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Role of physiology and immunology of the vector in disease transmission and effect of temperature and environment on the interaction of host and parasite

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Abstract

When an infection modifies the host's behavior of a way that benefits the pathogen, this is known as manipulation. Diseases spread by vectors are not an exception. As the name suggests, infections may directly disrupt host functions in order to regulate behavior. Nonetheless, these characteristics are probably influenced by the host's physiology and reaction to infection. Based on recent research on changed host-seeking in mosquitoes infected with malaria parasites, we emphasize the significance of taking host reaction and physiology into account. We contend that this broad approach will yield useful information across disease transmitted via vectors systems. We also point out how the malaria vector's humoral and cellular immune responses are influenced by ambient temperature. Phagocytosis and defensin production peaked at about 18°C, cecropin expression did not exhibit a significant influence of temperature or humoral melanization, and nitric oxide synthase production peaked at 30°C. Furthermore, intricate relationships between temperature, duration, and the kind of immunological challenge were seen in immune responses, which could rather than simply scale with temperature. As a result, immunological patterns seen in one set of circumstances don't give much information about how they would behave in even slightly different circumstances. Both the qualitative and quantitative effects of temperature have significant implications for the efficacy of various vector control methods and for extending organic or recombinant mechanisms for resistant from laboratory studies to the field, despite being frequently ignored in vector biology.

Keywords: Vector Immunity; Malarial Parasite; Melanization; Host–Parasite Interactions; Temperature

1. Introduction

From unicellular species like yeast, bacteria, and protozoa to eukaryotic beings like worms and insects, the realm of parasitism is extensive and includes a diverse range of taxa. To survive, these parasites have developed a variety of parasitism-related tactics. They frequently infect vertebrate hosts, which has a major effect on their ability to survive and procreate. Addressing basic biological issues and working in applied domains like epidemiological and medicine require an understanding of these tactics (Al-Musaedi, 2024).

In order to increase their fitness, parasitic organisms have developed manipulation techniques. *Platyhelminthes, Nematoda,* and *Arthropoda*, along with a variety of viruses, bacteria, fungi, and single-celled eukaryotes, are thought to contain at least some species that are able to manipulate their hosts' behavior (Poulin, 2010).

Examples of host exploitation abound. In one of the first examples, amphibians affected by larval acanthocephalan parasites showed atypical behavior and pigmentation, which increased their vulnerability to the parasite's next host (Hindsbo, 1972). Almost every major group of living animals has recorded this behavior. For example, the nematode

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called *Myrmeconema neotropicum* causes the tropical tree- dwelling ant *Cephalotes atratus* to have a vivid red abdomen (Poulin, 1999).

To improve their ability to spread and, in turn, their fitness, parasites may modify at least two phenotypic characteristics. Although behavioral changes often reduce the host's fitness by endangering survival, parasites that propagate through trophic chains by ingestion typically alter their intermediate hosts (Aeby, 2002). In order to attain sexual maturity and proliferate, parasites with complex life cycles often depend on a definitive host consuming their intermediate hosts (Lafferty, 2002). Numerous methods of morphological change assist this trophic transfer. For instance, the pointed antennae found in the snail *Succinea putris* are changed into vibrant, pulsing structures that resemble flashing lights by the parasitic spores of the digenean pathogen *Leucochloridium macrostomum* (Wesołowska, 2013). *Leucochloridium* species change the color and shape of their snail victims' tentacles, causing them to pulse in response their susceptibility to hunters (the definitive hosts) (Berdoy, 2000).

2. Parasite characteristics that impact host traits

The precise structure and mechanism of action of the proteins secreted by *Toxoplasma gondii*, for example, are yet unknown, despite their complex interactions that involve the host's immune system. In grasshoppers (*Meconema thalassinum*), substances released by the infectious hairworm *Spinochordodes tellinii* have an impact on the central nervous system development of its host., causing abnormal actions, such as jumping toward the water's surface where the parasite reproduces. (Biron *et al*., 2005).

The ways in which parasites modify host behavior are complex and poorly understood, despite the fact that some of the elements of these changes have been identified. One of the primary areas of cooperative study spanning the areas of parasitology, physiological science, and neurobiology is still unraveling all the possible pathways. The goal of current research is to comprehend how parasites influence animal behavior. The processes of manipulation are probably as varied as the wide taxonomic variety of parasites, which includes worms, parasitic insects, apicomplexans, viruses, and fungi (Poulin, 2010).

3. Humoral Immunity

The exoskeleton, which is made of a combination of chitin and proteins, provides insects with a comparatively high level of protection. The only fungus having enzymes capable of breaking down this hard matrix and entering insect hosts straight through the integument are parasitic fungi, including those belonging to the genera *Beauveria* and *Metarhizium* (Lefevre 2008). Unlike the immune system of vertebrates, the insect immune system is composed of well-defined layers of innate immunity and does not have the adaptive powers of memory cells and antibodies. Numerous proteins that identify peptidoglycans, lipopolysaccharides, and β (1,3)-glucans—components of microbial cell walls—are produced by insects (Rogers, 2007).

In insects, hemocytes like the plasmatocytes or granulation cells that circulate in the hemocoel phagocytose microbial propagules as part of cellular immunity. If the parasites are too big or too many for a single hemoglobin cell to consume, multilayered encapsulation and nodule development take place (Adamo, 2013). The imprisoned parasites, which are frequently melanized when the phenoloxidase cascade is activated, are surrounded by a multicellular envelope made up of nodules, which are collections of haemoglobin (Hurd, 2003). In insects, humoral immunity includes the manufacture of antimicrobial peptide and proteinase inhibitors, as well as the regulation of melanization through the stimulation of the phenoloxidase cascade (Helluy, 2013).

To fight illnesses from pathogens, animals have evolved humoral and cellular immune responses. Immunity, which is independent of particular immunological systems, is the ability to withstand or guard against a particular infection or germ (Lafferty, 2013). Only an innate, non-specific immune response is present in invertebrates. With the greatest number of species on the planet, insects primarily use their highly developed innate immune system to fight off a wide range of infections (Cator *et al*., 2013).

4. Disease-Transmitting Insects

Numerous species of parasites and pathogens that cause illnesses including infectious illnesses such filariasis, onchocerciasis, sleeping sickness, malaria, Chagas disease, and arboviruses are found in the orders Diptera and Hemiptera. Dipterans include mosquito (*Culicidae*), flying insects (*Simuliidae*), sand fly (*Phlebotominae*), tsetse fly families (*Glossinidae*), and roaches (e.g., *Sciaridae*). (Adamo, 2013). Many disease-causing agents, such as malaria, and

arboviruses are caused by mosquitoes belonging to Anopheles, the genus Aedes, and Culex. They are also responsible for the transmission of dengue virus, viral chikungunya, which causes illness, and filarial nematodes. (Patramool *et al*., 2012).

About 400,000 people are killed annually by mosquitoes alone, and malaria has been a major cause of mortality for many years. Malnourished children under the age of five are especially at risk. Additionally, 3.9 billion individuals in 129 countries are at risk of contracting this prevalent viral disease dengue, which is spread by the mosquito species *Aedes aegypti*, and *Aedes albopictus*. (Gulley, 2012). In Africa, the continent of America, the Persian Gulf, South Asia, and the Mediterranean, sandflies are the carriers of Leishmania. Infected individuals suffer irreversible deformity from leishmaniasis, onchocerciasis, and filariasis, which are spread by insects such as blackflies and mosquitoes, respectively. The infections *Trypanosftoma bruceirho* and *Trypanosoma brucei gambiense* are spread by tsetse flies in Africa, and infected individuals suffer from sleeping sickness. These illnesses generate a great deal of human pain and financial hardship (Villegas *et al*., 2015).

4.1. Common Insect Immune Situations

The basic insect immune response may be triggered by foreign invasion following damage or in the gastrointestinal tract, hemocoel, red blood cell, fat organs, glands that produce saliva, and other tissues following ingesting. The pattern recognition receptors (PRRs) on or inside the tissues of immunity system mediate recognition by binding to the molecular patterns associated with pathogens (PAMPS) of invasive microorganisms and parasites (Bahia, 2022). In addition to initiating many signaling pathways that produce immune effector genes and release antibodies and cytokines to eliminate invaders. (Vinayagam, 2023).

4.2. Antiviral and Antiparasitic Immunity in the Mosquito Gut

Mosquitoes are the deadly critters on the planet, killing at least 725,000 annually (Ahuja, 2025). Instead of acting as vicious killers, mosquitoes typically serve as the ostensibly harmless carriers of a wide variety of clinically significant infections that take advantage of the insects' feeding habits to finish their life cycles. The invertebrate host usually pays a price for the coexistence of the disease and mosquito (Christophides *et al*., 2004). In order to prevent the spread of deadly illnesses like dengue hemorrhagic fever and malaria, enormous attempts are being made to comprehend the genetic makeup of these particular insects and to create viable strategies for interfering with their ability to act as vectors (Crampton, 1994).

Before being transmitted to a human host, malaria parasites must undergo a number of intricate developmental changes within the mosquito vector. Male and female reproductive cells combine in the mid-gut lumen after being consumed by a mosquito, and over the course of around eighteen hours, they grow into a movable ookinete that will go to the gut epithelium and infiltrate a single epithelium cell. Before an infested cell is ejected from the epithelial layer, the ookinete must reach the basal lamina (Dantzer, 2004). When the parasite reaches the basal lamina, it transforms into an oocyst. Over a period of ten days, it generates countless sporozoites, which eventually escape into the mosquito's hemolymph. When a mosquito feeds on blood again, sporozoites can spread by migrating to and invading the salivary glands. The ookinete penetration into the gastrointestinal epithelium is a significant hurdle for Plasmodium development. The mosquito's lumen and epithelium immune responses are responsible for the bulk of the parasite removal (Speranc & Capurro, 2007).

Ookinetes can be lysed by the mosquito's complement-type effector protein TEP1 either inside the mid-gut epithelium cells' plasma membrane or outside of the cells in the basal lamina. (Kavaliers, 1999). The coloration of the first oocysts that develop in the midgut epithelium in specific strains of the mosquito vector *Anopheles gambiae* is caused by the target chemical phenoloxidase, which is generated from circulating hemoglobin. Furthermore, nitric oxide may be produced by epithelial cells, hemoglobin, and the fat body can kill early oocysts. (Gutierrez, 2009).

5. Immunity and Temperature

The cellular and humoral reactions that make up the insect's immune system work together to prevent the propagation of an infection. The selfless recognition signal is sent downstream by signal transduction cascades, which carefully control and coordinate these responses (Lefevre *et al*., 2008).

According to the studies, mosquitoes ought to display a range of resistance phenotypes in response to varying ambient temperatures. Both (i) the immediate impacts of host body temperatures on growth of parasites and (ii) the less researched indirect impact on the development of parasites, are two ways that temperature may influence the ability to resist phenotype and parasite growth (Cirimotich, 2010).

If the combined relative and/or full immune system's reaction of mosquitoes exhibit heat sensitivity, the current approach of characterizing innate immune system responses under normal laboratory conditions is insufficient to understand vector competency as it is shown in the field. We assessed humoral and immune cell responses in the Asian parasite vector, *Anopheles gonidi* at a variety of consistent temperatures in order to verify this claim (Little *et al*., 2005).

In this piece, we contend that mosquito immunity and consequently, resistance to parasites and diseases spread by vectors are significantly influenced by changes in the surrounding temperature (Will *et al*., 2020). We mainly focus on the temperature of the environment for four main reasons, even though many environmental factors may play a significant role in determining vector resistance (for instance, biotic factors like the larval as well as mature nutrition, larval competition, and abiotic factors like humidity and day length). First, despite the fact that the majority of mosquito habitats operate at 26–28 °C, parasites associated with malaria may spread between 16 and 35 °C, and other vector-borne illnesses (Kobayashi *et al*., 1981).

We summarize the current understanding of how temperature influences insect physiology and immunology below, as well as how parasite fitness is impacted by ambient temperature. To add substantial ecological realism to standard laboratory studies that explain the link between vectors and parasites, we argue that adjusting the ambient temperature is a feasible first step (Rahman *et al*., 2006)

We show that temperature may significantly affect mosquito immune responses in a variety of quantitative and qualitative ways, with potentially intricate interactions with time and immune challenge type. It should come as no surprise that temperature has an impact on immunological responses. The complexity and unpredictability of the impacts across several immunological measures challenges the prevailing disciplinary paradigm, which often ignores environmental variation (Rund *et al*., 2011).

6. Experimental

6.1. Material and method

We raised *An*opheles *gambiae* (Liston) in conventional insectary settings, which included a 12 L:12 D photo-period, 27 ± 1°C, and 80% humidity. We put mosquito eggs in 25 × 25 × 7-centimeter plastic trays that were filled with 1.5 liters of water. We separated freshly hatched larvae to guarantee an average density of two hundred per tray in order to reduce any possible variance in the size of the emerging adult mosquito body. For the first seven days after hatching, larvae were fed Liquifry; after that, they were provided Tetrafin flakes made from fish. About two weeks following egg hatching, pupae were taken from larva trays and put in experimental cages.

Adults were given an unlimited supply of a 10% glucose solution upon emergence. Five days after emergence, the mosquitoes employed in the humor melanin production and defensive gene expression tests were fed a blood meal from Wistar rats that were older than six weeks. Mosquitoes were put under anesthesia on ice on days 4-5 after emergence. After an immunological challenge, mosquitoes were randomly assigned to one of five reach-in incubators with temperatures of 10°C, 15°C, 20°C, 25°C, and 30 ± 0.5°C and relative humidity of 75 ± 3%. Several pilot investigations were conducted for each immune measure across a lower temperature and sample time point regime to guarantee that the effects of temperature, immunology challenge, and point of collection time on immunity response were consistent throughout the whole trial.

6.1.1. Immune challenge using Sephadex beads for melanization

Tyrosine hydroxylation and the oxidate polymers of indolequinones are the first two enzymatic and non-enzymatic processes that result in melanization. Total phenol oxidase activity, an essential enzyme in the melanin production reaction, has been employed as a stand-in for immune competence in several investigations up to this point. However, we decided to assess the melanin response directly due to phenol oxidases play a role in many other metabolic processes outside innate immunity. Melanization has been linked to the defenses of *Aedes aegypti* towards *Plasmodium gallinacean* sporozoites, and resistant *A. gambiae* (L35) strains from the rodent's pathogen eggs *Plasmodium berghei* and the newly discovered *Plasmodium falciparum*. One negative-charged CM-25 Sephadex bead was injected into females who were blood-fed in order to promote the melanization reaction. Only the tiniest Sephadex beads were visually chosen for inoculation; the beads' diameters vary between 30 to 100µm. To make the beads easier to see, they were placed in a DMEM mixture with 0.001% methyl green. We dispersed mosquitoes at random among temperature treatments and injected a single bead in a very small volume of fluid (less than 0.5µl). The mosquitoes that could walk were eliminated 24 hours after the immunological challenge, and the beads were separated in a phosphate-buffering saline solution dyed with 0.01 percent methyl green.

6.1.2. Phagocytosis: flu sphere-based immune challenge

Hemocytes recognize, engulf, and destroy tiny microorganisms and apoptotic cells as part of the cellular immune response known as phagocytosis. We used a Nanoject to inject about 50,000 yellowish-green carboxylate-treated flu spheres (1μm in diameter) into non-blood-fed females in order to promote phagocytosis. Ten mosquitoes were randomly assigned a single of four sample time points (1, 6, 12, and 24 hours) and a temperature treatment following immune challenge. Mosquitoes were extracted 1–24 hours after the immunological challenge. A combination of Vybrant CM-Di and nucleic acid staining was administered into each mosquito to label cells to fluorescently stain the hemoglobin in vivo. After that, ice-anesthetized mosquitoes' hemolymph was perfused onto a microscopic slide. After being fixed in 5% paraformaldehyde, hemocytes were cleaned with distilled water and a phosphate-buffer saline solutions with the pH of 7.5 and Molarity 0.2M. The degradation index and digesting capability for an overall of 50 count granulocytes were determined for each mosquito.

6.1.3. Gene expression: bacterial immune challenge

We investigated the effects of temperature on how to express of the genes for defensin 1 (DEF1), cecropin 1 (CEC1), and nitric oxide synthase (NOS) in response to heat-killed *Plasmodium gonderi* exposure, damage, and no manipulation. Two peptides with antimicrobial activity that are produced by localized barrier epithelia and the insect's fat body are encoded by DEF1 and CEC1. Both peptides have been connected to the death of Plasmodium. The effector molecule that NOS encodes, nitric oxide, has been shown to be a usual killer of a wide variety of illnesses and parasites. It has also been connected to a strong anti-malarial defense in mosquito mid-gut epithelia.

To stop bacterial development in mosquitoes maintained at different mean temperatures from being influenced by temperature, we employed thermally killed resistant to tetracycline GFP- carrying *Plasmodium gonderi* to be our challenge. At 37°C, *P. gonderi* were cultivated nighttime in incubator. The overnight culture was then used to create a serial dilution. We used a Nano Drop to record the absorbance value (OD600) from every dilution in order to estimate our injection dosage of *P. gonderi*. Before being put into their various temperature treatments, Ice-anesthetized mosquitoes were either left untreated (control mosquitoes) or injected with 0.2μl of sanitized LB (positive injury control) or 200 000 heat-killed *P. gonderi* following that, 15 mosquitos in every immune-challenge group were divided across four testing sessions (6, 12, 18, and 24 hours) and five temperatures.

6.1.4. Quantitative PCR, cDNA synthesis, and RNA collection

The mosquitoes were taken out of their temperature treatment after the immunological challenge, killed with chloroform, and promptly preserved in RNA later RNA stabilizing reagent at 4°C for upcoming molecular investigations. Five mosquitos from each of the treatment groups (a total of 200) were separated in -Mercaptoethanol and RLT buffer for lysis as soon as the experiment was over. The Qiagen RNeasy Small Kit was used to extract messenger RNA. A pool of four mosquitoes' mRNA was extracted to provide standards for the qualitative polymerase chain reaction (PCR).

Experimental treatment had an impact on ribosomal protein S7 expression. Three doubles of each cDNA standard, spanning six orders of magnitude, were used in each quantitative PCR experiment. Individual mosquitoes' cDNA values for every gene of interest were compared to the assay's standard curve. *Anopheles stephensi* and *Anopheles gambiae* sequencing were used to create primers and probes, and quantitative PCR was used to establish that DNA pollution in RNA samples was undetectable.

6.1.5. Analyses from statistics

PSAW 18.0 was used to conduct each statistical analysis for these studies. Non-significant interactions in the generalized linear model's (GLM) analysis were eliminated backwards to decrease full models. Using the Akaike information criteria, log likelihood values, and model deviance, we evaluated the final models' goodness of fit. GLMs contained covariates that were centered according to their grand mean.

6.1.6. Humoral melanization: the extent of melanization in beads

We evaluated the degree of melanization by categorizing retrieved beads into three groups: unmelanized, partially melanized (i.e., sections of the bead stayed unmelanized), and completely melanized. We conducted a logistic regression analysis to assess the impact of temperature on the probability that a bead would be assigned to a certain class using the total area of the beads as a covariate.

6.1.7. Phagocytosis: capacity and phagocytic index

We assessed how temperature and the sampling time point affected the average quantity of beads that granulation cells can absorb and the percentage of phagocytizing granulocytes using GLMs. Models incorporated temperature, sampling time point, and interaction with it as fixed factors for both response variables. To take into consideration a possible correlation between the mean amount of beads granulocytes ingest and the total amount of functioning granulocytes (with beads), the centralised phagocyte index was included as a covariate to the phagocytic capacity GLM.

6.1.8. Expression of genes

As our expression measure, we used the cDNA counts generated for each targeted genes using our traditional curve evaluation to analyze variations in overall gene expression across our treatment groups. Considering a distribution of gamma for the variable that is dependent, which was converted using a log link function, we used GLMs to analyze all expression data. To account for potential variations in assay efficiency and experimental sample independence, full multifactorial analyses were conducted for each gene independently. All models incorporated temperature, immunological challenge, and sample time point as fixed parameters.

7. Results

We specifically examined whether the rates of insects' characteristic humoral and immune-mediated responses were temperature-sensitive and if immune system responses were changed in essentially consistent ways across different immunological challenges and sampling time points. Since it has been shown that temperature influences pathogen performance, we will use non-living immunological stimuli in the upcoming research to isolate the effect of the temperature on the immune system.

7.1. Melanization of the humor

From mosquitoes kept at all experimental temperatures, we were able to retrieve 98% of the injected beads. The likelihood of retrieving unmelanized, partly melanized, or completely melanized beads was strongly impacted by temperature. More completely melanized bead than partially melanized ones were retrieved at 18°C compared to all other temperatures. As the temperature rose, the percentage of partly melanized beads compared to totally melanized beads peaked at 28°C. Conversely, the dimension of the infused bead (bead area) was unable to substantially predict bead status, and neither the temperatures nor beads size (area) had an impact on the likelihood of retrieving unmelanized beads. The melanization rate seems to peak at 18°C and decreases with increasing temperature.

7.2. Defensin activity

DEF1 expression was greatly impacted by temperature; the results were strongly influenced by immunological challenge and sampling time point. For instance, within the first 6–12 hours and 24 hours after being exposed to either an injury or a topical application of heat-killed P. gonderi, mosquitoes kept at 26°C showed elevated DEF1 expression. Mosquitoes kept at varying temperatures and exposed to the same immunological challenge, however, do not exhibit this pattern. DEF1 expression rises during the first 6 hours and rapidly decreases at succeeding testing intervals in mosquitoes kept at 34°C, whereas it peaks 12–18 hours after the immunological challenge in wounded insects kept at 18°C. In contrast, mosquitoes subjected to thermally killed *P gonidi* exhibit elevated DEF1 expression over the first six hours and decreases after 6–12 hours for mosquitoes kept at 18°C. DEF1 expression is also boosted during the first 6–12 hours for mosquitoes kept at a higher temperature (30°C and 34°C). In addition to the relationship between the temperature, the duration of sampling point, and immunological challenge, there was a significant main effect of temperature on DEF1 expression; mosquitoes put at 18°C expressed considerably more DEF1 altogether than mosquitoes put at warmer temperatures.

Table 1 GLM analysis final model outputs for DEF1, CEC1, and NOS.

	DEF1			***CEC1***			***NOS***		
Intercept	1	25954.69	<0.0001	1	24468.06	<0.0001	1	25699.21	<0.0001
Temperature	4	36.84	<0.0001	4	8.17	0.085	4	22.04	<0.0001
Sampling time point	3	46.91	<0.0001	3	31.68	<0.0001	3	146.27	<0.0001
Immune challenge	2	35.16	<0.0001	2	13.12	0.004	2	16.14	<0.0001
centered rpS7 cDNA counts	3	5.82	0.016	1	7.62	0.08	3	5.61	0.017
concentration of total RNA	2	4.91	0.019	1	6.83	0.039	2	155.63	<0.006
Temperature × time point of sample	12	76.66	<0.002	—	—	—	10	16.28	0.153
Immune challenge × sampling time point	6	10.88	0.092	—	—	—	6	6.87	0.788
Temperature ×immunological challenge	12	26.59	0.0005	8	24.45	0.017	8	294.01	<0.005
Temperature × time of sampling × immunological challenge	20	66.56	<0.0002	—	—	—	16	45.49	

7.3. *Cecropin* Activity

The gene expression of CEC1 was also strongly impacted by temperature, and this effect varied according to the kind of immunological challenge (table 1). The impact of temperature was independent of sampling time, in contrast to DEF1 expression. The highest levels of CEC1 expression were often seen in unaltered mosquitoes held at optimal to high temperatures (27°C, 30°C, and 35°C), injured insects housed at 18°C and 30°C, and thermally dead E. E. coli-treated mosquitoes kept at cooler to ideal temperatures.

7.4. Nitric oxide synthase activity

As with DEF1 expression, temperature had a substantial impact on NOS expression that changed with sample time point and immunological challenge (table 1). At later sample times, unmanipulated mosquitoes kept at lower temperatures (18°C: 24 h; 22°C: 18 h) expressed more NOS than insects kept at optimal or higher temperatures (26–34°C: 12 h) Twenty-four hours after the immunological challenge, mosquito challenged with heat-killed *P. gonidi* often showed elevated NOS expression.

8. Discussion

Studies on a variety of insect species and other ectotherms unequivocally show how temperature affects parasite virulence and host resistance. Here, we build on this research to show how a major malaria vector's humoral and cell immune system response rates may be strongly impacted by ambient temperature (Blanford, 2011). Additionally, there were notable temporal or rate effects for a number of the measurements, which differed based on the kind of immunological challenge and/or intricate relationships between variables. These pertinent nuances will be overlooked by the conventional methods that limit such experimental complexity.

We discovered that temperature had no discernible impact on monitoring gene expression above the background levels for CEC expression. Nevertheless, CEC expression did change with temperature based on whether *P. gonderi* was supplied after being injured or after being heat-killed. Therefore, even if temperature seemed unimportant at first, interactions with other "environmental" variability causes might result in complicated and unpredictable reactions (Kikankie *et al*., 2010;).

The temperature at which NOS expression peaked was somewhat higher than the mosquito's presumed optimal temperature; colonies are normally kept at about 27°C. An important anti-malarial defense found in the gut of *An. gambiae* is nitric oxide, a cytotoxic effector and cell signaling chemical that also contributes to the parasitic bottleneck

linked to ookinete movement within the gut epithelium. Additionally, since greater activity in the fatty tissue and circulatory granular cells when exposed to an infestation has been shown, it may be used as a last option against Plasmodium parasites. (Mittal *et al*., 1993).

Similarly, both phenoloxidase and the defensin peptide reside together at locations of melanin deposition in the mosquito *Aedes aegypti's* immunological responses against *P. gonderi*. Furthermore, they are frequently found in identical melanotic capsules, which may help to explain why DEF1 expression corresponds with the melanization pattern (Ren *et al*., 2004).

We have not linked temperature-induced changes in gene expression to functional tolerances or vector ability, as we have done in several previous transcriptional studies, despite the fact that temperature may significantly impact post-transcriptional regulation. Therefore, it is important to look at how temperature affects the synthesis of antimicrobial peptides, the activity of nitric oxide enzymes, and the removal of pathogens (Hoffmann *et al*., 2011). However, the interplay of temperature, immune challenge type, and post-immune challenge time point obviously makes it more difficult to interpret the numerous research carried out under a single set of circumstances (Hardie *et al*., 1994).

Temperature affects the methods used to manage the same disease species. More generally, complicated temperature–immune interactions could have far-reaching effects because mosquito resistance is crucial for the effectiveness of insect repellents (Patil *et al*., 1996), fungal synthetic pesticides, biological antibiotics, prospective transgenesis, paratransgenesis, and transmission tools in the field (Rivero *et al*., 2010;). Our results show that, given the ecologically varied context where the mosquito and pathogenic organisms live, it is imperative to begin conceptualizing vector immunity.

Compliance with ethical standards

Disclosure of conflict of interest

There is no conflict of interest.

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