



# Microbial symbionts expanding or constraining abiotic niche space in insects

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In addition to their well-studied contributions to their host's nutrition, digestion, and defense, microbial symbionts of insects are increasingly found to affect their host's response toward abiotic stressors. In particular, symbiotic microbes can reduce or enhance tolerance to temperature extremes, improve desiccation resistance by aiding cuticle biosynthesis and sclerotization, and detoxify heavy metals. As such, individual symbionts or microbial communities can expand or constrain the abiotic niche space of their host and determine its adaptability to fluctuating environments. In light of the increasing impact of humans on climate and environment, a better understanding of host-microbe interactions is necessary to predict how different insect species will respond to changes in abiotic conditions.

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## Introduction

Beneficial microbial symbionts are pervasive in insects and can provide functional traits to their hosts that allow them to adapt to novel ecological niches [1]. To date, the majority of microbial mutualists that have been functionally characterized in insects support their host in coping with challenges when confronted with biotic interaction partners, that is, food plants [2], pathogens, parasites, parasitoids, or predators [3]. Less is known, however, about the influence of microbial symbionts on insects' abiotic niche spaces, although evidence is accumulating that symbiotic microbes can affect the host's thermal tolerance [4\*,5] and desiccation resistance [6\*\*], as well as sensitivity toward inorganic toxicants [7]. For temperature in particular, it is becoming increasingly clear that microbes can expand the viable range of their

host by providing traits that enhance thermal tolerance but in other cases may limit adaptation to changing temperatures, for example, when obligate mutualists show a narrow window of temperature tolerance, or community members exhibit different responses to temperature fluctuations [4\*]. As such, microbial symbionts can both expand and constrain abiotic niche space, which has important implications for their hosts' adaptations to novel habitats and their response to environmental fluctuations, aspects that are important in light of increasingly rapid environmental change [5,8].

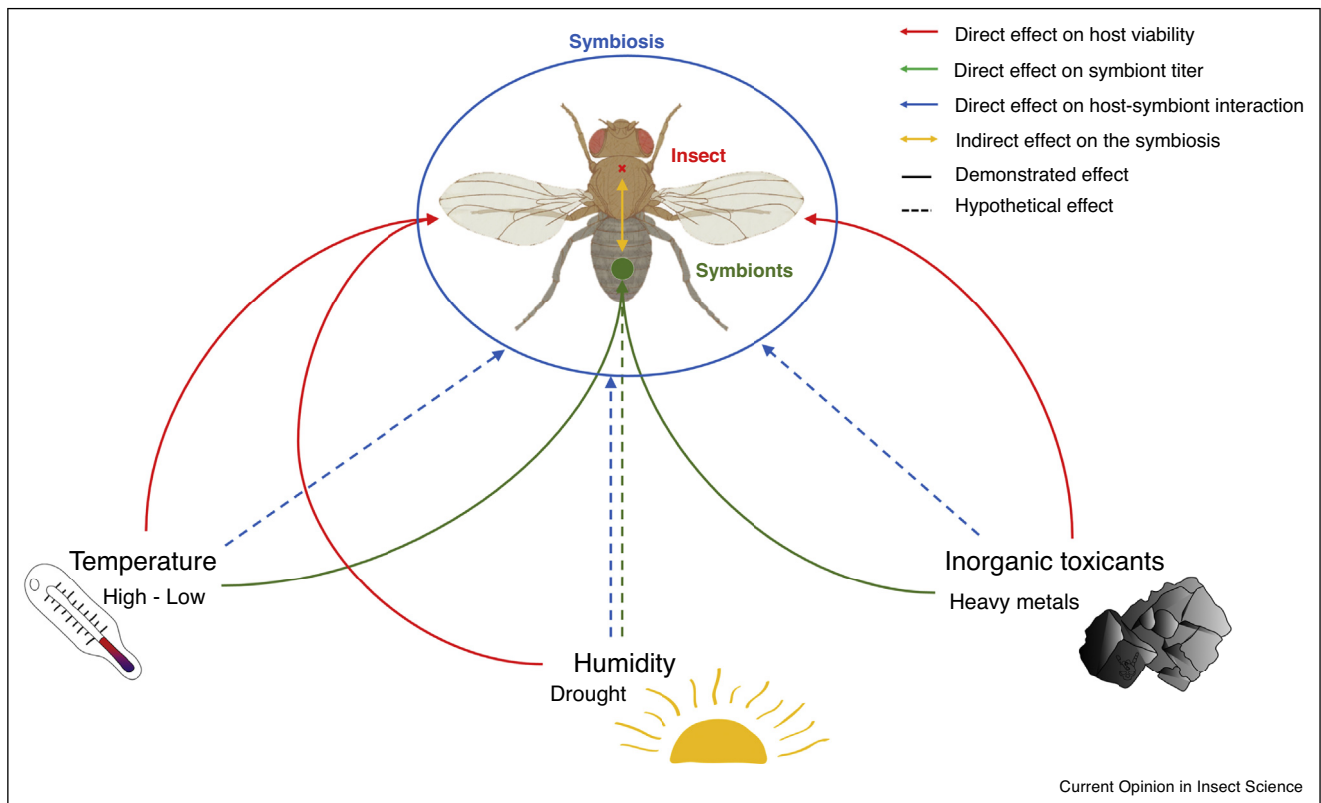
## Impact of temperature on the stability of symbioses

Temperature can affect both host and its microbial associates in a symbiosis [4\*]. Because of its effect on the speed of chemical reactions, thermal variation has a strong impact on host metabolism, and extreme temperatures threaten the host's survival and fertility [9]. In addition, obligate symbionts that provide essential services to their host can be depleted through thermal stress, which in most cases destabilizes the association, leading to a fitness decrease in both partners and thereby constraining the host's viable temperature range (Figure 1). In insects, such disruptions were observed in Hymenoptera [10], Hemiptera [11,12,13\*,14], Coleoptera [6\*\*,15,16\*\*,17], and Psocoptera [18], reflecting the severe impact of temperature on the stability of mutualistic associations. In the pea aphid, *Acyrtosiphon pisum* (Hemiptera, Aphididae), heat stress leads to the depletion of obligate endosymbionts (*Buchnera*), decreased fertility, and delayed developmental time [19]. Similarly, gut symbiont populations of stinkbugs are disturbed when exposed to high temperature, which leads to decreased growth and survival of the host. *Wolbachia*, which is estimated to infect more than half of all insect species [20], has been found to be highly temperature-sensitive in various insect hosts [21,22], possibly affecting the host-symbiont interaction. Recent reviews summarized temperature effects on symbiosis stability, revealing many obligate symbionts as their hosts' 'Achilles' heels' in the context of temperature change [4\*,5].

## Symbionts increasing their host's viable temperature range

Temperature-driven symbiont depletion, however does not necessarily result in the extinction of the host. The negative effects of thermal stress on the host-symbiont association can be mitigated by changes in the mutualist's own metabolic activity [23], through protection by

Figure 1



Effects of abiotic factors on insects, their associated microbial symbionts, and the symbiotic interaction. Changes in these abiotic factors can directly affect the host, microbial symbionts and/or intrinsic parameters of the interaction. Modification in one of the partner's viability or titer can affect the other's or can be reversed by the other partner's action, changing indirectly the nature of the symbiotic interaction. To our knowledge, a direct effect of an abiotic factor on a symbiotic interaction remains to be demonstrated, but a conceivable scenario could be that a temperature increase affects the functioning or stability of a transport protein, thereby disabling the supplementation of a metabolite from one partner to the other. In this hypothetical example, the modification of the transport protein does not directly affect the survival of the host or its symbiont, but impacts an intrinsic parameter of the symbiosis (i.e. metabolite exchange) and thereby the fitness effects of the association on the interaction partners.

secondary symbionts [24,25], or via symbiont-mediated modulation of host traits, such as gene expression [26] and behavior [27]. Such buffering of the consequences of thermal stress may thus enable an expansion of the insect's viable temperature range.

In *A. pisum*, two *Buchnera* symbiont strains co-exist with variable frequencies in natural populations. They differ in a single point mutation in a gene coding for a heat shock protein [28] that acts as a chaperone during stress events, such as heat exposure. This chaperone stabilizes proteins that ensure host cell homeostasis and integrity and thereby enhances host survival upon heat shock [29]. While the heat-sensitive variant is beneficial under intermediate temperatures, the tolerant one allows its host to perform better under higher temperatures. Those heat-sensitive variants are not homogeneously found in all aphid species resulting in heat tolerance for some (e.g. *Aphis gossypii*, Hemiptera, Aphididae) and possibly

explaining the presence of such aphids in warmer environments [30]. Thus, genetic variation in the aphid-*Buchnera* association allows it to adapt to fluctuating environments across geographic ranges and seasons [23,28,30,31].

In addition to obligate symbionts adapting directly to extreme temperatures, secondary symbionts can mitigate the impact of heat stress on the obligate symbionts and thereby maintain host survival. Again in the pea aphid, the secondary symbiont *Serratia symbiotica* has been found to compensate for *Buchnera* depletion after heat shock by lysis of part its own population, thereby releasing protective metabolites that partially rescue obligate symbiont titers and host fitness [25,32]. Similarly, heat-shocked eggs of *Diaphorina citri* (Hemiptera, Liviidae) mount an increased lysosomal degradation of some of their symbionts, which provides nutrients to the surviving symbionts as well as to the host [24].

Finally, microbial partners can modify host traits such as behavior, gene expression, and nutritional status to alleviate the consequences of thermal stress. For example, the thermal preference of *Drosophila melanogaster* (Diptera, Drosophilidae) is influenced by its respective *Wolbachia* strain [27<sup>\*</sup>], reflecting manipulation of the host's behavior by the symbiont to achieve its own optimal temperature. Although other studies also provide evidence that *Wolbachia* is involved in thermal tolerance mechanisms and modifies its host's viable temperature range, many factors such as pathogens, additional symbionts, and host traits modify host fitness in heat-exposed *Wolbachia*-infected hosts, thereby hindering a general understanding of the implications of *Wolbachia* infections for their hosts' viable temperature ranges [27<sup>\*</sup>,33,34]. In whiteflies, *Bemisia tabaci* (Hemiptera, Aleyrodidae), thermal tolerance is achieved by altering host gene expression through a secondary symbiont. *Rickettsia*, present in most of the body tissues in normal rearing conditions, induces expression of host cytoskeleton genes that support cell homeostasis under heat stress and thereby prevent cell lysis [26]. In *D. melanogaster*, disruption of its gut microbiota leads to decreased cold tolerance [35<sup>\*</sup>] that can be rescued by supplementing a single member of its natural microbiota, the yeast *Lachancea kluyveri* [36]. Although these results indicate that gut microbes affect cold tolerance in fruit flies, it remains to be investigated whether this is the result of a symbiotic relationship or a passive nutritional supplementation of metabolites from digested microbial cells.

In nature, adaptation to fluctuating temperatures is required for coping with seasonal changes and geographic range expansions. In this context, dynamic multipartite symbioses can confer adaptability by flexibly replacing symbionts with partners that allow the symbiotic association to persist under the given environmental conditions. In the multipartite ectosymbiosis between bark beetles and fungi, temperature plays a key role in determining the relative abundance of two mutualistic fungi [15]. One symbiont sporulates at warm temperatures, whereas the other one prefers cold temperatures. By swapping symbionts, the host is therefore able to expand its viable temperature range [15]. In Alaska, the Mountain pine beetle *Dendroctonus ponderosae* (Coleoptera, Curculionidae) that can endure both boreal temperatures (−20°C) and summer heat (up to 30°C) is associated with three different fungi that are found in different relative abundances depending on the season [37]. Similarly, Japanese chestnut weevils *Curculio sikkimensis* (Coleoptera, Curculionidae) harbor different endosymbiotic communities according to the geographic location across the island, with *Sodalis*, *Rickettsia* and *Wolbachia* being more abundant in high temperature sites [38]. The consequences of these multipartite symbioses for temperature acclimation have yet to be elucidated, but their dynamics may have implications for host fitness.

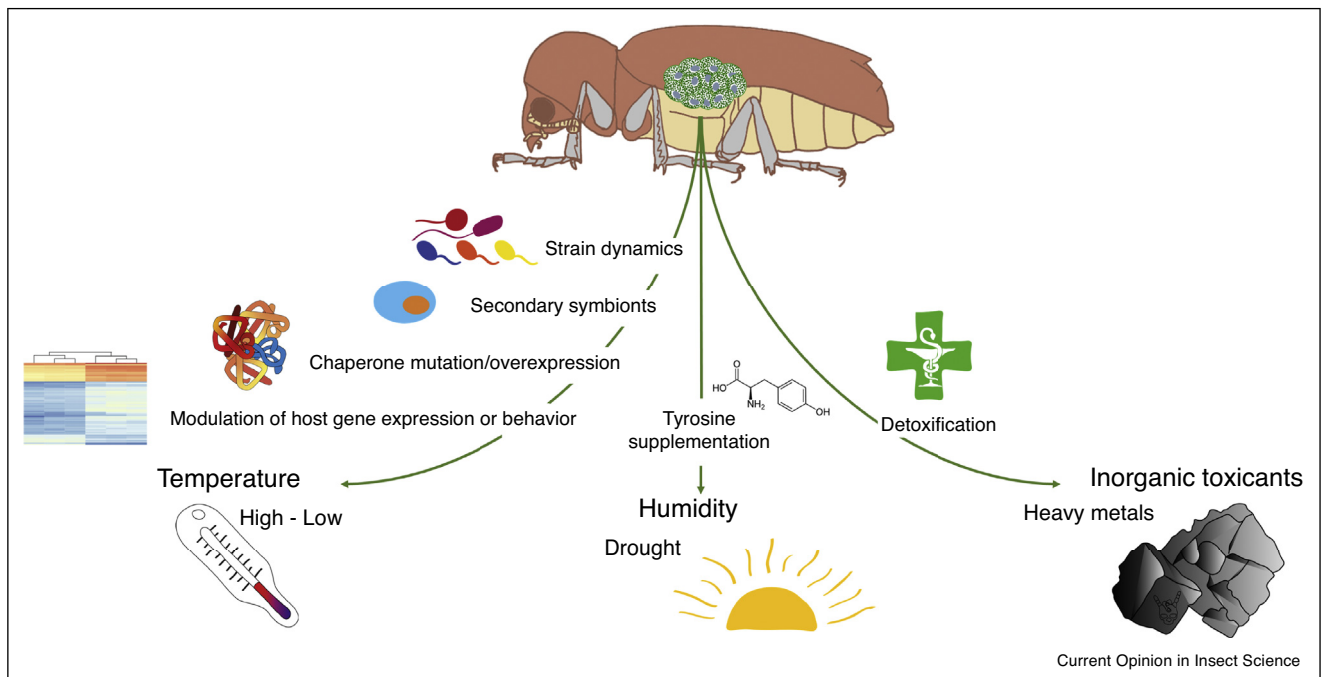
### Symbionts enhancing desiccation resistance

Low ambient humidity presents a substantial challenge to insects due to the high risk of desiccation. Compensatory adaptations to such dry conditions can be behavioral (staying in humid microenvironments, searching for water, controlled breathing to reduce water loss through open tracheae) or physio-chemical by improving the primary evaporation barrier — the insect cuticle [39]. The insect cuticle is primarily composed of chitin, polymer fibrils of *N*-acetyl-glucosamine, embedded in a matrix of proteins. It can be further modified through the two processes of melanization and sclerotization, that is, the incorporation of pigments and phenolic compounds that lead to increased coloration as well as stiffness and hardness, respectively [39]. The incorporation of these hydrophobic, aromatic compounds influences water evaporation, not only by increasing the thickness and density of the cuticle, but also by enhancing its hydrophobicity [40]. In addition, insects add another surface layer, the epicuticle, that typically consists mostly of hydrocarbons and primarily helps to prevent water loss, but secondarily often serves communication purposes [39]. While components of the epicuticle are mostly hydrocarbon-based and can easily be derived from carbohydrates and fatty acids within the insects' own metabolism [41], the phenolic compounds used for melanization and sclerotization of the exocuticle are derived from limited nutrients that insects are unable to produce *de novo*, particularly the aromatic amino acid tyrosine.

Tyrosine is usually considered to be semi-essential as insects can synthesize it from dietary phenylalanine, but they cannot produce it *de novo*. Particularly, plant-based diets are often low in tyrosine and phenylalanine content, effectively limiting availability of these aromatic amino acids for herbivorous insects. This is especially problematic during the important developmental phase of metamorphosis in holometabolous insects, when adults have to rapidly synthesize their new cuticle, as they would otherwise be unprotected for an extended period of time. Multiple studies provide evidence for phylogenetically distinct microbial symbionts across different insect taxa (including the beetle families Bostrychidae, Curculionidae and Silvanidae and the ant subfamilies Formicinae and Myrmicinae) that provide exclusively [16<sup>\*\*</sup>,42], or among other benefits [43–47,48<sup>\*\*</sup>], precursors for aromatic amino acids as essential building blocks for cuticle synthesis (Figure 2). Concordantly, reductions in cuticle thickness and hardening were observed in insects experimentally deprived of symbionts (*Camponotus fellah* (Hymenoptera, Formicidae) [49,50], *Sitophilus oryzae*, *Euscepes postfasciatus* and *Pachyrhynchus infernalis* (Coleoptera, Curculionidae) [16<sup>\*\*</sup>,48<sup>\*\*</sup>,51], and *Oryzaephilus surinamensis* (Coleoptera, Silvanidae) [6<sup>\*\*</sup>,52]).

The ecological benefits of a symbiont-mediated increase in cuticle thickness may include improved mechanical

Figure 2



Mechanistic basis of described symbiont-conferred adaptations to abiotic conditions including temperature, humidity and inorganic toxicants.

defenses against predators, parasitoids, and pathogens (especially entomopathogenic fungi that can actively penetrate the cuticle) as well as enhanced desiccation resistance. However, experimental studies assessing these effects remain scarce. While the mechanistic basis of symbiont-mediated tyrosine and L-DOPA supplementation as well as the implications for the host's growth, reproduction, and mortality have been characterized [16<sup>••</sup>,48<sup>••</sup>,51,53–55], only one study assessed the ecological consequences for the host: Engl *et al.* demonstrated that the thicker cuticle results in enhanced protection against water loss and increased population growth of symbiotic versus aposymbiotic insects upon exposure to low humidity [6<sup>••</sup>]. Thus, the supplementation of aromatic amino acids or their precursors can enable the host to survive unfavorable conditions, enabling it to invade ecological niches with otherwise prohibitively low ambient humidity, including anthropogenic habitats like grain storage facilities. While multiple grain pest beetle taxa live under dry conditions and likely benefit from enhanced desiccation resistance through the symbionts' effect on cuticle biosynthesis, other ecological benefits (e.g. defense) have been proposed but not demonstrated yet. Thus, we anticipate such symbiotic alliances to be more widespread and ecologically important than currently recognized, expanding the abiotic and biotic niche spaces of their hosts, as the insect cuticle serves as the primary barrier preventing water loss but also providing defense against antagonists.

### Symbiont modulation of host responses to inorganic chemicals

Inorganic chemicals constitute potential sources of energy and nutrition, but can also exert toxic effects on organisms. In marine invertebrates and terrestrial plants, it is well-established that microbial symbionts can allow for the exploitation of inorganic nutrient or energy sources that enable the host to colonize otherwise uninhabitable environments [56–58]. In insects, fixation of atmospheric nitrogen by host-associated microbes has been suggested for many taxa and may allow for the host's adaptation to nitrogen-poor diets. Despite numerous reports on diazotrophic microbes associated with insects, direct evidence for nitrogen fixation currently remains restricted to wood-feeding termites, beetles, and wasps, as well as leaf-cutter ants and fruit flies, and the contributions to the hosts' nitrogen budgets remain largely unknown [59,60]. As the literature on symbiotic nitrogen fixation in insects has been recently summarized in an excellent review [59], we focus here on symbiont-mediated effects on host resistance to inorganic toxicants.

Heavy metals occur in naturally or anthropogenically contaminated soils, and they can be taken up by plants [61] and transferred to insects and other animals through the food web [62]. There is evidence for rodents, earthworms and spiders that heavy metal exposure can alter the gut microbiota [63–66] and, in turn, microbes can detoxify or sequester toxic metals in mice and rats

[67–69]. By contrast, little is known about the interaction between heavy metal exposure and symbiotic microbes in insects, despite the deleterious impact of heavy metal accumulation on health, behavior, and fitness of insects, particularly pollinators [70]. In bumblebees, exposure to ecologically relevant concentrations of selenate resulted in changes in gut microbiota composition, as well as increased mortality in microbiota-deprived individuals [71]. Concordantly, two bumblebee-associated microbes (*Snodgrassella alvi* and *Lactobacillus bombicola*) were resistant to selenate *in vitro* and contained selenate detoxification genes in their genomes [71]. Similarly, the egg masses of aquatic chironomid midges (Diptera, Chironomidae) appear to be protected from heavy metals by detoxifying microbes, as revealed by the isolation of bacteria that detoxify heavy metals *in vitro* from eggs and larvae as well as bioassays showing reduced survival of symbiont-deprived individuals after exposure to lead or chromium as compared to symbiotic controls [7,72]. Finally, a selenate-reducing strain of *Alcaligenes faecalis* was recently isolated from the larval gut of the cerambycid beetle *Monochamus alternatus* (Coleoptera, Cerambycidae) [73], but the importance of selenate detoxification for the beetle remains unexplored.

## Conclusions

It is becoming increasingly clear that abiotic factors not only directly impact the fitness of insects but also affect the abundance and functionality of insect-associated microbes with indirect implications for the host's fitness. Conversely, symbionts can also expand the abiotic niche space of their insect host by improving its physiological state or by providing essential nutrients or protective enzymes. However, as compared to the wealth of knowledge on the impact of microbial symbionts on their hosts' interactions with biotic partners, the consequences of microbial associates for the hosts' expansion or contraction of abiotic niche spaces remain poorly understood, especially regarding the mechanistic basis and ecological implications of symbiont-provided protection from abiotic stress. In light of increasingly rapid changes in abiotic factors due to human activities (especially temperature and humidity, but also inorganic toxicants), a better understanding of microbial contributions to their insect host's viable range of abiotic conditions and adaptive potential is of high relevance for predicting insects' responses to anthropogenic environmental change.

## Conflict of interest statement

Nothing declared.

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