**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 two databases of different types of mosquito dissection experiments: one compiled by us, consisting of 131 detailed “physiological age” dissection studies; another, recently published dataset, consisting of 1490 studies on anopheline malaria vectors that used a lower resolution dissection method which determines a dichotomous measure of parity. These analyses allow us to produce, to our knowledge, the first ever species- and genus-level estimates of mosquito lifespan. Notably, for the major African malaria vector *Anopheles gambiae s.l.*, we estimate lifespans of lifespans ranging from 4.4 days (from MRR analysis) to 8.8 days (from dichotomous parity analysis). For the predominantly East-African vector *An. funestus s.l*., our lifespan estimates range from 4.2 days (MRR) to 13.2 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 dengue fever, chikungunya and Zika. In correspondence with laboratory studies, 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 determine that relatively few species within our databases indicate evidence of senescence. 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.

**Author summary**

Mosquitoes transmit some of the most important diseases aﬄicting humans, with malaria alone killing between 0.4-1.2 million people annually, chiefly children in low-income countries. The 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, explicitly target reductions in 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.

**Author contributions**

HCJG, AN and BL designed this study. BL was responsible for data curation and the formal analysis of the data. BL and AN developed the statistical methodology and conducted 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 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 with ongoing recapture efforts, 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 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 incasing ovaries changes irreversibly when ovaries first develop (Detinova, 1945). The proportion of parous individuals – those individuals that have borne offspring – can be determined by dissecting field-caught specimens and, by making assumptions of the duration of gonotrophic cycles, yields estimates of lifespan. In honour of the entomologist who first made this observation, this approach is known as Detinova’s method. The crude dissection technique needed to apply this method means it has been widely adopted, but its simplicity means it provides limited information about mortality. The next approach requires more sophisticated dissection and, rather than producing a dichotomous determination of reproductive status, yields a count of the number of reproductive cycles a mosquito has undergone. 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. After the scientist first observing these changes, this approach is known as Polovodova’s method. The challenges of this method include the amount of time and expertise it takes to collect data and lack of consensus regarding the type of oogenesis producing observable dilations, complicating interpretation of data. 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 (perhaps as a box)**

* MRR experiments – mark-release-recapture experiments, where mosquitoes are marked with a dye, released and ongoing captures of wild mosquitoes are made.
* 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 before.
* 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 (i.e. the time as measured by a timepiece).
* Detinova’s (dissection) method – dissecting mosquitoes to a 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**

In each of the three analyses, we estimate and report posterior mean lifespan, unless otherwise stated. Since we use a Bayesian approach to estimation, we determine distributions representing uncertainty in this quantity. Whilst in SOM, we provide detailed quantiles and summary measures, here we report only the posterior median – that is, the posterior median of mean lifespan. Any uncertainty measures provided in the main text are 25%-75% central posterior intervals.

**Chronological longevity estimated from MRR studies**

To begin, we estimated lifespan independently for each available MRR time-series (Figure 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 *Anopheles* *annulipes walker* (a species predominantly found in Australasia) 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, and we thus advise caution in their interpretation (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 are 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.

We next used Bayesian hierarchical models to compute species- (or species complex) and genus-specific estimates to subsume the variation within these taxonomic groupings. To ensure fair comparison, we present estimates for females that were not fed blood or sugar before release (Fig. 3*).* At the species level, the longest estimate was 18.3 days for the species *Ae. simpsoni s.l.* (an African vector of yellow fever). The smallest estimate was 0.8 days for *An.* *subpictus s.l. –* an Asian malaria vector – which is unfeasibly short 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 genera level, with *Culex* estimated to have the shortest lifespan (2.5 days) and *Aedes* the longest (6.9 days). *Anopheles* were estimated to live on average 5.0 days, while the average across all the available data covering the three genera was 4.6 days. Models incorporating grouping at the genus level fit the data better than when data was pooled across all genera (Fig. S1). Measures of model fit indicated that after the eﬀect of genus is accounted for, the incorporation of a species term conferred little additional predictive power. Taken together, this suggests that there is evidence of variation in lifespan across genera but that there is little within each.

We next consider the impact of three additional factors that could influence our estimates of lifespan: (i) mosquito sex, (ii) whether or not the mosquitoes are fed with blood or sugar before release, (iii) the spatial extent of the recapture zone.

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. 2). There was a consistent trend for females to live longer than males for each genus, with the greatest difference 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 data includes information on whether mosquitoes were pre-fed with sugar, blood, both blood and sugar, or alternatively unfed, which we used to determine the effects of feeding on female lifespan at the genus level and across all studies (Fig. S4). Since there were insufficient data on males that were fed with sugar versus unfed, we pooled all genera together to estimate a pooled effect of sugar-feeding (see SOM). Overall, we estimate that mosquitoes fed on sugar before release outlived those that were not fed: for females, the difference in lifespan was 0.6 days (p=0.15) and the pattern was consistent across all genera; for males, it was 0.5 days (p=0.15). The effect of blood-feeding on female mosquitoes was less marked, with blood-fed individuals living about 0.1 days longer (p=0.44) than unfed mosquitoes.

Following a release of marked mosquitoes, the rate of their recapture typically reduces in time because some mosquitoes die, and also 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 below the true lifespan then we should expect a positive correlation between the spatial extent of the recapture zone and lifespan. We found no such pattern (Fig. S2), although there was a slight (albeit insignificant) positive correlation between lifespan and trap density (Fig. S3).

**Reproductive longevity estimated from Polovodova’s dissection data**

Dissection allows the number of completed gonotrophic cycles to be counted and from this the mean number of cycles before death was estimated. Across the 131 studies, 95% of the individual time series estimates were less than 3 gonotrophic cycles (Fig. S7) and, overall, the mean number of cycles completed in a lifetime was 1.2 (Fig. 4; Table S2). The greatest number of cycles estimated was for *Anopheles sergentii* (2.5 cycles) which is adapted to desert conditions (it is known as the “oasis vector” of malaria; Sinka et al., 2010) and may have evolved greater longevity. The major African malaria vector *An. gambiae* *s.l.* was estimated to be the second longest living (1.9 cycles).The smallest estimated mean number of gonotrophic cycles was for *Anopheles* *bellator* (0.5 cycles; posterior mean) which transmits malaria in Brazil’s AtlanticForest. Using the posterior median point estimates, there were significant diﬀerences in estimated lifetime gonotrophic cycles amongst species (ANOVA: F24,106 =2.2, *p* <0.01; the non-parametric Kruskal-Wallace: , *p*<0.01).

The estimated lifetime gonotrophic cycles for the diﬀerent genera were *Anopheles,* 1.4; *Culex,* 1.0; *Mansonia*, 1.1; and *Aedes* 0.8 (Fig. 4; Table S2) and the diﬀerences between the genera were significant (ANOVA: F3,127 =3.4, *p* =0.02; the non-parametric Kruskal Wallace: , *p*<0.01).

**Anopheline reproductive longevity estimated from Detinova’s dissection data**

The database of anopheline bionomic quantities of Massey et al. (2016) contained 1490 dichotomous parity observations. As for the Polovodova dissection analysis, most estimates for the individual Detinova parity data were less than 3 cycles (81% of cases; Fig X).

To provide as much detail as possible, we estimated the number of gonotrophic cycles completed before death at the species-level if species was given (n=578 observations across 18 species; for example, *An. arabiensis*); else, we provided estimates at the level of morphospecies (n=1289 observations across 17 species-complexes; for example, *An. gambiae s.l.*). The two species with the lowest estimated lifespans belong to the *An. albitarsis* species complex – a malaria vector found throughout South America – with *An. albitarsis (*formerly *Sp. A*) and *An. albitarsis (Sp. B)* both estimated to complete 0.6 cycles in their lifetime (Fig. X). The longest-lived species also belonged to the *An. albitarsis* complex – *An. albitarsis marajoara* (formerly *Sp. C*; 3.7 cycles)– hinting at the extensive variation in this complex across the continent. Outside of the Americas, the longest-lived species were the major East-African vector *An. funestus*, estimated to live for 3.0 cycles, and, in Asia, *An. leucosphyrus* (2.4 cycles). Across the complexes, the shortest-lived group was *An. nuneztovari s.l.* – a primary vector species in areas of Colombia and Venezuela. The longest-lived complex was *An. funestus s.l.*

In addition to species and species-complex estimates, we also pooled all data to produce continent level lifespan estimates. The anopheline species in Africa were estimated to live longest (2.0 cycles; Fig. X), followed by Asian species (1.3 cycles) then the Americas (1.1 cycles). We note in passing that this pattern of lifespan roughly echoes that seen in the burden of disease, where Africa is subject to the highest burden followed by Asia and the Americas. Pooling all data, we estimated that overall average anopheline lifespan was 1.6 cycles.

To determine whether the differences in parity and, hence, lifespan were meaningful, we performed cross-validation (see SOM) to compare the fit of the model at the species, species-complex and continent-level. Across all continents, the species-level model had the highest predictive power on the hold-out dataset (log-likelihood=-18,132) followed by the complex level model (log-likelihood=-19,220; p<0.01 compared with species-level model) then the continent-level model (log-likelihood=-21,348; p=0.23 compared with complex-level model). These patterns were repeated within each continent, with the species model having a higher predictive accuracy than the complex model, which, in turn, better fit the data than the continent model. Therefore, in each case, there is evidence that lifespan varies by species and species-complex.

Is there variation between continents? Put in results.

**Gonotrophic cycle durations**

To convert reproductive longevity into chronological lifespan for each of the dissection methods, it is necessary to know the duration of the gonotrophic cycle (the reproductive cycle that female mosquitoes repeatedly undergo throughout their lives). Lacking consensus from the literature, we conducted a literature survey (see SOM), which was supplemented with gonotrophic cycle estimates from Massey et al. (2016), which was published during preparation of this manuscript. This resulted in 120 estimates of gonotrophic cycle duration obtained from a variety of methods: the two main approaches were laboratory observation of wild-caught specimens and their progeny (n=45), and MRR-based approaches (n=36). We recorded whether the duration reported was either for the 1st or subsequent cycles – if this was unspecified, the estimate was recorded for both cycle types.

There were significant differences in the raw estimates of 1st gonotrophic cycle duration amongst genera (ANOVA: F2,116 = 8.7, *p* <0.01; Kruskal-Wallis: , *p*<0.01), which led us to pool estimates for each genus separately. There were differences in estimates by experimental method but, without consensus over which of these is reliable, we pooled data across methods. Using a regression approach (see SOM), we determined *Anopheles* had the shortest gonotrophic cycle duration (with a mean of 3.7 days), followed by *Aedes* (4.6 days) then *Culex* (5.2 days). This ordering was maintained for subsequent cycle durations (see Fig X). Pooling data across all genera, the 1st cycle duration was estimated to be 4.0 days long and subsequent cycles, 3.6 days in duration.

**Comparison of longevity estimates from MRR and dissection studies**

We next compare the estimates of lifespan obtained from the MRR and dissection studies. To do so, we used our estimates of gonotrophic cycle durations to convert the dissection-based estimates of reproductive lifespan (Polovodova) or reproductive age (Detinova) into chronological lifespan as described in SOM. Table S3 and Table S provides posterior summaries of chronological lifespan for the species and genera in the dissection dataset (see also Fig. S10). For 12 species, we had enough data from both species to make a comparison, and there was a positive correlation (not statistically significant; Pearson correlation *ρ* = 0*.*34, *n* = 12, *p* = 0*.*27) between the two measures (Fig. 5), and in only one case – for *An. darlingi* - there was a significant diﬀerence in the time-series level lifespans (Table S4).

**The influence of weather on lifespan estimates**

Need to be careful about interpretation here since there is seasonal variation in population size, leading to changes in sampled lifespan. Can I control for season/geography in regressions?

To assess whether temperature affects lifespan 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 lifespan (overall or within genus; Fig. S5). 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*, *An. gambiae s.l.* and *An. culicifacies s.l.*; Fig. S6)

*An. gambiae s.l.* is the most important vector of malaria, and, since the Massey et al. (2016) dataset has many parity observations for this morphospecies (n=546), we decided to analyse these data in greater detail. First, we estimated NEED TO RERUN COUNTRY-LEVEL ESTIMATES AND K-FOLD.

**Estimates of the fraction mosquitoes capable of transmitting disease**

We can use the lifespan estimates from our 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. 8, 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 *An. subpictus* (posterior median; from the MRR analysis, as noted above likely to be due to the lifespan substantially underestimating lifespan) to 52% (posterior median) for the drought-adapted and long-lived *A sergentii.* The proportions surviving long enough to become infectious for *An. gambiae s.l.* were 10% (from MRRs) and 27% (from dissection studies); and for *An.* *funestus s.l.*, 9% (from MRRs). Using the individual time series estimates, thereevidence for a diﬀerence in EIP between the species (Kruskal-Wallis used due to non-normality of data; MRR: , *p* <0.01; dissection: = 38*.*9, p<0.01).

*Ae. aegypti* and *Ae. albopictus* are the main vectors of dengue, chikungunyaand Zika viruses. Because of their short intrinsic incubation periods a greater fraction of mosquito potentially live long enough to transmit diseases (Fig. 8), rising to a maximum of 84% for *Ae. albopictus* transmitting chikungunya.

**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. 6, we compare the predictive performance of the six models for describing lifespan in MRR studies of 33 species (see SOM for details). 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 *Ae. Aegypti*, the main vector of dengue fever, Zika and chikungunya. 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 76failed to detect age-dependence in the latter (Fig S11).

In Fig. 7, we compare the predictive performance of the six models for describing lifespan in dissection studies of 25 species, 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 (*An. gambiae s.l.* and *An. minimus*).

Overall, we conclude that the evidence for age dependence from MRR and dissection studies is mixed. From the two groups of species that were identified as exhibiting senescence from each meta-analysis, there was no overlap. Using the Gompertz model only, we compared the predictive accuracy versus the exponential for the 12 species with data from both analyses (Fig. S12) and only in one case (*An. subpictus s.l.*) there was consensus that the age-dependent model provided a better fit to the data. It is possible that some mosquito species do not live long enough in the wild to experience physiological decline. In support of this, a Spearman’s rank correlation test indicated that there was positive correlation between the ranked estimated lifespans of the species and the ranked mean predictive accuracy of age-dependent models for the MRR analysis (*ρ*=0.19, p=0.01), however this was not significant for the dissection analysis (*ρ*=0.07, p=0.43).

**Discussion**

In this study, we used a Bayesian hierarchical framework to analyse a database of mark-release-recapture experiments and two other databases of female mosquito dissection experiments. By applying a single framework, we pooled information from disparate experiments which, individually, estimate lifespan with considerable uncertainty. Across all meta-analyses, most estimated lifespans were less than 10 days, hinting that relatively few mosquitoes live long enough to transmit disease. We determined that lifespan 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, females outlive males. Pre-release feeding with sugar also lengthens lifespan across the three main genera, although this eﬀect is less marked than the sex diﬀerences. INCLUDE WEATHER VARIABLE DISCUSSION. By fitting a range of survival models to the data in two meta-analyses, we tested for age-dependent mortality and find mixed 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.

MRR experiments, in general, underestimate lifespan for two reasons. First, laboratory experiments have demonstrated that marking can reduce survival

(Verhulst, Loonen, and Takken, 2013; Dickens and Brant, 2014), and unfortunately we lacked the information to determine the significance of this effect. 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 trap range or trap density, however, hinting that this bias may be relatively minor. We conducted an *in silico* Monte Carlo 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). This 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 (Fig. S12). In light of this result, statistical power can be increased substantially by pooling data across experiments as we did using a Bayesian hierarchical model, although this does assume a degree of homogeneity between studies.

The two dissection methods assume reproductive age can be reliably determined by dissection. Polodova’s dissection method, in principle, offers more detailed information on the reproductive age of a mosquito (i.e. nulliparous, uniparous, biparous and so on) compared to Detinova’s approach that determines a simpler dichotomous measure (i.e. nulliparous and parous). This additional richness is a double-edged sword, however: there remains considerable debate concerning the interpretation of reproductive age estimated by this method (Fox and Brust, 1994); additionally, Polovodova’s method requires much more skilled dissection to find ovarioles termed ``diagnostic’’ of true reproductive age (Hugo et al., 2008). Indeed, it is believed that, as a mosquito ages, it is harder for a dissector to locate representative ovarioles, likely biasing estimates of lifespan downwards. Detinova’s method is simpler, so dissections can be carried out reliably and routinely by most field entomologists, which partly explains its popularity. It is possible that Detinova’s parity estimate may be biased upwards due to the methods used to trap mosquitoes – typically, these aim to catch mosquitoes when they attempt to blood-feed – but, to our knowledge, there are fewer concerns than with Polovodova’s method. This may explain why the estimates of lifespan derived from Detinova’s method typically exceed those from Polovodova’s.

The dissection methods also make further assumptions to estimate mosquito lifespan. They assume: (i) the relationship between physiological and chronological age is known; (ii) the population being sampled is in equilibrium (recruitment matches mortality); and, (iii) individual mosquitoes can be randomly sampled from the population. The exchange rate between physiological age and chronological age is the duration of gonotrophic cycles. Here, we carried out a literature search for published estimates of this duration, which was supplemented by anopheline data collected by the Massey et al. (2016) study. A range of methods are used to estimate gonotrophic cycle duration, including MRR studies and laboratory observation of field-caught specimens and their progeny. In our somewhat limited analysis of our literature-derived estimates, we found evidence of variation in duration according to the experimental method used and suggest further study of this aspect of mosquito ecology may be fruitful. Without clear consensus in the literature as to which of the experimental approaches is most reliable, we decided to pool all data in our analysis. From this, we were surprised to find that gonotrophic cycle duration appeared to differ substantially between genera, with culecine mosquitoes estimated to have longer cycles than aedines which, in turn, had longer cycles than anophelines. To our knowledge, this has not been reported elsewhere, and we used these gonotrophic cycle duration estimates to determine chronological age of mosquitoes. In respect to assumption (ii), any deviation from population equilibrium could result in an over- or under-abundance of young mosquitoes being sampled. To mitigate against this risk, we pooled data across time at each collection location (sometimes with multiple collection locations per study site) but recognise that more bespoke analysis examining those sites with collections at many time-points could produce more accurate, inter-annual, lifespan estimates. Field entomologists have challenged assumption (iii) – that collection methods produce random samples from 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 analysis, we chose to remove those nulliparous counts where there was an obvious under-abundance relative to the uniparous count. However, it is possible that an analysis that accounted for different collection methods could produce more reliable estimates.

Since our data span from well before the advent of widespread insecticidal bednets to present, it is possible that the populations more recently surveyed have artificially lower lifespans due to the killing effects of insecticides. Whilst neither the Guerra et al. (2014) datasets nor the data we collected ourselves noted whether vector control measures were in place at the time of experiment, the Massey et al. (2016) data includes this information. Using this, we estimated the impact on lifespan of indoor residual spraying (IRS) and insecticidal bednets (ITNs) for those species with sufficient observations and, unsurprisingly – given the success of these interventions (Bhatt et al., 2015) – found that both these interventions led to substantial reductions in lifespan. Since, however, the majority of our data were collected from before the year 2000 (X% for MRR, Y% for Polovodova-dissection and Z% for Detinova-dissection), when pyrethroid insecticides began to be widely distributed, this should limit the impact on our estimates.

In benign laboratory conditions, mosquitoes are expected to live longer than in wild populations, and laboratory lifespans can thus be considered upper bounds on lifespanIt is widely believed mosquitoes live artificially long under the benign conditions of the laboratory and their lifespans likely constitute an upper bound on wild populations. A large cage experiment of *Ae. aegypti* mosquitoesdetermined that females lived 32 days on average (Styer et al., 2007), and a similar study with female *A. stephensi* determined a median lifespan between 31 and 42 days (Dawes et al., 2009). These estimates are several multiples of the modal estimates from either of our analyses but without a gold standard method to measure mosquito lifespan in the field, it is diﬃcult to quantify the gap that exists between field and laboratory populations. 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.

In our analysis of the MRR data, we considered the effect of air temperature, sex and pre-release feeding status on lifespan. There is evidence mainly from laboratory studies that temperature modulates mosquito ecology and behaviour (Yang et al., 2009; Brady et al., 2013; Murdock et al., 2012; Beck-Johnson et al., 2013). The locations and times of year when the MRR studies were conducted encompassed a large range of average air temperatures, from approximately 10 oC to 35 oC yet we found no relationship between lifespan and temperature when pooling all series (Fig. S5) or for those individual species with the most data (Fig. S6). It is possible that by considering a raw average of air temperatures across the month and ignoring rainfall (whose historical data is likely less reliable for a given location), we obscured more complex interactions between temperature and lifespan. The observed laboratory relationship between lifespan and temperature, however, may not be as robust in the field if mosquitoes adjust their behaviour in reaction to changes in temperature (for example by seeking shade). As with laboratory studies (Styer et al., 2007; Dawes et al., 2009) our analysis indicates that females outlive male mosquitoes, although the magnitude of this difference is not as large in absolute or percentage terms. Ethical concerns mean it is now more common than historically for MRR experiments to release males opposed to females (Fig. SM2). Since differences in lifespan exist between the sexes, it is possible that other ecological parameters that can be determined by MRRs also differ, highlighting the need for field methods that directly measure these characteristics of wild females. Our estimates of lifespan indicate that mosquitoes sugar-fed prior to release lived longer than unfed individuals (Fig. S4) which may partly explain recent successes of vector control methods reducing access of the insects to sugar or using toxic sugar baits (Müller, Kravchenko, and Schlein, 2008; Müller, Junnila, and Schlein, 2010; Müller et al., 2010a; Müller et al., 2010b; Beier et al., 2012).

It is encouraging that our pooled estimates of lifespan from the MRR (6.0 days) and dissection (5.5 days) analyses are comparable and that there was a positive (although insignificant) correlation between corresponding species-level estimates. Across genera, the greatest discrepancy in estimates was for Aedes, with 8.1 days estimated from the MRR studies compared to 3.5 days from the dissection analysis. This was followed by Culex (2.9 days from the MRRs versus 4.9 days from dissection) with the smallest discrepancy for Anopheles (6.8 versus 6.4 days). Some of the diﬀerences in estimates between the two approaches are likely due to environmental and genetic diﬀerences between mosquitoes included in this analysis. However, we believe that part of the discrepancy can be explained by the assumptions required to analyse each field method and speculate that diﬀerences in dispersal rate may be responsible. Both Anopheles and Culex mosquitoes are generally thought to fly farther than Aedes, meaning that the estimates from MRR-based approaches will be most downwardly-biased for these genera. This is supported by our results since the Polovodova dissection estimates (themselves not reliant on assumptions about dispersal) are similar or exceed the MRR estimates for *Anopheles* and *Culex* mosquitoes, but not for *Aedes*.

Our conclusion that mosquito senescence is apparent in only a minority of cases across both experimental approaches contrasts with evidence from some laboratory studies (Styer et al., 2007; Dawes et al., 2009) and field experiments (Harrington et al., 2014). To determine if experimental characteristics were responsible for our inability to detect senescence, we conducted a power analysis of MRR experiments (see SOM). This work indicated that power to detect senescence senescence strongly depends on study length (Fig. S11B) but is insensitive to release size (Fig. S11C). 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 ‘mild’ senescence case population we consider in the power analysis (see SOM). 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 (Table SM2) this could partly explain our failure to detect senescence at the species level. It is possible however that laboratory studies overstate the magnitude of senescence since colony mosquitoes may survive long enough to experience physiological decline not felt in the wild. To our knowledge the MRR study of Harrington et al., (2014) on *Aedes* aegypti in Thailand has been the sole field experiment aiming to detect senescence and further studies are clearly needed.

In this work, we applied modern statistical methods to combine precious field data collected by entomologists past and present to produce lower bound estimates of mosquito lifespan. Although our approach to estimating mosquito lifespan is novel, its importance for disease transmission has long been recognized since even before 1957, when George Macdonald formulated his mathematical model of malaria transmission. Indeed, the recent declines in malaria prevalence in Sub-Saharan Africa were mainly due to interventions (insecticide-treated bednets and indoor residual spraying) that aim to reduce mosquito lifespan (Bhatt et al., 2015). Yet, there is ample evidence that mosquito resistance to pyrethroid insecticides has spread throughout the continent (World Health Organization, 2018) which may erode or reverse recent gains. A direct way to assess the performance of existing vector control methods is to estimate how mosquito lifespan responds to changes in measured resistance. The diﬀerent nature of the assumptions underpinning analysis of MRR and dissection studies means they oﬀer complimentary information on mosquito survival and lacking a gold standard method to estimate this quantity, we foresee continued reliance on these longstanding field entomological methods.

**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, more detailed species-level information was often available, and we estimate lifespans for both species and morphospecies.

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

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. See Table SM1 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 *NR* 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 increases the likelihood of false positives.

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). 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 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 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.

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 ± 1 degree, longitude ± 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**

**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 located with associated metadata is available as a Supplementary Online 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 relies on steady recruitment to the adult mosquito population. 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 *Anopheles, Aedes*, *Culex* and *Mansonia* genera.

The dissection data which we use provides a window onto the distribution of ages within each investigated 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 (completed on 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 to estimate the age of a given female mosquito, which results in a dichotomous observation for each specimen: nulliparous or parous. Like for the analysis for Polovodova’s method, we assume that there is steady recruitment to the adult mosquito population 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 of anopheline bionomic estimates, which includes 1490 observations of Detinova’s parity. 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 determine this characteristic, we conducted a meta-analysis of previously published studies that estimate the duration of the gonotrophic cycle (see SOM). This list was then supplemented with a list of 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 *Anopheles*, *Aedes* and *Culex* genera 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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