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Rethinking vector immunology: the role of environmental temperature in shaping resistance

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Abstract

Recent ecological research has revealed that environmental factors can strongly affect insect immunity and influence the outcome of host–parasite interactions. To date, however, most studies examining immune function in mosquitoes have ignored environmental variability. We argue that one such environmental variable, temperature, influences both vector immunity and the parasite itself. As temperatures in the field can vary greatly from the ambient temperature in the laboratory, it will be essential to take temperature into account when studying vector immunology.

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FURTHER INFORMATION Courtney C. Murdock and Matthew B. Thomas's homepage: http://www.thethomaslab.net

SUPPLEMENTARY INFORMATION See online article: S1 (table)
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Over the past 20 years, our understanding of the physiological and molecular interactions between parasites, such as *Plasmodium* spp. and dengue virus, and their mosquito vectors[1–3] has been radically transformed. Research has provided important insights into insect innate immunity[4], identifying a multitude of immunity genes and immune system pathways that influence resistance to vector-borne parasites, and has also revealed potential targets for genetic manipulation[5–7]. However, this body of work is incomplete. In particular, mosquito resistance to infection tends to be viewed as a static phenotype consisting solely of standard immune responses measured under a narrow set of laboratory conditions[1,8]. But vectors and parasites associate in a variable world. From work in other invertebrate–parasite systems, we expect that mosquito susceptibility to vector-borne parasites depends not only on genetic factors, but also on a wide range of biotic and abiotic factors. To complete our understanding of immunity and resistance, we need to consider this environmental variation.

In this Opinion article, we argue that variation in ambient temperature markedly shapes mosquito immunity and, in turn, resistance to vector-borne pathogens and parasites. Although many aspects of the environment might be important in shaping vector resistance (for example, abiotic factors such as humidity[9] and day length[10], and biotic factors such as larval[11] or adult nutrition[12] and larval competition[13]), here we focus primarily on environmental temperature for four main reasons. First, although most mosquito insectaries are set at 26–28 °C, malaria parasites can be transmitted across a temperature range of 16–35 °C, and transmission of other vector-borne pathogens such as dengue virus, chikungunya virus and filarial nematodes occurs across a similar temperature range. Second, mosquitoes are small-bodied, ectothermic insects. Many studies have already demonstrated that temperature can markedly affect diverse aspects of mosquito physiology and ecology, including the rate and viability of egg hatching[14], the rate of development[15–17], the propensity to feed and the rate of feeding[18], and survival[15]. It would be surprising if immune function were any different. Third, research on a wide range of invertebrates demonstrates that small, realistic changes in ambient temperature can significantly shape overall host resistance to a range of parasites by influencing, for example, parasite virulence, development rates, latency periods and clearance rates within the insect (see Supplementary information S1 (table)). Fourth, numerous studies have already found that the development rates of key vector-borne parasites are strongly temperature dependent; this has been shown for dengue virus[19], West Nile virus[20], yellow fever virus[21], chikungunya virus[22] and filarial nematodes[23,24], as well as human[25], rodent[26,27] and avian[28–30] malaria parasites. The extent to which temperature shapes vector resistance directly, through such effects on parasite biology, or indirectly, through effects on vector immunity, remains largely unexplored.

Below, we outline what is known about how temperature shapes insect immunity and physiology, and discuss how ambient temperature affects parasite fitness. We maintain that manipulating the environmental temperature is a tractable first step in incorporating important ecological realism into standard laboratory studies that characterize the vector–parasite interaction. A broader appreciation of the thermal ecology of vector–parasite interactions could have far reaching implications for understanding natural heterogeneity in mosquito resistance, modelling the transmission of vector-borne disease, predicting

transmission ecology in a changing climate and evaluating the efficacy of novel control tools involving genetic manipulation of mosquito physiology.

Temperature and immunity

The insect immune system consists of both cellular and humoral responses that interact to control the spread of an infection[31]. These responses are tightly regulated and coordinated through signal transduction cascades that transmit the non-self recognition signal downstream. The Toll, immune deficiency (Imd) and the lesser studied janus kinase–signal transducer and activator of transcription (JAK–STAT) pathways are three characteristic insect immune pathways that respond to a suite of parasitic challenges[32–34] (BOX 1). The end point of these signal cascades is typically the release of a transcription factor that triggers, for example, cell death or cell division, or the deployment of effector molecules such as antimicrobial peptides[32,35] (BOX 2 provides an illustrative example for mosquitoes and malaria, which represents the best described vector–parasite system). Changes in body temperature owing to variation in ambient temperature will significantly shift the metabolic rates of both insect vectors and the parasites that they host[36,37]. The consequences for various immune processes and for the resistance phenotype have not been extensively examined.

Effects of temperature on invertebrates in general

Invertebrates have been used widely by researchers in the field of ecological immunology to investigate the effects of environmental variation on the immune response and host fitness. Several studies have shown that temperature affects invertebrate immune traits. For example, following challenge with lipopolysaccharide (a bacterial product that activates immune responses), the larvae of the beetle *Tenebrio molitor* exhibit increases in phenoloxidase activity, antibacterial responses and metabolic rates, as well as weight loss, when maintained at a high temperature (30 °C) compared with when the larvae are maintained at lower temperatures of 10 °C or 20 °C[38].

Similarly, compared with crickets (*Gryllus texensis*) grown at their preferred temperature, those that are exposed to a simulated heat wave (5 °C above preferred temperature) for 6 days have increased fitness (increased egg laying, egg development and mass gain), phenoloxidase activity and levels of lysozyme-like enzymes, and exhibit resistance to the Gram-negative bacterium *Serratia marcescens*[39]. Interestingly, the resistance and fitness of crickets infected with the Gram-positive bacterium *Bacillus cereus* is actually lower at temperatures that deviate from the average field temperature (26 °C), suggesting a complex interplay between temperature and resistance to different pathogens[39]. By contrast, fruitflies (*Drosophila melanogaster*) reared at cooler than normal temperatures (17 °C) have enhanced survival when infected with either Gram-positive or Gram-negative bacteria (*Lactococcus lactis* or *Pseudomonas aeruginosa*, respectively) and increased expression of a suite of immunity genes compared with fruitflies housed at standard and warmer rearing temperatures (25 °C and 29 °C, respectively)[40]. This boost in immune performance at cooler temperatures might seem counterintuitive, but heat shock proteins (in particular, HSP83) might boost immune function at cooler temperatures in this species[36]. To date, only one study reports that temperature can act in combination with other aspects of environmental

variation, such as food availability and density of conspecifics, to affect phenoloxidase activity and total haemocyte counts[41].

Finally, there is evidence that being kept at fluctuating temperatures can have an effect on immunity. Butterflies (*Lycaena tityrus*) exposed to temperatures fluctuating around cooler (17.7 °C) and warmer (23.7 °C) than normal temperatures experience significantly different development times, phenoloxidase activity, responses to hot and cold stressors, and total haemocyte numbers compared with butterflies housed at a constant 17.7 °C or 23.7 °C mean temperature[42].

Effects of temperature on insect vectors

For insect vectors, we are aware of only two studies that specifically explore the effects of temperature on mosquito immunity[43,44]. The first study investigated how larval nutrition, adult body condition and ambient temperature affect the melanization response (an enzymatic cascade that results in encapsulation of parasites and pathogens with melanin) of adult *Anopheles gambiae* following challenge with Sephadex beads, and showed that melanization decreases progressively as temperature increases from 24 °C to 30 °C[43].

The second study measured how the performance of a suite of cellular and humoral immune responses varies in *Anopheles stephensi* across five different constant temperatures ranging from 12 °C to 34 °C[44] (FIG. 1). Temperature was shown to significantly affect all immune responses measured, but in different ways. Unexpectedly, melanization, phagocytosis and expression of the antimicrobial peptide defensin were all highest at 18 °C (8 °C below the standard rearing temperature for this mosquito). Expression of nitric oxide synthase (a key enzyme involved in defence against a multitude of parasites and pathogens, including malaria parasites) peaked at 30 °C, whereas expression of the antimicrobial peptide cecropin was not directly affected by temperature[44]. Interestingly, the temporal dynamics of many of the immune responses studied changed depending on both the ambient temperature and the type of immune challenge administered. Thus, the immune profile described across the course of an infection at one temperature would potentially be completely different if the same experiment were run at a different temperature[44].

Temperature and resistance

The overall effect of temperature on the ability of an insect to resist infection depends on not only the effects on elements of insect immune function and physiology, but also direct effects of temperature on the parasite itself. For example, recent research has shown that the effectiveness of mosquito immune responses against malaria parasites depends in part on the kinetics of host enzymes involved in the nitration and lysis of midgut cells infected with *Plasmodium* spp. parasites, and in part on the rate of parasite migration through the midgut epithelium[45]. Such host and parasite processes are not necessarily equally affected by temperature; the net effect of temperature on vector resistance and parasite fitness will depend on the relative thermal sensitivities of both host and parasite traits (BOX 2).

Temperature affects parasite physiology

Numerous studies have demonstrated the effects of temperature on diverse aspects of the host–parasite interaction, including host resistance, the latency period, the rate of parasite development, and parasite transmission in non-vector insect systems (see Supplementary information S1 (table)). For example, ambient temperature greatly shapes resistance of the crustacean *Daphnia magna* to infection with the bacterium *Pasteuria ramosa*[46]. When the host is housed under standard laboratory conditions (20–25 °C), the bacterium is highly virulent; by contrast, when the host is housed at lower temperatures (10–15 °C), it is much more resistant to infection. These lower temperatures, which are not optimal for the bacterium, represent thermal conditions that are commonly experienced by *D. magna* in the wild, a factor that might explain why this crustacean has not evolved resistance mechanisms against *P. ramosa* in the field[46].

Similarly, a study on the resistance of pea aphids to a pathogenic fungus of insects revealed that temperature significantly influences the susceptibility of aphid clones to infection. Further, how susceptibility changed with ambient temperature varied among clones. Two of the tested clones became more susceptible at optimal temperatures for vegetative growth of the fungus *in vitro*, whereas two other clones deviated considerably from this expected response, becoming less susceptible to infection at these temperatures. This effect, again, has implications for the pattern of co-evolutionary dynamics that is played out in the field[47]. Both of the studies discussed above clearly demonstrate that traits which are strongly influenced by genetics (that is, resistance and susceptibility) can be greatly influenced by environmental variability.

Temperature and the physiology of vector-borne parasites

With respect to vector-borne parasites, research on the direct effects of temperature has been limited, and the primary focus has been on the effect of temperature on development rates within the insect vector. This has been studied for malaria parasites of rodents[26,48], birds[28–30], lizards[49] and humans[25,50,51]; West Nile virus[20]; dengue virus[19]; and yellow fever virus[21]. In general, most of these studies show that warming temperatures are associated with a reduction in the development time of the parasite or pathogen. For example, the extrinsic incubation period of West Nile virus (strain NY99) in the mosquito *Culex tarsalis* decreases as ambient temperature warms from 10 °C to 30 °C[52]. Few studies have focused on how temperature shapes other aspects of parasite life history.

One recent study[53] examined the effects of temperature on the development rate and survival of a malaria parasite of rodents in the vector *A. stephensi*. In line with previous work, this study showed that parasite development rates increase linearly with increasing temperatures over a 20–26 °C temperature range, suggesting that parasite transmission potential should increase with warming temperatures. However, the proportion of mosquitoes that actually became infectious (that is, had sporozoites in the salivary glands) followed a nonlinear response, being highest at around 22 °C and substantially declining at warmer temperatures (FIG. 2). This pattern might be due to the effects of warming temperatures on vector physiology — such as increased heart rate and circulation (resulting in sporozoites circulating past the salivary glands too quickly to penetrate) and boosted

immune defences (for example, the production of nitric oxide) — or to direct negative effects of temperature on parasite survival (a development–survival trade-off). Whatever the mechanism, the result challenges the general assumption that 'vector competence' (the probability of a vector becoming infectious following an infectious blood meal) is temperature independent[53], and shows that small changes in ambient temperature can profoundly affect the force of infection (the rate at which susceptible individuals become infected). Furthermore, it demonstrates that temperature can have complex effects on different components of the vector–parasite interaction[54].

Implications

The complex effects of temperature on vector immune function have potentially far reaching implications. How and to what extent mosquitoes (species and populations) differ in their resistance to parasites is central to understanding natural variation in disease dynamics. Recent research indicates that *Anopheles* mosquitoes have the ability to finely discriminate between infection by *Plasmodium falciparum*, a malaria parasite of humans, and *Plasmodium berghei*, a malaria parasite of rodents, through the APL1 family of leucine-rich-repeat proteins, the encoding genes of which are located in a cluster of natural-resistance loci[55]. Specifically, APL1A is downstream of the Imd pathway, which recognizes *P. falciparum*, whereas APL1C is downstream of the Toll pathway, which recognizes *P. berghei*. These contrasting responses have been attributed to the different evolutionary histories of the mosquito–parasite associations[55,56]. However, these parasites have markedly different thermal sensitivities. Temperature differences in experimental conditions could themselves contribute to the differential expression of *APL1C* and *APL1A*, as well as to the variability in infection intensities among different *Plasmodium* spp.[57,58]. Although the development rates of *P. falciparum* and *P. berghei* are similar at their respective temperature optima[50,59], the changes in mosquito physiology (rates of immune enzyme activity and the timing of Imd pathway activation[60]) that are seen as temperature increases from 20 °C to 27 °C, as well as the differences in the infection intensities associated with each parasite species[60], could lead to differences in relative parasite growth rates.

At a more applied level, several of the current and prospective control tools being considered for use within integrated vector management (IVM) strategies[61,62] could be affected by temperature. For example, temperature-dependent toxicity has been demonstrated for a wide range of chemicals targeting a variety of insect pests (for example, blattaria (cockroaches)[63,64], lepidopterans (butterflies and moths)[65,66], dipterans (true flies)[67,68] and coleopterans (beetles and weevils)[65,69]). Although little work has been carried out to examine the effects of temperature on insecticide toxicity for disease vectors, some research suggests that the susceptibility of mosquito malaria vectors to pyrethroids, the most important class of insecticide in contemporary vector control, is affected by temperature[70]. Whether the temperature sensitivity of pyrethroid toxicity is due to changes in the expression or activity of detoxifying enzymes[71,72] or perhaps to alterations in nerve sensitivity[71] remains unknown.

Looking further forwards, it would be surprising if many of the novel transgenic technologies that are currently being developed in vectors to reduce parasite transmission

were not sensitive to temperature changes. Such technologies include the transgenic upregulation of antimicrobial peptides[73], the induction of Imd pathway transcription factors[74] and the expression of other factors such as SM1 and haemolytic C-type lectins[75,76]. SM1 is an effector protein that is secreted into the mosquito midgut after a blood meal and binds to the surface of the midgut and salivary glands, interfering with *Plasmodium* spp. invasion[75], whereas haemolytic C-type lectins lyse erythrocytes in the blood meal and inhibit the formation of *Plasmodium* spp. ookinetes[76]. As is the case for natural immunity, we anticipate that ambient temperature will have an impact on the effectiveness of these transgenic technologies, leading to variation in efficacy across space and time.

Moreover, the establishment and spread of transgenes through mosquito populations will be severely constrained if transgenic manipulation imposes any fitness costs to the vector. Two recent studies, which were carried out under standard insectary conditions, suggest that transgenic manipulation imposes minimal fitness costs to the mosquito vector[74,77]. However, physiologically mediated trade-offs between survival, reproduction and immunity often manifest under variable or stressful conditions (for example, heterogeneity in temperature and diet)[78] and might therefore be easily missed in optimal laboratory environments. It has been suggested that transient[74,79], rather than constitutive[79], upregulation of effector molecules using transgenic technologies will impose minimal fitness costs. However, even transient changes in immunity can have long-term physiological consequences (as seen with immune priming[80,81]), and more work is needed to definitively determine the fitness costs and benefits of these technologies.

Similarly, the success of new vector control tools involving the transinfection of mosquitoes with *Wolbachia* spp. could also be affected by temperature. In natural insect systems, variation in ambient temperature has been shown to significantly influence *Wolbachia* spp. transmission[82,83], dissemination[84,85] and cytoplasmic incompatibility[86,87], as well as their ability to shorten the life of their host[88]. Thus, the desirable transmission-blocking properties of vector infection with *Wolbachia* spp. (such as parasite interference and shortneing of the vector lifespan) that have so far been quantified under largely uniform laboratory conditions might vary considerably in vector populations distributed across thermally variable environments. It should be noted that the possible net outcomes could be very diverse, as they depend on interactions between the mosquito host, the parasite, *Wolbachia* spp. and the environment, all of which could respond in different ways to changes in ambient temperature.

Understanding the effects of temperature on parasite and vector fitness is also extremely relevant for modelling the transmission of vector-borne diseases and for predicting the consequences of future climate change. The risk of vector-borne disease transmission is frequently modelled using the ‘vectorial capacity’, which is a composite metric providing a measure of transmission intensity and is similar to the basic reproductive number, R_0. All the mosquito and parasite life history traits that constitute the vectorial capacity are temperature sensitive, and, consequently, vector-borne diseases such as malaria are expected to be highly responsive to increases in temperature that occur as a result of climate change. However, these traits do not exhibit identical temperature profiles (FIG. 2), so warming

temperatures might not necessarily correlate with an increase in malaria risk. More fundamentally, although numerous modelling studies (including some excellent recent work informing contemporary control strategies[89,90]) integrate aspects of the effects of environmental temperature, none includes the possible effects of temperature on vector resistance, which could be responsible for much of the variation in vector competence over time and space. Modelling work quantifying the spatial and temporal variation in the susceptibility of locusts and grasshoppers to fungal insect pathogens used in biological control provides an interesting illustration of the practical value of understanding temperature-dependent resistance and virulence[91,92].

Conclusions and perspectives

The resistance phenotype, comprising both vector immunity and the fitness of the vector-borne pathogen, is shaped by variations in ambient temperature. The conventional approach of studying vector innate immunity under a narrow set of laboratory conditions overlooks important biological complexity and is unlikely to adequately explain the variation in natural or transgenic resistance of vectors to parasites under field conditions. To move forwards, we need to begin framing our mechanistic understanding of vector immune systems in the ecologically variable world in which vectors and parasites associate.

Further work is needed to establish whether vector immune profiles quantitatively and qualitatively change across different mean temperatures and, if so, to determine the implications for vector resistance. We know of only one study that has explicitly measured the performance of a suite of immune responses across a range of constant temperatures in a vector system[44]. A first basic step should be to integrate relevant environmental variability, such as differences in ambient temperature, into laboratory experiments to evaluate whether vector immune function is sensitive or robust to ecological change. Even just systematically exploring the effects of a range of constant temperatures on vector resistance and on a wider suite of immune parameters, rather than studying vectors at the standard 26 °C or 27 °C, would be informative.

There is now strong evidence that, in addition to the mean temperature condition, the daily temperature fluctuation can be extremely important for a wide variety of ectotherm traits[93–96]. It will therefore be important to establish how fluctuations in daily temperature shape vector immune defences and resistance. Previous work[48] has shown that diurnal temperature fluctuations can greatly shape the fitness of *Plasmodium chabaudi* (a malaria parasite of rodents) in the mosquito vector *A. stephensi*, and that these effects vary depending on the extent of the fluctuation and the baseline mean temperature. Given the dynamic nature of immune responses across a thermal gradient, it is likely that short-term variation in temperature is an extremely important determinant shaping the immune phenotype and mosquito resistance.

Genetic variation in immune defence mechanisms and vector resistance to infection might also be maintained through adaptation to geographically different environments. For example, *D. melanogaster* genotypes sampled from tropical Africa have significantly lower resistance to bacterial infection (by *Providencia rettgeri*) at a low constant temperature (18

°C) than at a high constant temperature (28 °C), suggesting that these fruitflies have undergone adaptation to warmer temperatures. However, in the North American fruitfly populations, there is no evidence for local adaptation to different thermal environments[97]. In a recent study, Raffel *et al*.[98] demonstrated that thermal acclimation is important for resistance of the Cuban treefrog, *Osteopilus septentrionalis*, to the chytrid fungus *Batrachochytrium dendrobatidis*: unpredictable drops in ambient temperature slow down thermal acclimation and increase fungus-induced mortality. The extent to which mosquitoes or parasites become hot or cold adapted and what factors affect this remain largely unexplored.

One further challenge is to distinguish between the direct effects of temperature on parasite fitness and the indirect effects that are mediated through changes in vector physiology. One way to tease apart these effects is to compare parasite development *in vitro* with development *in vivo* across a variable thermal regime. For example, one study measured growth of a pathogenic bacterium both *in vitro* on nutritive plates and *in vivo* in *D. melanogaster*, and demonstrated that the increased resistance of *D. melanogaster* to bacterial infection at cooler temperatures is due to both slower bacterial growth and enhanced immune performance[40]. Similarly, studies on locusts and grasshoppers have shown that warmer body temperatures limit fungal infection owing to both direct negative effects of high temperatures on fungal development and enhanced host immune function[99].

A final step is to validate insights from the laboratory through the study of vector immune function, competence and fitness in field settings. For example, the elucidation of immune mechanisms through gene expression data should, when possible, be validated with measures of functional resistance. Changes in ambient temperature might also affect post-transcriptional processes, which in turn could alter the downstream products of mRNAs so that the levels or activities of these products do not correlate with the gene expression data. Comparative experiments assessing the thermal dependence of innate immune parameters, resistance and fitness for a suite of vector populations sampled from different geographical regions and microclimates would be invaluable in extending our understanding of the heterogeneities in natural refractoriness, and of how a changing climate and landscape might mediate the susceptibility of insect vectors[30,97].

As indicated previously, there are many sources of environmental variation (for example, diurnal and seasonal fluctuations in humidity[100], as well as variation in day length[10], pollution, altitude[101] and the extent of vegetation cover[102], and variation between indoor and outdoor environments[101]) that might interact with ambient temperature to mediate the range of actual temperatures experienced by an insect vector in nature. Predicting how these environmental variables combine to mechanistically influence the suite of factors involved in resistance is clearly a complex problem. That said, not all environmental parameters will have equal effects on vector fitness, physiology and resistance; evidence[14–18] (Supplementary information S1 (table)) suggests that temperature is a strong driver, whereas other variables can have little or no effect. Unravelling patterns and mechanisms from the complexity of natural systems requires an interdisciplinary perspective drawing on insights from ecology, immunology and molecular biology. Adding environmental variability to an

experimental system inevitably scales up the intricacies of that experimental work. But ignoring ecological realities does not make them go away.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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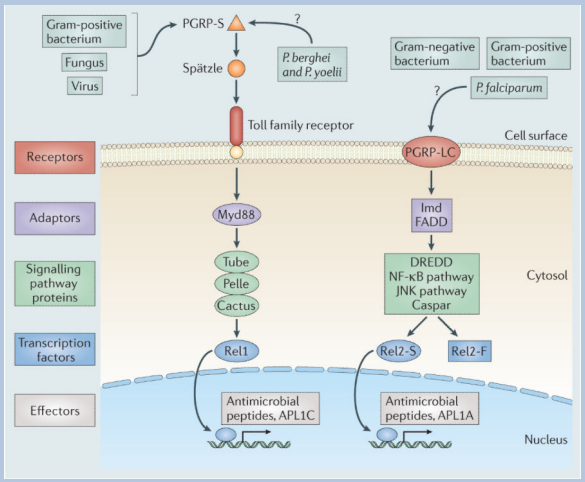
Box 1

Standard insect immune pathways

Different parasites and pathogens have antigens that are recognized as non-self by receptors on host cell surfaces, either directly or through intermediates (for example, Spätzle) (see the figure)[31]. This signal is transmitted into the interior of the cell through a cascade of adaptor and signalling proteins[31]. The Toll pathway is stimulated when a soluble peptidoglycan recognition protein (PGRP-S) binds either peptidoglycan, or an unknown ligand from rodent-infecting *Plasmodium* spp. such as *Plasmodium berghei* and *Plasmodium yoelii*, triggering a signal cascade that causes the ligand Spätzle to bind with the Toll family transmembrane receptor[55]. The immune deficiency (Imd) pathway is stimulated when the transmembrane protein PGRP-LC binds peptidoglycan or a *Plasmodium falciparum* ligand[35,55]. Both of these intracellular cascades, in turn, trigger the activation of transcription factors that pass through the nuclear membrane and initiate the upregulation of immunity genes and the synthesis of effector molecules that result in microbial killing[31]. In the mosquito, the Toll and Imd pathways end when the transcription factors Rel1 and Rel2-S, respectively, translocate into the nucleus and stimulate the expression of genes encoding antimicrobial peptides and genes involved in antimalarial defences (for example, the *APL1* family in the *Plasmodium* resistance island)[1,55].



Box 2

Mosquito–malaria parasite interactions

After a mosquito feeds on a vertebrate host infected with malaria parasites (*Plasmodium* spp.), gametocytes of all *Plasmodium* spp. enter first the anterior midgut and then the posterior midgut of the mosquito with the blood meal. Gametocytes become gametes shortly after entering the midgut, and male gametes fertilize female gametes to form zygotes[58]. Under standard laboratory conditions, zygotes become ookinetes within the first 12–24 hours post-infection and traverse the peritrophic matrix and midgut epithelium to form oocysts under the basal lamina[58]. Oocysts mature and rupture at approximately 14 days post-infection (under standard laboratory conditions), releasing sporozoites into the haemolymph; these sporozoites then migrate to the salivary glands[58].

Ookinetes can be lysed inside the cytoplasm of the midgut epithelial cells[45,103–105] or extracellularly in the basal lamina through the action of the mosquito compliment-like effector molecule TEP1 (REFS 33,45,56). In some strains of the mosquito vector *Anopheles gambiae*, the effector molecule phenoloxidase is released from circulating haemocytes and mediates the melanization of early oocysts that are established in the midgut epithelium[106,107]. Early oocysts can also be killed by nitric oxide produced by midgut epithelial cells, circulating haemocytes and potentially the fat body[108].

In addition to these malaria parasite-specific responses, the mosquito can defend itself from invading bacteria and other pathogens via its complement system and through phagocytosis of invading bacteria by circulating haemocytes (granulocytes); both of these processes require the opsonin TEP1 (REFS 108–110). Finally, antimicrobial peptides are produced systemically by fat body cells and secreted into the haemolymph, and are also produced locally by barrier epithelial cells[31].

It is clear that vector resistance is multifaceted and depends on many interacting traits of both the parasite (for example, growth rate, virulence and persistence strategies) and the vector (for example, immune pathways, stress responses and vector survival). Each trait potentially has a thermal performance curve. Furthermore, temperature can have an impact on parasite traits directly, indirectly (by affecting host defence mechanisms) or, most likely, a combination of both.

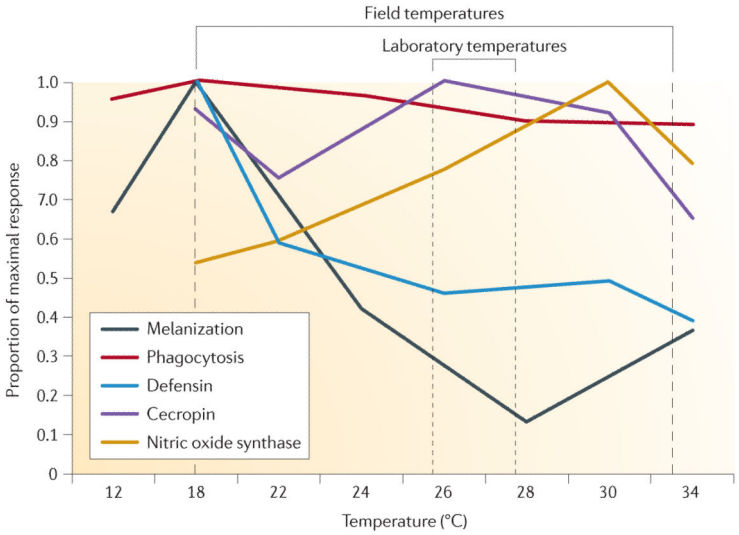


Figure 1. Environmental temperature profoundly affects the rates of a range of humoral and cellular immune responses

Rates of immune and humoral responses are represented as the mean proportion of the maximal response. Melanization and phagocytosis were measured as the proportion of Sephadex beads that were fully melanized or the proportion of haemocytes that had engulfed fluorescent beads, respectively, in mosquitoes housed at different constant temperatures. Expression levels (cDNA counts) of genes encoding two antimicrobial peptides, defensin and cecropin, and nitric oxide synthase are also shown for mosquitoes housed at different constant temperatures. These results clearly indicate that the immune phenotype described under laboratory conditions (26–28 °C) is not the same as the phenotype expressed across the range of possible field temperatures for parasite transmission (18–34 °C), and current laboratory studies on mosquito innate immune functions are missing potentially important biological complexity. Figure is modified from REF. 44.

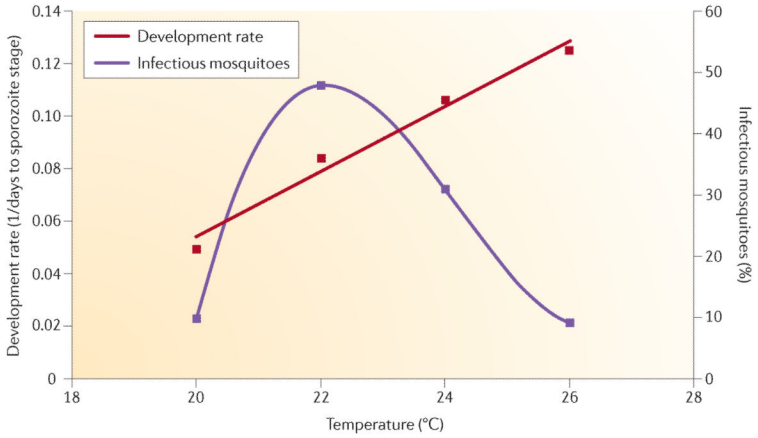


Figure 2. Changes in ambient temperature differentially affect two parasite traits

The proportion of infectious mosquitoes (that is, the proportion with sporozoites in their salivary glands) can be used as a measurement of parasite survival, and parasite development rate is measured as the reciprocal of the number of days taken to reach the sporozoite stage. Although parasites develop to the sporozoite stage at faster rates with increasing ambient temperature, the proportion of infectious mosquitoes sharply declines as the ambient temperature rises above 22 °C. The data shown are for *Plasmodium yoelii* infecting *Anopheles stephensi*. Figure is modified from REF. 53.