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How Environmental Change Will Impact Mosquito-Borne Diseases

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This Master's Project

HOW ENVIRONMENTAL CHANGE WILL IMPACT MOSQUITO-BORNE DISEASES

by

Arsal Khan

is submitted in partial fulfillment of the requirements
for the degree of:

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in
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Table of Contents

List of Tables

List of Figures

List of Acronyms

AR5	Fifth Assessment Report
ATV	All-Terrain Vehicle
AUC	Area Under Curve
BOCC	Board of County Commissioners
CDC	Center for Disease Control and Prevention
CNM	Correlative Niche Model
DTR	Diurnal Temperature Range
EU	European Union
FMCD	Florida Mosquito Control District
GCM	General Circulation Model
GDP	Gross Domestic Product
IPCC	Intergovernmental Panel on Climate Change
MIR	Minimum Infection Rate
NACCHO	National Association of County and City Health Officials
NCBI	National Center for Biotechnology Information
NUTS-3	Nomenclature of Territorial Units for Statistics
PFU	Plaque-Forming Unit
RCM	Regional Climate Model
RCP	Representative Concentration Pathway
SEI-SEIR	Susceptible Exposed Infectious – Susceptible Exposed Infectious Recovered
TSS	True Skill Statistic
UHI	Urban Heat Island
WHO	World Health Organization

Abstract

Mosquitos, the most lethal species throughout human history, are the most prevalent source of vector-borne diseases and therefore a major global health burden. Mosquito-borne disease incidence is expected to shift with environmental change. These changes can be predicted using species distribution models. With the wide variety of methods used for models, consensus for improving accuracy and comparability is needed. A comparative analysis of three recent modeling approaches revealed that integrating modeling techniques compensates for trade-offs associated with a singular approach. An area that represents a critical gap in our ability to predict mosquito behavior in response to changing climate factors, such as temperature, is evolutionary adaptive potential. Evolutionary studies for mosquitos have documented rapid evolutionary change in photoperiodic traits. Further research on evolutionary adaptive potential for mosquito thermal tolerances using longitudinal studies in conjunction with genomic approaches will allow for more realistic parameterization of mosquito biological processes. One of the primary factors driving disease patterns is urbanization. Urban areas are already highly impacted by climate-related health issues and offer a wide variety of potential aquatic habitats for breeding, thereby presenting vulnerable targets for mosquito populations. Mosquito-borne diseases have been historically underrepresented in urban health planning, and with projected increases in habitat suitability for temperate areas such as the U.S., promoting awareness of this issue constitutes a major health priority for the future. Integrating mosquito control policies into urban planning and design, such as concomitant strategies for elimination in green space development, will be highly beneficial in mitigating adverse health outcomes.

Introduction

In light of the recent COVID-19 pandemic, much attention has been directed towards the growing threat of zoonotic diseases (i.e., infectious diseases transmitted from vertebrate animals to humans) as a menace to global health. Zoonotic diseases account for an estimated 60% of all known infectious diseases and as high as 80% of newly emerging infectious diseases (Chala & Hamde, 2021; Salyer et al., 2017). These numbers equate to 2.5 billion cases of infection and 2.7 million deaths annually, qualifying zoonotic diseases as a substantial global health burden (Salyer et al., 2017).

Vectors (i.e., organisms that transmit infectious diseases from host to host) often bridge the gap between animal reservoirs for pathogens and human populations, representing an important link in the transmission cycle and another source of potential health risk. Most vector-borne diseases are zoonotic in origin, with few exceptions (e.g., dengue, malaria), accounting for 14% of known infectious diseases and a disproportionate number of emerging pathogens (mostly viruses) as well (Chala & Hamde, 2021). Likewise, patterns of emergence and re-emergence for vector-borne diseases have led to epidemic episodes and an overall increase in case load all around the world.

Mosquitos represent one of the most notable examples of vectors due to their ubiquitous presence globally (i.e., ≈100 trillion mosquitos) and track record as the most lethal species throughout human history. Mosquitos alone contribute to 500 million cases of infection annually, making them the leading cause of vector-borne diseases worldwide (Karypidou et al., 2020). Most of this disease burden lies in developing countries in the tropical belt, where housing and public health infrastructure are lacking in terms of providing protection to citizens, and climate conditions are conducive to mosquito persistence.

The sharp rise in disease prevalence seen today can likely be attributed to a multitude of factors that collectively encompass the concept of environmental change attributed to humans. These factors include phenomena such as climate change, loss of biodiversity, and sea level rise, all of which are happening at an unprecedented rate. The highly dynamic conditions dictating the disease landscape today are also expected to redistribute disease patterns into new areas. This shift runs the risk of affecting naïve populations that lack prior immune history, subsequently creating explosive epidemics.

Climate change in particular is a major focal point for research towards analyzing drivers of species distribution and ecological niche space shifts. This focus on climate change is due to the fact that all species have finite physiological limits associated with various lifecycle traits that are dependent on climate factors (e.g., critical temperature limits dictating functionality and survival or thresholds for humidity to prevent desiccation). These constraints hold especially true for ectotherms like mosquitos that rely on external heat sources to regulate body temperature. Many mosquito species are already nearing their upper limits for temperature suitability in the tropics and will likely be forced to migrate into temperate regions such as the U.S., mirroring the northward expansion seen in other species and increasing the risk of mosquito-borne disease endemicity in previously unaffected areas (i.e., once low winter temperatures no longer become a limiting factor).

In addition to climate change, the impact of globalization and the subsequent increase in international trade and travel has led to an increase in incidents where invasive species have been introduced to areas that would otherwise be inaccessible due to natural barriers. Likewise, changes in land use patterns and increasing urbanization have also resulted in higher contact rates between wildlife and people, as development begins to encroach upon natural habitats that are becoming increasingly fragmented and sparse. For many major cities in developed countries, the growing threat posed by mosquito-borne diseases has yet to be sufficiently addressed by urban health planners.

To anticipate how these various factors will culminate in range shifts for mosquito-borne diseases, predictive analytics techniques such as species distribution models are becoming more popular in scientific research. Modeling approaches have a high degree of flexibility depending on whether statistical or theoretical methods are used, what covariates are incorporated, the species being modeled, the spatial resolution and scope of the projection, and so on. Generally, all these factors vary based on the technology and information available and the research question being asked. Nonetheless, the lack of consensus on optimizing model performance and comparability across studies hinders the application of these models in terms of preparing health officials for and directing vector control organizations towards high-risk areas.

The goal of this paper is to address this disparity by establishing a framework for models that will aid in improving health outcomes and targeting hotspots associated with mosquito-borne diseases. Through a comparative analysis of three contemporary models, the strengths and weaknesses of each approach are highlighted to serve as a guide for providing recommendations for future models. Additionally, the concept of evolutionary adaptive potential for mosquitos and their pathogens represents a critical gap in our ability to forecast mosquito behavior and geographic distribution. A literature review of the current knowledge of mosquito thermal biology and general evidence of evolutionary adaptation addresses this issue and serves to inform recommendations towards bridging this gap going forward. Lastly, the need for prioritizing urban environments and the status of vector control programs in the U.S. are discussed in the final section of the paper, focusing on control practices within the state of Florida as a case study.

Methods

Reference material for all three sections was primarily obtained through a literature search of three library databases: Environment Complete, GreenFILE, and Scopus. Research was also supplemented through the use of Google Scholar. Key words and terms used during searches included “mosquito-borne disease”, “vector”, “disease model”, “evolutionary adaptation”, “thermal biology”, “phenotypic plasticity”, “mosquito control”, “climate change”, “urban mosquitos”, and “U.S.” in various combinations.

Section 1 consisted of a comparative analysis of three recent (i.e., 2020-2021) modeling approaches for mosquito-borne diseases taken from Q1 journals (i.e., *Climate Change*, *Nature Communications,* and *Parasites & Vectors*). Section 2 consisted of a literature review of mosquito thermal biology, alternative mechanisms for thermal adaptation such as phenotypic plasticity, and evolutionary adaptation research for both mosquitos and pathogens. Section 3 consisted of a literature review on mosquito control organizations within the U.S., identifying target vectors, common control practices, and areas in need of improvement. Studies on the impact of mosquitos in urban environments were also included to highlight the need for integrating control measures in urban planning and design. Lastly, a case study on the status of mosquito control in the state of Florida was conducted based on findings from two surveys that address issues such as budgetary constraints, equipment availability, staffing needs, and implementation levels for various tasks. Recommendations for all three sections were provided based on the information outlined above (i.e., improving model accuracy and establishing grounds for consensus in modeling approaches, designing adaptation studies on the potential for mosquitos to evolve higher upper thermal limits, and improving mosquito control practices throughout the U.S., respectively).

1. Standardizing and Improving Mosquito-Borne Disease Models

1.1 Overview of Model Types

Experimental methods, in which mosquitoes are subjected to varying conditions manipulated by the researcher, provide valuable insight into the process behind species behavioral or physiological response to climate change conditions. However, these findings alone are insufficient in describing how these response patterns will manifest themselves in real populations across space and time. Recent studies have described a variety of approaches for incorporating climate projections in models of arboviral (i.e., arthropod-borne virus) disease dynamics to predict spatiotemporal shifts for vectors and pathogens. Two broad modelling approaches that have been successfully employed thus far are phenomenological and mechanistic models (Ryan et al., 2019).

Phenomenological models are empirically based, where a statistical relationship is established between hypothetical covariates and field data to project trends into the future (Messina et al., 2015; Sillero et al., 2021). The term phenomenological is synonymous with correlative as these models make direct correlational inferences about the various factors involved in the analysis (Dormann et al., 2012). These models represent the most commonly employed method for species distribution modeling and have also been shown to replicate transmission data accurately, especially in terms of capturing the relationship between environmental factors that vary widely in time and space and disease occurrence (Dormann et al., 2012; Messina et al., 2015). Phenomenological models do not attempt to explain the underlying relationship between variables in terms of theoretically derived principles, but rather by using mathematical functions that best describe the data. This approach yields more narrow, specific ranges for predictions that are especially useful when analyzing factors with complex patterns of interaction that are not yet well defined (White & Marshall, 2019).

Mechanistic models, on the other hand, provide greater context for projections by incorporating vector physiology and viral transmission characteristics based on experimentally determined relationships with climate-related factors (e.g., how temperature interacts with mosquito thermal biology) (Messina et al., 2015). Mechanistic models are often used to make predictions based on the dynamic processes that influence species distribution, such as dispersal rates or the effects of disturbance events or shifting management practices for a given area (Dormann et al., 2012). Unlike phenomenological models, they do not require calibration with locally collected data to perform accurately (Caldwell et al., 2021). Instead, these models rely on equations derived from experimental data to parameterize certain biological functions that are critical for vector and viral development and persistence (Messina et al., 2015). The distinct qualities differentiating phenomenological and mechanistic model design are more clearly illustrated in Figure 1.

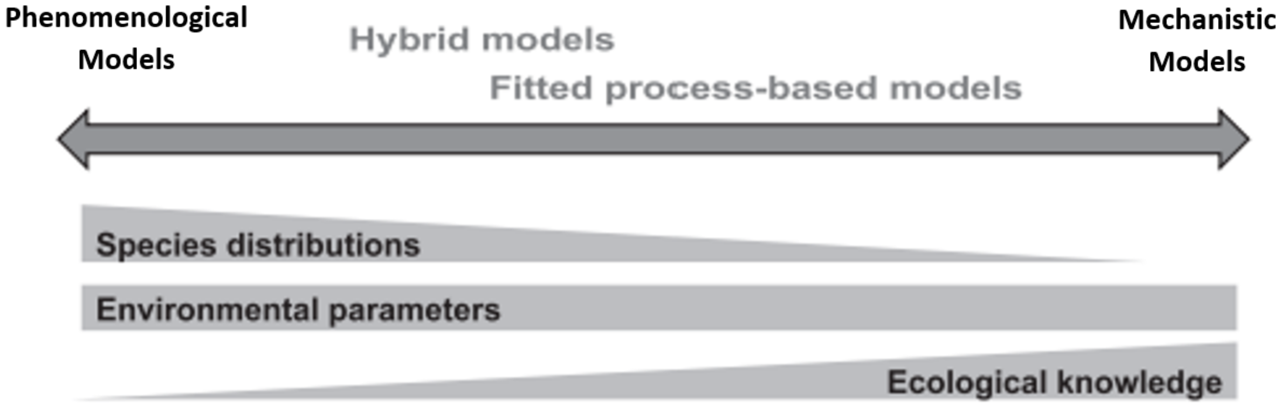


Figure 1 (modified from Dormann et al., 2012). The relationship between phenomenological and mechanistic models shown on a continuum. Phenomenological models do not require *a priori* ecological knowledge of the process being depicted but are instead dependent on the quality and abundance of available data for calibrating environmental parameters. At the opposite end of the spectrum, pure mechanistic models are independent of local data inputs and rely solely on well-defined ecological processes derived from experimental observations or theory. Hybrid models, such as fitted process-based models, combine elements from both ends of the spectrum (i.e., incorporating ecological knowledge with parameters calibrated with real world data). Integrated models, in which both general model types are applied simultaneously to a given dataset to generate predictions, are also a subset of hybrid models.

1.2 Temperature-based Mechanistic Model

As all aspects of mosquito biology relevant to either metabolic processes or transmission potential are linked with temperature, mechanistic models are a logical choice for projecting mosquito thermal suitability (Caldwell et al., 2021). Ryan et al. (2019) applied a model that estimated the basic reproduction number (R_0) of the *Aedes* genus of mosquitoes in response to projected temperature changes. Here, the role of temperature in determining viable habitat ranges was assessed independently from other factors impacting disease dynamics, such as precipitation, land use, or socioeconomic status. The study defined a threshold condition at which transmission could still occur ($R_0 > 1$) to determine transmission suitability worldwide in accordance with four different climate models, each forecasting the four separate representative concentration pathways (RCPs) discussed in the International Panel on Climate Change (IPCC) Fifth Assessment Report (AR5). Metrics for climate suitability and population density were overlayed on a raster surface to generate spatial projections illustrating transmission suitability and the number of people at risk (in billions) across the world as a function of time (see Figure 2). This marked the first study to attempt to model population risk associated with *Aedes*-borne disease in a seasonal context.

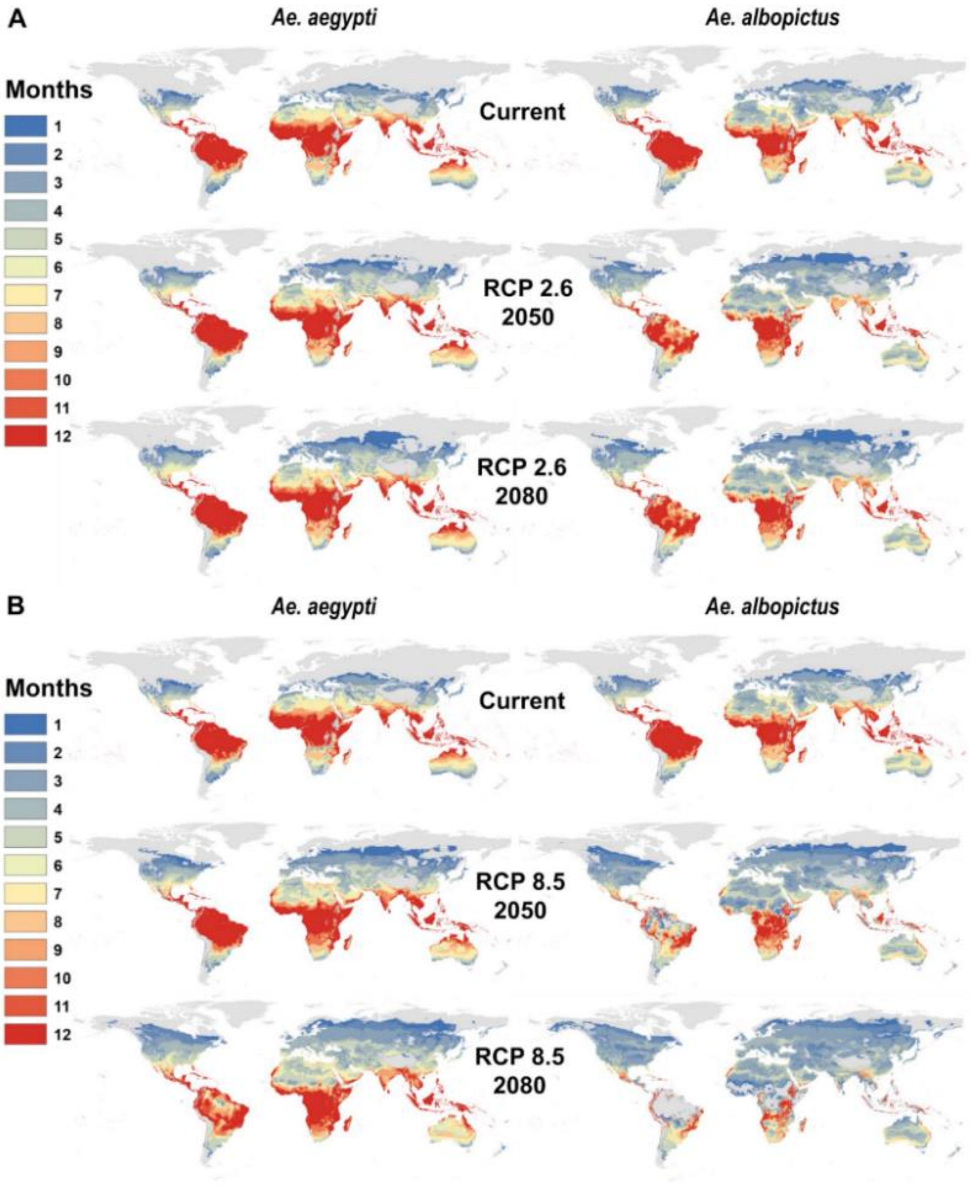


Figure 2 (from Ryan et al., 2019). Global thermal suitability maps for *Aedes aegypti* and *Ae. albopictus*. Suitability is defined in terms of the number of months for which mean temperature is above the threshold preventing the basic reproduction number (R_0) from reaching 0. Blue is indicative of areas with limited suitability windows (i.e., ≤ 6 months) whereas red is indicative of areas with longer suitability windows (i.e., ≥ 8 months). Projections were constructed for all four emission scenarios discussed in IPCC AR5 for 2050 and 2080. Only the projections for the lowest and highest emissions scenarios (i.e., RCP 2.6 and RCP 8.5, respectively) are shown here.

The results of the study indicated an overall increase in transmission risk and seasonal length associated with *Aedes*-borne diseases, particularly for Europe, high altitude areas in the tropics, the U.S., and Canada. Interestingly, the model predicted greater increases in transmission suitability for one species (i.e., *Aedes albopictus)* under the more moderate warming scenarios (i.e., RCP 4.5) in comparison with the more extreme scenarios where climate mitigation is minimal. This is because under these warmer scenarios, temperatures in certain areas (i.e., the tropics) are predicted to surpass the upper thermal limit of *Ae. albopictus*, whose thermal tolerance is relatively low in comparison to *Ae. aegypti* (i.e., 29.4˚C vs. 34.0˚C). This finding highlights the utility of models in uncovering the nonlinear and nuanced effects that varying degrees of mitigation can have on transmission suitability and across vectors with distinct physiological traits.

Although this study offers a sound foundation for temperature-based modelling for vector-borne diseases, there are several limitations hindering its direct application that should be addressed. First, the lack of inclusion of other covariates relevant to disease transmission means that the model is inherently over-simplistic in addressing transmission suitability. Even the inclusion of other, strictly climate-related factors such as precipitation and humidity would add to model robustness by assigning weights to each variable, thus balancing the respective influence of each. The logical reason for generating a solely temperature-based model is due to the relative abundance of research afforded towards studying mosquito thermal biology (Couper et al., 2021). From an experimental standpoint, temperature is relatively easy to manipulate and measure in a lab setting in relation to other climate variables. Additionally, the physical constraints imposed by temperature limitations are directly observable in comparison to some of the more complex or nonlinear affects associated with precipitation and humidity. This allows researchers to measure and quantify mosquito lifecycle processes under varying conditions more easily and create physiological response curves that can be incorporated into a mechanistic framework. More research should be allocated towards studying species physiology in response to other climate factors to improve parameterization of additional covariates. Lastly, the lack of validation measures for predicting current distribution patterns in this study means that the accuracy of the model is hypothetical. Validating model estimates against real-world disease occurrence or mosquito abundance data is vital in determining model performance contemporarily, while the use of historical records to generate model observations for the future would ground extrapolations to real world conditions.

1.3 Model Limitations and Integrated Models

In general, both phenomenological and mechanistic models have their limitations. Because phenomenological models are not derived from first principles, they have less explanatory power behind the how and why variables interact the way they do (White & Marshall, 2019). While ecological knowledge can guide the selection of variables in designing phenomenological models, the relationships and underlying processes dictating model behavior are implicit (Dormann et al., 2012). Phenomenological models also lack a strong biological component for predicting species behavior (Caldwell et al., 2021). In the case of developing vector control strategies, understanding the biological underpinnings of mosquito-climate interactions can help inform new methods for exploiting mosquito physiology and eliminating them from projected high-risk areas. For example, increasing insecticide resistance in some populations is an emerging issue threatening to minimize the efficacy of these chemicals as a control measure (Pu et al., 2020). Anticipating shifts in mosquito phenotypes in conjunction with changing climate conditions will be necessary to effectively cycle insecticide treatments and keep humanity a step ahead in the arms race.

Mechanistic models are associated with a higher potential for human error compared with empirical models because the biological parameters used are scaled up from controlled lab conditions with small sample sizes to whole populations in the wild that are subjected to more dynamic environmental conditions (Caldwell et al., 2021). This can introduce a level of observer error into projections through over generalization of lab results and subsequent parameterization inaccuracy. This is because the process-driven approach of mechanistic models is centered around functional relationships that are defined *a priori* and imply causation (i.e., temperature causes mortality at a certain threshold), which can potentially overlook confounding factors (Dormann et al., 2012). Mechanistic models are inherently inadequate for projecting variables that cannot be linked functionally to mosquito-borne diseases even if they are significant drivers of transmission, such as gross domestic product (GDP) per capita or urbanization trends (Messina et al., 2015). Due to these factors, mechanistic models can conversely be used to falsify rather than confirm hypothesis through formal comparison with real data (Dormann et al., 2012).

Accordingly, many studies utilize an integrated approach where modeling techniques (i.e., mechanistic and phenomenological) are combined to counterbalance the weaknesses associated with any single model type. Using statistical techniques to link abiotic environmental or other nonbiological factors with biologically based mechanistic equations have been shown to successfully reflect disease dynamics in the real world. Ultimately, a high capacity for variation exists across all model types depending on the covariates, datasets, and parameters utilized (i.e., different population metrics, RCPs, temporal and spatial scales, etc.). The lack of consensus on modeling approaches for mosquito-borne diseases implies that there is also a lack of consensus among the scientific community as to where these species will spread and where efforts towards increased vector surveillance should be directed (Messina et al., 2015). To improve model accuracy and comparability across studies, certain criteria for model design should be established for future research (Messina et al., 2015).

1.4 Need for Consensus and Standardization

A review article by Messina et al. (2015) explored modelling studies for dengue to make recommendations towards creating a standardized modelling framework. Comparing the performance of three mechanistic models to two statistical models, the article found that the mechanistic model projections showed more inconsistencies with the validation datasets. Similar to the Ryan et al. (2019) study, these mechanistic models were strictly based on temperature-dependent biological processes and predicted baseline transmission potential for several areas that had either not experienced an outbreak or were unlikely to experience an outbreak, such as the Sahara Desert. This highlights the distinction between defining thermal suitability versus transmission suitability and the need to incorporate additional climate and host related factors when modeling the latter.

For their recommendations, Messina et al. (2015) called for global health agencies (i.e., the WHO, the U.S. or European Centers for Disease Control) to set certain guidelines for disease models that are in line with international health policies and needs. These guidelines included using projecting at 30-year intervals (i.e., 2020, 2050, 2080) to coincide with climate projections from the IPCC Special Report on Emissions scenarios, combining and averaging climate variables across general circulation models (GCMs) for each scenario, using a minimal spatial resolution of 1 km for raster surfaces (unless policy measures specifically call for coarser resolution or finer resolution datasets become available), validating models using contemporary distribution data, providing uncertainty estimates for projections and covariates, and including variables relating to population and environmental characteristics. The authors also believe that an integrated modeling approach, where biological parameters derived from mechanistic models are combined with a suite of other variables in a statistical model, is preferred.

The foremost concern for modeling studies shouldn't be over whether phenomenological or mechanistic approaches are used, but rather with simply generating accurate predictions or testable hypotheses from sound model design (White & Marshall, 2019). I will now compare three different models that utilize distinct approaches with varying degrees of accuracy to highlight the strengths and weaknesses of each. By conducting a comparative analysis of these models, the goal of this research is to further contribute towards building consensus and standardizing model practices for future studies.

1.5 Model Comparative Analysis

1.5.1 Mechanistic Epidemiological Model for Two Equatorial Countries

Caldwell et al. (2021) created an epidemiological model that borrowed from previous mechanistic modeling work to predict key epidemic characteristics across two equatorial countries (i.e., Ecuador and Kenya). The compartmental SEI-SEIR model divides mosquito and host populations along different stages of the transmission cycle (i.e., SEI-SEIR acronym corresponds to categories such as susceptible, exposed, infectious, and recovered) based on parameterized mechanistic relationships between *Aedes* mosquito and arboviral life cycle traits and climate factors such as temperature, humidity, and rainfall (see Figure 3). The model was validated with *Ae. aegypti* occurrence data using samples collected by the authors and disease occurrence data for three arboviruses (i.e., dengue, chikungunya, and Zika) taken from local health agencies across the eight study sites.

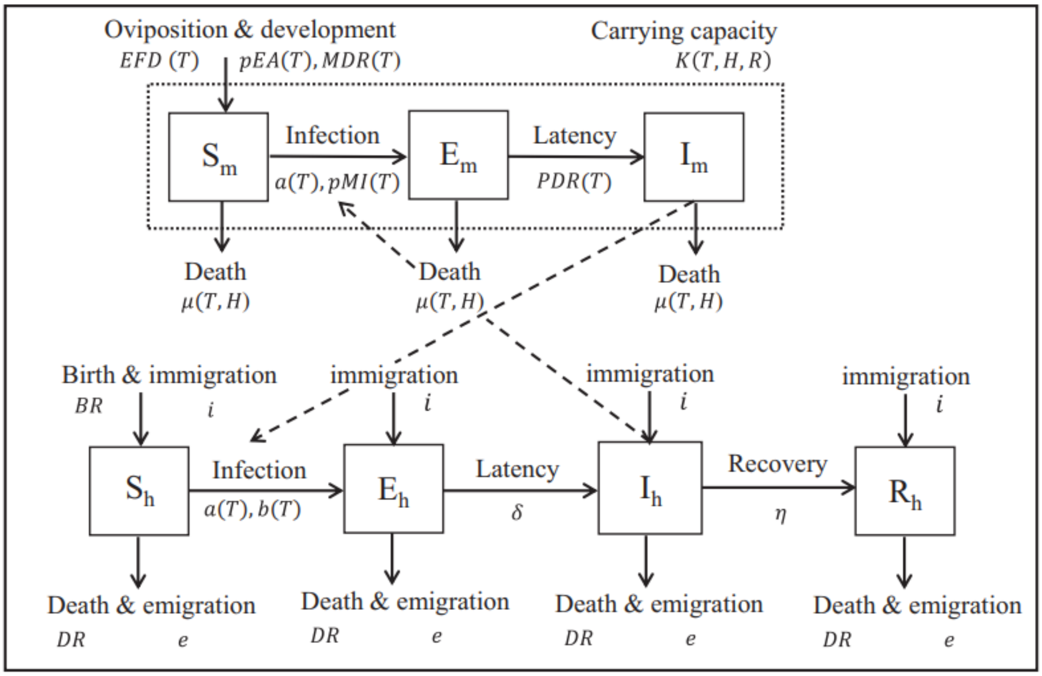


Figure 3 (from Caldwell et al., 2021). Epidemiological model divides mosquito and host populations into groups based on transmission potential and infection stages, respectively. Mosquito population groups are categorized as susceptible (S_m), exposed (E_m), and infectious (I_m). Host population groups are categorized as susceptible (S_h), exposed (E_h), infectious (I_h), and recovered (R_h). Solid lines indicate directional movement of individuals across compartmental groups, while dashed lines correspond to the direction of transmission effects based on climate-dependent rate parameters.

Predictions for mosquito presence significantly correlated with the validation data in all eight study sites, while predictions for disease occurrence significantly correlated with the validation data in seven of the eight sites. Defining the term outbreak as an uninterrupted period of time where the number of disease cases exceeded the mean number of cases plus one standard deviation, the model was successful in predicting the total number of outbreaks, the peak timing of outbreaks, and the length of outbreaks. However, the model was not able to accurately predict total outbreak size or the maximum number of infections. These results indicate that while climate may dictate certain spatiotemporal aspects of disease dynamics, the magnitude of outbreaks is more likely reliant on host availability. This finding emphasizes the need for parameterizing additional anthropogenic factors such as population immunity characteristics, vector control presence, and local movement and behavioral patterns relevant to susceptibility. Although data based on these conceptual metrics will be difficult to collect and delineate spatially, including even one overlooked driver of disease transmission may help capture additional epidemic characteristics as well.

The model performed slightly better across sites in Ecuador in comparison to Kenya overall. Ecuador is characterized by having seasonal epidemics while Kenya experiences low level endemic conditions. The authors reason that this because Ecuador has a larger proportion of coastal land, therefore experiencing more consistent seasonal cycles due to the buffering effects of the ocean. Inland areas are subjected to greater variation in climate, which is not conducive for generating large outbreaks. Kenya's climatic variation is a likely factor contributing to the model's decreased accuracy there.

Demographic and socioeconomic factors were also shown to influence disease dynamics. The model was less effective in predicting mosquito dynamics in areas with a high proportion of children under five (higher population mobility), which is more commonly observed in populations of lower socioeconomic status such as Kenya. Conversely, the model was less accurate in capturing disease dynamics in areas with higher percentages of indoor water pipes (lower human-mosquito contact rates) and houses built with cement (cooler indoor temperatures less suitable for mosquitoes), which are more common characteristics of the sites in Ecuador. All three traits relate to the difference in GDP between Ecuador and Kenya (i.e., $177 billion vs. $86 billion, respectively) and highlight the role socioeconomic factors play in dictating transmission potential. Nonetheless, the general success of the model across study sites with such distinct transmission characteristics is indicative of the comprehensiveness of the model design.

The inclusion of other climate factors besides temperature helped strengthen the predictive accuracy of the model for overall transmission potential. Humidity is vital to mosquito survival due to their elongated body structures (i.e., disproportionate surface area to volume ratio) which makes them susceptible to desiccation in arid environments. Rainfall creates aquatic habitats necessary for the three lifecycle stages of mosquito development prior to adulthood (i.e., egg, larva, and pupa), but excessive rain can lead to overflooding and subsequent flushing of oviposition sites. Because rainfall has been shown to interact with transmission dynamics in a variety of ways and at different time scales, three separate rainfall functions were utilized across study sites based on which function led to the highest degree of correlation between predictions and observations. The need to incorporate multiple rainfall mechanisms prompted Caldwell et al. (2021) to conceptualize a model for rain that influences transmission dynamics along different pathways (see Figure 4). This flexibility and innovation in capturing complex real-world dynamics will likely provide an edge over models that parameterize climate variables using a single function approach.

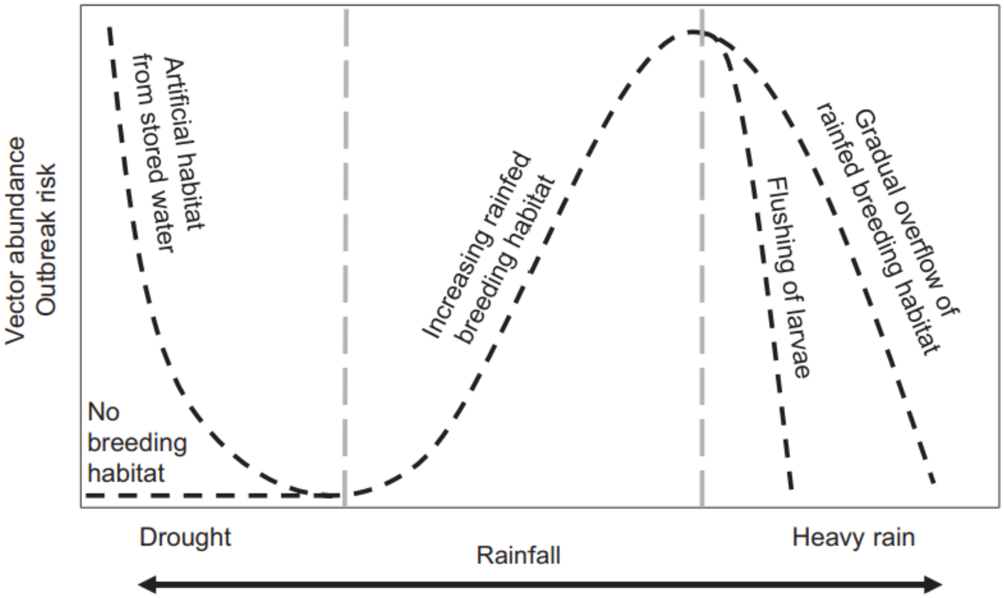


Figure 4 (from Caldwell et al., 2021). Conceptual rain model in which various mechanisms describing the effect of rainfall on disease dynamics are combined. Integrating several functional relationships into one model allows for rainfall to influence transmission potential in different ways (dashed lines) in response to varying rain conditions (x-axis). This dynamic approach is better suited for capturing the nonlinear and time delayed effects of rainfall.

1.5.2 Correlative Niche Models / Mechanistic Model for Greece

Karypidou et al. (2020) utilized an integrated modeling approach (i.e., applying both phenomenological and mechanistic techniques) to create a robust binary distribution map for the presence of *Anopheles sacharovi* in Greece, where it has been responsible for the recent reemergence of malaria (2009-2015). The study combined data generated from five correlative niche models (CNMs) with a recalibrated mechanistic model originally developed for another *Anopheles* species (i.e., *An. gambiae*). Correlative niche models are a type of ecological niche model that use geographical data (i.e., species occurrence) in conjunction with environmental predictors to create habitat suitability or occurrence probability maps (Sillero et al., 2021). Mosquito and viral life cycle processes, based on threshold temperature and rainfall conditions, were represented by the mechanistic vector-borne disease community model (VECTRI) to predict monthly averages for vector density. Both models were transformed into binary distribution maps, with a value of 1 representing mosquito presence and 0 indicating absence, and overlaid onto a single grid surface (see Figure 5). Defining transmission potential in this way, in which only areas that were designated as 1 by both models were categorized as malaria receptive, meant that mosquito activity had to exceed a certain threshold to be counted as present. Results were reported as the percent change in area projected to be suitable for transmission in relation to the baseline period (1971-2000).

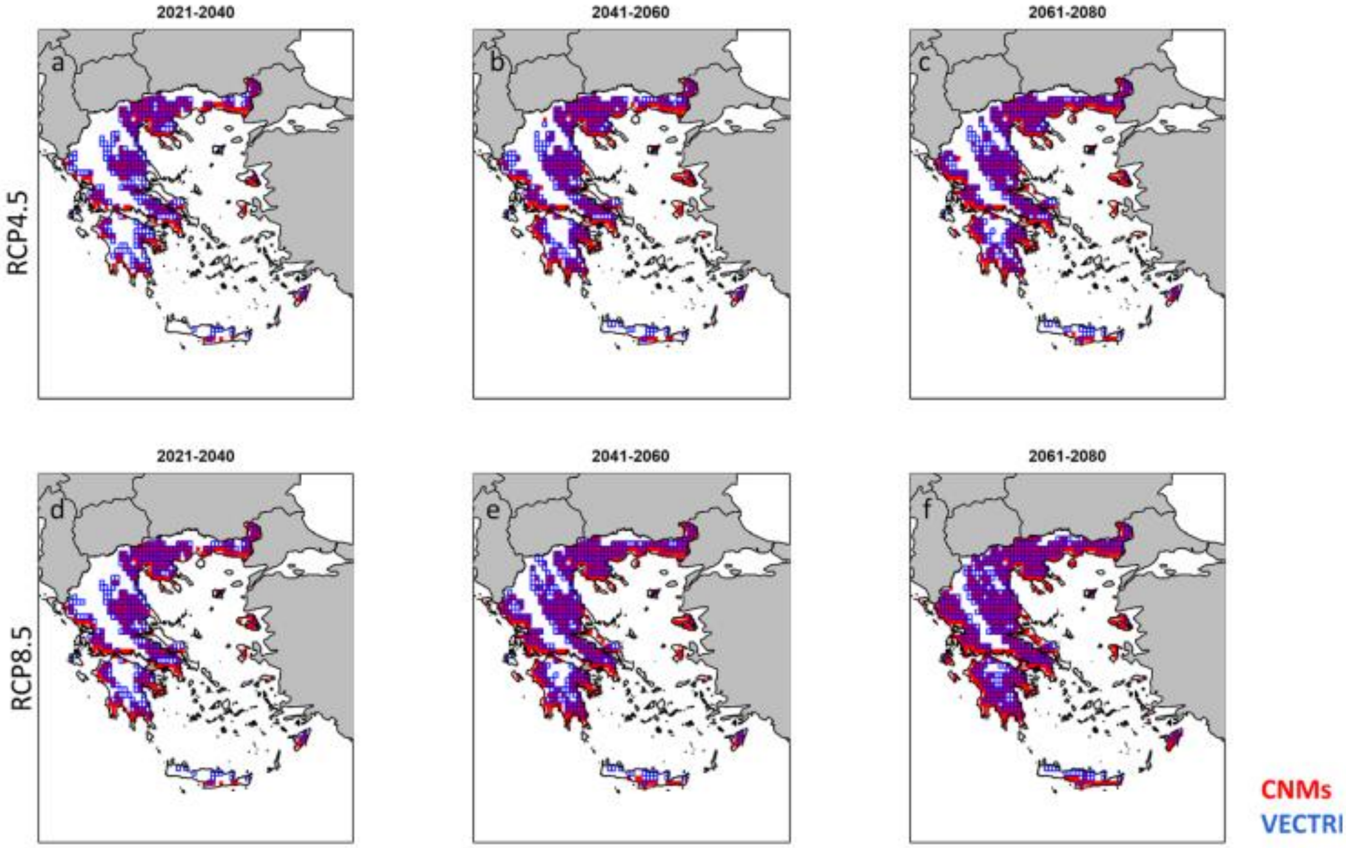


Figure 5 (from Karypidou et al., 2020). Integrated distribution map identifying malaria receptive areas generated from an ensemble of five correlative niche models (CNMs; red areas) and a mechanistic model (VECTRI; blue squares). Projections are given for RCP 4.5 (top row) and RCP 8.5 (bottom row) at three future periods (left to right): 2021-2040, 2041-2060, and 2061-2080. Both modeling approaches resulted in a high degree of spatial overlap.

Climate was modeled using an ensemble approach, averaging outputs from four separate regional climate models (RCMs) driven by four separate GCMs to control for uncertainty associated with model type. Data for maximum and minimum mean monthly temperatures and total monthly precipitation was fed into these models to generate data for 19 bioclimatic variables. These variables functioned as climate predictors for reproducing baseline conditions as well as estimating future conditions for three different projection periods (i.e., 2021-2041, 2041-

2060, and 2061-2080) in accordance with RCP 4.5 and RCP 8.5. After eliminating climate variables that were highly correlated, only four remained (i.e., mean diurnal temperature range, temperature seasonality, mean temperature of warmest quarter, and precipitation of warmest quarter). To serve as additional predictors, CNMs used land use data to identify aquatic habitats and calculate distance to water-covered areas, while the VECTRI model included population density as a measure for mosquito biting rate.

All model projections predicted a general increase in areas suitable for malaria transmission under both climate scenarios and for all three time periods. The estimates from the CNMs were nearly double those of VECTRI, both in terms of suitable area and population risk increase. Models were validated using historical malaria data taken from early 20th century maps and recent reemergence data obtained from the National Public Health Organization (NPHO) between 2009-2015. CNMs, VECTRI, contemporary data, and historical data shared considerable spatial overlap. Spatial boundaries were defined using the Nomenclature of Territorial Units for Statistics (NUTS-3) of the European Union (EU). Out of the 13 NUTS-3 administrative units identified as having reemergence cases, 10 were estimated to have at least 33% suitable area for transmission under even the most modest scenario. The fact that increased malaria suitability and population risk was predicted irrespective of the applied model type highlights the importance of this research as a form of preemptive vector surveillance. Karypidou et al. (2020) believe that given the shared climatic conditions and similar reemergence patterns observed in countries such as Italy and Spain, increased mitigation efforts should be directed toward other Mediterranean countries as well.

By incorporating two drastically different modeling approaches, the study was able to compare the results of each and combine them in a way that counterbalances the shortcomings of either method. For CNMs, models were run 1000 times each and evaluated using two performance metrics, area under curve (AUC) and true skill statistic (TSS). AUC is a measure of model accuracy (i.e., identifying and distinguishing between true presences and absences) while TSS corrects for model dependence on prevalence (i.e., sample size of the validation data) (Fukuda & De Baets, 2016; Karypidou et al., 2020). Only models that exceeded the threshold performance score were included in the ensemble. The quality control measures implemented in this study (i.e., multiple replicates and validation metrics) optimized model performance and offer a prime example of the standard that should be maintained for assessing future models.

Additionally, the study benefited from focusing on a smaller, more manageable spatial scale in Greece as opposed to the global projections from the Ryan et al. (2019) study. In terms of research applications, finer spatial scales are better suited for directing mitigation efforts from public health officials to local areas. The issue of scale is largely dependent on the availability of reliable data at the desired spatial resolution, and this is likely to become less limiting in the future with improvements in remote sensing technologies (Lorenz et al., 2020). Despite lacking any socioeconomic variables and only projecting for two of the four RCPs, Karypidou et al. (2020) provide an excellent modeling framework that illustrates the utility of integrating different model designs in a single analysis.

1.5.3 Machine Learning Ecological Niche Model for the Continental Americas

In light of recent outbreaks of eastern equine encephalitis virus and the detection of new strains of St. Louis encephalitis virus in multiple U.S. states, as well as the ever-growing threat of expansion of West Nile Virus, the need for improving our understanding of the distribution of the associated vectors is higher than ever. Contemporary information on the occurrence of the *Culex* mosquitos that transmit these arboviruses in the U.S. and Canada mainly stem from studies conducted at spatial scales at the state and provincial level from the early 1980s, further emphasizing the need for updated maps in line with subsequent shifts in environmental, climatic, and demographic trends. To address this issue, Gorris et al. (2021) turned to new breakthroughs in mathematical ecology through a machine learning ecological niche model known as Maxent to create habitat suitability maps for seven *Culex* species native to both North and South America.

To capture the variables associated with the distribution of *Culex* mosquitos, Gorris et al. (2021) conducted a literature review and made note of the environments where these species have been found as well as the factors that characterize those regions. These climatic and environmental variables were compiled from two sources (i.e., MERRAclim and EarthEnv) and included five metrics for temperature, three metrics for humidity, 12 land cover classifications, a measure for habitat heterogeneity, and four metrics for topography. Mosquito occurrence data covering the past 30 years was compiled from three data banks as well as two surveys conducted by public health departments. Together, these data inputs were aggregated and recalibrated to a shared spatial resolution of 30 km in order to be processed by Maxent on a single coordinate plane.

After establishing an environmental training area for each species, Maxent trained the models by cross-validating randomly selected background data points with the mosquito occurrence data. Models were filtered based on a series of threshold requirements (e.g., limiting bias in relation to omission rates) as a quality control measure to optimize model prediction accuracy without sacrificing model explainability in terms of identifying the ecological factors driving habitat suitability. Ten replicate models were then produced for each species and 80% of the mosquito presence data was bootstrapped for these replicates to produce a range of suitability values. This allowed researchers to identify both areas of high and low certainty in the model outputs. Mean permutation importance and mean percent variable contribution were calculated for the environmental predictors to describe and quantify the relative importance of each variable in terms of defining habitat suitability. Mean habitat suitability was determined for each species using the replicate models, which were then extrapolated to all of North and South America. Model outputs were normalized to a 0-1 scale, with 0 representing areas of low suitability and 1 representing areas of high suitability.

The results indicated differential habitat ranges for each *Culex* species that were mainly driven by distinct preferences to temperature, humidity, land cover, and vegetation conditions (see Figure 6). For example, the spatial distributions of *Cx. pipiens* and *Cx. quinquefasciatus* were mostly dependent on the urban/built-up land cover variable, while cultivated and managed vegetation was more important for *Cx. salinarius*, *Cx. tarsalis*, and *Cx. erraticus* (see Table 1). Due to the dependence of the model on species occurrence data, projected distributions were reflective of the fact that all seven species have been observed in North America and only three have been documented for South America.

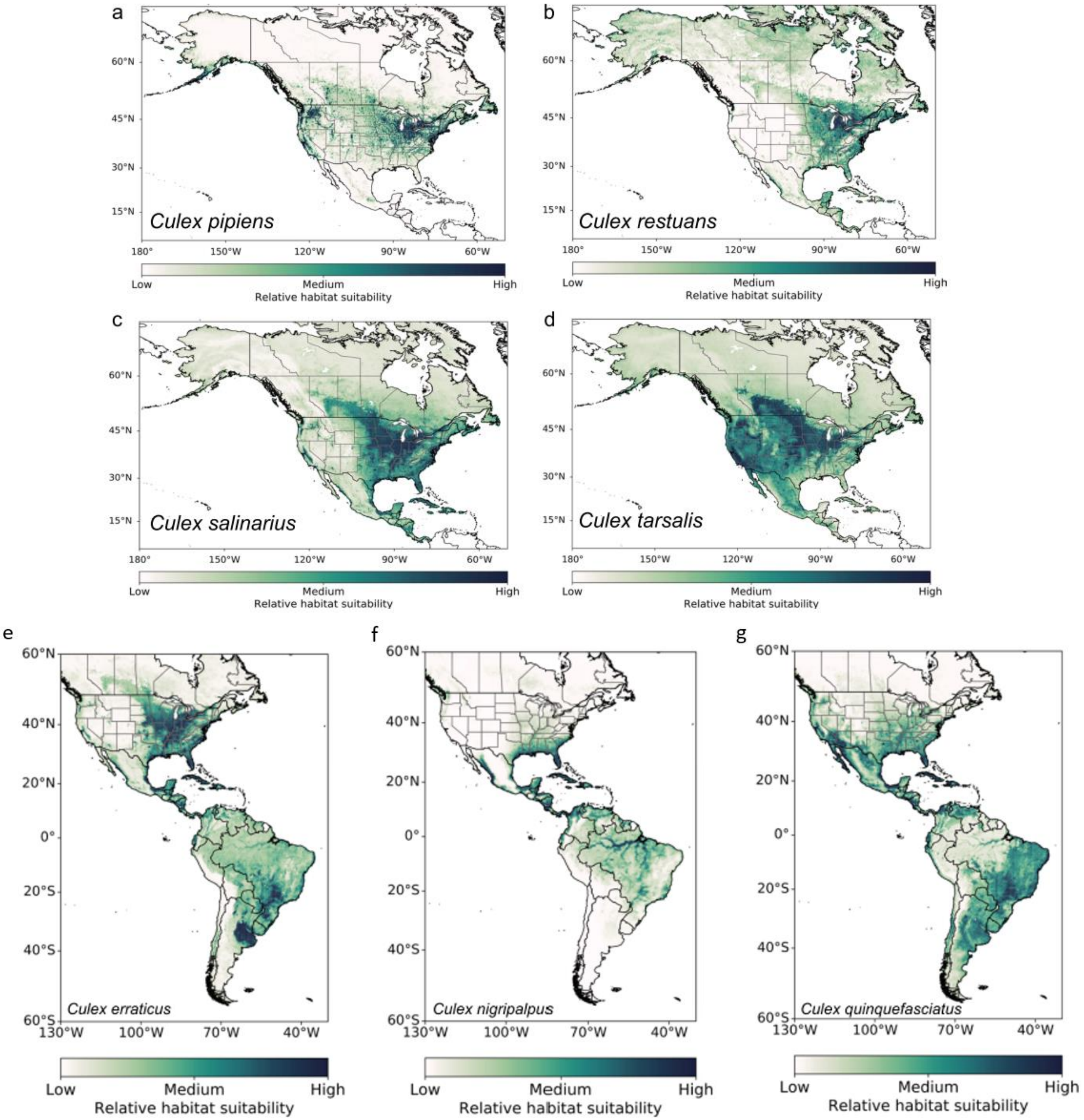
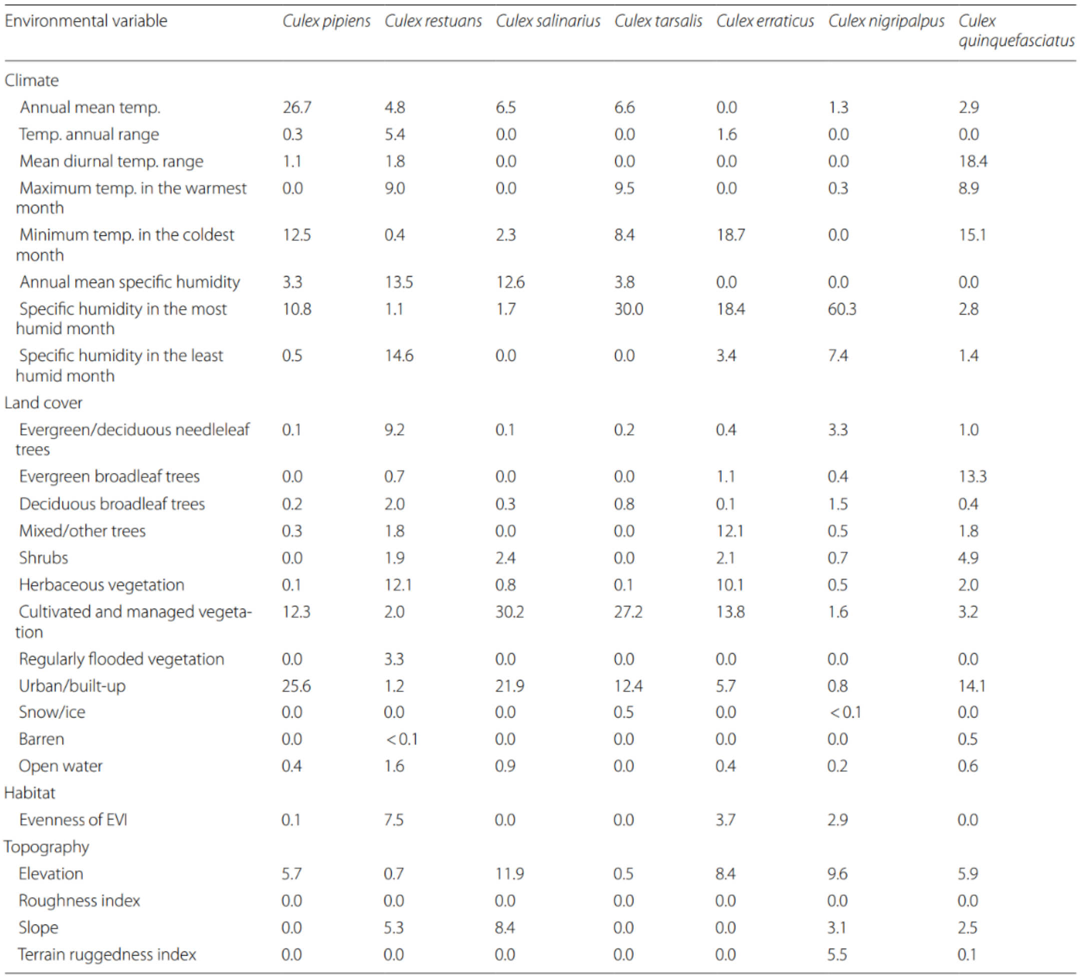


Figure 6 (modified from Gorris et al., 2021). Habitat suitability maps for seven predominant *Culex* species across the continental Americas as defined by Maxent. *Cx. pipiens*, *Cx. restuans*, *Cx. salinarius*, and *Cx. tarsalis* (a-d) have almost exclusively been documented in North America, while *Cx. erraticus*, *Cx. nigripalpus*, and *Cx. quinquefasciatus* (e-f) have ranges extending from North to South America.

Table 1 (from Gorris et al., 2021). Percent permutation importance for each of the 25 environmental variables entered into Maxtent to define habitat suitability ranges for seven *Culex* species in North and South America. Maxent determines percent permutation importance by randomly permuting the training data for a particular variable and calculating the change in model accuracy (i.e., AUC) that occurs as a result. This change is normalized to a percentage, the magnitude of which is indicative of the contribution of that variable to the performance of the model.



Environmental variable	*Culex pipiens*	*Culex restuans*	*Culex salinarius*	*Culex tarsalis*	*Culex erraticus*	*Culex nigripalpus*	*Culex quinquefasciatus*
Climate							
Annual mean temp.	26.7	4.8	6.5	6.6	0.0	1.3	2.9
Temp. annual range	0.3	5.4	0.0	0.0	1.6	0.0	0.0
Mean diurnal temp. range	1.1	1.8	0.0	0.0	0.0	0.0	18.4
Maximum temp. in the warmest month	0.0	9.0	0.0	9.5	0.0	0.3	8.9
Minimum temp. in the coldest month	12.5	0.4	2.3	8.4	18.7	0.0	15.1
Annual mean specific humidity	3.3	13.5	12.6	3.8	0.0	0.0	0.0
Specific humidity in the most humid month	10.8	1.1	1.7	30.0	18.4	60.3	2.8
Specific humidity in the least humid month	0.5	14.6	0.0	0.0	3.4	7.4	1.4
Land cover							
Evergreen/deciduous needleleaf trees	0.1	9.2	0.1	0.2	0.4	3.3	1.0
Evergreen broadleaf trees	0.0	0.7	0.0	0.0	1.1	0.4	13.3
Deciduous broadleaf trees	0.2	2.0	0.3	0.8	0.1	1.5	0.4
Mixed/other trees	0.3	1.8	0.0	0.0	12.1	0.5	1.8
Shrubs	0.0	1.9	2.4	0.0	2.1	0.7	4.9
Herbaceous vegetation	0.1	12.1	0.8	0.1	10.1	0.5	2.0
Cultivated and managed vegetation	12.3	2.0	30.2	27.2	13.8	1.6	3.2
Regularly flooded vegetation	0.0	3.3	0.0	0.0	0.0	0.0	0.0
Urban/built-up	25.6	1.2	21.9	12.4	5.7	0.8	14.1
Snow/ice	0.0	0.0	0.0	0.5	0.0	< 0.1	0.0
Barren	0.0	< 0.1	0.0	0.0	0.0	0.0	0.5
Open water	0.4	1.6	0.9	0.0	0.4	0.2	0.6
Habitat							
Evenness of EVI	0.1	7.5	0.0	0.0	3.7	2.9	0.0
Topography							
Elevation	5.7	0.7	11.9	0.5	8.4	9.6	5.9
Roughness index	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Slope	0.0	5.3	8.4	0.0	0.0	3.1	2.5
Terrain ruggedness index	0.0	0.0	0.0	0.0	0.0	5.5	0.1

One of the primary limitations of this study can be attributed to the coarse spatial resolution of the model projections. Because models were extrapolated to a continental scale to cover a greater distance, some of the nuanced heterogeneity in the geographic patterns of environmental variables or habitat suitability seen at finer scales is lost. Furthermore, projections are applied to regions that experience environmental conditions outside of the range of the background environmental training data, which inherently increases the uncertainty associated with interpreting results in areas such as the arctic or the desert. Also, the mosquito occurrence data used to inform the Maxent model are subject to their own sources of human error. For instance, only one of the three data sources utilized (i.e., VectorBase) identifies species based on genomics data. Mosquito species are often very morphologically similar, and many databases use highly generic terms and criteria for distinguishing one species from the next. This problem emphasizes the need for a centralized data repository for mosquito occurrence records based on genomic approaches for classification. Lastly, although all seven species belong to the same genus, they each occupy distinct ecological niches and would likely benefit from a more focused, individualized approach to distribution modeling rather than the broad overview provided here.

Despite these limitations and the contrasting lack of mechanistic parameters seen in the previous two studies, the results of this study are indicative of the application potential of a purely phenomenological approach. The effectiveness of this model can be attributed to the use of machine learning algorithms, an increasingly common method employed in species distribution modeling, in combination with environmental covariates selected through thorough background research. Simply consulting the literature to decide which factors were pertinent to the analysis allowed for Maxent to quickly process and analyze the patterns defining mosquito habitat in spite of the limited availability of reliable species survey data. The attention to detail in including multiple metrics pulled from a variety of data sources for the variables that were included in the model (i.e., including several different measures relating to humidity or mosquito data from five separate sources) meant that these factors were defined multidimensionally and likely compensated for other factors that were omitted. For instance, the lack of precipitation as a climate factor was not highly detrimental to model performance due to the inclusion of three different measures for atmospheric moisture as well as three separate land cover classes pertaining to aquatic habitats.

1.6 Summary: Model Recommendations

The respective strengths and weaknesses of the models from the previous section (see Table 2) highlight the broad range of options available in predictive spatial modeling. While model design and approach should ultimately be left to the discretion of researchers based on the questions they are attempting to answer, in terms of public health applications there are certain standards that will aid disease mitigation and vector control efforts. Focusing on model design, I believe an integrated or hybrid approach in which both mechanistic and phenomenological techniques are applied to a given dataset yields the best results. This is because mechanistic approaches lend models a defined, conceptual understanding of the phenomena being simulated that is rooted in biological theory, something that is lacking in phenomenological models. Conversely, the use of real-world data and the ability to link that data to a wide variety of ecological and anthropogenic covariates allows phenomenological models to capture aspects of disease dynamics that mechanistic models are blind to. Furthermore, machine learning algorithms offer a promising avenue to handle and interpret the multitude of datasets and interplay of factors relevant to transmission that would otherwise be too complex to define mathematically. Combining approaches would therefore be the logical choice for creating a more well-rounded model that covers most bases for drivers of disease transmission.

Table 2. Synthesis table for the three models discussed in the comparative analysis. Models range from a primarily mechanistic approach with limited calibration with environmental parameters (i.e., hybrid; Caldwell et al., 2021), to an integrated approach applying both mechanistic and phenomenological models to a dataset (Karypidou et al., 2020), to a strictly phenomenological approach (Gorris et al., 2021).

Study	Model Design		Climate Models / Factors	Additional Data	Strengths	Weaknesses
	Hybrid / Integrated					
	Mechanistic	Phenomenological				
Caldwell et al., 2021 Climate predicts geographic and temporal variation in mosquito-borne disease dynamics on two continents	SEI-SEIR epidemiological model incorporates mechanistic parameters (i.e., climate-dependent mosquito lifecycle traits) in correlation with environmental and anthropogenic factors (i.e., phenomenological) for a hybrid approach.		• 3 rainfall functions (Brière, inverse, and quadratic) • Mean temp. (˚C) • Mean relative humidity (%) • Mean annual rainfall (mm)	• Demographics (i.e., age, ancestry) • Housing quality (i.e., building materials) • Site characteristics (i.e., land cover, elevation) • Socioeconomics (i.e., GDP) • Arboviral & vector surveys	• Accurately predicts timing / duration of outbreaks • Multiple rainfall functions help capture nonlinear / time delayed effects	• Does not accurately predict magnitude of outbreaks • Generalizes trait characteristics for 3 distinct viruses
Karypidou et al., 2020 Projected shifts in the distribution of malaria vectors due to climate change	VECTRI model for *An. sacharovi* based on temp. and precipitation thresholds for vector biology and malaria transmission characteristics to predict vector density	Ensemble of 5 correlative niche models defines mathematical relationship b/w species occurrence records and environmental predictors to produce habitat suitability projection	• Ensemble of 4 regional climate models driven by 4 global climate models • 4 climate predictors (i.e., mean diurnal temperature range, temperature seasonality, mean temperature of warmest quarter, precipitation of warmest quarter)	• Topography (i.e., surface elevation, slope, aspect) • Distance to water-covered areas (derived from Coordination of Information on the Environment Land Cover database, 2012) • Malaria occurrence data provided by the National Public Health Organization (2009-2015)	• Integrated approach compensates for shortcomings associated w/ mechanistic & phenomenological models • Utilizes performance metrics (i.e., area under the curve & true skill statistic) • Higher spatial resolution than global / continental projections	• Lack of: humidity as a climate factor, socioeconomic factors, population age structure, urbanization trends, presence of vector control programs / measures, etc.
Gorris et al., 2021 Updated distribution maps of predominant Culex mosquitoes across the Americas	N/A	Machine learning ecological niche model (Maxent)	• Climate data from MERRAclim includes 5 measures for temperature and 3 measures for humidity	• Environmental data from EarthEnv • 12 land cover classes, 4 topographical measures • Mosquito occurrence data from 3 sources: VectorBase, VectorMap, National Ecological Observatory Network	• Most robust statistical approach of the 3 presented • Multiple metrics used to capture dynamic aspect of climate conditions • Permutation importance identifies which variables are most pertinent to model performance	• Course spatial resolution due to continental scale of projections • Inclusion of too many species undermines focus of study • Lacks anthropogenic factors and precipitation metric

Greater attention and resources must also be given to understanding the how critical mosquito lifecycle traits are impacted by climate factors outside of temperature. Although the dependence of mosquitos on humidity to avoid desiccation and precipitation to provide aquatic habitats for oviposition has been established, the more nuanced aspects of these relationships deserve further explorations. For instance, what is minimum threshold of humidity required for development and survival? Is there a maximum limit at which point humidity becomes detrimental and habitat suitability begins to decrease? Questions such as these will require extensive lab testing and field observation in order to approximate answers that will allow for more realistic parameterization of these factors. Merely incorporating metrics for climate that represent mean or min/max values is an oversimplification of how organisms experience climate conditions in nature. For this reason, future projections should look to implement dynamic models such as the rainfall model proposed by Caldwell et al. (2021) or multiple metrics capturing various aspects of each climate factor.

Other important covariates for mosquito-borne disease dynamics include factors relating to trade and travel (i.e., main driver for introduction of invasive species), socioeconomics (i.e., predictor of many aspects impacting human-mosquito contact rates such as housing infrastructure, vector control prevalence, population mobility, etc.), and population immune history (i.e., epidemiologically important to define populations at risk for “first exposure”; Caldwell et al., 2021; Messina et al., 2015; Ryan et al., 2019). While socioeconomic metrics such as GDP or median household income can readily be obtained for most areas, quantifying the amount of trade and travel or the baseline level of immunity for a particular area will likely require more effort and ingenuity on the part of the researcher. Efforts could entail obtaining local flight and shipping records and calculating annual rates associated with either, or combing through historical or hospital records documenting the occurrence of epidemics or case load for the disease of interest in the sample population.

Models should also aim to be as specific as possible in terms of predicting behavior for a particular mosquito vector because biological constraints differ from species to species and require distinct parameterization values. Parameters should be fine-tuned for the species in question rather than broadly applied to group of species, regardless of if they belong to the same genus. Additionally, models should be designed with a particular pathogen in mind and incorporate parameters based on that pathogen (i.e., extrinsic incubation period, replication rate, thermal optima, etc.) as well. Mechanistic models for mosquito-borne diseases often neglect the influence pathogens have in dictating transmission dynamics, instead focusing on the qualities of vertebrate and vector hosts.

Moving on from the theoretical aspects of model design and into the technical components, the highest spatial resolution available for datasets should be utilized to capture fine-scale variation at county and state levels. Enhanced resolution would better serve vector control organizations and hospitals alike as both rely on information at the local scale to make informed decisions based on where vector targets and vulnerable populations are situated, respectively. Messina et al. (2015) called for a minimum spatial resolution of 1 km due to the availability of population density and urban extent data at that resolution. However, advances in remote sensing and geospatial technology are likely to supersede this limit by capturing data at even finer scales.

For instance, the recently launched Landsat 9 satellite captures data at a resolution of 30 m and will be a useful tool for obtaining land cover data and tracking urbanization trends (NASA, 2021). Furthermore, advances in statistical methods and computational power have also improved our ability to estimate local population heterogeneity at higher spatial resolutions than before as well as to predict population sizes for unsurveyed locations (i.e., low-income areas with restricted access or limited capacity to conduct regular census surveys; Wardrop et al., 2017). In Nigeria for instance, small-scale microcensus data, high-resolution settlement maps, geolocated household surveys, and a multitude of predictors for human population density are used in combination as part of a cost-effective, bottom-up approach for spatially disaggregated population estimation to improve the efficacy of vaccine planning programs (Wardrop et al., 2017).

Regarding temporal scale, many climate projections cover a range of years (i.e., 2080-2099) or are given in intervals of 30 (i.e., IPCC) or more years. I believe predictions for transmission potential should be updated on an annual basis to simply cover the upcoming year. This approach would minimize the uncertainty associated with projecting too far into the future or extrapolating projections over a wide range of dates while still affording vector control programs and health officials ample time to plan accordingly. Even if model predictions are on the scale of several decades into the future, projections should concurrently be run for present day conditions and validated against mosquito abundance or disease occurrence data to ensure model integrity.

2. Evolutionary Adaptive Potential of Mosquito Vectors

2.1 Importance of Evolutionary Adaptation in Distribution Modeling

While considerable progress has been made in the methodology and computational power of mosquito-borne disease models, there are still critical gaps in our understanding of the mechanisms underlying vector disease dynamics. One fundamental principle dictating how species interact with their environment is evolutionary adaptation. Species distribution models commonly operate under the false assumption that species' biological response to environmental factors is fixed rather than adaptive (Couper et al., 2021). Basing predictions for future suitable habitat range on current biological constraints may underestimate actual distribution levels given the adaptive potential of the species in question (Couper et al., 2021). In the case of mosquitoes, r-selected organisms with high reproductive rates and short generation times, the likelihood of an evolutionary adaptive response to changing climate conditions is relatively high (Couper et al., 2021). This probability for adaptation is further heightened by the fact that rising temperatures associated with climate change are also expected to accelerate the mosquito lifecycle (Gorris et al., 2021).

For these reasons, conducting research toward quantifying evolutionary adaptive potential for mosquitos and incorporating this information into predictive models should be a high priority for improving model accuracy. According to Couper et al. (2021), understanding how population level genetic changes will emerge as a result of climate change depends on a multitude of factors. These include the climate factors directly influencing survival, the mosquito traits that are most sensitive to climate and relevant to fitness, the heritability of those traits, and the rate of evolutionary adaptation. In order to properly address the topic of mosquito evolutionary adaptative potential, an understanding of how mosquito biology relates to temperature must first be established.

2.2 Temperature and Mosquito Thermal Biology

The amount of research allocated towards studying mosquito response to temperature is disproportionate compared to other climate factors affecting species fitness (i.e., humidity and precipitation). However, given the direct influence temperature has on mosquito development, fecundity, and survival (due to the finite thermal ranges associated with each of these lifecycle processes) this may be the most important aspect limiting range expansion and population persistence in certain areas (Couper et al., 2021). While mosquito thermal breadth depends on the species ecological niche (i.e., temperate vs. tropical) and life history stage (i.e., juvenile vs. adult), lower critical limits are typically around 10-15 ˚C while the upper limit ranges between 32-38 ˚C (Butterworth et al., 2017; Couper et al., 2021). Accordingly, low winter temperatures are currently one of the primary factors preventing endemic transmission conditions in temperate areas such as the U.S., although future warming scenarios predict increasing habitat suitability during these colder months as well (Butterworth et al., 2017). However, in tropical regions where temperatures are already near the critical maximum for mosquitos, climate change is expected to surpass these upper limits and drastically reduce suitability for native populations (Couper et al., 2021; Ryan et al., 2019).

These estimates do not account for adaptive mechanisms that would enable mosquitos to withstand these temperature extremes, nor do they capture the range of temperature dynamics (e.g., diurnal variation, critical minimum or maximum, etc.) that impact the mosquito lifecycle because studies often rely on simply using annual or monthly mean values (Couper et al., 2021). In fact, a study looking at the influence of various climate factors (i.e., the 19 WorldClim Bioclimatic variables) on extinction rates for over 500 plant and animal species found that maximum annual temperature was the strongest driver of local extinctions (Román-Palacios *&* Wiens, 2020). This effect has been reflected in numerous studies showing high rates of mortality across mosquito species once temperatures surpass the thermal optima (i.e., temperature associated with peak trait performance) and approach the critical maximum (Couper et al., 2021).

In a study by Paaijmans et al. (2013), mosquito fitness was assessed under both constant and fluctuating temperature conditions to determine if diurnal variation played a significant role in thermal reaction norms. Fitness levels associated with ectotherm life-history traits as a function of temperature are visually represented as nonlinear asymmetrical curves that define the range between the critical minimum and maximum temperatures as well as the thermal optima. These curves are typically derived from experiments that model temperature under constant, lab-controlled conditions based on mean environmental temperatures that fail to reflect the dynamic conditions species face in nature and are therefore referred to as ‘fundamental’ performance curves. By incorporating diurnal variation into their analysis of *Anopheles stphensi,* Paaijmans et al. (2013) were able to construct ‘realized’ performance curves that were then compared to the fundamental curves to investigate the effects on the mosquito lifecycle (i.e., larval development, survival to adulthood, thermal optima, and critical temperature limits).

The results showed that while temperature fluctuation increased larval development and survival at low temperatures (i.e., 18 ˚C) compared to exposure at constant baseline conditions, trait performance was significantly diminished in response to fluctuation at higher temperatures (i.e., 32 ˚C, $p < 0.0001$), effectively lowering the critical maximum temperature. Likewise, fluctuation lowered the thermal optima, thereby decreasing the thermal safety margin (i.e., the temperature range between the temperature of the environment and the thermal optima) as well. The impact of temperature fluctuation was also found to be proportional to the degree of variability experienced (see Figure 7). The implications of these results are that short-term temperature variation impacts mosquito climate sensitivity and should therefore be factored into thermal performance studies to generate more realistic results. Additionally, this study demonstrates how different thermal responses can be obtained from organisms depending on the specific temperature aspect being modeled, which is why special consideration should be given towards determining which variables are most relevant to population persistence.

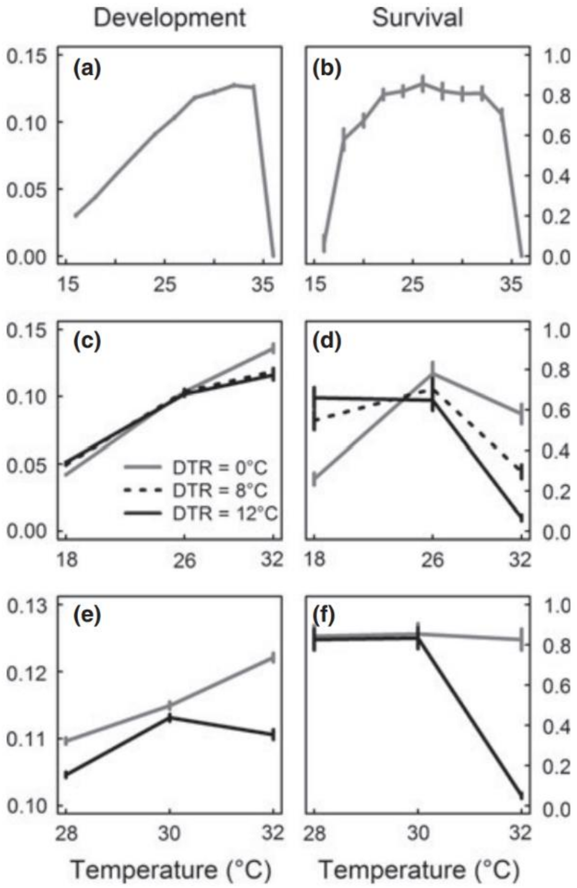


Figure 7 (from Paaijmans et al., 2013). Effects of constant and variable temperature conditions on mosquito daily development and juvenile survival rates. (a, b) Fundamental thermal performance curves under constant temperatures (i.e., 2˚C increments from 16 ˚C to 36 ˚C) for development and survival, respectively. (c, d) Mean development and survival rates at 18 ˚C, 26 ˚C, and 32 ˚C in response to diurnal temperature ranges (DTRs) of 0 ˚C (i.e., constant), 8 ˚C (e.g., 18 ˚C ± 4 ˚C), and 12 ˚C (e.g., 18 ˚C ± 6 ˚C). Variation significantly increased the rate of development at 18 ˚C (DTRs 8 ˚C and 12 ˚C, $P < 0.0001$) in proportion to degree of change. Variation significantly decreased the rate of development at 32 ˚C (DTRs 8 ˚C and 12 ˚C, $P < 0.0001$). (e, f) Mean development and survival rates at 28 ˚C, 30 ˚C, and 32 ˚C in response to DTRs of 0 ˚C (lighter color) and 12 ˚C (darker color). The DTR of 12 ˚C significantly reduced development and survival rates ($P = 0.014$ and $P < 0.0001$, respectively) at 32 ˚C.

2.3 Pathogen Thermal Dynamics

The ability to precisely measure how these various temperature metrics interact with disease dynamics is further obscured by the fact that temperature effects vary across biological scales (e.g., between vertebrate hosts, arthropod vectors, and pathogens) (Mordecai et al., 2019). While the literature on mosquito-borne diseases places greater emphasis on aspects of mosquito thermal biology, mosquitos are simply vehicles for the pathogens that are the actual cause of morbidity and mortality. Temperature-pathogen dynamics often show distinct response patterns compared with temperature-mosquito dynamics. For example, extrinsic incubation period (i.e., the time necessary for a virus in an infected mosquito to become transmittable to a host) decreases with warmer temperatures while adult mosquito mortality increases. While this disparity in thermal optima for vector competence (i.e., the ability of a vector to uptake and spread a given pathogen in a population) and pathogen development adds another layer of complexity for predicting climate impacts on disease distribution, pathogen thermal traits must also be considered when defining model parameters to ensure accuracy.

Although mosquitos are capable of transmitting parasitic infections, the pathogens with the highest evolutionary adaptive potential are single stranded RNA viruses (Bellone & Failloux, 2020). This chemical structure is common across the three main genera of arboviruses found in mosquitos (i.e., *Flavivirus, Alphavirus,* and *Phlebovirus*) and associated with high mutation rates (Bellone & Failloux, 2020). This can be attributed to the fact that RNA replication process lacks the accuracy and self-correcting mechanisms seen in DNA replication (i.e., higher polymerase error rate, lack of proofreading enzymes or mismatch repair; Bellone & Failloux, 2020). As a result, RNA viruses circulate between hosts and vectors as a dynamic mutant swarm that serves as a reservoir of genetic variability (Bellone & Failloux, 2020). This lends these viral populations a high degree of adaptability as variants are subjected to constant competition and selective pressures to produce high fitness strains that are more transmittable (Bellone & Failloux, 2020; Fay et al., 2021).

Nonetheless, non-vectored RNA viruses are still capable of mutating faster than arboviruses by a factor of 10 (Bellone & Failloux, 2020). One potential explanation for this disparity is the trade-off hypothesis, where viral fitness is constrained due to conflicting demands imposed by the need to alternate between species with vastly different physiological responses and thermal properties (Bellone & Failloux, 2020). Host-specific selective pressures have been shown to result in the emergence of more virulent strains (Bellone & Failloux, 2020). For example, a single amino acid change for a particular glycoprotein found in an African strain of Chikungunya resulted in atypical transmission (i.e., not through the primary vector, *Ae. aegypti*) by *Ae. albopictus* that caused 266,000 cases of infection during an epidemic from 2005-2006 (Bellone & Failloux, 2020).

Temperature has been shown to alter viral properties ranging from lipid profile molecular changes to protein and nucleic acid structure modifications that impact functional mechanisms such as replication (Bellone & Failloux, 2020). Despite some exceptions (i.e., West Equine Encephalitis Virus in *Culex tarsalis* has higher transmission rates at 18-25 ˚C than 32 ˚C), increasing temperature has generally shown to increase replication rates for viruses, as well as decrease the extrinsic incubation period in vector hosts. The higher the ratio of viral load to mosquito body weight and the shorter the extrinsic incubation period, the higher the vector competence of said mosquito (Bellone & Failloux, 2020).

A study by Fay et al. (2021) directly assessed the effects of temperature on the evolution of the most widely distributed flavivirus in the world today, West Nile Virus, using serial passage in *Culex tarsalis* cells. Passage was conducted at two temperature, 25 ˚C and 30 ˚C, and genetic changes during each successive pass was monitored using full-genome deep sequencing. Viral growth kinetic were determined using plaque titration 24 to 120 hours post infection at 24-hour intervals for each temperature. Viral load was measured by the number of viral genomes at each pass using real-time reverse transcriptase polymerase chain reaction.

The results (see Figure 8) showed that both growth kinetics (24-96 hours post infection) and viral output titers (passes 1-7, 9) were significantly higher ($p \leq 0.05$) at 30 ˚C than 25 ˚C. The accumulation of nonsynonymous substitutions (i.e., point mutations in nucleotide sequences that result in different amino acids being produced, altering protein structure) was also significantly higher for the 30 ˚C groups ($p \leq 0.05$) and is indicative of cell-specific adaptation in response to temperature induced selective pressure. This relatively high incidence of nonsynonymous substitutions corresponds with higher intrahost diversity of West Nile Virus strains compared with the 25 ˚C groups. In past studies, greater diversity in mutant swarms has been correlated with evolutionary trajectories leading to the development of high fitness strains through variant cooperation and co-infection. These findings highlight the role that rising temperatures associated with climate change could potentially play in shaping the evolution of arboviruses.

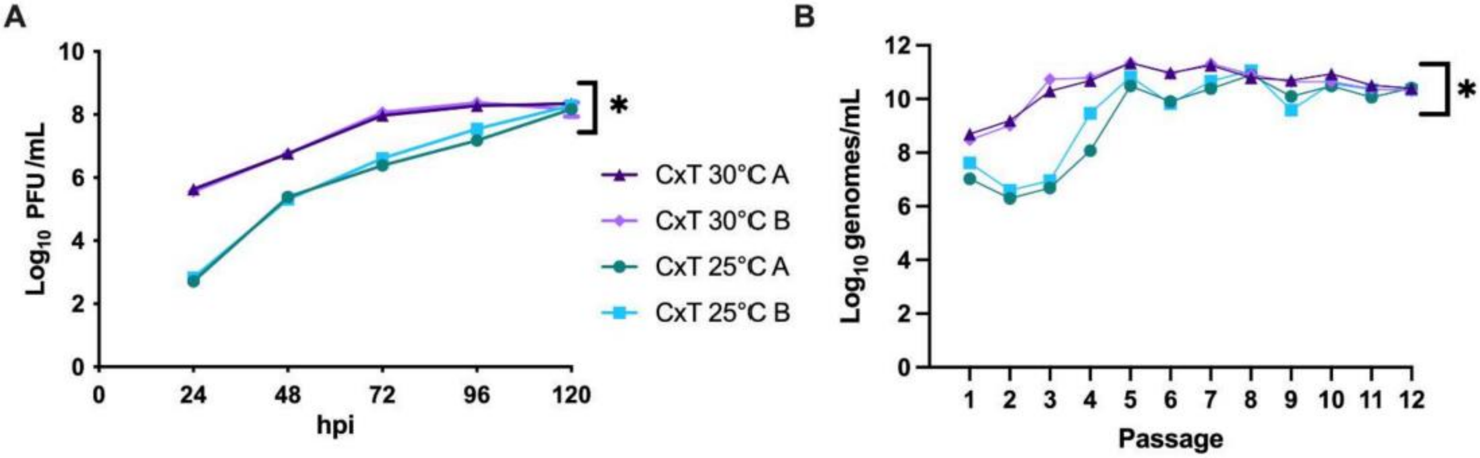


Figure 8 (from Fay et al. 2021). Cell serial passage results. A) Growth kinetics for two distinct lineages (A & B) of West Nile Virus in *Culex tarsalis* (C x T) cells exposed to two different temperature conditions (25 ˚C & 30 ˚C). Plaque titration was used to measure viral titers in terms of plaque-forming units (PFU) from 24 to 120 hours post infection (hpi) at 24-hour intervals. Viral titers were significantly higher for the 30 ˚C groups ($p \leq 0.05$) at 24-96 hpi, with all four groups converging with similar peak titer levels at 120 hpi. B) Viral load, measured by the number of West Nile Virus genomes using real-time reverse transcriptase polymerase chain reaction, at each successive pass for the four experimental groups. Viral load was significantly higher for the 30 ˚C groups ($p \leq 0.05$) during passes 1-7 and pass 9, before converging to similar levels at pass 12.

Temperature has also shown to influence mosquito microbiomes (i.e., the holobiont), which play a role in producing basal immune responses in the host species as a protective measure against foreign pathogens (Bellone & Failloux et al., 2020). Temperature can influence the microbial makeup and composition of the holobiont, therefore impacting infection resistance and consequently vector competency (Bellone & Failloux et al., 2020). For example, increasing temperature for *Culex pipiens* and *Culex restauns* mosquitos reduced levels of *Wolbachia* bacteria in the midgut, leading to enhanced susceptibility to West Nile Virus that subsequently caused higher infection rates in future generations due to vertical transmission (i.e., from adult females to offspring; Bellone & Failloux et al., 2020). Furthermore, correlations between immune system impairment and shifts in gene expression induced by thermal stress have been demonstrated in past studies, once again highlighting how temperature primes vector competency (Bellone & Failloux et al., 2020).

2.4 Phenotypic Plasticity

Besides genetic shifts associated with evolutionary change or shifting habitat range toward more tolerable climate zones, mosquitoes are capable of coping with thermal stress through phenotypic plasticity (Couper et al., 2021). Phenotypic plasticity encompasses behavioral, morphological, and physiological flexibility in response to climate change that is associated with a single genotype (West-Eberhard, 2008). This plasticity entails mechanisms for thermal acclimation, behavioral thermoregulation, and dormancy that have shown to be prevalent in other arthropods. Because phenotypic plasticity can be observed within a single generation, it offers a more rapid pathway to climate adaptation as opposed to natural selection.

Species thermal tolerance patterns coincide with climate patterns associated with latitudinal variation (Oliveira et al., 2021). Tropical zones are characterized by greater climate stability in comparison to subtropical and temperate zones, which is why tropical species generally have narrower thermal breadths as opposed to species at higher latitudes that are exposed to a wider range of conditions (Oliveira et al., 2021). However, the capacity for this phenomenon to manifest itself within a population at a single latitude over time has not been extensively explored (Oliveira et al., 2021). Accordingly, Oliveira et al. (2021) tracked seasonal related changes in thermal tolerance for a community of mosquitos in Florida to gauge acclimation plasticity over the course of the active season.

To define the timeframe for thermal tolerance acclimation, Oliveira et al. (2021) determined the mean environmental temperatures for each of the 30 days prior to mosquito capture and subsequent relocation to a lab. Then, using ordinary least-square models to determine which of those days had a temperature value that provided the best fit with the value for mosquito thermal tolerance, the amount of time necessary for thermal exposure to produce changes in mosquito tolerance was calculated. The study found that temperatures recorded 11 days before capture most accurately predicted the critical minimum temperature for mosquitos, while critical maximum temperature did not strongly correlate with a particular day. Therefore, an interval of 11 days following exposure was established for acclimation to occur.

The results of the study found seasonal shifts in both critical minimum and maximum temperatures that mostly coincided with patterns observed across latitudes, indicating parallel natural selection mechanisms acting across time and space. Critical minimum temperatures increased from spring to summer and then decreased from summer to fall. Critical maximum temperatures showed the opposite pattern by decreasing in the summer as opposed to the spring and fall. This effectively resulted in a decrease in thermal breadth during the summer compared to the rest of the year that is akin to how thermal tolerance decreases approaching the equator. According to the climate variability hypothesis, the narrowing of thermal breadth in tropical zones is because species in these regions experience comparatively stable and warm conditions. Conversely, the wider range of temperatures experienced by species in temperate zone results in broader thermal breadths. Therefore, the decrease in thermal tolerance during the summer is referred to as physiological tropicalization, while the increase in thermal tolerance during spring and fall is referred to as temperatization.

While the result for critical minimum temperature predictably tracked seasonal changes to increasing environmental temperatures, the countergradient pattern observed for critical maximum temperature was unexpected. Oliveira et al. (2021) believe that this effect (i.e., decrease in critical maximum temperature during the summer) is a result of stress from prolonged heat exposure that triggers behavioral thermoregulatory responses in mosquitos during the summer (i.e., seeking out cooler microhabitats as a buffer against high temperatures). By avoiding these conditions, the capacity for acclimation to high temperatures would presumably diminish. However, the effect of environmental temperatures on critical limits was more pronounced for critical minimum temperatures. Lower variability in upper thermal limits has been demonstrated for a variety of taxa, implying that there are greater developmental constraints on heat rather than cold acclimation.

In terms of assessing mosquito thermal physiology, this study highlights the importance of not only sampling across a species habitat range but also at different points in time during the active season. Measuring thermal tolerance based on samples collected during the summer would erroneously lead to underestimating thermal breadth and underfilling niche spaces for temperate areas that are still thermally suitable for survival. Additionally, this work elucidates the underlying role seasonal temperature variation plays in dictating vector competency for mosquitos, contributing to our ability to predict behavioral patterns and transmission risk. Combined with climate projections estimating increasingly longer and hotter summers, the results of this study support the hypothesis that mosquitos may also increasingly become more phenotypically tropicalized as a result of climate change.

As mentioned previously, thermal acclimation is but one of several phenotypically plastic responses mosquitos are capable of when facing adverse climate conditions. Another category falling under the broad umbrella of phenotypic plasticity is behavioral thermoregulation, which entails responses such as shifting microhabitat sites or activity patterns in favor of cooler areas and times, respectively. For example, when observing mosquito oviposition in tundra and taiga pools, Haufe and Burgess (1956) noticed that larvae would migrate down to greater depths whenever surface temperatures exceeded species thermal optima. Likewise, other studies have correlated increased mosquito presence in underground or shaded sites with rising temperatures, irrespective of the availability of unsheltered habitats (Couper et al., 2021). However, behavioral thermoregulation strategies centered around avoidance of temperature extremes can also result in fitness tradeoffs due to diminished opportunities for foraging or reproduction, thus constraining these behaviors (Couper et al., 2021). While few studies have addressed behavioral thermoregulation in the context of environmental temperatures, evidence of the functionality of such responses as a buffer against heat stress incurred during blood feeding has recently been discovered.

Hematophagy (i.e., blood feeding) can be a risky strategy for acquiring the energy for survival as it puts species in close contact with hosts and subsequent anti-parasitic defense measures (i.e., swatting or spraying) once detected (Benoit et al., 2019; Reinhold et al., 2021). However, blood has a much higher nutritional content (i.e., protein and lipid concentrations) in comparison to plant sources and therefore stimulates higher egg production in species that consume it (Benoit et al., 2019). In addition to the risk associated with host defenses, feeding on warm-blooded vertebrates has the potential to induce overheating as blood temperatures often range between 15-20˚C above mosquito core temperatures (Benoit et al., 2019; Reinhold et al., 2021). Therefore, understanding how mosquitos cope with this temperature disparity may also unlock a key piece to how behavioral thermoregulation will play a role in climate adaptation.

Reinhold et al. (2021) used a thermographic camera to monitor changes in body temperature in *Culex quinquefasciatus* in response to blood feeding. The study looked at temperatures at three different regions of interest across the mosquito anatomy (i.e., the head, thorax, and abdomen) and found that while body temperature closely tracked ambient temperature prior to feeding, temperatures at all three sites gradually increased as feeding began. Over the course of the meal, each region of interest began to develop distinct temperature profiles, a concept known as heterothermy. The mosquito body formed a temperature gradient with temperatures decreasing descending downward from the head to the abdomen, both of which differed significantly in temperature levels ($p < 0.002$).

Interestingly, the thermographic imaging managed to pick up the presence of a liquid droplet excreted from the tip of the abdomen during feeding in 15% of the sample population (see Figure 9). By retaining this droplet throughout the feeding process, mosquitoes were able to drop abdominal temperatures by 1.86 ± 0.2 ˚C. The formation of this droplet is a byproduct of a process called prediuresis, the aim of which is to rid the mosquito of excess water volume while simultaneously concentrating blood meal nutritional content (Lahondère & Lazzari, 2012). Here, we can see that prediuresis also serves an additional function by providing a mechanism for evaporative cooling during feeding (Benoit et al., 2019; Reinhold et al., 2021). In order to further study prediuretic response in the context of temperature as opposed to feeding, future research should monitor this behavior under varying temperature conditions. By doing so, scientists may uncover some of the molecular mechanisms underlying this process and therefore better understand if evaporative cooling will be a likely adaptation strategy to climate change.

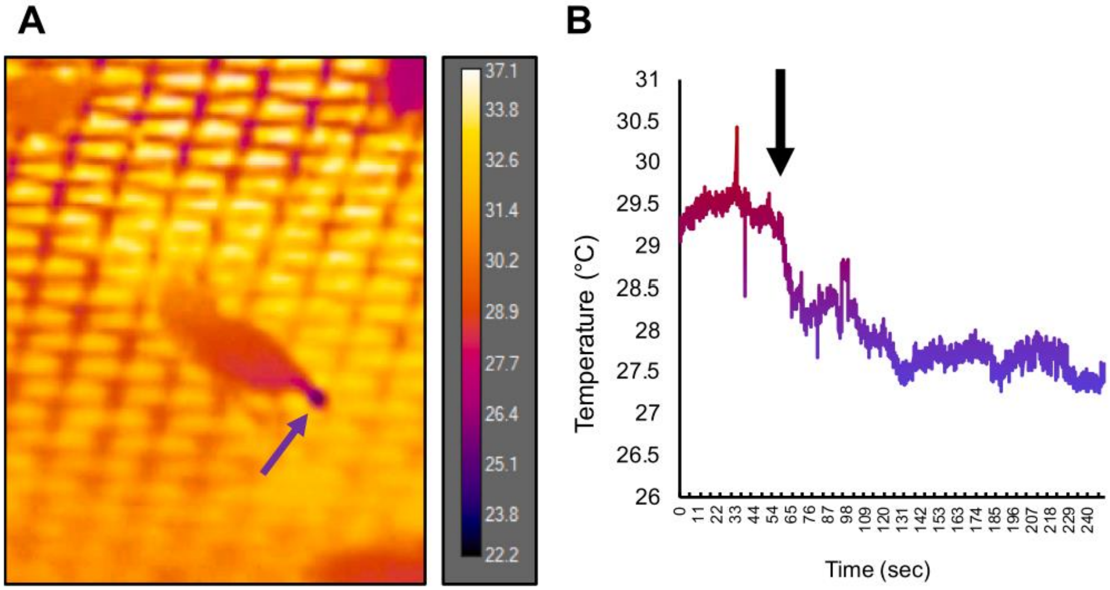


Figure 9 (from Reinhold et al., 2021). Mosquito prediuresis thermographics. A) Thermographic image of a female *Culex quinquefasciatus* mosquito during blood feeding. Arrow indicates the production of a prediuresis droplet consisting of fresh blood and urine used for thermoregulation. B) Fluctuation in *Cx. quinquefasciatus* abdominal temperature before and after droplet excretion. Point of excretion is indicated by the black arrow 60 seconds post-feeding and results in a decrease in temperature of 1.86 ± 0.2 ˚C.

2.5 Evolutionary Adaptation Studies

Evidence of evolutionary adaption in response to spatiotemporal climatic variation was demonstrated in a study from Urbanski et al. (2012). Through examining historical records of tire shipments and similarity in genetic markers between populations, the invasion of the species *Ae. albopictus* in the U.S. has been traced back to a shipment from Japan in 1985. By examining both the native and invasive populations of *Ae. albopictus* at various latitudes within their habitat ranges, the study was able to assess variation in photoperiodic traits in distinct populations across a latitudinal gradient.

After sampling mosquito populations in the field and maintaining them in the lab for several generations to control for environmental differences (i.e., common garden conditions), populations were divided into experimental groups and placed in different photoperiodic chambers with distinct patterns of light and dark exposure to measure trait values. The two photoperiodic traits assessed in this study include critical photoperiod (i.e., number of hours of sunlight needed to induce diapause in half of the population) and diapause incidence (i.e., the percentage of the population that undergoes diapause in response to unambiguous short-day length, which in this case consisted of 8 hours of sunlight). For both traits, the study was able to compare results using available data recorded from two distinct points in time (i.e., approximately 20 years apart for critical photoperiod and 10 years apart for diapause incidence), thus defining a time scale for evolutionary change.

The results of the study indicate that photoperiodism has evolved at a rapid pace over the study period in the U.S. populations. The slope for critical photoperiod a function of latitude for the U.S., while initially showing high residual variation, eventually converged with the slope associated with populations in Japan (see Figure 10). Diapause incidence also shifted spatially in the U.S., showing higher levels at northern latitudes that mirror patterns seen in Japan as well. The emergence of parallel patterns of clinal trait variation on two continents provides strong evidence for the role of climate-mediated selection in driving evolutionary adaptation. In addition, the high evolutionary rate observed for photoperiodism signifies its importance as a key adaptive response to climate factors. Future studies should be directed toward improving our understanding of the biological processes underlying photoperiodic regulation to better inform mechanistic models for mosquito-borne disease projections.

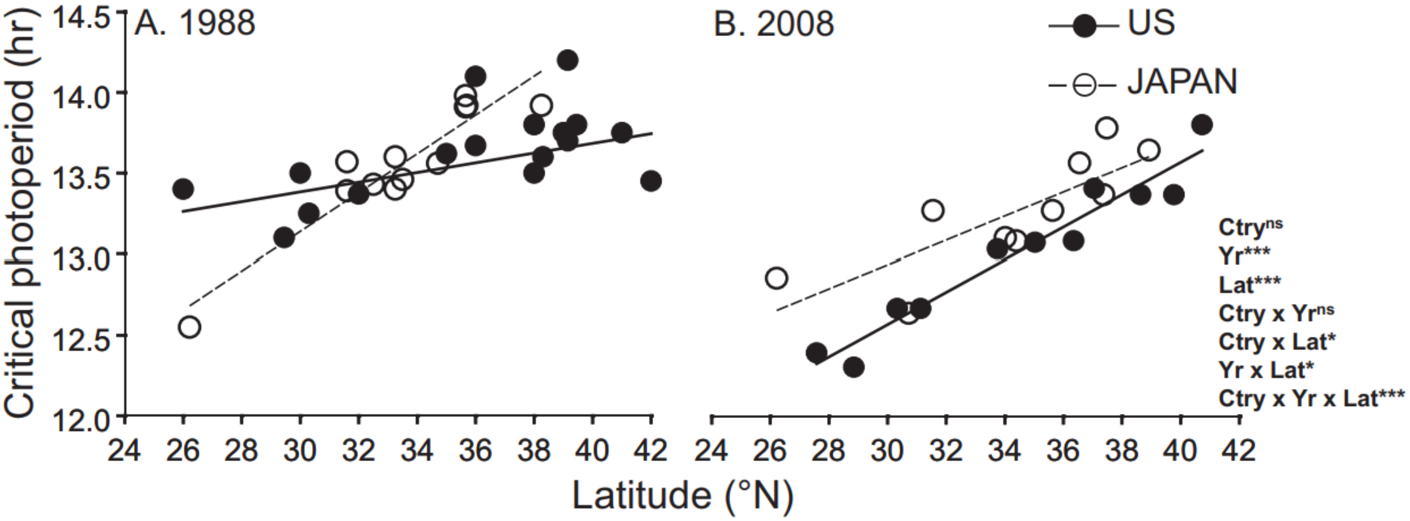


Figure 10 (from Urbanski et al., 2012). Critical photoperiod in native and invasive *Aedes albopictus* populations. Comparison of a photoperiodic trait, critical photoperiod (i.e., number of hours of sunlight needed to induce diapause response in 50% of the population), between invasive U.S. populations and native Japanese populations across a latitudinal gradient in A) 1988 and B) 2008. Critical photoperiod showed high residual variation (i.e., variability not attributed to differences in latitude) in the U.S. populations in 1988. In 2008, the slope for critical photoperiod of the U.S. populations approximated the slope of the Japanese populations. This data documents one of the most rapid rates of synchronic evolutionary divergence observed in the wild (≈ 5,158 Darwins).

This line of research was further pursued by exploring regional divergences in a suite of diapause and thermotolerance traits in U.S. *Ae. albopictus* populations (Batz et al., 2020). The study assessed differences in simulated winter cold tolerance, rapid cold shock tolerance, diapause duration, and post-diapause larval starvation tolerance in juvenile mosquitos as well as adult chill coma recovery time between populations collected from six northern sites (i.e., mean latitude: 39.8 ˚C) versus six southern sites (i.e., mean latitude: 34.0 ˚C) in the eastern U.S. A minimum of 40 male and 40 female mosquitos were assigned to each population and subsequently reared to the F_4 generation to control for confounding field effects, synchronize population within the lab, and produce sufficient quantities of egg stocks to complete the experiment. Three biological replicates were established for each population and F_5 egg samples collected from each replicate were used for the measurement of diapause traits. Molecular gene sequencing and investigation into non-diapause traits were incorporated into the study design as a way to determine whether or not differences in diapause traits are a result of natural selection processes or independent invasion events and population-level structural differences.

The results of the study (see Figure 11) showed that populations from northern regions had significantly greater survival after 185-day winter simulations (i.e., mirroring northern winter length conditions; $p = 0.023$), greater survival following rapid cold shock (i.e., 24-hour egg exposure at -9 ˚C; $p = 0.008$), longer time for diapause termination (i.e., 118.7 days vs. 111.5 days; $p < 0.001$), and greater starvation tolerance (i.e., number of days prior to mortality post emergence; $p = 0.019$) in comparison to southern populations. Additionally, adult chill coma recovery time was significantly shorter for norther populations as well (i.e., 19.4 min vs. 23 min; $p < 0.001$).

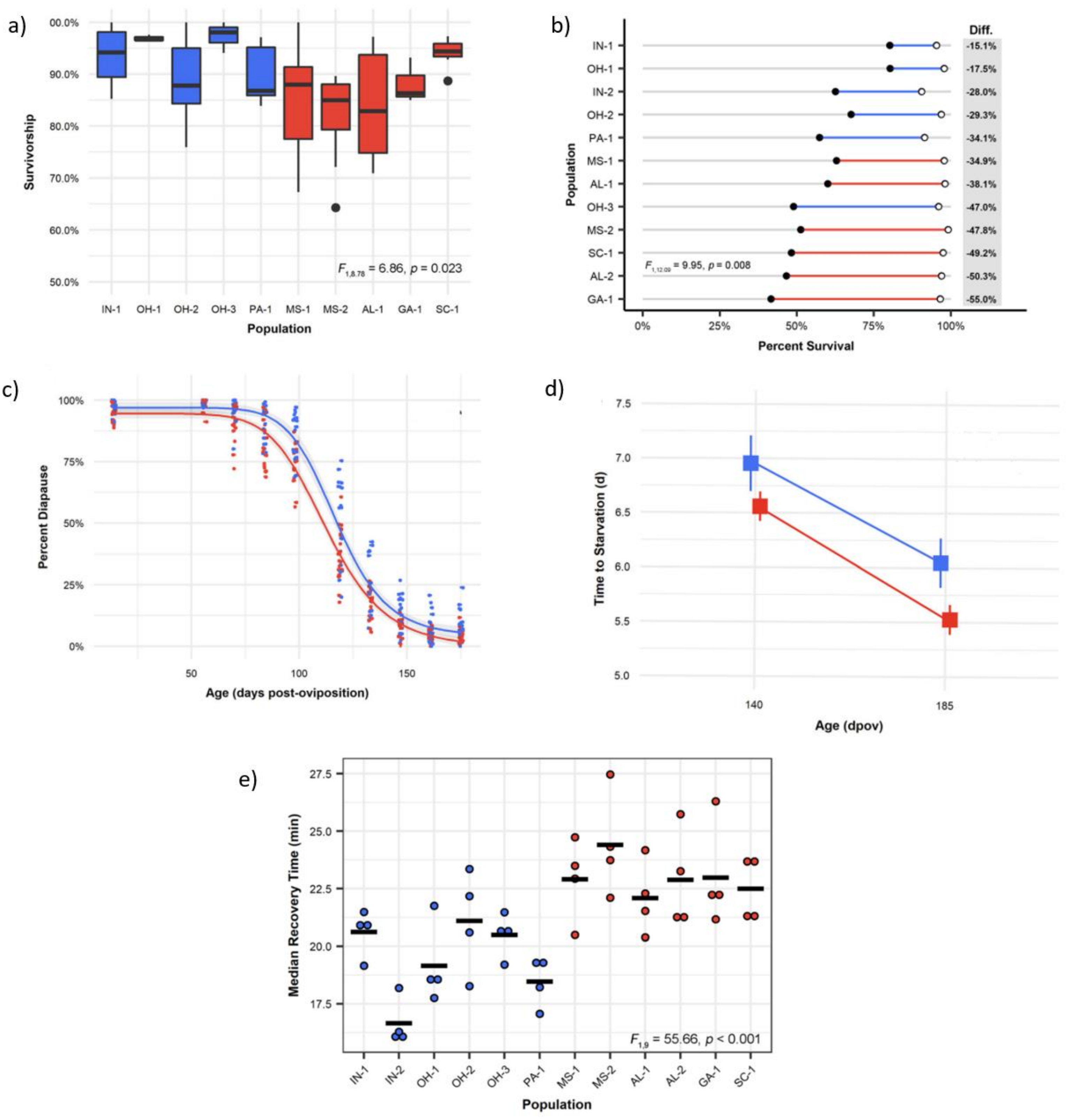

a)
00.0%
90.0%
80.0%
70.0%
60.0%
50.0%
Survivorship
IN-1
OH-1
OH-2
OH-3
PA-1
MS-1
MS-2
AL-1
GA-1
SC-1
Population
$F_{1,8.78}$ = 6.86, p = 0.023
b)
Diff.
IN-1 -15.1%
OH-1 -17.5%
IN-2 -28.0%
OH-2 -29.3%
PA-1 -34.1%
MS-1 -34.9%
AL-1 -38.1%
OH-3 -47.0%
MS-2 -47.8%
SC-1 -49.2%
AL-2 -50.3%
GA-1 -55.0%
Population
$F_{1,12.09}$ = 9.95, p = 0.008
0%
25%
50%
75%
100%
Percent Survival
c)
100%
75%
50%
25%
0%
Percent Diapause
50
100
150
Age (days post-oviposition)
d)
7.5
7.0
6.5
6.0
5.5
5.0
Time to Starvation (d)
140
185
Age (dpov)
e)
27.5
25.0
22.5
20.0
17.5
Median Recovery Time (min)
IN-1
IN-2
OH-1
OH-2
OH-3
PA-1
MS-1
MS-2
AL-1
AL-2
GA-1
SC-1
Population
$F_{1,9}$ = 55.66, p < 0.001

Figure 11 (modified from Batz et al., 2020). Comparison of thermotolerance across both diapause (a-d) and nondiapause traits (e) for six northern (blue) and six southern (red) populations of *Aedes albopictus* in the U.S. a) Survival rates were significantly higher following simulated northern winter conditions (i.e., 185 days) in northern (92.5%) than southern (86.2%) populations. b) Survivorship in diapause eggs following rapid cold shock (i.e., -9 ˚C for 24 hours) was significantly higher in northern (28.5% reduction in survival) than southern (45.9% reduction in survival) populations. Closed points represent treatment groups while open points are control groups (i.e., egg samples from the same biological replicate held at a constant 21 ˚C). Percentages indicate the difference in survival between the treatment and control. c) Time to reach 50% diapause was significantly higher in northern (118.7 days post oviposition) than southern (111.5 days post oviposition) populations. Lines represent dose response curves for each region while points represent the percentage of eggs that fail to hatch. d) Postdiapause starvation tolerance (i.e., number of days before mortality) decreases in older eggs but was significantly higher in northern than southern populations, regardless of age. e) Median adult chill coma recovery time (i.e., amount of time for individual to resume an upright position after being placed in an inverted position on ice for 2.5 hours) was significantly lower in northern (19.4 min) than southern (23.0 min) populations.

These results are reflective of the fact that northern sites experience longer winters, lower minimum temperatures, and later false springs (i.e., days with climate conditions that stimulate larvae emergence directly followed by lethal subzero days) than in the south. The results of the genetic analysis showed negligible regional differences across neutral molecular markers and non-diapause traits but consistent differences for diapause traits, providing further evidence of the strength of selection of temperature and the role of thermal tolerance as driving factors for evolutionary adaptation.

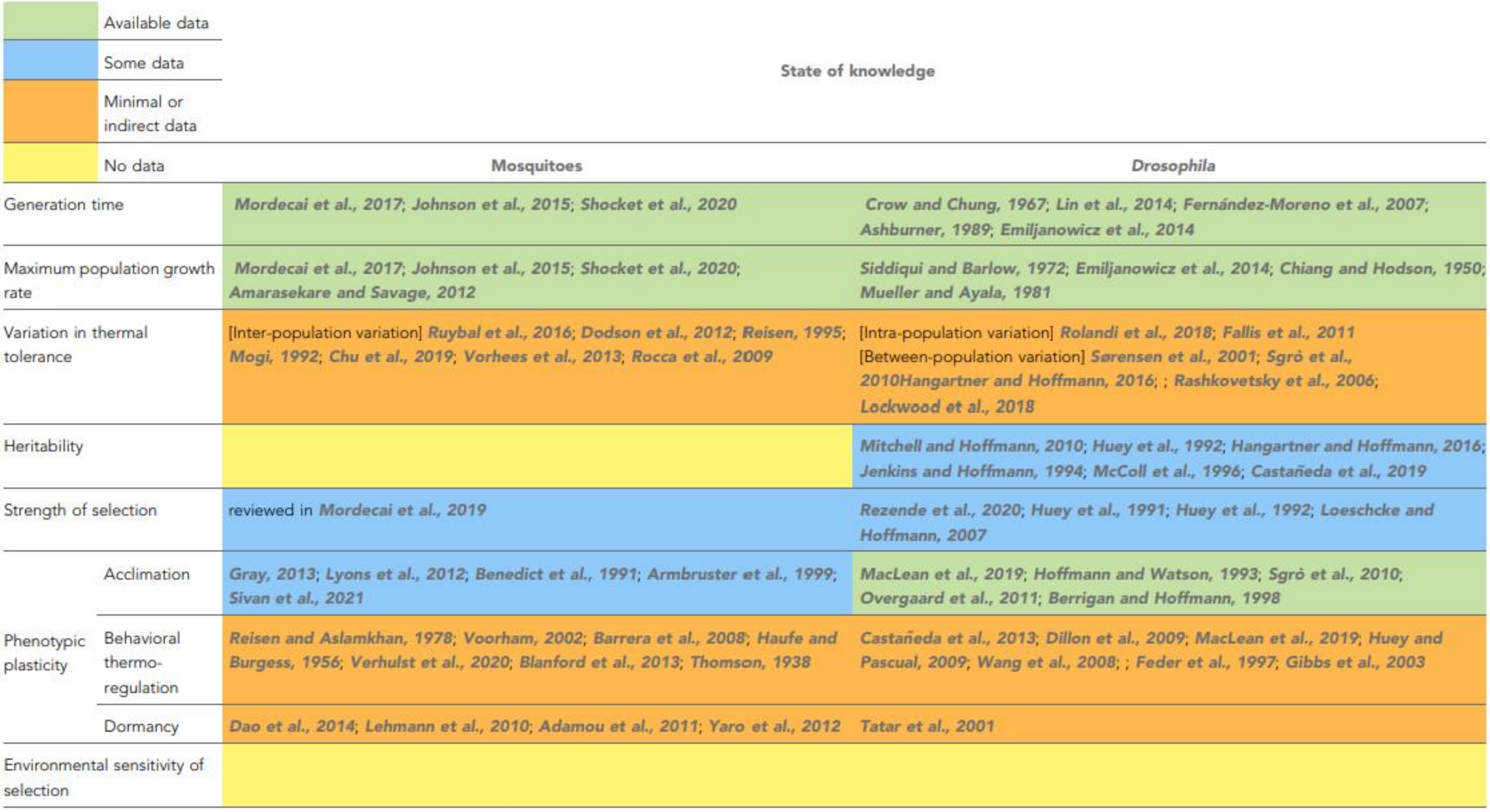
In presenting their findings, Batz et al. (2020) provide evidence for recent (≈35 years) geographic divergence across multiple traits within the "diapause program", defined here as a developmental pathway with certain molecular, physiological, and fitness-related outcomes that modify the mosquito lifecycle post-dormancy (i.e., into adulthood). Although literature on mosquito physiology acknowledges the dynamic mechanisms and biological processes occurring during diapause, ecological literature is largely behind on this concept and often describe diapause as a static state of 'developmental arrest' (Batz et al., 2020). Here, we see that selection for cold acclimation plasticity during diapause will play a pivotal role in defining mosquito overwinter survival rates, especially considering that mosquitos are immobile during this time and thus unable to engage in other forms of behavioral avoidance or thermoregulation to adapt (Batz et al., 2020).

While the previous two studies offer examples of evolutionary adaptation along the diapause program in response to changing levels of daylight or critical minimum temperatures associated with winter, research on evolving higher upper thermal limits in response to critical maximum temperatures has been more limited. The data for a variety of ectothermic species suggests that upper thermal limits for life history traits were less sensitive to temperature-induced selective pressure than lower limits (Couper et al., 2021). This implies that there are higher evolutionary constraints on upper limits that may stem from factors such as the increased metabolic cost of developing heat as opposed to cold tolerance (Couper et al., 2021). Obtaining empirical measurements for selection on genes associated with heat stress as well as trait sensitivity for upper thermal tolerance limits (i.e., the degree to which the trait shifts in response to the environment) should be top priorities for closing some of the gaps in our ability to forecast mosquito behavior and distribution in tropical areas (Couper et al., 2021).

2.6 Recommendations for Future Studies

The complex array of selective pressures and interactive effects that underly evolutionary adaptation make it impossible for any one study to cover them all. However, identifying the areas critical to enabling an evolutionary response for which data is currently limited or indirectly leveraged from related species, such as *Drosophilia*, is an important first step in directing research measures (see Table 3). Two areas that fit these criteria are heritability for thermal tolerance traits (i.e., how much phenotypic variance in a population can be attributed to genes from the previous generation) and thermal sensitivity of selection for fitness relevant traits (i.e., how the optimal phenotype varies in response to changing temperature conditions; Couper et al., 2021).

Table 3 (from Couper et al., 2021). State of knowledge for evolutionary adaptation parameters for mosquitos and a closely related genus, *Drosophilia*. References are for studies containing information relevant to the parameter they are listed under. Each parameter is color coordinated based on the level of information available: green indicates that data is readily available, blue indicates that some data is available but further research and collection is needed, orange indicates minimal or indirect (i.e., leveraged from closely related species or other adaptation mechanisms that are potentially related to thermal biology) data availability, and yellow indicates that no data is available (i.e., estimates for these parameters are unavailable at this time). Heritability and environmental sensitivity of selection represent the two areas most in need of research directives.



🟩	Available data
🟦	Some data
🟧	Minimal or indirect data
🟨	No data

		State of knowledge	
		Mosquitoes	*Drosophila*
Generation time		🟩 Mordecai et al., 2017; Johnson et al., 2015; Shocket et al., 2020	🟩 Crow and Chung, 1967; Lin et al., 2014; Fernández-Moreno et al., 2007; Ashburner, 1989; Emiljanowicz et al., 2014
Maximum population growth rate		🟩 Mordecai et al., 2017; Johnson et al., 2015; Shocket et al., 2020; Amarasekare and Savage, 2012	🟩 Siddiqui and Barlow, 1972; Emiljanowicz et al., 2014; Chiang and Hodson, 1950; Mueller and Ayala, 1981
Variation in thermal tolerance		🟧 [Inter-population variation] Ruybal et al., 2016; Dodson et al., 2012; Reisen, 1995; Mogi, 1992; Chu et al., 2019; Vorhees et al., 2013; Rocca et al., 2009	🟧 [Intra-population variation] Rolandi et al., 2018; Fallis et al., 2011 [Between-population variation] Sørensen et al., 2001; Sgrò et al., 2010Hangartner and Hoffmann, 2016; ; Rashkovetsky et al., 2006; Lockwood et al., 2018
Heritability		🟨	🟦 Mitchell and Hoffmann, 2010; Huey et al., 1992; Hangartner and Hoffmann, 2016; Jenkins and Hoffmann, 1994; McColl et al., 1996; Castañeda et al., 2019
Strength of selection		🟦 reviewed in Mordecai et al., 2019	🟦 Rezende et al., 2020; Huey et al., 1991; Huey et al., 1992; Loeschcke and Hoffmann, 2007
Phenotypic plasticity	Acclimation	🟦 Gray, 2013; Lyons et al., 2012; Benedict et al., 1991; Armbruster et al., 1999; Sivan et al., 2021	🟩 MacLean et al., 2019; Hoffmann and Watson, 1993; Sgrò et al., 2010; Overgaard et al., 2011; Berrigan and Hoffmann, 1998
	Behavioral thermo-regulation	🟧 Reisen and Aslamkhan, 1978; Voorham, 2002; Barrera et al., 2008; Haufe and Burgess, 1956; Verhulst et al., 2020; Blanford et al., 2013; Thomson, 1938	🟧 Castañeda et al., 2013; Dillon et al., 2009; MacLean et al., 2019; Huey and Pascual, 2009; Wang et al., 2008; ; Feder et al., 1997; Gibbs et al., 2003
	Dormancy	🟧 Dao et al., 2014; Lehmann et al., 2010; Adamou et al., 2011; Yaro et al., 2012	🟧 Tatar et al., 2001
Environmental sensitivity of selection		🟨	🟨

One feasible approach to studying these processes is through selection experiments (Couper et al., 2021). There are two general types of selection experiments (Fuller et al., 2005). The first is laboratory natural selection, in which the sample population is divided into treatment groups and subjected to varying levels of selective pressure associated with a particular environmental condition (i.e., temperature). By tracking changes in the response patterns for the trait of interest (i.e., thermal optima for adult survival) across successive generations, researchers can observe the strength of selection produced by various temperature conditions and the heritability of trait thermal properties first-hand (Couper et al., 2021; Fuller et al., 2005).

The second type of selection experiment is known as artificial selection, where the researcher chooses which individuals pass on to the next generation based on the desired criteria (i.e., mosquitos that pass a certain performance metric or trait measurement threshold). Here, selection pressure is enforced by the researcher rather than the treatment condition itself, allowing for greater control of the selection regime (e.g., strength, time scale, and consistency of selection).

Another promising approach to measuring the evolutionary parameters discussed (i.e., heritability and environmental sensitivity of selection) is through common garden experiments (e.g., Urbanski study on photoperiodism). These experiments expose distinct populations (i.e., sampled across a temperature gradient) to identical environmental conditions in a controlled setting to obtain trait measurements that can be attributed to inter-population genetic variation. Individuals are typically reared for one or more generations within the lab to control for confounding effects from the original environment (e.g., thermal acclimation to ambient habitat temperature or behavioral shifts due to vector control activities) and isolate genetic influences on trait performance. Likewise, this approach can be applied to a single population, measuring trait values at the individual level to ascertain intra-population genetic variation. Common garden experiments also present the opportunity to measure thermal sensitivity of selection if trait and fitness levels are measured concurrently. Furthermore, tracking thermal traits in both parents and offspring (i.e., housing mating pairs separately) allows for measurement of trait heritability based on the slope of parental and offspring trait values (see Figure 12).

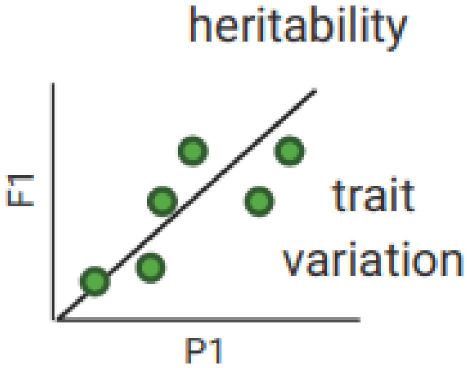


Figure 12 (from Couper et al., 2021). Conceptual graph for measuring heritability between successive generations. Slope of parental (P1) and offspring (F1) trait values provides a measure for heritability. High linear correlation between values corresponds to increased heritability in the trait of interest.

Both selection and common garden experiments can be combined with genomic approaches to observe and measure cross-generational genetic shifts in real time (e.g., the emergence of genetic mutations with climate-adaptive functionality or evolutionary rates). These approaches could include measures such as whole-genome sequencing or genome scans to identify potential gene candidates integral to mediating evolutionary adaptation. Although genomic techniques would be most feasible for species that have been studied more extensively and therefore have more readily available genetic data for reference (e.g., *Ae. albopictus*, *Ae. albopictus*, *An. stephensi*), this should not deter researchers from directing attention towards understudied mosquito vectors. On the contrary, improving representation of those species in genetic sequence databases should be a top priority for research going forward.

Regardless of what experimental type or methodology is employed, studying evolutionary processes presents a variety of challenges that must be addressed. First, designing temperature conditions that are relevant to future climate regimes experienced in nature will require insight into historical climate patterns and forethought in selecting future projections that are consistent with current trends. Temperature effects can vary depending on the time scale under which they are assessed (e.g., days, seasons, years) due to time lags between temperature conditions and mosquito or pathogen development (Mordecai et al., 2019). Therefore, longitudinal studies examining populations over the course of years or decades should supplement more short-term studies to better inform our understanding of the nonlinear effects of temperature on mosquito-borne disease dynamics. Since maintaining mosquito populations in a lab setting for such a prolonged period is not a viable option, tracking a single population in the wild and obtaining sample measurements at different points in time, similar to the aforementioned evolutionary studies on *Ae. albopictus*, represents the most practical option. This would entail collecting data on the target species as early as possible to establish a starting point for the evolutionary timeline of the study. Mixing up study designs to include both controlled laboratory and dynamic outdoor settings also serves the added purpose of capturing temperature effects in different contexts (i.e., isolated vs. interactively with other ecological factors).

Second, identifying the life-history traits that will serve as the focal point for the study is not an arbitrary choice but should be based on which traits are most pertinent to mosquito fitness as well as most strongly affected by thermal selection. Selection experiments on different fitness-relevant traits (e.g., adult survival, juvenile development, etc.) could be conducted to compare mortality rates across cohorts as a proxy for the fitness constraint posed by each trait. Furthermore, a potential starting point for identifying the most temperature sensitive traits would be through identifying which life-history trait has the lowest recorded critical thermal maxima based on previous research on mosquito thermal biology. For many mosquito species, these traits are adult lifespan and fecundity, indicating that research on thermal selection should be centered on the adult stage of the mosquito lifecycle.

Couper et al. (2021) drew from evolutionary rescue models typically used in conservation biology to develop an empirical framework for estimating population adaptive potential in response to climate change (see Table 4). Once baseline information for all the listed parameters is obtained through experimental research and observation, the model will be capable of predicting whether or not a mosquito population is capable of the required rate of evolution to persist in the face of sustained, directional environmental change. While this approach offers a relatively simplistic representation of evolutionary dynamics, more computationally intensive simulation tools are becoming increasingly widespread due to their ability to incorporate aspects such as demographic and environmental stochasticity, gene flow, dispersal, and carrying capacity.

Table 4 (modified from Couper et al., 2021). Evolutionary rescue model formula with a list of defined parameters. Evolutionary rescue models, typically used in conservation biology, utilize information on population genetic characteristics to calculate if the maximum sustainable rate of evolutionary change for that population exceeds the projected rate of change in the environment. Even with limited information or imprecise parameterization, these models can help place finite limits on species' capacity for climate adaptation and identify areas where further data collection is warranted.

Formula	Symbol	Parameter	Definition
$\eta_c = \sqrt{\frac{2r_{max}\gamma}{T}}\frac{h^2\sigma^2}{\lvert B-b\rvert}$	η_c	Maximum rate of environmental change	The highest sustainable rate of change in the environment that would still enable long-term population persistence
	r_{max}	Maximum population growth rate	Intrinsic rate of increase under ideal/optimal conditions
	γ	Strength of selection	Impact on fitness when trait values deviate from the optimum value associated with a given environmental condition
	T	Population generation time	Mean time between reproductive cycles in successive cohorts (for discrete, non-overlapping generations)
	h^2	Heritability	Proportion of phenotypic variance that is due to the cumulative genetic influence of previous generations
	σ^2	Phenotypic variance	The amount of variance in the trait of interest
	B	Environmental sensitivity of selection	The degree to which the optimum phenotype varies in response to changes in the environment
	b	Phenotypic plasticity	The capacity for a single genotype to produce alternative phenotypes under varying environmental conditions

For example, SLiM 2 is a software package for forward genetic simulation that offers researchers a high degree of flexibility in customizing the evolutionary scenario they wish to model (Haller & Messer, 2016). In a study aiming to identify the factors dictating coral metapopulation persistence in the face of global warming, the SLiM framework was successfully used to identify gene flow from migrating populations from warmer regions (i.e., coral with a higher proportion of heat-tolerance alleles) as the primary driver enabling long-term resilience and survival (Matz et al., 2020). This approach could similarly be applied to mosquito populations to identify the factors that would allow for persistence in tropical areas once temperatures exceed the upper thermal limits of species that currently reside there. As a greater number of genetic simulation packages become available to researchers, they represent a promising new avenue for capturing previously elusive aspects of evolutionary dynamics that will supplement findings from analytical work (Couper et al., 2021).

3. Vector Control in the U.S. and Emphasis for Urban Health Planning

3.1 Overview of Mosquito-Borne Disease Risk in Urban Environments

The primary application of research for improving mosquito-borne disease models, including gaining insight into the less well-understood aspects of disease dynamics such as evolutionary adaptation, is towards aiding public health officials, vector control organizations, and communities in anticipating and mitigating the risks associated with these diseases. While the bulk of this paper addresses mosquito-borne disease in the context of climate change, due to the relative ease of manipulating climate factors in a lab setting, the influence of non-climate related factors cannot be understated. In fact, climate-related factors were only reported to make up 10% of the driving influence for disease in comparison with the 26% attributed to land use change (Chala & Hamde, 2021). Specifically, rapid urbanization has shifted the vector-pathogen-host interface in a way that promotes increased transmission potential. This is because urban environments are associated with higher population densities, a wider range of potential oviposition sites (e.g., artificial containers that collect rainwater), and greater warming from the urban heat island (UHI) effect than surrounding rural areas (Chala & Hamde, 2021; Ligsay et al., 2021). The summation of these factors suggests that transmission risk associated with urban areas is disproportionately high.

For major cities in particular, high rates of international trade and travel could result in the inadvertent importation of an invasive vector species, making these areas focal points for future vector control and disease mitigation strategies. Despite acknowledgement by the World Health Organization (WHO) and subsequent efforts to make progress in this area, the management of urban mosquito-borne diseases has not yet been sufficiently addressed by the WHO Urban Health Initiative. As a result, strategies such as the use of green spaces to curb the urban heat island effect and associated health risks could also unwittingly provide suitable habitats for mosquitoes and thus increase transmission potential for mosquito-borne diseases (Ligsay et al., 2021). Coupled with the increasing trends of climatic suitability projected for temperate regions such as the U.S. and Europe, corresponding increases in transmission potential are likely across the urban centers of these countries. This section focuses on vector control within the U.S., synthesizing the literature on native mosquito vectors and mosquito-borne diseases in an urban context. The status of domestic mosquito control programs will also be assessed, using the state of Florida (where the burden of mosquito-borne diseases is high) as a case study. Based on this assessment, recommendations towards improving implementation measures will be provided.

3.2 Urban Mosquito Studies

Urban population growth presents a sizable and vulnerable target for mosquitos seeking viable hosts. Currently, over 50% of the population worldwide lives in cities, and that number is projected to rise an additional 20% by 2050 (Ligsay et al., 2021). Despite composing only 3% of the world's surface area, cities consume approximately 78% of the total energy and are responsible for over 60% of global CO_2 emissions (Ligsay et al., 2021). The thermal mass produced by cities is a byproduct of anthropogenic activity and low albedo, high population density, and general urban morphology and geometry all contribute to the heightened vulnerability of cities to climate-related health issues (Ligsay et al., 2021). In line with previous studies examining the influence of socioeconomic variables on health outcomes, the severity of sickness and disease is expected to be especially pronounced for socially disadvantaged groups living in low-income areas (Ligsay et al., 2021).

This disparity was highlighted in a study by Rothman et al. (2020) that investigated how the prevalence of infectious mosquitos varies with median household income across 5 neighborhoods in Baltimore, MD. To assess the number of infected mosquitos per neighborhood, the study used Minimum Infection Rate (MIR) as its primary metric (i.e., the ratio of infected mosquitoes to the total number of mosquitos tested). Sampling for 3 separate years (i.e., 2015-2017), the results showed a negative correlation between infection rates and income. The average MIR for mosquitos was almost twice as high for low-income (i.e., below Baltimore's 2012 median household income of $41,819) compared to high-income neighborhoods. This finding can be attributed to the fact that these low-income areas also had a higher proportion of abandoned buildings, vacant lots, and water containers (i.e., litter) per block that serve as convenient breeding grounds for mosquitos (Moyer, 2020). This study is among the first to measure infection rates at the neighborhood level in a major city and signifies the importance of mosquito surveillance at finer scales and in less accessible areas with high disease burden.

Other studies measuring mosquito wing length as an approximation of body size have similarly found that the mosquitos in low-income areas tend to be larger (Moyer, 2020). The implications for this finding are that larger mosquitos often live longer, thus enhancing vector competency by allowing them to bite and therefore infect more people (Moyer, 2020). Larger body size also correlates with higher egg production, which will further increase mosquito abundance in these areas (Moyer, 2020). One possible reason for this correlation is the lack of monitoring and maintenance of green spaces in these areas due to limited funding and support (Moyer, 2020). While urban greening represents a cost-effective and fast-acting solution for increasing albedo, improving health outcomes, and promoting the general well-being of surrounding communities, it may also inadvertently promote mosquito growth and abundance if neglected following implementation (Ligsay et al., 2021). For instance, planting trees and shrubs can lead to increased shading of oviposition pools and serve as nutritional stores for larvae in the absence of control services (Moyer, 2020).

Yang et al. (2019) explored how green space management practices, vegetation characteristics, and landscape composition influenced mosquito abundance across 16 vacant lots in 8 inner-city neighborhoods in Cleveland, Ohio. These lots, a byproduct of economic decline and the mass exodus of over 50% of the population, represent the perfect study sites for evaluating which conditions promote mosquito habitat suitability. Two lots from each neighborhood were assigned to either a control (i.e., management adhered to city guidelines and mowed the lot once a month) or treatment group (i.e., lots were mowed only once per year), and adult mosquito samples were collected from each area using baited traps every 4 weeks. Quadrat sampling was used to measure plant biomass and species diversity within each lot, while land cover data provided by the Cleveland City Planning Commission was used to characterize the surrounding city landscape at radii of 60 and 1000 m.

Although the results showed no significant differences in mosquito abundance based on the frequency of mowing, vegetation characteristics and landscape composition did have a significant effect. Both plant diversity and biomass were positively correlated with mosquito abundance within lots, while higher proportions of grass, shrubs, and tree canopy cover in the surrounding landscape also generally correlated with increased mosquito abundance. These results indicate that costly and intensive management practices such as frequent mowing not only decrease the biodiversity and conservation value of green spaces, but also fail to effectively control mosquito populations. Furthermore, urban conservation initiatives and planning should take mosquito surveillance data or historical occurrence records into account when designing green space to prevent these sites from becoming potential mosquito havens.

3.3 Mosquito Vectors in the U.S.

Mosquito-borne disease incidence in the U.S. has steadily increased over the past few decades, with some estimates reporting a tripling of cases when also accounting for flea and tick-related illnesses (Peterson et al., 2019). Accelerated by anthropogenic factors that have resulted in global ecological shifts, the multitude of available native and invasive mosquito species and associated pathogens is as expansive as ever (Peterson et al., 2019). Although most of the mosquitos in the U.S. fall under the category of nuisance mosquitos (i.e., those that do not transmit diseases), there are three main genera of mosquitos that are capable of infecting the public (CDC 2020). These include *Aedes* species (e.g., *Ae. aegypti* and *Ae. albopictus*), *Anopheles* species (e.g., *An. freeborni* and *An. quadrimaculatus*), and *Culex* species (e.g., *Cx. pipiens*, *Cx. tarsalis*, and *Cx. quinquefasciatus) (CDC 2020).*

Ae. aegypti and *Ae. albopictus* are believed to be responsible for approximately 5% of all global infectious diseases, as these species function as vectors for over 22 arboviruses (Khan et al., 2020). *Ae. aegypti* are active during the day, anthropophilic (i.e., preferring human hosts for feeding), and considered to be the primary vector for dengue, chikungunya, and Zika, with *Ae. albopictus* acting as a secondary vector (Caldwell et al. 2021; Khan et al., 2020). Dengue is the fastest spreading arbovirus worldwide and is responsible for approximately 390 million cases and 10,000 deaths per year and is endemic in over 120 countries (Colón-González et al., 2021).

While there is considerable overlap in the geographic ranges of both species, *Ae. aegypti* occupy a higher thermal niche than *Ae. albopictus (i.e.,* 21.3–34.0 ˚C vs. 19.9-29.4 ˚C) and are therefore more suited to tropical and subtropical conditions as opposed to temperate areas. *Ae. aegypti* prefers urban environments where there is a plethora of artificial containers to function as breeding sites as well as human hosts at their disposal (Ryan et al., 2019). Conversely, *Ae.*

albopictus has greater ecological flexibility and can thrive across a variety of rural and residential sites, feeding on other mammals and birds in addition to humans (Ryan et al., 2019).

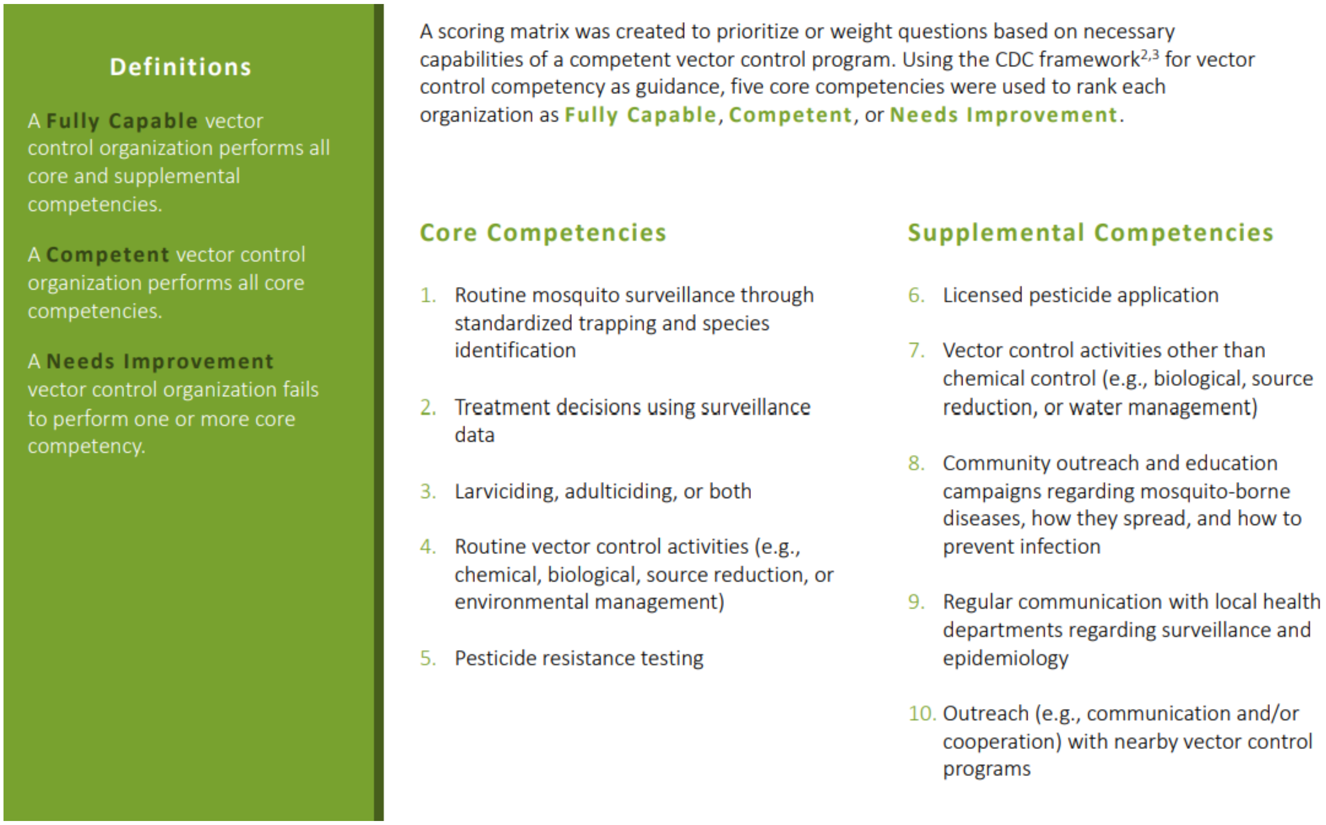
Multiple *Anopheles* species have been reported throughout the continental U.S. across 32 states and 1 territory, with at least 1 species in each area representing a competent malaria vector (CDC 2020; Dye-Braumuller & Kanyangarara, 2021). Malaria, which is caused by parasites in the *Plasmodium* genus, is responsible for approximately 229 million cases of illness and over 400 thousand deaths annually (Dye-Braumuller & Kanyangarara, 2021; Elmardi et al., 2021). It is therefore the deadliest mosquito-borne disease worldwide and is currently endemic in 87 countries (Elmardi et al., 2021). Malaria was previously endemic in temperate zones in the U.S. before being eradicated in the 1950s by the Office of Malaria Control in War Areas, the establishment of which led to the eventual creation of the Center for Disease Control and Prevention (CDC) (Dye-Braumuller & Kanyangarara, 2021). However, while these efforts greatly reduced malaria transmission, they did not completely eliminate the presence of competent *Anopheles* vectors from the U.S. (Dye-Braumuller & Kanyangarara, 2021). As a result, small-scale outbreaks have still occurred into the 2000s, with 2016 recording the highest incidence of malaria since 1972 with 2078 cases (Dye-Braumuller & Kanyangarara, 2021).

Culex mosquitos are known vectors for several arboviruses, such as West Nile Virus, St. Louis encephalitis virus, and both western and eastern equine encephalitis (Gorris et al., 2021). West Nile Virus was first introduced into the U.S. in 1999 and has since been responsible for over 50,000 cases of infection (Nasci & Mutebi, 2019). Because approximately 80% of West Nile Virus infections are asymptomatic, researchers believe that the true number of cases have been historically underreported and may be closer to one million (Rothman et al., 2020). Nonetheless, West Nile Virus is the most widespread flavivirus worldwide and the leading cause of mosquito-borne disease in the U.S. (Gorris et al., 2021; Kramer et al., 2019). Like *Ae. albopictus*, most *Culex* species feed on a range of different hosts and are more commonly found in temperate zones (Gorris et al., 2021).

3.4 Mosquito Control in the U.S.

Given the severity of and increasing health burden posed by these diseases, concerted effort at the local, state, and national level will be needed to prepare for future outbreaks. And with a lack of available vaccines or therapeutics for most mosquito-borne diseases, short-term improvements in the health sector should be aimed towards improving diagnostic methods to allow for early and accurate detection and treatment (de Souza et al., 2021; Peterson et al., 2019). This could entail identifying pathogens at the molecular level through genome sequencing or phylogenetic analysis (Chala & Hamde, 2021). In the context of health outcomes, the final goal of vector control should be for disease prevention rather than treatment.

The National Association of County and City Health Officials (NACCHO) outlines 10 criteria, based on the framework provided by the CDC, to rate the competency of vector control programs throughout the U.S. (see Figure 13). In a perspective piece on improving U.S. control infrastructure in light of the increasing threat posed by vector-borne diseases, robust surveillance of vector populations, lab detection of emerging pathogens and trends, and mitigation measures with proven effectiveness and scalability through testing were identified as fundamental qualities of an effective vector control program (Peterson et al., 2019). However, lack of national oversight and communication amongst organizations has produced a patchwork effect at the local level with standards and procedures varying greatly from operation to operation (Peterson et al., 2019). And while the NACCHO core competencies establish a sound basis for assessing and comparing programs, this metric would be more informative if categories were weighted so that programs that forgo insecticide resistance testing and programs that do not perform routine surveillance yield different composite scores when all else is equal (Dye-Braumuller & Kanyangarara, 2021).



Definitions

A **Fully Capable** vector control organization performs all core and supplemental competencies.

A **Competent** vector control organization performs all core competencies.

A **Needs Improvement** vector control organization fails to perform one or more core competency.

A scoring matrix was created to prioritize or weight questions based on necessary capabilities of a competent vector control program. Using the CDC framework[2,3] for vector control competency as guidance, five core competencies were used to rank each organization as **Fully Capable**, **Competent**, or **Needs Improvement**.

Core Competencies

1. Routine mosquito surveillance through standardized trapping and species identification
2. Treatment decisions using surveillance data
3. Larviciding, adulticiding, or both
4. Routine vector control activities (e.g., chemical, biological, source reduction, or environmental management)
5. Pesticide resistance testing

Supplemental Competencies

6. Licensed pesticide application
7. Vector control activities other than chemical control (e.g., biological, source reduction, or water management)
8. Community outreach and education campaigns regarding mosquito-borne diseases, how they spread, and how to prevent infection
9. Regular communication with local health departments regarding surveillance and epidemiology
10. Outreach (e.g., communication and/or cooperation) with nearby vector control programs

Figure 13 (from NACCHO, 2017). The 10 core competencies listed by the National Association of County and City Health Officials (NACCHO) for assessing vector control programs in the U.S. This list of competencies is tiered, with the first five criteria representing the primary core competencies while the next five are supplemental competencies. In order to be considered "Fully Capable", a program must adhere to all 10 competencies, while meeting only the core competencies earns a lower rating of "Competent". Failure to perform any of the core competencies means that the program is labeled as "Needs Improvement".

In a survey of 1,083 vector control programs nationwide, 84% were lacking in at least one of the five core capacities listed by the NACCHO and are therefore in need of improvement (Peterson et al., 2019). Of these programs, 98% lacked the training to conduct pesticide resistance testing (NACCHO, 2017). Over half of these programs were also lacking competency in conducting routine surveillance and directing treatment decisions based on the surveillance data (NACCHO, 2017). These limitations are compounded by the current shortage of trained entomologists, whose expertise on mosquito morphology and physiology could help guide field surveillance operations and lab research into novel control options, respectively (Peterson et al., 2019). Overall, only 8% of programs met the necessary criteria to be categorized as "fully capable" (Dye-Braumuller & Kanyangarara, 2021).

To address some of these current deficits in U.S. vector control infrastructure, the CDC has invested in 5 university-based research centers specializing in vector borne diseases through 2021 (Peterson et al., 2019). The primary function of these centers of excellence is to serve as communities of practice, provide the learning resources to train more entomologists, and conduct applied research on vector control practices (i.e., insecticide application methods that minimize the risk of developing resistance in the population) (Peterson et al., 2019). The CDC also provides funding to a range of state and local health departments federal agencies, nonprofit organizations, academic groups, community leaders, and innovators in both business and industry (Peterson et al., 2019). In doing so, the CDC is able to facilitate engagement across sectors and produce results at various spatial scales (i.e., creating partnerships between academia and industry with the shared goal of producing new vector control technologies or directing federal aid towards states with the highest disease burden to build frontline capacity; Peterson et al., 2019). This degree of collaboration is essential for creating an effective vector control network nationwide, which will likely continue to expand to include experts from a variety of scientific backgrounds (e.g., microbiologists, ecologists, epidemiologists, etc.) to meet growing information needs (Peterson et al., 2019). Through a case study, the current status of control efforts at the state level will be assessed through results obtained from two surveys detailing self-assessment responses from control organizations.

3.5 Case Study: Florida Mosquito Control

3.5.1 Control Priorities: Targets, Intervention Strategies, and Research Directives

The subtropical conditions and frequent storms which characterize the state of Florida make it one of the most prevalent mosquito hotspots within the U.S., as it currently hosts over 84 mosquito species (Kondapaneni et al., 2021). As a result, there are also 66 Florida Mosquito Control Districts (FMCDs) currently licensed by the state (Kondapaneni et al., 2021). While early control methods included clearing mosquito habitats with dynamite and diking wetlands, FMCDs have come a long way since and now perform an array of modern adulticiding, larviciding, surveillance, and community outreach practices (Kondapaneni et al., 2021). Control practices are tailored to each district based on the prevailing mosquito species and pathogens specific to the area and are often developed in close collaboration with research facilities. These partnerships allow for improved data analysis towards identifying mosquito habitats and optimizing the efficacy and safety of control approaches, resulting in advances in the field such as the creation of new chemical agents and deployment techniques (Kondapaneni et al., 2021). To identify control priorities, common approaches utilized, and directives for future research, Kondapaneni et al. (2021) conducted a survey capturing the responses of 34 FMCDs addressing these topics.

The predominant vector species listed as top priority targets for control fell under the *Culex* genus (mentioned by 79% of FMCDs) followed by the *Aedes* genus (mentioned by 44% of FMCDs). Specifically, the species *Cx. nigripalpus* was mentioned in the highest number of districts (i.e., 22) as it can be found in all 67 counties and is active year-round. Feeding off a wide range of hosts spanning from amphibians to humans, they represent a potential bridge for many zoonotic diseases and are the primary vector for St. Louis encephalitis virus. The other two species listed under the top three control priorities are *Ae. albopictus* and *Ae. aegypti*, which have widespread distribution not only in Florida but across the continental U.S. due to their ability to engage in hitchhiking dispersal mechanisms. Additionally, populations of these *Aedes* species have demonstrated high degrees of insecticide resistance, adaptability to breed in an assortment of water containers, and a propensity to infest indoor environments. Together, these factors make targeting these species problematic for FMCDs alone, emphasizing the need for educational outreach and community engagement in detecting and suppressing mosquito populations.

For larvicides, the most commonly used agents were Bti and Bs, biopesticides derived from microbes that only target certain species (i.e., “true flies” from the order Diptera or species under the Culicidae family). This allows for safe administration of these chemicals without incurring high mortality in other organisms compared to other larvicides. Following biopesticides, the insect growth regulator methoprene was the most popular chemical compound used to target juvenile mosquitos. Insect growth regulators mimic the mode of action of certain developmental hormones, thereby interfering with normal growth patterns, inhibiting maturation, or resulting in deformity or sterility in later stages. Methoprene has proven to be effective against a wide variety of mosquito species and is generally nontoxic to most mammals and invertebrates. Methoprene also has a short half-life of 10 days, making it an environmentally friendly option for control agencies.

In terms of adulticide options for control, FMCDs commonly employed organophosphates, such as naled and malathion. Despite the high level of success these compounds have shown in reducing mosquito population densities in the field, their toxicity poses health hazards to nontarget organisms and people alike. Accordingly, they are only used sparingly in situations where proximity to communities is low enough to minimize human exposure. Pyrethroids on the other hand offer a safer alternative to organophosphates and are now being used more commonly as a result, especially when applied in conjunction with piperonyl butoxide. Piperonyl butoxide has shown to increase the potency of pyrethroids to the point of effectively controlling resistant populations as well. As a further safety measure to counteract building levels of insecticide resistance, FMCDs will often cycle between pyrethroids and organophosphates depending on which compound has exhibited diminished efficacy. Nonetheless, all insecticides, including pyrethroids, have baseline levels of toxicity that can adversely impact surrounding flora and fauna and should therefore be supplemented with nonchemical strategies as well.

Nonchemical interventions aimed towards reducing larval populations include placing *Gambusia*, or mosquitofish, in waterbodies with sufficient depth in which larval presence has been detected. Mosquitofish are capable of consuming hundreds of larvae per day and are unaffected by most biopesticides and insect growth regulators, making them an ideal resource to utilize as part of an integrated approach. However, caution should be observed when working with mosquitofish due to their invasive nature (Cano-Rocabayera et al., 2019). Ideally, deployment should be limited to self-contained bodies of water where their capacity to displace other species is minimized and their eventual removal can be ensured (Cano-Rocabayera et al., 2019).

Perhaps the simplest yet most effective approach for eliminating mosquito larvae and potential breeding sites is through source reduction. This entails actions such as removing or dumping water containers such as garbage bins, lids, buckets, or trash that may serve as larval habitat following rain events. The advantage of source reduction as a control measure is that it does not require additional equipment or training to perform and has zero environmental impact, meaning that it can be carried out by professionals and everyday citizens alike. Source reduction therefore has high potential in terms of scalability and its subsequent impact on mosquito populations and emphasis should be placed on informing communities on the benefits of engaging in these behaviors, especially during the active season for mosquitos.

While there is an abundance of research available for two of the top three control priority species, considerably less research (i.e., less than the median number of publications for the top three studied species by a factor of 10) was available for 12 other notable vectors identified by FMCDs (see Figure 14). Because different species occupy distinct environmental niches that dictate when and where they are active, their level of abundance, and their physiological response to various control mechanisms, research priorities should align with studying species that have been historically underrepresented in the literature. In particular, *Cx. nigripalpus*, *Cs. melanura*, and *Ae. taeniorhychus* all constitute major vector species within Florida that demand greater attention for control strategies to be effective.

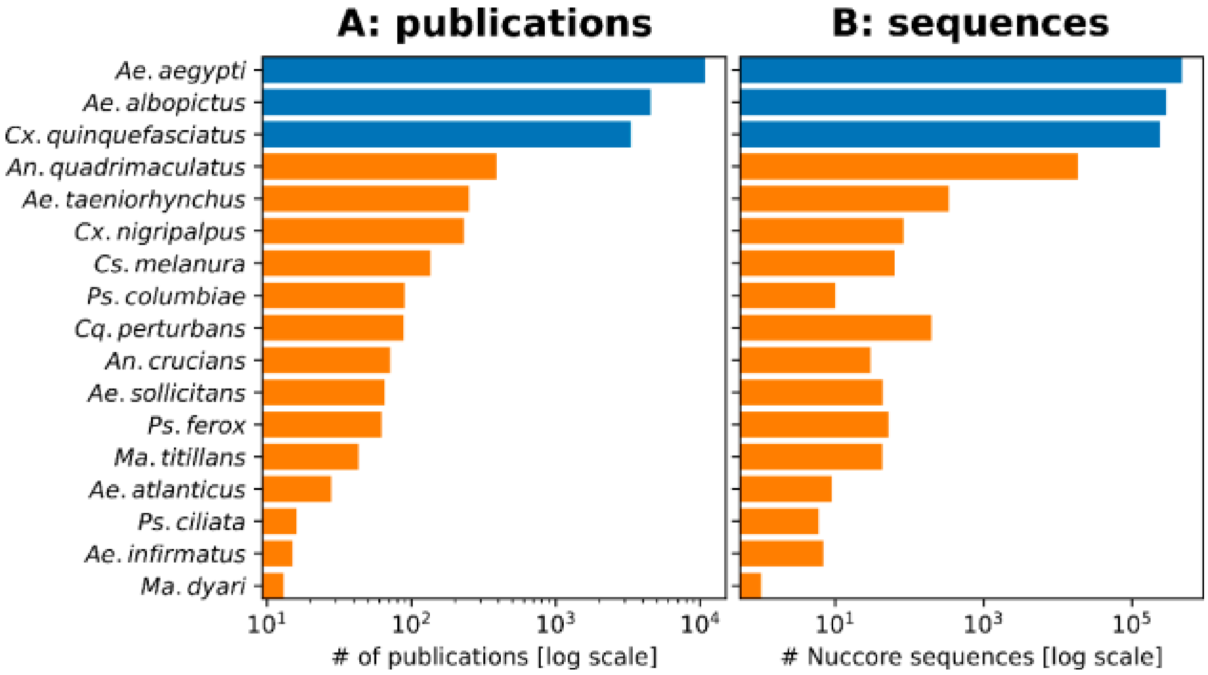


Figure 14 (from Kondapaneni et al., 2021). Publications for mosquito vectors in Florida. A) The number of PubMed publications and B) the number of nucleotide sequences published in the National Center for Biotechnology Information (NCBI) Nucleotide database for each of the 17 control priority species, 15 of which are known vectors, identified by Florida Mosquito Control Districts. The top three mosquito species with the highest global distribution and greatest number of publications are represented by the blue bars, while the orange bars represent understudied species. The threshold for being classified as understudied is if there are 10-fold less publications available for a species compared to the median number for the top three mosquito species.

Furthermore, because mosquitos do not adhere to the same geopolitical boundaries as municipalities or FMCDs, communication and cooperation between these districts will help to establish successful and enduring control strategies throughout the state. Currently, many FMCDs do work together to some degree but oversight through organizations such as the Florida

Mosquito Control Association Research Advisory Committee or the Florida Coordinating Council on Mosquito Control would improve coordination among the various parties to create a cohesive plan of action. This would include sharing research data on novel control methods, the emergence of resistant populations, shifts in outbreak patterns, or the arrival of newly invasive species. Additionally, FMCDs with budget constraints could rely on help from other districts with more ample resources during the peak of mosquito season or spikes in disease occurrence.

3.5.2 Assessing Capacity: Budgetary Constraints, Staffing Needs, and Equipment Availability

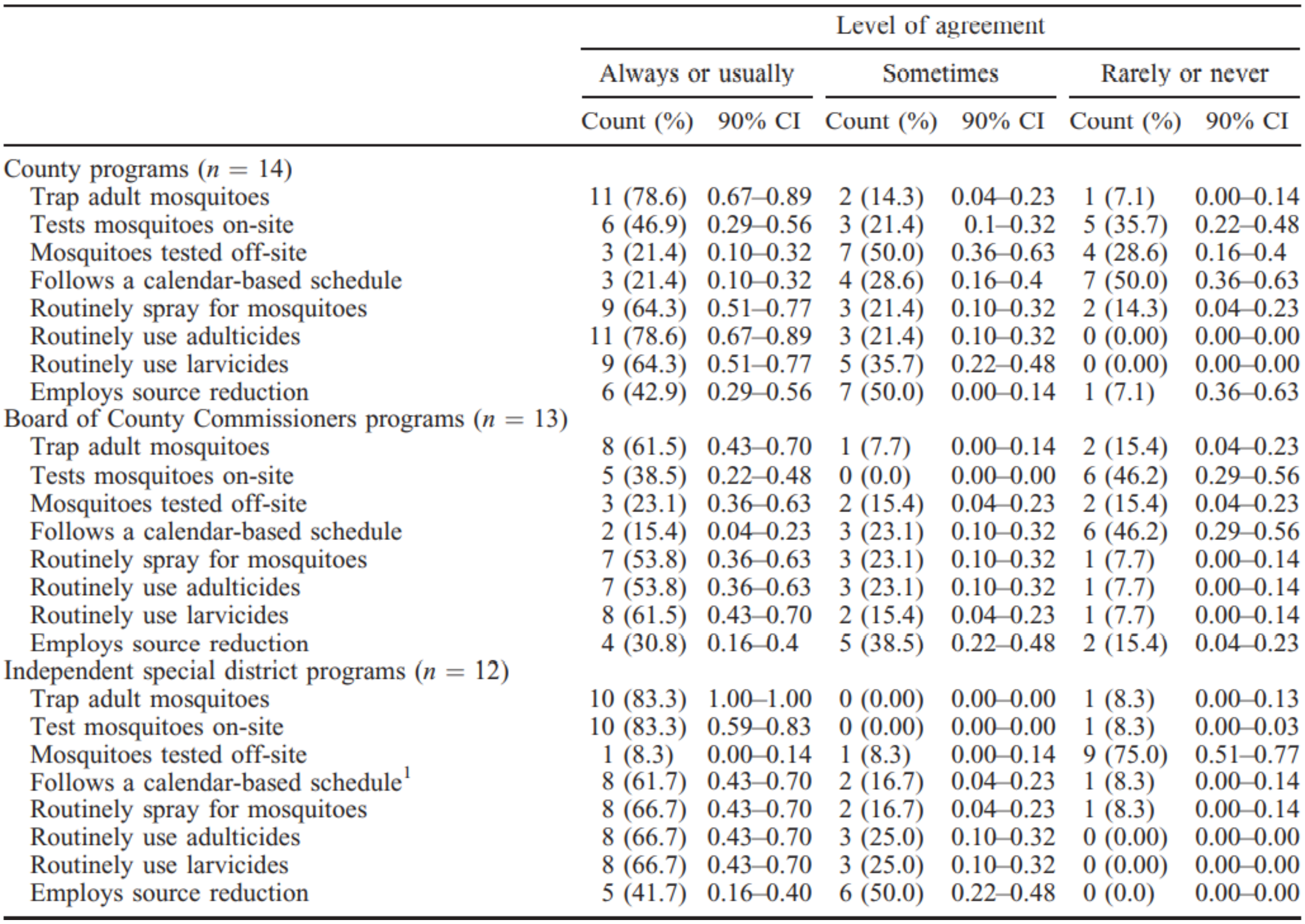
In another survey conducted across the state of Florida, FMCDs self-assessed their capacity to perform in terms of budget, staff and equipment availability, standard operating procedures, and general competency to implement tasks (Moise et al., 2020). The survey was able to obtain responses from 44 of the districts and open programs out of the 90 that were contacted. Of these programs, the majority operate under the Board of County Commissioners (BOCC) and county (i.e., 33% and 31%, respectively) while the rest were mostly independent special taxing districts.

Funding for mosquito control programs is mainly provided by county or city governments, with only 1-2% coming from the state budget based on a three-tier system. Tier I programs have an annual budget of over $3 million and receive minimal support from the state, while Tier III programs have budgets under $1 million and receive the most support. The county programs were initially calculated as having the smallest average annual budget at $697,402 and are therefore grouped under Tier III. Conversely, the independent programs had an average annual budget of $6,253,148, resulting in a Tier I classification. 67.5% of the programs captured in the survey indicated either not having or not knowing of a contingency fund in the case of an outbreak emergency.

Responses related to staffing availability and needs showed that although personnel were adequately trained and competent, the majority reported not have sufficient staffing numbers to properly carry out surveillance or control measures (i.e., 61% and 52%, respectively). The two professions for which staffing shortages were most apparent were medical entomologists (i.e., representing 5% of personnel) and laboratory technicians (i.e., representing 11% of personnel). Overall, the operational capacity for the respondent districts was calculated as under two-thirds (i.e., 63.7%) of the perceived need for workers.

Implementation of control tasks were consistently higher for independent districts, followed by county, and then BOCC programs (see Table 5). For example, independent districts adhered to a calendar-based schedule and tested mosquitos for arboviral presence much more consistently than county or BOCC programs. While most responded with "always or usually" for carrying out mosquito trapping and utilizing adulticides and larvicides, source reduction was the least performed activity across the board, representing an area in need of improvement.

Table 5 (from Moise et al., 2020). Survey responses indicating the level of implementation for a variety of mosquito control and surveillance measures for county, Board of County Commissioners, and independent special taxing district programs in Florida. Implementation was generally higher for independent districts, while the task of source reduction represents an area with the highest potential for improvement across all three district types.



	Level of agreement					
	Always or usually		Sometimes		Rarely or never	
	Count (%)	90% CI	Count (%)	90% CI	Count (%)	90% CI
County programs (*n* = 14)						
Trap adult mosquitoes	11 (78.6)	0.67–0.89	2 (14.3)	0.04–0.23	1 (7.1)	0.00–0.14
Tests mosquitoes on-site	6 (46.9)	0.29–0.56	3 (21.4)	0.1–0.32	5 (35.7)	0.22–0.48
Mosquitoes tested off-site	3 (21.4)	0.10–0.32	7 (50.0)	0.36–0.63	4 (28.6)	0.16–0.4
Follows a calendar-based schedule	3 (21.4)	0.10–0.32	4 (28.6)	0.16–0.4	7 (50.0)	0.36–0.63
Routinely spray for mosquitoes	9 (64.3)	0.51–0.77	3 (21.4)	0.10–0.32	2 (14.3)	0.04–0.23
Routinely use adulticides	11 (78.6)	0.67–0.89	3 (21.4)	0.10–0.32	0 (0.00)	0.00–0.00
Routinely use larvicides	9 (64.3)	0.51–0.77	5 (35.7)	0.22–0.48	0 (0.00)	0.00–0.00
Employs source reduction	6 (42.9)	0.29–0.56	7 (50.0)	0.00–0.14	1 (7.1)	0.36–0.63
Board of County Commissioners programs (*n* = 13)						
Trap adult mosquitoes	8 (61.5)	0.43–0.70	1 (7.7)	0.00–0.14	2 (15.4)	0.04–0.23
Tests mosquitoes on-site	5 (38.5)	0.22–0.48	0 (0.0)	0.00–0.00	6 (46.2)	0.29–0.56
Mosquitoes tested off-site	3 (23.1)	0.36–0.63	2 (15.4)	0.04–0.23	2 (15.4)	0.04–0.23
Follows a calendar-based schedule	2 (15.4)	0.04–0.23	3 (23.1)	0.10–0.32	6 (46.2)	0.29–0.56
Routinely spray for mosquitoes	7 (53.8)	0.36–0.63	3 (23.1)	0.10–0.32	1 (7.7)	0.00–0.14
Routinely use adulticides	7 (53.8)	0.36–0.63	3 (23.1)	0.10–0.32	1 (7.7)	0.00–0.14
Routinely use larvicides	8 (61.5)	0.43–0.70	2 (15.4)	0.04–0.23	1 (7.7)	0.00–0.14
Employs source reduction	4 (30.8)	0.16–0.4	5 (38.5)	0.22–0.48	2 (15.4)	0.04–0.23
Independent special district programs (*n* = 12)						
Trap adult mosquitoes	10 (83.3)	1.00–1.00	0 (0.00)	0.00–0.00	1 (8.3)	0.00–0.13
Test mosquitoes on-site	10 (83.3)	0.59–0.83	0 (0.00)	0.00–0.00	1 (8.3)	0.00–0.03
Mosquitoes tested off-site	1 (8.3)	0.00–0.14	1 (8.3)	0.00–0.14	9 (75.0)	0.51–0.77
Follows a calendar-based schedule[1]	8 (61.7)	0.43–0.70	2 (16.7)	0.04–0.23	1 (8.3)	0.00–0.14
Routinely spray for mosquitoes	8 (66.7)	0.43–0.70	2 (16.7)	0.04–0.23	1 (8.3)	0.00–0.14
Routinely use adulticides	8 (66.7)	0.43–0.70	3 (25.0)	0.10–0.32	0 (0.00)	0.00–0.00
Routinely use larvicides	8 (66.7)	0.43–0.70	3 (25.0)	0.10–0.32	0 (0.00)	0.00–0.00
Employs source reduction	5 (41.7)	0.16–0.40	6 (50.0)	0.22–0.48	0 (0.0)	0.00–0.00

Besides the disparities in implementation levels, availability of resources in terms of control equipment also varied widely across mosquito districts. Although only 20% of respondent districts possessed foggers or mist blowers for insecticide applications, this equipment was the most consistently owned overall. Many BOCC programs lacked key pieces of equipment such as granule applicators or vehicles such ATVs and helicopters that would allow for greater coverage, while some independent and county programs owned all the equipment addressed in the survey. This reinforces the need for promoting communication between districts and improving funding and allocation of resources towards programs in need of support.

3.6 Management Recommendations for U.S. Mosquito Control

As evidenced by the surveys mentioned in the previous sections, mosquito control efforts in the U.S. are consistently lacking in key areas that diminish their overall impact. To build upon the mosquito control infrastructure already in place and address these key areas in need of improvement, I believe that the actions that will be most beneficial fall into four general categories. These categories are: 1) securing funding for staffing, equipment, and training needs, 2) improving awareness and education on vector control issues in communities and urban planning, 3) compiling baseline data on mosquito-vector and pathogen occurrence, 4) and promoting cross-collaboration between mosquito-control organizations and professionals from various scientific backgrounds.

Many of the deficiencies associated with mosquito control programs on an individual level likely stem from insufficient funding. The influence of budget on program efficacy is reflected by the fact that one of the primary shortcomings identified across mosquito control districts according to NACCHO criteria was the inability to conduct pesticide resistance testing, which can be attributed to a lack of training on utilizing techniques such as bottle bioassays (i.e., measuring mortality rates of mosquitoes placed in bottles coated with a particular insecticide; CDC, 2022). While resistance testing methods themselves are not resource intensive in terms of requiring expensive equipment or state-of-the-art lab facilities to carry out, more rigorous training and assessment protocols for staff necessitate greater budget allocation. This financial need is further demonstrated in the Moise et al. (2021) survey where implementation levels across FMCDs correlated with the level of funding provided. Budget availability also correlated with staffing operational capacity, which was mainly limited in regard to employees with specialized scientific or technical backgrounds. Although this issue has been addressed by agencies such as the CDC by establishing centers of excellence that serve as training grounds for mosquito control professionals and aspiring entomologists, funding was limited to $50 million until the end of 2021 (CDC, 2022). Lobbying for continued funding for projects such as these is of vital importance for ensuring future reserves of mosquito control experts and research and development towards new innovations in the field (i.e., synthesizing more target specific pesticides, releasing sterile male mosquitos into the wild, etc.).

Because mosquito-borne diseases have such a widespread distribution that involve a complex and ever-changing interplay of factors, relying solely on mosquito-control organizations to shoulder the brunt of this problem is unrealistic. Educating the public on the threat and adverse outcomes posed by mosquito-borne diseases as well as the precautionary measures they can take to protect themselves is necessary to supplement professional efforts and improve overall control coverage. Having communities actively engage in source reduction or utilize personal protective measures (i.e., insect repellent, window screens) during the active mosquito season will greatly diminish the chances of large-scale epidemics by minimizing contact rates between vectors and hosts.

This push for increased awareness for mosquito-borne diseases also extends up the ladder to government officials and city planners to acknowledge and integrate this issue in public policy and urban design. For example, this could entail directing more manpower for trash collection in abandoned lots (i.e., minimizing the accumulation of potential mosquito breeding sites) or incorporating ponds with mosquitofish in green space projects. Moreover, environmental justice projects aimed towards maintaining neglected spaces in low-income neighborhoods as urban gardens or recreational areas would be beneficial to the community at large as adult mosquitos do not discriminate along socioeconomic lines and will simply spill over or migrate into more affluent areas if left undisturbed.

Data regarding mosquito or disease occurrence records represent one of the primary informational needs of mosquito control organizations and health officials to establish baseline risk assessments from site to site (e.g., what mosquito-pathogen combinations are historically associated with the area, when did the most recent outbreaks occur, etc.). The availability of high-quality data can vary widely from state to state due to the widespread absence of long-term monitoring efforts, an issue which is only further compounded by the lack of a centralized data repository for mosquito-borne disease distribution on a national scale. Efforts towards engaging in more vigilant data collection and the initiation of longitudinal monitoring programs should be ramped up as soon as possible to enhance our ability to discern and anticipate disease patterns.

Improvements in high-resolution remote sensing technologies offer a promising avenue for identifying mosquito habitats that are often difficult and time-consuming to detect with the naked eye.

Mosquito-borne disease dynamics involve a complex interplay of factors that are collectively beyond the breadth of any one area of expertise. Even stripped down to its most basic form, mosquito-borne diseases encompass a three-party system (i.e., host, vector, and pathogen) where outcomes are not only dictated by interactions between players but by the myriad of environmental forces acting on each of them individually. The scope of this issue has prompted some researchers to consider an ecosystem approach to disease prevention, in which human health is considered in parallel with environmental health (Mills et al., 2010). This perspective necessitates the formation of multidisciplinary relationships between wildlife biologists, ecologists, entomologists, public health officials, and medical researchers (Mills et al., 2010).

Furthermore, the disjointed nature of mosquito control organizations in the U.S. not only creates health disparities among communities but also undermines regional health as a whole since infected mosquitos are capable of crossing borders into unsuspecting areas. For this reason, establishing a communication network among organizations within states would strengthen efforts on a larger scale. Effective communication allows for more transparent and prompt access to information on current distribution patterns for vectors and pathogens as well as the ability to redistribute resources and equipment to districts with more limited funding during unexpected disease outbreaks or mosquito blooms. One potential way of introducing a channel of communication would be by creating oversite committees that uphold regular meetings amongst control organizations for each state. In this scenario, these organizations can keep one another updated on the latest control priorities and techniques, creating a composite image of the status of mosquito-borne diseases throughout the state in the process. Continued collaborations with universities will also be integral in pushing towards new innovation and breakthroughs in control and surveillance technologies, diagnostics, therapeutics, and predictive modeling, all of which collectively mitigate adverse impacts associated with mosquito-borne diseases (Mills et al., 2010; Peterson et al., 2019).

Conclusions

As modeling techniques become more sophisticated in projecting spatial patterns for climate and disease, they will prove to be an invaluable asset to guiding preemptive control and preventative healthcare measures. Establishing standardized methods for the public health application of such models constitutes a fundamental step towards improving confidence in their predictions, subsequently promoting more widespread application of modeling approaches. For example, models that incorporate aspects of both mechanistic and phenomenological approaches and as many relevant covariates as possible (i.e., demographic structure, socioeconomics, multiple metrics for each climate factor, etc.) are more likely to approximate real world disease dynamics. Minimizing the scale of projections to capture variation at local levels that coincide with the scales at which public health departments and vector control organizations operate would go a long way in translating model outputs into realistic targets. Likewise, reducing the temporal scale of outputs to an annual basis would also yield more accurate maps (e.g., errors associated with climate projections compound exponentially the further you extrapolate in time) with more practical timelines for developing intervention strategies.

Continued progress in model performance will not only entail technological innovation but further research into empirical gaps for disease dynamics such as evolutionary adaptive potential, which will be greatly aided by the implementation of longitudinal studies that will allow for adaptive processes to play out over longer timespans. These studies include selection and common garden experiments that simulate evolutionary processes and assess the genetic component of phenotypic variance, respectively. Combining these studies with genome sequencing techniques will allow researchers to quantify the magnitude of genetic change, identify the specific candidate genes underlying thermal adaptation, and measure the level of environmental sensitivity and heritability of those genes.

Lastly, emphasizing the need for specific mosquito-control protocols in urban health planning in the U.S. will be imperative to ensuring a prompt and effective response to mitigating the emerging threat posed by mosquito-borne diseases. Historically, mosquito-borne disease has not been considered as a major health burden to American cities, and as a result the infrastructure for dealing with these diseases is severely lacking in many urban areas. If urbanization trends continue, the majority of the world population is set to be living in cities over the next few decades, presenting a considerable target for host-seek mosquito vectors. The physical characteristics and qualities of urban environments also provide a wide array of manmade containers and debris that can potentially become aquatic habitats for mosquito oviposition. Moreover, the increased rates of international trade and travel particularly associated with major cities further increases the probability of inadvertently introducing invasive vector species. These risk factors, coupled with climate regime shifts that coincide with species migration, highlight the need to create policies that will tailor control practices and infrastructure to meet the needs of urban environments.

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