Predicting Age in Aedes aegypti (Diptera: Culicidae) Females to Monitor Changes in Transmission Potential

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Research

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Abstract

**Background:** The *Aedes aegypti* mosquito is a vector of several viruses including dengue, chikungunya, zika, and yellow fever. Vector surveillance and control are the primary methods used for the control and prevention of disease transmission, however, there is an overreliance on measures of population abundance in surveillance programs as a trigger for initiating control activities. At the northern edge of *Ae. aegypti*’s geographic range, survival seems to be the factor limiting disease transmission. In this study, we sought to test the utility of using body size as an entomological index to surveil changes in the age structure of field collected, female *Aedes aegypti*.

**Methods:** We collected female *Ae. aegypti* mosquitoes using BG sentinel traps in three cities at the northern edge of their geographic range. Collections took place during their active season over the course of three years. Female wing size was measured as an estimate of body size and reproductive status was characterized by examining ovary tracheation. Chronological age was determined by measuring transcript abundance of an age-dependent gene. These data were then combined with weather data from the estimated larval development period and adulthood (one week prior to capture). Two sources of weather data were tested to see which was more appropriate for evaluating impacts on mosquito physiology. All variables were then used to test models for predicting age via structural equation modeling.

**Results:** We found that there was a bias in the body size of mosquitoes collected alive from the BG sentinel traps that favored large females. In comparing city-specific NOAA weather data and site-specific data from HOBO remote temperature and humidity loggers, we found that HOBO data was more tightly associated with body size. We found that body size itself was not associated with age. Of all the variables measured, we found that temperature during development, body size, and relative humidity in the one week prior to capture produced the strongest model for predicting age. The strength of models improved drastically when testing one city at a time, with Hermosillo having the strongest model for predicting age.

**Conclusions:** Body size increased the strength of weather-based models for predicting variation in age. Importantly, we found that variability of the factors measured was greater within cities than between cities, meaning that age predictions must be made on a city by city basis. These results contribute to efforts to use weather forecasts to predict changes in the probability of disease transmission by mosquito vectors.

**Introduction**

The *Aedes aegypti* mosquito is a vector of several viruses including dengue, chikungunya, zika, and yellow fever. Due to its prevalence and immense economic burden, dengue is the most important of the arboviral diseases affecting humans [1,2]. The global incidence of dengue fever has been increasing dramatically, more than doubling every ten years, with nearly half of the world’s population at risk of
infection [3]. Vector surveillance and control continue to be integral for preventing disease transmission due to the wide variety of pathogens spread by mosquitoes, the absence or lack of available vaccines [3], and the emerging fact that viral infections do not necessarily lead to lifelong immunity as had long been assumed [4–6]. It is highly likely that rapid warming occurring in temperate regions of the planet is affecting the physiology, seasonality, and geographic distribution of mosquito populations and their associated pathogens. Changes in disease distribution are especially problematic because the introduction of disease into immunologically naive populations increases the intensity of pathogen replication and the severity of illness and infectiousness [7]. For these reasons, improved surveillance and control activities that prevent disease transmission are greatly needed.

Currently, vector surveillance and control activities are triggered by reports of high mosquito abundance. However, mosquito abundance is not always the primary factor limiting disease transmission in an area. There are several physiological and behavioral factors which regulate the likelihood that a mosquito will transmit disease, otherwise known as vector capacity. These factors include the mosquito’s survival rates, biting frequency, likelihood of encountering a human, reproductive rate, susceptibility to infection, and the incubation period of the pathogen [8]. A good example of where mosquito abundance is not the factor limiting transmission can be found in cities in the Sonoran Southwest. Despite abundant populations of *Ae. aegypti*, the frequent travel and trade occurring between cities with and without dengue transmission, and similar human behavior and living conditions, local dengue transmission has been infrequent in Nogales, Sonora, Mexico and has not occurred at all in Tucson, Arizona. Both cities experience a significant amount of travel and trade from Hermosillo, Sonora, Mexico which has regular seasonal dengue transmission. A recent publication showed that *Ae. aegypti* abundance is actually higher in Nogales compared to Hermosillo [9], which begs the question, “what is actually limiting dengue transmission in this region?” It is of great public health interest to increase our understanding of how disease transmission is regulated in order to identify appropriate surveillance methods for that area.

While surveilling changes in population abundance may work for anticipating disease risk in some areas, this is clearly not the case for the Sonoran Southwest. It is also unlikely that genetic differences are responsible for the varying transmission rates in this region. Previous sampling of *Ae. aegypti* that included Nogales, Hermosillo, and Tucson found that all three cities’ populations belong to the same genetically distinct sub-group, differentiating them from other populations tested in Arizona, Texas, and Florida [10]. This is likely the result of frequent human trade and travel between locations. Ernst et al. (2016) concluded that the stark difference in age structure of *Ae. aegypti* females between Nogales and Hermosillo was likely responsible for the differences in transmission dynamics and not larval, pupal, or adult abundance. Females in Hermosillo appeared to have higher survival rates than those in Nogales. The survival rate of a female mosquito is an important factor in transmission potential because after consuming an infected blood meal, the female must outlive the incubation period of the virus before being able to transmit the infection. This means that only the oldest subset of a given population is capable of disease transmission. Unfortunately, testing individual field-collected mosquitoes for age to get an idea of survival rate is a costly and time-consuming process. Health departments are typically functioning under significant financial constraints and cannot incorporate costly new methods for vector
surveillance into their control programs. Where survival is a limiting factor regulating dengue transmission, it would be extremely useful to identify a simple and sustainable proxy for characterizing age in field populations.

One potential indicator for longevity in field mosquitoes is body size. Mosquitoes are holometabolous insects which means that their size in adulthood is determined by environmental conditions during larval development. Once they emerge as adults, they will remain the same size throughout adulthood. The size of a mosquito has significant impacts on many of the physiological and behavioral components that determine vector capacity [11]. Beyond affecting adult body size, developmental conditions are known to alter resource allocation during adulthood between reproduction, immunity, and lifespan through changes in the regulation of several biochemical pathways [12–18]. For example, a large, well-provisioned female mosquito is more likely to invest a greater proportion of her blood meals into reproduction. Conversely, a small, ill-provisioned female is less likely to invest as much into reproduction and more likely to allocate incoming resources to self-maintenance [19].

Since mosquitoes are ectotherms, size is also determined by temperature during development. Warmer temperatures speed up the rate of development, reducing the time available for feeding, thereby producing smaller mosquitoes. The opposite is also true, where colder temperatures slow down the rate of development allowing more time for feeding and producing larger mosquitoes. As a consequence, size can be used as an indicator for how an insect’s life-history strategies are prioritized [18,20]. For example, size can play a role in modulating longevity, survival and fecundity [20–24]. If body size in *Ae. aegypti* can be linked with differential mortality in areas at risk of emergent dengue transmission, we could gain a valuable tool for public health surveillance.

In this study, we tested whether variation in body size, along with temperature and humidity data, can predict age in field-collected, adult female *Ae. aegypti*. The cities in the study were sampled over a period of three years, have robust populations of *Ae. aegypti*, and have very different rates of local dengue transmission despite their geographic proximity. We also determined chronological age using a technique based on measuring the abundance of an age-dependent gene, SCP-1 [25]. Contrary to the findings of several studies that have looked for associations between mosquito body size and age/survival, we expected to find that the inclusion of measurements of size along with weather data would strengthen our ability to predict changes in transmission risk. Previous field studies that tested the association between longevity/survival and body size were based in tropical locations where temperature-mediated variation in size and longevity/survival would be minimal compared to locations at the edge of *Ae. aegypti*'s geographic range [26,27]. We hypothesized that range-edge populations are distinct in the degree to which environmental conditions impact survival/longevity and as a consequence, their ability to transmit disease.

**Methods**

**Study area**
Adult mosquitoes were collected from households in 3 cities over a four-day period, once a month. Each city had between 15 and 40 trapping sites (Fig. 1b, 1c, 1d, Supp. Table 1). The study cities occupy a latitudinal transect at the northern edge of the geographic range of *Ae. aegypti* (Fig. 1a). At the southern end of this transect is Hermosillo, Sonora, Mexico (29.0989° N, 110.9542° W); a city where the *Ae. aegypti* population has maintained local, seasonal transmission of the dengue viruses. At the center of the transect is Nogales, Sonora, Mexico (31.1907° N, 110.5645° W) which saw its first cases of local transmission in 2014, during the study period; At the northern end of this transect is Tucson, Arizona, USA, (32.2217° N, 110.9264° W) which has no documented cases of locally-acquired dengue fever before or during the study period. This transect of the Sonoran Desert occupies 394.2 km and a range in elevation from 210 m (Hermosillo, Mexico) above sea level to 1,199 m (Nogales, Mexico). Collections were limited to the 3 months of the monsoon season, July, August, and September (sometimes in October), due to the significant seasonal increase in mosquito abundance following summer monsoon precipitation events (Supp. Table 2).

**Mosquito sampling and testing for bias**

Biogents BG-Sentinel traps were baited with octanol and lactic acid lures and were either connected to a battery or to a household electric supply. BG Sentinel traps have been found to be about as efficient as human landing rate or backpack aspirators and more efficient than oviposition traps for evaluating abundance in the field [28–30]. BG Sentinel traps have a slight bias for host-seeking *Ae. aegypti* females and the location of the trap is a potential source of bias against nulliparous females [31]. Also, adult mosquito abundance is not affected by the use of insecticides indoors [32]. In essence, known trapping biases would result in over-sampling of our target group of blood-fed females, which is preferable for public health surveillance.

Adults collected from traps were aspirated into containers and taken to the laboratory for analysis. Dead adult females were counted and included in abundance data but not included in any parity or age analyses. Live females were stored in a -80º C freezer until processing (N= 3,920 measures of individual size and N = 4,739 individuals analyzed for parity status). Since traps were only checked once a day during the collection periods, some mortality occurred in the field-collected females that could have caused a bias in the body size of the surviving females. To test if there was a size bias due to differential survival in the trap, dead females from Tucson (N = 60) were measured and compared to live females (N = 78) from the same subset of sites and months.

**Mosquito age and parity assessments**

Ovaries were dissected to determine parity. Visual inspection of trachea in the ovaries allowed us to determine whether a female had completed a reproductive cycle, or not [33,34]. Tracheae that are tightly coiled are considered nulliparous, having never completed a reproductive cycle. Individuals with extended tracheae are considered parous, since once the tracheae extend to transport oxygen to developing eggs they will not recoil. Individuals determined to have completed a reproductive cycle, and/or had a visible, undigested blood meal, and/or eggs were all considered as parous. Parity serves as a physiological
marker of age and for observing changes over time in biting persistence and the human/mosquito contact rate, for a given location [34–36].

Classification of individuals into age groups was done with a genomic age-grading technique using real-time PCR assays of an age-dependent gene, SCP-1 [25]. Females tested for age could be classified categorically as being either 0-5, 6-14, or ≥15 days old. A continuous measure of age was also adapted from the abundance values of SCP (Ernst, unpublished data), and was used for testing the regression models.

**Wing measurements and weather data**

Wings were removed from field-collected females and affixed onto glass microscope slides with a drop of water. Samples were secured onto the slides with a glass cover slip fixed with tape on the sides. Length was measured from the proximal to the distal end for each wing, as described in [37].

Seven-day averages of temperature, diurnal temperature range, average daily maximum and minimum temperature, and percent relative humidity were estimated using city-specific, historical weather data from the National Oceanic and Atmospheric Association (NOAA) and using site-specific (sites within cities) averages from remote climate loggers (HOBO Pro v2, Onset). Weather averages from NOAA and the HOBOs were each tested against wing length to determine which data source was a better fit.

Using our sample-specific age data, we also tested a new technique for estimating when a particular female developed. This technique involves back-casting by different periods of time starting from the date a sample was captured, based on the results of the age-dependent gene expression analyses (Supp. Table 3). Estimating the developmental period of individual mosquitoes in order to study the impact of environmental factors on adult longevity is a novel approach, considering previous studies typically assign the same estimated development period to all mosquitoes sampled [38].

A number of dates are missing HOBO data: Nogales 2013 August, Age group 1 was generated from 6 days (missing one day of weather data). No HOBO data exists for Hermosillo 2015 or 2013 August and Nogales 2013 August for age groups 2 and 3, or July 2013 for any city.

**Statistical Analysis**

All data was analyzed on R 1.0.143 [39] and JMP [40]. ANOVA and linear regression were used to test the impact of the explanatory variables *temperature during development, wing length, and relative humidity in the 1 wk prior to capture* and *temperature in the 1 wk prior to capture* on the response variable, *age*.

To test direct and indirect effects of explanatory factors on wing length and age at death, we used a combination of factor analysis and regression analysis known as multivariate path analysis or structural equation modeling. R was used to do the path analyses on *average temperature during development, wing length, relative humidity in the one week prior to capture and temperature in the one week prior to capture*, and *age*. The strength of the models tested were evaluated by comparing their AIC values which
take into account indirect effects and impose a penalty for each additional variable used. Using AIC enables prioritization of simplicity in model selection.

Results

The wing lengths of field-collected females were tested for normality and found to be non-normally distributed (Shapiro-Wilk test; \( P < 0.01, N = 138 \)). Wing length was log transformed and tested again but remained non-normally distributed (Shapiro-Wilk test; \( P < 0.01, N = 138 \)). Wing length averages and standard deviations are reported in Supp. Tables 4 and 5.

Size bias associated with trapping method/collection times.

A Student’s paired t-test of females that were dead vs. alive showed that smaller females were more likely than larger females to have perished in the traps before being collected, \( t = 5.14, df = 136, p < 0.0001 \) (Fig. 2). Females collected alive and used for parity and age testing had a mean wing length of 2.73 mm and those that were dead upon collection and unable to be used for further analysis had an average wing length of 2.47 mm.

HOBO vs. NOAA weather data.

Linear regression analysis of average temperature during development using HOBO data and NOAA data showed that NOAA data overestimates the site-specific HOBO data (Fig. 3). Weather averages from both sources were also regressed against wing length to see which was more closely associated with length and by proxy, which is more closely associated with conditions actually experienced by larvae during development. HOBO data produced the strongest model for length (Table 1a).

Wing length and temperature during development.

Linear regression analyses of wing length and average temperature during development using HOBO data showed that statistical significance improved when analyzing females separately by their parity status. Nulliparous females had a stronger relationship between wing length and average temperature during development compared to parous females. Nulliparous; Adj. \( r^2 = 0.134 \), RMSE = 0.298, df = 163, \( p < 0.0001 \). Parous; Adj. \( r^2 = 0.045 \), RMSE = 0.287, df = 946. All females; Adj. \( r^2 = 0.055 \), RMSE = 0.291, df = 1,119, \( p < 0.0001 \).

Repeating the same analyses using NOAA data also showed that wing length and average temperature during development are more closely related in nulliparous females. Nulliparous; Adj. \( r^2 = 0.116 \), RMSE = 0.306, df = 215, \( p < 0.0001 \). Parous; Adj. \( r^2 = 0.058 \), RMSE = 0.285, df = 1,289. All females; Adj. \( r^2 = 0.067 \), RMSE = 0.291, df = 1,526, \( p < 0.0001 \).

Wing Length and Age
We used linear regression to test whether *length* was associated with *age* and found no statistical significance, Adj. $r^2 = 0.0002$, RMSE = 5.274, df = 1,545, $p = 0.253$. We then tested each city individually, which were also not significant; Hermosillo: Adj. $r^2 = 0.002$, RMSE = 5.317, df = 521, $p = 0.949$. Nogales: Adj. $r^2 = -0.002$, RMSE = 4.89, df = 374, $p = 0.643$. Tucson: Adj. $r^2 = 0.000$, RMSE = 5.41, df = 648, $p = 0.258$.

**Path analysis for wing length.**

A path analysis for wing *length* using temperature during development, temperature in the 1 wk prior to collection, and percent relative humidity in the 1 wk prior to collection showed that all three variables had a significant effect on wing *length* (Fig. 4). This model for predicting length had the lowest AIC score compared to the other models tested (Table 1a).

**Path analysis for age.**

The set of variables used for constructing the path analysis for testing direct and indirect effects on age for all 3 cities was done using the set of variables that produced the strongest model based on AIC scores (Table 1b). This model used HOBO weather averages and had an AIC of 6,847.56 using temperature during development, percent relative humidity in the 1 wk prior to collection, and wing length and had an $r^2$ of 0.014 (Fig. 5). However, using this model for each city separately proved more effective for predicting age (Table 2). The model for predicting age in Hermosillo was the most robust with an AIC of 1,622.46 and an $r^2$ of 0.19 (Fig. 6).

**Discussion**

In this study, we aimed to use a combination of weather variables and measurements of wing length to predict variation in age. We found that for the 3 cities sampled, the best variables for predicting age were average temperature during development, wing length, and average relative humidity in the 1 wk prior to capture. Although combining all 3 cities in the model provided the greatest range in temperature and relative humidity, we found that replicating this model for each city individually produced stronger models for *age*. This is likely because the variation in wing length between cities was greater than the variation within cities. Hermosillo, the only city with regular dengue transmission, produced the strongest model which predicted about 19% of the variation in *age*. Interestingly, a similar study done by this study’s authors found the same results when testing models to predict age in a controlled laboratory setting. Temperature during development, relative humidity during adulthood, and wing length were the best variables (to the exclusion of crowding during development and temperature during adulthood) and predicted 15% of the variation in age at death (Jeffrey Gutiérrez, *In Press*).
Our modeling in the current study was limited by mortality in the traps which selected against smaller mosquitoes. This biased the subset of females being tested for age as only females that were collected alive were suitable for processing. This study is the first to demonstrate a size-dependent bias in adult trapping using BG Sentinel traps. In fact, a previous study that tested for a bias in body size in BG Sentinel traps did not find any [41].

We also tested whether using remote weather sensors at each collection site actually provides improved data for mosquito surveillance compared to using more readily accessible data such as those made available by the NOAA. We found that indeed, models using site-specific HOBO data were stronger than those using city-specific NOAA data. This information is important for validating the additional cost and effort associated with monitoring weather at multiple sites within a city.

**Conclusions**

Although we were able to predict only 14% of the variation in age using our model, it is important to note that using weather data and size measurements requires very little technical expertise, can be measured quickly, and does not require expensive equipment or materials compared to other methods used for characterizing age structure in field mosquitoes. These benefits make size measurements more likely to be taken up in surveillance protocols by health departments compared to other methods for determining chronological age in field-collected mosquitoes like characterization of gene transcription profiles [25], analysis of cuticular hydrocarbons using mass chromatography/mass spectrometry [42], or quantification of pteridine fluorescence [43]. Importantly, our results demonstrate that including measurements of wing length improve estimates of age over using weather data alone.

Although wing length itself is not associated with age and cannot serve as its proxy, it is a much needed on-the-ground entomological variable that can be collected as a part of routine surveillance activities to strengthen weather-based risk-prediction models. This study contributes to efforts to use weather forecasts to predict changes in the potential for mosquito-borne disease transmission. As stated in the introduction, mosquito abundance is not always a limiting factor for disease transmission, therefore new methods for predicting changes in other aspects of vectorial capacity are needed. Future research should focus on improving our understanding of the links between weather and vector physiology.

**Declarations**

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.
Availability of data and materials

The city-specific climate datasets used and analyzed during the current study are available the National Oceanic and Atmospheric Association's Climate Data Online Search Tool at https://www.ncdc.noaa.gov/cdo-web/search.

Other datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors’ contributions

EHJG worked in the field collections, managed the datasets, did the analyses, and wrote the manuscript. MAR, KRW, KCE, and GD provided guidance and critique on the methodology, analyses, and conclusions. All authors read and approved the final manuscript.

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**Tables**

**Table 1: Comparing AIC values between models.**
| Explanatory Variables                                      | NOAA          | HOBO          |
|-----------------------------------------------------------|---------------|---------------|
| Avg. Dev. Temp., Prev. 1wk. RH, Prev. 1wk Temp.           | 439.596*      | 413.4185*     |
| Avg. Dev. Temp., Prev. 1wk. RH.                          | 525.7594      | 415.9599      |
| Prev. 1wk. RH, Prev. 1wk Temp.                           | 729.2559      | 668.7226      |
| Avg. Dev. Temp., Prev. 1wk. Temp.                        | 440.2945      | 421.3252      |
| Avg. Dev. Temp., Prev. 1wk. RH, Prev. 1wk Temp., # females/site | 441.444       | 423.298       |
| Avg. Dev. Temp., Prev. 1wk. RH., # females/site          | 527.7311      | 427.8371      |
| Prev. 1wk. RH, Prev. 1wk Temp., # females/site           | 566.2172      | 680.3344      |
| Avg. Dev. Temp., Prev. 1wk. Temp., # females/site        | 442.3052      | 433.7088      |
| Explanatory Variables                                      | HOBO   | NOAA   |
|----------------------------------------------------------|--------|--------|
| Avg. Dev. Temp., Prev. 1wk. RH, Prev. 1wk Temp.          | 8,641.58 | 9,818.04 |
| Avg. Dev. Temp., Prev. 1wk. RH                           | 8,639.58 | 10,791.66 |
| Prev. 1wk. RH, Length                                     | 7,097.97 | 8,555.46 |
| Avg. Dev. Temp., Prev. 1wk. RH, Length                   | 6,847.56* | 8,553.65 |
| Avg. Dev. Temp., Prev. 1wk. RH, Length, # females/site.  | 6,848.57 | 8,555.00 |
| Avg. Dev. Temp., Prev. 1wk. RH, # females/site.          | 8,641.21 | 10,793.58 |
| Avg. Dev. Temp., Prev. 1wk. RH, Prev. 1wk Temp, # females/site. | 8,643.16 | 9,820.04 |
| Avg. Dev. Temp., Prev. 1wk. RH, Prev. 1wk Temp, # females/site, Length. | 6,850.29 | 7,762.23 |
| Avg. Dev. Temp., Prev. 1wk. RH, Prev. 1wk Temp., Length. | 6,849.47 | 7,760.67* |

**Table 2: Comparing AIC values between models for each city.**

| Explanatory Variables                                      | Statistic | Tucson | Nogales | Hermosillo |
|----------------------------------------------------------|-----------|--------|---------|------------|
| Avg. Dev. Temp., Prev. 1wk. RH, Length.                  | AIC       | 3,340.54 | 1,845.70 | 1,622.46*  |
|                                                          | df        | 549    | 313     | 263        |
|                                                          | Adj. $r^2$ | 0.07   | 0.07    | 0.19       |

**Figures**
Figure 1

Study cities and their collection sites. A) Study cities, Tucson, Nogales and Hermosillo. B) Collection sites within Tucson, Arizona. C) Collection sites within Nogales, Sonora, Mexico. D) Collection sites within Hermosillo, Sonora, Mexico.
Average wing size of dead vs. alive females. Mean wing length of dead and alive females from four sites and three months, Tucson 2014. Comparison of means using ANOVA and Turkey-Kramer HSD found that they are significantly different with dead females being significantly smaller than those that were collected alive.
Figure 3

NOAA vs. HOBO temperature averages. We compared both sources of data from the week prior to collection. NOAA’s one-week temperature averages are significantly higher than HOBO averages, making HOBO data more accurate for predictions regarding the physiological responses of mosquitoes to weather variability.

Figure 4

Path analysis for wing length. The model for predicting wing length that had the lowest AIC value used HOBO data and included avg. temp. during development, and avg. relative humidity and temp. in the 1 wk. prior to capture.
Path analysis for age, all cities. The model for predicting age that had the lowest AIC score used HOBO data and included avg. temp. during development, wing length, and avg. relative humidity in the 1wk prior to collection. This model had an r2 of 0.014.
Path analysis for age, Hermosillo only. City-specific models for age prediction were more robust than the model including all cities. The strongest model for predicting age had an $r^2$ of 0.19 for Hermosillo.

**Supplementary Files**

This is a list of supplementary files associated with this preprint. Click to download.

- SuppTable1Sitelocations.docx
- SuppTable4Avgwinglength.xlsx
- SuppTable2Mediancollectiondates.docx
- SuppTable3Devperioddates.docx
- Supp.Table5SDwinglength.xlsx