

Vector competence of *Aedes aegypti* mosquitoes for filarial nematodes is  
affected by age and nutrient limitation

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## Abstract

Mosquitoes are one of the most important vectors of human disease. The ability of mosquitoes to transmit disease is dependent on the age structure of the population, as mosquitoes must survive long enough for the parasites to complete their development and infect another human. Age could have additional effects due to mortality rates and vector competence changing as mosquitoes senesce, but these are comparatively poorly understood. We have investigated these factors using the mosquito *Aedes aegypti* and the filarial nematode *Brugia malayi*. Rather than observing any effects of immune senescence, we found that older mosquitoes were more resistant, but this only occurred if they had previously been maintained on a nutrient-poor diet of fructose. Constant blood feeding reversed this decline in vector competence, meaning that the number of parasites remained relatively unchanged as mosquitoes aged. Old females that had been maintained on fructose also experienced a sharp spike in mortality after an infected blood meal (“refeeding syndrome”) and few survived long enough for the parasite to develop. Again, this effect was prevented by frequent blood meals. Our results indicate that old mosquitoes may be inefficient vectors due to low vector competence and high mortality, but that frequent blood meals can prevent these effects of age.

Keywords: ageing, *Brugia malayi*, mosquito, nutrition, refeeding syndrome, survival

## 1 Introduction

Age is a critical factor affecting the ability of mosquito vectors to transmit diseases due to the incubation period parasites require to develop before they are transmitted (Macdonald, 1956). This period can be so long that few mosquitoes survive to ages where transmission occurs (Garrett-Jones and Shidrawi, 1969). Malarial parasites can take from 9 to 15 days between being ingested in a blood meal to become fully developed and migrate to the mosquito's salivary glands for transmission (Warrell and Gilles, 2002). The dengue fever virus (Chan and Johansson, 2012) and filarial nematodes (Erickson et al., 2009) have similar incubation periods. For these diseases, the rate of transmission will depend on the age structure of the vector population, and this can be targeted by vector control programmes aiming to reduce transmission (Cook et al., 2008).

Senescence of a variety of traits may alter rates of disease transmission as mosquitoes age. For example, there can be declines in rates of blood feeding and flight (and therefore host-seeking behaviour) (Christensen et al., 1986; Sylvestre et al., 2013). Similarly, *Aedes aegypti* mosquitoes infected with the dengue-fever virus take longer to blood feed as they age (Sylvestre et al., 2013), and this may have a negative effect on the vector's fitness since the host's defensive behaviour can kill the mosquito or prevent a blood meal from being taken (Walker and Edman, 1985).

In many organisms it is common to find that immune system function declines with age, a process known as immunosenescence (Nikolich-Žugich and Čičin-Šain, 2010), and this has the potential to increase rates of disease transmission by mosquitoes. Immunosenescence has been best documented in *Drosophila* (Eleftherianos and Castillo, 2012; Felix et al., 2012; Katewa and Kapahi, 2011; Mackenzie et al., 2011; Remolina et al., 2012), but it also seems to be common mosquitoes (Christensen et al., 1986; Chun et al., 1995; Desowitz and Chellappah, 1962; Hillyer et al., 2005; Li et al., 1992; Wang et al., 2010). Older *Culex*

*pipiens fatigans* became more susceptible to the filarial nematode *Brugia sp*, showing a higher parasite load than younger individuals (Desowitz and Chellappah, 1962). As mosquitoes age an increase in parasitemia, as well as the size of parasites was observed in older *Anopheles quadrimaculatus* infected with the filarial nematode *Dirofilaria uniformis* (Duxbury et al., 1961). Melanisation, a process that involves deposition of melanin on the parasite as part of its immune response (Michel et al., 2006), was observed to be reduced in older *Anopheles gambiae*, the primary malaria vector (Chun et al., 1995), as well as in insecticide-resistant *Culex pipiens* (Cornet et al., 2013), and *Ae. aegypti* infected with *Dirofilaria immitis* (Christensen et al., 1986). The number of haemocytes, a cell type involved in melanisation and phagocytosis of parasites, also declined when *An. stephensi* (Foley, 1978) and *Ae. aegypti* aged (Hillyer et al., 2005).

Across many species diet alters rates of senescence in many traits, with dietary restriction generally reducing the rate of senescence and extending lifespan (Austad, 1989; Joy et al., 2010; Masoro, 2005). Similarly diet can alter senescence of the immune system (Ponton et al., 2011). In disease vectors, diet is also known to directly affect parasite development, including *Ae. aegypti* mosquitoes infected with the filarial nematode *Brugia pahangi* (Sneller and Dadd, 1981), *An. stephensi* infected with the rodent malaria parasite *Plasmodium yoelii yoelii* (Lambrechts et al., 2006), and the kissing bug *Rhodnius prolixus* challenged with *Enterobacter cloacae* (Feder et al., 1997).

In nature adult mosquitoes commonly feed on two foods with very different nutritional content - blood and sugars such as nectar. Fructose from nectar is a source of energy for female mosquitoes in the wild and is likely to be especially important when suitable vertebrate hosts are scarce (Foster, 1995). When *Ae. aegypti* female feed only on sugar they can have increased lifespan, similar to the effect seen when other species are maintained on a restricted diet (Joy et al., 2010). Therefore, this form of dietary restriction

provides a way to both manipulate and understand aging in mosquitoes, and may also be relevant to disease transmission when access to blood meals in the wild is restricted, as it might occur in night-feeding species if bed nets are widely used.

*Aedes aegypti*, the primary vector of dengue and yellow fever viruses, and the filarial nematode *B. malayi* have been used as a laboratory model to investigate host-parasite interactions (Macdonald, 1962). *Brugia malayi* causes lymphatic filariasis in humans, also known as elephantiasis, it occurs in South East Asia and is responsible for 10% of the total cases, while *Wuchereria bancrofti* causes the remainder (Ichimori, 2010). Lymphatic filariasis is a highly debilitating disease, which affects 120 million people in the world (Ichimori, 2010). The infected patient may develop lymphoedema and scrotal hydrocele after several years of infection, which makes lymphatic filariasis the second leading cause of chronic disability worldwide (Ichimori, 2010). In the mosquito, the filarial nematode develops in the indirect flight muscles until fully developed to a third instar larva when it migrates to the mosquito's proboscis to infect another human host in the next blood meal (Beckett and Macdonald, 1971). Most known natural populations of *Ae. aegypti* are resistant to the filarial nematode, with the exception of populations found in peri-domestic and forested areas of East Africa (Paige and Craig, 1975; Rodriguez and Craig Jr, 1973). Resistant individuals are able to kill the parasites during its early development, approximately two days after infection (Magalhaes et al., 2008; Rodriguez et al., 1984).

Despite its medical importance, little is known about *A. aegypti*'s ageing mechanisms, how senescence affects the mosquito's vectorial capacity (Christensen et al., 1986; Hillyer et al., 2005; Sylvestre et al., 2013), and how diet affects vector competence when mosquitoes age. In this study we investigated how the age of *Ae. aegypti* influences two key traits affecting disease transmission — susceptibility to the parasite and the probability of surviving sufficiently long after blood feeding for the parasite to be transmitted. We also examined how

these traits were altered by dietary restriction, where mosquitoes were maintained on sugar and not allowed to blood-feed before exposure to the parasite. This form of dietary restriction is known extend lifespan in *Ae. aegypti*, so it is useful to see if changes in vector competence are governed by a similar mechanism. Furthermore, wild mosquitoes may also sometimes have restricted access to blood. We found that very young *Ae. aegypti* are highly susceptible to the filarial nematode *B. malayi*. Contrary to the expected outcome of immunosenescence, females became less susceptible with age, but frequent blood feeding before infection reversed the susceptibility decline in older mosquitoes. We did not find parasites in a genetically resistant line at any age, suggesting that genetic resistance to *B. malayi* does not senesce. Therefore, age and the history of blood-feeding can substantially alter the susceptibility of *Ae. aegypti* to *B. malayi*.

## **2 Material and Methods**

### **2.1 Ethics statement**

This study was approved by the Animals in Science Regulation Unit from the Home Office Science, United Kingdom, under the license PIL 70/25044. The study adhered to the principles of the Animals (Scientific Procedures) Act 1986.

### **2.2 Mosquito lines**

To investigate how susceptibility to *B. malayi* and other fitness parameters change as *Ae. aegypti* mosquitoes age, we used a susceptible (LVP-S) and a resistant line (LVP-R) that are derived from the same laboratory strain (Liverpool). This strain originated from West Africa and has been maintained in the lab since 1936 (Macdonald, 1962). Susceptibility to *B. malayi* was originally segregating in the Liverpool strain, but in order to sequence the mosquito genome several rounds of inbreeding were required (Nene et al., 2007) and

resistance became fixed (Juneja et al., 2014). Susceptible LVP-S stocks have been maintained by the NIAID/NIH Filariasis Research Reagent Resource Centre (FR3, Atlanta, Georgia, USA) to culture *B. malayi*. We obtained LVP-S from the FR3 and LVP-R from the Malaria Research and Reference Reagent Resource Centre (MR4, ATCC, Manassas, Virginia, USA).

### 2.3 Experimental design

To test if age affected the susceptibility of mosquitoes to *B. malayi*, we infected mosquitoes of six different ages. For the LVP-S line we hatched eggs every week to create a time series of ages from 5 days to 6 weeks (age of adult mosquitoes was calculated from emergence). The lifespan of female mosquitoes in the laboratory is approximately eight weeks, so we chose this range because five days is when the majority of females have mated and are prone to take a blood meal, and we needed to keep mosquitoes for a further 2 weeks after infection to check the development of the parasites. Therefore we had LVP-S adult mosquitoes of 6 different ages: six, five, four, three, two weeks and five days old. For the LVP-R lines we only investigated old (six and five weeks old) and young (five days old) mosquitoes. We omitted some time points in this treatment because the aim was to test whether resistant individuals would become susceptible with age: having young and old treatments would suffice to explain this.

We hatched eggs and first instar larvae, which were density controlled to 150 larvae/1500 ml water at 24 hours after eclosion. We gave 1 g of liver powder to larvae every two days after emergence and water was changed at the same rate. Adult females and males were kept in cages (32.5 x 32.5 x 32.5 cm, BugDorm), with two replicates per treatment.

To test if blood feeding prior to the infected blood meal affects how susceptibility changes with age, we maintained the mosquitoes on either fructose or fructose and blood, with two cages per treatment for each time-point. The fructose was fed to mosquitoes via

cotton wool soaked with 10% w/v fructose and 0.1% w/v p-amino benzoic acid (PABA, Sigma-Aldrich) on top of cages, with the cotton wool pads being frequently replaced. For the blood treatment, we fed females once a week with human blood (NHS Blood and Transplant, Addenbrooke's Hospital, Cambridge, UK) using an artificial feeder (PS6, Hemotek). Females that failed to have a blood meal were discarded after each feeding. There was natural mortality after adult emergence and approximately 90% of two and three-week old females were alive to be infected. Of the older treatments, 65% of six and five-week old females survived until the day we infected all females. Mosquitoes in the youngest (five-day) age group were only exposed to the fructose treatment because mosquitoes would not take more than one blood meal before this age. The majority of mosquitoes from both cages of four-week old LVP-S fed on blood died one week post adult emergence; therefore we have no data for this time point. We did not repeat this because losing this time point did not affect the overall aim of the study. We recorded the survival of females every day after the infected blood meal.

## 2.4 Infections

*Brugia malayi* was harvested from a euthanised infected gerbil by injecting 30 ml of sterile saline solution into the peritoneal cavity of the animal. We made an incision on the abdomen of the gerbil and using a funnel we collected the fluid containing microfilariae (mf), which was mixed to human blood. All mosquitoes, resistant and susceptible of all ages, were fed on the same day and time with blood containing approximately 1000 microfilariae per 20  $\mu$ l of blood. Unfed females and males were discarded and fully fed females were kept in cages.



## 2.5 Blood meal size

To estimate the size of the blood meal taken by infected females we measured the absorbance of haematin - a product of the digestion of haemoglobin that is defecated after feeding (Briegleb, 1980). A subset of the fully-fed females from all treatments (see results for exact numbers) was individually placed into 30 ml glass vials soon after the infected blood meal was taken to collect defecated material. Three days later, we transferred live females to a cage (so we could later check parasite load) and added 1 ml of 1% w/v  $\text{LiCO}_3$  solution (Sigma-Aldrich) to the vials. We did not estimate the blood meal size of females of the 4 week treatment because adults died soon after emergence, as mentioned above. The solution containing the eluted excrement was read in a spectrophotometer at 387 nm, using the  $\text{LiCO}_3$  as a blank.

## 2.6 Parasite load

We measured the parasite load of all live females on either the 10<sup>th</sup> or 11<sup>th</sup> days post infection, when the parasites had fully developed into L3 larvae, the infective stage (Erickson et al., 2009). We separated the thorax from the abdomen of each female using entomological blunt forceps and incubated this in 50  $\mu\text{l}$  phosphate buffered saline (PBS) at 37°C for 1 hour, which causes L3s to exit the carcass. The supernatant was transferred to a microscope slide and the number of parasites was counted.

## 2.7 Statistical analysis

We used R version 2.15.1 (R Foundation for Statistical Computing, Vienna, Austria) to perform all tests and the package ggplot2 to design the graphs (Wickham, 2009). In all cases we checked for non-linear effects of the age by testing if polynomial functions better fitted the data. To explain the number of parasites infecting females we fitted a linear mixed-effect

model with a Poisson error distribution with the package lme4 (Bates et al., 2012). The equation was as follows:

$$n_{i,j,k,l} = \log^{-1}(\alpha_k + \theta_l + \sigma_j + \varepsilon_{i,j,k,l})$$

where  $n_{i,j,k,l}$  is the number of worms infecting female  $i$  from cage  $j$  of age  $k$  and food treatment  $l$ ,  $\alpha_k$  is a fixed effect of age as a covariate (a continuous variable),  $\theta_l$  is the food treatment given to females prior to the infected blood meal,  $\sigma_j$  is a random effect of the cage the mosquitoes are housed in and  $\varepsilon_{i,j,k,l}$  is the residual error to allow over-dispersion within each cage.

We fitted a linear mixed-effect model to investigate differences in blood meal size given age and treatment. Our equation was as follows:

$$B_{i,j,k,l} = \alpha_k + \theta_l + \sigma_j + \varepsilon_{i,j,k,l}$$

Where  $B_{i,j,k,l}$  is the absorbance of haematin in the faeces of female  $i$ , from cage  $j$ , age  $k$  and food treatment  $l$ ,  $\theta_l$  is the food treatment females received before the infected blood meal,  $\sigma_j$  is a random effect of the cage the mosquitoes were housed in and  $\varepsilon_{i,j,k,l}$  is the residual.  $\alpha_k$  is a fixed effect of the age of females, and because the effect of age was not linear, this was fitted as a second order polynomial. We analysed data of susceptible and resistant lines separately because we failed to get data from older resistant mosquitoes fed on fructose before the infections; these individuals died before we measured the blood meal size. Therefore for the LVP-R line our equation was similar to the above, but without  $\theta_l$ .

The effect of age and food source on survival rates was analysed using a Cox's proportional hazards mixed effect model, which accounted for between-cage variation in survival rates. The hazard for the  $i^{\text{th}}$  female from cage  $j$  at time  $t$  post infection was modelled as:

$$H_{ij}(t) = H_0(t)e^{Xi\beta + bj}$$

Where  $H_0(t)$  is the baseline hazard at time  $t$ ,  $X_i$  is a vector of the fixed effects,  $\beta$  is the corresponding vector of coefficients, and  $b_j$  is a random effect of cage  $j$  nested within time post infection. The fixed effects comprised strain, food treatment and day of infection. Mosquitoes that were still alive at day 10 post infection were censored. The model was fitted by maximum likelihood using the `coxme` (Therneau, 2012) package.

### 3 Results

#### 3.1 Susceptibility to *B. malayi* declines with age, but frequent blood-feeding can reverse the decline

When mosquitoes were maintained on a diet of fructose, their susceptibility to *B. malayi* declined rapidly with age (Figure 1). Five-day-old mosquitoes that fed on infected blood became infected with an average of 3.3 L3 worms, and 80% ( $N=71$ ) of the mosquitoes carried at least one worm. When two-week-old mosquitoes were infected, the average number of parasites that developed had dropped to 2.0 (69 % infected,  $N=54$ ), and by five weeks this had declined further to 0.9 (58%,  $N=14$ ).

If the mosquitoes were regularly fed on blood, then the decline in susceptibility with age was considerably less. At two-weeks old, the previously blood-fed and fructose fed mosquitoes developed similar parasite burdens, which were reduced compared to five-day old mosquitoes (Figure 1). However, this decline was reversed in older mosquitoes that received a blood meal prior to infections. This diet-specific effect of age on parasite load is highly significant (Figure 1; diet-age interaction:  $Z = -3.95$ ,  $p < 0.0001$ ), with a smaller main effect of diet (main effect diet:  $Z = 2.2$ ,  $p = 0.02$ ).

Most *A. aegypti* genotypes in nature are genetically resistant to *B. malayi*, so we also tested if the extent of this resistance changed with age in a laboratory line, but did not find any parasites in the 180 resistant individuals we dissected (Table 1).

### 3.2 Younger females engorge more blood than older females

Changes in parasite burden as mosquitoes age could reflect changes in the amount of blood and therefore microfilaria that are ingested. Therefore, we tested if the size of the blood meal was affected by age and the diet mosquitoes had been maintained on by measuring the amount of haematin that they excrete after having the infected blood meal. However, care is needed whilst interpreting these results as infected or old animals might process haemoglobin differently and we did not have means to test this. In both the genetically resistant and genetically susceptible mosquitoes, five-day old females engorged a larger blood meal than older females (Figure 2A; LVP-S main effect age:  $F_{(2-123)} = 5.14, p < 0.01$ , LVP-R main effect age:  $F_{(1-61)} = 13.19, p < 0.001$ ). The differences in blood meal size are largely driven by five-day old females taking the largest blood meals (Figure 1A). To confirm this we did pair wise comparisons of all the blood-meal sizes taken at different ages and on different diets. For the LVP-S mosquitoes, the only individually significant differences were between five-day old mosquitoes and the treatments with the three smallest blood meals – two-week old females fed on blood (Tukey,  $Z = 3.56, p < 0.01$ ), three-week old females fed on fructose (Tukey,  $Z = 3.46, p = 0.01$ ) and five-week old females fed on blood (Tukey,  $Z = -3.74, p < 0.01$ ).

### 3.3 Fructose-feeding can result in high mortality in old mosquitoes

The survival of mosquitoes after infection is crucial for parasite transmission. If females die before the complete development of the parasite, they can no longer infect the

human host. We found that there was no significant overall tendency for older mosquitoes to die at a higher rate in the 10 days after they had been infected (Figure 3; main effect of the covariate age:  $Z = 0.44$ ,  $p = 0.66$ ). However, there was a dramatic increase in the mortality of older mosquitoes if they had been maintained on just a fructose diet before their infective blood meal - all the six-week old LVP-S females died compared none of the five-day old mosquitoes (Figure 3; diet-age interaction:  $Z = 4.56$ ,  $p < 0.00001$ ).

This mortality among the older fructose-fed mosquitoes was largely attributed to a spike in mortality rates between days two and four after feeding on the infected blood meal; after day four the survival of females fed on both diets tended to follow the same pattern (Figure 3). This is supported statistically, as when each 24 hour period is analysed separately, most of the significant differences in mortality rate occur between days two and four (Figure 3). We observed a significant difference in mortality between days two and three in five-week old LVP-S (Fisher's exact test,  $p = 0.008$ ), in five-week old LVP-R (Fisher's exact test,  $p = 0.007$ ) and in six-week old LVP-R (Fisher's exact test,  $p = 0.04$ ). In six-week old LVP-S the difference in mortality rate was observed between days three and four (Fisher's exact test,  $p = 0.003$ ) as well as between days seven and eight (Fisher's exact test,  $p = 0.01$ ).

Mortality does not seem to be attributed to the parasite as the rate in which females died did not differ between genetically susceptible and genetically resistant females (main effect of genotype:  $Z = -0.82$ ,  $p = 0.41$ ). In addition, three-week old females of both diet treatments died at a similar rate, but the difference in parasite burden in this time point is high (Fig 1). These observations also indicate that differences in parasite burden (Fig 1) are not explained by parasites killing infected individuals in some treatments and resulting in lower overall infection rates.

## 4 Discussion

We found that the vector competence of *Ae. aegypti* was heavily influenced by the combination of age and diet. Rather than showing signs of the immune system senescing, females harboured fewer *B. malayi* as they aged. However, this was only the case if they were maintained on fructose prior to the infected blood meal, as old females that had frequently blood-fed remained as prone to harbour the filarial nematodes as younger females. Genetically resistant mosquitoes also showed no signs of immunosenescence, as no worms developed regardless of whether they were young or old.

The diet of mosquitoes has been shown to affect their susceptibility to parasites in other species. Similar results to ours were found when different-aged *An. gambiae* were maintained on either fructose or blood before receiving a blood meal infected with *Plasmodium falciparum* – the number of parasites declined with age in the fructose-fed females, but remained stable in the blood-fed females (Okech et al., 2004). This suggests that there may be changes in the nutritional environment inside the mosquito that either prevent or delay worm development. There are several reasons why maintaining mosquitoes on a nutrient-poor diet might cause them to be prone to harbour less *Brugia* as they get older. It could be a direct effect of nutrient limitation affecting the parasite, as the parasite must acquire nutrients from the mosquito to develop and grow (Combes, 1997). If mosquitoes do not take a blood meal they will become deprived of nutrients such as amino acids, proteins, and cholesterol (Briegel, 1990; Caragata et al., 2014; Gary and Foster, 2001; Ziegler and Van Antwerpen, 2006), which might impair the development of *B. malayi* larvae. Alternatively, many other aspects of the mosquito physiology, immunity and microbiota are changed by blood feeding (Boissière et al., 2012; Castillo et al., 2011; Kokoza et al., 2001), and these could affect parasite development in older mosquitoes.

Our results suggest that vector competence of *Ae. aegypti* may remain largely unchanged as they age in areas where they have unrestricted access to blood meals. This is likely to be the case in most populations with a high human density, where females rarely feed on nectar (Edman et al., 1992). However, in areas with low human population density or, in the case of night other night feeding mosquito species, where there are high levels of bed net use prevent blood meals being taken, mosquitoes may decline in susceptibility as they grow older. The implication of this can be a reduction in the disease transmission not only as a direct consequence of preventing blood feeding but also due to a reduction in the survival or vector competence of females due to low nutritional diet. Whether this is ever important in nature would require studies of the nutritional status of wild mosquitoes.

For old females that had been maintained on fructose, there was a spike in mortality two to four days after feeding on infected blood. This meant that few of these females would have survived long enough to transmit the parasite. However, when mosquitoes had been maintained on blood, then the majority of the oldest females were alive at day 10 post-infection, allowing the full development of the parasite. These observations are supported by a previous study which observed that *Ae. aegypti* females that were offered only one blood meal had their lifespan halved comparing to females that were blood fed once a week (Putnam and Shannon, 1934). Therefore the rate at which mosquitoes survive is strongly dependant on their diet, and this will in turn affect the vector capacity of the population. This can be particularly important for mosquito control strategies that are aimed to prevent mosquitoes from taking a blood meal, such as bed nets. The alternative sources of food for females are either sugar nectar (which is the male's only diet) or blood from other vertebrates. Therefore, understanding how diet affects the survival of mosquitoes of various ages can be important for such strategies.

The parasite does not seem to be causing mosquitoes to die in these experiments, as mortality rates for both genetically resistant and susceptible females are similar. It is common for models of disease transmission to assume that mortality rates are constant with age (Novoseltsev et al., 2012; Styer et al., 2007), but our results suggest that the extent to which this will be the case depends on the diet of the mosquito.

The sharp increase in mortality that occurs a few days after a fructose-fed mosquito blood feeds for the first time is a form of refeeding syndrome. This syndrome was first observed in World War I, when malnourished soldiers were re-fed and developed abnormal medical conditions (Keys, 1950). After a period of starvation, if a nutritious meal is given to a starved patient it can lead to malfunctions of kidney, nerve, cardiac and skeletal muscle cells, which can ultimately lead to death (Khan et al., 2011). It tends to manifest in the first few days after the feeding re-initiation, and happens due to changes in electrolytes in serum that can affect the cell membrane potential (Crook et al., 2001). It is possible that these old and partially starved mosquitoes may experience similar metabolic stresses. The higher mortality of older females occurred during the period when females would be producing eggs. A blood meal triggers a cascade of metabolic events that culminate with egg production four days later (Putnam and Shannon, 1934), and it is possible that these substantial metabolic requirements contributed to the mortality rate (Briegel et al., 2002, 2001).

We conclude that the effects of the mosquito age on parasite development and survival critically depend on the quality of the mosquito's diet. Frequent blood meals earlier in life are critical in maintaining the parasite load and keeping the mosquitoes alive until the parasite fully develops. If females feed only on fructose, then parasitemia and survival after infection are impaired. In this model system, mosquitoes remain effective vectors as they age, as long as females are well-nourished.



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Table 1: Parasite load of genetically resistant females of different ages, fed on either fructose or blood

Age	Fructose	Parasite load		
		<i>N</i>	Blood	<i>N</i>
5 days	0	90	-	-
5 weeks	0	6	0	37
6 weeks	0	15	0	31

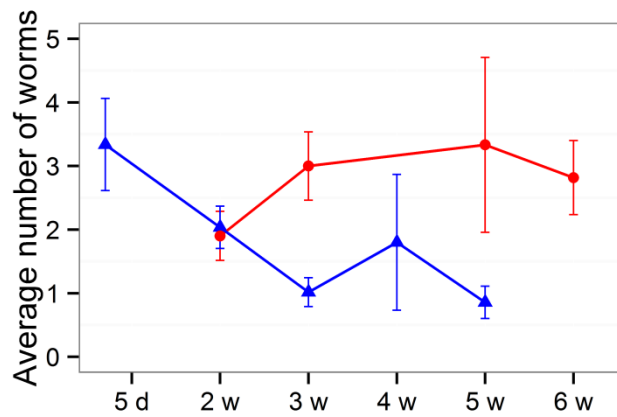


Fig. 1. Changes in parasite burden in genetically susceptible (LVP-S) females of *Ae. aegypti* females. Mosquitoes of different ages were fed on either blood and fructose (red) or fructose (blue) prior to the infected blood meal. Age is given in days (d) or weeks (w). Vertical bars represent standard errors.

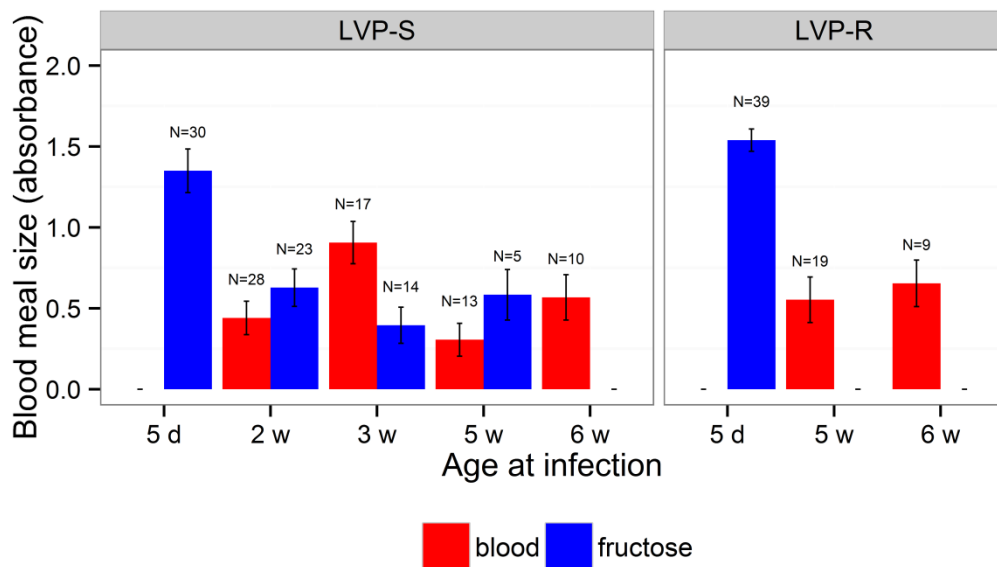


Fig. 2. Blood meal size of genetically susceptible (LVP-S) and genetically resistant (LVP-R) females of *A. aegypti* mosquitoes. The amount of haematin in mosquito faeces is used as a proxy of blood meal size, measured as absorbance at 387 nm. Mosquitoes of different ages

were fed on either blood and fructose (red) or fructose (blue) prior to the infected blood meal.

Age is given in days (d) or weeks (w). Vertical bars represent standard errors. Numbers above bars represent sample size.

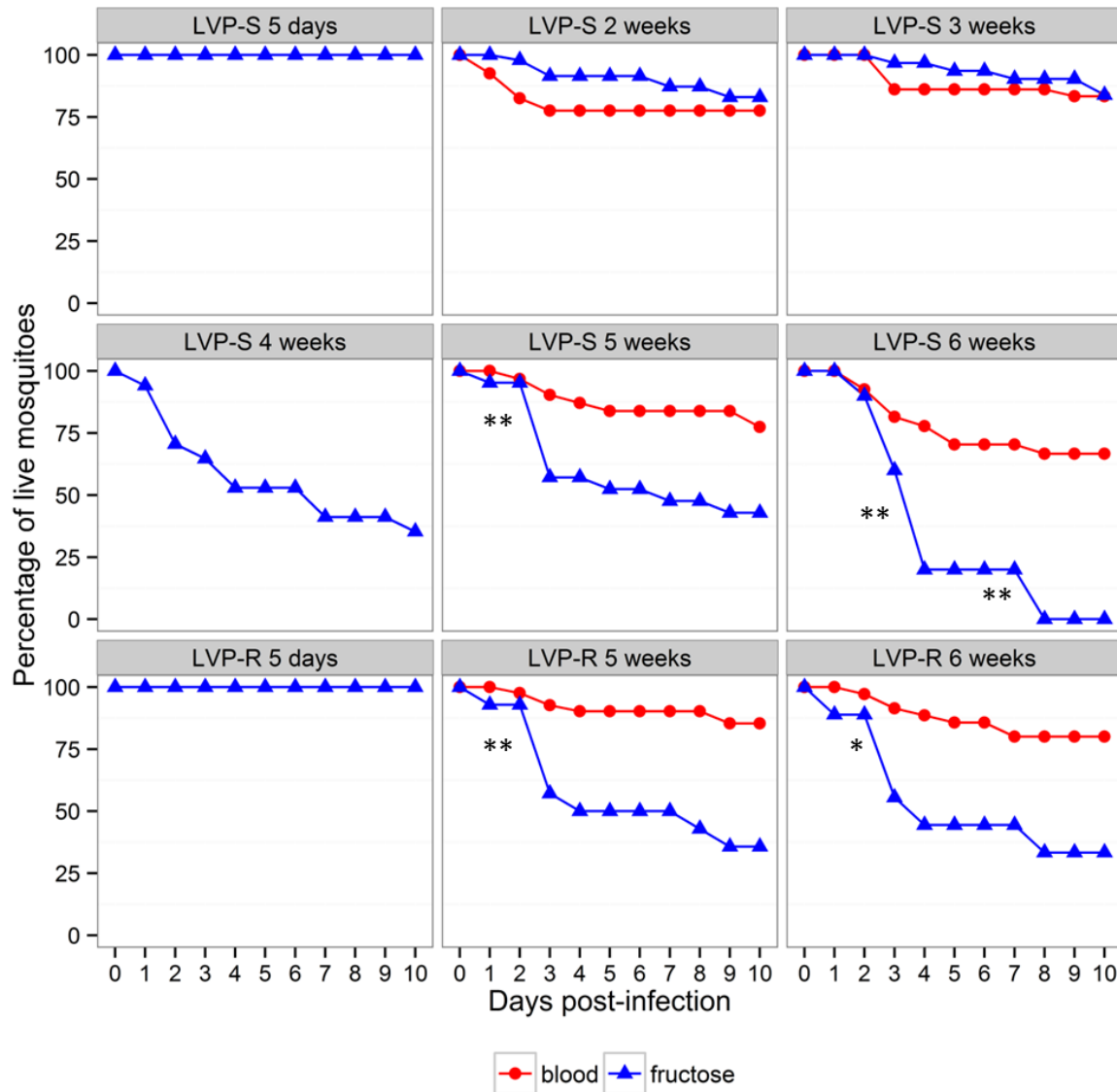


Fig. 3. Survival of *Aedes aegypti* females of different ages and diet after a blood meal containing *B. malayi* microfilaria. Genetically susceptible (LVP-S) and genetically resistant (LVP-R) females were fed on either blood (red) or fructose (blue) before being given the

infected blood meal (day 0). We performed Fisher's exact test to investigate if there were differences in daily mortality between females fed on different diets. Significant differences are indicated on the graph (\*  $p < 0.05$ ; \*\*  $p < 0.01$ ).