**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 animal, yet we still know relatively little about their ecology. Mosquito lifespan is a key determinant of the force of transmission for the diseases they vector, but the field experiments and dissection methods used to determine this quantity produce estimates with high uncertainty. In this paper, we use Bayesian hierarchical models to analyse a previously published database of 232 mark-release-recapture (MRR) experiments and two databases of different types of mosquito dissection experiments. One, compiled by us, consisted of 131 detailed estimates of the number of egg-laying (gonotrophic) cycles, the other, a recently published dataset of 1490 studies of parity (whether a mosquito has laid eggs or not) in anopheline malaria vectors. We analysed or reanalysed all studies with the same methodology and used Bayesian hierarchical statistics to obtain estimate at the species and genus level. For the major African malaria vector *Anopheles gambiae s.l.*, we estimate lifespans ranging from 4.4 days (from MRR analysis) to 10.3 days (from parity analysis). For the predominantly East-African vector *An. funestus s.l*., our lifespan estimates range from 4.2 days (MRR) to 7.1 days (dichotomous parity analysis). We estimate lifespans ranging from 4.7 days (physiological age analysis) to 6.2 days (MRR) for *Aedes aegypti*, and a lifespan of 11.6 days for *Ae. Albopictus* (only present in MRR data) – the predominant vectors of key arboviruses. Additionally, we estimate that female mosquitoes outlive males by 1.2 days on average (mean estimate; 25%-75% CI: 0.3-1.6 days). By fitting a range of survival models to the data, we found relatively little evidence for senescence in the field. Our analyses, supplemented by power analyses, indicate the comparatively weak evidence for mosquito lifespans in the field. We conclude further progress will require larger and longer experiments or the development of novel new methodologies.

**Author summary**

Mosquitoes transmit some of the most important diseases aﬄicting humans, with malaria alone killing between 390,000 and 46,000 people annually in 2019, chiefly children in low-income countries. The force of transmission strength of these diseases depends critically on the duration of mosquito lifespans, and some of the most successful disease control interventions, including insecticide-treated bednets, work because they reduce mosquito longevity. In this study, we conduct meta-analyses of two important classes of field experiments used to estimate wild mosquito lifespan: mark-release-recapture studies, where mosquitoes are marked with dye then released with the number of marked mosquitoes caught monitored over time; and experiments involving dissection of wild-caught females, whose reproductive anatomy is used as a biological clock to determine physiological age. In all analyses, we estimate that most mosquito species live less than 10 days on average, which suggests that relatively few mosquitoes live sufficiently long to transmit disease. We find evidence of variation in mosquito mortality across species, with the estimates of lifespan obtained from each method largely corresponding for the few species with data from both experiments. Finally, by fitting a range of survival models to the data, we conclude that, for most species, mosquitoes do not experience strong age-related increases in mortality. We critique the quality of the existing evidence base about mosquito lifespans in the field and suggest how it may be improved.

**Author contributions**

BL, AN and HCJG designed this study. BL was responsible for data curation, developing the statistical methodology and conducting the investigation. All authors were involved in drafting the original manuscript and revising it.

**Keywords**

mosquitoes, mortality, meta-analysis, 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 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 longevity. The first is through mark-release-recapture (MRR) experiments, a technique that is widely applied to estimate lifespan 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 attempts 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 significantly increases ambient population levels) raises important ethical issues.

The second approach is specific to female mosquitoes and makes use of their gonotrophic cycle and involves two distinct dissection-based techniques. The simplest and most widely used approach is based on the observation that the appearance of the fine tracheoles encasing ovaries changes irreversibly when ovaries first develop (Detinova, 1945). The proportion of parous individuals – those individuals that have produced offspring – can be determined by dissecting field-caught specimens and, by making assumptions of the duration of gonotrophic cycles, an estimate of lifespan can be derived. In honour of the entomologist who first made this observation, this approach is known as Detinova’s method. The straightforward dissection technique needed to apply this method means it has been widely adopted, but the assumptions that need to be made limit the information that can be derived about mortality. The second technique requires more sophisticated dissection and involves counting the number of reproductive cycles a mosquito has undertaken. 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 (Polovodova, 1949). A skilled dissector can determine the number of such dilations, so providing richer data on longevity. This approach is known as Polovodova’s method after the scientist who first observed these changes. The challenges of this method include the amount of time and expertise it takes to collect data. There is also concern that ovarioles may not produce visible dilations during oogenesis meaning many need be dissected to uncover those few which correspond with the number of gonotrophic cycles a mosquito has experienced (Fox and Brust, 1994). Both dissection approaches are specific to females and require conversions 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).

An issue with all 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, to identify correlates of lifespan and to learn lessons for further studies. Here, we apply a common statistical methodology to analyse data from 232 MRR experiments, 1490 observations of parity obtained through Detinova’s method, and 131 studies that used Polovodova’s method to determine physiological lifespan. For both MRR and Detinova’s method, we make use of valuable published databases: for MRR, we use that published by Guerra et al. (2014); for Detinova’s parity determination, we use a study of anopheline malaria vectors assembled by Massey et al. (2016). In addition, we extracted data from studies that used Polovodova’s method ourselves via a literature search. We concentrated on the three major genera of mosquito vectors, *Anopheles,* *Aedes* (in its traditional sense) and *Culex*, which constitute the majority of thedata.

**Glossary (as a box)**

* MRR experiments – mark-release-recapture experiments, where mosquitoes are marked with a dye or equivalent, released and then subject to potential recapture.
* Gonotrophic cycle – the sequence of searching for a host, blood-feeding, egg maturation and oviposition for a female mosquito.
* Parity rate – the proportion of female mosquitoes that have laid eggs.
* Nulliparous – a female that has not laid eggs.
* Parous – a female that has laid eggs.
* Uniparous / biparous / triparous – a female that has undergone 1 / 2 / 3 gonotrophic cycles.
* Physiological or reproductive age / time – the number of gonotrophic cycles a female has undergone throughout their life / over a period of time.
* Chronological age / time – age or time measured in calendar time (e.g. days).
* Detinova’s (dissection) method – dissecting mosquitoes to determine whether a female is nulliparous or parous (i.e. providing a dichotomous measure of reproductive status).
* Polovodova’s (dissection) method – dissecting female mosquitoes to determine the physiological age.

**Results**

We report estimates of the lifespan of different mosquito species (the mean unless otherwise stated) obtained using the techniques described in the Introduction. We used a Bayesian approach to parameter estimation which provides posterior distributions describing uncertainty in the lifespan estimates. In the Supplementary Online Material (SOM), we provide detailed quantiles and summary measures but here report only the posterior median of mean lifespan with 25%-75% central posterior intervals given as uncertainty measures.

***Most estimates of mosquito lifespan from MRR studies are less than ten days, though there is considerable variation***

We first estimated lifespan independently for each available MRR time-series (Fig. 1; Methods). The estimates varied substantially both within and among species, though a majority were less than ten days (187 of 236 time-series point estimates). In comparison, mosquito longevity in laboratory conditions is typically found to exceed 30 days (e.g. Styler et al., 2007). Our estimates ranged from 0.7 days from a study of the predominantly Australasian *Anopheles* *annulipes* to 38.3 days from a study of *Aedes aegypti*. It is likely that the very short longevity estimates reflect dispersalout of the recapture zone or a violation of the assumptions of our analyses (see Discussion). There are multiple data sets for the most important vector species such as *An. gambiae s.l.* (malaria)*, Ae. aegypti* and *Ae.* *albopictus* (yellow fever, dengue and Zika viruses) and *Culex tarsalis* (West Nile Fever, Western Encephalitis), all of which show considerable variation. For example, there were 54 estimates of lifespan 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.

***MRR data show more evidence of lifespan variation at the genus than species level***

We next combine the data from individual studies using a suite of Bayesian hierarchical models. The first model pools studies by species, the next by genus, and a final model pools all studies together. To ensure comparability, we exclude studies where females were blood or sugar fed before release (Fig. 2 and Table S1), while later we will investigate the role of feeding *per se.* We also set a threshold of at least two time series for inclusion in the species level analysis, which is why some species present in Fig. 1, for example *An. annulipes,* are not included in Fig. 2. The most long-lived species was *Ae. simpsoni s.l.* (an African vector of yellow fever), and the most short-lived was *An.* *subpictus s.l.* (an Asian malaria vector), although this estimate was unfeasibly low and almost certainly reflects dispersalout of the recapture zone or a violation of the assumptions of our analyses. There were also differences in longevity at the genus level, with *Culex* estimated to have the shortest lifespan and *Aedes* the longest.

The considerable variation in these results probably stems from a combination of biological differences at each of the levels in the hierarchy and idiosyncratic study-level variation. To better understand the relative roles of these factors, we compared the fit of the different hierarchical models. Since models with more parameters will naturally fit the data more closely, we used a cross-validation approach that explicitly accounts for this when comparing models (see SOM).The genus level model fitted the data significantly better than the overall model, yet the species model did not significantly improve the fit over the genus model (Table S1). This suggests that lifespan differed across the three genera, but species-level variation did not help to further explain variation observed at the study level.

***MRR data suggest females tend to live longer than males***

The MRR studies included male-only and female-only releases, and mixed releases of both sexes, allowing us to estimate male and female lifespan at the genus level (Fig. 3). Each genus showed a trend for females to live longer than males, with the greatest difference for *Aedes* (2.5 days; *p*<0.01, where *p* is the fraction of pairwise posterior samples in which males outlive females), 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). It is possible that these differences in lifespan are due to sex-specific variation in dispersal, but, to our knowledge, there is limited evidence to suggest this.

***MRR data suggest that sugar feeding modestly increases the lifespan of marked mosquitoes, yet blood-feeding of females makes little difference***

MRR experiments differ in whether mosquitoes are fed or not before their release, and if so whether with sugar, blood, or both. We studied the effects of feeding on female lifespan at the genus level and across all studies (Fig. S1). Since there were insufficient data at the genus level on males that were fed with sugar versus unfed, we combined all genera to estimate a pooled effect of sugar-feeding. For both sexes and across all genera, we found modest evidence that sugar feeding prior to release extended life-expectancy, by an average of 0.6 days for females (*p*=0.15, where *p* is the fraction of pairwise posterior samples in which unfed outlived fed) and 0.5 days for males (*p*=0.15). The effect of blood-feeding on female mosquitoes was not significant, with blood-fed individuals living about 0.1 days longer than unfed mosquitoes (*p*=0.44).

***Lifespan estimates from MRR data were not affected by spatial scale of the experiment as would be expected if dispersal out of the study area was causing severe bias***

Following a release of marked mosquitoes, the rate of their recapture typically reduces over time because some mosquitoes die and because some disperse out of the recapture area. These factors are indistinguishable in spatially averaged recapture data, which is why our estimates are lower bounds on lifespan. If dispersal out of the recapture area commonly reduces the lifespan estimate below the true lifespan then we should expect a positive correlation between the spatial extent of the recapture zone and lifespan. This pattern was not apparent in our data, suggesting that dispersal may not be responsible for severe underestimation (Fig. S2). However, we suspect that dispersal bias may have affected a number of individual studies where we obtained unfeasibly short lifespan estimates, for example the case of *An.* *annulipes* noted above.

***Detinova dissection data show that the use of insecticides reduces anopheline longevity***

The Massey et al. (2016) database contained 1490 parity observations across 26 anopheline species-complexes (henceforth ‘Detinova data’). Insecticide based control measures were in use at the time and place of the study in 519 cases, not in use in 364 cases, while this was unspecified in the remaining 607 cases. Since insecticides act by killing mosquitoes, their use should reduce mean lifespan and we investigated whether this was detectable in the data. We analysed 867 observations (509 with insecticide and 358 without) representing all data from the 16 species complexes with 5 or more observations (see SOM). The effect of insecticides was large in all cases (Fig. S3), on average reducing lifespan by 56% at the species level (51%-58%; difference in posterior median estimates of mean lifespan).

In the following analyses of the Detinova data, we omitted those cases where insecticide was known to be in use, leaving 1126 observations. It is possible that insecticides were used in some of the 607 cases where this was not recorded. However, 76% of these studies took place before 2000, when bednets impregnated with pyrethroid insecticides began to be widely distributed across the African continent (Bhatt et al., 2015; elsewhere, their distribution remained low) suggesting that the majority of these results will be unaffected by insecticide.

***Detinova dissection data suggest most female anopheline mosquitoes complete fewer than three gonotrophic cycles, though this varies considerably between species***

Detinova dissection determines parity, which indicates the fraction of female mosquitoes that have laid eggs. Assuming regular gonotrophic cycles throughout a mosquito’s life, this allows us to convert parity into lifespan (see SOM). We first estimated lifespan for each study independently (Fig. S4). In 78% of cases, fewer than three gonotrophic cycles were completed before death. We next combine these data into Bayesian hierarchical models which pool the studies by species, then species-complex, then continent (here, including Africa, Asia, and Americas), and finally all the studies are pooled together. The lifespan estimates, now in terms of gonotrophic cycles rather than chronological time, are shown in Fig. 4 and Table S2. Two of the species with the lowest estimated lifespans belong to the *An. albitarsis* species complex (Sp. A & Sp. B), which is a malaria vector found throughout South America. The taxon with the greatest lifespan was *An. albitarsis marajoara*, which is part of the same complex (formerly Sp. C), indicating extensive variation in this complex across the continent. Outside of the Americas, the species with the longest estimated lifespans were the major African vector *An. funestus*, and, in Asia, *An. acontinus*. Across the species complexes, the shortest lifespans were estimated for *An. aquasalis s.l.,* which is a dominant malaria vector species in the Amazon (Sinka et al., 2010), and *An. gambiae s.l..*The anopheline species in Africa were estimated to live longest, followed by those in Asia and then the Americas.

To help understand these results, we compared the fit of our hierarchical models at different taxonomic levels in a similar manner to our analysis of variance in the MRR data. Across all continents, the species-level model had the best fit followed by the species-complex level model then the continent-level model (Table S1). Introducing grouping at the species-complex level led to similar gains in the estimated fit over the continent-level model as introducing species did over the species-level model. Overall, this provides evidence that lifespan, at least as measured by number of gonotrophic cycles, varied according to species-complex and, within this grouping, according to species. This differs from our MRR results, a matter we will return to in the Discussion.

***Polovodova dissection data show no variation in lifespan by species or genus***

We estimated lifespan from 131 studies reporting Polovodovan data (Fig. 5 and Table S3), again using Bayesian hierarchical models to pool information across studies. This data included species in four genera (*Anopheles*, *Mansonia*, *Culex*, and *Aedes*), and we thus used species, then genus, and finally all species as the hierarchy levels.

The mean number of cycles completed in a lifetime was estimated to be 1.2, and ranged from 0.45 for *An.* *bellator*,which transmits malaria in Brazil’s AtlanticForest, to 2.45 for *An. sergenti*, which is adapted to desert conditions (it is known as the “oasis vector” of malaria; Sinka et al., 2010) possibly accounting for its greater longevity.

At the genus level, *Anopheles* were estimated to go through the most gonotrophic cycles (1.4) followed by *Mansonia* (1.1), *Culex* (1.0), and Aedes (0.8).

As for the MRR and Detinova dissection data, we compared the fit of the models at each taxonomic grouping (species-level, genus-level and all data pooled) using a cross-validation approach. In contrast to the other two datasets, the models grouped at each of the three levels all fit the data equally well (Table S1).

***There is evidence that gonotrophic cycle duration varies across mosquito genera***

To convert reproductive lifespan estimates into calendar time, it is necessary to estimate the duration of the gonotrophic cycle. We conducted a literature survey of gonotrophic cycle duration measurements including 45 estimates based on laboratory observations of wild-caught specimens and their progeny, and 36 estimates based on MRR methods, where mosquitoes of known age are released and by examining the parity of collected species, an estimate of the gonotrophic cycle duration obtained. In 20 cases, separate estimates for the first gonotrophic cycle were reported which we included in our analysis. We combined these data using a regression approach (SOM).

We found *Culex* mosquitoes to have the longest first gonotrophic cycles (with a mean of 5.2 days; Fig. 6), followed by *Aedes* (4.5 days) then *Anopheles* (3.7 days). These differences among genera were significant (ANOVA: F2,116 = 8.7, p<0.01), and the genus ordering was maintained for subsequent cycle durations. Pooling data across all genera, the first cycle duration was estimated to take 4.0 days and subsequent cycles were estimated to take 3.6 days.

***There are no significant correlations in species-level lifespan estimates using the three different methodologies***

We used our estimates of gonotrophic cycle durations to convert the dissection-based estimates of reproductive lifespan into chronological lifespan as described in the SOM (Tables S4 and S5 provide summaries of chronological lifespan estimates for the species and genera in the Detinova and Polovodova analyses). This allowed us to compare the lifespan estimates from the dissection studies and the MRR studies, where data from the same species were available (Fig. 7). Comparing the two dissection methods (Fig. 7A), there was a positive correlation across six species. For the ten species with both Detinova and MRR estimates there was a slight negative correlation (Fig. 7B), while there was a positive correlation between the Polovodova estimates and those from MRR (Fig. 7C; n=12). In no cases were these correlations statistically significant, though the sample size for each comparison is small.

***The Detinova data does not support an effect of average temperature on the lifespan of African malaria vectors, yet temperature daily range may be important***

*An. gambiae s.l.* and *An. funestus s.l.* are the two most important malaria vector complexes in Africa, and the Massey et al. (2016) dataset has many parity observations for these taxa allowing us to analyse the effects of climate on lifespan (after removing cases where insecticide was used, there were n=257 studies for *An. gambiae s.l.* and n=109 for *An. funestus s.l.*). We collected environmental data on daily temperature mean and range for the sites and dates where studies were conducted and included them as covariates in our analysis (see SOM). We did not detect a significant association between lifespan and average temperature for either complex. However, there was a domed relationship between daily temperature range and lifespan for *An. gambiae s.l.*, suggesting these mosquitoes live longest when the range is approximately 1.5ºC (Fig. 8, based on a quadratic regression model). Our estimates suggest that at a daily temperature range of 0.5ºC, *An. gambiae s.l.* lifespan is approximately 9.1 days, which increases by nearly 50% if the daily temperature range increases by 1ºC (change of 4.3 days: 95% CI: 2.2-6.4 days). We did not detect a relationship between temperature range and lifespan for *An. funestus s.l.*.

***Most mosquitoes do not live long enough to transmit disease***

The extrinsic incubation period of a vector-borne disease (EIP) is the time required for a pathogen ingested in one blood meal to become transmissible during a future feeding event. In order to transmit a disease, a mosquito must live for at least as long as the EIP, with additional time required for finding a host and possibly a mate. We next use our lifespan results, from all three methodologies, to estimate how many mosquitoes are likely to survive beyond this lower bound for different vector-disease combinations (Fig. 9).

Our results suggest that for most anopheline species only a small proportion of individuals live long enough to transmit the malaria pathogen. For the major malaria vector *An. gambiae s.l*., lifespan estimates from the Detinova, Polovodova and MRR methods suggested that 38%, 21% and 10% of individuals survived long enough potentially to transmit malaria, the differences reflecting. For the longest lived species, *An. koliensis* and *An. punctulatus*, 52% of individuals were predicted to live longer than the EIP.

*Ae. aegypti* and *Ae. albopictus* are the main vectors of dengue, chikungunyaand Zika viruses. These viruses have short EIPs in comparison with malaria, and hence we estimate that a greater fraction of mosquitoes will live long enough to transmit the diseases. The highest estimate (obtained from the MRR analysis) was 86% for *Ae. albopictus* transmitting chikungunya.

***The MRR and Polovodova data suggests that mosquito senescence may occur in some but not all free-living mosquito populations***

So far, we have assumed that mosquitoes die at a constant rate throughout their adult lifetimes. If mosquitoes tend to senesce, however, we should expect a better fit to the data from using models which allow the mortality rate to increase with age. We tested this using species level lifespan estimates obtained from MRR (Fig. 10), and the Polovodova method (Fig. S5). We included five senescence models in addition to the constant mortality model, allowing a wide range of age-dependent functional forms to be tested simultaneously. To handle ambiguities caused by the multiple comparisons, we created a ranking representing relative evidences for or against senescence at the species level: if all senescence models fitted the data better, we took this to represent evidence in favour of senescence; if the constant mortality model outperformed one or more of the senescence models, we took this to mean both senescence or constant mortality models could explain patterns seen in the data; if none of the senescence models fitted the data better, we took this to be evidence for age-independent mortality.

We detected evidence for and against age-dependent mortality in 8 and 14 species respectively, and mixed evidence in 11 species (Fig. 10) using MRR estimates. Age-dependent mortality was supported in species including *Ae. aegypti*, the main vector of dengue fever, Zika and chikungunya.

Using Polovodova method estimates, we detected evidence of senescence in only two out of 25 species (*An. gambiae s.l.* and *An. minimus*; Fig. S5). There was no overlap between the species exhibiting evidence for senescence from each meta-analysis.

It is possible that some mosquito species do not live long enough in the wild to experience senescence. In support of this hypothesis, we detected a positive correlation between the ranked estimated lifespans of the species and the ranked mean predictive accuracy of age-dependent models for the MRR analysis (Spearman’s rank correlation test:, p<0.05). However, this was not significant for the Polodova method analysis (,p=0.44).

**Discussion**

In this study, we analysed a database of mark-release-recapture experiments and two other databases of female mosquito dissection experiments. Our approach enabled us to pool information from disparate experiments, which individually estimate lifespan with considerable uncertainty, to obtain estimates at the species and genus levels. Most of the estimated lifespans were less than 10 days, which is shorter than the EIPs of several important mosquito borne diseases including malaria and Zika. This suggests that relatively few mosquitoes live long enough to transmit these diseases, and highlights the importance of interventions that act to reduce adult lifespan. A further result, from Detinova dissection data, was that applying insecticides in the environment markedly reduces the lifespan of adult Anopheline mosquitoes. Taken together, these results help to explain the success of insecticide treated bednets in sub-Saharan Africa, which are thought to be largely responsible for malaria prevalence halving between 2000 and 2015 [Bhatt et al 2015].

Despite the large number of studies in each of our three meta-analyses, there remains considerable variation in lifespan estimates for the same species. There are two potential explanations for this variation. First, there may be true differences in lifespan among populations, perhaps due to differing genetics or differences in the local environment. Second, there is inevitably a degree of residual measurement noise affecting our results, that was not fully eliminated by pooling the underlying studies.

We used Bayesian hierarchical models to partition the variance in the data across different taxonomic ranks. The MRR analysis suggested that most variance in lifespan occurs at the level of the genus rather than the species, yet the reverse was true of the Polovodova analysis. Moreover, these two analyses did not agree on the ordering of genera in terms of lifespan, with MRR data indicating that *Culex* mosquitoes are on average the shortest lived and *Aedes* the longest, while Polovodova finding *Aedes* to be the shortest lived and *Anopheles* the longest. The Detinova data, which was limited to Anopheline species, indicated that there is more variation at the species than species-complex level. These inconsistencies, while perhaps unsurprising in the view of the heterogeneous nature of our source data, suggest that differences in environment may be more important than phylogeny in determining the lifespan of mosquitoes in a given population.

We used meteorological records to investigate the effect of temperature on lifespan which has been shown to important in laboratory studies (Yang et al., 2009; Brady et al., 2013; Murdock et al., 2012; Beck-Johnson et al., 2013). Specifically, we determined the average air temperatures for all the sampling localities and times of Detinova dissection studies, which included the *An. gambiae s.l.* and *An. funestus s.l.* complexes. Average temperatures varied from 9oC to 29oC but were not correlated with lifespan. We found no relationship which may be because our climate estimates were too crude, ignoring weather variation around average temperatures. Alternatively, the effects of temperature may be smaller in the field than the laboratory if mosquitoes adjust their behaviour to buffer themselves against temperature extremes, for example by seeking out cool, shady micro-habitats during periods of high temperature. We did find that longevity was significantly correlated with the daily temperature range in *An. gambiae s.l.* (there was a similar non-significant trend in *An. funestus s.l.*) but are uncertain how to interpret this finding.

The contribution of measurement noise to our results can be decomposed into two components: sampling noise, i.e. the unbiased noise associated with sampling of a population; and methodological biases, that are specific to each type of empirical study. MRR studies are particularly affected by sampling noise because the fraction of released mosquitoes that are recaptured is typically small, while sample sizes tend to be larger in the case of dissection methods. The unbiased nature of sampling noise means that pooling studies, as we have done here, may help to reduce its role. Methodological biases, by contrast, can affect disparate studies in a similar manner, and are therefore important to identify.

MRR experiments have a tendency to underestimate lifespan for two reasons. First, laboratory experiments have demonstrated that marking can reduce survival (Verhulst, Loonen, and Takken, 2013; Dickens and Brant, 2014). It is difficult to quantify the effect of this on our results because the studies included in the meta-analysis used a variety of marking compounds and methods whose impacts may differ. Further laboratory studies to investigate specific effects of marking would be helpful, particularly when performed in tandem with MRR experiments. Second, a marked mosquito that dies and another that disperses out of the study area are both not recaptured, meaning that lifespan will be underestimated by analysis of spatially-pooled data. In this study, we did not find a significant association between trapping area and lifespan estimate, which would be expected if this was a major source of error. However, we note that many of the MRR studies included in this analysis will not have been performed with the primary aim of estimating longevity, and therefore may not have paid particular attention to minimising these issues.

To help interpret our results, we conducted a Monte Carlo simulation study to determine how accurately mosquito lifespan could be estimated in an “ideal’’ MRR experiment, where mosquitoes are not affected by marking nor do they emigrate out of the study area (see SOM for full details). Our analysis showed that many experiments included in the database had such short study durations or released so few marked mosquitoes that lifespan would be inaccurately estimated due to overwhelming sampling noise (Fig. S6). This may explain why there is so much study level variance in our MRR meta-analysis. Though statistical power can be increased by pooling data across experiments, as we have done here, this is an inefficient approach to future research into mosquito longevity. Our Monte Carlo simulations suggest that it is more worthwhile to perform a small number of ambitious MRR experiments, where many mosquitoes are released and recapture efforts last for at least two weeks, than a large number of more modest experiments.

The two dissection methods assume reproductive age (the number of gonotrophic cycles) can be estimated by examining ovariole structure. Polovodova’s method provides a direct count of the number of cycles and requires highly skilled dissection techniques (Hugo et al., 2008); even then the accuracy of the method has been questioned (Fox and Brust, 1994). In particular, it becomes harder for a dissector to distinguish the ovariole remnants of previous gonotrophic cycles as a mosquito ages, which may lead to the underestimation of lifespans. Detinova’s method provides less information, yet it is simpler and dissections can be carried out reliably and routinely by most field entomologists, which probably explains its more widespread use.

Lifespan estimates from both dissection methods are sensitive to the assumption that population size is stable; lifespan will tend to be underestimated if the mosquitoes are collected from a growing population and vice versa. We will have reduced, but not eliminated, this bias by pooling data that were collected at different times of year for each study site where multiple collections were made. It is also possible that lifespan estimates will be affected by the trapping method, which may over-represent some life-stages at the expense of others. Typically, mosquitoes are caught when they attempt to blood-feed, and there are differing opinions about whether nulliparous females are more (Clements and Paterson, 1981) or less (Gillies and Wilkes, 1965) likely to be sampled. A further challenge to estimating chronological lifespan from dissection data is uncertainty in the length of the gonotrophic cycle. This is typically calculated using MRR studies or by observation of wild-caught females, though we found evidence that the method chosen influenced the estimated duration. We found significant variation in gonotrophic cycle length across the three mosquito genera for which we had data.

There is evidence that mosquito mortality rates rise with age (senescence) from laboratory studies (Styer et al., 2007; Dawes et al., 2009) and a single field experiment (Harrington et al., 2014). By fitting a range of survival models to the data in two meta-analyses, we tested for age-dependent mortality. We found limited evidence for senescence. evidence for it: in the MRR experiments, data were better fit by models allowing senescence in 8 of 33 species; in the analysis of Polovodova-type dissection experiments, we detected senescence in only 2 of 25 species. To help interpret these results, we conducted a simulation power analysis of a typical MRR study to understand the factors affecting the likelihood of detection of senescence (Section S3). This revealed that study duration is very important, but the initial release size much less so. For the intermediate strength of senescence that we modelled, the recapture efforts must continue for at least 18 days to detect senescence 80% of the time, yet the median duration of experiments in the MRR dataset was only 10 days. It may be that mosquitoes in the field seldom live long enough to experience the senescence observed under laboratory conditions, but if they do then carefully designed and extended MRR experiments will be required to detect it. Clements and Patterson (1981) conducted a meta-analysis of MRR and dissection field experiments and determined that mortality increased with age at a rate comparable to the ‘mildly’ senescent population we consider in the power analysis (Fig. S7). In this case, detecting senescence with a power of 80% required a study length of at least 18 days. Since the median study duration for experiments included in our analysis was 10 days this could partly explain our failure to detect senescence at the species level. To our knowledge, the MRR study of Harrington et al., (2014) on *Ae. aegypti* in Thailand has been the sole field experiment that has detected senescence: here, laboratory-reared mosquitoes of different ages were marked and released. It is possible that this study was able to detect senescence because mosquitoes of ages up to 20 days old were released, which considerably exceeds our estimates of wild lifespan.

As has been found in laboratory studies (Styer et al., 2007; Dawes et al., 2009), our analysis of the MRR data indicates that female mosquitoes outlive males in the field, although the magnitude of this difference is not as great. Because of ethical concerns about releasing female vectors, many recent MRR experiments use only males (Fig. SM1). Our results suggest caution in extrapolating results from one sex to the other.

**Conclusion**

We applied modern statistical methods to combine field data collected over 99 years to produce lower bound estimates of mosquito lifespan. Our results indicate that there is considerable variation among populations, though most mosquitoes are estimated to live for less than 10 days. Though estimates from individual MRR and dissection studies tend to be imprecise, in the absence of alternative methods we foresee continued reliance on these approaches. In the case of MRR studies, we have used simulations to show that large scale experiments are generally preferable to a large number of small-scale experiments.

**Materials and 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). In the MRR and Polovodova-dissection analyses, most data were collected before molecular techniques allowed these taxa to be separated, and, for these, we work chiefly with morphospecies. In the Detinova-dissection analyses, more detailed species-level information was often available, and we estimate lifespans for both species and morphospecies. A detailed description of methods is provided in the SOM file.

**Mark-release-recapture (MRR)**

Data from MRR experiments in the Guerra et al. (2014) database were examined. 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. See Tables SM1 & SM2 for a summary of data characteristics.

We analysed all MRR experiments within the same statistical framework. In the simplest case, mosquitoes are released on day zero and the probability that they remain inthe 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 negativebinomial sampling model with mean (*NR* − *Y* (*t* − 1)) *S* (*t*) *ψ*, 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(−*λt*). We utilised this form extensively but in testing for senescence used five other models where *λ*(*t*) varies with time so that,

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 also increased the likelihood of false positives.

We used two analyses to estimate lifespan. One considered each study separately; the other involved a hierarchical approach which grouped studies according to taxonomy. Parameters were estimated using Bayesian techniques with relatively uninformative priors for *κ* and the parameters of *λ*(*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).

Using the hierarchical model, we estimate distributions of lifespan at the species and the genus levels, and across a dataset where experiments with fewer than six recaptures and those species within only a single study were removed. 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 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 covariates such as diﬀerences in experimental methodology. Posterior distributions were derived using Markov Chain Monte Carlo (MCMC) methods with convergence assessed using the statistic (Gelman and Rubin, 1992). The predictive power of the modelwas 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 (Lambert, 2018) 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.

**Dissection**

**Polovodova’s method**

Studies using Polovodova’s dissection method to determine reproductive age 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 included with associated metadata is available as a Supplementary data file.

Most dissection studies recorded the distribution of reproductive age (nulliparous, uniparous, biparous and so on) in wild-caught mosquito samples collected over a specific period of time. Overall, we found 568 physiological age cross-sections recorded at distinct times in 72 published articles. Our statistical approach assumes stable population sizes. To guard against the eﬀect of fluctuating population sizes on our analysis, we aggregated the data at a given location across cross-sections taken at diﬀerent times. 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*.

The dissection data which we use provides information on the distribution of ages within each population. By assuming that population sizes were fixed throughout the period of investigation, this allows us to estimate mean lifespan using a statistical model of mortality incorporating the probability of mosquito capture. We modelled the number of mosquitoes found by dissection to be of age *a* using the negative binomial distribution with mean Ψ*S*(*a*) and shape parameter *κ*, where Ψ is the product of the recruitment rate of adult mosquitoes 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). In data sets where the fraction of nulliparous females was less than 90% the uniparous females (completed one gonotrophic cycle), we excluded the nulliparous observation. Data was analysed using a Bayesian framework similar to that used to analyse the MRR data, with minor diﬀerences in the specification of the priors (see SOM).

**Detinova’s method**

Detinova et al. (1962) provide an alternative dissection method to estimate the age of a given female mosquito, which results in a binary observation for each specimen: nulliparous or parous. As for the analysis of dissection data from Polovodova’s method, we assume that constant population sizes are constant and with knowledge of a given gonotrophic cycle duration (see below), we can estimate mean population lifespan (see SOM).

Massey et al. (2016) provides a database estimates of different anopheline bionomic variables, which includes 1490 observations of parity using Detinova’s method. As for the other two analyses, we use a Bayesian framework. The likelihood assumed is a binomial distribution with sample size given by the number of specimens dissected and probability parameter representing the proportion parous in the wild population. The probability parameter is allowed to vary according to experiment but are assigned hierarchical beta priors (see SOM) that allow partial pooling of observations according to a grouping (species, morphospecies, genus and so on).

**Gonotrophic cycle duration estimates**

To compare lifespan estimates from the MRR and dissection analyses, we need to convert physiological age into chronological age. To do this, we conducted a meta-analysis of previously published studies that estimate the duration of the gonotrophic cycle (see SOM). This was supplemented by references in the review by Silver (2007). Whilst compiling our dataset on gonotrophic cycles, Massey et al. (2016) published a database of bionomic quantities for malaria vectors (that is, including only anopheline species). Included in this dataset were estimates of gonotrophic cycle duration. After removing duplicates with our dataset, we were left with 120 estimates of gonotrophic cycle duration.

Most published estimates of gonotrophic cycle duration were obtained by observing wild-caught specimens or their progeny in the laboratory or by dissecting females recaptured in MRR studies. Studies diﬀered greatly in how (if at all) they represented uncertainty in their estimates. 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 of this distribution. Using the quantiles of the normal distribution, we estimated its mean and standard deviation separately for the genera *Anopheles*, *Aedes* and *Culex* by regression (see SOM).

We converted physiological age to chronological age by sampling from this distribution to obtain a particular gonotrophic cycle length for each mosquito (see SOM).

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