human feeding could potentially be reduced through selective application of control measures. For example, evidence reviewed here indicates that the host choice of some mosquito species is correlated with the reproductive success obtained from that host. Successful development of a vaccine that reduces the survival and fecundity of mosquitoes after feeding on humans (Billingsley et al., 2008) could be one means of reducing this advantage. Similarly, if it can be proven that humans have relatively poorer defensive behaviour than other available hosts, control measures that increase mortality associated with trying to feed on people (e.g ITNs) may be the quickest way to select against anthropophily. Efforts must also be made to identify the genes involved in mosquito host choice. If a mosquito host species shift cannot be generated through selection, a transgenic approach based on driving genes for zoophily into vector populations could also be effective.

A starting point for either of these enterprises is identification of the selective forces that cause mosquitoes to specialize on humans. This will require experimental investigation of mosquito-host interactions of similar thoroughness to those conducted on herbivore-plant systems (Jaenike, 1990). Assessing the mosquito fitness consequences of interactions with human hosts presents logistical and ethical challenges not applicable in studies of plants; especially if the mosquitoes are disease vectors. Development of large-scale semi-field systems (e.g. Ferguson et al., 2008) will make it possible to experimentally observe human, animal and mosquito behaviour under relatively natural conditions without risking exposure to parasites. These systems will make it possible to compare the defensive behaviour of humans and animals to mosquito biting, and measure mosquito fitness under varying host availability and environmental conditions. Use of such tools, in combination with rigorous field investigation of genetic and environmental variation in mosquito feeding behavior, will help reveal the basis of their host species choice and guide the development of new disease control strategies based on sustained modification of mosquito behaviour.

1.8 Aim and objectives

Overall, my PhD thesis aimed to identify the selective forces that may explain why major African malaria vectors (*An. arabiensis* and *An. gambiae s.s*) specialize feeding on humans or cattle over alternative vertebrate hosts (e.g. dogs, goats and chickens). In attempt to address this general question, the following specific objectives were identified:

1) To establish a colony of *An. arabiensis* for use in comparison of their fitness and behaviours with that of *An. gambiae s.s*.
2) To experimentally investigate whether host species choice influences mosquito fitness.
3) To identify selective pressures that may be linked to the variation in mosquito fitness.
4) To predict the impact of host species choice on mosquito total lifetime reproductive success.
5) To explore the circustances (e.g. host species diversity and use of interventions) under which host species choice may be manipulated for malaria control strategies.

2 Establishment of a semi-field and laboratory colony of *Anopheles arabiensis* from a wild population in Tanzania

2.1 Introduction

The objective of this thesis was to examine the host feeding behaviours and fitness of two African malaria vectors under relatively realistic conditions, and test a series of distinct hypotheses to explain their differential host species preference. Ideally, these experiments could be done directly on wild mosquitoes, but there are number of logistical and ethical issues (e.g. exposure of human volunteers to potentially infected mosquitoes, and the need for a sustained supply of large numbers of mosquitoes for experiments) that made this impossible. The best alternative to work with colonized mosquitoes that can be reared under realistic conditions in large numbers, but guaranteed infection free, to facilitate ethical execution of experiments. At the start of my Ph.D., the Ifakara Health Institute (IHI) in Tanzania where I based my field work already had *An. gambiae s.s* colony available, but not one of *An. arabiensis* (despite this species being an important malaria vector within the region, Russell et al., 2010). Therefore, my task was to establish *An. arabiensis* colony under both semi-field conditions in IHI and then later in laboratory at the University of Glasgow (UG). The colonization of mosquitoes from wild population can be difficult, and mosquitoes may undergo bottlenecking and changes in their fitness and genetic structure during the colonization process (Norris et al., 2001, Arias et al., 2005). The aim of this study was to establish two new colonies (laboratory and semi-field) of *An. arabiensis* for use in experiments here, and examine changes in the life history development and phenotype through the colonization process.

The colonization of mosquitoes under laboratory conditions from wild populations generates selection that preferentially favours individuals that can reproduce and survive under these artificial holding conditions (Benedict et al., 2009, Benedict et al., 2006). The most common changes observed during the colonization of wild mosquitoes under laboratory conditions include reduced flight ability (Clarke et al., 1983), blood feeding success (Barnett and Gould, 1962, Mpofu et al., 1993, Keegan et al., 1964), mating competence(Barnett and Gould, 1962, McDonald et al., 1979, Lardeux et al., 2007b), oviposition (McDonald et al., 1979, Klein et al., 1982), egg viability (Mpofu et al., 1993) and survival (Soelarto et al., 1995). These changes are generally observed during the first few generations of colonization, and suggest that wild mosquitoes suffer fitness costs when initially exposed to laboratory conditions which may be overcome as subsequent generations become adapted to these conditions (Bangs et al., 2002). Not all mosquito species can adapt laboratory conditions, and some important vectors (e.g. *An. funestus*) continue to prove difficult to establish. Particular challenges to colonization of haematophagous insects include the inability to collect a sufficient number of wild blood fed individuals to establish a population, and the sub - optimality of environmental rearing conditions (e.g. temperature, humidity and food supply) that may substantially reduce the fitness (Westbrook et al., 2010, Jones and Foster, 1978, Veronesi et al., 2009, Armstrong and Bransbyw, 1961). The fitness of both adults and immature stages of mosquitoes under laboratory conditions is known to be heavily dependent on temperature (Fike Knop et al., 1987, Westbrook et al., 2010, Barnett and Gould, 1962, Aytekin et al., 2009, Impoinvil et al., 2007, Soelarto et al., 1995). Temperature can influence mosquito fitness by affecting their rate of egg and larval development (Bayoh and Lindsay, 2003, Impoinvil et al., 2007, Bayoh and Lindsay, 2004), and subsequent adult body size (Aytekin et al., 2009, e.g. Anopheles superpictus and Aedes albopictus Westbrook et al., 2010). Water temperatures in the range of 22 – 27ºC have been associated with optimal larva development and adult body size of various *Anopheles*, *Culex* and *Aedes* mosquitoes (Impoinvil et al., 2007, Westbrook et al., 2010, Fike Knop et al., 1987, Barnett and Gould, 1962, Aytekin et al., 2009), whereas exposure to extreme low or high temperature values outside of this range significantly reduces mosquito fitness (Impoinvil et al., 2007, Westbrook et al., 2010). This suggests that changes in ambient temperature impose strong selection pressures on mosquito fitness and development.

Within endemic malaria transmission settings in Africa, the temperature of both larval and adult malaria vector habitats can vary substantially on a daily, weekly, and seasonal basis (Himeidan et al., 2009, Minakawa et al., 2006). However, laboratories rear colonies under constant temperature conditions (as reviewed by Benedict et al., 2009). Other environmental factors that are known to influence mosquito fitness in the wild but which are typically standardized under laboratory conditions include humidity (Kessler and Guerin, 2008) , and food quality and quantity (Grech et al., 2007). While standardizing mosquito rearing conditions may optimize mosquito production under laboratory conditions (Benedict et al., 2009) , by reducing variation in mosquito development and fitness this procedure may give rise to mosquitoes whose life-history is very different from those observed under natural field conditions.

A major aim of this thesis was to measure the fitness and behaviours of adult Anopheles vectors in the presence of different host types. The large numbers of guaranteed malaria free adult mosquitoes needed for these experiments required they be supplied from a colony rather than wild population. Ideally, however, these mosquitoes should be as representative as possible of wild mosquitoes in their fitness and behaviours. Consequently, the decision was taken to establish a new colony of *An. arabiensis* mosquitoes for these experiments within semi-field conditions, under which mosquitoes were reared in cages as typical settings in laboratory but exposed to the ambient climatic conditions of malaria endemic setting in Southern Tanzania. The establishment of a new *An. gambiae s.s* colony under semi-field conditions was not similarly possible for this work as this species has suffered marked declines throughout the the study region (Kilombero valley) in which these studies were based (Russell et al., 2010), and sufficient numbers can no longer be collected to initiate a new colony. Consequently, experiments with *An. gambiae s.s* relied on an existing laboratory colony established at the IHI. In addition to the semi-field colony, I also aimed to establish a standard laboratory reference colony of *An. arabiensis* at the University of Glasgow. Here I describe the procedures followed to establish both the laboratory and semi-field colony of *An. arabiensis* from a wild population in southern Tanzania, and changes in the respective development and life - history of individuals through the respective colonization process.

2.2 Methods

2.2.1 Establishment of An. arabiensis colony at IHI.

2.2.1.1 Mosquito collection and species identification

Wild blood fed *Anopheles gambiae s.l* were repeatedly collected from Sagamaganga village from June –Sept 2007, with supplemental collections to bolster the fledgling colonies made in March 2008. Sagamaganga village was chosen as an appropriate location for the source of the mosquito population because of its accessibility (only 20 km from the Ifakara Health Institute (IHI), where this study was conducted) and because its particular environmental characteristics make it suitable habitat for large numbers of *An. arabiensis* mosquitoes. Specifically, the residents of this village are mainly pastoralists from the Barabeig and Sukuma tribes who keep large numbers of cattle and practice rice cultivation. It is known that *An. arabiensis* are commonly found in the presence of livestock (Mutero et al., 2004, Mutero et al., 1999), and that rice fields provide larval breeding sites for this mosquito species (Ijumba et al., 2002, Mwangangi et al., 2007, Jarju et al., 2009, Mutero et al., 2000). During collection of wild *Anopheles* mosquitoes, blood fed females that were visually identified as belonging to the *An. gambiae s.l* group were aspirated from inside of cattle sheds and houses during early morning catches and kept inside mosquito cages before being transported alive to the semi-field insectary at the IHI (Figure 2.1).

On arrival at the semi-field insectary (Figure 2.2), individual blood fed females were transferred from the collection cage into individual paper cups (4 cm diameter × 8 cm depth) to allow for oviposition under natural light, temperature and relative humidity conditions. Each paper cup was lined with filter paper at the bottom and filled with water (1-2 mm depth) to allow for oviposition. The tops of the paper cups were covered with mesh netting, on which a cotton wool pad soaked in 10% glucose solution was placed as a source of water and sugar. All cups were checked on a daily basis and the number of eggs laid within a sub-sample was counted under a dissecting microscope. All mosquitoes that laid eggs were subsequently killed by ether, kept inside eppendoff tube with silica gel, and sent to the IHI molecular laboratory for identification of species by Polymerase Chain Reaction (PCR) (Scott et al., 1993). The eggs of all wild caught mothers confirmed to be *An. arabiensis* were pooled together to form the founder populations for the establishment of the *An. arabiensis* colony under semi-field insectary conditions at the IHI.

Figure 2.1 Collection of *An. gambiae s.l* using aspirator and tourch from a cow shed at Sagamaganga village.

2.2.1.2 Rearing of *An. arabiensis* under semi-field insectary at IHI.

The semi-field insectary is located in a section of the large semi-field system recently developed at the IHI (Ferguson et al., 2008). Adult mosquitoes are contained in cage held within an area made of black cloth walls with six windows, and larvae in basins within an area for egg and larval rearing. These mosquitoes are exposed to ambient temperature that range from 22.60 to 34.43ºC (Ferguson et al., 2008).

The eggs of wild *An. arabiensis* laid into individual paper cups at the IHI hatched into first instars within 2-3days. After this time, eggs from approximately 6 distinct clutches were pooled together into one larval basins filled with 2 cm depth of water (33.6 cm diameter × 15 cm height) with no more than 500 first instar in each rearing basin (Figure 2.2). First instars were provided with approximately 25 mg of finely ground fish food flakes (Tetramin ®) once per day. After two days, the water depth in basins was increased by 1 cm depth and the daily food allocation was increased approximately to 35 mg. This amount of larval food added to basins twice a day (making a total of 70 mg per day) during the 3rd instar period of development. When larvae reached the 4th instar stage, 6 – 7 days after hatching, water depth was increased to 4 cm. At the onset of pupation (within 7 – 8 days from hatching), daily food allocation was reduced to 35mg. Water in larval basins was replaced after every two days to maintain freshness, and reduce the build up waste products. A Tinytag ® (Gemini, UK, Tinytag Ultra2, Place) temperature probe was placed inside one rearing basin to record water temperature on an hourly basis during larval development. The average monthly water temperature in larval basins was calculated from these daily records.

Pupae were typically observed to appear in larval basins within 6 – 8 days from the time 1st instars. Pupae were collected on a daily basis from larval basins and transferred by Pasteur pipette into paper cups (4 cm diameter × 8 cm depth) into mosquito cages (45 × 45×45 cm) for emergence. Approximately 350- 1500 pupae were added into cages over 3 day period to give rise to approximately 1050– 4500 adults per cage. When in cages, adult mosquitoes were provided with a 10% glucose solution through wicks of filter paper that were inserted into universal tubes. The offspring of wild parents collected in were defined as the F1 generation from which all subsequent generations were propagated.

Adult mosquito cages were contained within a large black tent (512 × 278 × 253 cm) with 6 windows (each measuring 80 × 80 cm) to provide them with natural light and darkness (Figure 2.3). Mosquitoes inside this tent were exposed to ambient temperature and relative humidity. In nature, *An. gambiae s.l* mosquitoes mate in aerial swarms at dusk within 24 hours after emergence from their breeding sites and before blood feeding (Charlwood et al., 2002). Within the semi-field insectary, mosquitoes were exposed to ambient light conditions including the natural timing of sunset and dawn through six large windows that were created on the sides of the holding tent (Figure 2.3). The design of the adult room was intended to maximize the efficiency of two key mosquito life-history feeding processes: with the exposure to ambient dusk and dawn conditions enhancing swarming behaviour, and the flexibility to shut out light intended to enhance blood-feeding success of mosquitoes exposed to human hosts during daylight hours.

Adult females within the semi-field insectary were first given the opportunity to blood feed at an age of 3-4 days old. Mosquitoes were starved of glucose and water for 12 hours before blood feeding to increase their hunger and motivation to blood feed. Initially, mosquitoes were exposed to a human blood source twice per day to enhance their probability of feeding, and mimic host exposure patterns during typical rural settings where humans are now most commonly available early in the evening (7:30 pm) before they go to sleep under bed nets, and early in the morning when people get up (Russell et al., 2010, Killeen et al., 2006).

In comparison to their sister species *An. gambiae s.s*, *Anopheles arabiensis* are relatively large mosquitoes that need a larger amount of blood to produce eggs (Hogg et al., 1996). Therefore, during establishment of the *An. arabiensis* colony, one of the biggest challenges was to ensure feeding was sufficiently frequent to maximize reproductive success. Blood was provided to mosquitoes from the forearm of human volunteers (insectary technicians) for three consecutive days, to ensure that most individuals obtained at least one blood meal. The forearm was inserted inside adult cages for 15 – 20 minutes. After these 3 days of host exposure, egg bowls (7.8 cm diameter × 4 cm depth) filled with water to 2 cm of water were placed in each adult cage to allow for oviposition. To facilitate the continuous collection of eggs, mosquitoes were given a further opportunity for 3 consecutive nights of blood feeding two days after the first cycle finished. This blood-feeding regime was adopted to maximize egg production while adapting mosquitoes to the semi-field conditions.

Eggs laid by mosquitoes in egg bowls were observed for hatching on a daily basis, with 1st instars typically appearing 2 – 3 days after oviposition. First instars were transferred into rearing basins and larvae were reared as described above. The monthly average water temperature, development time (egg to adult stage), the average number of pupae for 27 generations and the body size of emerging adult (only up to 8th generation) of the semi-field mosquitoes were recorded.

Figure 2.2 **Semi-field insectary : The rearing area exposed to ambient conditions**

Figure 2.3 **Semi-field insectary: An area made with black cloth walls and six windows within which adult mosquitoes where reared.**

2.2.2 Establishment of An. arabiensis colony at UG.

2.2.2.1 Colonization of *An. arabiensis* under insectary conditions at UG

After 6 generations, eggs from the *An. arabiensis* semi-field insectary I established at the IHI in Tanzania were transported to the University of Glasgow (UG) and used to establish a new colony under laboratory conditions of 26 ± 1ºC, 80% relative humidity. These conditions are similar to the rearing protocol used for other African *Anopheles* mosquitoes in the UG insectaries. The eggs were transported to Glasgow on wet filter paper that was taken from egg bowls inside the IHI semi-field stock cages. Eggs were carried by individuals travelling from the IHI to UG within a 2-day period (e.g. 1 day of road travel from IHI to Dar es Salaam, 2nd day of air travel between Dar es Salaam and Glasgow). Once the eggs arrived at UG, they were transferred into standard larval rearing trays (5 × 16 × 16 cm) and allowed to hatch under insectary conditions. The water depth in larval rearing trays was set at 2 cm for first instars and increased by 1 cm after every two days as described for the semi-field insectary above. Larvae were fed with Tetramin ® (approximately 0.25 mg) once per day. Larval trays were inspected daily and pupae observed within them were collected on a daily basis and transferred into small bowls (9.5 cm diameter × 4 cm depth) for emergence into standard stock cages (20 × 20 × 20 cm) to establish the F1 laboratory generation.

The light condition inside the insectary was set at 12:12 hour light/dark cycle to reflect the time of sunset and sunrise in tropical African countries. The standard procedure for conducting experimental infection studies of *Anopheles* in the UG insectary involves feeding mosquitoes with human blood mixed with cultured gametocytes through an artificial membrane feeding device (Carter et al., 1993). Therefore, in establishing the *An. arabiensis* laboratory colony at UG, I aimed to adapt mosquitoes to feed and produce on blood provided by an artificial membrane feeding system. As mosquitoes usually have low blood feeding success on artificial membranes when first brought into laboratory colonies (Benedict et al., 2006), initially mosquitoes were split into two groups, one which were provided blood through arm feeding to which they were accustomed (e.g. to maintain colony production during the transition to membrane feeding), and another group that was selected for membrane feeding ability. Offspring of mosquitoes generated from arm feeding were transitioned to membrane feeding, and those generated from membrane fed parents were kept separate to form a membrane-adapted line. Prior to blood feeding (by either means) mosquitoes were starved of glucose and water for 12 hours as in the semi-field insectary in Tanzania. Mosquitoes were blood fed for 20 – 30 minutes at 8 am in the morning, just after the light turned on in the insectary. Initially, mosquitoes exposed to membrane feeders (2.5 cm diameter × 3.5 cm height) had very poor feeding and reproductive success. Consequently, membrane-feeding opportunities were provided for 5 days consecutively to maximize their probability of feeding and obtaining enough blood to reproduce. Egg bowls (9.5 cm diameter × 4 cm depth) were first introduced into membrane fed cages (20 × 20 × 20 cm) on 3rd day after the start of feeding, and kept inside for continuous collection of eggs over subsequent days.

Over time, the number of mosquitoes maintained on arm-feeding was intentionally reduced while those exposed to blood via membrane feeders was increased to make the transition to a colony maintained entirely on membrane feeding. After the *An. arabiensis* colony had been successfully transitioned to exclusive membrane feeding, adults were shifted to being provided with a 10% glucose solution to a 5% glucose/0.05 % para-amino-benzoic acid (PABA) as consistent with the standard rearing protocol for other African *Anopheles* mosquitoes (e.g. *An. gambiae s.s*) maintained at UG. This transition was made gradually by first reducing the concentration of glucose from 10% to 7.5%, and then further to 5% (with a week in between). Data on the average number of pupae produced from arm-fed and membrane fed lines were collected throughout colonization process to track the rate of adaptation to the artificial feeding system and insectary conditions at UG.

2.3 Results

2.3.1.1 Mosquito collection and species identification.

During initial collections in Sagamaganga village, approximately 1,360 blood fed *An. gambiae s.l* were collected resting inside cattle and houses. Subsequent analysis of ovipositing females confirmed that 87 – 94% of individuals in weekly collections were *An. arabiensis* (Table 2.1). Measurements taken from a subsample of wild females from 3 collections indicated that the average wing length ranged from 3.13 ± 0.03 to 3.19 ± 0.02 mm (Table 2.1). The average number of eggs laid by a subset of ovipositing females from the first 3 collection raised from 73.45 ± 2.87 to 99.62 ± 6.24 eggs (Table 2.1). Eggs from *An. arabiensis* obtained from 6 distinct collections carried out between June - Sept 2007 and March 2008 were used to establish the semi-field colony (Table 2.1).

Date	Collection	Total mosquitoes	Proportion of *An. arabiensis*	Average number of eggs	Average wing length (mm)
1-07-07	1	267	0.87	73.45 (2.87)	3.13 (0.03)
18-08-07	2	134	0.94	82.97 (4.44)	3.19 (0.02)
31-09-07	3	70	0.90	99.62 (6.24)	3.13 (0.06)
22-02-08	4	225	0.86	-	-
11-03-08	5	351	0.87	-	-
25-03-08	6	314	0.86	–	–

Table 2.1. Abundance of wild *An. arabiensis* in Sagamaganga village and their fitness traits. Numbers in brackets indicate one standard error of the mean (1 s.e.m).

2.3.1.2 Expansion of the SFS colony of *An. arabiensis*.

Thousands of eggs from wild *An. arabiensis* were pooled and successfully reared in the semi-field insectary under ambient temperature and relative humidity. The adaptation of wild *An. arabiensis* to semi-field condition was evidenced by the increasing survival of larva and pupation (Figure 2.4), increased rates of blood feeding over the first few generations. The average number of pupae produced per generation per day in the colony as a whole fluctuated through time (Figure 2.4). When the colony of *An. arabiensis* was between 5 and 27 generations under semi-field conditions, the average number of pupae per generation never dropped below 360.

Variation in the number of pupae produced within the semi-field insectary may be associated with the seasonal changes in average water temperature per day. Over the first two years of *An. arabiensis* colonization in semi-field conditions, average daily water temperature in larval habitats fluctuated between 21.55 ± 0.50 ºC to 30.21 ± 0.54 ºC (Figure 2.4). Restricting analysis to the average number of pupae produced between generation 5 and 27, average daily water temperatures was divided into three equal groups of 21 – 24, 25 – 28, and 29 – 31ºC per day, and their relationship with the average number of pupae produced per day was analysed using general linear model in R statistical software (Crawley, 2007). The average number of pupae was significantly related with the average water temperature per day ($F_{2, 20} = 4.51$, $R^2 = 0.31$, $P = 0.02$,Figure 2.4 ,Figure 2.5), with the number of pupae per day being substantially greater in water temperature of 21 – 24 ºC than in 25 – 28 ºC ($T = -2.70$, $P = 0.03$) and 29 – 31 ºC per day ($T = -2.83$, $P = 0.03$), but did not differ significantly between water temperature of 25 – 28 ºC and 29 – 31 ºC ($T = -0.10$, $P = 0.99$) per day (Figure 2.5).

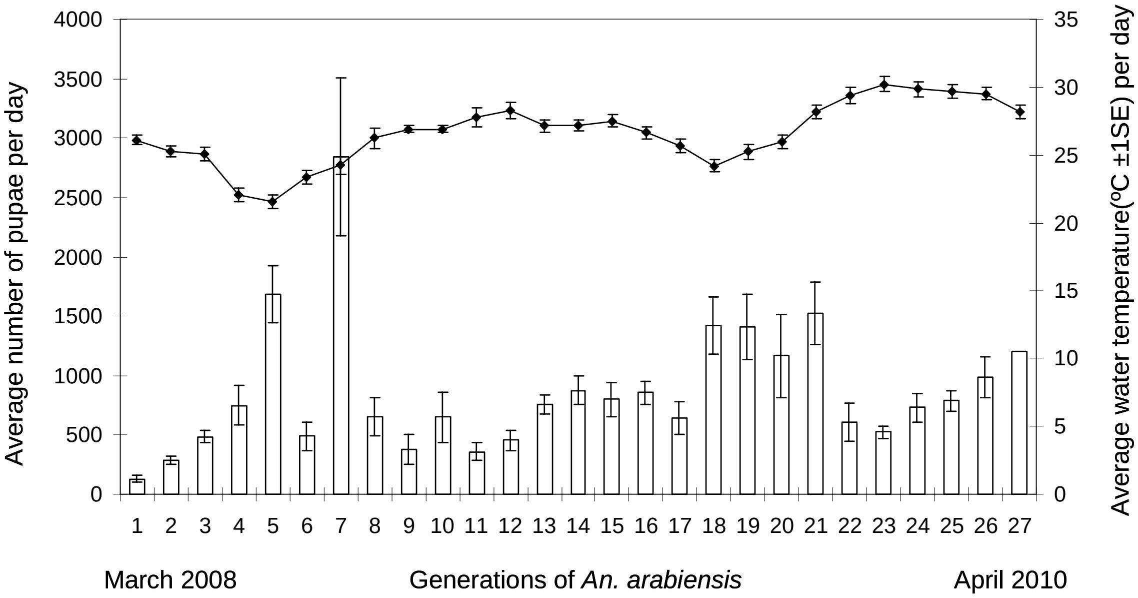

Figure 2.4. Relationship between the average number of *An. arabiensis* pupae per day and average water temperature per day in larval rearing habitats over a 2 –year period of colonization within a semi-field insectary at the Ifakara Health Institute. The error bars indicate the standard error of mean.

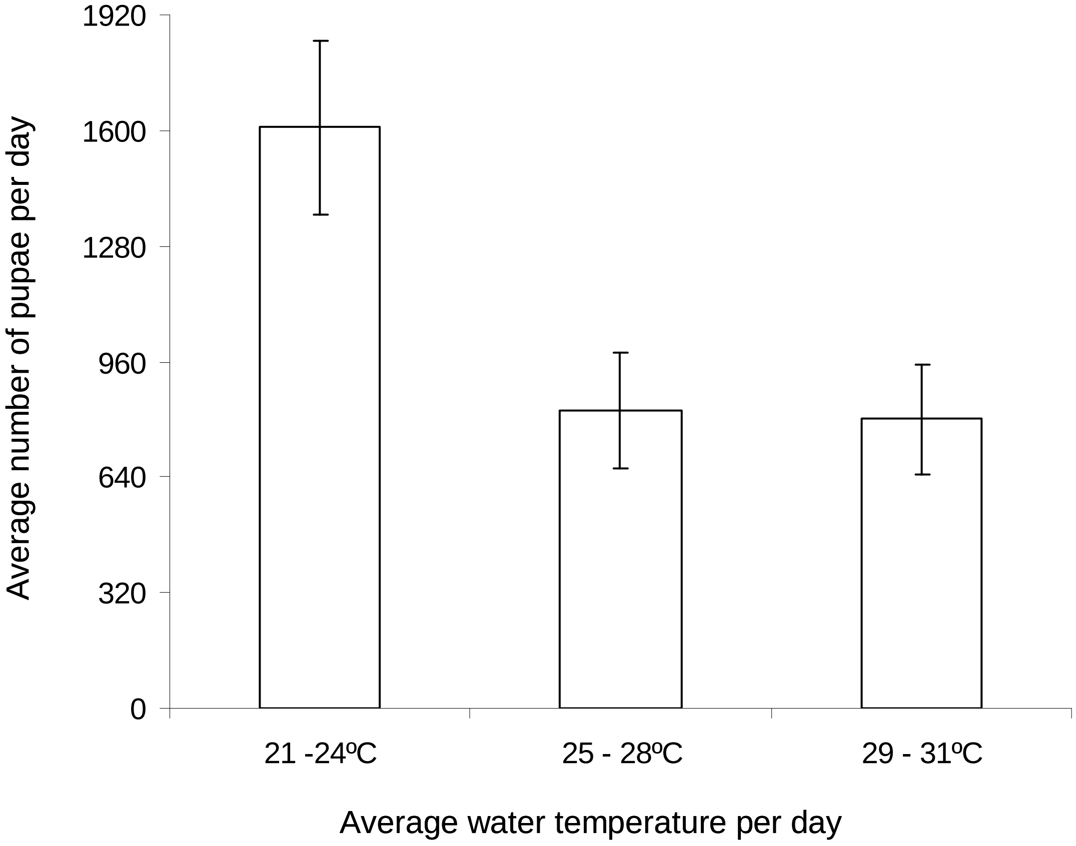

Figure 2.5. Variation in rearing water temperature and the average number of pupae produced per day during colonization of *An. arabiensis* in the semi-field insectary conditions. The error bars indicates one standard error.

Although variation in average larval water temperature substantially influenced the production of pupae per generations, there was no clear evidence of an effect on the body size of emerging adult mosquitoes under semi-field conditions. Variation in body size of adult *An. arabiensis* between generations was analysed using general linear model(lm) in R the statistical software (Crawley, 2007). Here, generation was considered as 'explanatory variable', and wing length as an index of body size was considered as the 'response variable'. Across generations from the wild parents to F8, the average body size of female *An. arabiensis* reared within the semi-field insectary temperature varied significantly between generations ($F_{8, 372} = 2.08$, $P = 0.04$, Figure 2.6), but showed no evidence of a consistent increase or decrease through time. There was no significant difference in the body size of wild parents and any generation of their offspring (up to F8) ($P > 0.05$ in all two-ways comparisons,Figure 2.6).

During rearing of the first eight generations in the semi-field insectary, the average water temperature per day fluctuated from 21.55 ± 0.50 to 26.26 ± 0.73 ºC (Figure 2.6). This range of water temperature generated adults of similar body size in the semi-field insectary (3.05 ± 0.05 mm and 3.25 ± 0.05 mm). This suggests that the semi-field system mimicked the average water temperature of larval habitats of *An. arabiensis* as in their natural environments.

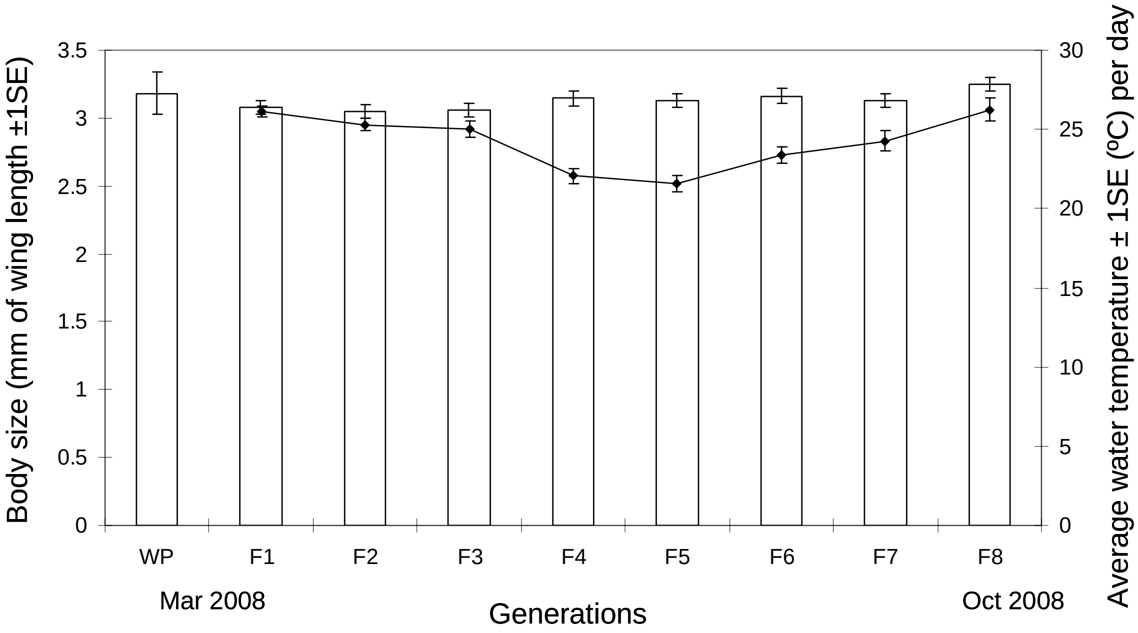

Figure 2.6. Relationship between the average water temperature and variation in the the average of adult body size of *An. arabiensis* across 8 generations in the semi-field insectary. WP = wild parental generation.

2.3.1.3 Colonization of *An. arabiensis* under insectary conditions

Several thousands of *An. arabiensis* eggs from the IHI semi-field colony were used to establish a new colony of *An. arabiensis* at the University of Glasgow. Initially, several challenges were encountered when trying to select *An. arabiensis* for membrane feeding in the UG insectary. During the first 2 attempts of adapting semi-field *An. arabiensis* from arm feeding to membrane feeding device, feeding success was very poor and consequently very low number of pupae were produced (an average of 15.67 ± 3.95 to 17.33 ± 9.33 pupae per day). Gradually, over 18 months in insectary conditions, the feeding success of *An. arabiensis* exposed to artificial membrane was observed to increase and the average number of pupae increased to 373.61 ± 45.10 per day but never dropped below 53.47 ± 7.44 per day. This rate of pupal productivity under membrane was similar to that observed from arm feeding in the UG insectary (39.72 ± 6.29 to 223 ± 26.68, Figure 2.7a & b). These results indicate that the *An. arabiensis* colony become adapted to membrane feeding system after 5 generations in UG the insectary conditions (i.e. approximately 6 months of membrane feeding).

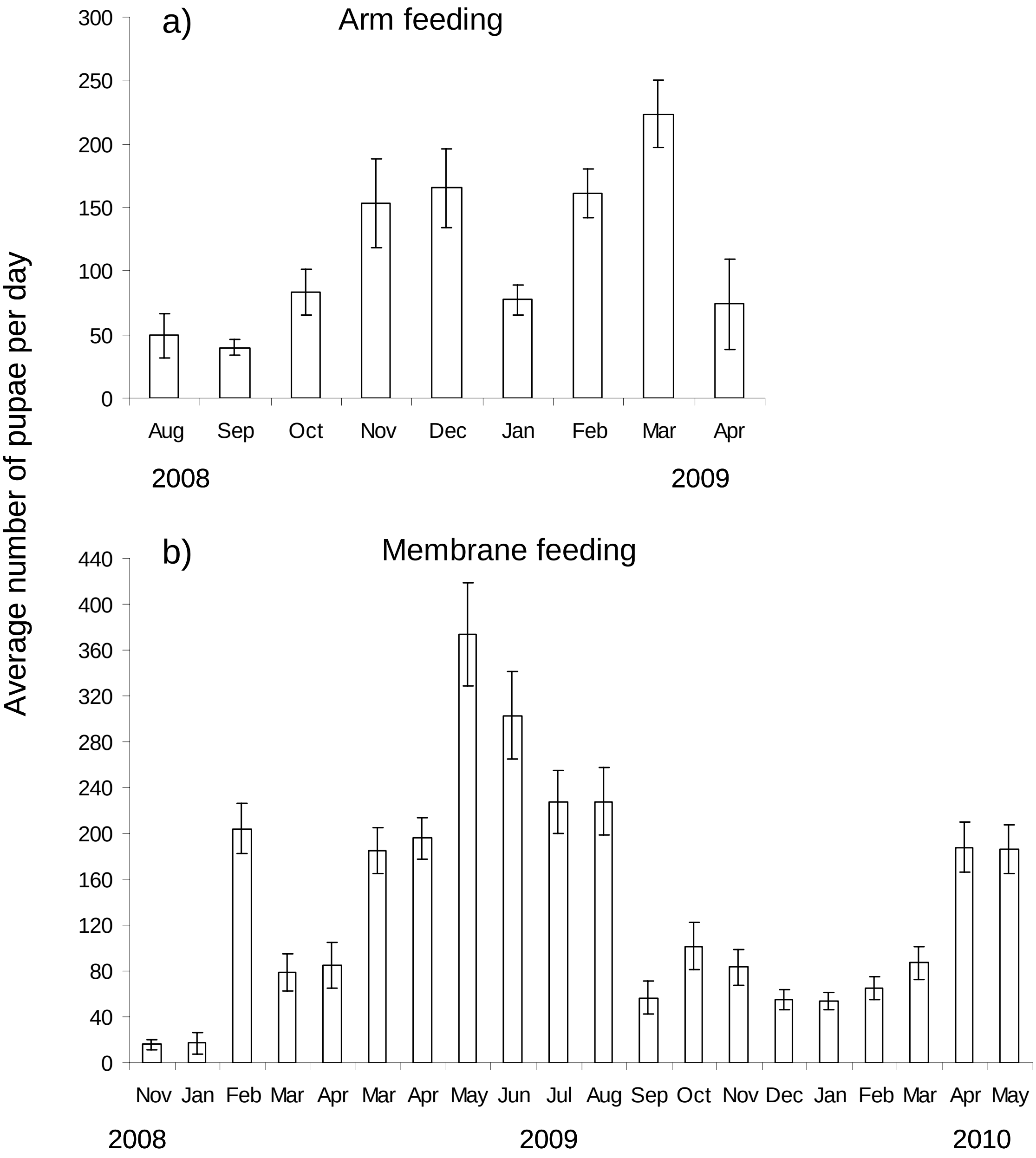

Figure 2.7. Pupae production during colonization of *An. arabiensis* from the IHI semi-field insectary to laboratory conditions at the University of Glasgow. 'a' indicates pupal production from arm feeding, and 'b' the production from membrane feeding.

2.4 Discussion

Anopheles arabiensis from a wild population in Tanzania were successfully colonized under semi-field insectary at the IHI and insectary conditions at UG. While *An. arabiensis* has been successfully colonized in the laboratory before (Mpofu et al., 1993), to my knowledge this represents the first successful colonization of this species under semi-field conditions. Under semi-field conditions, I hypothesize colonization success was enhanced by allowing vectors to feed on a host species they typically feed on in nature (humans), and by initially establishing a blood feeding regime that is similar to their prevailing biting time on humans within the villages where they were collected (e.g. early evening and morning when people are unprotected by bed nets). Although initially *An. arabiensis* fed under these conditions generated few pupae, the productivity of mosquitoes reared under these conditions increased through time, with their average body size remain similar to wild parental generation. A similar process of gradual increase in colony production was observed in the *An. arabiensis* population established under laboratory conditions at UG. Although mosquitoes reared under these conditions initially had poor feeding success on the artificial membrane feeding system and produced very few offspring, within approximately 5 generations of colonization there was evidence that *An. arabiensis* had adapted to increase their blood feeding and resultant reproductive success. Overall, this study indicates that the feeding and reproductive success of *An. arabiensis* under both realistic semi-field and laboratory conditions are reduced within the first few generations, but rises over time to generate adult mosquitoes with similar body size to that of wild populations.

The dynamics of wild mosquitoes including *An. arabiensis* depend on environmental factors such as temperature (Himeidan and Rayah, 2008), humidity (Bangs et al., 2002, Afrane et al., 2006), rainfall (Koenraadt et al., 2004, Charlwood et al., 1995) and the availability of blood meal (Minakawa et al., 2002a), that consequently influence larval density and pupation rate (Mwangangi et al., 2008, Muturi et al., 2008, Himeidan et al., 2009, Minakawa et al., 2006, Mwangangi et al., 2007). In agro-ecosystems where *An. arabiensis* are abundant, larvae are typically found in the breeding sites close to their preferred hosts (e.g. humans and cows Minakawa et al., 2002a), which may minimize the amount of energy expended on searching for oviposition sites after feeding. Typically water temperatures in these rice paddies range from 18.4 – 37° C (Mwangangi et al., 2006, Mwangangi et al., 2007, Jarju et al., 2009, Minakawa et al., 2006), suggesting this range is suitable for larval development. The ability of the semi-field and laboratory conditions used here to mimic these micro-climatic and host conditions may have enhanced the efficiency of the colonization process.

In addition to mimicking natural conditions that are appropriate for *An. arabiensis*, the success of colonization under semi-field conditions was probably facilitated by excluding sources of mortality that exist in the wild. For example, the survival of *An. arabiensis* larvae and pupae under field conditions is known to depend on the quality of water in terms of food, depth, predation and water temperature (Minakawa et al., 2005, Minakawa et al., 2006, Muturi et al., 2008, Koenraadt et al., 2004, Munga et al., 2007). Here under semi-field conditions, there was no risk of predation during larval development, and water depth was regulated on the basis of what was thought to capable of achieving suitable water temperature and food density conditions. Furthermore, in contrast to natural conditions, water in larval rearing habitats was exchanged on a regular basis to avoid the build up of waste products or toxins that may impede growth. The addition of fresh water and standardized food was observed to prevent the formation of dirt scum on the surface of larval water, while giving rise to relatively rapid and efficient larval development. Optimizing the amount of food provided to larvae at different stages minimized the larval mortality during the colonization of *An. maculates* (Bangs et al., 2002). This suggests that optimizing larval food provision as appropriate for specific larval development stages may be required to increase efficiency of colonization.

Water temperature is one of most important exogenous factors affecting the development of eggs, larval and pupae, and consequently their abundance and distribution in the natural environments (Benedict et al., 2006, Himeidan and Rayah, 2008). In nature, high larval density populations of *An. arabiensis* are often associated with dry lowlands where larval habitats include sunlit paddies and pools without vegetations that range in temperatures from 24 – 25ºC, and may be optimal for egg, larvae, and pupal development (Minakawa et al., 2002b, Minakawa et al., 2006, Jarju et al., 2009, Muturi et al., 2008, Munga et al., 2009). In contrast, in cooler highland areas *An. arabiensis* experience higher larval mortality and lower pupae productivity which may be due to suboptimal water temperature (19-20 ºC) in most of larval habitats (Minakawa et al., 2006). The present study indicated that high pupal productivity of *An. arabiensis* under semi-field insectary conditions was obtained under ambient temperature and relative humidity conditions with optimal temperature for pupal production being between 21 – 24 ºC per day. These water temperatures under semi-field conditions are within the range associated with highest larval and pupal density in nature (Mwangangi et al., 2007, Minakawa et al., 2006).

In addition to its influence the survival and development of larvae, water temperature is also known to influence the body size of emerging adult mosquitoes (Lyimo et al., 1992, Aytekin et al., 2009). This phenotypic trait is directly related to adult fitness traits including feeding success, fecundity, mating competence and survival (Ameneshewa and Service, 1996, Lyimo and Takken, 1993, Briegel, 1990, Ng'habi et al., 2008). Laboratory studies indicate that larvae reared at high water temperatures produce relatively small adults, whereas those reared at cooler temperatures produce relatively larger mosquitoes (Westbrook et al., 2010, Aytekin et al., 2009, Impoinvil et al., 2007). In the present study, there was no evidence of variation in average body size of *An. arabiensis colony* between generations reared under the fluctuating water temperature (21.55 ± 0.50 to 26.26 ± 0.73 ºC) in semi-field insectary, and between semi-field mosquitoes and their wild parents.

In addition to the blood-feeding regime and larval habitat temperature, another factor that may have enhanced colonization success under semi-field conditions was exposure to appropriate conditions of photoperiodicity for the stimulation of host-seeking and mating. *Anopheles arabiensis* adults were kept inside an area surrounded with black cloth walls, in which six large windows were present to allow for light entry and the creation of darkness. By keeping these windows open, adults in the colony were exposed to natural sunset periods which are known to stimulate swarming and mating activities in mosquitoes (Reisen et al., 1977, Charlwood et al., 2002). Furthermore, having windows in place provided flexibility to create darkness within the system during blood-feeding periods, which is thought to stimulate host-seeking behaviours (e.g. *An. arabiensis* bite late night in their natural environments, Taye et al., 2006).

One of the largest obstacles in the colonization of mosquitoes in the laboratory is their poor blood feeding efficiency under artificial conditions (Benedict et al., 2006). Similarly, a substantial challenge to the establishment of the *An. arabiensis* laboratory colony was the initially poor feeding and reproductive success of these mosquitoes on the artificial membrane feeding system. Over a period of 6 months (after 5 generations), the proportion of *An. arabiensis* that fed from the membrane system gradually increased, followed by an increase in the number of offspring they produced. However, pupal production continued to vary even after relatively high and constant rates of membrane feeding were achieved (e.g. maximum production of pupae observed in period of May to June, with lower production of pupae from September to February of the next year). Overall, this variation could be due to changes in other unknown environmental or handling conditions in the insectary during this time, or possibly to seasonality in temperature.

Despite the initially poor productivity of *An. arabiensis* under semi-field and laboratory settings, relatively large and stable colonies of this mosquito were successfully established within both these conditions within 20 – 27 generations of the wild population. While both colonies can provide a valuable source of mosquitoes for experimental work, I hypothesize that the more natural rearing and environmental conditions of the semi-field colony generated individuals that are more similar in behaviour and life-history to their wild counterparts, and thus more suitable for the experimental study of mosquito ecology and fitness. The similar body size of *An. arabiensis* females within the semi-field colony and the natural field populations suggest these mosquitoes are exposed to similar environmental constraints and selective pressures during their development. This contrast with the finding that *An. gambiae s.s* in laboratory conditions are significantly smaller than their counterpart in wild population (Huho et al., 2007). Overall, this study reinforces the need to incorporate natural feeding behaviours and environmental conditions into insectary rearing protocols as much as possible to both enhance the viability of colonies, and help to maintain their behavioural similarity to wild population as is required for relevance in evolutionary and ecological studies.

3 The evolutionary consequences of host species choice for African malaria vectors: Could untreated bed nets select for a host shift?

3.1 Introduction

The evolution of resistance by pathogens and the invertebrates that transmit them poses one of the greatest biological impediments to the sustainable control of infectious disease (Roberts and Andre, 1994). Consequently, the conventional paradigm is that evolutionary change by pathogens and their vectors has only detrimental impacts on disease control, and that stopping or slowing this process is the only strategy of sustaining control (Read et al., 2009, Koella et al., 2009). A lesser explored possibility is that interventions could be designed to generate selection upon disease-transmitting agents that provides a fitness reward for adopting new phenotypes that reduce their ability to transmit disease (Kurzban and Egeth, 2008, Ferguson et al., 2006). The advantage of such an approach is that it could harness selection and use it to drive and sustain evolutionary changes that reduce disease transmission. Once put into place, such an approach would be reinforced instead of undermined by natural selection, and providing an opportunity to exert ‘Darwinian prevention’.

Such an approach could be particularly amenable for diseases that are indirectly transmitted by arthropod vectors. While vectors normally complete their life cycle on a wide variety of host species, the pathogens that are transmitted by them are frequently restricted to only one host species. Consequently, if the fitness landscape can be manipulated to provide vectors with higher reproductive success from feeding on non-permissive host species, natural selection will be generated upon vectors to avoid pathogen-susceptible hosts, and thus inevitably reduce pathogen transmission.

One disease for which such an approach could be particularly useful is malaria; the vector-borne disease responsible for the greatest loss of human life (Snow et al., 2005). Although international efforts to reduce transmission have made substantial progress (Coll-Seck et al., 2008), this disease continues to be a major source of morbidity and mortality in the developing world (Snow et al., 2005). This disease is caused by *Plasmodium* parasites that are transmitted between people by female *Anopheles* mosquitoes during blood feeding. The host range of *Anopheles* mosquitoes shows extensive variation with some species using a wide range of mammalian and avian hosts and others being largely restricted to only one host species (Lyimo and Ferguson, 2009). The tendency of A*nopheles* vectors to specialize on humans (anthrophily) is one of the most important biological determinants of malaria transmission (Kiszewski et al., 2004). The *Anopheles gambiae s.s* mosquitoes that transmit malaria in Africa are amongst the most anthrophilic vector species ever described; and this behaviour is thought to be largely responsible for the disproportionate burden of malaria in this continent (Kiszewski et al., 2004). The *Plasmodium* parasites causing malaria in humans generally do not infect any other species (with *P. knowlesi* being the recently confirmed exception). Consequently, a shift in malaria vector host choice from humans onto animals generally reduces transmission(Philip et al., 2009).

While it is expected that the host species choice of haematophagous insects has been shaped by natural selection, few empirical tests have been conducted to evaluate if and how host choice is correlated with fitness in any of the medically important vector species. Furthermore, there has been little elucidation of the selective pressures that cause anthrophilic vectors to specialize their feeding on humans. There has been some investigation of the potential to reduce malaria transmission by using alternative animals to lure mosquitoes away from humans (Rowland et al., 2001, e.g. Zooprophylaxis, Philip et al., 2009); but is unknown whether this or any other intervention in use or under development has the potential to trigger long-term evolutionary changes in vector host species choice.

Currently, the most widely used intervention against malaria vectors in Africa is the bednet. Bednets have the greatest impact on reducing human exposure to malaria when they are treated with insecticides (ITN's) (Lengeler, 2004) that not only prevent biting but also reduce the size of vector population. However, bednets provide some personal protection from mosquito bites and disease exposure even when untreated (Clarke et al., 2001). Although the prevalence (coverage) of ITN's is expected to increase dramatically in the near future due to the planned expansion of ITN's distribution programme (Roll Back Malaria Partnership, 2008), the vast majority of bed nets currently in use throughout Africa are untreated (Killeen et al., 2007b). In some settings, the widespread use of untreated nets has been associated with significant reduction in the anthropohily of local malaria vectors (Burkot et al., 1990) but it is unclear whether this change may have been the product of behavioural plasticity or selection on mosquito host choice. To our knowledge, there has been no investigation of evolutionary implications of net use on mosquito fitness and its potential to select for host species switch.

In order to both predict the potential utility of this or other interventions to generate selection upon malaria vectors to shift their host preference away from humans, some fundamental questions must be addressed: (1) is the fitness of malaria vectors dependent on their host species choice? (2) is the inherent preference of vectors for humans associated with a fitness advantage?, and (3) are there interventions which diminish the fitness benefits of anthrophily to the point it becomes more profitable for the vectors to switch to other alternative animal hosts? I experimentally investigated these questions within two species of *Anopheles* vectors that are responsible for the bulk of malaria transmission in sub-Saharan Africa: *An. gambiae s.s* and *An. arabiensis*. These sibling species are distributed widely throughout Africa, but vary in their inherent host preference. While *An. gambiae s.s* is highly anthrophilic and feeds exclusively on humans in most of its range (Lyimo and Ferguson, 2009), *An. arabiensis* feed readily on humans and cows (Sharp and Lesueur, 1991, Hadis et al., 1997, Tirados et al., 2006, Taye et al., 2006); and generally prefer the latter when available (Killeen et al., 2004, Duchemin et al., 2001).

Here I experimentally tested a series of hypotheses to explain the variation in host preference between these two mosquito vectors, and evaluated whether the use of untreated nets could reduce the fitness value of this human host below that accrued from feeding on other animals commonly available in the same environment. The specific hypotheses I tested were: (1) the feeding success, survival, egg production and their resultant lifetime reproductive success of mosquito vectors is dependent upon host species (2) mosquito fitness is highest when feeding on preferred host species (e.g. humans for *An. gambiae s.s* and cows for *An. arabiensis*), and finally (3) protecting humans with an untreated bed nets representative of those in operational use can reduce the fitness value of humans below that of other commonly available animals hosts.

Until recently, the experimental study of malaria vector host species choice and subsequent fitness under natural conditions has not been possible due to the inherent difficulties in tracking the fate of individual vectors during and subsequent to interaction with hosts without risking the exposure of human volunteers to potentially infectious mosquito bites. To overcome these limitations, this study made use of a large-scale experimental Semi-Field System (SFS) at the Ifakara Health Institute in Tanzania. Within this contained Anopheles biosphere, it was possible to experimentally observe behavioural interactions between African malaria vectors and their hosts using population of mosquitoes known to be malaria free. This SFS is one of only a few such facilities worldwide for the study of *Anopheles* ecology (Ferguson et al., 2008), and is an invaluable research tool for experimentally testing hypotheses about the ecology and evolution of human disease vectors that would otherwise be logistically and ethically unfeasible.

3.2 Methods

3.2.1 Study site

The study was conducted at the Ifakara Health Institute (IHI) in the Kilombero valley of Tanzania. High levels of malaria transmission are sustained year-round in this area by vectors *An. gambiae s.s*, *An. arabiensis* and *An. funestus*. Throughout the region, livestock and domestic animals including cows, calves, goats, chickens and dogs are commonly kept in or near houses.

3.2.2 Mosquito colonies

Experiments were conducted from May to September 2007 and 2008 using female *An. arabiensis* and *An. gambiae s.s* reared in colonies at the IHI. The *An. arabiensis* colony was established with individuals from the village of Sagamaganga in 2007 and 2008 (~20 km from the IHI) and is maintained in an outdoor semi-field insectary (Ferguson et al., 2008), at 25 - 32ºC and relative humidity of 51 – 90%. The *An. gambiae s.s* colony was established at the IHI with individuals from Njage village in 1996 (~70 km from IHI) and is maintained in an indoor insectary at 26 ± 2.5ºC and relative humidity of 80 ±10%. Mosquitoes in both colonies are maintained on human-blood provided thrice weekly by arm feeding.

3.2.3 Experimental set up

An experimental hut (3.5 X 4 X 2.5 m) was constructed in one netting enclosed chamber (9.1 X 9.6 X 3.7 m) of the IHI semi-field system (SFS) (Ferguson et al., 2008), (Figure 3.1). Mosquitoes released into the chamber could enter the hut through the open eaves fitted with an inside baffle that allowed mosquitoes to easily enter but not leave by the eave. Mosquitoes could also enter the hut by one of six windows, all that were fitted with exit traps that allowed mosquitoes to enter the hut and trapped them while trying to leave (Prior and Torr, 2002).

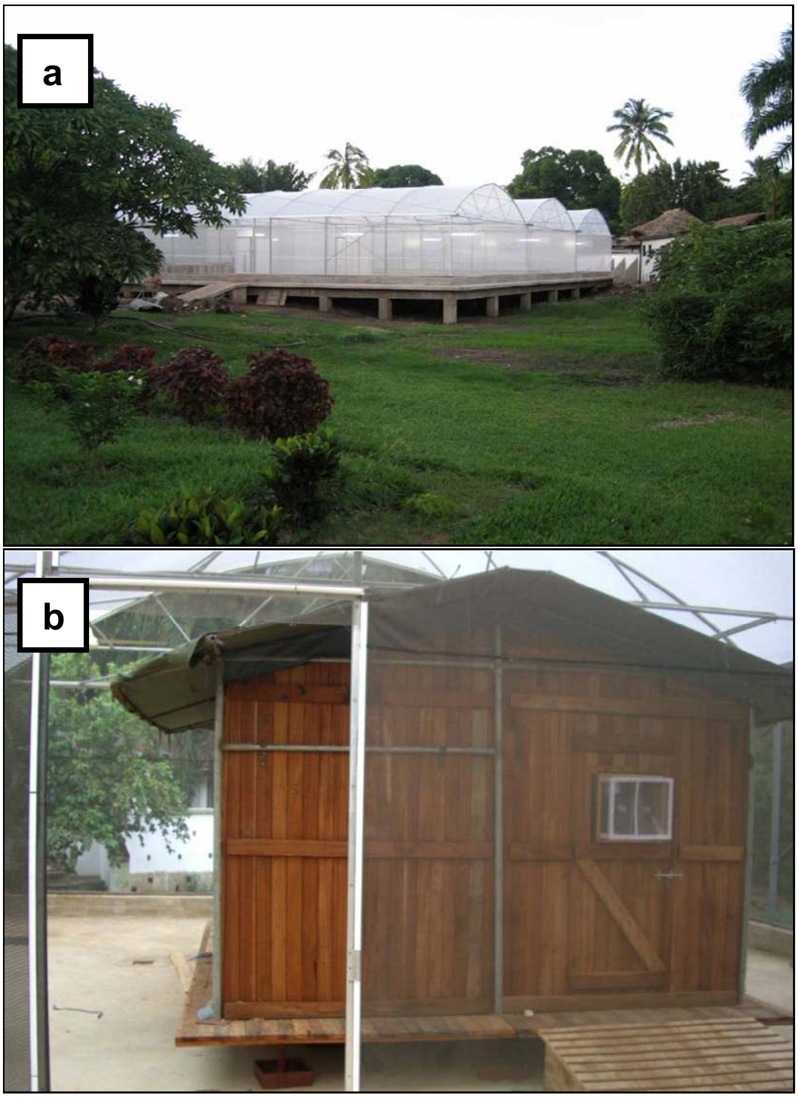

Figure 3.1. Experimental approach at the Ifakara Health Institute. ‘a’ indicates a semi-field system (SFS) and ‘b’ indicates an experimental hut within one chamber of SFS.

Mosquito-feeding success was evaluated on humans and 4 other animal species that are commonly available in or near houses in the Kilombero Valley: chickens, cows, dogs and goats. Two sub-categories of cattle were tested: cows and calves. Within other host types, animals were roughly of the same age and size. Human hosts were presented either exposed or sleeping under an untreated bed net (to contrast mosquito fitness of humans both the conditions under which this host – mosquito association arose, and under which humans are more likely to be found currently, e.g. under untreated net). Standardized 'typical' untreated nets were created following the W.H.O standard protocol for simulating the average condition of nets currently in use, by cutting 6 moderately sized holes into the wall of the net (4 X 4 cm) (WHO, 2005).

On each night of the experiments, one individual from one of the 7 potential host types was placed inside the experimental hut at dusk. Human volunteers were provided with a simple bed and sheet, and animals were not provided with beddings as in most rural areas. Human volunteers were instructed to sleep as usual, and react normally to mosquito biting (e.g. killing or brushing them away if wanted). After the host had entered, two hundred female mosquitoes of either *An. arabiensis* or *An. gambiae s.s* (4-6days old) were released in the corner of the chamber (maximum distance of 4.5 m from host). Mosquitoes were neither given water or neither glucose solution (i.e starved for 6hours) nor blood fed prior to experiments, to maximize their physiological demand for blood and motivation to host seek upon release. The next morning, all areas of experimental chamber and hut were intensively searched to recapture (by aspirator) mosquitoes. Recaptured mosquitoes were identified as being fed, unfed, live or dead. Six replicates (consisting of an assay with a different host individual) were performed for each of the 7 host treatments, for both of the mosquito species (84 trials in total). Experiments were run in blocks of one week, within which seven nights of consecutive trials were performed using a different host type each night (12 week-long blocks, with a different individual of each host type represented in each block).

3.2.4 Fitness measurements

Three metrics of host dependent feeding success were calculated: (1) the proportion of recaptured mosquitoes that were alive and had blood fed, (2) the proportion of recaptured mosquitoes found dead after the host-seeking, and (3) the size of bloodmeal obtained by fed mosquitoes. To measure bloodmeal size, fed mosquitoes were moved into individual 30ml plastic tubes on morning of their recapture and kept there for 3 days (while fed 10% glucose solution *ad-libitum*). After three days, these mosquitoes were moved into individual paper cups for oviposition and the hematin content of the bloodmeal excreta deposited in the initial holding tube measured to provide an index of the mass of blood ingested (Briegel, 1980).

Once transferred into individual holding cups for oviposition (lined with damp filter paper), mosquitoes and the number of eggs they laid counted under a dissecting microscope. Daily checks of all blood fed mosquitoes continued after oviposition to record the exact day of death following blood meals from different hosts. In these holding cups, mosquitoes were also provided a 10% glucose solution as an additional source of nutrition. The wing lengths of a subsample of mosquitoes from some experimental blocks were measured to provide an index of body size.

3.2.5 Ethical considerations

Mosquitoes used in these experiments had not been blood fed prior to use and thus were guaranteed to be free of malaria and other directly transmitted blood borne pathogens. All human hosts were adult volunteers from the research team. They provided written informed consent prior to participation, and were tested for malaria by Rapid Diagnostic Test (RDT) a few hours before their scheduled trial to ensure malaria parasites were not inadvertently introduced into the SFS. Any volunteer who tested positive was provided with treatment and did not participate in trials. The animals used in these trials were borrowed voluntarily from local community members after the purpose of the experiments explained and informed consent provided. Only animals that had not been treated with any topical insecticide within 2 – 3 months prior to the proposed experiment were selected. This study was reviewed and approved by the Institutional Ethical Review Board (IRB) of the IHI (IHRDC/IRB/No.A015) and the Medical Research Coordination Committee of the Tanzania National Institute for Medical Research (NIMR1HQ/R.8a/Vol.IX/708).

3.2.6 Statistical Analyses

Statistical analysis was used to evaluate the impact of host species choice on 6 key indicators of mosquito foraging success and fitness. Three of these response variables were binomial in form: blood feeding success, probability of death during host seeking, and probability of producing eggs, and the other three were continuous: blood meal size, number of eggs laid (fecundity), and post-feeding survival (days). Binomial response variables were analysed using generalized linear mixed effect models (glmer) in R statistical package (Crawley, 2007), with ‘host species’ and ‘mosquito species’ treated as fixed effects and ‘host individual’ as a random effect (6 individuals per host species). For each response variable, a base model was fit that included only the random effect of host individuals. The significance of the additional fixed effects of host and mosquito species, and their interactions, was tested by sequentially adding them to this base model and applying Likelihood Ratio Test (LRTs) to examine if they significantly decreased the negative log likelihood of the model ($P < 0.05$). For variables in which host species was identified as being statistically significant, Dunnett’s post hoc tests (adjusting for multiple comparisons) was used to identify all statistically significant two-way differences between the unprotected human reference group and all other host types. This procedure was then repeated using humans with an untreated net as a reference group to estimate how use of this intervention influenced the fitness value of humans relative to animal hosts.

The continuous response variables of blood meal size, and the number of eggs laid (excluding individuals that did not lay) were also analyzed using generalized linear mixed effect models (lmer) in R(Crawley, 2007). As with proportion data, ‘host species’ and ‘mosquito species’ were treated as fixed effects, and ‘host individual’ as a random effect. The significance of fixed effects were evaluated by sequentially adding them to a base model including only the random effect of host individual as described above.

The Cox Proportional Hazards Model (coxph) was used to estimate the impact of host species on the post-feeding survival of *An. gambiae* s.s. and *An. arabiensis*. In this model, a frailty function (Hougaard, 1995), was used to incorporate the random effects arising from use of different host individuals in different experimental blocks, and host and mosquito species were fitted as main effect using R statistical software (Crawley, 2007). As described above, these two fixed effects and their interaction were sequentially added to a base model including only the random individual effect to test if they significantly improved the likelihood.

All reported chi-square values refer to the output of generalized linear mixed models, and all reported z-values are for Dunnett’s two-way test between a human reference group and another host species (adjusted for multiple comparisons). Values defined as ‘OR’ are odds ratios from Cox proportional hazard models (coxph).

3.2.7 Modeling the impact of host species on lifetime reproductive success

Models were constructed on the basis that to produce eggs, a female mosquito must successfully acquire a blood meal during one night of seeking on the j^{th} host type(with probability β_j), survive through the subsequent egg development period of d_{ov} days (with a daily survival probability $s_{ov,j}$,) and oviposit (with probability γ_j); with ovipositing females laying 'F_j' eggs. I assume unfed females who do not succeed in feeding on one night of seeking (with probability= $1 - \beta_j$) can attempt to feed again on 'k' successive nights until they succeed or die. After laying eggs, females can initiate a new feeding cycle. However, both fed and unfed females who have not laid eggs (with probability= $1 - \beta_j * \gamma_j$) will attempt to feed again before starting a new feeding cycle. The daily survival probability of unfed mosquitoes (s_f) is independent of host type, whereas during the period between blood consumption and oviposition it varies with host type with probability $s_{ov,j}$.

The expected number of eggs resulting from multiple feeding cycles $R(j)$, is thus: $\mathrm{R}(j) = \sum_{k=0}^{\infty} [(1-\beta_j\gamma_j)s_f]^k \beta_j (s_{ov,j}^{d_{ov}} \ \gamma_j F_j)$ and the lifetime reproductive success (R_{0j}) expected from multiple feeding cycles i is given by $R_{0j} = \sum_{i=1}^{\infty} s_f^{i-1} R(j)$, assuming age-independent survival parameters. The survival of host seeking and blood fed mosquitoes under natural conditions may be influenced by varieties of selective pressures (e.g. predation, ITN's and IRS, Roitberg et al., 2003, Anderson and Roitberg, 1999, Charlwood et al., 1997, Russell et al., 2010, Musawenkosi et al., 2004). Under these conditions, female mosquitoes may survive at least 5 feeding cycles (Charlwood et al., 2000, Killeen et al., 2000). I assume more than 5 feeding cycles and age independent fecundity in order to test for the impact of host species choice on mosquito fitness under semi-field conditions where there are no sources of extrinsic mortalities (e.g. predation, ITN's and IRS). Values for host-specific mosquito fitness traits were directly estimated from experiments described above, with the exception of daily survival between feeding and oviposition (s_{ovj}). Our analysis estimated the odds of survival during this period relative to an unprotected human. These ratios were used to adjust published values of the survival of human-fed mosquitoes in the field (Appendix 1) to obtain relative values for other host types.

Confidence intervals around the expected mean values of R_{0j} were generated using the R software package. A 1000 simulations of the total lifetime reproductive success (R_0) of an individual *An. arabiensis* or *An. gambiae* s.s. when feeding exclusively on each of the 7 investigated host types were performed. Uncertainty within each simulation run was introduced by selecting the value of each host-specific parameter randomly from either a binomial (probability of feeding, surviving and oviposition) or normal distribution (number of eggs laid) with a mean and standard error as estimated from the appropriate statistical model previously described. Host-independent parameters in this model (daily survival when unfed, duration of egg development) were estimated from published literature (Appendix 1).

To test for any statistically significant differences in R_0 between host types (within a mosquito species), bootstrapping analyses were performed on each of the 21 possible two-way host comparisons between the 7 host types. At the start of a simulation to compare two host types, one value of R_o for host type 1 and type 2 were randomly drawn from their simulated distributions. A count was started to record every time that the R_o for host type 1 was greater than host type 2, with the entire procedure being repeated 10,000 times. The proportion of these 10,000 runs in which the R_o of one host type was greater than the other was used as an estimate of the probability that the lifetime reproductive success of mosquitoes on these host types was significantly different (if $p < 0.05$). The above procedure was repeated with the direction of comparison reversed (e.g how many times host type 2 > host type 1, instead of host type 1 > host type 2) to obtain (a two-tailed) test of statistical differences between host types in either direction.

3.3 Results

The feeding success and subsequent fitness of 16,517 *Anopheles* vectors (50.96% *An. arabiensis*, 49.14% *An. gambiae s.s*) were recorded over 84 nights of experiments. Of the 200 female mosquitoes released in each trial, an average of 45.5% and 73.7 of *An. arabiensis* and *An. gambiae s.s* were recaptured the next morning respectively (Figure 3.2a & b). The proportion of mosquitoes recaptured did not vary between host species in either *An. arabiensis* ($\chi^2_6 = 9.76$, $P = 0.13$) or *An. gambiae s.s* ($\chi^2_6 = 9.49$, $P = 0.15$). The impact of host species on mosquito fitness measured are detailed below.

3.3.1 Mosquito feeding success

Combining across host types, the probability of a mosquito obtaining a blood meal during one night's host seeking was almost three times greater for *An. gambiae s.s* than *An. arabiensis* (Table 3.1). As expected, use of simple untreated net reduced but did not completely block feeding of mosquitoes on humans (Figure 3.2c & d). Host species was a significant predictor of the feeding probability of both *An. arabiensis* ($\chi^2_6 = 52.80$, $P < 0.001$) and *An. gambiae s.s* ($\chi^2_6 = 23.89$, $P < 0.001$) although the pattern of response varied between mosquito species (Mosquito*Host interaction: $\chi^2_6 = 115.63$, $P < 0.001$, Figure 3.2c & d). In accordance with its observed feeding preference in nature, *An. arabiensis* were substantially more likely to obtain a blood meal from cows than unprotected humans ($Z = 3.90$ $P < 0.001$,Figure 3.2 c). This vector also tended to feed upon calves at higher probability than on humans, although this effect was of marginal statistical significance ($Z = 2.55$, $P = 0.05$). In contrast to their anthrophily in the field, *An. gambiae s.s* had a similar probability of obtaining blood meal from all host types in these experiments ($P > 0.05$ in each case,Figure 3.2 d) except for chickens which mosquitoes very rarely obtain a meal from (<9% , $Z= -4.00$, $P < 0.001$).

Protecting human hosts with an untreated net widened the biological and statistical differences in the feeding success of *An. arabiensis* between human and bovid hosts (test of difference relative to humans: cows, $Z = 6.10$, $P < 0.001$,calves: $Z = 4.74$, $P < 0.001$), but did not significantly change the ranking of humans relative to any alternative animals (differences observed were similar to when human were not using a net). In *Anopheles gambiae s.s* , providing human hosts with untreated nets generated a moderate but not statistically significant reduction in feeding probability in comparison to unprotected controls($Z = 1.49$, $P = 0.47$,Figure 3.2 d). However, the use of untreated net did alter feeding success of *An. gambiae s.s* on humans relative to animal hosts. Whereas the unprotected humans were fed upon with equal probability to all host types except chickens. When nets were used *An. gambiae s.s* were less likely to obtain a blood meal from protected humans than from dogs ($Z = 2.68$, $P = 0.04$).

Of the 4,088 and 1,954 *An. gambiae s.s* and *An. arabiensis* recaptured in the experimental hut, only 161 and 3 were found dead respectively. There was no significant effect of host species on the proportion of mosquitoes found dead on recapture (*An. arabiensis*: $\chi^2_6 = 1.89$, $P = 0.93$, and *An. gambiae s.s*: $\chi^2_6 = 8.21$, $P = 0.22$,Figure 3.2 e & f).

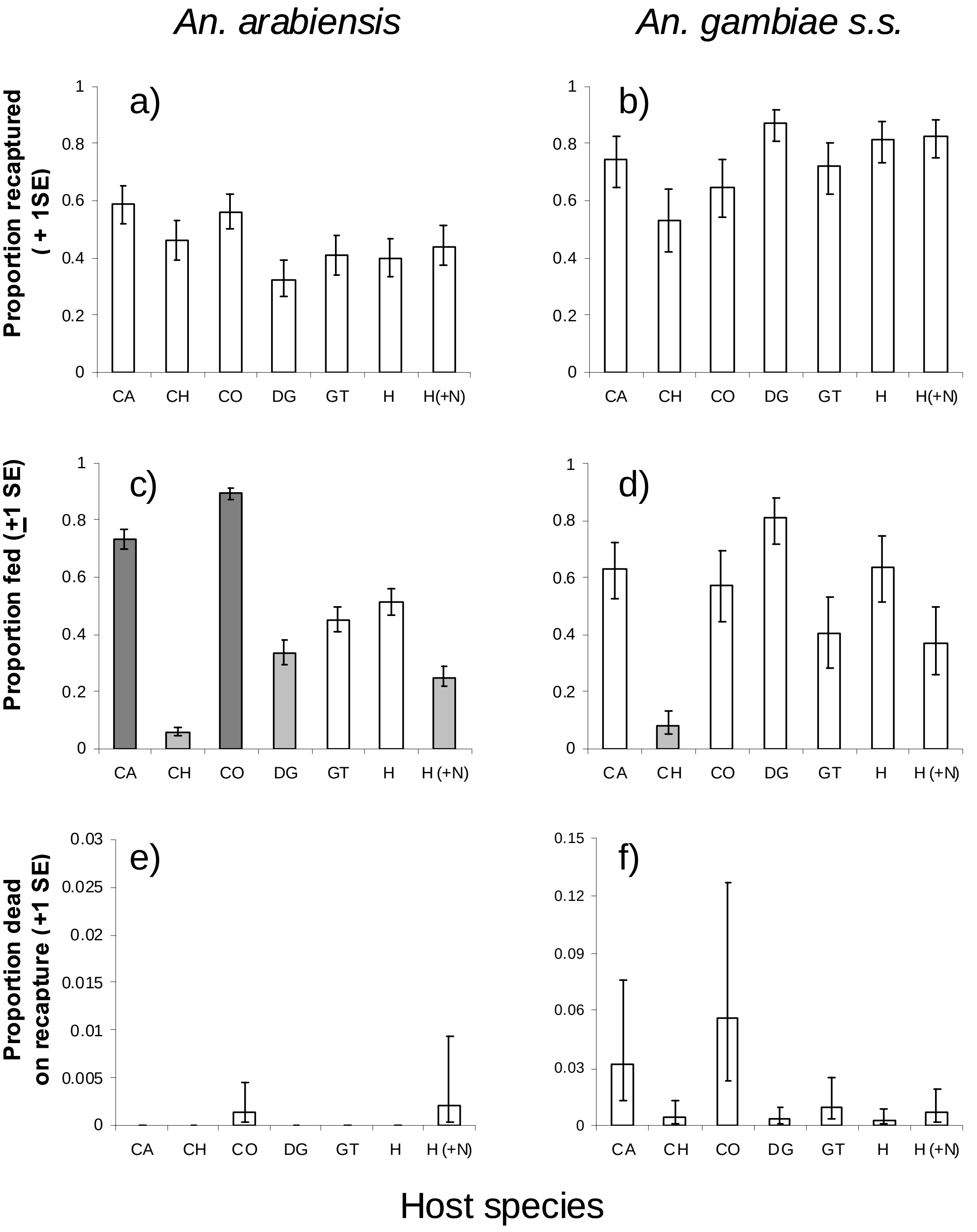

Figure 3.2. Estimated proportions (± 1 s.e.m) of *An. arabiensis* (left column) and *An. gambiae* s.s (right column) that were recaptured in each trial (a, b), obtained blood meals in one night of host seeking (c, d), and that died during the host seeking period (e, f). Host types are: CH = chicken, CA = calf, CO = cow, DG = dog, GT = goat, H = unprotected human, and H+N = human sleeping under an untreated net. Colours indicate the nature of statistical differences between the "human without a net" reference group and all other host treatments (as determined by Dunnett's post hoc test, adjusting for multiple comparisons). Dark grey indicates treatments that had a statistically higher value of the trait than the human (without a net) reference group, light grey indicates treatments that had a statistically lower value of the trait than the reference group, and white refers to treatments that were not significant different from the reference group.

A total of 4,216 haematin samples were collected from mosquitoes that fed in these trials and used to estimate host-species dependent blood intake (*An. arabiensis*: n = 1,755, *An. gambiae s.s*: n = 2,461). On average, the size of blood meal obtained by *An. arabiensis* was more than two times of that taken by *An. gambiae s.s* (Table 3.1). This is not surprising as *An. arabiensis* is a substantially larger mosquito than *An. gambiae s.s* and there is widely documented positive correlation between body size and blood intake (reviewed by Lyimo and Ferguson, 2009). The effect of host species on blood meal size varied significantly between mosquito species (Mosquito*Host interaction: $\chi^2_6 = 113.44$, $P < 0.001$, Figure 3.3a & b). Whereas the size of blood meals obtained by *An. arabiensis* was relatively uniform across host types ($\chi^2_6 = 3.28$, $P = 0.77$, Figure 3.3a), the blood intake of *An. gambiae s.s* did vary between host species ($\chi^2_6 = 22.50$, $P < 0.001$, Figure 3.3 b). *Anopheles gambiae s.s* obtained substantially larger blood meals from unprotected humans than from any other animal species ($P< 0.05$ in all cases) except cows ($Z = -1.76$, $P = 0.31$). *Anopheles gambiae s.s* mosquitoes that were able to feed on humans protected by a net did obtained blood meals of similar size to those who fed on exposed people ($Z = 1.08$, $P = 0.77$). Mosquitoes who were able to feed on humans sleeping under a net obtained larger blood meals than those feeding on goats ($Z = -2.78$, $P = 0.03$) and chickens ($Z = -2.92$, $P = 0.02$).

3.3.2 Mosquito reproductive success

Of 4,216 mosquitoes that blood fed in these experiments, 1,569 went on to lay eggs (*An. arabiensis*: n = 347, An. *gambiae s.s*: n = 1,222). Host species influenced the probability of oviposition by both *An. arabiensis* ($\chi^2_4 = 14.85$, $P < 0.02$, Figure 3.3c) and *An. gambiae s.s* ($\chi^2_6 = 21.03$, $P = 0.0018$, Figure 3.3d). In *Anopheles arabiensis* the probability of producing eggs after feeding on dogs was significantly higher than on calves ($Z = -2.88$, $P = 0.02$) or goats ($Z = -3.10$, $p = 0.01$). Oviposition rates were similar after feeding on dogs, chickens, cows, and humans (exposed or under a net) ($P > 0.05$ in all cases, Figure 3.3c). *Anopheles gambiae s.s* had a higher probability of laying eggs after feeding on goats ($Z = 2.57$, $P = 0.01$), calves ($Z = 3.98$, $P < 0.001$), and dogs ($Z = 4.31$, $P < 0.001$) than on unprotected humans. Oviposition rates on cows, chickens and humans (exposed and protected by a net) were not significantly different ($P > 0.05$ in all cases, Figure 3.3d).

Considering mosquitoes that did lay eggs after feeding, *An. arabiensis* was significantly more fecund than *An. gambiae s.s* ($\chi^2_1 = 156.563$, $P < 0.001$, Table 3.1). This fecundity advantage is likely a consequence of the larger body size and associated blood intake of *An. arabiensis* (Table 3.1). Restricting analysis to mosquitoes that laid at least one egg, host species did not have statistically significant impact on the number of eggs produced by either *An. arabiensis* ($\chi^2_6 = 1.46$, $P > 0.05$, Figure 3.3e) or *An. gambiae s.s* ($\chi^2_6 = 5.73$, $P > 0.05$, Figure 3.3f).

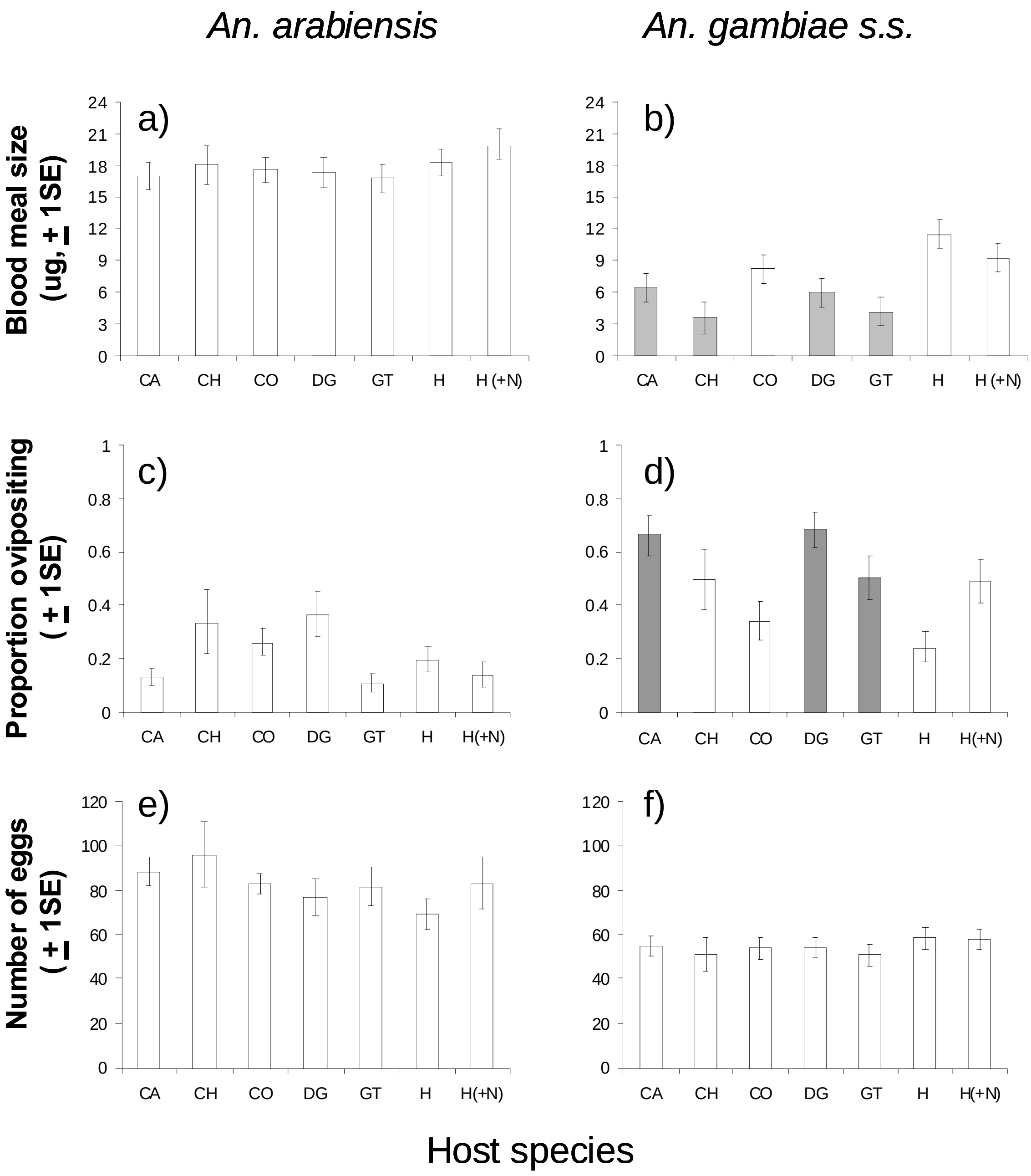

Figure 3.3. Estimates (± 1 s.e.m) of the mean blood meal size (μg of hematin, a, b), oviposition rate after one blood feed (c, d), and number of eggs laid (e, f) by *An. arabiensis* (left column) and *An. gambiae s.s* (right column) after feeding on different host types. Host types are: CH = chicken, CA = calf, CO = cow, DG = dog, GT = goat, H = unprotected human, and H+N = human sleeping under untreated net. Colours indicate the statistical differences between the "human without a net" reference group and all other host types (as determined by Dunnett's post hoc test, adjusting for multiple comparisons). Dark grey indicates treatments that had a statistically higher value than the "human without a net" reference group, light grey indicates treatments that had a statistically lower value of the trait than the reference group, and white refers to treatments that were not significant different from the reference group.

3.3.3 Mosquito survival

Pooling across host types, the survival *An. arabiensis* was substantially higher than *An. gambiae s.s* (Table 3.1), with the odds of mortality of *An. arabiensis* being only 1/3 that of *An. gambiae s.s* (Odds ratio: 0.33, 95%CI: 0.31 – 0.36, $\chi^2_1 = 621$, $P < 0.001$). The impact of host species on post-feeding survival varied between vector species (Host*Mosquito species interaction): $\chi^2_6 = 48$, $P < 0.001$, Figure 3.4 a & b). In *Anopheles arabiensis,* post-feeding survival did not vary between host species ($\chi^2_6 = 8.6$, $P = 0.2$, Figure 3.4 a). However, *An. arabiensis* fed on cows ($\chi^2_1 = 4.29$, $P = 0.04$) and goats ($\chi^2_1 = 4.75$, $P = 0.03$) had moderately lower survival than on unprotected humans (Figure 3.4 a). The survival of *An. arabiensis* who fed on humans sleeping under a net was not different from any other host type ($P > 0.05$ in all 2-ways comparisons,Table 3.3). In contrast with *An. arabiensis*, the survival rates of *An. gambiae s.s* varied significantly with host species ($\chi^2_6 = 106.4$, $P < 0.001$, Figure 3.4 b). The survival rates of *An. gambiae s.s* after feeding on unprotected humans was significantly higher than for any other host types except cows ($\chi^2_1 = 1.13$, $P = 0.29$, Table 3.2). The survival advantage from human blood was particularly apparent from day 12 onwards, where the proportion of mosquitoes alive in the unprotected human group was consistently higher than all other host species treatment. Providing humans with an untreated net was associated with significant reduction of post-feeding survival of *An. gambiae s.s* relative to those who fed on unprotected human controls (Table 3.3, Figure 3.4b). *Anopheles gambiae s.s* who succeeded in feeding on humans using net had poorer survival than those who fed on calves, cows dogs and goats (Table 3.3).

Mosquito fitness trait	***An. arabiensis***	***An. gambiae s.s***
*Mean body size (mm)	3.40 ± 0.02	2.81 ± 0.04
Proportion feeding	0.28 (0.23– 0.33)	0.71 (0.69 – 0.72)
*Mean bloodmeal size (μg)	17.11 ± 0.51	7.33 ± 0.22
*Mean fecundity	80.10 ± 2.03	54.97 ± 1.51
Median survival(days)	13 (12-14)	7 (7-8)

Table 3.1. Fitness traits of *An. arabiensis* and *An. gambiae s.s* combined over all host species and individuals. Numbers in bracket are 95% confidence intervals and values of parameters with * are mean ± standard error (s.e.m).

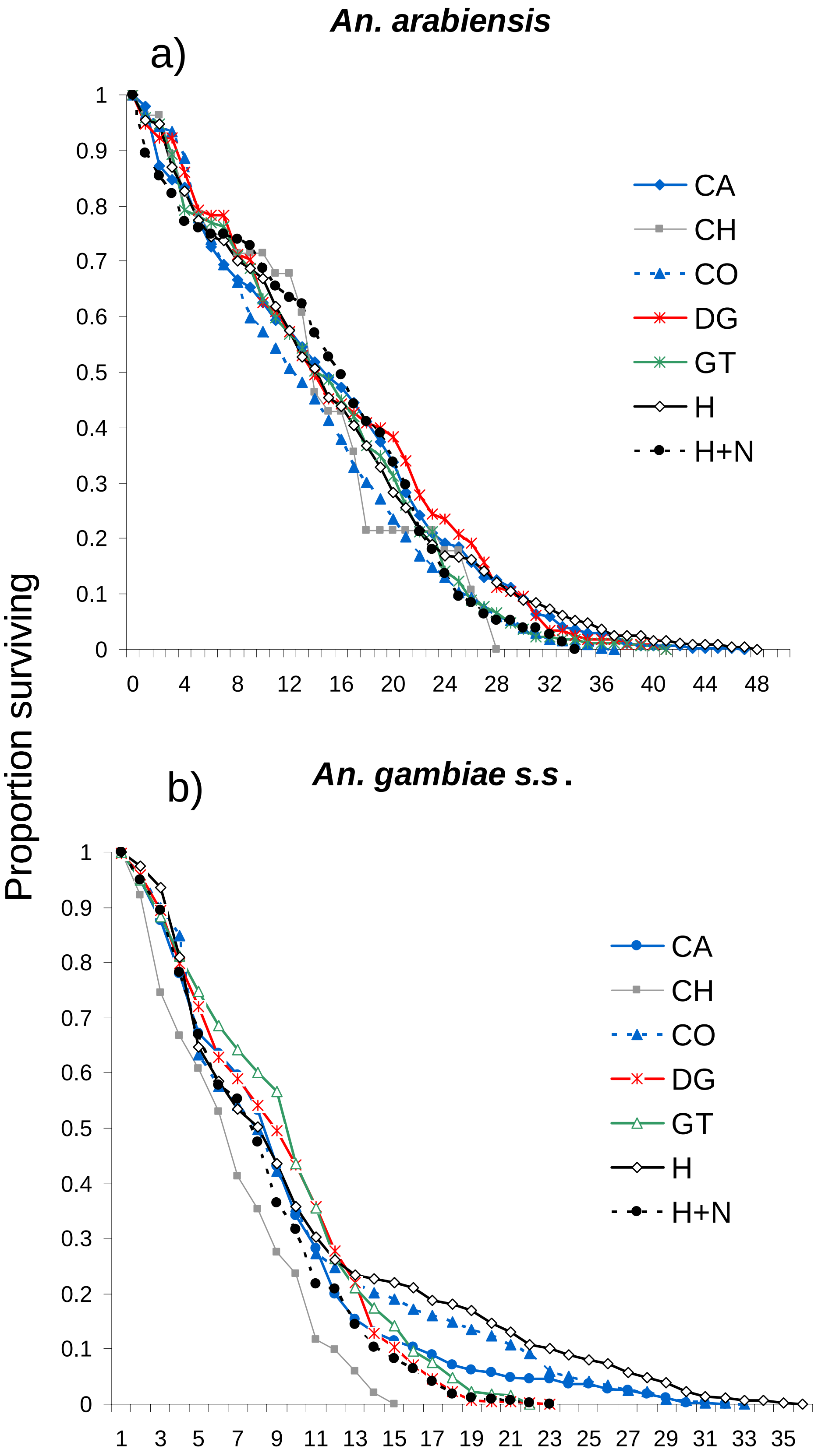

Figure 3.4. Survival of (a) *An. arabiensis* and (b) *An. gambiae s.s* after taking one blood meal from different host species. Lines represent the survival function as estimated from the fitting Cox proportion hazard model (controlling for random variation between individuals from the same host species).

	Odds Ratio of mortality (relative to human without a net)	
Host species	*An. arabiensis*	*An. gambiae s.s*
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 holed net	1.08 (0.85 – 1.38)	1.83 (1.56 – 2.14)

Table 3.2. Relative odds of mortality of *An. arabiensis* and *An. gambiae s.s* after feeding on different host species, relative to the human without a net (reference group). Odds ratio were obtained after fitting Cox regression model while controlling for the variation between individuals within host species. Numbers in bracket are 95% confidence intervals. Groups with odds ratio that are higher than one and whose 95% confidence interval that does not include one exhibit significantly higher mortality than the human without a net reference group.

	Odds Ratio of mortality (relative to human with a net)	
Host species	*An. arabiensis*	*An. gambiae s.s*
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)

Table 3.3. Relative odds of mortality of *An. arabiensis* and *An. gambiae s.s* after feeding on different host species, relative to human with a net (reference group). Odds ratio were obtained after fitting Cox regression model while controlling for the variation between individuals within host species with human under untreated holed net as the reference group. Numbers in bracket are 95% confidence intervals. Groups with odds ratio that are higher than one and whose 95% confidence interval that does not include one exhibit significantly higher mortality than the human without a net reference group.

3.3.4 Mosquito lifetime reproductive success.

When modeled as the function of both feeding probability and post-feeding fitness, the lifetime egg production of both *An. arabiensis* and *An. gambiae s.s* was predicted to vary between host types (Figure 3.5a & b). *Anopheles arabiensis* was predicted to produce a greater number of eggs during their life on cows than any other host type (Figure 3.5a, Table 3.4). For this vector, the lifetime egg production on cows was similar to calf, dog and unprotected human host, but significantly higher than on chickens, goats and human sleeping under a net. In *Anopheles gambiae s.s*, chickens were associated with a significantly lower lifetime egg production than cows, calves, dogs and unprotected humans (Figure 3.5b, Table 3.4). The expected lifetime egg production of *An. gambiae s.s* on all mammalian hosts was similar. When the effect of host-specific feeding probability was removed, neither the lifetime egg production of *An. arabiensis* nor *An. gambiae s.s* was predicted to vary significantly between any host species.

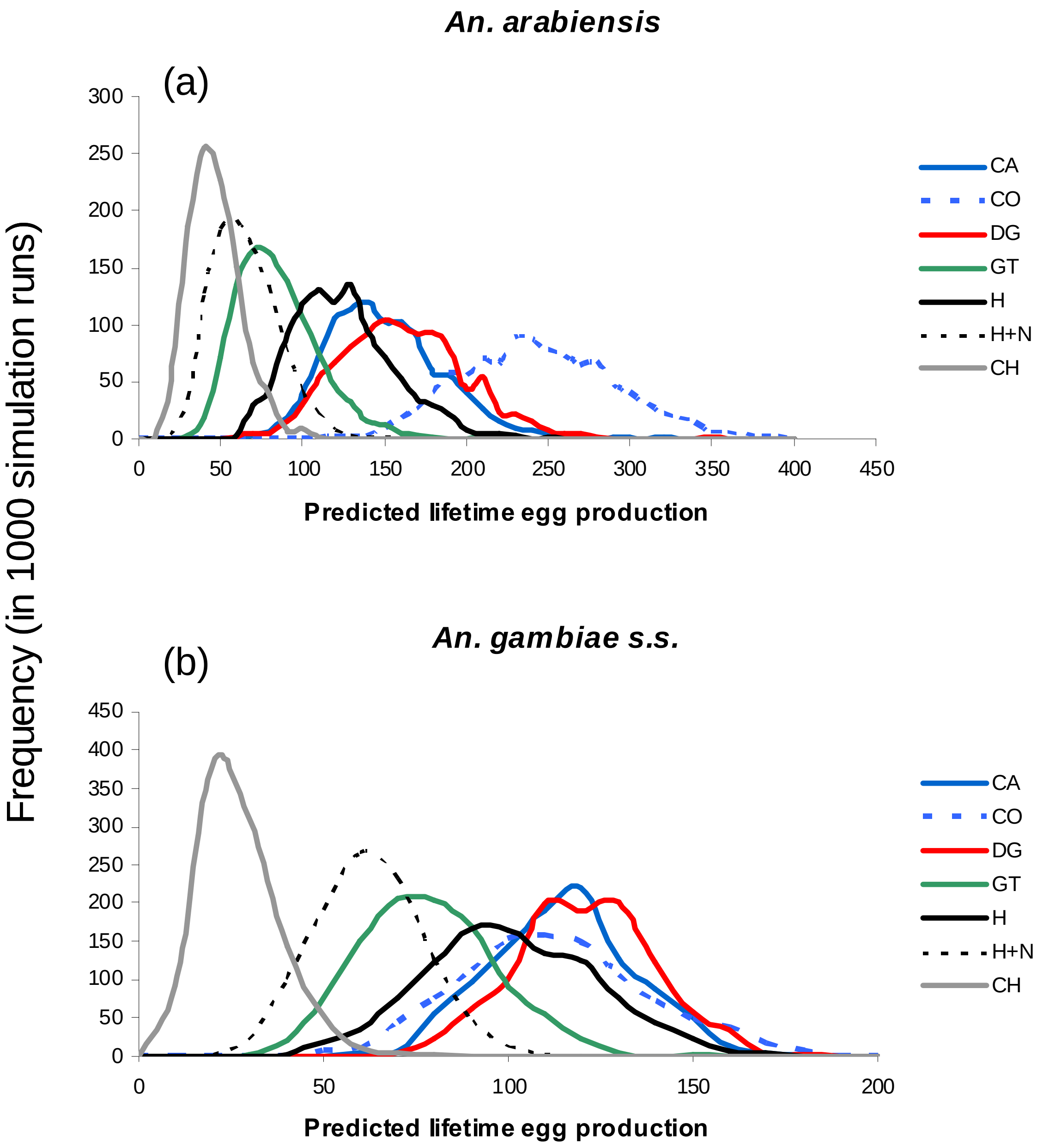

Figure 3.5. Predicted distributions of the lifetime egg production of (a) *An. arabiensis* and (b) *An. gambiae s.s* when feeding on hosts of different species (based on 1000 simulations). Predictions were obtained from a life-history model based on host – specific estimates of mosquito feeding success and fitness as measured in experiments. Host types are: CH = chicken, CA = calf, CO = cow, DG = dog, GT = goat, H = unprotected human, and H+N = human sleeping under an untreated net.

Anopheles arabiensis

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							--

Anopheles gambiae s.s.

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							--

Table 3.4. Proportion of times (in 10,000 runs) that a randomly selected value of the predicted lifetime egg production of mosquitoes on one host type (left hand column) was higher than on another (right hand row). To test for statistically significant differences between groups, the standard cut-off value for significance ($\alpha = 0.05$) was adjusted for 21 possible multiple comparisons. Consequently, mosquito lifetime egg production was considered to be significantly higher on one host type (left hand column) than another (right hand row) if the probability was greater than 0.9976, and significantly lower on one host type (left hand column) than another (right hand row) if the probability was less than 0.0024.

3.4 Discussion

Despite the critical importance of insect vector host choice to disease epidemiology and control, our understanding of the selective pressures that shape this phenotype is very limited. Here we present results from the first experimental investigation of the fitness consequences of host species choice for one of the world's important insect vectors, the *Anopheles* mosquitoes that transmit malaria in Africa. Uniquely these results have been obtained under a realistic semi-field setting, where hosts and vectors are able to interact naturally. This innovative approach has allowed us to test fundamental theoretical predictions about the relationship between host specialization and fitness in this system, and evaluate the nature of selection generated by common malaria-protective measures (e.g. untreated bednets) for malaria vectors to reduce their feeding on humans. Such information is a vital first step for prediction of the conditions under which malaria control efforts could be enhanced by environmental management that not only reduces vector abundance, but generates selection for them to reduce their contact with humans.

At least one of the 6 fitness traits we measured in each malaria vector species was significantly influenced by host species. However, the types of traits that were influenced varied notably between vectors. In *Anopheles arabiensis*, host species was a primary determinant of their probability of getting a blood meal, but not of their subsequent fitness. In contrast, under the 'no choice' experimental assays conducted here, the typically anthrophilic *An. gambiae s.s* was equally likely obtain a blood meal from all mammalian hosts, and used the blood of different species for reproduction and survival with varying efficiency. Notably, although both vector species performed poorest on chickens, there was no one host species that was universally optimal to either vector. This suggests there may be trade-offs in the value of host resources for different life-history processes (e.g. survival versus fecundity), and that study of single fitness traits may yield unreliable predictions of the net impact of host choice on mosquito fitness.

These results challenge some previous hypotheses about the nature of selection governing the host species choice of disease vectors. Specifically, defensive behaviour has been widely postulated to explain the innate preference of mosquitoes for particular host species (Edman and Kale II, 1971, Edman et al., 1974, Day and Edman, 1983, Day and Edman, 1984, Darbro and Harrington, 2007). The nocturnal, anthrophilic feeding behaviour of *An. gambiae s.s* mosquitoes is frequently postulated to be an adaptation to avoid host defensive behaviour (Bockarie et al., 1996, Killeen et al., 2006, Day and Edman, 1984). While a negative correlation between host defensive behaviour and mosquito feeding preference has been found in some systems (Day and Edman, 1984, Day and Edman, 1983, Edman and Kale II, 1971, Edman et al., 1974, Darbro and Harrington, 2007), here we found no evidence that the preferred host of either vector (e.g. cows for *An. arabiensis,* humans for *An. gambiae s.s*) was associated with lower mosquito mortality during host seeking. Thus, host defensive behaviour may not play the pivotal role in malaria vector host species choice that has been previously assumed. Alternative explanations for host species-specific variation in mosquito fitness include haematological, and physiological properties. Properties of host blood such as blood amino acid concentration (Chang and Judson, 1979, Harrington et al., 2001), red cell density and haemoglobin concentration (Taylor and Hurd, 2001, Shieh and Rossignol, 1992), have been implicated as potential determinants of mosquito fitness. Follow-up investigation of host individual and species-level variation in these traits and their link to mosquito vector fitness is described in Chapter 5. However, we note that host preference within the *Anophelines* is extremely diverse (Lyimo and Ferguson, 2009), and encompasses a range of species with significant haematological and physiological variation. Consequently, I hypothesize that the relative importance of such host properties varies between mosquito species, and is unlikely to provide a universal explanation for variation in host species choice.

Evolutionary theory predicts that the fitness of specialists is highest when preferred resources are consumed (Levins, 1962, Pyke et al., 1977, MacArthur and Pianka, 1966), such that preference and performance should be positively correlated. My life history model indicates this prediction is met in *An. arabiensis,* whose lifetime egg production was estimated to be substantially higher on their naturally preferred cow hosts. However, no association between the host species preference and lifetime reproductive success of *An. gambiae* s.s. was predicted. There are several possible reasons why this prediction was not met in this vector. First, failure to detect a relationship between host preference and fitness is not uncommon in other insect-host systems (Agrawal et al., 2002). The phenomenon has been studied primarily in phytophagous insects where there is general support for a positive relationship between preference and performance, but also many exceptions (Gripenberg et al., 2010). Failure to detect a performance-preference relationship in some of these cases has been attributed to ecological variation that modifies the quality of hosts in different environments (Gripenberg et al., 2010). Such environmental effect could also have influenced our ability to detect host-associated fitness differences here. In these experiments, hosts deliberately presented to mosquitoes in the same microhabitat (inside an experimental hut) to disentangle the effects of host species from differential habitat use. However, in the field humans are usually found inside whereas animals are sometimes kept inside (although in this region, calves and goats are kept overnight inside sheds). If there is an additive effect of host microhabitat on vector fitness, the benefits of anthrophily for *An. gambiae* s.s. may only be evident when compared with animals situated outdoors. This interesting possibility suggests that it is not necessarily the physiology or behaviours of human hosts themselves, but rather suitability of their domestic environments that needs to be altered to minimize selection for human preference. Further experimental investigation is planned to test this possibility.

Few studies have investigated preference-performance relationships in haematophagous insects, and these have been done almost exclusively under laboratory conditions where hosts are anaesthetized or restrained during mosquito feeding. While some studies have found insect fitness to be highest on preferred host species, others have found no association (Lyimo and Ferguson, 2009). Interpretation of these studies is clouded by fact that frequently comparisons were made not between preferred hosts and alternatives that are found in the same environment, but with novel host species encountered only in the laboratory (Harrington et al., 2001, Shroyer and Siverly, 1972, Downe and Archer, 1975, Kweka et al., 2010). Such comparisons are unlikely to accurately represent the host fitness landscape faced by vectors in nature, and may overestimate the relative advantage of ‘preferred’ (and natural) hosts.

The impacts of host species choice on vector fitness described here may also influence their ability to transmit parasites. Human blood was associated with enhanced long –term survival in *An. gambiae s.s*; an effect which could significantly benefit malaria parasites. *Plasmodium falciparum*, the most common human malaria parasite in Africa, requires a minimum of 10 days development within vectors before it can infect new hosts (Beier, 1998). In the wild, most African vectors die this incubation period is complete (Chege and Beier, 1990), thus hosts that enhance vector longevity will also enhance their vectorial capacity.

Our results indicate that even when they do not completely prevent mosquito biting, untreated nets have the potential to reduce the fitness that malaria vectors derive from humans, and alter the value this host relative to animal alternatives. The lifetime reproductive success of *An. arabiensis* was predicted to be higher on cows than on any other host, but the relative advantage over humans only became statistically significant when they were using untreated nets. Although the use of untreated nets was not predicted to significantly reduce the lifetime reproductive success of *An. gambiae* s.s. on humans, this intervention did significantly reduce their longevity and thus vectorial capacity. Thus on the basis of this small-scale empirical investigation of vector-host interactions, the widespread use of this simple intervention would be predicted to reduce the transmission potential of *An. gambiae* s.s., and also generate selection for *An. arabiensis* to become more zoophilic. Large-scale epidemiological studies provide some, but not universal, support for these predictions.

When in good condition, untreated nets can significantly reduce malaria infection risk (Mwangi et al., 2003, D'Alessandro et al., 1995, Clarke et al., 2001), and in some locations their widespread use has been shown to reduce transmission (Hii et al., 2001, Burkot et al., 1990). The widespread use of untreated (Lefevre et al., 2009), insecticide treated nets (ITNs) (Lindblade et al., 2009, Quinones et al., 2000, Bogh et al., 1998, Kaburi et al., 2009), and indoor residual spraying (IRS) (Gillies and Furlong, 1964) in and around houses have been associated with a reduction in anthrophily, although whether these changes were due to phenotypic behavioural shifts or evolutionary changes in host preference is unknown. Other studies have found no change in vector feeding behaviour in response to interventions (Quinones et al., 1997, Smith, 1966, Gillies and Smith, 1960). In contrast to these experiments, in nature vectors that are prevented from biting by an untreated net, can move onto a nearby unprotected host in the same evening (Genton et al., 1994, Burkot et al., 1990). Thus in nature, the ability of untreated or treated nets to select for a change in vector behaviour likely depends on the proportion of the population that is covered and local availability of alternative animal hosts. Another crucial requirement for evolutionary change is that host choice behaviour has a genetic basis. Although the relative contribution of genetics to mosquito host preference remains largely unexplored, studies of African malaria vectors and other haematophagous insects suggest this trait is at least partially genetically controlled (Coluzzi et al., 1979, Rebollar-Tellez et al., 2006, Mukwaya, 1977, Gillies, 1964, Lefèvre et al., 2009). In several malaria endemic regions of Africa including the Kilombero Valley, the majority of people are now sleeping under an untreated net (Killeen et al., 2007b), a coverage of 91% within Kilombero Valley (Russell et al., 2010). As coverage levels of these nets and their more effective insecticide-treated counterpart grows, so too will the selection pressure they impose. Here I highlight the need to also recognize the opportunities presented by increasing coverage of insecticide treated nets to select for zoophily as well as insecticide resistance (e.g. behavioural and metabolic resistance). In particular, the presence of alternative host species of high fitness value to vectors (e.g. cows for *An. arabiensis*) in areas where these interventions are widely utilized may promote selection for reduced anthrophily, and delay the emergence of insecticide resistance (Kawaguchi et al., 2004). Where possible, opportunities to reduce human biting either on a short-term (e.g. by zooprophylaxis) or through long-term selection on anthrophily should be exploited as a means to improve the effectiveness and sustainability of current frontline interventions.

Recent increases in the coverage of ITNs in Tanzania and Kenya have been correlated with a shift in malaria vector species composition from the highly anthrophilic *An. gambiae s.s*. to more zoophilic *An. arabiensis* (Bayoh et al., 2010, Russell et al., 2010). The disproportionate impact on *An. gambiae* s.s. has been attributed to their enhanced predisposition to feed on humans indoors, where the likelihood of contacting an ITN is highest (Bayoh et al., 2010). Our results suggest that even when nets are untreated, *An. gambiae* s.s. may pay an additional survival cost from feeding in their presence that *An. arabiensis* avoids. The reduced survival of *An. gambiae* s.s. relative to *An. arabiensis* in the presence of net barriers would compound their vulnerability to domestic net-based strategies.

While we conclude that results from small-scale semi-field studies can contribute to understanding of larger-scale epidemiological and evolutionary processes, some important aspects of vector-host interactions could not be captured in these experiments. First, in order to accurately measure the fitness consequence of interaction with specific host species, bioassays were conducted under ‘no choice’ conditions where only one host was available to mosquitoes at a time. However in nature, mosquitoes forage in environments where multiple host species may be simultaneously available. Consequently, the host-specific feeding probabilities estimated here do not reflect host selection patterns in nature, but the capacity of vectors to exploit these different species when necessary. Second, the requirement for large numbers of similarly aged and guaranteed malaria-free mosquitoes in these experiments necessitated the use of insectary-reared rather than wild mosquitoes. Both vectors species used here came from colonies established at the IHI, where they are maintained on human blood through arm-feeding, under ambient temperature and light conditions. While the *An. arabiensis* colony was only a few generations removed from the wild (< 7 generations), the *An. gambiae* s.s. colony has been running for 14 years (recent declines in this species throughout the region prevented fresh collection). The colonization process can increase the ability of vectors to exploit novel hosts (e.g. if they are the only source of blood made available, Benedict et al., 2006), and may reduce their tendency to discriminate between host species (Lefèvre et al., 2009). However extensive field studies have shown that these vectors feed primarily on humans in this area (Mayagaya et al in preparation), and thus they were maintained on their natural host in colony. Finally, our predictions of mosquito lifetime reproductive success were based on consideration of a few key life-history traits: their ability to acquire blood, and subsequent reproduction and survival. While these traits are undoubtedly strongly associated with mosquito lifetime reproductive success, additional traits not measured here may also influence the overall fitness (e.g. ability to avoid predation, hatching and development success of eggs). If such traits vary between host species and have a large impact of mosquito fitness, our current model predictions will be inaccurate. The most important and yet untested assumptions of this model are that the fitness consequences of host choice as estimated from the first host encounter (as experimentally estimated here) apply to all subsequent blood meals, and are independent of mosquito age or previous feeding history. Further experimental investigation of additional life-history traits and these model assumptions are planned to further refine this model.

In conclusion, this study highlights the advantages of complementing field-based study of vector-borne disease epidemiology and control with experimental investigation of vector ecology and evolution. The last decade has been a proliferation of infectious disease ecology and evolution research, however to date this approach has received little application to public health (Stearns and Koella, 2008, Restif, 2009, Nesse and Stearns, 2008). Moving the powerful insights obtained from this basic research discipline into real world disease settings where they can guide and improve control will require a closer integration of theory, experimentation with natural systems, and field observation. Novel approaches such as the use of semi-field systems can make a valuable contribution by permit a much wider range of experimental manipulation than would otherwise be possible under relatively natural conditions (Ferguson et al., 2008). Here we show that such approaches can be adopted even within remote, disease endemic field settings, and used to experimentally evaluate a range of hypotheses regarding the stability of vector-host system. Where possible we advocate a broader application of such approaches to help identify control strategies that can provide both short-term epidemiological benefits, and guide the management of longer-term evolutionary processes that mediate transmission.

4 The influence of host defensive behaviours on the fitness of African malaria vectors: implications for the evolution of host choice

4.1 Introduction

Many organisms including plants and animals actively defend themselves from ectoparasites by chemical responses and / or behavioural avoidance (Bryant et al., 1991, Hart, 1992, Hart, 1990, Jaenike, 1990). Variation in the strength of defensive behaviours between and within host species has been hypothesized to be a major source of selection acting on host specificity (Fox, 1981, Bryant et al., 1991), which in the case of parasites is a critical predictor of their transmission and stability (Poulin, 1998, Lehane, 2005). Perhaps on account of their massive economic impact, the role of plant defensive behaviours (e.g. chemical compounds) in driving the host specificity of phytophagous insect pests has been extensively investigated (Jaenike, 1990, Stout et al., 2006). Detailed cross-taxa investigations in these systems generally indicates that plant host defensiveness is one of the most important selection pressures shaping the host species preference of phytophagous insects (Despres et al., 2007). In contrast, relatively little is known about the importance of defensive behaviours mounted by vertebrate hosts in generating selection for host specificity in the insect vectors of disease (Edman and Scott, 1987), especially with respect to those capable of transmitting pathogens to humans. Given the tremendous impact of insect vector borne disease on public health, there is a need to identify both the ecological and evolutionary forces that drive host specificity, and particularly those that generate selection for specialization on humans.

Of all the insect vectors of human disease, mosquitoes are probably responsible for the greatest loss of life and morbidity through their central role in the transmission of malaria, dengue and other arboviruses (Kiszewski et al., 2004, Weaver and Reisen, 2010). The frequency with which mosquito vectors feed on humans and other pathogen-susceptible host species, and their long-term survival are considered to be key determinants of disease transmission (Killeen et al., 2000). Both of these phenomena may be influenced by host defensive behaviours. First of all, as host defensive behaviours can prevent mosquito biting (Edman and Kale II, 1971, Waage and Nondo, 1982), and/or interrupt blood feeding (Hodgson et al., 2001), it may act to reduce parasite transmission by reducing host – vector contact rates and increasing the fitness costs of host-seeking to mosquitoes (Anderson and Roitberg, 1999, Darbro and Harrington, 2007). Alternatively, if host defensiveness generally acts not to kill mosquitoes but divert them to other hosts, it may increase pathogen transmission by increasing the number of different hosts that vector contact during their lifetime (Davies, 1990, Hodgson et al., 2001). Consequently, host defensive behaviours could impose substantial limitations on the fitness of both important mosquito vector species and the parasites they transmit, and correspondingly generate selection for specificity on poorly defensive host species or individuals.

Measuring the impact of host defensiveness on mosquito blood acquisition ability is vital not only to estimate its potential to shape host – vector species associations, but also to assess the evolutionary viability of disease control strategies based on manipulating the defensiveness of target host types (e.g. humans, by selectively protecting them with insecticide treated nets or house screening, Lindsay et al., 2002, Killeen et al., 2006). If host defensive behaviour has a minimal impact of vector fitness and does not vary significantly between potential hosts, the targeted protection of one host type by an intervention could be expected to generate strong directional selection for a host shift onto species that are relatively much easier to obtain blood from. If, however, host defensiveness is a substantial source of mosquito mortality and is negatively associated with their host species preference (e.g. preferred hosts are the least defensive, Day and Edman, 1984, Hodgson et al., 2001, Darbro and Harrington, 2007), then it may be difficult to generate selection for vectors to shift onto alternative host species whose defensive behaviours may exert a similar or higher costs to mosquito fitness as do the protective measures used by humans.

Animals defend themselves against mosquitoes and other ectoparasites by variety of means including protective tissues (Lehane, 2005), immune responses (Khokhlova et al., 2008, Billingsley et al., 2006), physical movements and avoidance of their habitats (Hart, 1992, Hart, 1990). Additional artificial defensive measures developed by humans including repellents(Katz et al., 2008), screening houses (Kirby et al., 2009), indoor residual spraying (IRS) (Musawenkosi et al., 2004) and insecticide treated nets (ITNs) (Killeen et al., 2006), are also highly effective means of reducing the blood feeding success and survival of mosquitoes. Studies of mosquitoes and other haematophagous insects have shown that their feeding success on animal hosts can be significantly reduced by defensive behaviours (Hodgson et al., 2001, Schofield and Torr, 2002, Darbro and Harrington, 2007). The effectiveness of defensive behaviours has been shown to vary between host species (Day and Edman, 1984), individuals (Anderson and Brust, 1997) , and in response to additional factors such as host parasite infectious status (Darbro et al., 2007), and vector density (Kelly and Thompson, 2000). Studies of other ectoparasites indicate that the consequences of host defensiveness may be non-linearly related to vector fitness; with the feeding and reproductive success of fleas being reduced by strong host behavioural and immune defenses (Hawlena et al., 2007, Khokhlova et al., 2008, Bize et al., 2008), but moderate defensiveness enhancing their blood intake and survival (Bize et al., 2008). Consequently, the net impact of host defensive behaviours on the fitness and consequent population dynamics of vectors may be context-specific, and therefore must be assessed within the context of the diversity of host defensive behaviours they are most likely to encounter within natural settings. However, most investigations of the impacts of host defensiveness on mosquito fitness have been drawn from observations made under relatively artificial laboratory conditions (e.g. Edman and Kale II, 1971, Waage and Nondo, 1982, Kweka et al., 2010, Darbro and Harrington, 2007), and/or using host species that are unlikely to be encountered by them under natural conditions (Kweka et al., 2010). Consequently, very little is known about the comprehensive fitness impacts of the defensive behaviour of natural hosts, including humans, under realistic conditions of vector – host contact that are typical of disease transmission settings.

Most of previous studies of host defensive behaviours have been based on study of *Aedes* and *Culex* mosquitoes under controlled experimental conditions where their behaviours when exposed to (frequently atypical) hosts within small holding cages has been observed (e.g. Day and Edman, 1984, Edman and Kale II, 1971, Waage and Nondo, 1982, Kweka et al., 2010, Darbro and Harrington, 2007). Comparable studies are lacking for the mosquito vectors that pose the biggest risk to human health, and consequently knowledge of the importance of host defensive behaviours in driving selection for human preference, and hence disease exposure, is absent. Here, an experimental semi-field-system (SFS) situated within an area of endemic malaria transmission in Tanzania was used to quantify and compare the impact of defensive behaviour by humans and other equally available animal hosts on the feeding efficiency and fitness of the two most important African malaria vectors *Anopheles arabiensis* and *An. gambiae s.s*. Use of the SFS allowed investigation of the mosquito – host interactions under relatively natural conditions but without risking the exposure of human volunteers to malaria infection (as mosquitoes used in assays could be guaranteed to be uninfected). To assess the efficiency of defensive behaviours in humans and other naturally occurring host species, paired trials were conducted in which the feeding success and subsequent fitness (reproduction and survival) of mosquito vectors was assessed when exposed to hosts under natural foraging conditions (during one night of host seeking, when hosts were free to exhibit behavioural responses against mosquito bites), and when hosts were physically restrained to prevent them from exhibiting behavioural defenses. The following specific hypotheses were tested: (1) mosquito feeding success and subsequent fitness is significantly enhanced when natural host defensive behaviours are restricted, and (2) variation in the relative efficiency of host defensive behaviour between host species (as estimated by the difference in mosquito feeding success on hosts when free and restrained) is correlated with mosquito host preference in nature. A final aim was to evaluate whether the fitness costs imposed by host defensive behaviour could impede selection upon these malaria vectors to switch their host choice from humans onto other commonly available animal alternatives.

4.2 Methods

4.2.1 Study site and mosquito colonies

Experiments were conducted on the two African malaria vectors *An. arabiensis* and *An. gambiae s.s*. Mosquitoes used in these trials were obtained from insectary colonies maintained at the Ifakara Health Institute, as described in Chapter 2 and 3. Both of these mosquito colonies are routinely maintained on human blood provided by direct arm-feeding (3 times each week). Mosquitoes used in these experiments were 4-6 day old females who had not been previously blood fed.

4.2.2 Mosquito feeding assays

As described in Chapter 3, a series of trials were conducted in which the feeding success and subsequent fitness (probability of obtaining a blood meal, blood meal size) and subsequent fitness (fecundity and survival) of cohorts of *An. arabiensis* and *An. gambiae s.s* were estimated after they were released inside a chamber (9.1 × 9.6 × 3.7 m) of a netting – enclosed semi-field system, in which an experimental hut (3.5 × 4 × 2.5 m) containing one of six different host types was present (humans, cows, calves, dogs, goats, and chickens). Released mosquitoes were allowed to spend one night (7 pm – 7 am, coincident with their natural host seeking activity, (Killeen et al., 2006) attempting to forage on the host within the experimental hut, who was free to mount any natural anti-mosquito defensive behaviours (e.g. swatting and tail flapping). The following morning, all released mosquitoes (living and dead) were recaptured, their feeding success recorded. The subsequent longevity and reproduction of surviving mosquitoes within a semi-field insectary was measured (Chapter 3). Six replicates using different individuals were conducted for host species for both vector species (6 individuals/host species × 6 host species × 2 vectors = 72 trial nights).

An additional round of trials was conducted using the same host individuals that participated in the natural semi-field assays (when hosts were unrestrained as described above), but this time hosts were restrained from moving during mosquito feeding. During these trials (conducted during the day), a transparent cup containing 10 unfed female *An. arabiensis* or *An. gambiae s.s* females (4-6 day old, previously unfed) were directly applied to the skin surface of a restrained host for a period of 15 minutes. During these experiments, human hosts (volunteers) were asked to apply a cup directly to the skin of their forearm and refrain from moving until the trial was complete. Animal hosts were physically restrained from movements by a variety of methods. Dogs were muzzled while their owners held their head, one technician applied gentle but restraining pressure on their body, and another applied the mosquito cup to the dog's flank, thigh or neck. Calves, cows and goats were restrained from moving by placing them within metal restraining stall, while cups containing mosquitoes were applied to their flank, neck, thigh or ears. Chickens were held in a lateral recumbent position on a table by one technician, with mosquito cups being applied to their body. Experiments were replicated by using 30 different mosquitoes per host individual to yield a total sample size of 2160 mosquito feeding rates measured (10 mosquitoes/cup × 3 cups/host individual × 6 host individual/host species × 6 host species/vector × 2 vectors). Statistical analysis (as described below) was conducted to test how preventing hosts from making physical defensive movements influenced the feeding success and subsequent fitness of both malaria vector species.

4.2.3 Fitness measurements

Four measurements of feeding success and fitness were measured in the mosquitoes that succeeded in feeding in the trials described above. Blood meal size was estimated indirectly on the basis of the amount of haematin excreted within 3 days after feeding (for details, (for details see Chapter 3 and Briegel, 1980). Oviposition rate and fecundity were measured as the proportion of mosquitoes that laid eggs and their number of eggs respectively. The survival of these mosquitoes was measured as the duration (total number of days) after blood feeding until the day of their death.

4.2.4 Ethical considerations

Ethical procedures were followed as in Chapter 3.

4.2.5 Statistical analyses

Statistical analysis was conducted to assess the impact of preventing host defensive behaviour (through physical restraint) on five key mosquito fitness parameters: their probability of obtaining a blood meal, blood meal size, oviposition rate (probability of laying eggs), fecundity (number of eggs laid) and long-term survival. Two of these parameters were binomial variables (e.g. measured as proportions): probability of feeding (feeding success) and the probability of producing eggs (oviposition rate), and the other three were continuous variables: blood meal size (mass of haematin excreted), fecundity and the number of days mosquitoes survived after feeding. The proportion data (e.g. feeding success and oviposition rate) were analysed using generalized linear mixed effect models (glmer) using the R statistical software (Crawley, 2007). Previous analysis of mosquito fitness under these experimental conditions showed that the impacts of host species were highly variable between vector species (see Chapter 3). Consequently, an apriori decision was made to analyse each mosquito species separately in order to simplify analysis and interpretation of how each vector species was affected by experimental conditions (restraint status). During analysis for each vector species, ‘host species’ and ‘restraint status’ (unrestrained or restrained) were tested as main effects. Within host species, the unit of replication was ‘host individual’ (6 per host species) which was fit as a random effect. The significance of the main effects of ‘host species’, ‘restraint status’ and their interaction were tested by sequentially adding them to a base statistical model that contained only the random effect of ‘host individual’. The importance of these factors was evaluated by testing if their addition to the base model (in the case of the main effects), or to a model including both main effects (in the case of the interaction) led to a statistically significant improvement in the amount of variation explained as assessed by the use of likelihood ratio test (LRT). When the interaction term was significant, the main effect of ‘host restraint’ was analysed separately for each host species to test its significance on feeding success and oviposition rate as described above. The continuous variables of blood meal size and fecundity (based on mosquitoes that laid at least 1 egg) were analysed using generalised linear mixed effect models (lmer) with the R statistical software (Crawley, 2007). Similar to the analysis of proportion data as described above, the statistical significance of the fixed effects of ‘host species’, ‘restraint status’ and their interaction were tested through sequential addition to a base model that contained only the random effect 'host individual'. In trials were *An. gambiae s.s* were fed on restrained hosts, there were no mosquitoes that laid eggs after feeding on chickens and goats (probably few mosquitoes fed on these hosts). Consequently, these two host species were not included in the analysis of how host restraint influenced *An. gambiae s.s* fecundity.

Mosquito survival was analysed using the Cox Proportional Hazard Model (coxph) in the R statistical software (Crawley, 2007). In this analysis, the six different replicates (e.g. host individuals) conducted for each host species treatment were treated as random effects. A frailty function was used to incorporate the random effect of host replicate into the Cox model while evaluating for the additional impact of host species, restraint status, and their interaction on the post – feeding survival of mosquitoes. Initially, all three factors including the main effects and their interaction were fitted in the same statistical model. When the interaction term was significant, the main effect of 'host restraint' was analysed separately for each host species to test its significance on mosquito survival.

4.3 Results

4.3.1 Impact on feeding success of mosquito vectors

The impact of host restraint status on the feeding success of both vector species (e.g. probability of getting a blood meal) varied between host species (host species × restraint status: *An. arabiensis*, $\chi^2_5 = 126.94$, $P < 0.001$, Figure 4.1 a – f, *An. gambiae s.s*, $\chi^2_5 = 31.25$, $P < 0.001$, Figure 4.2a - f). For *An. arabiensis*, the probability of obtaining a blood meal when hosts were restrained was significantly higher for chickens ($\chi^2_1 = 140.46$, $P < 0.001$, Figure 4.1 a), dogs ($\chi^2_1 = 24.92$, $P < 0.001$, Figure 4.1 b), goats ($\chi^2_1 = 4.36$, $P = 0.04$, Figure 4.1 c), and humans ($\chi^2_1 = 27.66$, $P < 0.001$, Figure 4.1 e). Notably, host restraint did not improve the feeding success of *An. arabiensis* on the host species which it most commonly feeds upon in nature (bovids). This vector had similarly high feeding success on free and restrained calves ($\chi^2_1 = 0.62$, $P = 0.43$, Figure 4.1 d), and actually had a higher probability of obtaining a blood meal from unrestrained than restrained cows ($\chi^2_1 = 13.39$, $P < 0.001$, Figure 4.1 f). The feeding success of *An. gambiae s.s* was significantly improved by host restraint only in the cases of chickens ($\chi^2_1 = 94.26$, $P < 0.001$, Figure 4.2a) and cows ($\chi^2_1 = 7.82$, $P = 0.005$, Figure 4.2f). Their probability of obtaining a blood meal was unaffected by the restraint status of all other species (e.g. dogs: $\chi^2_1 = 0.08$, $P =$

0.77, Figure 4.2b, goats: $\chi^2_1 = 2.10$, $P = 0.15$, Figure 4.2c, calves: $\chi^2_1 = 0.62$, $P = 0.43$, Figure 4.2d, and humans: $\chi^2_1 = 0.46$, $P = 0.50$, Figure 4.2e).

Mosquito blood meal size was also significantly influenced by the interaction between host species and restraint status (host species × restraint status: *An. arabiensis*, $\chi^2_5 = 38.34$, $P < 0.001$, Figure 4.3a - f, and *An. gambiae s.s*, $\chi^2_5 = 73.15$, $P < 0.001$, Figure 4.4a - f). Contrary to expectation, *An. arabiensis* obtained significantly larger blood meals from unrestrained than restrained hosts of all species ($P < 0.001$ in all cases, Figure 4.3a - f), although the magnitude of difference varied between host species. In contrast, *An. gambiae s.s* acquired similarly sized blood meals from restrained and unrestrained chickens ($\chi^2_1 = 47.00$, $P = 0.49$, Figure 4.4a), calves ($\chi^2_1 = 0.01$, P 0.91, Figure 4.4d), and humans ($\chi^2_1 = 0.96$, P 0.33, Figure 4.4e). This vector was able to obtain slightly larger blood meals from restrained than unrestrained dogs ($\chi^2_1 = 6.83$, $P = 0.01$, Figure 4.4b), and goats ($\chi^2_1 = 5.17$, $P = 0.02$, Figure 4.4c), but surprisingly acquired larger blood meals from cows in experiment where they were unrestrained ($\chi^2_1 = 72.93$, $P < 0.001$, Figure 4.4f).

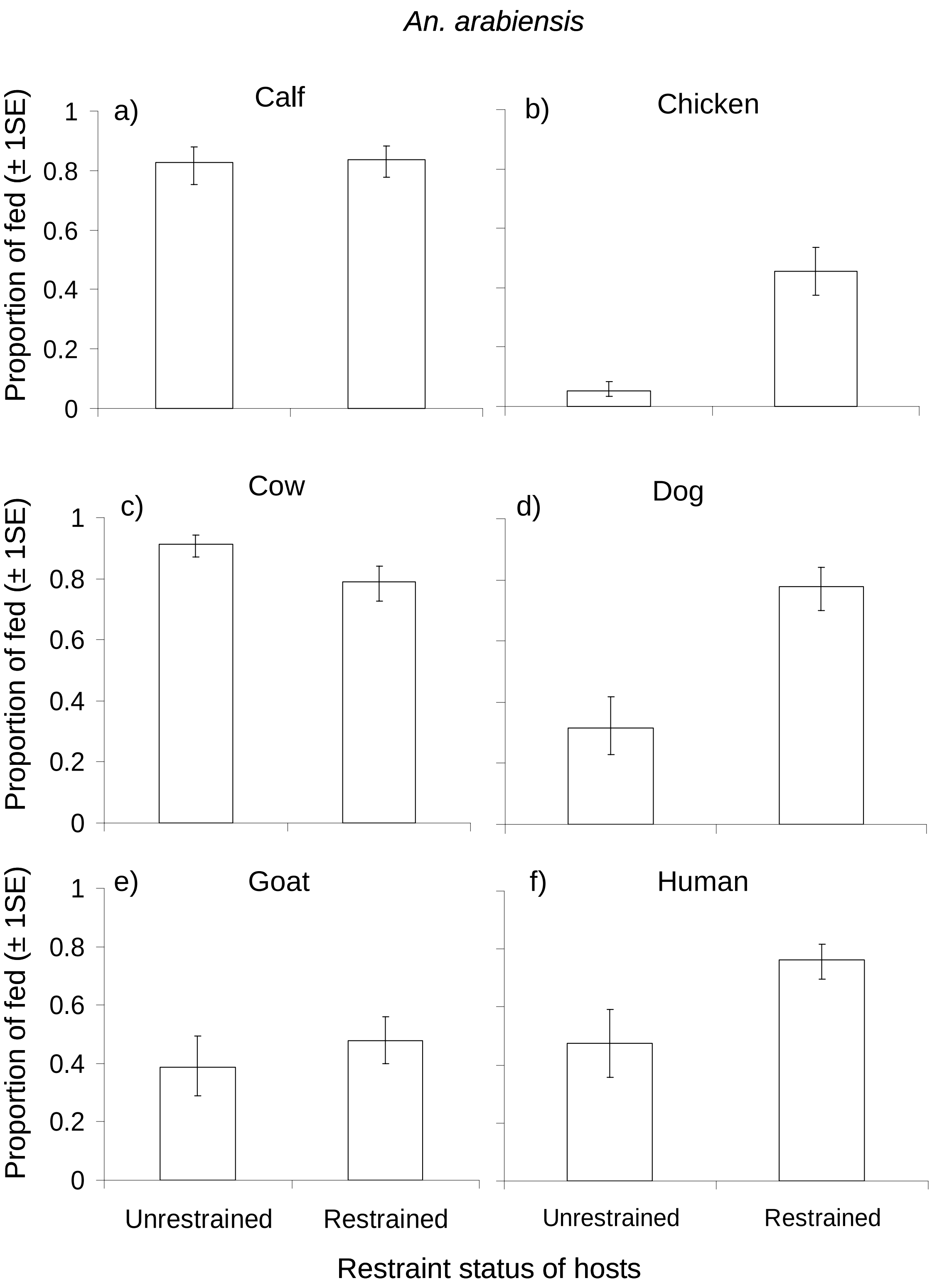

Figure 4.1. Estimated proportion (± 1 s.e.m) of *An. arabiensis* that succeeded in obtaining a blood meal from either unrestrained or restrained hosts. (6 different host individuals per host species).

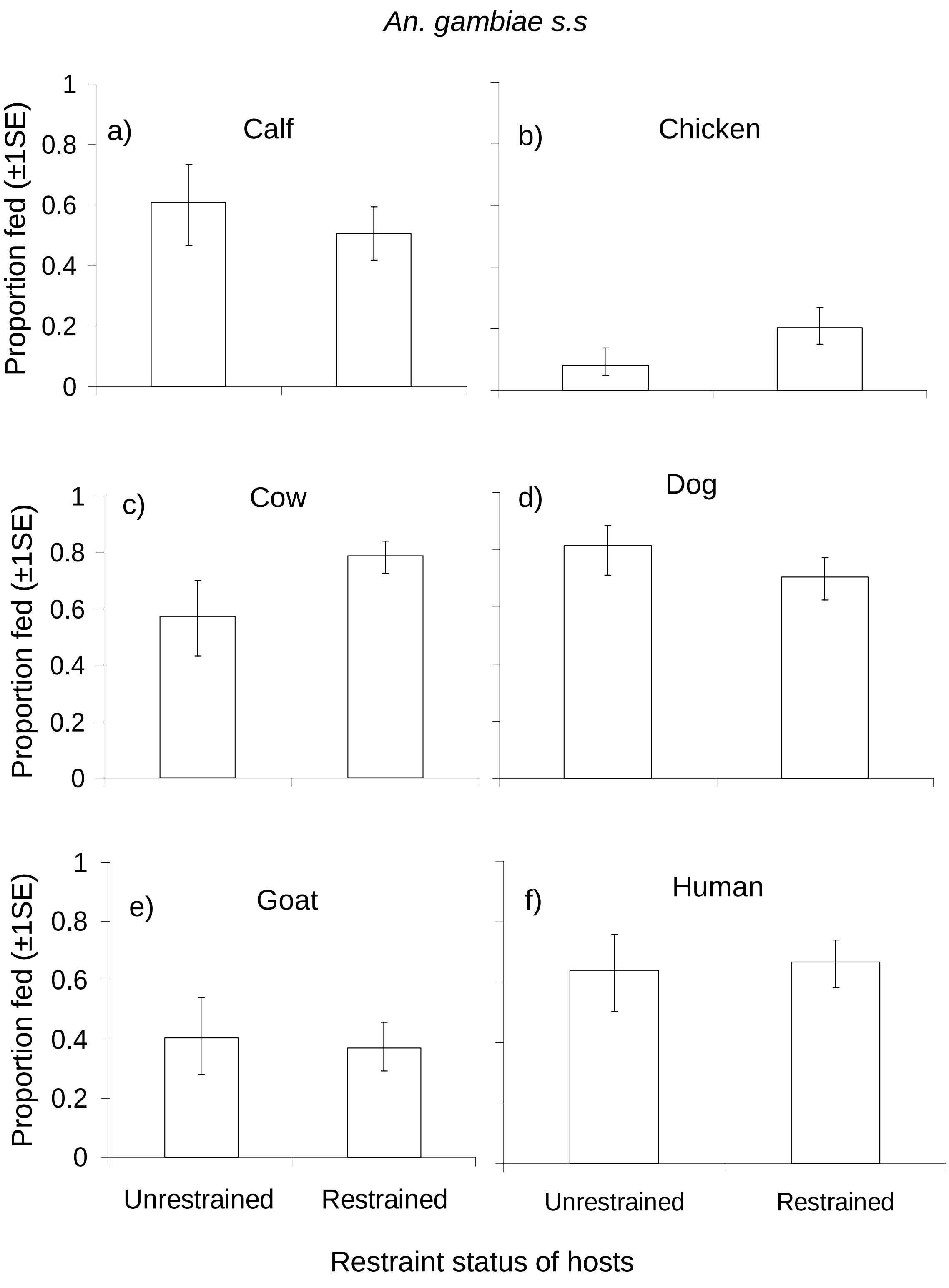

Figure 4.2. Estimated proportion (± 1 s.e.m) of *An. gambiae s.s* that succeeded in obtaining a blood meal from either unrestrained or restrained hosts. (6 different host individuals per host species).

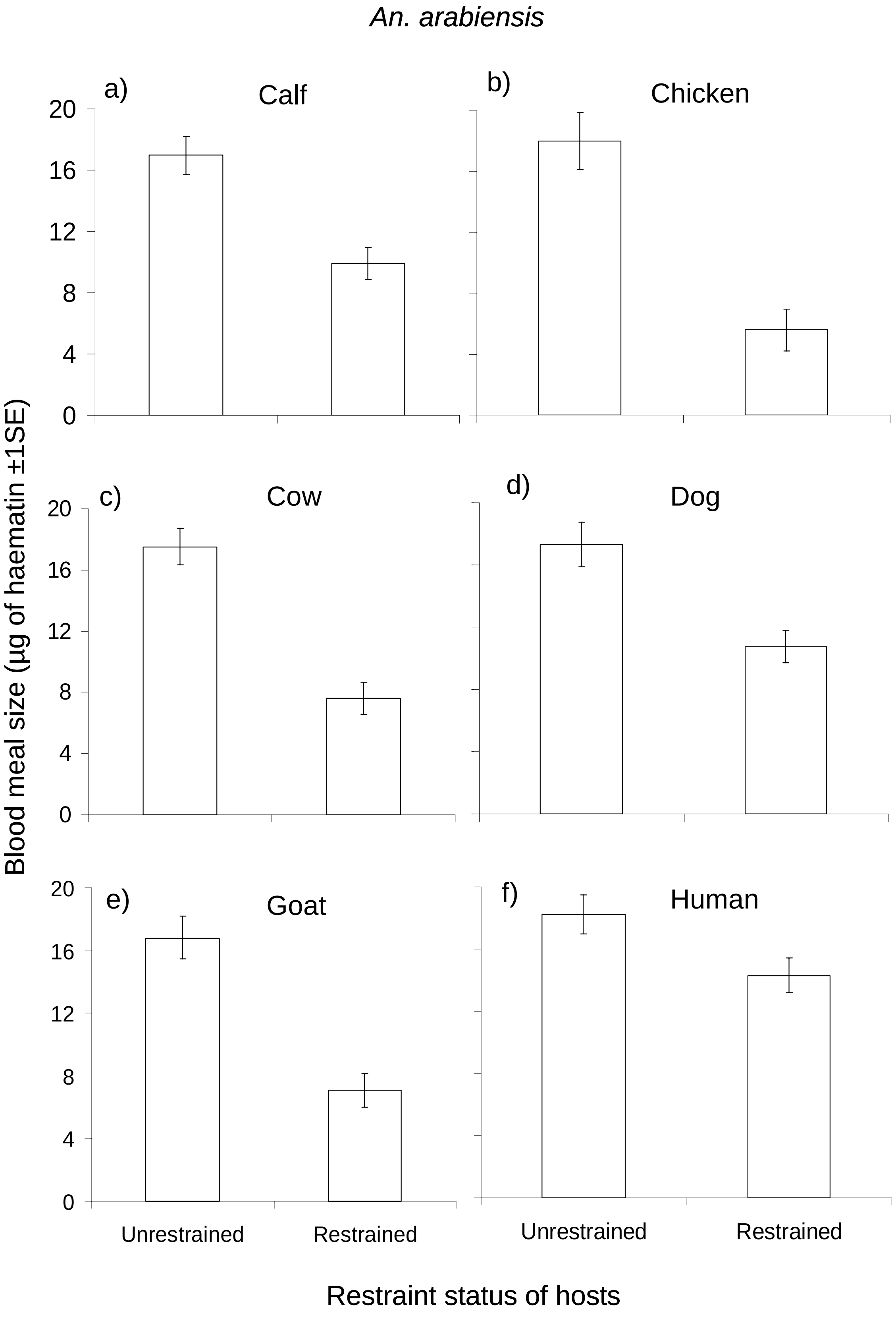

Figure 4.3. Estimated mean (± 1 s.e.m) blood meal size of *An. arabiensis* fed on either unrestrained or restrained hosts of different species. (6 different host individuals per host species).

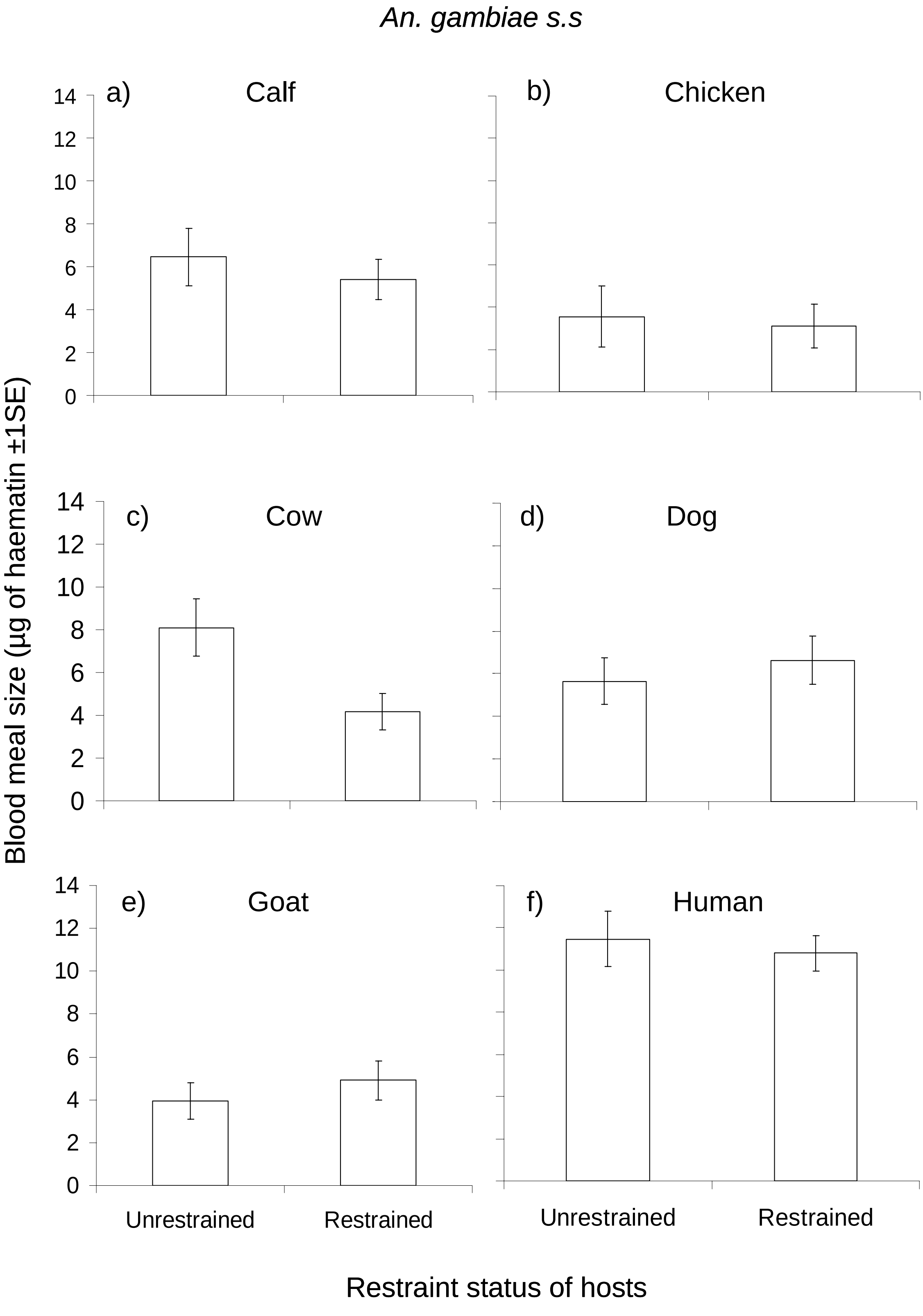

Figure 4.4. Estimated mean (± 1 s.e.m) blood meal size of *An. gambiae s.s* fed on either unrestrained or restrained hosts of different species. (6 different host individuals per host species).

4.3.2 Impact on mosquito reproductive success

For mosquitoes that successfully acquired a blood meal, their probability of laying eggs (oviposition rate) also depended on the interaction between host species and restraint status (host species × restraint status: *An. arabiensis*, $\chi^2_5 = 18.37$, $P = 0.002$, Figure 4.5a – f, *An. gambiae s.s*, $\chi^2_5 = 87.48$, $P < 0.001$, Figure 4.6a-f). *Anopheles arabiensis* had a higher probability of producing eggs after feeding on unrestrained than restrained in 3 out of 6 species: chickens ($\chi^2_1 = 7.51$, $P = 0.006$, Figure 4.5a), dogs ($\chi^2_1 = 20.60$, $P < 0.001$, Figure 4.5b), and cows ($\chi^2_1 = 10.46$, $P = 0.001$,Figure 4.5c). In contrast, host restraint status did not influence the oviposition rate of *An. arabiensis* feeding on goats ($\chi^2_1 = 0.51$, $P = 0.47$, Figure 4.5e), calves ($\chi^2_1 = 0.15$, $P = 0.69$, Figure 4.5d), and humans ($\chi^2_1 = 0.11$, $P = 0.74$, Figure 4.5e). The oviposition rate of *An. gambiae s.s* was greater on unrestrained than restrained hosts of all species ($P > 0.05$ in all cases, Figure 4.6a – f).

Restricting consideration to mosquitoes that laid at least one egg, the fecundity of *An. arabiensis* was unaffected by host restraint status in 5 out of 6 host species (dogs, goats, calves, humans and cows: $P > 0.05$ in all cases, Figure 4.7b – f). *Anopheles arabiensis* fecundity, however, was slightly reduced on chicken hosts when they were restrained ($\chi^2_1 = 4.82$, $P = 0.03$, Figure 4.7a). No data were available on *An. gambiae s.s* fecundity after feeding on restrained chickens and goats. Across the remaining 4 host species, the fecundity of *An. gambiae s.s* was similar on restrained and unrestrained hosts (dogs, calves and cows: $P > 0.05$ in all cases, Figure 4.8b, d & f). In contrast, *An. gambiae s.s* fecundity was significantly lower in experiments where human hosts were restrained than free ($\chi^2_1 = 8.96$, $P = 0.003$, Figure 4.8e).

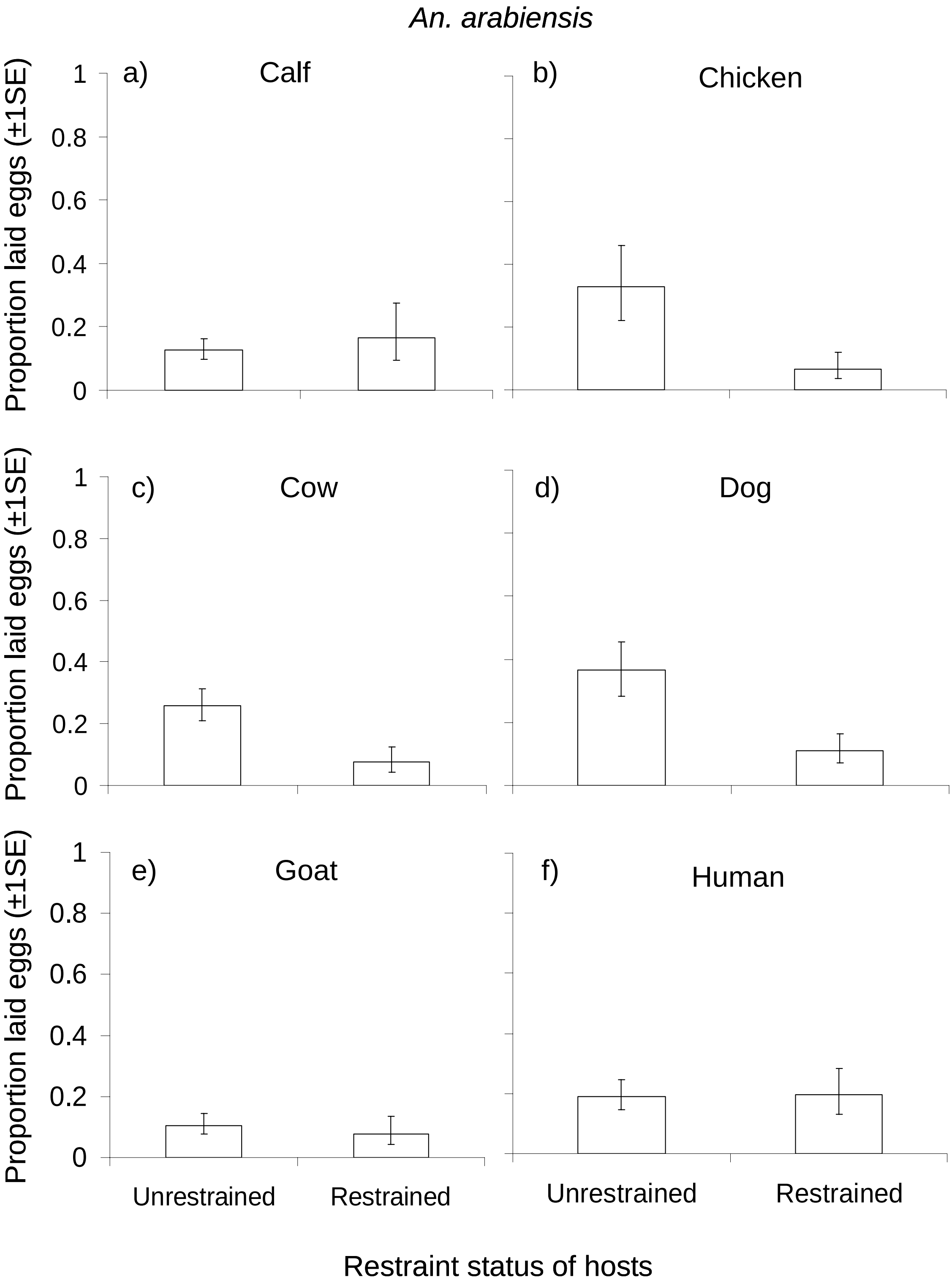

Figure 4.5. Estimated proportion of *An. arabiensis* that laid eggs (± 1 s.e.m) after feeding on either unrestrained or restrained hosts of different species. (6 different host individuals per host species).

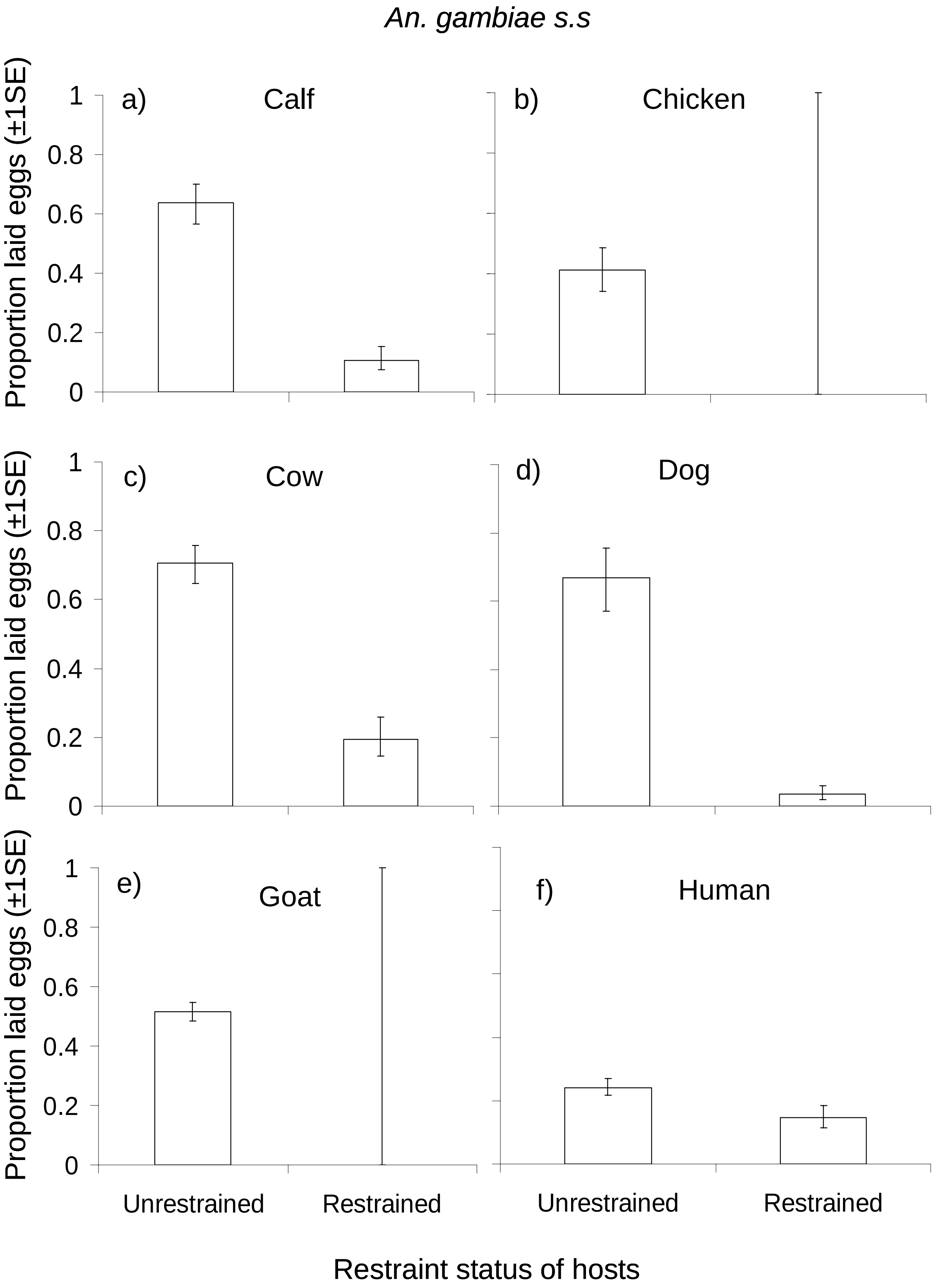

Figure 4.6. Estimated proportion of *An. gambiae s.s* that laid eggs (± 1 s.e.m) after feeding on either unrestrained or restrained hosts of different species. (6 different host individuals per host species).

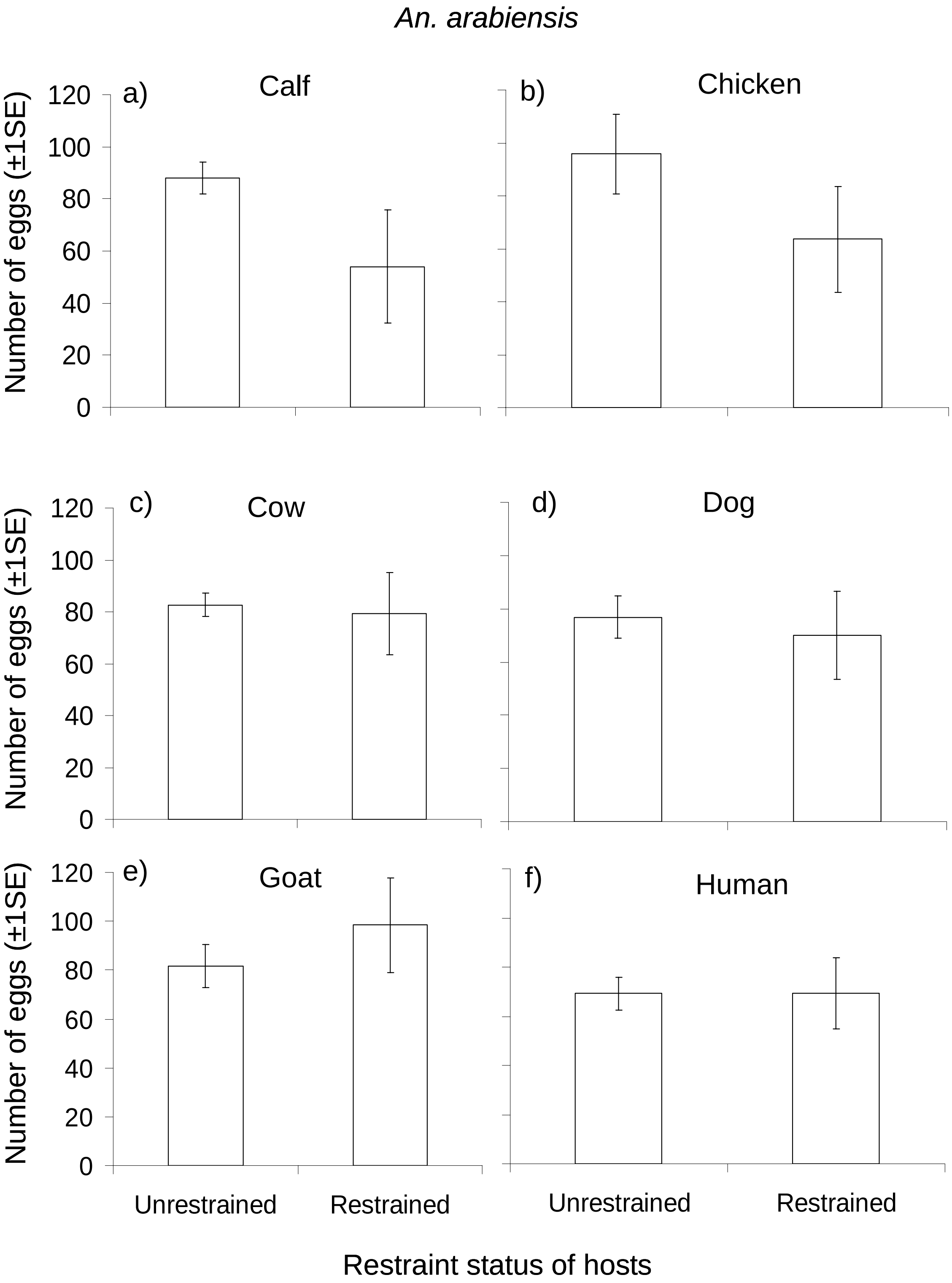

Figure 4.7. Estimated mean (± 1 s.e.m) number of eggs laid by *An. arabiensis* after feeding on either unrestrained or restrained hosts of different species. (6 different host individuals per host species).

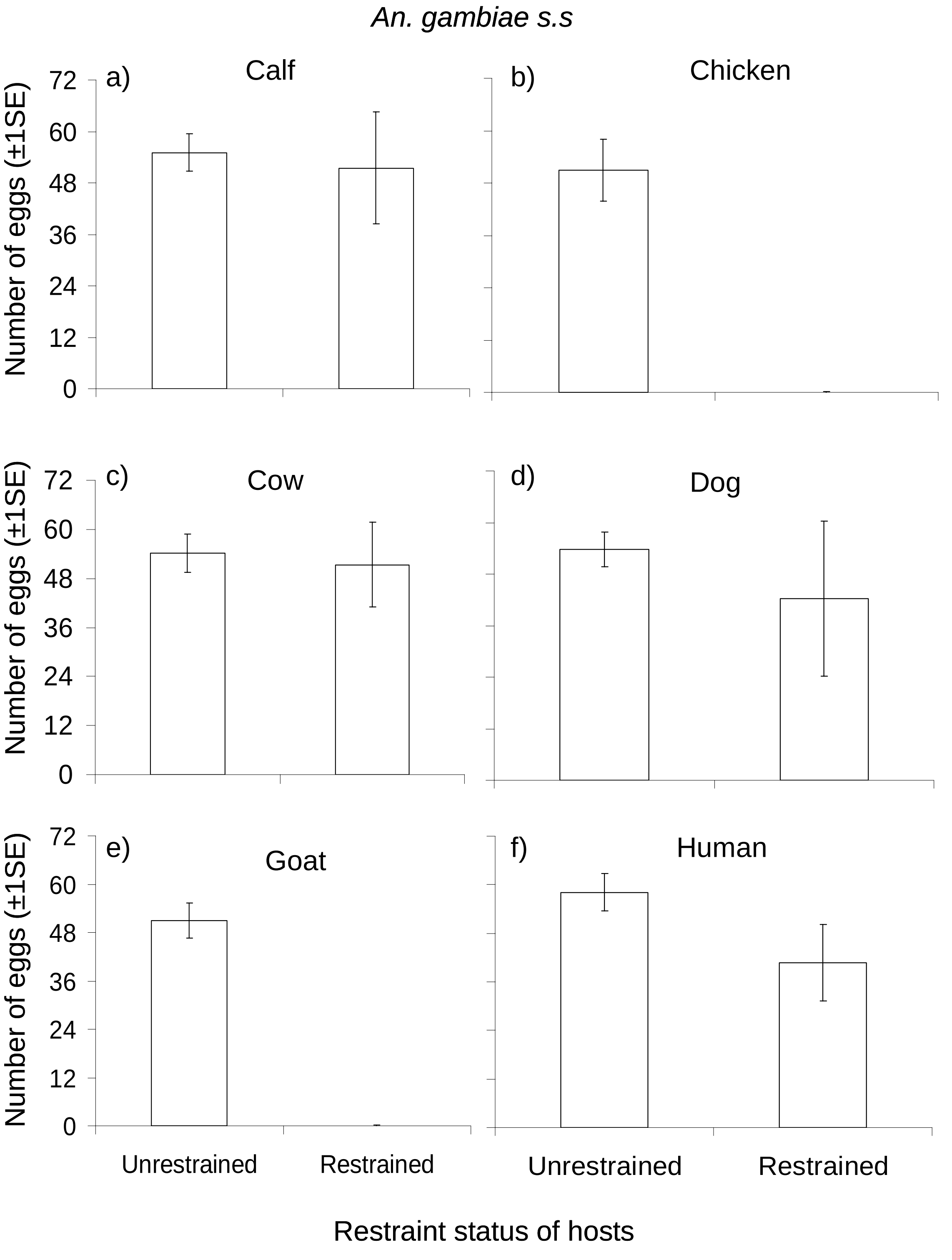

Figure 4.8. Estimated mean (± 1 s.e.m) number of eggs laid by *An. gambiae s.s* after feeding on either unrestrained or restrained hosts of different species. (6 different host individuals per host species).

4.3.3 Impact on the survival of mosquitoes

Overall, the median survival of mosquitoes was greater after feeding on unrestrained than restrained hosts (*An. arabiensis* by 2 -7days, *An. gambiae s.s* by 1 -4 days ,Table 4.1). In *An. arabiensis*, the impact of host restraint on their survival did not vary significantly between host species (host species × restraint status: $\chi^2{}_5 = 6.66$, $P = 0.30$, Figure 4.9a – f). The reduction in *An. arabiensis* survival after feeding on restrained relative to unrestrained hosts was significant for all host species ($P < 0.05$) except calves ($\chi^2{}_1 = 2.85$, $P = 0.1$, Figure 4.9d), and varied in magnitude from 42 – 58% (Table 4.2). In contrast, the impact of host restraint on *An. gambiae s.s* survival varied significantly between host species (host species × restraint status: $\chi^2{}_5 = 18$, $P = 0.003$, Figure 4.10a – f, Table 4.2). These mosquitoes had higher survival after feeding on unrestrained than restrained chickens ($\chi^2{}_1 = 7.94$, $P = 0.005$, Figure 4.10a), dogs ($\chi^2{}_1 = 45.5$, $P < 0.001$, Figure 4.10b), calves ($\chi^2{}_1 = 6.85$, $P = 0.01$, Figure 4.10d), and cows ($\chi^2{}_1 = 19.2$, $P < 0.001$, Figure 4.10f). However, host restraint status did not influence the post-feeding survival of *An. gambiae s.s* feeding on goats ($\chi^2{}_1 = 2.66$, $P = 0.1$, Figure 4.10c) or humans ($\chi^2{}_1 = 0.11$, $P = 0.74$, Figure 4.10e).

Estimated median survival days				
	An. arabiensis		*An. gambiae s.s*	
Host species	Unrestrained	Restrained	Unrestrained	Restrained
Calf	15 (13 – 18)	9 (7 – 13)	8 (7 – 8)	5 (4 – 7)
Chicken	14 (13 – 18)	12 (10 – 15)	7 (4 – 9)	3 (2 – 6)
Cow	13 (11 – 14)	6 (4 – 8)	7 (6 – 8)	5.5 (5 – 7)
Dog	14 (12 – 20)	9 (8 – 11)	8 (8 – 9)	4 (4 – 4)
Goat	15 (12 – 18)	10 (6 – 14)	9 (9 – 9)	5 (3 – 10)
Human	15 (13 – 17)	8 (5 – 12)	7 (6 – 8)	8 (6 – 9)

Table 4.1. Estimated median survival (days) of *An. arabiensis* and *An. gambiae s.s* after feeding on hosts when they were either free to exhibit natural defensive behaviours or physically restrained from movement during feeding. (6 different replicates per host species). The numbers in brackets are 95% confidence intervals of the median survival

Odds of mortality (OR) on unrestrained relative to restrained hosts		
Host species	*An. arabiensis*	*An. gambiae s.s*
Calf	0.80 (0.61 – 1.04)	0.68 (0.51 – 0.91)
Chicken	0.54 (0.30 – 0.96)	0.43 (0.24 – 0.78)
Cow	0.56 (0.45 – 0.69)	0.55 (0.42 – 0.72)
Dog	0.42 (0.29 – 0.60)	0.47 (0.38 – 0.58)
Goat	0.41 (0.29 – 0.58)	0.74 (0.51 – 1.06)
Human	0.58 (0.45 – 0.74)	0.96 (0.74 – 1.24)

Table 4.2. The estimated odds of mortality in *An. arabiensis* and *An. gambiae s.s* after feeding on hosts when they were free to exhibit natural defensive behaviours relative to when hosts were physically restrained from movement. The numbers in brackets are the 95% confidence intervals of odds ratio (OR).

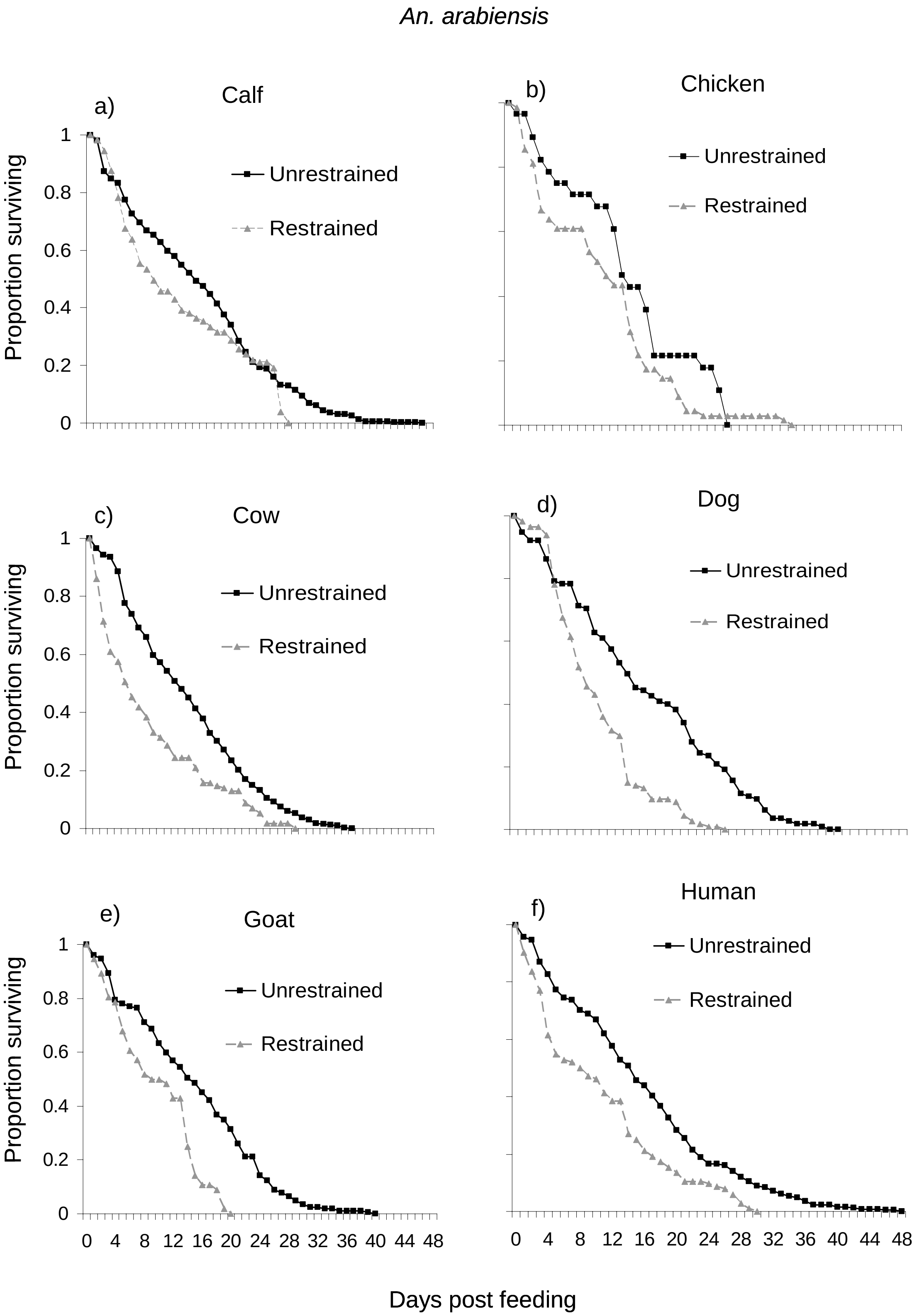

Figure 4.9. Survival of *An. arabiensis* after feeding on hosts of different species when that either were free to exhibit natural defensive behaviours or physically restrained from movements during blood feeding. Lines indicate their predicted survival through time, with black line indicating survival after feeding on unrestrained hosts, and grey after feeding on restrained hosts. The survival function was estimated from the Cox proportional hazard model (COXPH) accounting for variation between 6 different replicates per host species.

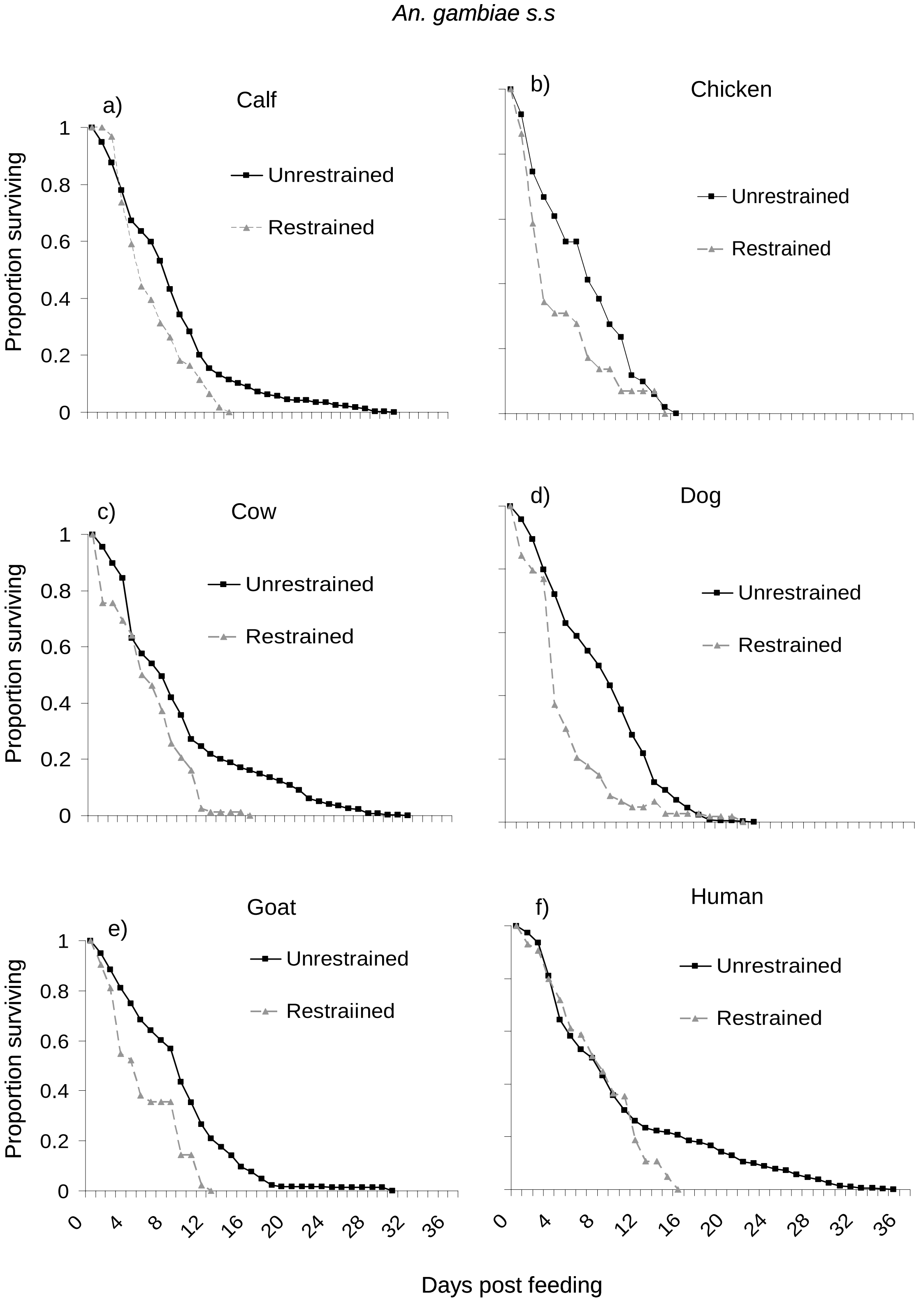

Figure 4.10. Survival of *An. gambiae s.s* after feeding on hosts of different species when that either were free to exhibit natural defensive behaviours or physically restrained from movements. Lines indicate their predicted survival through time, with black lines indicating survival after feeding on unrestrained hosts, and grey after feeding on restrained' hosts. The survival function was estimated from the Cox proportional hazard model (COXPH), accounting for variation between 6 different replicates per host species.

4.3.4 Summary of results

In summary, host restraint generally improved the probability of obtaining a blood meal in *An. arabiensis* (on 4 out of 6 host species), but less so in *An. gambiae s.s* (2 out of 6 host species, (Table 4.3). However, the only impact of preventing host defensive movements on the subsequent fitness of blood fed mosquitoes was to reduce it, with evidence of reductions in the oviposition rate, fecundity and survival of both mosquito species after feeding on restrained versus unrestrained hosts (Table 4.3).

Impact of host restraint on mosquito fitness

Fitness traits	*An. arabiensis*						*An. gambiae s.s*					
	CA	CH	CO	DG	GT	HU	CA	CH	CO	DG	GT	HU
Probability of feeding	0	+	–	+	+	+	0	+	+	0	0	0
Blood meal size	–	–	–	–	–	–	0	0	–	+	+	0
Oviposition rate	0	–	–	–	0	0	–	–	–	–	–	–
Fecundity	0	–	0	0	0	0	0	na	0	0	na	–
Survival	0	–	–	–	–	–	–	–	–	–	0	0

Table 4.3. A summary of the impact of host restraint (on different host species) on various fitness traits of *An. arabiensis* and *An. gambiae s.s*.Host species are abbreviated as : CA - calf, CH - chicken, CO - cow, DG - dog, GT - goat, and HU - human. Symbols indicate impact of host restraint on mosquito fitness, e.g: '+' indicate that mosquito fitness was higher when hosts were restrained, '–' indicate that mosquito fitness was lower when hosts were restrained, and '0' indicate that there was no significant difference in fitness between restrained and unrestrained hosts. 'na' indicates that no data were available for the given mosquito fitness trait in the particular host and vector species combination.

4.4 Discussion

This study provides the first demonstration of the impact of defensive behaviours by humans and other alternative animal hosts in limiting the feeding success and subsequent fitness of African malaria vectors *An. arabiensis* and *An. gambiae s.s*. By contrasting the fitness of host seeking mosquitoes under natural conditions when hosts were free to exhibit natural behaviour with when they were physically restrained, this study was able to estimate the relative effectiveness of defensive behaviours by the hosts that these mosquitoes are most likely to encounter in nature, and evaluate whether their preference for particular host species (including humans) is linked with poor defensiveness. Consistent with initial prediction, there was some evidence that mosquito-feeding success (probability of obtaining a blood meal) was significantly improved when hosts were physically restrained. However, this result was not consistent across both vector species on all host species. More surprisingly, I found that the subsequent fitness of mosquitoes that were able to obtain a blood meal under natural conditions was generally greater than those that fed on restrained hosts (oviposition rate and survival). These results suggest that natural physical defensive behaviours made by host species including humans do not impose a substantial fitness costs on malaria vectors, and may be unlikely to restrict their host species choice.

As expected, *An. arabiensis* was generally more likely to obtain a blood meal from most host species when they were physically restrained from moving (e.g. for chickens, dogs, goats, and humans). A notable exception to this rule however, was the feeding success of *An. arabiensis* on their naturally preferred bovid hosts (Muriu et al., 2008), which was similarly high regardless of host restraint status, and generally greater than on any other host species. This suggests either that bovids have considerably poorer defensive behaviours than the other host species assayed here, or that *An. arabiensis* has evolved strategies to more effectively evade the defensive behaviour of this host type. In contrast to *An. arabiensis*, the feeding success of *An. gambiae s.s* was generally unaffected by host restraint status in both naturally preferred (e.g. humans) and rarely exploited host species (e.g. dogs, goats and calves). For *An. gambiae s.s.*, the host species whose physical movements were associated with reduction in their feeding success were chickens and cows. For both vector species, improvements in feeding success due to host physical restraint were most pronounced for chicken hosts. This suggests, as has been reported elsewhere (Chapter 3 and Darbro and Harrington, 2007), that these avian hosts have considerably more effective anti-mosquito behaviours than mammals, and may explain why these hosts are rarely exploited by these vectors in nature (Muriu et al., 2008), despite occurring at relatively high densities throughout their range.

Previous studies have indicated that calves are more likely to defend themselves against biting insects than adult cows (Torr et al., 2007). Here I found that host physical restraint did not affect feeding success of *An. arabiensis* upon calves, but was associated with a relatively small reduction of feeding probability on cows. This confirms that movements by these two host types may have differential impacts on *An. arabiensis* fitness, with movement by calves having little impact on mosquito biting success, and movement by cows perhaps enhancing it. However, experiments on *An. gambiae s.s* suggested that adult cows may be more defensive than calves; as physical restraint of the former significantly increased feeding success. Consequently, no general conclusions about age specific differences in bovid defensive behaviours towards malaria vectors can yet be made.

Restricting consideration to mosquitoes that succeeded in obtaining a blood meal there was no evidence for either vector species that their blood meal size was improved after exposure to restrained host species. In fact in most cases (summarized inTable 4.3), mosquito blood meal size was actually greater after feeding on hosts under natural conditions than when they were physically restrained. As host defensive behaviours have been shown to both interrupt the feeding process and cause mosquitoes to abandon feeding before obtaining a full blood meal (Hodgson et al., 2001), these results were surprising. Studies of the mosquito *Ae. aegypti* and other ectoparasites under laboratory conditions have indicated that host defensive behaviour reduces blood meal size (Hodgson et al., 2001, Hawlena et al., 2007, Khokhlova et al., 2008, Bize et al., 2008). One reason for the unexpected impact of host restraint on mosquito blood meal size observed here could include in experiments with unrestrained hosts, mosquitoes were exposed to hosts overnight for a 12 hour period (7 pm – 7 am). In contrast, mosquitoes were exposed to restrained hosts for a period of 15 minutes, with the latter been selected because pilot work indicated that this more than enough time for mosquitoes to finish one complete blood meal (Chapter 5). The enhanced blood meal size of mosquitoes in the natural feeding bioassays suggest that these vectors may take repeated feeds from the same host throughout the night, intaking a higher total volume of blood than can be consumed in just one feed. A second possibility is that mosquito blood intake rate increased with the blood flow rate of their host during feeding (Daniel and Kingsolver, 1983), and that unrestrained hosts have a higher rate of blood flow than restrained ones. Host movements may be associated with increase in metabolic activities that generate sweat, rise body temperature, and increase blood flow rates and hence vasodilatation; these physiological changes which are speculated to enhance both host attractiveness to mosquitoes and blood intake (Nacher, 2005, Lacroix et al., 2005). Finally, the enhanced blood meal size of mosquitoes on unrestrained hosts may be because under natural conditions, mosquitoes preferentially choose to feed at sites on the body that are more easily to obtain blood from (e.g. where blood vessels are easily accessible and/or skin is relatively thhin, Li and Rossignol, 1992) than the places which mosquitoes were experimentally applied on restrained hosts here. For example, in restrained experiments here mosquitoes were applied to human forearms, whereas under natural conditions they preferentially bite feet (Dekker et al., 1998). Similarly, evidence suggest that *An. arabiensis* preferentially lands and feeds on cow legs (Habtewold et al., 2004), where here they were exposed to a variety of sites on the body of bovids (e.g. flanks, and thigh muscles). Further experiments are required where both the length and site of exposure to hosts under natural conditions and when they are physically restrained will be necessary to distinguish these hypotheses.

As mosquito blood meal size is strongly and positively correlated with their reproductive success (Briegel, 1986, Edman and Lynn, 1975) and the acquisition of energy reserves critical for long-term survival (Nayar and Sauerman, 1975), host defensive behaviours that limit blood meal volume are expected to reduce mosquito survival and reproductive success (Anderson and Roitberg, 1999). As I found no evidence that the restriction of host defensive behaviours (through physical restraint) enhanced mosquito blood meal size, it is thus not surprising that it also did not enhance mosquito oviposititon rate or fecundity. More surprising was the fact that the relatively larger blood meals mosquitoes acquired from feeding on free rather than restrained hosts did not translate into correspondingly greater reproductive success. In *An. arabiensis*, the relatively larger blood meal sizes associated with feeding on freely moving hosts were correlated with an increase in oviposition rate in only 3 out of 6 assayed host species, and increased fecundity in only 1 out of 6. *Anopheles gambiae s.s* blood meal size was found to be significantly larger on unrestrained hosts in only 2 out of 6 species (Table 4.3), but across all host species variation in blood meal size due to host restraint status (both positive and negative effects) were not consistently associated with changes in their oviposition and fecundity. Considering mosquitoes that did lay eggs, the relative uniformity in their fecundity regardless of host species or restraint status suggests that although there was variation in the efficiency with which they acquired blood, in almost all cases those that were able to obtain the minimum volume of blood necessary to initiate oviposition (Takken et al., 2002) also obtained a sufficient amount to maximize egg production. Mosquito fecundity is known to be linearly related to blood volume only above a minimum threshold (below which no eggs are produced) and below a maximum threshold (above which no further eggs are produced) (Roitberg and Gordon, 2005).

While the larger mosquito blood meals observed in trials where hosts were free to move did not give rise to greater reproductive success, they were associated with a statistically significant and considerable enhancement of longevity on most host types (by 32 – 59%, Table 2). Conventionally, it is assumed that mosquitoes use blood resources for egg production and not survival (Briegel, 1985, Zhou et al., 2007), however some previous studies indicate that the survival of mosquitoes and other ectoparasites may increase with the size of ingested blood meals (Hawlena et al., 2007, Harrington et al., 2001, Bize et al., 2008), suggesting that they use blood proteins to synthesize energy reserves for their survival (Nayar and Sauerman, 1975, Ziegler and Ibrahim, 2001). I note that the survival effects here describe only the long-term survival of mosquitoes after they have successfully taken a blood meal and do not incorporate any immediate feeding associated mortality risk during the feeding process . In a previous study I measured the number of mosquitoes found dead during one night of host-seeking in the presence of different host species, and used this as an indirect estimate of feeding-associated mortality (Chapter 3). Here I found that the feeding associated mortality was relatively low for both vector species and did not vary between host species (Chapter 3). Combining this finding with data here showing that preventing hosts from mounting defensive behaviours does not enhance their longer-term survival, these results suggest that host defensive behaviours do not significantly reduce the survival of these mosquito vectors.

Here I sought to estimate the relative importance of host defensive behaviour to the fitness of malaria vector mosquitoes, and ultimately to their host choice in nature, by examining the relative change in their performance when hosts were prevented from exhibiting it. With respect to explaining the preference of these malaria vectors for certain host species, results from this comparative analysis are mixed. It is widely assumed that the preference of highly anthrophilic African malaria vectors such as *An. gambiae s.s* has arisen as a product of selection driven by host defensive behaviour (Kelly, 2001), with the reduced capacity of human hosts to mount defensive behaviour while sleeping explaining why the peak of mosquito biting activity occurs at 10 pm -2 am (e.g. when most people in endemic areas are sleeping, Taye et al., 2006, Killeen et al., 2006). Results obtained provide partial support for the hypothesis that preferred host species may mount less effective defensive behaviours than other available species. For example, physically restraining hosts significantly improved the feeding success of *An. arabiensis* on all host species except their preferred bovid (cows and calves) hosts, on which they were able to feed with similar success when free or restrained. This suggests that the natural defensive behaviour of this host type does not impose a significant impediment to the feeding success of *An. arabiensis*, whereas that of all other hosts considered does. However, no such association was apparent for *An. gambiae s.s*. Limiting the ability of its naturally preferred human hosts to mount defensive behaviours did not influence feeding success of *An. gambiae s.s*, however nor did it influence the ability of this mosquito to obtain blood from several other host species such as calves, dogs and goats. In both vector species, the host type whose defensive behaviour appeared most effective at preventing bites was chickens, which is the host species that both are least likely to feed upon in nature (Muriu et al., 2008). Consequently, variation in host defensive behaviours may be generating selection for mosquitoes to avoid certain host species, but does not appear to consistently explain their choice of preferred host. Furthermore, if host defensive behaviour is acting to shape the host preference of malaria vectors, it is doing so by influencing the relative ease of acquiring blood from certain species, and not the value of that blood meal subsequent to ingestion (as post-feeding estimates of mosquito fitness were consistently higher or similar on unrestrained than restrained hosts).

Results presented here on the importance of host defensive behaviours to malaria vectors may also have implications for the epidemiology and control of malaria. Here, host restraint status was shown to influence the probability of mosquito feeding, the size of blood meals and their subsequent survival; all of which are important determinants of mosquito population dynamics and malaria transmission potential (Killeen et al., 2000). Specifically, I wished to evaluate whether other host species that are commonly found in the same domestic environment as humans are relatively more or less defensive against biting by malaria vectors. The upscaling and widening of distribution of the ITNs throughout sub-Saharan Africa (Roll Back Malaria Partnership, 2008), combined with improvement in housing (Kirby et al., 2009) and use of other protecting measures such as ‘repellents’(Katz et al., 2008), means that the relative ‘defensiveness’ of humans within the available community of potential host species is significantly increasing. This increased protection of humans inevitably yields a clear epidemiological advantage (Lengeler, 2004), and may also select for evolutionary changes that could impact how mosquitoes interact with humans, with either positive or negative effects for disease control. Examples of detrimental evolutionary change in response to growing ITN use include the development of insecticide resistance (Ranson et al., 2009, Hemingway et al., 2002), or mosquito behavioural shifts to biting outside and/or early in the evening when nets are not in use (Sochantha et al., 2010, Bockarie et al., 1996, Bockarie and Dagoro, 2006, Killeen et al., 2006, Trung et al., 2005). In contrast, evolutionary changes in mosquito behaviour that could be beneficial for control include a shift in host preference away from anthrophily. Such changes are most likely to occur when there is no substantial fitness cost to mosquitoes from shifting from humans to animal alternatives. Here, with the exception of chickens, I found no evidence that the natural defensive behaviours of animal species likely to be kept in and around households are more costly to malaria vectors than those of humans. In fact in the case of *An. arabiensis*, I found that they may be less likely to encounter effective defensive behaviours on bovids than humans, and thus would do better to switch to the former host type (especially if humans are even more highly protected with a net or insecticide). I thus hypothesize that variation in defensive behaviour between host species should not prevent malaria vectors from exploiting alternative host species when humans are protected. There is some evidence that malaria vectors decrease their feeding on humans in presence of IRS (Service et al., 1978, Gillies and Furlong, 1964) or ITNs (Kaburi et al., 2009), but in other studies no such changes have been observed (Smith, 1966, Quinones et al., 1997). Further investigation is required to confirm whether the failure of vectors to become increasingly zoophilic in the presence of human-protective measures is due either to a lack of opportunity (e.g. low availability of animal alternatives in these settings), and /or a hard-wired behavioural predisposition that causes vectors to select only humans despite the increased risk it may entail.

5 The influence of host haematological properties on feeding efficiency and survival of African malaria vectors

5.1 Introduction

Haematophagous insects rely on resources from host blood to fuel their reproduction and survival (Prasad, 1987, Lehane, 2005). Consequently, variation in host haematological properties may influence the feeding efficiency and fitness of these insects, and the transmission ability of those that are vectors of pathogens (Marshall, 1981, Lehane, 2005). Consistent variation in haematological properties between potential host species may generate host species-specific fitness rewards that could cause selection for insect vectors to preferentially select some host types over others. Many important insect disease vectors including those responsible for several human diseases are known to have highly specific host species preference (Lyimo and Ferguson, 2009). The evolutionary and ecological causes of this specialization are not well known, but one hypothesis is that specialism has been driven by variation in host haematological properties that has led vectors to feed preferentially on hosts whose blood provide the highest fitness reward (Ward, 1992, Poulin, 1998). However, whether there is sufficient variation in the nutritive value of blood between host species to influence insect vector fitness, and possibly drive their host species preference, remains unknown.

Several aspects of the feeding efficiency and fitness of many haematophagous insects and vectors are known to vary between host species (Lehane, 2005, Krasnov et al., 2007, Ulloa et al., 2005, Akoh et al., 1993, Xue et al., 2009, Harrington et al., 2001, Krasnov et al., 2002, Krasnov et al., 2003, Krasnov et al., 2004, Khokhlova et al., 2007, Williams, 1993, Lyimo and Ferguson, 2009). For example, the feeding efficiency and subsequent fecundity and survival of some flea species is host species dependent with their performance frequently being higher on naturally-preferred than on rarely encountered host species (Krasnov et al., 2007, Krasnov et al., 2004, Krasnov et al., 2003, Khokhlova et al., 2007, Williams, 1993) but not all cases (Khokhlova et al., 2008). The fecundity of several mosquito species (e.g. *Aedes* and *Culex* species) have also been shown to vary between host species (Lyimo and Ferguson, 2009, Akoh et al., 1993, Xue et al., 2009, Downe and Archer, 1975, Shroyer and Siverly, 1972, Ulloa et al., 2005). Additionally, the feeding success (probability of feeding) and subsequent survival of mosquitoes has also been shown to be host-species specific (Harrington et al., 2001). Several hypotheses have been proposed to explain host-dependent variation in insect fitness (Lyimo and Ferguson, 2009), yet few have been tested and none have been conclusively demonstrated to explain this phenomenon. First, it has been widely hypothesized that host defensive behaviour may be the most important determinant of the fitness of haematophagous insects (Darbro and Harrington, 2007, Davies, 1990, Walker and Edman, 1985, Walker and Edman, 1986, Edman et al., 1974, Edman and Kale II, 1971, Waage and Nondo, 1982, Day and Edman, 1984), with insects acquiring the largest blood meals and experiencing the lowest risk of death on poorly defensive species and or individuals (Day and Edman, 1984, Walker and Edman, 1986, Hodgson et al., 2001, Hawlena et al., 2007, Khokhlova et al., 2008, Darbro and Harrington, 2007). A second possibility is that variation in the haematological properties are responsible for host-species specific differences in insect vector fitness as has been investigated in a variety of laboratory settings (Krasnov et al., 2004, Krasnov et al., 2007, Harrington et al., 2001, Williams, 1993, Downe and Archer, 1975, Daniel and Kingsolver, 1983, Sant'Anna et al., 2010, Bennett, 1970, Sarfati et al., 2005, Baylis and Mbwabi, 1995, Krasnov et al., 2003). Finally, it is possible that it is not intrinsic variation in host behavioural or physiological properties that influences their value to haematophagous insects, but macro-ecological variation in their relative availability within the environment with haematophagous insects becoming most efficient at extracting and using the blood resources of the host species they are most likely to encounter (Chaves et al., 2010, Guarneri et al., 2009). Investigations presented elsewhere in this thesis aimed to test for host species –specific differences in fitness of African malaria vectors (Chapter 3), and its relationship to their defensive behaviour (Chapter 4). The aim of the study presented here was to evaluate the impact of haematological variation on the fitness of these vectors, and explore whether these differences could account for their pattern of host choice in nature.

Of all the insect vectors of disease, the ones whose host species choice has the most direct impact on human health and well-being are the *Anopheles* mosquitoes who transmit malaria in Africa (Coetzee, 2004). Of all insect vectors, these are responsible for the great loss of human lives (Smith et al., 2001, Smith et al., 2005), a phenomena that has been attributed to their highly specialized feeding on humans (Kiszewski et al., 2004). The major African malaria vectors include *An. arabiensis* and *An. gambiae s.s* (Coetzee, 2004). Whereas *An. gambiae s.s* feeds almost exclusively on humans in most African settings (Kiszewski et al., 2004), *An. arabiensis* is more opportunistic and can readily switch their feeding from humans to cows when available (Tirados et al., 2006, Kent et al., 2007). In fact, when both host species are available, *An. arabiensis* will preferentially feed on cows over humans (Duchemin et al., 2001). My previous experimental work (Chapter 3), measured the fitness of these vectors on a variety of different species (humans and domestic animals) that they are likely to encounter in their natural environment. These experiments provided the first demonstration that under relatively realistic semi-field conditions several aspects of the fitness of these vectors (e.g. feeding success, and survival) varies substantially between host species. The observed variation in mosquito fitness between these host-species did not appear to be correlated with indirect estimates of their host defensive behaviours (e.g.Chapter 3 & 4), suggesting that there is a need to identify other factors that may explain this. Consequently, here I sought to test an alternative explanation for host-species dependent variation in mosquito fitness: differences in haematological properties between host species that influence the uptake of blood and its conversion into resources for fecundity and survival.

Many properties of host blood such as the density of red blood cells (as measured by packed cell volume (PCV), white blood cells, haemoglobin concentration (Hb), and the concentration of plasma proteins, glucose, fibrinogens, leucocytes, and amino acids vary both within and between host species (Nemi, 1986, Wintrobe, 1933, Hawkey et al., 1991, Hawkey, 1991). Several of these factors have been implicated as influencing mosquito feeding and fitness under controlled laboratory settings (Taylor and Hurd, 2001, Shieh and Rossignol, 1992). Here I focused on investigating two key haematological properties that I hypothesize are most likely to influence mosquito feeding efficiency and fitness: (1) the density of red blood cells (PCV), and (2) haemoglobin concentration (Hb). These properties could influence the nutritive values that mosquitoes obtain from a blood meal in several different ways. First, both PCV and Hb are indicators of the amount of protein per unit of blood volume (as protein is stored in haemoglobin, which is contained within red cells). Protein from haemoglobin is the primary resource required by mosquitoes for egg production (Zhou et al., 2007), thus as the content of this resource rises in blood (through increased levels of Hb per red cells and/or increased PCV), so too may the reproductive value of blood to mosquitoes. Thus, if mosquito fitness (e.g. fecundity) is maximized by the haemoglobin content of host blood, they may evolve a preference for the host species with the highest density of red blood cells (PCV) relative to other available alternatives (Vaughan et al., 1991, Daniel and Kingsolver, 1983). Alternatively, the PCV and/or Hb of host blood may still be a predictor of mosquito protein intake, although these haematological properties may be negatively correlated with one important aspect of mosquito feeding efficiency: the rate at which they can imbibe blood. Studies have shown that the viscosity of blood increases with PCV and Hb (Stone et al., 1968). Mosquitoes may have difficulty imbibing very viscous blood (Taylor and Hurd, 2001, Daniel and Kingsolver, 1983), and laboratory studies of the mosquitoes *Anopheles stephensi* and *Aedes aegypti* have shown that mosquito blood intake per meal (as indexed by average blood meal size) is highest on blood of intermediate rather than low or high PCV (Taylor and Hurd, 2001, Shieh and Rossignol, 1992). Consequently, it is possible that host species and individuals with intermediate PCV and Hb provide the greatest fitness reward to mosquitoes (Baylis and Mbwabi, 1995, Daniel and Kingsolver, 1983, Taylor and Hurd, 2001), and natural selection may drive mosquito host preference towards host species with these haematological characteristics. As the density of red blood cells per unit blood volume (PCV) is generally highly correlated with haemoglobin concentration (Posner et al., 2005), it is hypothesized that these traits will have similar impact on mosquito feeding success and fitness, although one may be more successful at explaining small-scale variation in these mosquito traits than the other.

This study tested whether naturally occurring variation in the PCV and Hb concentration within and between host species can explain the differential fitness benefits that African malaria vectors acquire from feeding on a variety of commonly encountered host species (as described in Chapter 3). The ultimate aim was to evaluate the relative importance of host haematological properties in structuring vector – host species associations by testing for a correlation between haematological properties that appear to optimize vector fitness and the natural host species preference of these vectors in nature. In examining the impact of variation in host PCV and Hb on the fitness of the African malaria malaria vectors *An. arabiensis* and *An. gambiae s.s*, the following specific hypotheses were tested: (1) there is significant variation in the PCV and Hb concentration between the host species commonly available in their environment, (2) naturally-occurring variation in PCV and Hb between host species influences the feeding efficiency and subsequent fitness of malaria vectors, (3) vector feeding efficiency and fitness is maximized on host species with either (i) intermediate or (ii) high values of PCV and Hb, and (4) host species with optimal haematological properties for feeding success and fitness are the ones naturally preferred by these vectors.

5.2 Methods

5.2.1 Study site and mosquito colonies

The study was conducted at the Ifakara Health Institute (IHI) situated within the Kilombero valley of southern Tanzania (7º44'-9º26'S/35º33'-36º56'E). Each of the 3 main African malaria vectors *An. gambiae s.s*, *An. arabiensis* and *An. funestus* are found in this area where they contribute to one of the highest rates of malaria transmission on record (Smith et al., 1993, Charlwood et al., 1998, Haji et al., 1996, Charlwood et al., 1995). Other details of vector ecology and malaria epidemiology in this area can be found in Chapter 2. These experiments were conducted on the two main vector species in this region *An. arabiensis* and *An. gambiae s.s*. Mosquitoes used in these experiments were obtained from insectary colonies maintained at the IHI as described in Chapter 2 and 3.

5.2.2 Mosquito feeding assays using unrestrained hosts

The fitness of both vector species was evaluated after exposure to 1 of 6 different host types in bioassays conducted under semi-field conditions (for details, see Chapter 3). The host species investigated were adult humans, and four other animal species that are abundant within domestic environments of the Kilombero Valley and other areas of east Africa where these vectors are found: chickens, cows, dogs and goats. Two different categories of human host were investigated: (1) exposed (e.g. unprotected by a bed net to simulate the conditions under which the *Anopheles*-human association originally evolved), and, (2) protected by a typical, untreated net following WHO standard procedures (WHO, 2005), to represent the conditions under which vectors typically encounter humans in this region (Russell et al., 2010, Killeen et al., 2006). Additionally, separate groups of cattle were investigated: cows and calves (see Chapter 3).

On each night of experiments, one vertebrate host of a randomly selected species was placed inside an experimental hut within the semi-field system (SFS), and a cohort of either 200 female *An. gambiae* s.s or *An. arabiensis* were released into the SFS outside of the experimental hut where the host was held (Chapter 3). During the night, mosquitoes had the potential to enter and feed on the host in the hut (Chapter 3). The next morning the entire area of the SFS chamber including both inside and outside of the experimental hut and its exit traps were intensively searched for mosquitoes. All detected mosquitoes were recaptured by mouth aspirator, and identified as being fed or unfed, live or dead. Six different individuals from each host treatment group were used in these experiments (2 vector species x 7 host types x 6 individuals/host type = 84 trial nights). After each trial, all alive, blood fed mosquitoes were recaptured and individually monitored to estimated their blood meal size, fecundity and survival (see Chapter 3).

5.2.3 Assessment of packed cell volume (PCV) and haemoglobin concentration (HB)

PCV and Hb measurements were taken from all hosts that participated in mosquito feeding trials (approximately 2 hours before hosts entered into the experimental hut). A blood sample of about 1-2 ml was withdrawn from each host into a heparinized vacutainer tube. The haemoglobin (Hb) concentration of this blood was measured from a 10µl sample aliquoted into a standard disposable microcuvette and that was measured in a HemoCue® hemoglobinometer (Haemoglobin Whole Blood Test System, HemoCue Limited, Derbyshire, UK) (Posner et al., 2005). The PCV of host blood was also measured using the micro - centrifugation method (MicroHematocrit Centrifuge, Hettich Instrument L. P., Tuttlingen, Germany)(Al-Odeh et al., 1994). Blood was collected in a 10 µl micro-capillary tube that was sealed at the bottom using clay and centrifuged for 10 minutes at 3000 rpm. The centrifuged capillary tube was then aligned along the scale of a haematocrit reader to measure the percentage of the sample composed of packed red cells (Brian et al., 2000).

5.2.4 Estimating the rate of blood intake by mosquitoes

To estimate the influence of variation in haematological properties on the rate of blood intake by vectors, an additional round of experiments was conducted in which the time required by mosquitoes to feed to repletion on different host individuals and species was directly measured. On account of the need to directly observe and time the duration of feeding, these experiments were conducted on hosts that were restrained from moving during the mosquito feeding process. These experiments were conducted on the same host individuals (6 per host type) that participated in the natural feeding bioassays. During these experiments, human hosts (volunteers) were asked to apply a transparent cup containing one mosquito directly to the skin of their forearm and refrain from moving until the experiment was complete. Animal hosts were physically restrained from movement by a variety of methods (as described in Chapter 4).

During all experiments, one unfed female mosquito (4-6 days old) that had been previously starved of glucose and water for 6 hours was directly applied to the surface of host skin. An observer holding a stopwatch started timing mosquito feeding as soon as it began probing the skin and their abdomen began to turn red, and stopped when the mosquito became fully engorged and withdrew its proboscis. In these experiments, humans were considered as one host group (e.g. not with and without a bed net). Experiments were replicated by using 6 different mosquitoes per host individual to yield a total sample size of 432 mosquito feeding rates (6 mosquitoes/host individual × 6 host individuals/host type × 6 host types × 2 mosquito species).

5.2.5 Ethical considerations

Ethical procedures were followed as described in Chapter 3 & 4.

5.2.6 Statistical analyses

5.2.6.1 Haematological variation between host species

Variation in the Hb and PCV between host species was analysed by generalized linear mixed models (lmer) in the R statistical software package (Crawley, 2007). In this analysis, ‘host species’ was treated as a main effect. PCV and Hb measurements were taken from each host individual at two different time points: once during the experiment with *An. arabiensis*, and once with *An. gambiae s.s*. Consequently, experiment type (*An. arabiensis* or *An. gambiae s.s*) was used as a surrogate for the time of measurement and treated as a random effect. The main effect ‘host species’ was added into a null model containing only the random effect to yield the full model. The significance of ‘host species’ was tested by comparing a null consisting only of the ‘random effect’ and full model consisting of the ‘random effect’ and ‘host species’ using likelihood ratio test (LRT) in R. When host species was found to have a statistically significant effect on either PCV or Hb, a Tukey post hoc test was conducted to identify which pairs of host species were significantly different from one another. The linear relationship between PCV and Hb was also tested using generalized linear mixed models, with PCV specified as the ‘response variable’ and Hb as the independent explanatory variable.

5.2.6.2 Impact of haematological properties on mosquito feeding and fecundity

Statistical analysis was conducted to evaluate whether variation in the mosquito feeding efficiency and fitness between host individuals and species could be accounted for by variation in host PCV and Hb. Previous study demonstrated that mosquito feeding success (blood meal size) is maximized at an intermediate level of PCV (Taylor and Hurd, 2001), suggesting that relationship between the haematological variables and mosquito fitness would be curved rather than linear. The appropriate analysis would be based on categorizing explanatory continuous variable into three discrete groups to test for both monotonic and curvilinear relationship between the haematological properties and mosquito fitness traits. Prior to analysis, the continuous values of PCV and Hb were converted into categorical variables of ‘low’, ‘medium’ and ‘high’ by dividing their range (from lowest to highest value) into 3 equally spaced intervals. The influence of these two categorical explanatory variables on 4 mosquito feeding and fitness traits was then investigated: duration of feeding (in seconds), blood meal size (µg of haematin), blood intake rate (µg haematin/second) and the number of eggs laid. As above, associations between explanatory and response variables were tested using generalized linear models in R statistical software (Crawley, 2007). Here the variables of ‘host species’, ‘PCV’ and ‘Hb’ were treated as fixed categorical effects whereas the host individual from which multiple measurements were treated as a random repeated measures effects. The approach taken was to compare a range of alternative statistical models based on different combinations of these 3 potential explanatory variable models, and identify which could account for the most variation in mosquito feeding and fitness. Comparisons between alternative statistical models were made on the basis of the Akaike Information Criterion (AIC) (Burnham and Anderson, 2002); a measure describing the amount of information explained by a particular statistical model relative to the total information available in the data set (Burnham and Anderson, 2002).

A multi-step model selection procedures was used to identify the best statistical model of mosquito feeding and fitness. In the first step, each of the 3 potential explanatory variables (host species, Hb and PCV) was individually added to a null model that contained only the random effect, and the negative likelihood in ANOVA procedure used to test their statistical signifance (Crawley, 2007, Burnham and Anderson, 2002). Next, the ‘best’ statistical model out of these four competing alternatives (the null and 3 single-variable models) was identified on the basis of differences in their AIC ($\Delta_{i,}$ calculated as the AIC_i of a particular model (i) minus the AIC of the model with lowest value ($\Delta_i = AIC_i - AIC$). Differences in AIC were then scaled to obtain the weighted Akaike values ($wAIC_i$ values ranging from 0 – 1) which is an indicator of the strength of statistical support for a particular model relative to alternatives in the competing set (Burnham and Anderson, 2002).

$$wAIC_i = \frac{\exp\left(-\frac{\Delta_i}{2}\right)}{\sum_{r=1}^{R}\exp\left(-\frac{\Delta_r}{2}\right)}$$

Alternative models were ranked based on descending order of Akaike weights ($wAIC_i$); with the strength of support of two different models considered as being tied if their $wAIC_i$ differed by less than 0.2 (Burnham and Anderson, 2002). A model was selected as ‘best’ when it had the highest wAICi and if was a statistically significant improvement over the null model (ANOVA test).

A second step of model selection was conducted to test if the explanatory power of the best model identified in the 1st round of selection (containing only 1 of 3 potential explanatory variables) could be improved further by the addition of other explanatory variables not initially selected. Here, the two remaining explanatory variables (of Hb, Host species or PCV) were individually added to the best first-round model. The likelihood ratio test (ANOVA procedure) was used to test whether these variables were statistically significant when added to the best model from the first round, and their wAICi calculated. In this 2nd round, a model was selected as best if it had both the highest wAICi and was a statistically significant improvement from the best model identified in the 1st round. Where warranted, a third and final round of model selection was conducted following the same procedure employed during the 1st and 2nd step, to test if addition of the remaining unselected explanatory variable led to further statistically significant improvement of the model.

5.2.6.3 Impact of haematological properties on mosquito survival

The impacts of host species, PCV and Hb on the post–feeding survival of mosquitoes were tested using the Cox proportional hazards model (coxph) in R (Crawley, 2007). A frailty function was incorporated into the Cox Proportional Hazard Model (coxph) that allowed ‘experimental replicate’ (6 within each host species treatment) to be fitted as a random effect. Initially, the statistical significance of host species, PCV and Hb were individually tested when added to a null model that included only random effect of ‘experimental replicate’ using a likelihood ratio test. The maximum likelihood scores for each of the three competing single-variable Cox models were used to compute the AIC values of each single-variable models (on the basis of the negative log-likelihood value (Burnham and Anderson, 2002, Crawley, 2007); which were then used for the computation of their $wAIC_{i.}$ Models were then ranked on the basis of this weighting procedure as described above (Burnham and Anderson, 2002), and a 'best' model out of 3 competing single-variable alternatives was identified. After identifying which of the 3 potential explanatory variables provided the best model of mosquito survival, further rounds of model selection were conducted following the protocol described above to test if further improvements could be made by incorporating additional, previously unselected variables.

5.3 Results

A total of 16,517 *Anopheles* vectors (49.14% *An. gambiae s.s*, 50.96% *An. arabiensis*) were observed over 84 nights of semi-field experiments. A further 338 mosquitoes (51% *An. gambiae s.s,* 49% *An. arabiensis*) were exposed to the same host individuals used in these semi-field assays in further experiments (when they were restrained) to estimate the effect of host haematological factors on blood intake rate.

5.3.1 Variation in haematological properties between host species

The PCV of host blood varied significantly between host species ($\chi^2_5 = 47.59$, $p < 0.001$, Figure 5.1) with the red cell density of human hosts being 1.5 to 1.8 times higher than any other host type ($p < 0.001$ in all two-way comparisons). There was no statistically significant difference between the PCV of animal (non-human) hosts ($p > 0.05$ for all two- way comparisons (Figure 5.1). Blood Hb levels also varied significantly between host species ($\chi^2_5 = 41.75$, $p < 0.001$,Figure 5.2). The Hb of human hosts was generally greater than of animal hosts ($P < 0.001$ in all two-way comparisons). Within animal hosts, the only observed difference in blood Hb was between chickens and goats, with the former having a significantly higher Hb concentration than the latter ($T = -3.27$, $P = 0.01$). Pooling over all host species, there was significant positive correlation between the PCV and Hb of host individuals ($\chi^2_1 = 99.03$, $p < 0.001$, $r^2 = 0.96$ (Figure 5.3).

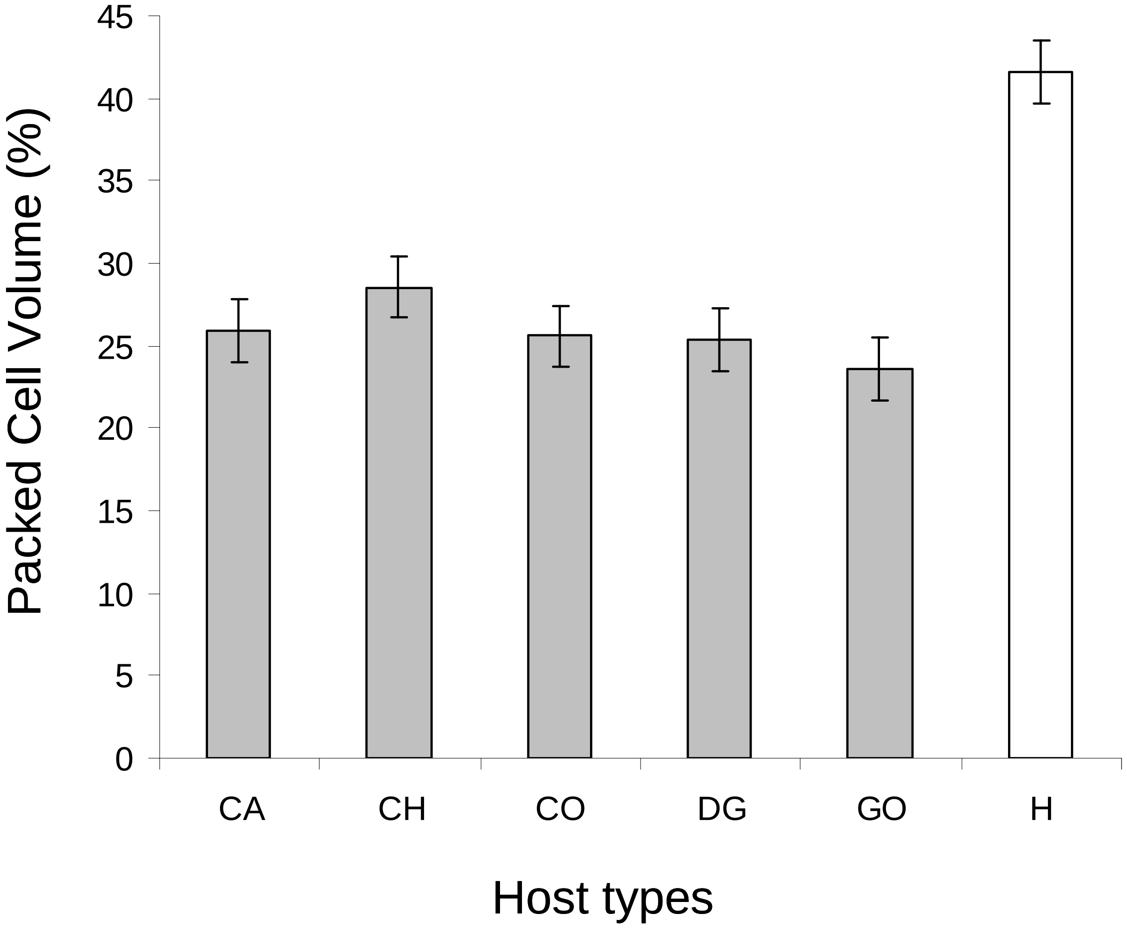

Figure 5.1. The mean packed cell volume (PCV, ± 1 s.e.m) of host species fed upon by mosquito vectors. The x-axis indicates different host species: CA - calf, CH - chicken, CO - cow, DG - dog, GO – goat, H – human. Bars of similar colour are not statistically different from one another, bars of different colours represent groups that are significantly different.

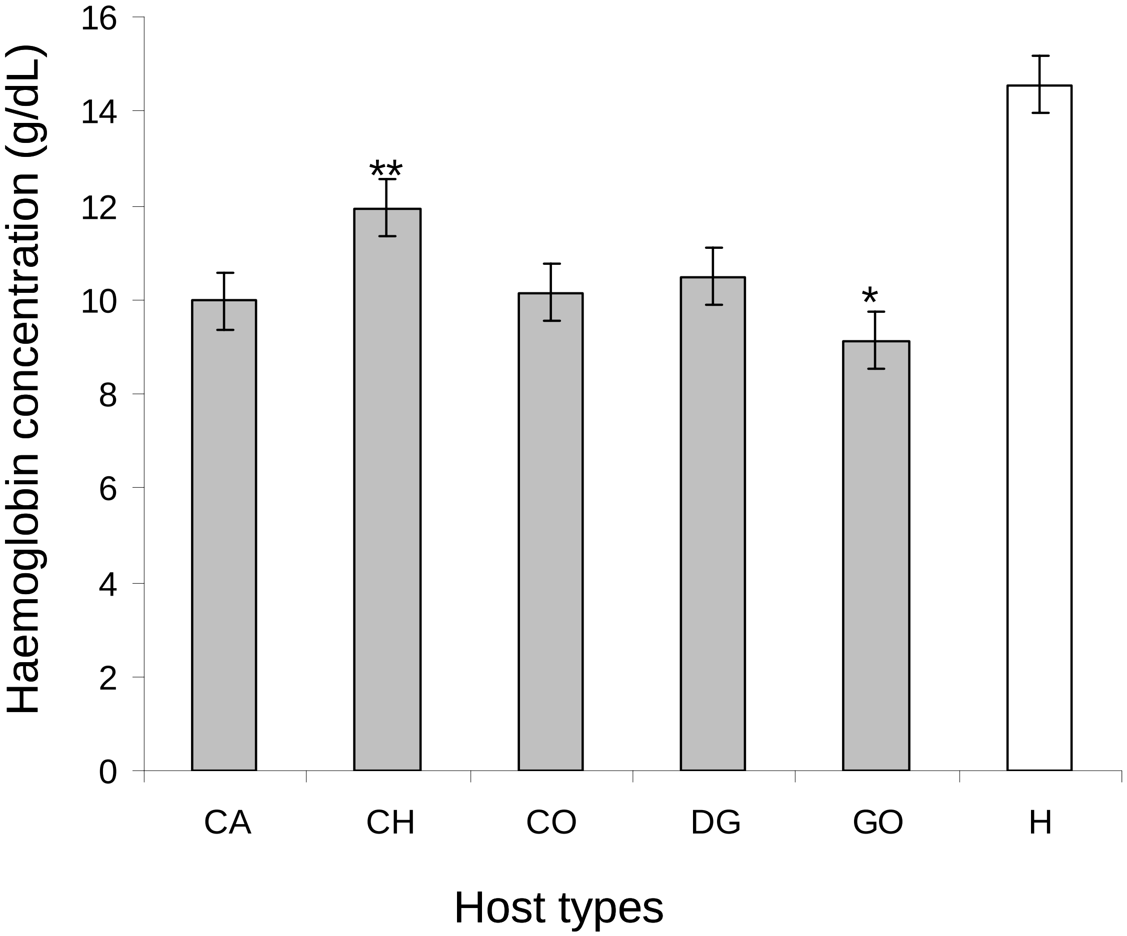

Figure 5.2. The mean haemoglobin concentration (Hb, ± 1 s.e.m) of host species fed upon by mosquito vectors.The x-axis indicates different hosts: CA - calf, CH - chicken, CO - cow, DG - dog, GO – goat, H – human. Bars of similar colour are not statistically different from one another except for two groups with and *, bars with different colours represent groups that are significantly different.**

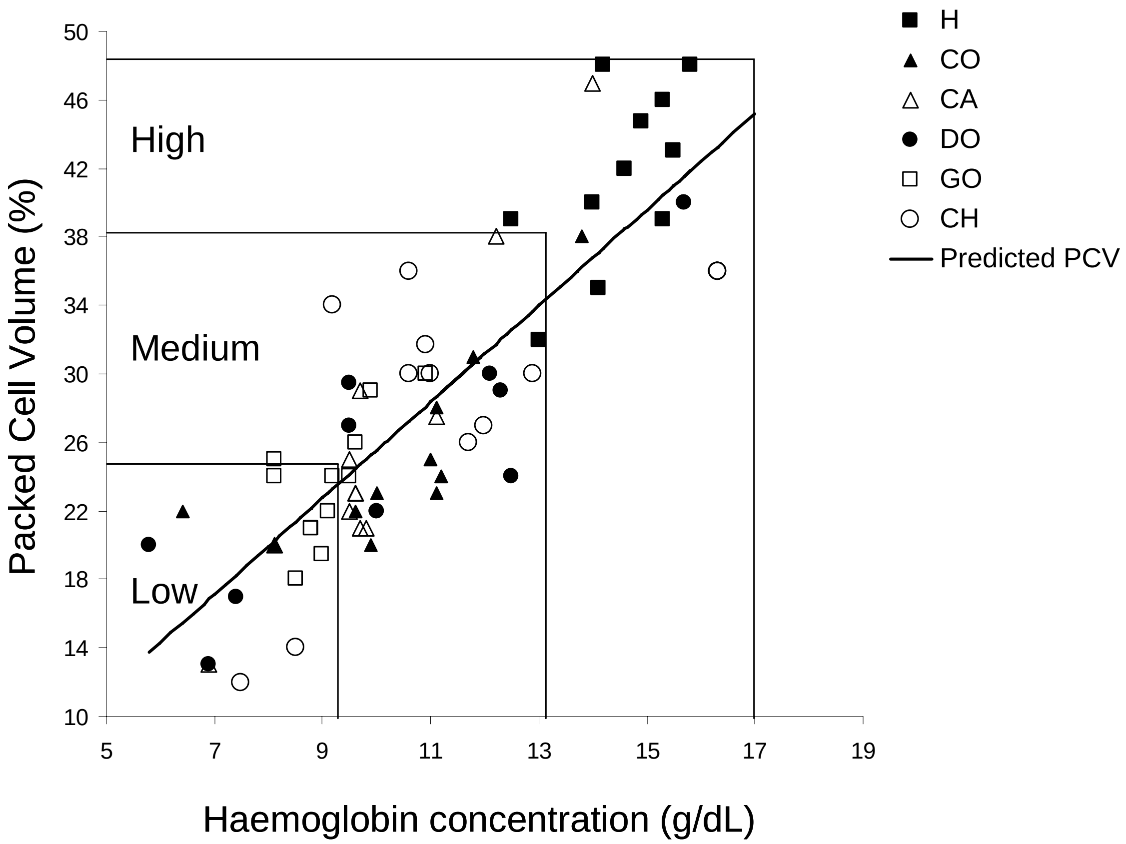

Figure 5.3. Best fit linear relationship between the haemoglobin concentration (Hb) and packed cell volume (PCV) of hosts individuals used in these experiments. Symbols represent the different host species from which measurements ere taken.

5.3.2 Impacts on mosquito feeding efficiency

In feeding experiments with restrained hosts, the amount of time it took mosquitoes to feed to repletion did not vary between host species in either mosquito species (*An. arabiensis*: $\chi^2_5 = 5.98$, $P = 0.31$, *An. gambiae s.s*: $\chi^2_5 = 5.23$, $P = 0.39$, Table 5.1, Table 5.2, Figure 5.4). Similarly, the feeding duration of *An. arabiensis* was not significantly related with variation in host Hb ($\chi^2_2 = 2.07$, $P = 0.35$) or PCV ($\chi^2_2 = 4.88$, $P = 0.09$, Figure 5.4a). However, the duration of feeding in *An. gambiae s.s* was significantly longer on hosts in the high PCV category than in the low and medium groups ($\chi^2_2 = 13.70$, $P < 0.001$, Figure 5.4b), but did not vary between hosts in the medium and low PCV groups ($z = -0.94$, $P = 0.61$). A similar trend of longer feeding duration on hosts within the high PCV group was observed for *An. arabiensis*, but did not reach statistical significance ($P = 0.09$, Figure 5.4b). The duration of *An. gambiae s.s* feeding was unrelated to variation in Hb ($\chi^2_2 = 3.80$, $p = 0.15$). When added to the PCV- only model of *Anopheles gambiae s.s* feeding duration, neither host species ($\chi^2_5 = 1.95$, $P = 0.86$) nor Hb ($\chi^2_2 = 0.83$, $P = 0.66$) were statistically significant (Table 5.1).

Blood feeding duration: model comparison and selection

Vector species

	An. arabiensis					*An. gambiae s.s*				
Model	AIC	Δ_i	ω_i	Ranking	P-value	AIC	Δ_i	ω_i	Ranking	P-value
Step 1										
Null	604.67	0.88	0.33	2		575.90	9.70	0.01	2	
HS	608.69	4.90	0.04	4	0.31	580.67	14.47	0.001	3	0.39
PCV	603.79	0	0.51	1	0.09	566.20	0	0.98	1	0.001
Hb	606.60	2.81	0.12	3	0.35	576.10	9.90	0.01	2	0.15
Step 2										
PCV	-	-	-			566.20	0	0.82	1	
PCV+Hb	-	-	-	-		569.37	2.03	0.17	2	0.66
PCV+HS	-	-	-	-		574.25	3.44	0.01	3	0.86

Table 5.1. Comparison and selection of statistical models explaining variation in the blood feeding duration of two malaria vector species. The explanatory variables tested were: HS – host species, PCV – packed cell volume and Hb – host haemoglobin concentration (Low, Medium and High). At each stage of model selection, models were ranked on the basis of Δ_i values and the Akaike weights (ω_i) as decribed in the text.

Host species	**Duration of feeding (second)**		**Blood meal size (µg)**	
	An.arabiensis	*An.gambiae s.s*	*An. arabiensis*	*An. gambiae s.s*
Human	256.80 ± 36.00	283.70 ± 41.35	13.54 ± 1.99	15.23 ± 1.85
Calf	216.00 ± 34.80	222.91 ± 40.33	5.68 ± 1.72	3.73 ± 2.22
Chicken	210.00 ± 30.00	169.99 ± 40.51	4.96 ± 1.57	2.94 ± 1.82
Cow	252.60 ± 35.40	233.87 ± 40.76	6.72 ± 2.21	3.46 ± 2.23
Dog	225.60 ± 35.40	246.10 ± 40.76	13.40 ± 2.28	5.58 ± 2.04
Goat	166.20 ± 38.40	181.08 ± 44.50	6.06 ± 2.21	3.26 ± 2.07

Table 5.2. The average time required (seconds) for two malaria vector species to blood feed to repletion on different host species (hosts restrained during feeding).

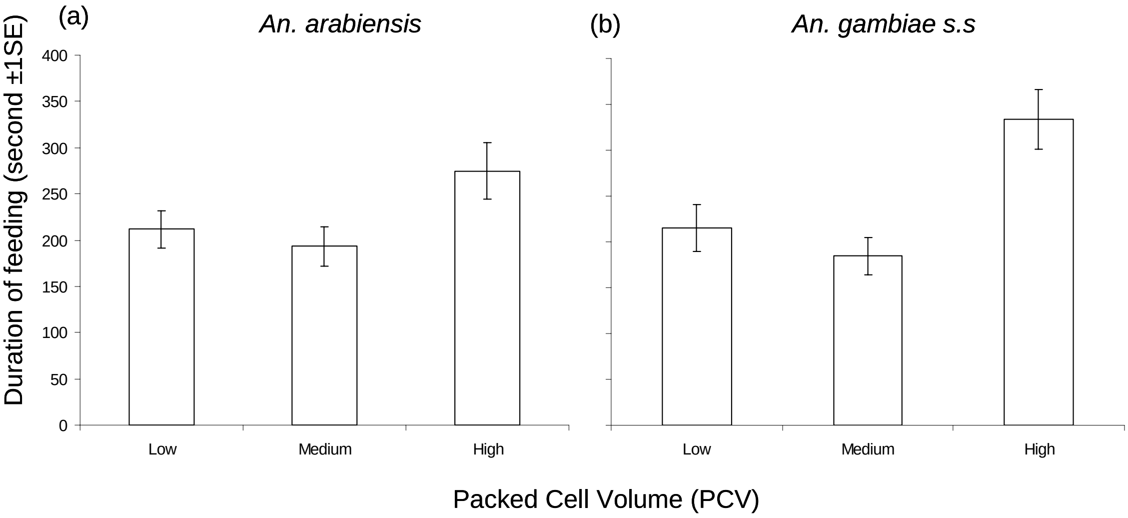

Figure 5.4. Estimated duration of feeding (mean, ±1 s.e.m) of two mosquito species to feed to repletion on hosts in different packed cell volume categoriesa).*An. arabiensis*, and b) *An. gambiae s.s*.

Blood intake rate was calculated by dividing the size of a blood meal obtained by an individual mosquito (on a restrained host) by the time it took them to acquire it (number of seconds). The blood intake rate of both mosquito species varied significantly between host species in both *An. arabiensis* ($\chi^2_5 = 25.13$, $P < 0.001$, Table 5.2, Figure 5.5a) and *An. gambiae s.s* ($\chi^2_5 = 30.09$, $P < 0.001$, Figure 5.5b). The blood intake rate of *An. arabiensis* was significantly higher on humans than on all other host types ($P < 0.001$, in all 2-way comparisons) except for dogs on which it was similar (human versus dogs: $T = -1.64$, $P = 0.32$, Figure 5.5a). Additionally, when considered independently of host species, there was a significant association between the blood intake rate of *An. arabiensis* and host PCV *(*$\chi^2_2 = 12.39$, $p = 0.007$,Figure 5.5c, Table 5.3), but not with their Hb concentration ($\chi^2_2 = 3.20$, $p = 0.07$,Figure 5.5e, Table 5.3). *Anopheles arabiensis* had a greater intake rate on hosts in the high PCV category than on those in the medium ($T = 3.60$, $P < 0.001$) and low groups ($T = 3.45$, $P = 0.002$). Of all 3 single- variable models of *An. arabiensis* blood intake rate, the one containing host species had the highest degree of statistical support ($\omega_i = 0.96$, Table 5.3). This 'host-species' only model could not be significantly further improved by the addition of either PCV or Hb (Table 5.3).

The blood intake rate of *An. gambiae s.s* was significantly higher on humans than all other host species ($P < 0.001$,Figure 5.5 b). Blood intake rate also varied significantly between hosts in different PCV ($\chi^2_2 = 7.16$, $P = 0.03$) and Hb groups ($\chi^2_2 = 10.75$, $P = 0.005$). Of all 3 alternative models of *An. gambiae s.s* blood intake, the host species model had the greatest strength of statistical support ($\omega i = 0.99$, Table 5.3). This host species-only model was significantly improved by the further addition of host PCV ($\chi^2_2 = 12.26$, $P = 0.002$, Figure 5.5d), but not Hb concentration ($\chi^2_2 = 0$ $P = 1$, Figure 5.5f). After accounting for variation due to host species, *An. gambiae s.s* blood intake was predicted to be highest on hosts within the low ($T = -2.91$, $P = 0.01$) and medium PCV ($T = -3.42$, $P = 0.001$) than high PCV group, but did not vary between hosts in the low and medium PCV groups ($T = 0.19$, $P = 0.98$). This model of *An. gambiae s.s* blood intake (incorporating both host species and PCV) was not significantly improved by the further addition of Hb ($\chi^2_2 = 2.55$, $P = 0.28$,Table 5.3).

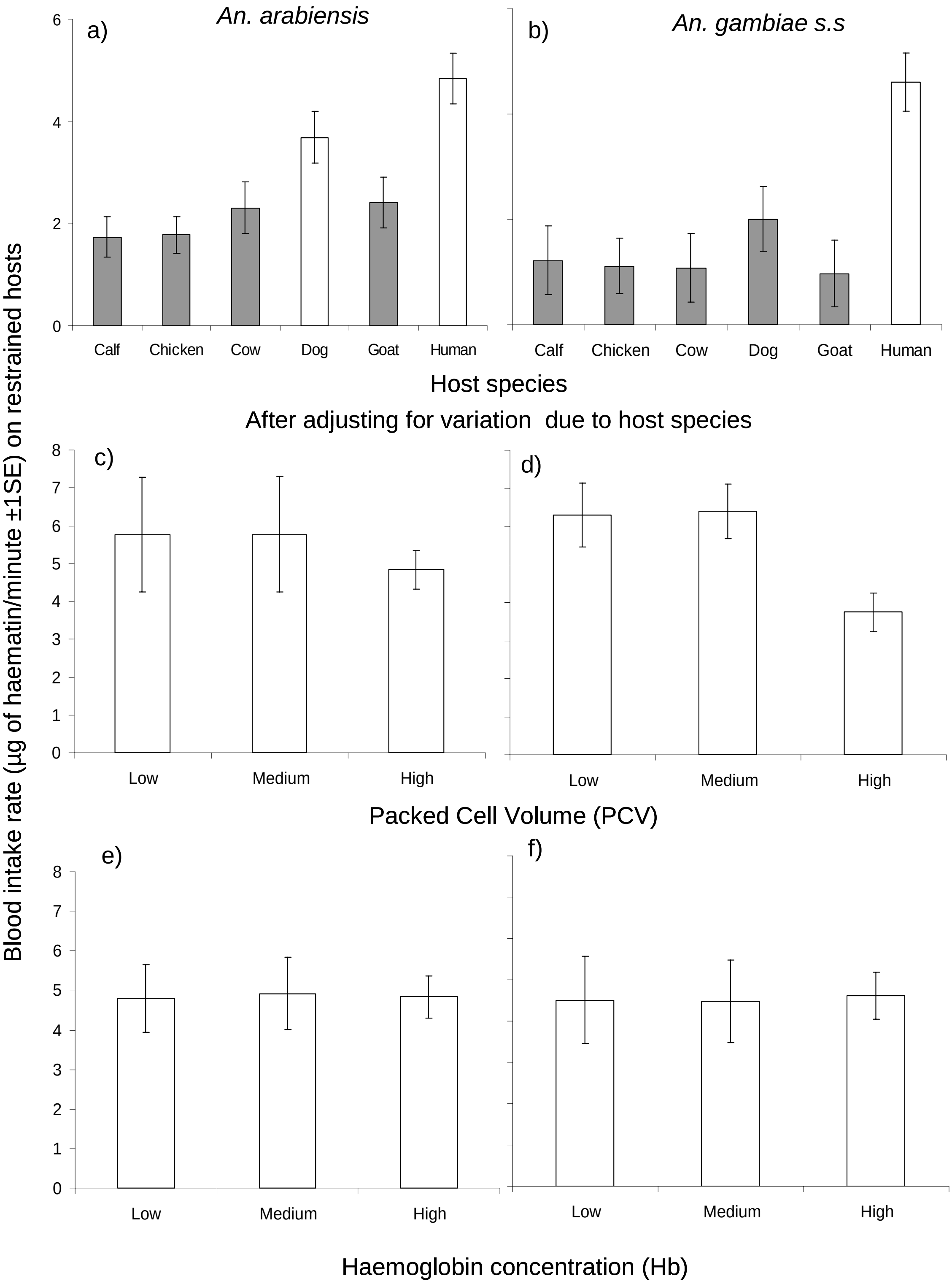

Figure 5.5. The blood intake rate (mean, ± 1 s.e.m) of two species of malaria vectors (*An. arabiensis*, a, c & d, *An. gambiae s.s*, b, d, & f) in relation to variation in host species (a, b), packed cell volume (c , d) and haemoglobin concentration (e, f). Note that the predicted impacts of PCV and Hb are as estimated after controlling for variation in host species.

Blood intake rate on restrained hosts: model comparison and selection

Vector species

	An. arabiensis					*An. gambiae s.s*				
Model	AIC	Δ_i	ω_i	Ranking	P-value	AIC	Δ_i	ω_i	Ranking	P-value
Step 1										
Null	524.72	15.13	0.0005	4		464.10	20.1	$4.3X10^{-5}$	4	
HS	509.59	0	0.96	1	<0.001	444	0	0.99	1	<0.001
PCV	516.34	6.75	0.03	2	0.006	460.94	16.94	0.0002	3	0.03
Hb	521.53	11.94	0.002	3	0.07	457.34	13.34	0.001	2	0.005
Step 2										
HS	509.59	0	0.83	1		444	8.26	0.02	2	
HS+Hb	514.48	4.89	0.07	3	1	448.35	12.61	0.002	3	1
HS+PCV	513.76	4.17	0.10	2	1	435.74	0	0.98	1	0.002
Step3										
HS+PCV	-	-	-	-	-	435.74	0	0.81	1	
HS+PCV+Hb	-	-	-	-	-	437.20	1.46	0.19	2	0.28

Table 5.3. Comparison and selection of statistical models to explain variation in the blood intake rate of two malaria vector species. The explanatory variables tested were: HS – host species, PCV – packed cell volume and Hb – host haemoglobin concentration. At each stage of model selection, models were ranked on the basis of Δ_i values and the Akaike weights (ω_i) as decribed in the text.

A total of 4,216 blood meal size measurements were taken during experiments where mosquitoes were allowed to feed naturally on unrestrained hosts (*An. arabiensis*: n = 1,755, *An. gambiae s.s*: n = 2,461). Previous analysis indicated that under these conditions, the blood meal size obtained by *An. arabiensis* did not vary between host species ($\chi^2_6 = 3.28$, P = 0.77, Chapter 3). Further analysis here indicated that *An. arabiensis* blood meal size was also unrelated to host PCV ($\chi^2_2 = 2.63$, P = 0.27) or Hb groups ($\chi^2_2 = 4.41$, P = 0.11,Table 5.4). In contrast, the blood meal size of *An. gambiae s.s* (when feeding on unrestrained hosts) was significantly related to host species ($\chi^2_6 =$ 22.502, P < 0.001, Chapter 3), PCV ($\chi^2_2 = 10.23$, P = 0.01, Figure 5.6a), and Hb ($\chi^2_2 =$ 9.46, P = 0.01, Figure 5.6b). When feeding on unrestrained hosts, *An. gambiae s.s* took larger blood meals from hosts in the high PCV versus medium group (T = 3.28, P = 0.003, Figure 5.6a), but their blood meal size did not vary between hosts in high and low PCV groups (T = -1.26, 0.42). With respect to host Hb, *An. gambiae s.s* took larger sizes of blood meal from hosts in the high than the medium (T = 2.62, P = 0.02) and low groups (T = 2.69, P = 0.02, Figure 5.6b). Comparison of the three statistically significant single variable models of *An. gambiae s.s* blood meal size indicated that the 'host species' model had the strongest degree of statistical support ($\omega i = 0.83$,Table 5.4). Neither the further addition of PCV ($\chi^2_2 = 2.10$, P = 0.35), or Hb ($\chi^2_2 = 0$, P = 1) significantly improved the explanatory power of this 'host species' only model (Table 5.4); suggesting that the putative differences in blood meal size due to PCV and Hb when these variables were considered in isolation may have actually been driven by underlying variation in host species (with blood meal size being highest on the host species that also had the highest PCV and Hb levels, e.g. humans).

Bloodmeal size from unrestrained hosts: Model comparison and selection

Vector species

	An. arabiensis					*An. gambiae s.s*				
Model	AIC	Δ_i	ω_i	Ranking	P-value	AIC	Δ_i	ω_i	Ranking	P-value
Step 1										
Null	12044	0.4	0.37	1		14477.8	10.5	0.004	4	
HS	12052.8	9.2	0.004	3	0.77	14467.3	0	0.83	1	<0.001
PCV	12045.4	1.8	0.18	2	0.27	14471.6	4.3	0.10	2	0.01
Hb	12043.6	0	0.45	1	0.11	14472.4	5.1	0.10	2	0.01
Step 2										
HS	-	-	-	-	-	14467.3	0	0.67	1	
HS+ PCV	-	-	-	-	-	14469.2	1.9	0.26	2	0.35
HS+Hb	-	-	-	-	-	14471.8	4.5	0.07	3	1

Table 5.4. Comparison and selection of statistical models to explain variation in the blood meal size of two malaria vector species obtained from from un-restrained hosts.The explanatory variables tested were: HS – host species, PCV – packed cell volume and Hb – host haemoglobin concentration. At each stage of model selection, models were ranked on the basis of Δ_i values and the Akaike weights (ω_i) as decribed in the text.

An. gambiae s.s

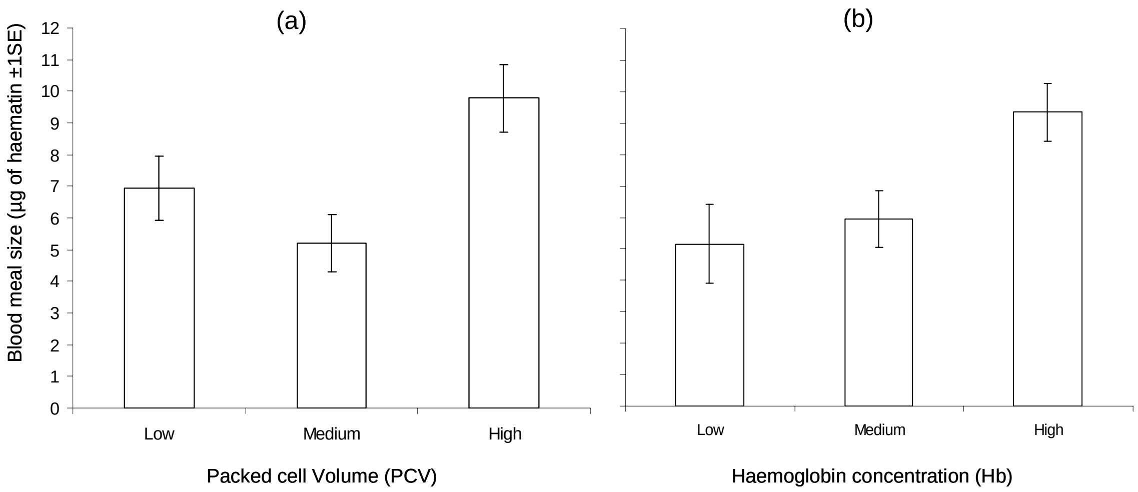

Figure 5.6. Estimated blood meal size (mean µg of haematin, ±1 s.e.m) of *An. gambiae s.s* from unrestrained hosts

5.3.3 Impacts on mosquito fecundity

Previous analysis indicated that the number of eggs laid by mosquitoes after feeding on unrestrained hosts did not vary significantly between host species in either vector species (*An. arabiensis*: $\chi^2_6 = 5.63$, $P = 0.47$, *An. gambiae s.s:* $\chi^2_6 = 1.44$, $P = 0.96$, Chapter 3). Further analysis here indicates that the egg production of *An. arabiensis* was similarly unrelated to host PCV ($\chi^2_2 = 2.92$, $P = 0.23$) or the Hb group ($\chi^2_2 = 0.54$, $P = 0.76$, Table 5.5 & 5.6). Similarly, the fecundity of *An. gambiae s.s*. was also unrelated to host PCV ($\chi^2_2 = 4.70$, $P = 0.09$,) or Hb concentration ($\chi^2_2 = 3.84$, $P = 0.15$, Table 5.5 & 5.6).

Fecundity of two vector species on unrestrained host species		
Haematological Properties	*An. arabiensis*	*An. gambiae s.s*
PCV:Low	80.46 ± 3.89	52.47 ± 2.98
Medium	86.98 ± 4.82	52.21 ± 2.76
High	74.96 ± 5.15	60.19 ± 3.01
Hb: Low	82.83 ± 3.50	50.81 ± 3.53
Medium	81.83 ± 6.19	53.03 ± 2.77
High	77.40 ± 5.29	58.77 ± 2.71

Table 5.5. Relationship between the number of eggs (mean ± s.e.m) produced by two mosquito species and different levels of PCV and Hb of unrestrained host species.

Mosquito fecundity on unrestrained hosts: model comparison and selection										
Vector species										
	An. arabiensis					*An. gambiae s.s*				
Model	AIC	Δ_i	ω_i	Ranking	P-value	AIC	Δ_i	ω_i	Ranking	P-value
Step1										
Null	3441.9	5.9	0.04	4		11051	0.7	0.30	2	
HS	3441.3	5.3	0.05	3	0.47	11061.6	11.3	0.001	3	0.96
PCV	3436	0	0.70	1	0.23	11050.3	0	0.43	1	0.09
Hb	3438.4	0.23	0.21	2	0.76	11051.2	0.9	0.27	2	0.15

Table 5.6. Comparison and selection of statistical models to explain variation in the number of eggs produced by two malaria vector species after feeding on unrestrained hosts.The explanatory variables tested were: HS – host species, PCV – packed cell volume and Hb – host haemoglobin concentration.

5.3.4 Impacts on mosquito survival

Previous analysis (Chapter 3) indicated that the post-feeding survival of *An. arabiensis*, was similar after feeding on all host species ($\chi^2_6 = 8.6$, $P = 0.2$), however that of *An. gambiae s.s* varied between host species ($\chi^2_6 = 106.4$, $P < 0.001$), with survival being highest on humans than on any other host species except cows. Further analysis indicated that the post-feeding survival of *An. arabiensis* was also significantly related to host PCV ($\chi^2_2 = 11.6$, $P = 0.003$) and Hb concentration ($\chi^2_2 = 16$, $P = 0.0003$, Table 5.7, Table 5.8). Of all statistically significant single-variable models of *An. arabiensis survival*, the one with the highest degree of statistical support was that containing Hb ($\omega_i = 0.89$, Table 5.7). The further addition of host PCV ($\chi^2_6 = 8.5$, $P = 0.01$, Table 5.7) to the Hb-only model was statistically significant. The further addition of 'host species' as an explanatory variable did not significantly improve the statistical model of *An. arabiensis* survival based on host Hb and PCV variation ($\chi^2_6 = 12.8$, $P = 0.05$, Table 5.7). In this final 'best' model of *An. arabiensis* survival, their mortality was predicted to be lower after feeding on hosts in the high than low Hb group (Hazard Ratio: 0.62, 95% CI = 0.44 – 0.86, P = 0.004,Table 5.8), and similar on hosts in the low and medium group. In contrast to the effect of Hb, *An. arabiensis* survival was predicted to be higher after feeding on hosts in the low PCV than medium (Hazard Ratio: 1.26, 95% CI = 1.04 – 1.53, P = 0.02) and high PCV group (HR: 1.50, 95% CI = 1.09 – 2.09, P = 0.01, Table 5.8).

The post-feeding survival of *An. gambiae s.s* was also significantly related to host species ($\chi^2_6 = 106.4$, $P < 0.001$, Table 5.7 & Chapter 2), PCV ($\chi^2_2 = 18.4$, $P< 0.001$, Table 6 & 7) and Hb concentration ($\chi^2_2 = 12.8$, $P = 0.002$, Table 5.7, Table 5.8). Of the 3 potential single-variable models of *An. gambiae s.s* survival, the host species only model had a much higher degree of statistical support than other two alternatives ($\omega_i = 1$, Table 5.7). Both host PCV ($\chi^2_2 = 31.4$, $P < 0.001$) and Hb category ($\chi^2_2 = 11.1$, $P = 0.004$, Table 5.7) were statistically significant when added to this host-species model (Table 5.8). In the second round of model selection, the two-variable model containing host species and PCV had the greatest degree of statistical support ($\omega_i = 0.99$, Table 5.7). The further addition of Hb concentration to this model was also statistically significant ($\chi^2_2 = 78.6$, $P < 0.001$, Table 5.7), indicating that all 3 explanatory variables were predicted to influence *An. gambiae s.s* survival. After adjusting for variation due to Hb and PCV, *An. gambiae s.s* survival was predicted to be significantly higher on cows than on humans, and similar on humans, calves, dogs and goats (Table 5.8). Survival was predicted to be lower after feeding on net – protected humans and chickens than on the unprotected human control group. When mosquito survival rates were not adjusted for underlying variation in host PCV and Hb, *An. gambiae s.s* survival is predicted to be highest on humans (Chapter 3) suggesting that the advantage of anthrophily observed here may be driven by host species-specific variation in these haematological traits. As with *An. arabiensis*, host Hb and PCV were predicted to have differing impacts on mosquito mortality in the final model of *An. gambiae s.s* survival. Here after adjusting for variation due to host species, *An. gambiae s.s* survival was predicted to be positively associated with host PCV; with survival being significantly higher on hosts in the medium and high PCV groups than in the low group (61% and 54% respectively,Table 5.8). In contrast, medium and high levels of Hb were associated with reduced survival in *An. gambiae s.s* relative to the low group (Table 5.8).

Post –feeding survival on unrestrained hosts: Model comparison and selection

Vector species

An. arabiensis						*An. gambiae s.s*					
Model	AIC	Δ_i	ω_i	Ranking	P-value	Model	AIC	Δ_i	ω_i	Ranking	P-value
Step 1						Step 1					
HS	17765.2	7.2	0.02	3	0.2	HS	30880.2	0	1	1	<0.001
PCV	17762.8	4.8	0.08	2	0.003	PCV	30967	86.8	$1.4X10^{-19}$	3	0.002
Hb	17758	0	0.89	1	0.0003	Hb	30972.8	92.6	$7.8X10^{-21}$	2	<0.001
Step 2						Step 2					
Hb	17758	6.8	0.02	3		HS	30880.2	30.4	$2.5X10^{-7}$	3	
Hb+PCV	17751.2	0	0.63	1	0.01	HS+PCV	30849.8	0	0.99	1	<0.001
Hb+HS	17752.4	1.2	0.35	2	0.3	HS+Hb	30870.2	20.4	$3.7X10^{-5}$	2	0.004
Step 3											
Hb+PCV	17753.2	9.6	0.02	2		HS+PCV	30851.8	78.4	$9.4X10^{-18}$	2	
Hb+PCV+HS	17743.6	0	0.98	1	0.047	HS+PCV+Hb	30773.4	0	1	1	<0.001

Table 5.7. Comparison and selection of statistical models to explain variation in the post-feeding survival of two malaria vector species fed on unrestrained hosts. The explanatory variables tested were HS – host species, PCV – packed cell volume and Hb – host haemoglobin concentration. At each stage of model selection, models were ranked on the basis of Δ_i values and the Akaike weights (ω_i) as decribed in the text.

	An. arabiensis			*An. gambiae s.s*		
Haematological Properties	Odds Ratio (OR)	95% CI	P- values	Odds Ratio (OR)	95% CI	P- values
Full model						
Host species: Human *	-	-	-	-	-	-
Calf	-	-	-	0.98	0.78 – 1.24	0.8
Chicken	-	-	-	1.65	1.18 – 2.30	0.003
Cow	-	-	-	0.53	0.40 – 0.70	<0.001
Dog	-	-	-	0.84	0.59 – 1.18	0.3
Goat	-	-	-	1.24	0.93 – 1.66	0.1
Protected human	-	-	-	1.63	1.39 – 1.91	<0.001
Hb: Low*	-	-		-	-	-
Medium	1.01	0.81 – 1.27	0.91	2.24	1.76 – 2.86	<0.001
High	0.62	0.44 – 0.86	0.004	1.62	1.18 – 2.21	0.003
PCV: Low*	-	-		-	-	-
Medium	1.26	1.04 – 1.53	0.02	0.39	0.30 – 0.52	<0.001
High	1.50	1.09 – 2.09	0.01	0.46	0.30 – 0.68	<0.001

Table 5.8. The odds of mortality in *An. arabiensis* and *An. gambiae s.s* as estimated from a statistical model of their survival based on variation in host species, haemoglobin concentration (Hb), and packed cell volume (PCV). Estimates are given only for parameters that were statistically significantly related to survival.

5.4 Discussion

This study provides the first quantitative test of the impact of haematological variation in host PCV and Hb on the feeding efficiency and subsequent fitness of the African malaria vectors *An. arabiensis* and *An. gambiae s.s*, under relatively natural semi-field conditions. Overall, there was some evidence that these haematological traits influenced the blood intake efficiency of *An. gambiae s.s*, but not of *An. arabiensis* which performed similarly well regardless of variation in these host traits. In general, the blood feeding efficiency of *An. gambiae s.s* was highest on its preferred human hosts than any other species. However, neither variation in host PCV and Hb were significantly related to mosquito fecundity in either vector species. In contrast, both these haematological traits were predicted to have independent effect on mosquito post-feeding survival and in the case of *An. gambiae s.s* may provide a mechanistic explanation for why their survival is higher on human than animal hosts. This finding challenges the traditional assumption that host blood is a primarily a resource for reproduction and not long-term mosquito survival, and suggests that vector longevity may be more dependent on intrinsic host factors than previously assumed.

Here I observed significant variation in the PCV and Hb of host commonly found in and around domestic environments in rural Tanzania, with humans generally having the highest values of these haematological traits than other animal host species. Observations made here are broadly consistent with reference haematological indices reported for domestic animals and humans (Vasquez and Villena, 2001, Nemi, 1986) that indicate humans and dogs generally have higher values of PCV and Hb than cows, sheep, goats, and chickens (Nemi, 1986). However, the Hb and PCV values observed in the sample of dogs used here were below the reported normal ranges (e.g. by 1.13 – 6.91 for Hb, and 13.52 – 27.94% for PCV), which may either be the sample of dogs used here not being sufficiently representative, or of the published values in veterinary records not reflecting the average condition of dogs in rural African settings. Thus, with the possible exception of dogs, I conclude that the host species-specific haematological variation observed in these experiments here are representatives of the average values associated with these host species in nature.

The impact of host haematological variation on mosquito feeding efficiency differed between vector species. Specifically, neither the duration of blood feeding, rate of blood intake per second, nor final blood meal size of *An. arabiensis* was significantly impacted by either host Hb or PCV. In contrast, pooling across all host species, *An. gambiae s.s* were observed to take significantly longer to obtain a full blood meal from hosts in the high PCV group than all others. Neither host species nor Hb levels could further explain variation in *An. gambiae s.s* feeding duration. A potential explanation for this phenomenon is that the increased viscosity of blood in high PCV group reduces its flow rate through the *An. gambiae s.s* proboscis (Stone et al., 1968), delaying the rate at which it can be imbibed (Shieh and Rossignol, 1992) and consequently requiring mosquitoes to feed for longer before obtaining a full blood meal (Baylis and Mbwabi, 1995, Daniel and Kingsolver, 1983). Blood viscosity may not have been so restrictive to *An. arabiensis.* This species is significantly larger than *An. gambiae s.s* (Hogg et al., 1996), and has a food canal of a wider diameter (Daniel and Kingsolver, 1983), which may allow it to imbibe a wider range of blood than *An. gambiae s.s* with similar efficiency regardless of its viscosity. While extending the duration of blood feeding on hosts with relatively viscous blood (e.g. high PCV) could be a compensatory mechanism that allows mosquitoes to obtain a full blood meal, prolonging the feeding encounter may expose mosquitoes to other costs including increasing feeding associated mortality (Anderson and Roitberg, 1999) and / or predation (Roitberg et al., 2003). One way to avoid these costs would be to feed on hosts at times when they least likely to mount defensive behaviour, which has been hypothesized as the reason why *An. gambiae s.s* has evolved to feed on humans at night time when they are asleep (Day and Edman, 1984).

In addition to its effects on feeding duration, host PCV was also found to influence the blood intake rate (mass of haematin/second) of *An. gambiae s.s*. Host species was also a significant predictor of *An. gambiae s.s* blood intake rate, with the average intake of protein per second being highest on their preferred human hosts. After adjusting for variation due to host species, the blood intake rate of *An. gambiae s.s* was predicted to be higher on hosts in the low and medium than high PCV group. Again, the most plausible explanation for this effect is the relatively reduced viscosity of blood in the low and medium PCV groups, which may enhance its flow rate during *An. gambiae s.s* feeding (Vaughan et al., 1991). This would be consistent with studies of *An. stephensis* that show their blood intake rate is highest on hosts with intermediate than high packed cell volume (Taylor and Hurd, 2001, Daniel and Kingsolver, 1983)

While natural feeding of *An. gambiae s.s* (overnight feeding on unrestrained hosts), both ‘host species’ and ‘PCV’ were significantly predictors of *An. gambiae s.s* blood meal size when tested on their own, when PCV was added to a statistical model that already included the effect of 'host species' it was not found to be a significant. A possible interpretation for this is that the apparent significance of 'PCV' to *An. gambiae s.s* blood meal size (when investigated on its own) is an artifact of the association between high PCV' and 'human host', with the latter factor being the cause and the former a correlate of large blood meal size in *An. gambiae s.s*. Alternatively, it is possible that the PCV values of human hosts was responsible for the blood meal size effects, and exceptionally high that no further impact of host PCV could be detected after accounting for this because the majority of variation in PCV is contained between and not within host species, and mosquitoes adjust their time of feeding. Further investigation of *An. gambiae s.s* blood meal size after feeding on human blood spanning a wider range of PCV will be necessary to separate these hypotheses.

Despite the reported impacts of PCV on the feeding efficiency of *An. gambiae s.s* (but not An. *arabiensis*), neither this host haematological trait nor haemoglobin concentration had any significant effect on the fecundity of either vector species. Laboratory studies indicate that mosquito blood meal size is a strong predictor of their fecundity (Edman and Lynn, 1975, Lea et al., 1978, Hogg et al., 1996). Consequently, neither host species, Hb nor PCV influenced the blood meal size of *An. arabiensis*, it is unsurprising that these factors were also unrelated to its fecundity. However, as A*n. gambiae s.s* obtained significantly larger blood meals from humans than any other host, it is surprising that this host type was not associated with a fecundity advantage. Similar results have been reported for *Aedes aegypti* that had similar fecundity on humans and chicks despite taking substantially larger meals from the former (Harrington et al., 2001). A possible explanation for these results could be that although blood meal size and fecundity are positively correlated over the lower ranges of blood intake, once a minimum threshold of blood has been exceeded mosquitoes produce the same number of eggs regardless of further intake (Takken et al., 2002, Roitberg and Gordon, 2005, Lea et al., 1978).

Although host haematological variation was found to be unrelated to mosquito fecundity in these experiments, it could possibly impact other aspects of mosquito reproduction that were not measured here. For example, it has been hypothesized that mosquito-feeding success is increased on hosts made anemic by parasitic infection, because they are rendered unable to mount effective defensive behaviours and may be more attractive to mosquitoes (Nacher, 2005, Lacroix et al., 2005, Nacher, 2002). In contrast, increasing host PCV by treating them for anaemia has been associated with reducing their attractiveness to mosquitoes (Nacher et al., 2003). Thus, perhaps PCV influences mosquito fecundity not by influencing the number of eggs per blood meal, but their probability of getting a blood meal.

From an evolutionary point of view, mosquitoes should maximize their feeding efficiency by selecting host species that maximize their lifetime reproductive success. Results presented here and in Chapter 3, however, do not indicate that mosquito fecundity is maximized on their naturally preferred host species (e.g. cows for *An. arabiensis*, and humans for *An. gambiae s.s.*). However, there is some evidence that another component of mosquito lifetime reproductive success, their long-term survival is maximized on preferred host species, possibly as a consequence of their haematological properties. The post-feeding survival of *An. gambiae s,s* was greatest after feeding on their preferred human hosts (Chapter 3). Analysis here indicates that *An. gambiae s.s* survival is positively correlated with host PCV, and thus the characteristically high red cell densities associated with humans may be responsible for the longevity advantage they provide. After adjusting for variation to host species and PCV, host Hb was predicted to be negatively related to *An. gambiae s.s* survival. The influences of haematological variation on *An. arabiensis* survival were more clearly separated from those of host species, which had no detectable impact on *An. arabiensis* survival. However, the nature of these effects were opposite from what was predicted in *An. gambiae s.s*. Specifically, *An. arabiensis* survival was predicted to be negatively associated with host PCV, and positively with Hb. It is unclear why host PCV and Hb were estimated to have such contrasting effect on survival of *An. arabiensis* and *An. gambiae s.s*; however, these results indicate that these haematological traits can have opposite and independently effects on mosquito fitness.

The strong impact of host haematological traits on the survival of these vector species is surprising as blood resources have been primarily linked to mosquito fecundity (Woke, 1937b, Downe and Archer, 1975, Bennett, 1970, Takken et al., 2002, Ulloa et al., 2005, Chang and Judson, 1979, Jalil, 1974, Briegel and Horler, 1993, Harrington et al., 2001, Woke, 1937a), but rarely to their lifespan (Harrington et al., 2001). Lipids are thought to be the most important energetic resource for mosquito long-term survival. The association of PCV and Hb with mosquito survival here suggests that blood may be used both as a source of lipids and proteins in these mosquitoes. Laboratory experiments have demonstrated that proteins contained in red blood cells can also be used as the resources for the synthesis of lipids in mosquitoes (Nayar and Sauerman, 1975, Ziegler and Ibrahim, 2001), suggesting that they may accumulate more lipids for their survival when feeding on hosts with high levels of PCV.

Mosquito survival is one of the most important determinants of malaria transmission (Billingsley et al., 2008, Killeen et al., 2000) and any host factor that influences their longevity could be of epidemiological relevance. Here I found that *An. arabiensis* survival was negatively associated with host PCV, and positively correlated with Hb concentration. In contrast, the survival of *An. gambiae s.s* was positively correlated related to host PCV, but negatively with Hb. Anaemia (characterized as reduction in PCV and Hb (Bergsjo et al., 1996, van den Broek et al., 1999, WHO, 1992) is a common health problem of women and children in sub-Saharan Africa (van den Broek, 2001, Kitange et al., 1993) and is one of the primary symptoms of severe malaria (Phiri et al., 2008, Schellenberg et al., 2003). The prevalence of anaemia within human populations in Africa has been related to malaria transmission. If results from the small small-scale experiments conducted here were to scale up to the population level, it is possible that interventions that reduce the prevalence of anaemia in humans could also influence mosquito survival. However, the net consequences of such a phenomena for malaria transmission are unclear, as PCV and Hb are predicted to have opposite effects on mosquito survival here whereas both would be increased by reducing anaemia.

A general goal of this study was to investigate whether the host species preference of these African vectors can be attributed to specific haematological characteristics, which optimize mosquito fitness. Can the variation in host Hb and PCV observed here help explain why these vector species preferentially select some host species over others? Evidence for this is mixed. Under natural conditions, *An. arabiensis* will preferentially feed on cows over humans (Duchemin et al., 2001), and on humans over all other animal species investigated here (Taye et al., 2006, Hadis et al., 1997, Sharp and Lesueur, 1991). Neither host Hb or PCV influenced fecundity of this vector, but their survival was greatest on hosts with medium and high Hb groups, and in the low PCV groups. Neither their primary or secondary preferred cow and human hosts fall in the category of low PCV and medium –High Hb group; suggesting these preferred hosts do not have the optimal combination of these haematological traits for *An. arabiensis* survival. In contrast, the survival of *An. gambiae s.s* was predicted to be maximized on hosts with high PCV, which is a characteristic of their preferred human hosts. However, human hosts were also associated with relatively high Hb, which, (after adjusting for variation due to host species and PCV), was predicted to be negatively correlated with *An. gambiae s.s* survival. Thus, there is no clear evidence that haematological properties of the preferred host species of these mosquito vectors are optimal for their fitness. Further investigation of other haematological properties that are known to influence the fitness of blood feeding insects (e.g. nutrients carried in blood plasma including amino acids, glucose and lipids, Nayar and Sauerman Jr, 1977, Spielman and Wong, 1974, Harrington et al., 2001) is required before the ultimate role of host haematology in driving selection for host species choice can be evaluated.

6 The impact of uniform and mixed species blood meals on the fitness of the mosquito vector *An. gambiae s.s:* Does a specialist pay for diversifying its host diet?

6.1 Introduction

Many organisms exhibit substantial variation in their degree of diet specialization, with some exploiting a wide variety of resources and others concentrating on only one (Poulin, 1998, Jaenike, 1990, Futuyma and Moreno, 1988, Timms and Read, 1999). Consumers that exploit only one or a limited number of resources are referred to as specialists (Gandon and Michalakis, 2002, Gandon et al., 1996), whereas those switching between different resources are referred to as generalists (Márcio et al., 2010, Abrams, 2006a, Abrams, 2006b). Evolutionary theory predicts that specialism should evolve when there is a greater fitness advantage from concentrating feeding on only one dietary resource rather than a mixture (Timms and Read, 1999, Robinson and Wilson, 1998, Levins, 1962, Pyke et al., 1977, Ward, 1992, MacArthur and Pianka, 1966). In contrast, generalism is predicted to evolve when there are only moderate differences in the fitness obtained from feeding on different dietary sources such that there is no net advantage to being selective (Abrams, 2006b, Egas et al., 2004, Abrams, 2006a). Numerous studies have attempted to test for the existence of dietary fitness trade-offs associated with specialist and generalist feeding, with the majority focusing on experimental study of phytophagous insects (Futuyma and Moreno, 1988, Via, 1990, Price et al., 1980, Jaenike, 1990). The feeding strategies of insects vary extensively between extreme specialism and generalism (Jaenike, 1990). In support of theoretical predictions, experimental studies provide some evidence that some generalist phytophagous insects (e.g. grasshoppers and beetles) have greater fitness (fecundity and survival) when feeding on a variety of resources than when selectively feeding on a single plant host (Michaud and Jyoti, 2008, Allard and Yeargan, 2005, Bernays et al., 1994); and that specialists experience a reduction in fitness when they switch to a novel host resource and/or mix their diet (Thomas et al., 2010). These studies also suggest that the impact of dietary diversity on both specialist and generalist insects depends on the specific type of dietary resource being consumed as well as whether it is fed on exclusively or in combination with other resources. For example, there is some evidence that both specialist and generalist phytophagous insects may have similar fitness benefits from feeding on mixed and single host diets (Bernays et al., 1994, Hauge et al., 1998, Nielsen et al., 2002), which has been hypothesized to be because overall the specific combination of resources in these diets provide a similar quantity of nutrients. Thus understanding the selective pressures that promote generalism and specialism requires consideration of the nutrient value of both selective and mixed feeding, and of the specific types of resources consumed.

As with phytophagous insects, the dietary range of other organisms that rely on living hosts for their survival, including parasitoids (Jervis and Kidd, 1986, Kawecki, 1998), parasites (Poulin, 1998) and ectoparasites (Lyimo and Ferguson, 2009) are also known to vary significantly between specialism and generalism. Overall, specialism is thought to be more prevalent than generalism within these classes of parasitic organisms (Lehane, 2005, Poulin, 1998), however, relatively few empirical tests of the role of fitness trade-offs in limiting their diet range have been conducted. Consequently, the role of natural selection in shaping the host species diversity of these organisms is relatively unknown.

Extreme specialization on human hosts, a feeding strategy known as anthrophily is a common biological characteristic of many important insect vectors of human disease, where variation in the extent anthrophily can predict the stability and transmission intensity of diseases they transmit (Kilpatrick et al., 2006, Kiszewski et al., 2004, Kilpatrick et al., 2007). In particular, malaria in Africa is the clearest example of a devastating human disease whose stability is driven primarily by the anthrophilic behaviour of the *Anopheles* mosquitoes that transmit it (Kiszewski et al., 2004). The major aim of research presented in this thesis has been to test a variety of predictions from evolutionary theory that may account for why African vectors have been selected to specialize their feeding on humans (e.g. host-species dependent fitness in Chapter 3, host defensive behaviour in Chapter 4, and variation in blood nutrient value in Chapter 5). The aim of this study was to investigate the more general issue of whether mosquitoes that concentrate feeding on the same host species (uniform diet, e.g. humans) throughout their life have a greater cumulative fitness than those that switch between host species.

Perhaps due to inherent logistical challenges of conducting experiments where ectoparasites are allowed to repeatedly feed on humans and other host species throughout their life, little is known about the fitness consequences of host switching in these organisms relative to phytophagous insects (Lyimo and Ferguson, 2009). In haematophagous insects such as the mosquito vectors of malaria, trade-offs in their fitness from feeding on uniform and mixed host species diets could arise from variation in their phenotypic traits of those of their hosts that allow them to locate (Li and Rossignol, 1992) and access blood vessels before being interrupted by host defensive behaviours (Day and Edman, 1984, Walker and Edman, 1986, Edman and Scott, 1987, Davies, 1990, Hodgson et al., 2001, Darbro and Harrington, 2007) in several ways. First, it has been observed that some mosquitoes that switch their feeding between humans and animals (e.g. zoophilic *An. arabiensis*) have a relatively larger body size, proboscis and a greater number of maxillary teeth than anthrophilic mosquitoes (Howard, 1924, Daniel and Kingsolver, 1983), suggesting that there may be specific mosquito phenotypic traits associated with generalism. For example, having a large proboscis and a relatively high number of maxillary teeth may be necessary to efficiently penetrate the relatively tough skin of animal hosts such as bovids, but may be more likely to elicit pain and thus defensive behaviour in relatively thinner skinned hosts such as humans. Consequently, phenotypic adaptations to thicker-skinned hosts could be traded off against increased host seeking mortality on thinner-skinned ones. Second, during the blood feeding process the efficiency with which mosquitoes filter and retain red blood cells (primary source of nutrients) from host serum is known to depend on erythrocyte size, a trait that varies between host species (Nemi, 1986, Wintrobe, 1933, Hawkey et al., 1991). The efficiency with which red cells are filtered by the mosquito pyloric armature depends on the relative spacing of its spicules and spines relative to the size of host red cells (Vaughan et al., 1991), and adaptations that maximize filtration efficiency on host species with relatively large red blood cells could reduce efficiency on hosts with relatively small ones (Vaughan et al., 1991). Finally, the chemical digestion of blood is known to be energetically costly for mosquitoes as it involves production of enzymes that can hemolyse red blood cells, digest specific blood proteins and detoxify metabolites (e.g. toxicity of heme, Graca-Souza et al., 2006, Sarfati et al., 2005). Consequently, there are several mosquito phenotypic traits that influence their ability to extract and digest the blood of different host species, and could generate fitness trade-offs that would account for the evolution of specialist versus generalist feeding strategies.

Mosquitoes in the genus *Anopheles* are responsible for malaria transmission to humans (Kelly-Hope et al., 2009). Globally, there is substantial variation in the feeding habits of *Anopheles* mosquito species ranging from those that switch between a range of mammals and bird species to those that feed on only one host species such as humans (Lyimo and Ferguson, 2009). Of all *Anopheles* species, probably the one exhibiting the most extreme anthrophilic behaviour is the African malaria vector *An. gambiae s.s* which feed almost exclusively on humans throughout their range. Here, experiments were conducted to investigate the fitness consequences of host species switching in this anthrophilic mosquito with the aim of testing the general prediction from evolutionary theory that specialists pay a fitness cost for diversifying their host species diet. The first specific hypothesis I tested was whether the lifetime reproductive success of *An. gambiae* *s.s* (as estimated over 2 gonotrophic cycles) is greater after feeding on a uniform blood diet of their preferred host species (e.g. humans) in comparison to switching between the blood of humans and other common animal species that are readily available in the same environment (e.g. cattle, chickens and dogs) as these malaria vectors. Lastly, I tested whether the fitness consequences in anthrophilic *An. gambiae s.s* depends on host species mixed in their blood meal.

6.2 Methods

6.2.1 Mosquito rearing

The *An. gambiae s.s* Keele strain (Hurd, 2005) was used in this study. Mosquitoes were obtained from a laboratory colony maintained under standard insectary conditions of 26 ± 1ºC, 80% relative humidity, and a 12:12 hour light/dark cycle at the University of Glasgow. Larvae were reared in plastic trays (16 × 16 × 5 cm) filled with distilled water to a depth of 2 cm and fed on ground fish food (Tetramin®) once a day. Pupae were collected daily as they developed in larval trays and placed into netting enclosed stock cages (30 × 30 × 30 cm) for adult emergence. Adult mosquitoes were provided with a solution of 5% glucose/0.05 % para-amino-benzoic acid (PABA) from the wick of filter paper that was inserted in a small glass bottle containing solution. For maintenance of the colony, female mosquitoes were membrane fed twice a week on fresh washed human erythrocytes that were resuspended to 40% haematocrit in heat-inactivated pooled human AB serum (erythrocytes and serum obtained from the blood transfusion service). Small plastic bowls (9.5 cm diameter × 4 cm depth) lined with filter paper and filled with distilled water to a depth of 1cm were placed in mosquito stock cages 2-3 days after each blood feed to allow for oviposition. Eggs laid in these bowls were allowed to hatch within them and the resulting first instar larvae were then transferred into standard plastic rearing trays as described above.

6.2.2 Preparation of mosquitoes for experiments

A total of 1000 and 1500 pupae of *An. gambiae s.s* were collected over a period of three consecutive days from the colony (described above) and placed in an adult stock cage for experiments 1 and 2 respectively. Two days before experiments, 500 and 582 adult females were transferred from the stock cage into one of three waxed cartons (9.5 cm diameter × 9 cm depth) that were covered with mesh. The wax cartons contained a minimum of 100, and a maximum of 200 female mosquitoes. Mosquitoes were maintained under insectary conditions for 2 days and offered their first blood meal when 5 – 7 days old.

6.2.3 Membrane feeding of mosquitoes

Prior to blood feeding, baudruche membrane (Joseph Long Inc., Belleville, New Jersey, USA) were secured across the open-ended bottom of glass membrane feeders (2.5 cm diameter × 3.5 cm height) with rubber bands. A series of three glass feeders connected by rubber tubing were clamped in a retort stand, and then attached to a circulating waterbath set at 37ºC to mimic the body temperature of a human host. For their first blood meal, all *An. gambiae s.s* were fed on human blood. The three different groups (minimum of 100, and maximum of 200 female mosquitoes) held in different wax cartons were then placed under a membrane feeder (one carton per feeder). The feeders were then lowered onto the top of each carton so that the membrane surface was in full contact with the mesh opening of the carton. Once in contact with the cartons, 1.5 ml of human blood (supplied by Patricell Company, Bio- City Nottingham, Nottingham, UK) was put into each membrane feeder (with blood from a different human donor being placed in each of the 3 feeders). Membrane feeders were kept in contact with the top of cartons for 20 -30 minutes to allow mosquitoes within them to blood feed. Two hours after the feed, cartons were inspected and all unfed mosquitoes were removed and killed.

Blood fed mosquitoes were kept in these original holding cartons for a further 3 days after their first feed. On day 3, survivors were transferred into individual universal tubes (2.3 cm diameter × 9 cm depth) filled with approximately 1cm of distilled water to allow them to lay their eggs. The numbers of eggs laid were counted to establish the oviposition rate (proportion laying eggs) and fecundity of mosquitoes (number of eggs laid) after this first blood meal. One day after being given opportunity to oviposit, all mosquitoes were offered a second blood meal (Gillies, 1953). All mosquitoes, regardless of whether they laid eggs or not after their first feed, were offered a second blood meal. For the second blood meal, mosquitoes were randomly allocated into one of 4 different treatment groups corresponding to the host species whose blood they would be given: human (supplied by Patricell Company, Bio- City Nottingham, Nottingham, UK), cow, dog, or chicken (supplied by Harlan Laboratories Ltd, Belton, UK). Within each of these 4 host treatments groups, blood samples from 3 different host individuals (per species) were used (giving rise to 12 different feeding groups: 3 replicates/host diet treatment × 4 host diet treatments). Each feeding groups contained 10 – 25 mosquitoes (randomly allocated from the survivors of the first feed).

On the day of the second feed, mosquitoes were transferred from individual oviposition tubes into one of 12 different holding cartons corresponding to the different feeding groups as described above. Mosquitoes in these cartons were blood fed using the membrane feeding procedures as described above (3 series of 4 feeds at a time), to give rise to 3 replicates of 4 different host treatment groups: (1) uniform human, (2) mixed human + chicken, (3) mixed human + cow and (4) mixed human+ dog. As during the first blood meal, on their second feed mosquitoes were allowed to feed for 20 – 30 minutes after which their feeding success (proportion that took a blood meal) was recorded. All mosquitoes that were observed to have fed (through visual inspection 2 hours after the feed) were held in the cartons for 3 days and then transferred into individual universal tubes for oviposition. Tubes were observed for eggs for 3 -4 days from the first day on which oviposition was possible, and the number that were laid were counted under a dissecting microscope. All mosquitoes were transferred into dry universal tubes after 4 days in oviposition tubes and maintained there until the end of the experiment. From the first day after the second blood feed, all mosquitoes were checked on a daily basis to monitor their survival for further 18 days (23 -25 days from emergence). This entire experimental procedure was replicated twice.

6.2.4 Statistical analyses

Statistical analysis was conducted to assess the impact of uniform and mixed host species diets on 3 key measures of mosquito fitness: oviposition rate, fecundity and survival. One of these measures was a binomial variable: the proportion of mosquitoes that laid eggs (oviposition rate) after the second blood meal. The two other fitness indices were measured as continuous variables: the number of eggs laid after the second blood meal, and the number of days mosquitoes survived after their second blood meal. The two replicates of this experiment (blocks) were analysed separately.

Whereas the impact of host species diversity on the fitness arising from single meals were evaluated in previous chapters (e.g Chapter 3), the aim of this study was to test whether diversity in host diet across multiple blood meals throughout an adult female's life influenced her long – term survival and cumulative net reproductive success (as estimated from the distribution of eggs laid over 2 blood meals). First,I investigated whether the proportion of mosquitoes that laid eggs after the second blood feed was influenced by the host species whose blood they received using generalized linear mixed effect models in the R statistical software (Crawley, 2007). Here 'host species diet treatment' was taken as the main effect, whereas the different host individuals within a host species treatment (3 per host species) were considered as a random effect. A base statistical model including the random effect of host individual was constructed, to which the main effect of 'host species diet treatment' was then added to form the full model. The significance of 'host species treatment' was tested using negative log likelihood (likelihood ratio test) which measured whether there was a statistically significant improvement in the amount of variation explained when this explanatory variable was added to the base model.

The continuous variable of mosquito fecundity (the number of eggs laid) was analysed using generalized linear mixed effect models (lmer) in R statistical software (Crawley, 2007). Initially, the effect of host species on the number of eggs laid after the second blood feed was investigated, restricting analysis to mosquitoes that laid at least one egg. Here 'host species diet treatment' was taken as the main effect, whereas the different host individuals within a host species treatment (3 per host species) were considered as a random effect. As with the analysis of oviposition rate, the main effect of 'host species diet treatment' was added into a base model containing random effect of 'host individual'. The significance effect of the host diet treatment on fecundity after the second blood meal was tested using likelihood ratio test (LRT) as described above.

In addition to examining how variation in host species influenced mosquito fecundity on the second blood meal, analysis were also conducted to investigate the cumulative impact of host diet diversity over two blood meals on their total reproductive success. In these experiments it was not logistically possible to measure the lifetime egg production of individual mosquitoes, because they had to be pooled into groups for blood feeding within which they could not be individually recognized. This made it not possible to associate the known reproductive success of an individual mosquito on its first blood meal with its performance on the second blood meal. Thus the impact of host diet diversity was investigated from the distribution of reproductive success in cohorts of mosquitoes fed on either uniform or mixed host species diets. To do this, first the distribution of the number of eggs laid by mosquitoes after the first blood meal (on humans) was computed. The observed range of egg- laying after the first blood meal (experiment 1: 0 – 103 eggs, experiment 2: 0 – 159 eggs) was split into 11 intervals that each spanned maximum of 15 eggs units. As all mosquitoes took their first blood meal from humans, it was assumed that their fecundity after the first blood meal was similarly distributed. Second, the distribution of eggs laid by mosquitoes on their second blood meal was computed for each of the 4 different host species treatment groups in the same way as described for the first blood meal (different distributions for each host species group, pooling across all 3 host individuals per host diet treatment group). Finally, the observed distribution of eggs laid on the first and second blood meal were summed to give a cumulative distribution of reproductive success of mosquitoes in all 4 host species diet treatments over two blood meals. The impact of host diet treatment on this cumulative measure of mosquito fecundity was assessed using a Kruskal-Wallis test to evaluate whether there were statistically significant differences between host species diet treatments. Then a post hoc comparison using Mann-Whitney test with Bonferroni correction was used to identify whether the cumulative number of eggs varied between the mixed diet treatments.

The impact of host species diet treatment on mosquito survival was analysed using Cox Proportional Hazard Model (COXPH) in the R statistical software. (Gillies, 1953) Differences in survival between treatment groups was assessed from the day after the second blood meal onwards. In this analysis, ‘host species diet treatment’ was considered as a main effect, and the three different host individuals within each host species treatment treated as a random effect. A frailty function was used to incorporate the random effect of host individual (within a host species diet treatment) into the Cox model. Then the main effect of ‘host species diet treatment’ was added to this base model to test for statistical differences in the survival of mosquitoes.

6.3 Results

Overall, a total of 257 and 494 mosquitoes were offered both first and second blood meals during experiment one and two respectively. Of these, a total of 140 and 200 mosquitoes took a second blood meal and survived at least another 3 days further to produce eggs.

6.3.1 Mosquito reproductive success

The oviposition rate of *An. gambiae s.s* after their second blood meal did not vary between host diet treatments in experiment one (χ^2_3 = 5.37, P = 0.15, Figure 6.1a), or experiment two (χ^2_3 = 2.18, P = 0.53, Figure 6.1b). Furthermore, the number of eggs laid by ovipositing *An. gambiae s.s* after the second blood meal was also similar irrespective of host species diet treatments in experiment one (χ^2_3 = 1.78, P = 0.62, Figure 6.1c), and two (χ^2_3 = 3.84, P = 0.28, Figure 6.1d). Finally, summing across both the first and second blood meals, there was no evidence that the cumulative number of eggs laid by *An. gambiae s.s* over two blood meal varied between host species diet treatments in either experimental block (experiment 1: χ^2_3 = 0.12, P = 0.99, Figure 6.2a – d, experiment 2: χ^2_3 = 0.10, P = 0.99, Figure 6.3a - d). Similarly, a post hoc comparison (Mann-Whitney test with Bonferroni corrections) showed that there were no significant differences in cumulative number of eggs produced by these mosquitoes on 3 mixed host species diets (P > 0.05 in all two – way comparisons). The shape of distribution of numbers of eggs produced by these mosquitoes was right skewed for all host species.

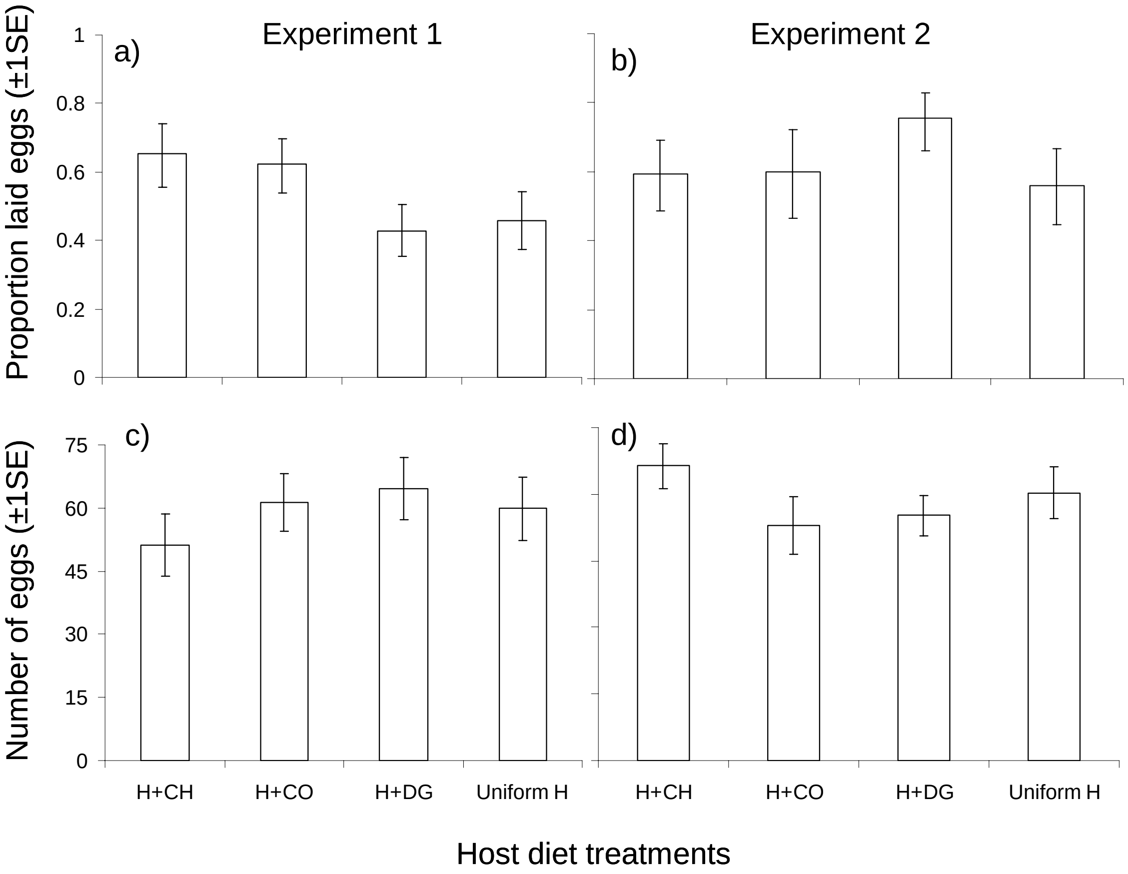

Figure 6.1. The reproductive success (±1 s.e.m) of *An. gambiae s.s* after their second blood meal on hosts of different species. Th host species diet treatment are represented by abbreviation: H + CH – mixed human + chicken, H + CO – mixed human + cow, H + DG – mixed human + dog, and UniformH- human blood. Figure a& b give the oviposition rate (proportion of mosquitoes that laid eggs) after taking a second blood meal from different host spcies, an figure c & d show the average number of eggs laid by mosquitoes who oviposited after their second blood meal.

Experiment 1

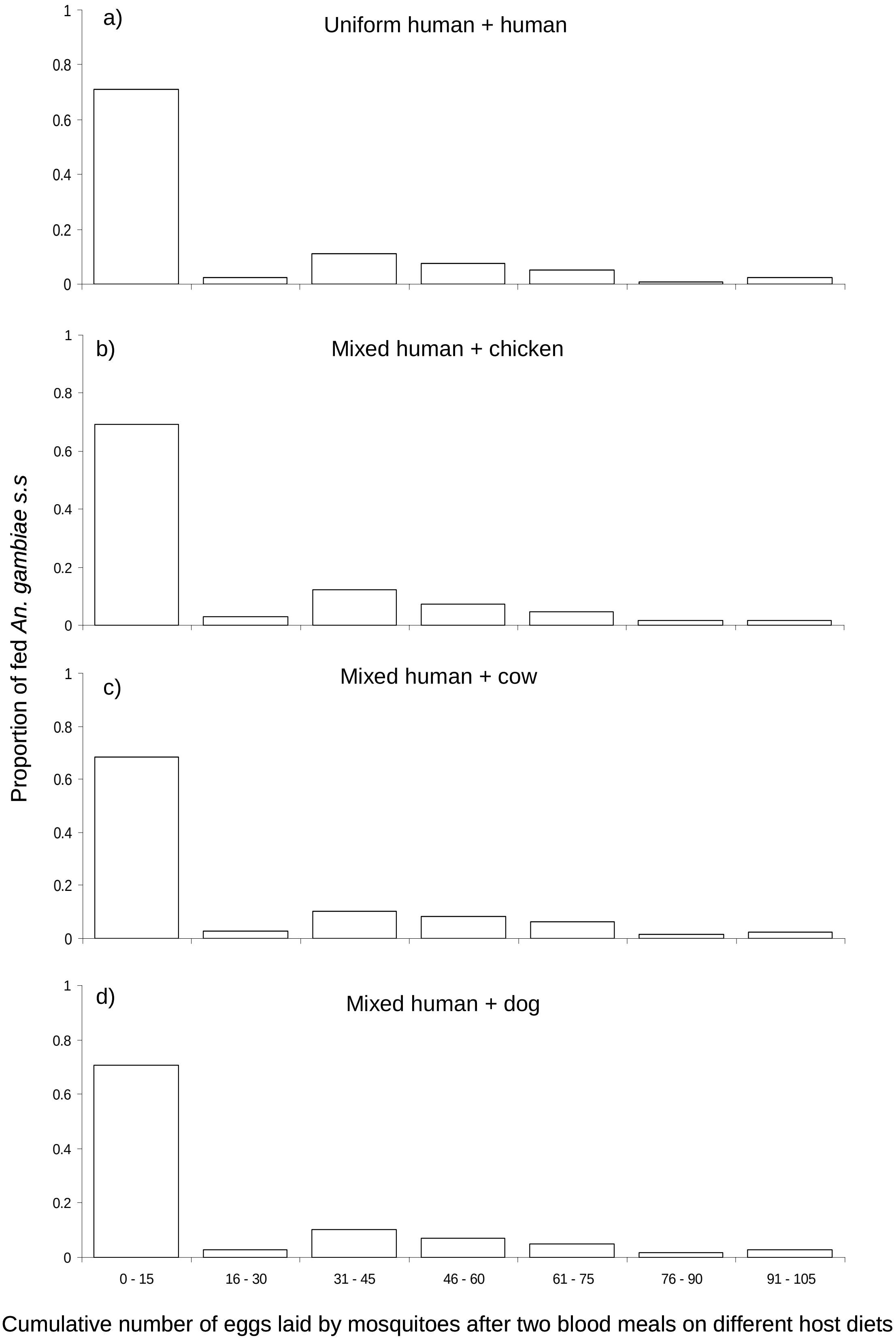

Figure 6.2. Distribution of the cumulative number of eggs laid by *An. gambiae s.s* over two blood meals on either uniform or mixed host species in experiment 1.

Experiment 2

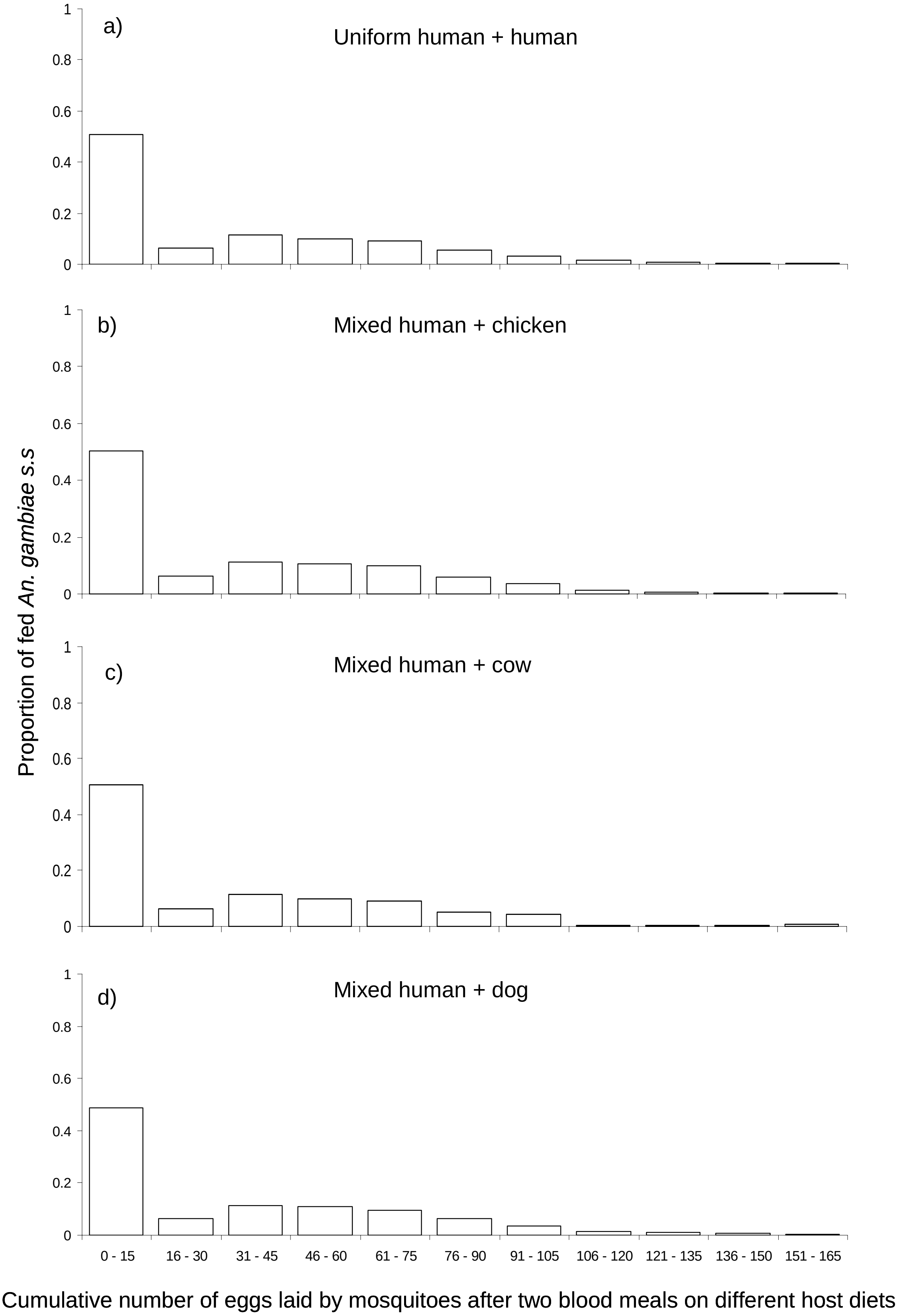

Figure 6.3. Distribution of the cumulative number of eggs laid by *An. gambiae s.s* over two blood meals on uniform and mixed host species in experiment 2

6.3.2 Mosquito survival

The long-term survival of *An. gambiae s.s* mosquitoes did not vary between host diet treatments in either experiment (experiment 1: χ^2_3 = 1.28, P = 0.73, Table 6.1, Figure 6.4a, experiment 2: χ^2_3 = 0.88, P = 0.83, Table 6.1, Figure 6.4b). The odds of mortality after feeding on uniform human blood only were not different from from feeding on any of the 3 mixed host species diet (Table 6.1). Similarly, no significant differences were observed between the 3 mixed host species diets.

Odds of mortality (OR) of *An. gambiae s.s* relative to a uniform human diet		
Host diet treatment	Experiment 1	Experiment 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)

Table 6.1. The estimated odds of mortality of *An. gambiae s.s* after taking two blood meals from different host species combinations relative to a uniform human blood diet.The numbers in brackets are the 95% confidence intervals of odds ratio (OR).

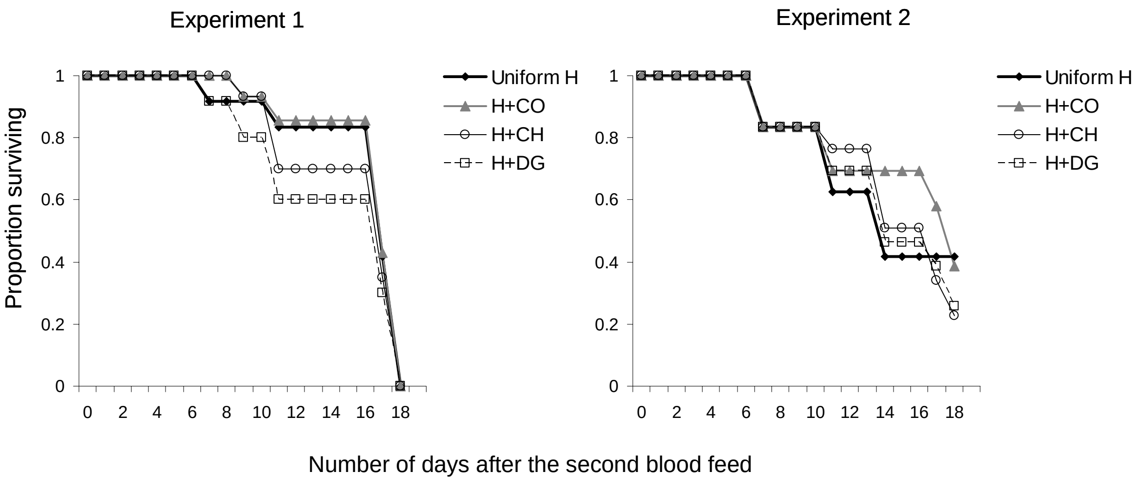

Figure 6.4. Survival of *An. gambiae s.s* after feeding on either uniform human or mixed host species dietsThe lines indicate their predicted survival after two blood meals. The survival function was estimated from the Cox proportional Hazard Model (COXPH).Host species diet treatments are abbreviated: Uniform H- uniform human, H + CO – mixed human+cow, H + CH – mixed human + chicken, and H + DG – mixed human + dog.

6.4 Discussion

This study tested for a fitness cost of a generalist blood feeding strategy with respect to reproductive success and survival, in the highly specialized African malaria vector *An. gambiae s.s*, which blood feeds almost exclusively on humans under natural conditions. Overall, under the controlled laboratory conditions used here, mosquitoes were found to have similar reproductive success and survival after feeding on a uniform human-only, or mixed host species diets. This suggests that at least from the point of imbibing blood and onwards, these mosquitoes obtain relatively equal fitness rewards from the blood of different host species, and that there is no evidence of trade-off between the ability of mosquito to digest and utilize blood resource from different host species.

The reproductive success of mosquitoes (oviposition rate and fecundity) is known to be influenced by the quantity of blood imbibed during a blood feed , and some studies have also shown it to vary between hosts of different species (Lyimo and Ferguson, 2009). However, here I found no evidence that reproductive success of mosquitoes after their second blood meal was influenced by host species. These results are consistent with the findings from my previous semi-field experiments (Chapter 3) which showed that under more natural conditions where *An. gambiae s.s* fed on live hosts, there was no impact of host species on the reproductive success of mosquitoes after their first blood meal; either when hosts were free to mount defensive behaviours (Chapter 3), or physically restrained from movement during feeding (Chapter 5). Although haematological properties such as red blood cell size, density and amino acid composition are known to vary between the host species investigated here (Hawkey, 1991, Nemi, 1986, Wintrobe, 1933, Hawkey et al., 1991), a possible explanation for why this variation did not impact mosquito reproductive success here is that in all cases it was not of sufficient magnitude to make a major impact on their fitness, or perhaps that mosquito compensate for poorer blood quality by adjusting the total volume they intake or time they spend feeding (which was measured in chapter 4 but not here). Further work is needed to characterize both the biochemical composition of blood from these different host species, and mosquito feeding dynamics upon them, to test these possibilities. These results do suggest that biochemical variation in the blood composition of different host species is not likely to account for selection towards exclusive human feeding in this mosquito vector. Therefore, on the basis of these results and those reported in other chapters, human preference and the adoption of specialist versus mixed host strategy by this species may be related to larger-scale ecological factors that either govern the relative availability of different host types and nutrient value they provide.

Even though the reproductive success of *An. gambiae s.s* after one blood meal (e.g. first meal, Chapter 3 &, second blood meal investigated here) is not significantly different on humans and other host species, it is possible that there could be minor variation between host species that if consistently exhibited could add up over successive gonotrophic cycles to generate a net difference in their lifetime reproductive success. However, at least over the two gonotrophic cycles examined here, I found no evidence of a statistical difference in the cumulative number of eggs laid by mosquitoes on human-only and mixed species diets. In nature, the majority of *An. gambiae s.l* do not survive for more than 2 gonotrophic cycles (e.g. median survival of *An. gambiae s.l* corresponding to 4 - 5 days, Chege and Beier, 1990), thus the time scale used here appropriately represents average reproductive period of mosquitoes in the wild. In these experiments, I had to estimate differences in cumulative reproductive success of mosquitoes not at the level of individuals, but of cohorts, and this may have reduced the ability to detect impact of human-only and mixed species diets. Although there were limitations on the way reproductive success could have been investigated here, if there are differences in cumulative egg production on single and mixed host diets, they are unlikely to be of very high magnitude.

Although human-only and mixed host diets did not influence *An. gambiae s.s* fecundity here, blood diets have been demonstrated to influence the fitness of other mosquito species in previous laboratory studies (Woke, 1937b, Mather and DeFoliart, 1983, Shroyer and Siverly, 1972, Downe and Archer, 1975, Bennett, 1970, Woke, 1937a). Whilst here *An. gambiae s.s* fed on blood only from their preferred human or other commonly available animal hosts, most other laboratory studies have contrasted mosquito on a range of unnatural host species (Bennett, 1970, Harrington et al., 2001, Nayar and Sauerman Jr, 1977, Shelton, 1972, Downe and Archer, 1975, Woke, 1937a).

In this study, the survival of *An. gambiae s.s* after two blood meals was not influenced by the host species composition of their previous meal (uniform human or mixed diet). This result differs from previous work conducted under semi-field conditions that found the survival of *An. gambiae s.s* after one blood meal was significantly higher on human and cow blood than that of dogs, chickens and goats (Chapter 3). The greater survival of *An. gambiae s.s* on human blood in these experiments was hypothesized to be a product of the relative high packed cell volume of human blood relative to other host species (Chapter 4). A possible explanation of impact of host species on the survival of *An. gambiae s.s* under semi-field but not laboratory conditions could be that human blood is such a good resource for mosquito long - term survival, that as long as they consume it on their first blood meal the impact of host diversity in later blood meals become negligible. Alternatively, it could be that under the artificial laboratory conditions used here the impact of host skin, defensive behaviours and blood haematological traits were removed, where the impacts of host species diversity are underestimated. Further work is required to disentangle these hypotheses.

Although the existence of fitness trade-offs in resource exploitation have been widely predicted for dietary specialists (Levins, 1962, MacArthur and Pianka, 1966, Pyke et al., 1977), similar to this study their existence has failed to be confirmed in several empirical studies of phytophagous insects (Thomas et al., 2010, Hauge et al., 1998, Agrawal et al., 2002, Bernays, 1999, Rapport, 1980). In these studies, it has been hypothesized that the lack of fitness differences between single and mixed host diets is because the plant resources incorporated in both meal types had similar nutritional value (Rapport, 1980, Hauge et al., 1998). Although I found no evidence of an overall fitness advantage from feeding only on human blood here, it is premature to dismiss the possibility that trade-offs in host exploitation ability exist under more natural conditions that may explain why this mosquito has evolved a specialist rather than generalist feeding strategy. Firstly, the specialism of *An. gambiae s.s* could be driven by the fact that their human host is much more abundant (Chaves et al., 2010), and that this has generated correlated selection for *An. gambiae s.s* to become more efficient at detecting and responding to human host odours (Lefèvre et al., 2009), even though there is no underlying difference in the fitness value of the blood of different host species. Here, blood was presented to mosquitoes under standardized conditions in which the effects of variation in host odour, defensive behaviour, and skin thickness had been removed. This design was used to specifically isolate the impact of host blood on mosquito fitness and the fact that his resource on its own appears to be a poor predictor of the host preference of *An. gambiae s.s* suggest that it is these other ecological factors that may be responsible for driving selection for (human) host specialization. Other studies have shown that the feeding success of *An. gambiae s.s* on artificial membrane can be improved by immersing them with a human body odour (Andreasen et al., 2004), and it is possible that if the host blood sources here had been pre-conditioned to smell like their natural host species I would have observed a clearer fitness advantage associated with human-only feeding. Further experimental investigation is required to test whether other ecological factors such as these could generate fitness trade-offs in host exploitation ability that could account for the evolution of human specialism in *An. gambiae s.s*. Additionally, comparative study of more generalist vector species such as *An. arabiensis* is required to test whether their fitness is less influenced by host species choice than specialists, as predicted by evolutionary theory (Pyke et al., 1977, MacArthur and Pianka, 1966, Levins, 1962). Such investigations could provide a clearer understanding of the evolution of host specialization in important insect vector species such as *An. gambiae s.s*, and possibly highlight strategies for minimizing selection towards host preference behaviours that fuel disease transmission.

7 General discussion

7.1 Overview

The host species choice of malaria vectors is one of the key biological determinants of global transmission patterns. However, our understanding of the ecological and evolutionary factors that drive host species choice in malaria vectors is very limited. My PhD thesis has aimed to experimentally investigate the potential determinants of host species choice of the African malaria vectors, who are amongst the most highly host specialized and efficient vectors on the planet, with the aim of identifying how they could be manipulated to reduce anthrophily. As a starting point, I first established a colony of *An. arabiensis* under semi-field insectary to ensure an adequate supply of this mosquito for comparative experiments with the anthrophilic *An. gambiae s.s* (already available in colonies in Tanzania and Glasgow). Then I experimentally established whether the fitness of these mosquitoes is dependent upon the host species they encounter and feed upon, contrasting amongst the host types most likely to be encountered in their natural environments. The remainder of my thesis involved experimentally testing a series of evolutionary hypotheses to account for the variation in the preference and fitness of *An. arabiensis* and *An. gambiae s.s* mosquitoes on different host species. The aim of this work was to identify what attributes (e.g. behavioural, haematological) make some host species more beneficial to mosquito fitness than others, and to examine if common vector control methods could alter the value of humans relative to other available animal hosts. Finally, I tested the general issue of how host diet diversity (single versus mixed host species) influences resource intake and fitness of a specialist malaria vector (*An. gambiae s.s*). The key findings from this investigation are briefly highlighted below.

7.2 Host species-specific fitness of African malaria vectors.

In chapter 3, I investigated if and how the fitness of African malaria vectors is influenced by host species choice. My initial prediction was that the highly specialized feeding behaviour of these vectors (*An. arabiensis* on humans and cattle, *An. gambiae s.s* on humans) is a product of natural selection whereby mosquito fitness is highest on their naturally preferred host types. This prediction was partially met in that I confirmed that the fitness of these mosquitoes does vary between host species. I found that the feeding and expected lifetime reproductive success (predicted egg production) of *An. arabiensis* was greater on their naturally preferred bovid hosts than any other host species. In contrast, although I found *An. gambiae s.s* took larger blood meals and survived longer on their preferred human hosts than on most other animals, the mathematical model of their overall reproductive success based on empirically – derived estimates of all fitness components did not indicate that human hosts provided a significant lifetime reproductive advantage as would be required by natural selection to generate a preference for humans.

There are several potential reasons why I did not detect any evidence of a fitness benefit associated with anthrophily in *An. gambiae s.s* in the experiments conducted here. The first may simply be that the preference of this vector for humans under natural conditions is largely a product of their greater availability compared to the alternatives surveyed here, than to any specific intrinsic biological attributes of humans that makes them better hosts. An alternative possibility is that the fitness benefit *An. gambiae s.s* acquires from anthrophily arises because of human habitats, and not their biological properties, are highly conducive to their reproduction and survival (discussed further below). For example, it may be that *An. gambiae s.s* does best when foraging inside houses, where humans are more commonly found. An additional explanation for why predictions from evolutionary theory about the relationship between host preference and fitness were met for *An. arabiensis* but not *An. gambiae s.s* could be the colonization history of these vectors prior to experimentation. Whereas the *An. arabiensis* used here had been only recently colonized (< 7 generations from the wild population at the time of experiments), the *An. gambiae s.s* used here has been maintained under colonization for almost 14 years. During this time, *An. gambiae s.s* had been maintained exclusively on its preferred human host so would not be expected to lose its ability to exploit this host type, but may have reduced its ability to discriminate between preferred and non-preferred host species. Ideally, this possibility could have been avoided by working with *An. gambiae s.s* that were similarly closely related to wild populations as were *An. arabiensis*. However, given the recent and rapid decline in *An. gambiae s.s* throughout the study region (Russell et al., 2010), it was not logistically possible to establish a new colony of *An. gambiae s.s* for experiments described here.

7.3 Intrinsic host properties influencing mosquito fitness

Chapter 3 indicated that there were clear differences in mosquito fitness (in terms of survival and feeding success) between host types, even if not always in the direction predicted by evolutionary theory. The aim of work presented in Chapter 4 and 5 was to test what the intrinsic host properties of host species may explain their differential impacts on malaria vector fitness. Specifically I sought to assess the relative importance of host defensive behaviours and intrinsic haematological traits (e.g. haemoglobin concentration, and packed cell volume) which may influence the quality of mosquito blood meals.

7.3.1 Host defensive behaviours

In chapter 4, I evaluated the relative impact of host defensive behaviour by comparing mosquito fitness under circumstances where it could be exhibited and where it was restricted. My initial prediction was that mosquito feeding success and fitness would be the highest in the absence of host defensive behaviour, and more specifically, that host species with the least effective defensive behaviour would be the most highly preferred in nature. As predicted, the preferred host species of *An. arabiensis*, calves and cows, appeared to have the least effective defensive behaviour. Specifically, *An. arabiensis* had higher feeding success on these hosts than on any other types, and unlike on any other host species, the feeding success of this vector on livestock was not improved by physically restraining them from mounting defensive behaviours. It is possible that bovid hosts simply have poorer defensive behaviours than all other host species, and/or that *An. arabiensis* have evolved specific mechanisms to evade their defensive behaviours. In contrast, the feeding success of *An. gambiae s.s* was unaffected by restraining host defensive behaviours; either in their naturally preferred humans or rarely exploited animal alternatives (with the exception of chickens).

Surprisingly, both *An. arabiensis* and *An. gambiae s.s* obtained larger blood meals from feeding on hosts under natural conditions when hosts were free to exhibit defensive behaviours than when they were restrained. Possible explanations for this are that blood flow is enhanced in mobile hosts, which may increase the speed and volume of blood intake. Alternatively it could be that under natural conditions mosquitoes select feeding sites on the host body from which blood vessels are more accessible than from those which they were given access to in the restrained host experiments here (although I tried to mimic the known natural feeding sites on the host body as much as possible). My finding that mosquito blood meal size and subsequent reproductive success and survival is not enhanced by physically restraining hosts during feeding suggests that if natural host defensive behaviours are acting to shape the host species preference of malaria vectors, they do so by influencing the probability of getting a blood meal, but not by reducing the value of blood meals (e.g. blood meal size) that are taken.

7.3.2 Host haematological properties

In chapter 5, I investigated whether the variation in nutritive value of blood in terms of Packed Cell Volume (PCV) and haemoglobin concentration (Hb) between host species could explain observed differences in their fitness value to malaria vectors, and is correlated to with their host species preference in nature. Necessary conditions for this are the existence of significant variation in these haematological traits between host species, of sufficient magnitude to influence mosquito fitness. The first condition was met in that there was statistically significant variation in PCV and Hb between the host species assayed here, that were consistent with reported differences in medical and veterinary standards. However, evidence that variation in these traits between and within host species had a strong impact on mosquito feeding and reproductive success was mixed. The feeding success (rate of blood intake and blood meal size) of *An. arabiensis* was unrelated with host haematological traits of PCV and Hb. In contrast, the feeding success (duration of blood feeding, rate of blood intake and blood meal size) and fitness of *An. gambiae s.s* were more sensitive to variation in PCV between host species than *An. arabiensis*. *Anopheles gambiae s.s* took a shorter time to obtain a full blood meal from host individuals with low and medium PCV levels than those in the high PCV category; after adjusting for the effect of host species.

Initially, I predicted that if there were any effects of host haematological variation on mosquito fitness, they would most likely be evident in terms of their fecundity (as protein from blood is the primary resource for egg production) than in long-term survival. However, whereas I found that neither Hb nor PCV had any significant effect on mosquito fecundity in either vector species, both factors were significantly related to *An. arabiensis* and *An. gambiae s.s* survival. Surprisingly, variation in host PCV and Hb were predicted to have opposite effects on the survival of *An. arabiensis* (i.e. positive relationship with Hb, negative with PCV), and *An. gambiae s.s* (i.e. positive relationship with PCV, negative with Hb). The greater survival of *An. gambiae s.s* on hosts with high PCV may be associated with their intrinsic ability to synthesize large quantities of lipids from host red blood cells (Harrington et al., 2001), but it is unclear why an opposite pattern should be predicted for *An. arabiensis.*

Overall, there was no clear evidence that the haematological traits that give rise to highest fitness in these vectors are at optimal levels in their preferred host species. The survival of *An. arabiensis* was greatest on hosts in the medium – high Hb group, and in the low PCV groups. However, neither their primary nor secondarily preferred host species (e.g. cow and human respectively) fall in the category of low PCV and medium – high Hb group, suggesting these hosts do not have optimal combination of these haematological traits for *An. arabiensis* survival. In contrast, the survival of *An. gambiae s.s* was predicted to be maximized on hosts with high PCV, which is a characteristic of their preferred human hosts. However, human hosts were also associated with relatively high Hb, which after adjusting for variation due to host species and PCV was predicted to be negatively correlated with *An. gambiae s.s* survival. Therefore, there is no clear evidence that host-species specific variation in Hb or PCV could be responsible for generating selection for the host species preference of *An. arabiensis* or *An. gambiae s.s*. Further experimental investigation is required to test whether other haematological properties including glucose, amino acids and lipids that may also influence mosquito fitness before the potential impact of haematological factors on driving the host species preference of malaria vectors can be fully evaluated.

7.4 Impact of vector control on malaria vector fitness and the evolution of host species preference.

To my knowledge, my Ph.D. research is the first to experimentally measure the selection pressures generated by the use of untreated net by humans for malaria vectors to switch their host species choice to more (readily available) animal alternatives (Chapter 3). My results indicated that simple untreated bed nets in the condition they are most likely to be encountered in typical African communities (e.g. with some holes) can reduce the fitness value of human hosts to the highly anthrophilic *An. gambiae s.s* below that of other commonly available animal hosts. Similarly, the use of untreated nets by humans widened the fitness differential between humans and bovid hosts for *An. arabiensis*. Furthermore, my life history model that combined all the impacts of host species on mosquito fitness (e.g. probability of feeding, reproduction and survival) predicted that the relative advantage of bovid over human hosts for *An. arabiensis* in terms of their lifetime reproductive success only achieves statistical significance when humans are using untreated bed nets. Although the use of untreated nets was not predicted to significantly reduce their lifetime reproductive success, the survival of *An. gambiae s.s* that succeeded in blood feeding on people in the presence of a net was significantly lower than after feeding on most of alternative animal hosts, suggesting even this simple and flawed intervention does increase some of the costs of anthrophily in this vector. Therefore, on the basis of my small scale empirical investigations, I hypothesize that the widespread use of untreated nets by humans has the potential to generate selection on *An. arabiensis* to increase its zoophily, and to a lesser extent, possibly for *An. gambiae s.s* to reduce its anthrophily (e.g. in environments where animal alternatives are readily available). Overall, the sustainability of conventional vector control strategies such as ITNs and IRS may be enhanced by integrating them with environment management strategies that increase the availability of alternative animal hosts (e.g. zooprophylaxis) as this could help generate selection for reduced anthrophily and not just for insecticide resistance which could rapidly undermine these control measures (Stump et al., 2004, Yadouleton et al., 2010, Casimiro et al., 2006, Coleman et al., 2008).

7.5 Fitness consequences of specialism versus generalism

Finally in Chapter 6, I investigated the general issue of whether a specialist feeding strategy (feeding on only one preferred host species throughout life) is more profitable to mosquitoes than a generalist strategy combining preferred (e.g. humans) and other readily available animal species. Unlike all other work, this experiment was done in a laboratory setting in which mosquitoes were provided with blood from different species from an artificial membrane feeder. Thus, it could address only the fitness impacts arising from blood composition and not other ecological factors that may influence mosquito-feeding success under more natural conditions (e.g. odour, defensive behaviour, and skin thickness). Under these conditions, I found that the survival and reproductive success (oviposition, fecundity) of *An. gambiae s.s* fed either on a uniform human or mixed host species diet (e.g. human plus cow, chicken or dog) was similar. This suggests that there were no strong trade-offs in the ability of mosquitoes to handle and digest blood from different vertebrate host species that could generate selection for adopting a specialist rather than mixed host species feeding. Several laboratory studies of other insect – host systems have similarly failed to find clear evidence of fitness advantage from a uniform versus mixed host species diet (Bernays, 1999, Bernays et al., 1994, Thomas et al., 2010, Hauge et al., 1998), so this finding is not unusual. Perhaps, the fitness advantage of a uniform versus mixed host feeding strategy would be detected when studying mosquitoes foraging under more natural field conditions, where additional ecological factors that could determine the benefit of host species switching (e.g. relative availability and distance between different host species, and suitability of their microhabitats) could be considered.

7.6 Implications for the epidemiology and control of malaria.

Host species and their intrinsic behavioural and haematological properties were shown to influence some aspects of the probability of feeding, blood meal size and longevity of the two African vector species investigated here, all of which are important determinant of malaria transmission potential. Specifically, feeding probability determines the probability a mosquito will encounter a potentially infected host, blood meal size determines the number of parasites the mosquito will ingest, and survival whether the mosquito will live long enough to transmit parasites to a new host (based on the length of the extrinsic incubation period). From a disease control perspective, environmental control of malaria could be conducted not only implementing measures to divert mosquitoes away from humans (e.g. bed nets, zooprophylaxis), but also by making human hosts less profitable to malaria vectors than alternative animal species such that selection is generated for them to reduce their anthrophily. My life history model predicted that the reduced survival of anthrophilic *An. gambiae s.s* fed on humans protected by untreated net was not sufficiently large to significantly reduce their lifetime reproductive success relative to animal alternatives. In contrast, the life history model predicted that the use of untreated nets by humans could generate a statistically significant reduction in the total lifetime reproductive success of *An. arabiensis* such that they would gain a significant advantage from becoming more zoophilic (on bovid hosts). With the exception of chickens (which are rarely fed on by either vector species in nature), I also found no evidence that natural defensive behaviours of the animals assayed here (e.g. those most likely to be kept in and around houses) are more costly to malaria vectors than those of humans. This suggests that under environments were animal alternative hosts are available, it is unlikely that mosquitoes will be restricted from switching to feed on them because of increased defensiveness (relative to humans).

Recent studies from a variety of locations in East Africa provide evidence that in addition to significantly reducing malaria transmission (Lengeler, 2004), the widespread use of ITNs is increasingly associated with a shift in malaria vector composition from *An. gambiae s.s* to *An. arabiensis* (Russell et al., 2010, Bayoh et al., 2010, Lindblade et al., 2009). These results have been interpreted as evidence that ITNs are much more effective at targeting *An. gambiae s.s* than *An. arabiensis*; presumably, because the former is more endophilic than the latter (Boreham and Port, 1982, Coluzzi et al., 1979, Petrarca et al., 1991). My results suggest further that the apparent greater resilience of *An. arabiensis* may also be due to the fact that they pay less of a fitness cost from switching from humans to animal hosts than *An. gambiae s.s*, a strategy which may allow them to avoid contact with ITNs without compromising their reproduction. Further investigation is required to identify if and how the greater effectiveness of ITNs against *An. gambiae s.s* is related not only to their mass killing effect, but the increased fitness costs they impose on the mosquitoes that they divert away from protected humans. This will enable identification of whether there are any conditions under which the sustained use of ITNs could induce *An. gambiae s.s* to reduce its anthrophily (either through phenotypic plasticity or selection that generates genetic changes) and assess what the long-term implication for control would be.

7.7 Recommended directions for future research

In this thesis, I was able to experimentally test the impact of variation in host species, defensive behaviours, and the haematological traits of Hb and PCV on malaria vector fitness, and evaluate their potential to explain the host species preference of *An. arabiensis* and *An. gambiae s.s*. While several of these traits were found to have an impact on mosquito fitness, I could not identify any one trait that was consistently related to the host species preference in both these vectors. Were I to extend my studies in this area, below are some of the key hypotheses I would prioritize for investigation to further resolve the determinants of host species preference evolution in these African malaria vectors.

7.7.1 Mosquito genetic factors

The evolution of a trait including host species preference in mosquito vectors requires pre-existing genetic variation for it within a population (Roff, 1992), as well as that its fitness consequences vary between available host species (Poulin, 1998). The contribution of mosquito genetic factors to the host species choice of malaria vectors is not well understood, but previous studies have demonstrated that the host species preference of *An. gambiae*, *Aedes simpsoni* and *Aedes aegypti*, and *Culex quinquefasciatus* may have some genetic basis (Kilpatrick et al., 2007, Mukwaya, 1977, Gillies, 1964). Under experimental conditions, selection experiments generated divergent feeding preference in

An. gambiae for humans or cows within the 5 – 6 generations (Gillies, 1964), whereas cross-mating experiments between zoophilic *Aedes simpsoni* and anthrophilic *Aedes aegypti* have been shown to generate hybrids with an intermediate preference (Mukwaya, 1977). It is possible that the host species preference shown by the mosquitoes studied here are the results of co-evolutionary processes generated by historical selection pressures, which have caused genes for anthrophily (e.g. in *An. gambiae s.s*) to reach fixation in most African populations. As a first step to assessing this possibility, I could conduct experiments on natural populations that exhibit variation in host species preference (e.g. zoophilic versus anthrophilic populations of *An. arabiensis*) to assess the heritability of host species preference by comparing the preference of the maternal and F1 generation under semi-field conditions. If a genetic basis was established, further cross mating and quantitative genetic analyses could be conducted to estimate the relative contribution of environmental versus mosquito genetic factors to the overall preference phenotype.

7.7.2 Host species availability

In addition to mosquito genetic factors, the host species preference of mosquitoes may be additionally shaped by environmental factors. Specifically, preference may evolve in response to their relative abundance of particular host types in the environment instead of just their specific physiological or behavioural properties (Kilpatrick et al., 2007, Chaves et al., 2010). For example, *Ae. aegypti* and *Ae. albopictus* preferentially feed frequently on humans (Harrington et al., 2001, Ponlanwat and Harrington, 2005), but their host species preference can be somewhat modified in response to the relative availability of alternative host species in the environment (Ponlanwat and Harrington, 2005, Richards et al., 2006). Similarly, it has been suggested even the highly anthrophilic *An. gambiae* s.s may reduce their feeding on humans in areas where their availability has been reduced by high community coverage of bed nets (Lefevre et al., 2009), and *Culex nigripalpus* shift their feeding from birds to humans during the time of year when avian abundance decreases due to seasonal migration (Clements, 1999). The role of host species availability in selecting for host species preference and/or behavioural plasticity in the vectors investigated here remains unknown. This could be tested by examining how the host species preference of these vectors varies in communities where the relative abundance of humans and other animal alternative differs Such studies are currently being conducted by another colleague in our team (Valeriana Mayagaya, M.Sc. student, IHI) and will complement the results of my studies.

7.7.3 Microhabitat

In natural environments, mosquito vectors often exhibit a preference for feeding on hosts either inside (endophilic) or outside (exophilic). The ability of mosquitoes in the *Anopheles gambiae* complex to exploit hosts in these microhabitats has been linked with the existence of chromosomal inversions (Coluzzi et al., 1979, della Torre et al., 2002). The preference of mosquito vectors for certain microhabitats may not be independent of the type of host species they are likely to find there, for example, *An. gambiae s.s* is known to be both highly anthrophilic and endophilic (Boreham and Port, 1982, Coluzzi et al., 1979, Petrarca et al., 1991, Aniedu, 1993). In contrast, the more zoophilic *An. arabiensis* is known to be more exophilic (Petrarca et al., 1991, White et al., 1972). The more humid conditions of indoor environments, as well as the presence of human hosts, may be advantageous to *An. gambiae s.s* (who are more sensitive to desiccation than *An. arabiensis*, Gray and Bradley, 2005), and may have generated selection for endophily. However, it is unclear how much the innate host species prerefence of African malaria vectors is a factor of the suitability of their microhabitats. For logical reasons as well as the need to separate the intrinsic properties hosts from their habitats, in my semi-field experiments all hosts were presented to mosquitoes within 'indoor' environments (e.g. an experimental hut). If the fitness benefits of anthrophily in *An. gambiae s.s* are primarity due to the suitability of indoor environments, they may not have been detected here. To test this possibility, further experiments could be conducted to disentangle the impacts of host and habitat properties by conducting experiments where host types are assayed both when in and outside an experimental hut.

7.7.4 Evolution of a host species shift in African malaria vectors

Another critical area I would like to investigate further is the general prediction that has emerged from my PhD thesis that it may be possible to implement vector control strategies that not only reduce malaria vector populations but also select for behavioural shifts that reduce their contact with humans. The degree of anthrophily in malaria vectors, a key determinant of transmission intensity, could be modified by increasing the uptake of vector control measures that selectively protect humans. I have found that selectively protecting humans using untreated net reduces the longevity of *An. gambiae s.s* on human hosts and make them less profitable hosts than all other animal species except cows, and increases the relative fitness benefits of *An. arabiensis* on their naturally preferred bovid hosts. Similarly, some field studies have demonstrated that the sustained use of untreated

(Lefevre et al., 2009), and insecticide treated bed net ITNs (Kaburi et al., 2009, Lindblade et al., 2009), and spraying insecticide on house walls IRS (Gillies and Furlong, 1964), has been associated with a reduction in anthrophily in malaria vectors, as well as behavioural shifts from feeding inside to outside houses. Whether such behavioural shifts are due to phenotypic plasticity or evolutionary changes in host species preference is unknown. To test this possibility, behavioural surveillance of wild malaria vectors during a long-term control programme could help to test if intervention select for behavioural shift in malaria vectors. This area of research is one of the objectives of an European Union (EU) grant to be conducted at IHI in which I am involved, so I may have an opportunity to further investigate the association of long-term vector control programme and the selection for mosquito behavioural shift.

In addition to changes in host-species preference of malaria vectors in response to interventions, another area of interests that has emerged from my PhD thesis is how host species may influence broader aspects of mosquito fitness, and specifically not just the production of eggs but the subsequent development and competitive success of larvae that hatch from them. Competition during the larval stage is known to have a strong impact on larval survival and species composition (Bagny et al., 2009, Murrell and Juliano, 2008, Juliano, 2009). If mosquitoes obtain better resources from preferred hosts to provision their eggs, then the advantage of being selective could be enhanced larval competitive success which I did not get the opportunity to test here. In nature, *An. arabiensis* and *An. gambiae s.s* are known to use similar larval habitats (Koenraadt et al., 2004, Chen et al., 2008), and compete for resources within them (Paaijmans et al., 2009). Therefore, I would like to further investigate if and how host species influences larval competition between *An. gambiae s.s* and *An. arabiensis* and consequently influence their abundance in the environment. My starting prediction would be that the larval competitive success of these vectors is enhanced when their mothers feed on preferred host species (e.g. cows for *An. arabiensis*, and humans for *An. gambiae s.s*). To test this possibility, I would conduct experiments in which cohorts of *An. arabiensis* and *An. gambiae s.s* were fed either on human or cow blood, with their resulting offspring (eggs) mixed and allowed to develop together in a common habitat. I would collect all the pupae that emerged, allow them to develop into adults and then perform PCR on them to identify the species composition. I would then test whether the ratio of *An. arabiensis* to *An. gambiae s.s* that emerged from these habitats (a measure of the outcome of competitive success, if they started at a 50:50 ratio) was influenced by the host species on which their mothers fed. I have proposed these experiments as part of a one year postdoctoral fellowship offered through the IHI

(Hassan Mshinda Career Development Fellowship) for which I have been awarded and hope to being working on in November 2010.

7.8 Conclusions

To my knowledge, my Ph.D. research is the first experimental investigation of fitness consequences of host species choice in the African malaria vectors. The results indicated that the fitness of African malaria vectors depends on the host species they encounter and feed upon. The intrinsic behavioural and haematological properties of these host species were shown to influence some aspects of probability of feeding, blood meal size and longevity of *An. arabiensis* and *An. gambiae s.s*, but the effect was not consistent for both vector species. Finally, my Ph.D. research demonstrates that the use of even simple interventions that selectively protect humans (e.g. untreated bed nets) may be sufficient to generate selection for reduced anthrophily in areas where this strategy is integrated with environmental management to increase the availability of alternative animal hosts (zooprophylaxis). These novel results suggest that there is a possibility of devising control strategies that work by not only reducing vector populations, but also driving ecological and evolutionary changes within them that may enhance the long-term effectiveness of interventions. I would like to further explore the feasibility of such ecological and evolutionary – based strategies during the next stage of my career.

Appendix 1: Parameters in mosquito life-history model

Parameters	Symbol	Value	Source
Fixed			
Daily survival during host seeking	s_f	0.8	(Killeen et al., 2007a)
Daily survival between feeding and oviposition after feeding on an unprotected human	$S_{ov(h)}$	0.9	(Killeen et al., 2007a)
No. days between feeding and oviposition	d_{ov}	3	(Gillies, 1953)
No. days between oviposition and seeking new host	d_f	1	(Gillies, 1953)

Glossary

Anautogeny: Mosquito egg production depends upon consumption of blood at the adult stage.

Anthropophilic: A preference for blood feeding on humans.

Autogeny: The ability to produce one or more egg batches without feeding on blood. Some mosquitoes are autogeneous, with energetic resources for egg production being taken from larval nutritional reserves instead of from blood feeding at the adult stage.

Human blood index (HBI): The proportion of blood fed mosquitoes in a given population sample that test positive for human blood.

Fitness: This term is notoriously difficult to define and is given numerous different definitions in the literature. In this article it is defined as the total number of offspring produced by a certain type (phenotype or genotype) relative to that of another type.

Zoophilic: A preference for blood feeding on non-human animals.

Specialist: This term used for animal species that feed entirely on one type (uniform) dietary resource.

Generalist: This term used for animal species that feed on variety (mixed) of dietary resource.

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