



Thermal biology of invasive *Aedes* mosquitoes in the context of climate change

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The increasing incidence of arboviral diseases in tropical endemic areas and their emergence in new temperate countries is one of the most important challenges that Public Health agencies are currently facing. Because mosquitoes are poikilotherms, shifts in temperature influence physiological functions besides egg viability. These traits impact not only vector density, but also their interaction with their hosts and arboviruses. As such the relationship among mosquitoes, arboviral diseases and temperature is complex. Here, we summarize current knowledge on the thermal biology of *Aedes* invasive mosquitoes, highlighting differences among species. We also emphasize the need to expand knowledge on the variability in thermal sensitivity across populations within a species, especially in light of climate change that encompasses increase not only in mean environmental temperature but also in the frequency of hot and cold snaps. Finally, we suggest a novel experimental approach to investigate the molecular architecture of thermal adaptation in mosquitoes.

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Current Opinion in Insect Science 2022, 51:100920

This review comes from a themed issue on **Vectors and medical and veterinary entomology**

Edited by **David Poumo Tchouassi, Jandouwe Villinger and Amy Morrison**

For complete overview about the section, refer “**Vectors and medical and veterinary entomology (August 2022)**”

Available online 11th April 2022

<https://doi.org/10.1016/j.cois.2022.100920>

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Introduction

Aedes spp. mosquitoes transmit viruses responsible for human diseases such as dengue, chikungunya, Zika, and

yellow fever, adversely affecting millions of people each year [1]. Despite substantial efforts to prevent the spread of arboviral diseases, our current vector control methods are challenged by the rise of insecticide resistance in adults and changes in the choice larval development sites, from small containers and vernal pools to underground and other cryptic habitats [2,3]. Mosquitoes are poikilotherms, thus environmental temperature (T_a) directly affects their overall physiology, fitness, and seasonal activity [4]. Additionally, with global climate change, the increase in average T_a favors the geographic expansion of important mosquito vectors, thereby increasing the risk of disease outbreaks [2,5–7]. Climate change is a strong selective force, known to cause behavioral and physiological adaptations in *Drosophila* flies and other insects. Additionally, several traits associated with thermal adaptation (see Table 1), such as critical thermal limits, metabolic rate and body size, were shown to be genetically inherited and subjected to genetic adaptation over microevolutionary timescales [8–11]. Despite the direct impact on mosquito reproductive success, population dynamics, and, by extension, on public health, mechanisms underlying thermal adaptation remain poorly understood in disease vector mosquitoes, both phenotypically and genetically.

Here, we review and discuss the current knowledge on *Aedes* mosquito thermal biology in comparison to laboratory and field studies conducted in other dipterans, primarily *Drosophila* spp. We focus on two of the most invasive species in the world and major vectors of pathogens, *Aedes aegypti* and *Aedes albopictus*, as well as species that have more recently invaded Europe and the US, *Aedes japonicus* and *Aedes koreicus*. *Aedes japonicus* and *Ae. koreicus* are raising concerns because of their rapid spread and ability to vector both arboviruses and *Dirofilaria immitis* [12] (Figure 1). *Aedes japonicus* is found mostly in rural habitats, whereas *Ae. koreicus* is an anthropophilic species mostly associated with urban settings; both species are cold tolerant and overwinter as eggs. *Aedes japonicus* and *Ae. koreicus* are adapted to higher altitude than *Ae. albopictus*, thus increasing areas at risk for *Aedes*-borne diseases [13,14]. Insights on the impact of T_a on life-history traits of both species have been highlighted as a way to assess their likelihood of invading new areas in Europe [15]. Phenotypic traits, however, have not been thoroughly studied and/or linked to genotypes yet and modeling remains the main tool to estimate future distributions [16,17].

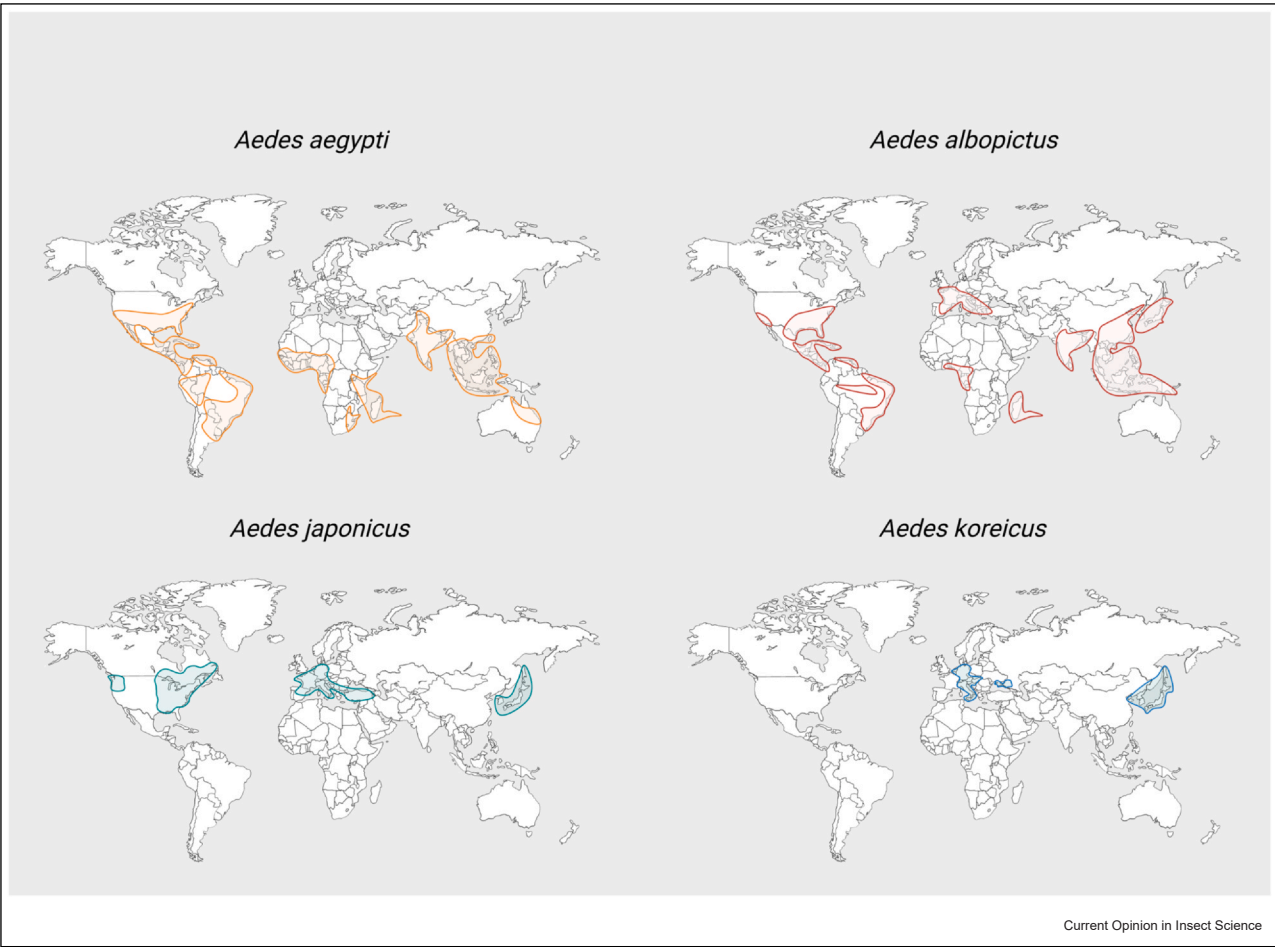
Table 1

Definition of concepts and factors in thermal biology. These traits can be affected by many biotic factors including, but not limited to, the insects' age, physiological and reproductive status.

Traits	Definition
(T_p)	Resting temperature chosen by an organism when provided with a range of temperatures
(T_t)	Ability to cope with high and/or low temperatures. (T_t) can be genetically inherited or acquired after exposures to high/low temperatures.
Thermal adaptation	Ability to cope with prolonged exposures to high/low temperatures without physiological or behavioral stress.
Thermal sensitivity	Degree to which an organism responds to changing environmental temperatures and how it affects thermal performance.
Thermal performance	Range of temperatures within which a given behavioral or physiological trait can occur with a critical minimum (CT_{min}) and maximum (CT_{max}) temperature beyond which the performance becomes impossible (i.e. defining the tolerance range) and the temperature at which the performance is considered optimal (T_{opt}).

(Modified after [16,30]).

Figure 1



Current global distribution of *Aedes aegypti*, *Aedes albopictus*, *Aedes japonicus*, and *Aedes koreicus*. Rare occurrences have not been represented for clarity.

Sources: European Centre for Disease Prevention and Control and European Food Safety Authority; Center for Disease Control and Prevention; [4,22,71–74].

Physiological and behavioral traits associated with thermal adaptation

In mosquitoes, T_a impacts larval developmental time, adult longevity, body size, egg hatchability and overall population dynamics, as reviewed in Reinhold et al. [4]. Moreover, T_a can also affect the replication and dissemination of arboviruses within the vector's body [18–20] and alter the composition of the microbiota [21–23], resulting in a feedback effect on mosquito behavior, physiology, and pathogen transmission [24,25]. Finally, metabolic rates are sensitive to body temperature and, as temperature alters metabolism and energy budgets, it can impact the ability of a species to cope with changing climatic conditions [26]. Insects facing rapid changes in T_a use several physiological and behavioral strategies to avoid the risk of thermal stress and associated cellular damages including behavioral thermoregulation and the synthesis of heat shock proteins [27–29]. However, prolonged exposure to high or low T_a can lead to profound and lasting behavioral and physiological changes allowing species (or populations) to develop, survive and reproduce under otherwise unfavorable conditions (i.e. thermal adaptation) [16,30] (see Table 1). The existence of climatic adaptation has been shown by the identification of a range of physiological and behavioral responses/phenotypes, which are correlated with the geographic distribution of a species across ecological gradients (e.g. elevation, latitude, and climate) [31–33].

Thermotolerance (T_t) and thermopreference (T_p) can vary significantly *between* different mosquito species [6]. For example, *Aedes canadensis*, which develops in snow-melt (vernal) pools in early Spring, and *Ae. japonicus* are considered cold tolerant [13]. In the latter species, T_p is shifted towards cooler temperatures in comparison to tropical species such as *Ae. aegypti* [34] where adults show a lower T_t for high temperature [13]. Interestingly, *Culex* spp. T_p also greatly varies between species and while some species such as *Culex quinquefasciatus* exhibits lower T_p , others including *Culex tarsalis* and *Culex territans* have a much higher T_p which may be linked to T_a in these species' native habitat and host preference [35]. *Aedes aegypti* is native of sub-Saharan Africa and does not have the ability to overwinter in temperate areas such as the Northern US, except for one population in Washington DC, which uses underground-warmer-niches to avoid cold stress [36] (Figure 1). Additionally, one study showed that *Ae. aegypti* eggs can survive low and sub-zero temperatures for a short time period, suggesting the ability to rapidly acclimatize to new conditions [37]. The globally invasive *Ae. albopictus* is native to tropical South East Asia, it is overall considered more cold-resistant and exhibits an overall higher ecological plasticity than *Ae. aegypti*. Temperate populations of *Ae. albopictus* undergo photoperiod diapause and overwinter as eggs, suggesting

extensive physiological adaptations to cooler climates. The physiological and molecular mechanisms of photo-periodic diapause have been extensively studied in this species [38,39], providing an understanding of the factors influencing the onset of the mosquito season in Spring. However, global climate change includes not only an increase in average T_a , but also in the frequency of extreme weather events such as heat waves and/or cold snaps. These climatic events may occur out of season and thus be disjoint from changes in day length, one of the main cues initiating diapause. We argue that in light of climate change, a precise quantification of thermal traits such as the egg freezing point, the adult critical upper and lower thermal limits (CT_{max} and CT_{min}) and the adult T_p , which could influence overwintering, and/or ability to overcome cold/hot snaps will be crucial to better understand the mechanisms underlying the invasive success of *Aedes* mosquitoes.

In contrast to differences in thermal sensitivity across species, variations *within* mosquito species (i.e. at the strain/population level) remain understudied [40], in particular in *Aedes* spp. Experimental evidence from other insect species supports the hypothesis that population-level differences in T_t and thermal performance exist in relation to the species distribution range. For example, in *Drosophila melanogaster* heat tolerance is lower in temperate populations compared with tropical ones [41] and temperate populations also have a shorter recovery time after a chill coma than tropical ones [42,43]. In the mosquito *Culex tarsalis*, differences in CT_{max} (see Table 1) between populations positively correlate with maximum temperatures at native sites [44]. Moreover, populations from high-altitude/cooler sites have significantly higher metabolic rates compared to populations from warmer sites, which is due to thermal compensation in organisms living in cold climates [44]. In *Wyeomyia smithii*, effects of latitude and season on T_t have been demonstrated [45]. Indeed, populations from northern regions have a higher cold T_t compared to populations from the south but no difference in heat T_t has been found [45]. In *Ae. albopictus*, strains from northern Asia and North America have higher overwintering survival rates than those from tropical Asia and Brazil [46]. The same study showed that none of the tested *Ae. aegypti* strains survived overwintering under northern US climatic conditions, highlighting the contrast between these two species. Conversely, inhibition of egg hatching in response to a short-day photoperiod has been noted in a strain of *Ae. aegypti*. This highlights the behavioral and physiological plasticity of certain strains in response to changes of abiotic factors, which could lead to the expansion of this species to colder climates [47]. Importantly, as differences in DENV-2 virus development have been found between populations of *Ae. aegypti*, it is critical to

consider T_a as a potential factor influencing infection rates which can have consequences on the magnitude of outbreaks [7,18].

Identification of the mechanisms for physiological and behavior shifts observed in *Aedes* populations will be critical for accessing the potential impact of climate change on these factors. *Drosophila* spp., which has been extensively studied provided a framework for future studies with *Aedes* populations including the genetics of thermal adaptation described in the following section.

Genetics of thermal adaptation

Evaluating thermal adaptation requires the quantification and understanding of both specific phenotypic characters directly related to temperature (i.e. egg freezing point, CT_{max} and CT_{min} , and T_p) and fitness and metabolic features such as longevity, body size, expression of heat shock proteins and enzyme kinetics, which can all be affected by T_a . Moreover, each of these traits may be polygenic, even in the absence of a continuous, normally distributed phenotype within a population, and epigenetic changes or transcriptomic/phenotypic plasticity (i.e. the ability of a genotype to change its expression/phenotype when environment changes) may contribute in shaping adaptive responses to current increase in T_a . To predict how mosquitoes respond to climate change, we first need to identify the phenotypic traits that could be mostly controlled by T_a , such as T_f (e.g. CT_{max} and CT_{min}) and T_p , and the methodologies to quantify these traits [6], and then to mechanistically understand the effects of genetic and environmental variation on these traits. A powerful methodology to disentangle genetic variation from plasticity, test plasticity whether thermal-related traits are genetically inherited and if adaptive genetic changes occur has recently emerged from studies in *Drosophila* spp. [8,32,48–50]. This approach, often referred to as ‘evolve and re-sequence [E&R], includes the combination of *omics* techniques (e.g. whole-genome sequencing [WGS] and RNA-seq) to samples from common-garden experiments (i.e. comparison of genetically distinct strains/populations which are maintained under identical environmental conditions) [51]. RNA-seq and WGS approaches generate lists of either differentially expressed genes or genomic regions harboring ‘evolved/selected’ variants. These lists will include regulatory regions controlling gene expression besides coding sequences because thermal adaptation is a complex trait, which is expected to be governed by genes working in interconnected regulatory networks [52]. Because of this cellular intricacy with core genes affecting and being affected by secondary genes, where ‘core’ and ‘secondary’ refer to the respective contribution on the phenotype, genome-wide analyses are prone to false positives [52]. Despite this complexity, a meta-analysis of 28 studies on thermal-adaptation in *Drosophila* spp., using both RNA-

seq and WGS-based, highlighted concordance on the gene ontologies, such as oxidoreductase, glutathione metabolism enzymes, heme-binding proteins, peptidase, cytoskeleton-related proteins, and enzymes involved in the metabolisms of fatty acids and chitin [53]. These data suggest that thermal adaptation in *Drosophila* spp. is mediated by numerous genes, each with a limited effect; alternatively, genetic redundancy could also explain convergence in functions with different lists of genetic elements [53]. Concordance on gene ontologies allows pinpointing to candidate orthologous groups for functional validation. Using this approach, it was recently demonstrated that exposure to high temperatures results in adaptive evolution of key dopamine synthesis and transport genes, and of one gene regulating their expression (i.e. *Hr38*), in *Drosophila simulans* [54]. *Omics* approaches could also be applied to *ad hoc* collected natural samples and, albeit still controversial, similar gene expression profiles could be observed between natural and laboratory-evolved *Dr. melanogaster* populations suggesting that garden experiments can mimic natural evolution [50,51]. In *Drosophila* spp., genetic adaptation to climate can be rapid, within 60–100 generations of populations exposed to a novel temperature regime, with also examples of fluctuating selection between favorable alleles during either hot or cold seasons [49,50]. Additionally, after exposure to a high-temperature regime for 100 generations, a different expressional profile was observed in *Dr. simulans* males *versus* females, with most differences being observed in highly expressed genes of the fat body, involved in lipid metabolism, that were overexpressed in males and downregulated in females, suggesting that the two sexes have different requirements when exposed to novel temperature conditions [50]. Investigating these phenomena (i.e. inheritance of thermal-related traits, evolutionary adaptation, and sex differences) is paramount in *Aedes* spp. mosquitoes given their invasiveness and the different role of males and females in arboviral disease transmission. Evolutionary adaptive genetic changes in response to increase in T_a are plausible in *Aedes* mosquitoes given their short generation times, large populations and their dependency on T_a to regulate activity and physiology [6]. Supporting a genetic contribution to thermal adaptation in mosquitoes, chromosomal inversions that correlate with latitudinal or aridity clines have already been identified in *Anopheles gambiae* [55,56]. Additionally, we compared phenotypic differences in fitness (i.e. fecundity and wing-length, a proxy for body size) and thermal traits between tropical and temperate strains of *Ae. albopictus* and identified significant differences in body size, fecundity, and nutrient levels (Carlassara, unpublished). These results in both invasive temperate and native tropical strains support a correlation with the climatic conditions of the strain native range and could indicate either a polygenic inheritance and/or extensive plasticity for *Ae. albopictus* thermal adaptation.

Conclusion and knowledge gaps

Because mosquitoes are ectotherms, T_a directly affects their physiology, fitness, seasonal activity, and, ultimately, their adaptability to new environments. Moreover, experimental evidence is accumulating across a broad range of insect species on physiological, behavioral, and genetic-based adaptive responses caused by global climate change. Despite its direct impact on mosquito biology, thermal adaptation remains poorly understood in disease vector mosquitoes, both phenotypically and genetically. Overall, a better understanding of how T_a affects *Aedes* spp. biology and of the potential heritability of T_a associated traits is critical to identify key factors contributing to their invasive success and may lead to innovative strategies of population control.

In *Drosophila*, the E&R approach has been shown useful to understand the molecular mechanisms underlying thermal adaptation by allowing us to observe, in a controlled environment and through evolutionary times, changes at multiple levels (i.e. physiological, behavioral, and genetic). To avoid biases and be able to disentangle the multiple factors contributing to thermal adaptation, evolution experiments are often set up with established insect colonies instead of freshly collected specimens, of which the genetic background, the time of introduction and origin may be unknown. Validation of results from E & R studies based on laboratory-adapted strains using wild populations will further lead to a more refined understanding of the species thermal adaptation dynamics as adaptation to laboratory conditions may reduce genetic variation and lead to changes in behavior and physiology [57]. E&R studies can also be associated with strain crosses to identify whether observed correlations between thermal-related traits, such as T_p and T_n , are due to genetic correlations (i.e. thermal traits controlled by linked genetic regions) or correlated selection across strains/populations [58].

In conclusion, studying how mosquitoes are responding to global warming can provide more accurate data to implement in models of their future distribution and could lead to the identification of novel targets for population control.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

CL was supported by the Department of Biochemistry, The Fralin Life Science Institute, and The Global Change Center at Virginia Tech. Funding was also provided to CL by the USDA National Institute of Food and Agriculture HATCH project VA-160160 and the National Science

Foundation under Grant IOS-2114127. Funding was provided to MB by the Human Frontier Science Program Research Grant (RGP0007/2017), the Italian Ministry of Education, University and Research FARE-MIUR project R1623HZA5 and the European Research Council Consolidator Grant (ERC-CoG 682394) under the European Union's Horizon 2020 Programme. The Department of Biology and Biotechnology 'L. Spallanzani' also received financial support from the Italian Ministry of Education, University and Research (MIUR) through the Dipartimenti d'Eccellenza Program (2018–2022). Funding sources had no role in study design; in the collection, analysis, and interpretation of data; in the writing of this review; and in the decision to submit the manuscript. Figure 1 was created with Biorender.com.

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