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Mutualism meltdown in insects: Bacteria constrain thermal adaptation

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Abstract

Predicting whether and how organisms will successfully cope with climate change presents critical questions for biologists and environmental scientists. Models require knowing how organisms interact with their abiotic environment, as well understanding biotic interactions that include a network of symbioses in which all species are embedded. Bacterial symbionts of insects offer valuable models to examine how microbes can facilitate and constrain adaptation to a changing environment. While some symbionts confer plasticity that accelerates adaptation, long-term bacterial mutualists of insects are characterized by tight lifestyle constraints, genome deterioration, and vulnerability to thermal stress. These essential bacterial partners are eliminated at high temperatures, analogous to the loss of zooanthellae during coral bleaching. Recent field-based studies suggest that thermal sensitivity of bacterial mutualists constrains insect responses. In this sense, highly dependent mutualisms may be the Achilles' heel of thermal responses in insects.

Introduction

Current models of climate change predict a 1.8 – 4°C increase in global mean surface temperature by 2100 [1]. Consequences will be complex and vary by region, season, and latitude. Expected outcomes include severe precipitation events, habitat destruction and fragmentation, and extreme temperatures. For countless species, climate change has already impacted the distributions, timing of growth and reproduction, fitness, and interactions [2]. Whether and how organisms will adapt to the more severe changes yet to come present critical questions for biologists and environmental scientists. In recent years, it has become increasingly clear that microbial symbionts often mediate or constrain adaptation to environmental fluctuations. Given this, our ability to predict how species and communities will respond to climate change may hinge on understanding the ecological significance and versatility of microbial partners.

As small-bodied poikilotherms, insects are tremendously susceptible to extreme temperatures [3]. Thermal tolerance and adaptation in insects is complex, involving a combination of behavioral, physiological, and cellular responses. In addition, insects are prone to establishing associations with bacterial partners, which also influence adaptation to a changing environment. Broadly speaking, microbial symbionts mediate ecologically important traits and therefore may facilitate adaptation to environmental stresses [4]. However, highly dependent interactions tend to be vulnerable to environmental degradation, and the breakdown of such mutualisms through mutualist abandonment or coextinction may accelerate the loss of biodiversity under global change [5]. Long-term, bacteriocyte-associated mutualists of insects exemplify this vulnerability. We have long known exposing insect hosts to heat stress can eliminate these critically important bacteria. Recent work suggests that the thermal tolerance of the fragile bacterial partner may limit the tolerance of the host.

Below, I argue that microbial symbionts are important parts of the puzzle of understanding the drivers, as well as the constraints, of insect adaptation. In particular, this review focuses on limitations imposed by insects' reliance upon heat-susceptible mutualists. I address several questions: Why are these mutualists so vulnerable to heat stress? Is their heat-fragility rooted in predictable, if not inevitable, genomic changes in highly specialized long-term mutualisms? In light of known genomic constraints, what do experimental and ecological data tell us about symbiont heat sensitivity and its impact on host fitness, species distributions, and adaptive potential? Finally, how might symbiont population dynamics, including acquisition of secondary symbionts or symbiont replacement, compensate for the meltdown of an obligate mutualism? Recent studies have a clear message: microbial symbionts can profoundly impact thermal adaptation in insects, both for better and for worse. Predicting specific outcomes will require a deeper understanding of how microbe-host interactions function and evolve under a wide range of ecological scenarios. I focus on studies published in past two years (2010–2011), but not exclusively so.

Thermal responses in insects

As small-bodied animals whose internal temperature varies with the ambient environment, insects are enormously susceptible to extreme temperatures. Lower temperature limits are known to shape distributions of some insect species [6], and so shifts in lower temperatures under climate change are expected to influence insect distributions. Climate change models also predict a higher frequency of extreme heat events. The specific effects of heat waves on insect mortality and distributions are difficult to predict, partly because heat responses in insects are so varied and complex. Plastic responses may involve behaviors to avoid high temperature, as well physiological and cellular mechanisms to cope with heat stress such as expression of heat shock proteins (HSPs) [3]. Thermal sensitivity may vary tremendously across life stages [7] and can depend on the composition of larval diet [8]. Influenced by combined effects of behavior, physiology, and evolutionary history, thermal tolerance can vary widely within insect groups, as recently shown in ants [9].

In addition to plastic responses, genetic changes underlying evolutionary adaptation may include a wide range of behavioral or physiological traits [2]. For species that are living close to their physiological tolerance and are unable to disperse to suitable habitats, adaptation may be the only way to avoid extinction. Genetic changes in heat shock proteins or their regulators may be important in thermal adaptation across a range of species, including insects [10]. In a recent study, a QTL implicated as contributing to thermal adaptation in the lab and field occurs in a genomic region near a known regulator of HSPs [11]. Predicting how insects respond and adapt to increased temperature requires an integrated view of the organisms' evolutionary history, ecology, and physiology.

Microbial contributions to thermal responses

Layered upon this intricate portrait of thermal response is a diverse array of microbial symbionts inhabiting insects. Although rarely considered in studies of thermal adaptation, microbes may facilitate or constrain responses to a changing environment, including temperature extremes. Microbial symbionts may accelerate adaptation by virtue of providing a broadened metabolic repertoire. Many symbionts also provide mechanisms of plasticity through rapid evolution of existing microbial partners or replacement by novel associates. Given their potential contributions to ecological versatility, it is not surprising that microbial partners often expand the range of temperatures in which hosts can thrive. In insects, examples include cold-tolerant fungi that enable winter fungiculture by leafcutter ants, thereby expanding the ecological niche of this association northward [12]. In addition,

heritable, facultative mutualists provide aphid hosts with protection against heat stress [13–15].

However, just as microbial symbionts may facilitate innovation and adaptation, other highly specialized associations may be vulnerable to environmental fluctuations and ultimately constrain adaptation of the host. Theory tells us that mutualistic interactions may be threatened by environmental degradation, and their breakdown may accelerate impacts of global change [5]. Highly dependent mutualisms, in which evolutionary fate of host and symbiont are inexorably tied, are predicted to be among the most vulnerable.

Bacterial mutualists of insects include some of the most specialized and highly constrained mutualisms known in the animal world (recently reviewed by [16]). Obligate mutualists, the main focus of this review, are highly integrated with their hosts, live within specialized host cells (called bacteriocytes), and provide essential nutrients to ~10–15% of insect species [17]. Often the bacteria supplement very specific nutrients that are missing in an unbalanced diet of the host (such as plant sap or vertebrate blood), while also performing more general functions such as nitrogen recycling. These bacteria allow insect hosts to thrive on diets and live in habitats that would be insufficient otherwise. The bacteria are transmitted maternally, typically via the egg. As expected from this transmission mode, the bacteria have coevolved with their respective insect group, often for hundreds of millions of years. Given their highly dependent nature, these associations are exactly the types of mutualisms that may be fragile in the face of a changing environment. Indeed, as discussed below, the evolutionary trajectory of bacteriocyte-associated mutualists leads to consistent patterns of genome degradation, which may constrain adaptation the bacteria and insect host alike and leave these associations vulnerable to climate change.

Genome evolution – a link between lifestyle constraints and heat-sensitivity

Studies of molecular variation in bacteriocyte-associated mutualists, including numerous full genome sequences, have revealed overall trajectory of genome reduction. Typically these mutualist genomes are <800 kb, and often much, much smaller [18]. Patterns of genome reduction and associated sequence features reflect lifestyle-related shifts in fundamental evolutionary processes. For instance, maternal transmission and recurrent population bottlenecks in endosymbionts is coupled to a significant reduction in effective population size (N_e), or the pool of individuals contributing offspring to the next generation [19–22]. This reduction in N_e is thought to accelerate the fixation of slightly deleterious mutations [23]. In addition, while host-dependent microbes include species with high recombination rates, obligate mutualists of insects show reduced recombination (even complete asexuality) that may reflect the loss of recombination genes, mobile DNA, and opportunities for gene exchange due to their host-associated lifestyle [24] and that exacerbates effects of genetic drift under reduced N_e . While notable exceptions exist (highlighted in [18]), these mutualists usually show extreme AT base compositional biases, first documented by Ohtaka and Ishikawa [25]. These mutualists lack many DNA repair functions, so their genomes may manifest greater exposure to the universal GC→AT biased mutation in bacteria explained by high rates of C/G to T/A transitions [26]. Finally, obligate mutualists that have lived for millions of years within insect cells have surely experienced relaxed selection on metabolic functions that are redundant in a host cellular environment.

At a protein and DNA sequence level, an expected consequence of genome-wide accumulation of deleterious mutations is reduced stability of encoded proteins and structural RNAs. Supporting this idea, the first molecular evolutionary analyses of insect mutualists revealed an unusually low structural stability of 16S rRNA and excess of nonsynonymous substitutions in proteins [23]. Assessing this effect genome-wide, Van Ham [27] found that

proteins in obligately intracellular bacteria, including long-term insect mutualists, had destabilizing amino acid substitutions expected to reduce the efficiency of protein folding, leading to possible misfolding and aggregation. These mutualists constitutively over-express the chaperonin GroEL, called 'symbionin' when discovered in *Buchnera* of aphids due to its high abundance in cells of this mutualist [28], perhaps as a compensatory mechanism to cope with degraded protein structures [23].

Constraints on gene exchange in obligate mutualists have important implications for their adaptive potential. Unlike most bacteria that experience frequent gene acquisition from foreign sources, these mutualists seem constrained to a one-way road of gene loss and, therefore, must live with the functions they have. In the absence of gene acquisition, once a function is lost, it is forever lost in that particular lineage. Genome sequences have shown the surprising absence of nutritional functions in certain mutualist lineages (e.g. *cys* genes in *Buchnera* of the green bug aphid *Schizaphis graminum* [29], loss of *glnA* in *Blochmannia* of the ant *Camponotus vafer* [30]). This irreversible gene loss may constrain the evolutionary potential of the symbiont and host alike. Moreover, a substantial loss of regulation genes explains why mutualists show only a modest shift in transcript abundances in response to heat shock [31], distinct diet treatments [32] and host developmental stages [33].

In sum, the specialized lifestyle of obligate mutualists in insects alters fundamental processes of genome evolution, thereby reducing protein stability, limiting the generation of evolutionary novelty via gene exchange, and narrowing functional plasticity via transcriptional responses. Each of these features may constrain the response of the insect-bacterial partnership in the face of changing environmental parameters, including temperature.

Heat-sensitivity of mutualists, in the lab and field

The prediction that symbionts are heat-sensitive has been borne out by lab and field experimental studies. We know that many bacteriocyte-associated endosymbionts have reduced densities or are lost entirely when the insect host is exposed to high temperatures. Results are difficult to compare across studies, due to variation in experimental conditions (diet fed, duration of heat treatment, humidity, etc.), the potential contributions of facultative symbionts that may or may not have been screened, and genetic variation among host and symbiont strains. Despite this variation in experimental conditions, results clearly point to heat-sensitivity of several bacteriocyte-associated mutualists (Figure 1).

Buchner [17] noted that high temperatures reduce symbiont survival across a range of insect hosts. Following this, early work showed that aphid nymphs treated at 37°C for up to 3 hours had slower growth and, depending on duration of heat treatment, did not produce progeny and lacked 'normal' *Buchnera* [34]. Further work showed that temperatures as low as 25–30°C caused densities of *Buchnera* in aphids to decline [13]. Longer-term heat treatment of weevils (35°C for 1 month) was used to eliminate the *Sitophilus oryzae* (cereal weevil) primary endosymbiont, or SOPE [35]. In cockroaches, various heat treatments were found to destroy some bacteria, but the authors did not identify conditions that completely eliminated bacteria without also killing the roach host (presumably due to heat stress) [36]. More recently, Sacchi et al. [37] found that cockroach endosymbionts deteriorate under heat stress of 39°C for several (>18) days. While additional experiments in diverse host-symbiont associations are needed to test the generality of this trend, current data indicate that many primary symbionts are thermosensitive. As a point of contrast, the thermal niche (or, range over which 75% of maximal growth rate occurs) for the related free-living bacteria *E. coli* is 28.5–41.0°C [38], and *E. coli* can survive up to 55°C.

While the mechanisms underlying heat-susceptibility of obligate mutualists of insects are unclear, they may involve the distinct features of these reduced bacterial genomes (Figure 2). Despite high levels of GroEL in endosymbiont cells, degraded mutualist proteins may be susceptible to destabilization under heat stress. Moreover, low stability of structural RNAs [23] could contribute to thermal sensitivity. In addition, the typically extreme AT-rich DNA of mutualists (though fascinating exceptions exist [18]) could be fragile to heat, due to the lower thermostability of AT pairs versus GC pairs, and perhaps far more important, the destabilizing effects of AT pairs on DNA stacking [39]. Finally, the loss of cell-surface proteins means that endosymbiont cells may be quite fragile. Many mutualist lineages have a minimal cell wall and membrane or have lost these entirely and rely on host-derived membranes. While beyond the scope of this review, heat treatment in the range of 35–40°C can also eliminate facultative symbionts such as *Wolbachia* and *Spiroplasma* (e.g., [40] and [41], respectively), suggesting a general mechanism of heat susceptibility. Notably, these transient symbionts show many of the same genomic features of obligate mutualists (genome reduction, AT bias, etc.), though typically less severely.

It is also possible that heat susceptibility of endosymbionts involves disruption of host functions that are involved in symbiont maintenance. In fact, the relatively moderate GC content of (54% [42]) of the heat-sensitive weevil endosymbiont (SOPE) suggests alternative, or additional, mechanisms of temperature vulnerability. While the precise mechanisms remain uncertain, it is increasingly clear that vulnerability to heat stress impacts insect-bacterial symbioses in natural field settings, as detailed below.

Real world examples

Insect-bacterial symbioses have proved to be useful models to understand bacterial contributions to animal nutritional physiology and development, as well as the impact of endosymbiosis on bacterial genome evolution. From an ecological standpoint, these associations are increasingly visible models to explore integrated responses to environmental fluctuations, including climate change.

A recent study of pentatomid stinkbug species [43] explored temperature sensitivity of bacterial symbionts that live within crypts in the animals' hindguts. The lifestyle of these hindgut symbionts differs somewhat from that of bacteriocyte-associated symbionts. The symbionts of pentatomids, while vertically inherited, are transmitted when females smear of bacteria onto the egg, rather than via transovarial transmission [44]. Host species carry one major bacterium in their midgut, and symbiont monophyly occurs within host genera; however, the bacterial group is polyphyletic and symbionts apparently are more evolutionary labile than bacteriocyte associated mutualists [45]. (Some stinkbug symbionts show patterns of genome degradation that mirror that in bacteriocyte-associated mutualists [46], but this has not been demonstrated in pentatomid hosts.) Despite some differences in lifestyle compared to bacteriocyte-associated mutualists, I describe the stinkbug system here because it illustrates how microbial symbionts can mediate the impact of increased temperature on host fitness and, potentially, mediate shifts in host species distributions.

Specifically, Prado et al. [43] found that a 5°C elevation in temperature (from 25°C to 30°C) resulted in symbiont elimination from two pentatomid stinkbug species. Symbiont loss, in turn, caused a significant reduction in the fitness of the insect host. In this example, the impact of temperature on host fitness is a clear consequence of symbiont death rather than a direct effect of heat on host biology. This striking result informs our interpretation of recent ecological studies of this insect host. Notably, in the past 50 years, two pentatomid species in Japan have shifted to cooler areas that are northward, or at higher elevations, compared to the previous species range [47]. In addition, field studies simulated climate change and

confirmed the deleterious impact of heat on host fitness [48]. Combined, these results present the intriguing possibility that heat-fragile bacterial symbionts shape the thermal response of the insect host. As noted by Musolin et al. [48] and echoed by Prado et al. [43], mechanisms underlying the deleterious effects of heat on the stinkbug host are unknown, but may relate to malfunction of the gut symbiotic bacterial fauna under high temperature extremes. These findings suggest that symbiont loss under high temperatures may influence insect fitness and drive shifts in species distributions.

Second, a heat-sensitive mutation in *Buchnera* illustrates how genetic variability among mutualists can influence the fitness of hosts in a temperature-related manner. In *Buchnera*, a recurrent mutation at a homopolymer within regulatory region of the gene *ibpA* disrupts the expression of this small heatshock protein. This disruption further reduces the (already modest) heat shock response of *Buchnera*. This mutation affects host-fitness in a temperature-dependent fashion [49]. Aphids containing *Buchnera* with this mutation have an advantage under cool temperatures, but are more vulnerable to thermal stress in lab [49] and field [50] settings. The distinct fitness outcomes at low versus high temperatures highlight a potential trade-off in thermal adaptation [51]. In a study of aphid strains representing diverse natural populations, Burke et al. [52] found that the heat sensitive mutation occurs rarely, in <3% of aphids screened. This result is consistent with selection favoring *IbpA* production. Combined, these studies illustrate that genetic variation in *Buchnera* can allow aphids to adapt to current temperature regimes. The fact this endosymbiont mutation is not more common in natural aphid populations suggests that hosts experience strong selection for tolerance of warm or otherwise stressful conditions.

Rescuing effects of other players

As noted above, facultative mutualists provide aphid hosts with context-specific benefits. In particular, *Serratia symbiotica* and *Hamiltonella defensa* protect aphid hosts against heat stress [13] [14,15]. Not surprisingly, the infection of pea aphids with *S. symbiotica* reaches up to 80% in warm habitats, such as the Central Valley of California where this aphid species is a pest [15]. The mechanism by which facultative symbionts protect aphids from heat stress is uncertain. It may involve shielding *Buchnera* from effects of heat. Supporting this, the presence of *S. symbiotica* increases the persistence of bacteriocytes (and therefore probably *Buchnera*) in aphids under heat stress [13]. Alternatively, facultative symbionts may compensate for the loss of obligate mutualists due to heat [53].

On evolutionary timescales, such compensation of obligate mutualist functions by a facultative symbiont might be the first step in the adoption of a second stable mutualist in insects, or the wholesale replacement of an existing mutualist lineage. For instance, replacement of obligate mutualists in weevils is well documented [54], and recent data suggest a *Sodalis*-like bacterial associate may be involved [55]. In addition, a recent study of the Cedar aphid highlights an evolutionarily stable integration of a *S. symbiotica*-related lineage with *Buchnera* in a dual symbiosis [56].

The long-term coevolution of many insect-bacterial mutualisms (as long as 200 MY in the aphid-*Buchnera* partnership) encompassed periods when the Earth experienced hotter temperatures. For example, intense and abrupt intervals of global warming occurred during the late Paleocene ~53 MYA [57]. It is unclear how these historical warming events influenced insect-bacterial partnerships, or whether secondary symbionts might have played some role during these periods.

Conclusions

Climate change is already influencing the distributions, growth and reproduction, fitness, and interactions of countless species. The more severe changes yet to come include increased frequency of extreme temperatures and precipitation, and associated habitat fragmentation and destruction. Biologists and environmental scientists are now faced with the daunting task of developing predictive models for whether and how organisms will respond and adapt to these changes. Increasingly, these models account for the widespread and profound importance of symbiotic interactions.

The wide diversity of bacteria associated with insects offer valuable models to explore how microbial symbionts might facilitate and constrain adaptation to a changing environment. Many recent studies illustrate that symbiosis can drive ecological innovation and accelerate adaptation. However, the exceptionally stable, long-term mutualists of insects are vulnerable to thermal stress. We have long known that high temperatures can eliminate these mutualistic partners from insect hosts, analogous to corals' loss of zooxanthellae that provide up to 90% of host nutritional requirements, or coral 'bleaching' [58]. More recently, studies have shown that heat-induced loss of symbionts may constrain the thermal niche of insect hosts. For example, heat-vulnerability of vertically transmitted stinkbug symbionts may explain the shift in host species distributions toward cooler regions.

Valuable directions for future work will include integrating results from lab-based studies with field-based sampling and experiments. Apart from a few studies, there has been little attention to measuring and testing the effects of ecologically relevant temperatures on symbiont densities and functions. While we know that high temperatures can eliminate obligate mutualists in controlled environments, are those ecologically realistic in natural insect populations? To what extent does the insect host adjust its behavior to avoid heat stress? Although obligate mutualisms are constrained ecologically and genetically, what mechanisms for adaptation exist? In addition, facultative mutualists are relatively poorly studied outside of aphids. What is the diversity and frequency of facultative mutualists in other host groups, and what effects do they have on hosts? Do they confer heat protection, and if so, by what mechanism? Only by understanding the contributions of microbial symbionts to insect physiology and ecology can we hope to predict how these animals will respond and adapt to a hotter climate.

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Figure 1. Examples of conditions under which obligate mutualists are eliminated from insects
Examples shown are detailed in the text.

Figure 2. Potential mechanisms underlying heat-susceptibility of obligate mutualists

Heat sensitivity may be rooted in the distinct features of these reduced bacterial genomes. Clockwise: Despite high expression of GroEL, degraded proteins may have unstable structures and be susceptible to destabilization by heat stress. Moreover, thermolabile structural RNAs [23] could contribute to heat sensitivity. In addition, the typically extreme AT-rich DNA of mutualists (though fascinating exceptions exist [18]) could be unstable under high temperatures, due to the lower thermostability of AT pairs or destabilizing effects of AT pairs on DNA stacking [39]. The loss of membrane proteins may reduce the stability of endosymbiont cells. In addition to these features, it is also possible that heat susceptibility of endosymbionts involves disruption of host functions that are involved in symbiont maintenance.