A Meta-analysis of Longevity Estimates of Mosquito Vectors of Disease

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Abstract

Mosquitoes are responsible for more human deaths than any other organism, yet we still know relatively little about their ecology. Mosquito lifespan is a key determinant of transmission strength for the diseases they vector, but the field experiments used to determine this quantity – mark-release-recapture (MRR) studies and wild-caught dissection of female mosquitoes – produce estimates with high uncertainty. In this paper, we use Bayesian hierarchical models to analyse a previously-published database of 232 MRR experiments and compile then analyse a database of 131 dissection studies to produce the first ever species- and genus-level estimates of mosquito lifespan. Due to the assumptions required to analyse the field data, we term our estimates lower bounds on lifespan (LBL). Notably, for the major African malaria vector *Anopheles gambiae s.l.*, we estimate LBLs of 4.5 days (mean estimate; 25%-75% CI: 3.8-5.1 days for unfed female mosquitoes from the MRR analysis) and 9.5 days (mean estimate; 25%-75% CI: 5.2-11.0 days from the dissection analysis); and an LBL of 4.3 days (mean estimate; 25%-75% CI: 3.6-4.8 days, only present in the MRR database) for the predominantly East-African vector *A. funestus s.l.* We estimate LBLs of 7.0 days (mean estimate; 25%-75% CI: 4.5-8.5 days from the MRR analysis) and 5.0 days (mean estimate; 25%-75% CI: 3.5-5.1 days from the dissection analysis) for *Aedes aegypti*; and 12.1 days (median estimate; 25%-75% CI: 10.0-13.7 days from the MRR analysis) for *Ae. albopictus* – the predominant vectors of dengue fever, chikungunya and Zika. Our estimates indicate that there is significant variation in lifespan across species, with most variation explained by differences between genera. In correspondence with laboratory studies, we estimate that female mosquitoes outlive males by 0.9 days on average (mean estimate; 25%-75% CI: 0.3-1.6 days). We fit models incorporating mosquito senescence to the data, which allows us to assess evidence for age-dependent mortality in mosquitoes across different species. We determine that 8 of 33 species included in the MRR database indicated evidence for senescence, versus only 2 of 25 species from the dissection database. Our analysis applies a common framework to the analysis of databases of MRR and dissection-based experiments, allowing us to produce

robust estimates of lower bounds on lifespan. It also enables us to critically appraise each field method, highlighting a need for alternative field methods for measuring this important mosquito characteristic.

Keywords

mosquitoes, mortality, senescence, mark-release-recapture, vector-borne disease, Bayesian, hierarchical model.

Introduction

Some of the most important infectious diseases afflicting humans are transmitted by mosquitoes (Gates, 2014), including pathogens such as the causative agent of malaria that have been associated with humans throughout our evolutionary history (Carter and Mendis, 2002) as well recently emergent infections such as the Zika virus (World Health Organisation, 2016). Most mosquito species have a "gonotrophic cycle" involving successive episodes of vertebrate blood feeding, egg maturation and oviposition (Silver, 2007). In order for a mosquito to transmit a pathogen it must feed on an infectious person and live long enough to complete at least one gonotrophic cycle and feed on an uninfected and susceptible individual. Adult lifespan is thus a critical determinant of the ability of a mosquito population to allow the persistence of an indirectly transmitted infection (Macdonald, 1957). Lifespan can of course be straightforwardly assessed in the laboratory, but it is generally accepted that measurements under relatively benign laboratory conditions are likely to have limited relevance in the field, and much effort has been directed at estimating this parameter in the vector's natural environment (Clements and Paterson, 1981; Guerra et al., 2014). Most work has focused on assessing average daily mortality rates, and the simplest assumption is that these do not vary with mosquito age – in this case longevity is simply the reciprocal of mortality. Testing this assumption and discovering whether mosquitoes senesce or show other types of age-dependent mortality has also been studied in the field (Clements and Paterson, 1981; Harrington et al., 2008; Hugo et al., 2014).

There are two main strategies to estimate mosquito mortality rates and longevity. The first is through mark-release-recapture (MRR) experiments, a technique that is widely applied to estimate these parameters in many types of animal. As applied to mosquitoes, insects are caught in the field or reared in the laboratory and then marked, typically with fluorescent dust. The mosquitoes are then released into the field and then efforts are made to recapture them, for example using human baits or light traps, usually over an extended period of time. Mortality rates can be statistically estimated from the numbers of recaptures given certain assumptions (Silver, 2007). The main challenges with MRR is ensuring

the marking technique does not affect recapture probability, and distinguishing mortality from mosquitoes dispersing out of range of being recaptured. Also, releasing insects that can transmit disease (especially if this increases ambient population levels) raises important ethical issues.

The second technique is specific to mosquitoes and makes use of their gonotrophic cycle (Polovodova, 1949; Detinova, 1962). The mosquito ovary is made up of ovarioles, each of which typically produces one egg every gonotrophic cycle. After the egg passes into the oviduct the distended ovariole does not completely recover its previous form but a discrete dilation remains which can be detected by dissecting the female reproductive organs. Data on the fraction of females that have oviposited provides some information about mortality rates. However, a skilled dissector can distinguish the number of dilations from multiple gonotrophic cycles so providing much richer data on longevity. The challenges of this method include the amount of time and expertise it takes to collect the data, establishing the relationships between physiological and chronological time (though the distribution of the number of gonotrophic cycles wild-caught mosquitoes have gone through is of direct epidemiological relevance) and the fact that it only applies to females.

An issue with both methods is that they require logistically difficult and expensive field campaigns. There is thus value in conducting a meta-analysis of existing data to explore consistency across studies, identify correlates of lifespan and to learn lessons for further studies. Here we analyse data from 232 MRR and 131 dissection studies using a common statistical methodology. For MRR we make use of a very valuable database of 394 mosquito studies assembled by Guerra et al. (2014) while the dissection studies we extracted from the literature ourselves. We concentrated on the three major genera of mosquito vectors, *Anopheles, Aedes* (in its traditional sense) and *Culex*, which constitute the majority of the data.

Methods

In recent years many important vectors of disease have been shown to be complexes of closely related species, biotypes or forms that cannot be distinguished morphologically (for example the morphospecies *Anopheles gambiae sensu lato* is now separated into the widespread *gambiae, coluzzii, arabiensis* and a number of more local species). As the majority of studies analysed here took place before molecular techniques allowed these taxa to be separated we work here chiefly with morphospecies.

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Mark-release-recapture

Data from MRR experiments in the Guerra et al. (2014) database were examined and those with fewer than six recaptures and species with only a single MRR study were excluded for the hierarchical analysis. Of the 232 data sets, 179 involved only females, 35 males, and 18 both sex releases. For 102 data sets the age of the released mosquitoes was known (the average age of released mosquitoes was 4.0 days) while in the other cases it was unknown or unrecorded; in these cases we assumed the mosquitoes were newly emerged at the time of release and return to this assumption later. See Table S1 for a summary of other data characteristics.

We analysed all MRR experiments within the same statistical framework (for full details see the Supplementary Online Material (SOM)). In the simplest case N_R mosquitoes are released on day zero and the probability that they remain in the recapture area until day t is $S(t)$ when they are recaptured with probability ψ. We model the number of mosquitoes recaptured on day t using a negative binomial sampling model with mean $(N_R - Y(t-1))S(t)\psi$, where $Y(t-1)$ is cumulative captures before day t, and shape parameter κ. The negative binomial has been used previously in analyses of mosquito count data (Service, 1971; Nedelman, 1983) because of its ability to represent temporal over-dispersion in recaptures most likely caused by variable weather. A slight modification was required for studies with multiple releases (see SOM).

The simplest model for $S(t)$ assumes there is a constant probability (λ) that a mosquito dies or leaves the recapture area so that the numbers remaining after time t are given by the exponential distribution, $\exp(-\lambda t)$. We utilised this form extensively but in testing for senescence used five other models where $\lambda(t)$ varies with time so that,

$$S(t) = e^{-\int_0^t \lambda(\tau)\mathrm{d}\tau}.$$

Details of the five models (Gompertz, Weibull, Gompertz-Makeham, Logistic and Logistic-Makeham), which vary in their ability to detect different forms of age-dependent mortality, are given in the SOM. Using multiple different types of models increased our chances of detecting senescence though, as discussed below, also increases the likelihood of false positives.

Parameters were estimated using Bayesian techniques with relative uninformative priors for κ and the parameters of $\lambda(t)$, but assuming a prior for ψ indicating a low recapture probability (bounded in part by knowledge of the maximum daily recapture rates; see SOM). We used a Bayesian hierarchical model to estimate distributions of lifespan at the species and the genus levels, and across the complete data set. This procedure assumes that there is a distribution of lifespan parameters for each species from which those governing individual MRR time series are sampled, and similarly a distribution at the genus level from

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which those for individual species are derived (rather akin to random effects in classical statistics). Within this framework we can also allow the parameters for individual time series to be influenced by co-variates such as differences in experimental methodology. As in the estimation of the parameters of the individual experiments, relative uninformative priors were set for the parameters of the hierarchical models except for ψ where again a distribution representing low recapture probabilities was assumed. Posterior distributions were derived using Markov Chain Monte Carlo (MCMC) methods with convergence assessed using the $\hat{R}$ statistic (Gelman and Rubin, 1992). The predictive power of the model was assessed using K-fold cross validation which tests the ability of the model fitted to part of the data to predict the rest using multiple different partitions. Further details of the prior specification, fitting and validation through posterior predictive checks are given in the SOM.

Two studies of *Anopheles balabacensis* reported capture rates increasing with time, presumably reflecting a violation of our assumption of constant recapture probabilities. We omitted this species from the analysis.

The Guerra et al., (2014) database included the latitude and longitude of each study along with the date when the study began. We used this information to find estimates of the air temperature for each study using the European Centre for Medium Range Weather Forecasts' ERA Interim Daily historical database. For each study we calculated the mean monthly temperature across a spatial area of (latitude $\pm$ 1 degree, longitude $\pm$ 1 degree), for the month at which each study was carried out. The records for this database begin in 1979, which pre-dates the study date for 65 of our 232 MRR time-series. For these time-series, we chose to estimate the air temperature by an average of the corresponding monthly temperatures over the years 1979-89.

Dissection

Studies using dissection to estimate mosquito longevity were located in literature databases using relevant keyword, citation and author searches, and by checking previous studies cited by the papers located (see SOM). The list of studies located with associated metadata is available as a Supplementary Online File.

Most dissection studies recorded the distribution of the number of gonotrophic cycles in mosquito samples collected over a specific period of time. Overall, we found 568 time series in 72 published articles. Because seldom were sufficient ancillary data available to analyse trends over time, we aggregated the data from each time series. We further omitted time series with fewer than 100 mosquitoes and for species with only one data set leaving 131 studies of mosquitoes in the genera *Anopheles, Aedes, Culex* and *Mansonia.*

To compare lifespan estimates from dissection and MRR studies we need to convert physiological age (the number of gonotrophic cycles) into chronological age. Using a literature search and a review by Silver (2007) we found 79 estimates

in 42 published articles. Most estimates were obtained by dissecting females recaptured in MRR studies or by observations in the laboratory, the latter tending to give longer durations. Studies differed greatly in how (if at all) they represented uncertainty in their estimate of the duration of the gonotrophic cycle. Where confidence limits were given we treated these as the relevant quantiles of a normal distribution, where a range was stated (e.g. "4-6 days") we interpreted the bounds as the 2.5% and 97.5% quantiles of a normal distribution, and where a single figure was quoted we assumed this was the mean this distribution. Using the quantiles of the normal distribution, we estimated its mean and standard deviation by regression (see SOM). Initially we calculated distributions of gonotrophic cycle lengths at the species and then genus levels, but because of the paucity of data for many species and the lack of significant differences we aggregated the data into a single distribution. We converted physiological age to chronological age by sampling from this distribution to obtain a particular gonotrophic cycle length for each mosquito (we also explored sampling from this distribution to obtain the duration of *each* gonotrophic cycle which increased the uncertainty in lifespan estimate but did not affect any of the conclusions).

We modelled the number of mosquitoes found by dissection to be of age a using the negative binomial distribution with mean $\Psi S(a)$ and shape parameter κ, where Ψ is the product of the recruitment rate of adult mosquitoes, which we assume is constant over time, and the probability of being captured for dissection, and $S(a)$ is the probability of surviving until age a. We used the number of females that have yet to lay eggs (nulliparous) to estimate the recruitment rate as described further in the SOM. Initial examination revealed that in some data sets the number of nulliparous females was anomalously low, something that has been noticed before (Gillies and Wilkes, 1965). As some studies have suggested that the first gonotrophic cycle tends to be longer than the subsequent ones, this is probably due to differences in capture probability. In data sets where the fraction of nulliparous females was less than 90% the uniparous (completed on gonotrophic cycle) we excluded the nulliparous observation.

Data was analysed using a Bayesian framework as with the MRR data with minor differences in the specification of the priors (see SOM). Some published studies do not distinguish the number of gonotrophic studies beyond a threshold (the more ovariole dilations there are the harder it is to count them) which is akin to censoring the data. Because this censoring involves only a relatively small number of mosquitoes in each time series (median = 2%), and because of the technical difficulties of allowing for censoring in our Bayesian estimation procedures, we assume the females died at the threshold age.

Results

Lifespan estimates from MRR

MRR estimates the length of time a mosquito remains alive and is still in the area available for recapture. It thus provides a lower bound to mean lifespan which we shall refer to as LBL. In 187 of the 230 MRR time series the estimated LBL was less than 10 days (Fig. 1). The smallest estimate was 1.1 days for the Asian malaria vector *Anopheles subpictus s.l.* which is unfeasibly short and almost certainly reflects dispersal out of the recapture zone or a violation of the assumptions of our analyses. The longest estimate was 26.9 days for the temperate species *Aedes simpsoni s.l.* which is a vector of yellow fever in Africa. There are multiple data sets for the most important vector species such as *Anopheles gambiae, Aedes aegypti* and *albopictus* and *Culex tarsalis* all of which show considerable variation. For example, there are 54 estimates of LBL for *Ae. aegypti* which range from 2.2 days to 38.3 days with a mean of 8.3 days and coefficient of variation of 0.7. There were significant differences in LBL amongst species (ANOVA on median LBL controlling for sex and pre-release feeding: $F_{37,194} = 2.5$, p <0.01; the non-parametric Kruskal Wallace: χ^2_{38}, p<0.01).

The estimated mean LBL for female mosquitoes not pre-fed with blood or sugar before release for *Culex, Anopheles* and *Aedes* were 2.9, 6.8 and 8.1 days respectively with an overall estimate of 6.0 days (Fig. 2; Table S8). Differences between genera were significant (ANOVA on median LBL controlling for sex and pre-release feeding: $F_{2,229} = 12.4$, p<0.01; Kruskal Wallace: $\chi^2_2 = 30.8, p < 0.01$). K-fold cross validation suggests that after the effect of genus is accounted for the incorporation of a species term provides little predictive power (Fig. S#; in part explained by the latter model over-fitting the data where there are few time series per species).

We reasoned that if dispersal out of the recapture area was reducing the LBL below the true lifespan then there should be a positive correlation between the spatial extent of the recapture zone and LBL. We found no such pattern (Fig. S23), although there was a positive correlation between LBL and trap density (Fig. S24).

The MRR experiments included a mixture of male-only and female-only releases, and releases of both sexes. We estimated average male and female LBL at the genus level (Fig. 3; there were too few studies to make comparisons at the species level). There was a consistent trend for females to live longer than males for each of the genera, with the difference largest for *Aedes* (2.5 days; fraction of pairwise posterior samples of females versus males where difference was less than zero, p<0.01), followed by *Anopheles* (2.0 days; p=0.17) and *Culex* (0.3 days; p=0.34). Overall, female mosquitoes were estimated to live 0.9 days longer than males (p=0.10).

The MRR experiments included information on whether mosquitoes were pre-fed

with sugar (41 time series), blood (71), both (4) or alternatively unfed (116). We estimate that female mosquitoes that were fed on sugar pre-release lived on average for 0.7 days longer than those that were not fed (Fig. S26; a pattern that was consistent across the genera). There were insufficient males that were either fed or unfed with sugar prior to release to make a meaningful comparison. Females that were blood-fed prior to release on average lived 1.5 days longer than those who were not fed for *Aedes* but this trend was reversed for *Anopheles* meaning that there was little difference overall.

To access whether temperature is associated with LBL we used weather records to calculate average temperatures at the MRR sites (see Methods). Using both linear and quadratic temperature terms in regressions, we found no significant relationship between study-site temperature and LBL (overall or within genus) for the 238 datasets we analysed (Fig. 4). This result held if, instead of pooling results from all time series, we considered the four species with the most data individually (*Ae. aegypti*, *Cx. tarsalis*, *A. gambiae s.l.* and *A. culicifacies s.l.*; Fig. S25).

Number of gonotrophic cycles estimates from dissection

Dissection allows the number of completed gonotrophic cycles to be counted and from this the mean number of cycles before death was estimated. Overall, the mean number of cycles completed in a lifetime was 1.3 (posterior mean; Fig. 5; Table S9) and across the 131 studies, 95% of the individual time series estimates were less than 3 (Fig. 6). The estimated greatest number of cycles was for *Anopheles sergentii* (3.0 cycles; posterior mean) which is adapted to desert conditions (it is known as the "oasis vector" of malaria) and may have evolved greater longevity. The important African malaria vector *A. gambiae s.l.* was estimated to be the second longest living (2.4 cycles; posterior mean). The smallest estimated mean number of gonotrophic cycles was for *Anopheles bellator* (0.6 cycles; posterior mean) which transmits malaria in Brazil's Atlantic Forest. There were significant differences in estimated lifetime gonotrophic cycles amongst species (ANOVA: $F_{24,106} = 2.2$, $p < 0.01$; the non-parametric Kruskal Wallace: $\chi^2{}_{24}$, $p < 0.01$).

The estimated lifetime gonotrophic cycles for the different genera were *Anopheles,* 1.6; *Culex,* 1.2; *Mansonia,* 1.1; and *Aedes* 0.8 (Fig. 5; Table S9) and the differences between the genera were significant (ANOVA: $F_{3,127} = 3.4$, $p = 0.02$; the non-parametric Kruskal Wallace: $\chi^2_3 = 21.7$, $p < 0.01$).

Comparison of longevity estimates from two methods

To compare the two methods, we converted numbers of gonotrophic cycle (physiological age) into lifespan (chronological age) as described in the SOM using estimates of gonotrophic cycle duration (Fig. S21). For ten species, we had

enough data from both species to make a comparison, and there was a positive correlation (not statistically significant; Pearson correlation $\rho = 0.42$, $n = 10$, $p = 0.23$) between the two measures (Fig. 7), and in only one case – for *A. darlingi* - (Table S#) there was a significant difference in the time-series level LBLs.

Evidence for age-dependent mortality

The survival model upon which the above analyses are based is the single-parameter exponential model which assumes an age-invariant mortality hazard. We also fitted five multi-parameter models that allow, in different ways, mortality to vary with age. We did this to maximise our chance of detecting age-varying mortality (though aware of the risks of false positives with multiple estimations).

In Fig. 8, we compare the performance of the six models for describing lifespan in MRR studies of 33 species using K-fold cross-validation. We categorised the evidence for age-dependent mortality in each species according to the performance of the five age-dependent models versus the exponential: '+' indicated that all age-dependent models outperformed the exponential; '?' indicated that the exponential outperformed one or more age-dependent models; and '-' indicated that the exponential performed at least as well as all other models. Overall, we estimated that there were 8 '+' species, where age-dependent mortality fit the data better; 11 '?' species where the evidence was mixed; and 14 species where constant mortality models performed at least as well. The species where age-dependent mortality best fit the data included the major vector of dengue fever, Zika and chikungunya, *Ae. Aegypti*. These studies also tended to include multiple release MRR studies which, on average, were conducted over a longer period of time than the others, which may be why we failed to detect age-dependence in the latter (Fig S28).

In Fig. 9, we compare the performance of the six models for describing lifespan in dissection studies of 25 species using K-fold cross-validation, and categorise the evidence in the same way as for the MRR analysis. By our metric, we determined that there were only two species with evidence for age-dependent mortality (the major African malaria vector *A. gambiae s.l.* and *A. minimus*, a malaria vector in Asia).

Overall, we conclude that there is mixed evidence for age-dependent mortality from studies of mosquitoes in the field. It is possible that some of the sampled mosquito species did not live long enough in the wild to experience physiological decline. A Spearman's rank correlation test indicated that there was a correlation between the ranked estimated LBLs of the species and the ranked mean predictive accuracy of age-dependent models for the MRR analysis (ρ=0.19, p=0.01), however was not significant for the dissection analysis (ρ=0.07, p=0.43). Similarly, a recent study determined that the degree of senescence varies according to season for semi-wild populations of *Ae. aegypti* (Hugo et al., 2014), and it is

possible that by pooling data from different geographies and seasons that we failed to detect age-dependent mortality in some cases.

Estimates of the fraction mosquitoes capable of transmitting disease

We can use the posterior parameter estimates from our Bayesian analysis to estimate the fraction of mosquitoes that live beyond a certain age. In order to transmit a disease, a mosquito must live longer than the length of the intrinsic incubation period (the time taken for a pathogen ingested in one blood meal to be ready to be transmitted during a future feeding event). This is a lower bound as it does not include the waiting time to find a host after feeding or egg maturation. In Fig. 10, we plot the fraction of the mosquito population that pass this threshold using estimates from both MRR and dissection studies for vector species (see SOM for references used to identify species as vectors) and their most significant diseases.

For malaria, estimates of the minimum fraction of the population that can transmit the disease vary from <0.1% for *A. subpictus* (from the MRR analysis, as noted above likely to be due to the LBL substantially underestimating lifespan) to 52% for the drought-adapted and long-lived *A sergentii.* The proportions surviving long enough to become infectious for the two major African malaria vectors were: for *A. gambiae s.l.*: 10% (MRR) and 27% (dissection); and for *A. funestus s.l.*: 9% (MRR). Using the individual time series estimates, however, there was inconclusive evidence for a difference in EIP between the species (MRR ANOVA: $F_{14,58} = 1.8$, p=0.07; MRR Kruskal-Wallis: $\chi^2_{14} = 30.2$, p <0.01; dissection ANOVA: $F_{11,64} = 1.8$, p<0.01; dissection Kruskal-Wallis: $\chi^2_{11} = 38.9$, p<0.01).

Ae. aegypti and *Ae. albopictus* are the main vectors of dengue, chikungunya and Zika viruses. Because of their short intrinsic incubation periods a greater fraction of mosquito potentially live long enough to transmit diseases (Fig. 10), rising to a maximum of 84% for *Ae. albopictus* transmitting chikungunya.

Discussion

In this study, we applied a Bayesian hierarchical framework to the analysis of a database of mark-release-recapture experiments and a database of mosquito dissection studies to estimate mosquito lifespan. By applying a single framework, this allows us to effectively synthesise information from the disparate experiments which, individually, estimate lifespan with considerable uncertainty. Due to the assumptions required to analyse the field data, our estimates represent lower bounds on lifespan (LBL). Across both meta-analyses, the estimated LBLs were mostly less than 10 days, hinting that only a small proportion of mosquitoes

may live long enough to transmit disease. We determined that LBL varies across species and genera, although most variance is explained by genus. The MRR analysis includes experiments conducted on each sex individually, and we estimate that, on average, males live shorter lives than females. Pre-release feeding with sugar also lengthens lifespan across all three genera, although this effect is less marked than the sex differences. In contrast to a number of lab-based experiments, temperature was not determined to significantly impact lifespan. By fitting a range of survival models to the data in both meta-analyses, we could assess evidence for age-dependent mortality. Overall, we conclude that the evidence is mixed: in the MRR experiments, in 8 of 33 species we found evidence for mosquito senescence, whereas in only 2 of 25 species included in the dissection analysis were better fit by a model incorporating an increasing risk of mortality with age.

MRR experiments are known to produce downwardly-biased estimates of lifespan. Lab experiments have demonstrated that marking can negatively impact survival (Verhurst et al., 2013; Dickens and Brant, 2014), resulting in artificially depressed survival. MRR studies typically cannot differentiate between a mosquito dying and dispersal from the study area meaning that lifespan will be underestimated. In this study, we found a positive correlation between lifespan estimates and the density of traps, indicating that better trapping coverage likely raises estimates towards their real value. We conducted an *in silico* Monte Carlo study to determine how accurately we could estimate mosquito lifespan given study parameters in an ideal MRR experiment, where the assumptions of no emigration and harmless marking are fully satisfied (see SOM for full details). This work indicated that for many of the experiments, the short study lengths or typical numbers of mosquitoes released, results in considerable uncertainty in lifespan estimates (Fig. S27). This indicates that statistical power can be substantially increased by pooling data across experiments as we did using a Bayesian hierarchical model.

The key assumptions of dissection based methods to determine chronological age are: (i) physiological age can be accurately determined by dissection of female specimens (unlike MRR, this method can only be applied to one sex), (ii) the relationship between physiological and chronological age is known, (iii) the population being sampled is in equilibrium (recruitment matches mortality) and (iv) individual mosquitoes can be randomly sampled from the population. The reliability and accuracy of dissection has been questioned. The objections include the impracticality of dissecting more than a small proportion of ovarioles (Hoc and Wilkes, 1995), particularly in African vector species (Gillies and Wilkes, 1965), the related issue of locating ovarioles whose count of dilations represents true physiological age (Fox and Brust, 1994), and the variation in numbers of ovariolar dilations for mosquitoes of the same, known, physiological age (Kay, 1979; Russell, 1986; Hugo et al., 2008). Indeed there is considerable uncertainty concerning the fundamental question of how dilations in ovarioles form in the first place. Whilst the 'Old School' of thought (a term coined by Fox and Brust, 1994) headed by Polovodana (Polovodova, 1949) and Detinova (Detinova, 1962)

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considers dilations to result from normal oogenesis, a 'New School' headed by Lange and Hoc (Lange and Hoc, 1981) has challenged this assertion. The New School believe that only abortive oogenesis results in follicular dilations because normal oogenesis destroys the sack-like structures (Fox and Brust, 1994). This means that Polovodana's method requires dissecting large numbers of ovarioles to uncover those with the most dilations, where abortive oogenesis has occurred in each gonotrophic cycle. They deem these ovarioles 'diagnostic' since only in these cases the number of dilations equals the number of gonotrophic cycles that have occurred. As a mosquito ages, the number of diagnostic ovarioles diminishes, since the random occurrence of normal oogenesis in a particular ovariole means its dilation count does not equal the number of gonotrophic cycles undertaken. This increased difficulty of finding diagnostic ovarioles as a mosquito ages would elevate the chance of age 'hypodiagnosis' for older specimens (Fox and Brust, 1994), and likely biases lifespan estimates downwards. The difficulty of locating diagnostic ovarioles has been investigated using lab populations of *Culex* and *Aedes* mosquitoes by Hugo et al., 2008, who conclude that only a small percentage of ovarioles are diagnostic. The exchange rate between physiological age and chronological age is the duration of gonotrophic cycles. Two methods are commonly used to estimate the duration of gonotrophic cycles: MRR studies (see, for example, Gillies and Wilkes, 1965), where marked mosquitoes are recaptured and dissected to determine the number of gonotrophic cycles occurring since release; and laboratory-based observations of colonies of (typically) wild-caught females, or their progeny (see, for example, Afrane et al., 2005). Whilst it is unclear how each method could bias estimated gonotrophic cycle duration, in our analysis, laboratory-based studies indicated a longer gonotrophic cycle (Fig. S22). The distributions we used to convert physiological age into calendar age were calculated by pooling data across both approaches, to incorporate uncertainty from both experimental procedures. It is possible, however, that this aggregate approach may induce biases in estimates and an approach more entrenched in experimental knowledge would fare better. If a population of mosquitoes is shrinking, this leads to a relative under-abundance of young mosquitoes, and a flattening of the survival curve, resulting in over-estimates of lifespan. For stable populations, periods when shrinking occurs must result in equal changes in the population size compared to those when it expands. If mosquito collections occur with equal frequency in each of these two modes, then aggregating the data across all sampling times and estimating a single model, as we do here, should yield an approximately unbiased estimate of lifespan. The additional uncertainty of a fluctuating population size, however, could lead us to understate the uncertainty in estimates. Field entomologists have challenged the assumption of random sampling the mosquito population, although there are conflicting opinions as to whether this results in a relative paucity (Gillies and Wilkes, 1965) or abundance (Clements and Paterson, 1981) of nulliparous individuals. In our database, there are cases where there was an obvious deficit of nulliparous individuals, which has previously been ascribed to the differing distribution of resting females between indoor and outdoor traps (Detinova, 1962; Gillies and Wilkes, 1965). We chose to not include those counts of nulliparous individuals in our analysis

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where their number was less than 90% of the uniparous. Whilst we see no obvious differences in lifespan according to collection method (data not shown) or location, it is possible that the assumption of random sampling is violated, although the directionality of the bias induced by this is unclear. Overall, the assumptions underpinning estimates from dissection studies indicate that our estimates represent lower bounds on lifespan. The alternative dissection-based approach of Detinova (Detinova, 1962), based on dichotomous categorisation of female mosquito specimens as 'parous' or 'unparous' relies on fewer assumptions, and is widely used. Further work examining parity rates in field specimens may be fruitful although, in principle, it offers less information on the age structure of a population than Polovodova's approach.

By applying a common method to analysing all studies in our databases, it is possible that we may have missed patterns of mortality that would have been evident from using a more bespoke approach. As our *in silico* analysis of MRR experiments indicates, however, the overdispersed data from single experiments results in high measurement error (Fig. S27). By applying different methods to each study, this could lead us to falsely detect patterns when none are present, and we prefer a pooled approach.

The different nature of the assumptions of each of the two methods means they offer complimentary information on mosquito survival. We also note that Polovodova's dissection-based studies require specialised expertise which will often be unavailable, whereas MRR methods can more readily be used. Furthermore, most if not all dissection methods that have been used previously are only applicable to female mosquitoes, whereas MRR can be applied to either sex and can additionally be used to determine other ecological parameters (for example, population size and dispersal). Although dissection data gives detailed of age-structure, we thus foresee a continued reliance on MRR experiments in field entomological experiments. Efforts to use both approaches concurrently will be particularly useful and will allow quantification of the biases induced by the assumptions of each. Similarly, MRR experiments releasing large numbers of marked mosquitoes and recording spatiotemporally-disaggregated captures of wild and re-caught marked mosquitoes will be especially useful to estimate lifespan and dispersal.

To compare estimates of lifespan derived from MRR with those from dissection-based methods, we display the estimates of lifespan from those ten species occurring in both databases in a single plot (Fig. 7). In is reassuring that there is correlation between estimates from both approaches, although the small sample size likely hindered our ability to determine statistical significance. In both cases, we estimate that *A. sergentii* was amongst the longest lived of the anopheline species with a posterior median LBL of 8.7 days (median estimate; 25%-75% CI: 5.9-13.8 days) from the MRR analysis and 10.0 days (median estimate; 25%-50% CI: 7.6-14.0 days) from the analysis of dissection studies. This species is a major vector of malaria in the Sahara (Sinka et al., 2010), where to act as a disease vector it must persevere through these hard

conditions. It is reasonable to hypothesise that this species should live longer than those in environments where the potential for blood-feeding and oviposition is greater. The species with the greatest discrepancy in the estimates was the major African malaria vector *A. gambiae s.l.*, where we estimated LBLs of 4.4 days (median estimate; 25%-75% CI: 3.8-5.1 days for unfed female) from the MRR analysis and 7.6 days (median estimate; 25%-75% CI: 5.2-11.0) from the dissection analysis. Across genera, the greatest discrepancy in estimates was for *Culex* (a posterior median of 2.5 days from the MRR versus 4.4 days from the dissection analysis) with the smallest discrepancy for *Anopheles* (4.8 versus 5.0 days). By contrast for *Aedes* the estimates from the MRR studies (6.9 days) exceed those of dissection-based studies (3.5 days). Across all studies we estimate from the MRR analysis that mean mosquito lifespan is 4.6 days versus 5.1 days from the dissection-based studies. Some of the differences in these group-level estimates between the two approaches is likely due to environmental and genetic differences between mosquitoes in the experiments that were analysed in each meta-analysis. However, we believe that part of the discrepancy can be explained by the methodological differences in approaches. We speculate that differences in dispersal rate can explain some of the discrepancy. Both *Anopheles* and *Culex* mosquitoes are generally thought to fly farther during their lifetimes than *Aedes* [Charles, do you have a reference here?], meaning that the estimates from MRR-based approaches will be most downwardly-biased for these genera. This is supported by our results since the dissection-based estimates (themselves not reliant on assumptions about dispersal) exceed the MRR estimates for *Anopheles* and *Culex* mosquitoes, but not for *Aedes*.

It is widely believed mosquitoes live artificially long under the benign conditions of the laboratory. We find it informative to consider estimates of lifespan derived from observations of such populations as they constitute an upper bound on the lifespan of wild populations. Also, since the numbers of mosquitoes involved in large cage experiments often numbers in the thousands, these estimates have lower uncertainty than those from field experiments although are typically conducted on highly inbred mosquito strains. Styer et al. (2007), using colonies of 45,054 female and 55,997 male *Ae. aegypti*, determined that females lived nearly twice as long as males; the median lifespan was estimated as 31.69 ± 0.06 days for females and 16.39 ± 0.03 days for males. A similar study by Dawes et al., 2009 with a lab colony of over 1000 female *A. stephensi* found similar estimates for median lifespan (31-42 days). These estimates are many multiples of the average estimates that result from our analysis of field data which, as discussed, represent lower bound estimates. Without an unbiased method to measure mosquito lifespan, however, it is difficult to quantify and explain the gap that exists between field and laboratory lifespans. The development of additional methods to estimate mosquito age, such as 'Near-Infrared Spectroscopy' (Mayagaya et al., 2009; Sikulu et al., 2011; Lambert et al., 2018), if they are proven to work in the field, may be of considerable worth here.

We conducted a power analysis of MRR experiments to determine whether typical experimental characteristics could detect senescence. Here we calculated

the power of a maximum likelihood estimator of the 'senescence parameter' β of the Gompertz survival function (see Table S#) for case study populations with three different levels of senescence (Fig. S28A). This analysis indicated that power to detect senescence strongly depends on study length (Fig. S28B) but is insensitive to release size (Fig. S28C). Clements and Patterson (1981) conducted a meta-analysis of MRR and dissection-based field experiments and found evidence of an increasing risk of mortality hazard with age that is similar in magnitude to that of the 'mild' case considered above. For this case, detecting senescence with a power of 80% requires a study length of at least 18 days. Since the median study length for experiments included in our analysis was 10 days (Table S#) this could partly explain our failure to detect senescence at the species level. A number of experiments have found evidence of age-dependence in laboratory populations (Styer et al., 2007; Dawes et al., 2009). However, the artificially benign environment of the laboratory means mosquitoes live considerably longer than in the wild, where they may die because of exogenous factors, before the effects of physiological decline have had time to manifest. Field experiments have also found evidence for age-dependent mortality. Harrington et al. (2008) conducted a field experiment where mosquitoes reared under laboratory conditions were marked and released at different ages. Analysis of the resultant MRR time-series indicated that mosquito mortality increases with age at release. It is possible, however, that this field experiment suffers from the same biases as laboratory-based approaches, because the released mosquitoes were often of ages considerably higher (up to 20 days) than typical estimates of wild mosquito lifespan.

As ethical concerns of contributing to disease burden are more often considered, it is now less common for MRR experiments to release female mosquitoes versus males than historically (Fig. S2). Our analysis indicates that females outlive male mosquitoes by approximately 0.9 days (Fig. 3), meaning that differences between the sexes may exist for other ecological parameters determinable by MRR. This suggests that continued field entomological work on contained releases of mosquitoes in semi-field sites or large microcosms may be a valuable source of information on female mosquitoes.

Our estimates of LBL indicate that mosquitoes that were sugar-fed prior to release lived on average 0.7 days longer than those that were unfed (, suggesting the potential value of this underappreciated aspect of the mosquito ecology to the insects. It may also partly explain the recent successes in the use of Attractive Toxic Sugar Baits (ATSB) as a vector control intervention (Schlein and Muller references). More research is needed, however, to identify the sugar-feeding frequency and food sources for wild populations.

There is considerable evidence from the laboratory that temperature modulates mosquito ecology and behaviour (Matt Thomas references). The locations and times of year over which the MRR studies were conducted encompassed a large range of average air temperatures, from approximately 10 °C to 35 °C and, within this, we determined no relationship between lifespan and temperature

across all time series (Fig. 4) or, for any of the species with the most data (Fig. S25). It is possible that by considering a raw average of air temperature across the month, this ignored, more complex, interactions between temperature and lifespan. It is also possible that by ignoring the effects of rainfall (the historical data on rainfall is less likely to be reliable for a given location), that this masked a more complex interaction between longevity and temperature. The observed laboratory relationship between lifespan and temperature, however, may not be as robust in the field if mosquitoes adjust their behaviours (such as, by seeking shade) in reaction to changes in temperature. More work exploring the relationship between mosquito ecology and temperature in semi-field experiments may be useful in probing these interactions further.

In this work, we have used modern statistical methods to synthesise precious field data conducted by entomologists past and present, to produce lower bound estimates of mosquito lifespan. The importance of vector mortality for disease transmission has long been recognised, however, since even before 1957, when George Macdonald formulated the now famous Ross-Macdonald equation of R_0 for malaria. Indeed, the recent declines in malaria prevalence in Sub-Saharan Africa were likely due to upscaling of interventions (insecticide-treated bednets and indoor residual spraying) that aim to reduce mosquito lifespan (Bhatt et al., 2015). Worryingly, resistance to pyrethroids, the only class of insecticide used in current insecticide-treated bednets and likely the only product to come to market in the near future, has been determined to be widespread and increasing in intensity across Sub-Saharan Africa (WHO reference 2018). This alarming trend highlights the need for continued MRR and dissection-based studies to monitor the effectiveness of bednets and determine whether more expensive alternatives, such as nets incorporating piperonyl butoxide be deployed. It also emphasises the need for investment in new tools for real time monitoring of mosquito populations. In recent years, there has been considerable funding allocated to molecular and genomic research into mosquitoes that strengthens existing interventions and suggest novel control strategies. Without commensurate funding allocated to applied vector ecology, our lack of knowledge in this area threatens our opportunity to capitalise on molecular advances and potentially hinders our ability to control of mosquito-borne disease.

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