Opinion

A perspective on insect–microbe holobionts facing thermal fluctuations in a climate-change context

Corentin Iltis [ID],[1*†] Kévin Tougeron [ID],[1,2†]
Thierry Hance [ID],[1] Philippe Louâpre [ID][3] and
Vincent Foray [ID][4]

[1]*Earth and Life Institute, Biodiversity Research Center, Université catholique de Louvain, Croix du Sud 4-5, Louvain-la-Neuve, 1348, Belgium.*

[2]*UMR CNRS 7058 EDYSAN (Ecologie et Dynamique des Systèmes Anthropisés), Université de Picardie Jules Verne, 33 rue St Leu, Amiens, 80039, France.*

[3]*UMR CNRS 6282 Biogéosciences, Université Bourgogne Franche-Comté, 6 Boulevard Gabriel, Dijon, 21000, France.*

[4]*UMR CNRS 7261 Institut de Recherche sur la Biologie de l'Insecte, Université de Tours, Parc Grandmont, Tours, 37200, France.*

Summary

Temperature influences the ecology and evolution of insects and their symbionts by impacting each partner independently and their interactions, considering the holobiont as a primary unit of selection. There are sound data about the responses of these partnerships to constant temperatures and sporadic thermal stress (mostly heat shock). However, the current understanding of the thermal ecology of insect–microbe holobionts remains patchy because the complex thermal fluctuations (at different spatial and temporal scales) experienced by these organisms in nature have often been overlooked experimentally. This may drastically constrain our ability to predict the fate of mutualistic interactions under climate change, which will alter both mean temperatures and thermal variability. Here, we tackle down these issues by focusing on the effects of temperature fluctuations on the evolutionary ecology of insect–microbe holobionts. We propose potentially worth-investigating research avenues to

(i) evaluate the relevance of theoretical concepts used to predict the biological impacts of temperature fluctuations when applied to holobionts; (ii) acknowledge the plastic (behavioural thermoregulation, physiological acclimation) and genetic responses (evolution) expressed by holobionts in fluctuating thermal environments; and (iii) explore the potential impacts of previously unconsidered patterns of temperature fluctuations on the outcomes and the dynamic of these insect–microbe associations.

Introduction

Mutualistic relationships with microorganisms are pervasive in insects and undoubtedly participated in the evolutionary success of this highly diversified group, by enabling the colonization of ecological niches otherwise difficult to exploit (Henry *et al*., 2013; Sudakaran *et al*., 2017). Many of these microorganisms, whether obligatory or facultative (i.e. required or not for successful insect growth and reproduction), have clear consequences on the phenotype of their individual hosts while also being heritable through vertical transmission (Moran *et al*., 2008; Oliver *et al*., 2010; Feldhaar, 2011). These remarkable features led some authors to coin the term 'holobiont' to describe a new biological and evolutionary entity comprised of the host and the microbial consortium that resides within (microbiome) (Margulis and Fester, 1991). This concept gave rise to the hologenomic theory of evolution, which envisions holobiont phenotypes as the consequence of interactions between the genetic information borne by each partner (host and microbiome) and the environment (Zilber-Rosenberg and Rosenberg, 2008). The hologenome evolutionary framework elicited counterarguments and debates, stating that the holobiont concept may not apply to all forms of mutualistic interactions. This should depend on whether the evolution of host and symbiont traits might reciprocally impact their fitness and whether their fitness interests align to make the holobiont the primary unit of selection (Moran and Sloan, 2015; Theis *et al*., 2016).

The way the host and its microbiome interact when facing ecological pressures is illustrated by insect–microbe associations responding to temperature, which influences the metabolism and population growth rate of both insect and microbial associates (Farrell and Rose, 1967; Huey and Stevenson, 1979; Angilletta, 2009). Temperature affects not only the host and the symbiont independently but also their behavioural and physiological interplay. Thermal conditions experienced by the interacting organisms can be influenced by processes expressed by insect hosts, such as thermoregulatory behaviour and microhabitat selection (Arnold *et al*., 2019; Truitt *et al*., 2019; Hague *et al*., 2020). Conversely, layered upon their intrinsic thermal sensitivity, insect ability to cope with different thermal conditions can be shaped by relationships with microbial partners capable of modifying the thermal tolerance of the holobiont – for better and for worse – through diverse metabolic and physiological pathways (Burke *et al*., 2010; Brumin *et al*., 2011; Wernegreen, 2012). Thus, the holobiont thermal response is shaped by complex bidirectional influence between insect and symbiont abilities to tolerate and adapt to different thermal environments.

An extended focus has been devoted to the responses of insect–microbe partnerships to either (i) constant developmental temperatures (exposing the holobiont to static thermal environments over several days or even the whole insect lifetime), or (ii) a single brief exposure to temperatures out of the permissive range for several hours (hereafter defined as a thermal shock) (Wernegreen, 2012; Corbin *et al*., 2017; Lemoine *et al*., 2020). Thus, most of the previous experimental investigations ignored the complexity of thermal dynamics experienced by insects and their symbionts in nature, where temperatures fluctuate across multiple spatial (mosaic of microclimatic conditions or geographic gradients) and temporal scales (diurnal or seasonal temperature cycles) (Colinet *et al*., 2015; Pincebourde and Woods, 2020). Such temperature fluctuations have specific effects on multiple fitness components in insects (e.g. development, longevity, reproduction) and microbes (e.g. biomass yield, population growth rate) (Colinet *et al*., 2015; Saarinen *et al*., 2018). They could, as such, constitute a major selective pressure driving the evolution of holobiont phenotypes and symbiont prevalence dynamics. For these reasons, we argue that the available body of knowledge provides a blinkered view of the thermal ecology of holobionts, making its potential relevance for predicting the evolution of mutualistic interactions in field conditions and under climate change scenario somewhat uncertain (Corbin *et al*., 2017; Higashi *et al*., 2020). Indeed, climate change is expected to alter thermal variability as warming is not occurring evenly across latitudes and time periods (day and night, or winter and summer) (IPCC, 2014). There is, therefore, an urgent need to develop the current understanding about the mechanisms allowing all constituents of holobionts to keep up with altered fluctuating thermal environments and the consequences of temperature fluctuations for the evolutionary and ecological dynamics of insect–symbiont interactions.

We intend to synthetize and discuss the current knowledge background about the thermal ecology of insect–microbe mutualisms, with a special focus on fluctuating temperatures. Our aim is to pinpoint worth-investigating research avenues to develop a more comprehensive overview of three major points of discussion. The first will evaluate the relevance of concepts used to predict the impacts of thermal fluctuations on organismal performance when applied to holobiont systems. The second will pertain to the ability of insect–microbe holobionts to express plastic (behavioural thermoregulation or physiological acclimation) or genetic (evolutionary) responses to temperature fluctuations. The third will deal with known and potential impacts of various patterns of thermal fluctuations on the outcomes and the dynamic of insect–symbiont interactions.

From constant to fluctuating thermal environments: on the application of Jensen's inequality to mutualistic interactions

The thermal biology of a poikilothermic organism is traditionally described through the lens of thermal performance curves conceptualizing the relationship between a fitness correlate (e.g. development, growth rate, reproductive output) and environmental temperature (Huey and Stevenson, 1979; Angilletta, 2009). As temperature increases, performance gently rises up to a maximal value reached at optimal conditions, before abruptly collapsing once this optimum is exceeded (Fig. 1). According to Jensen's mathematical inequality, thermal fluctuations should improve organism performance in the accelerating portion of the curve (for a mean temperature below the optimum), while the reverse pattern should occur in the decelerating part (for a mean temperature close or higher than the optimum). However, fluctuations in particularly warm conditions might sometimes benefit the organism by enabling recovery from damage sustained during heat stress (Ruel and Ayres, 1999; Martin and Huey, 2008; Colinet *et al*., 2015). Such predictions are intrinsically related to the position (thermal optimum) and the shape (thermal breadth, that is, the temperature range over which performance remains positive) of thermal performance curves (Foray *et al*., 2014; Colinet *et al*., 2015).

Predictions derived from Jensen's inequality could be challenged and vary according to insect symbiotic status
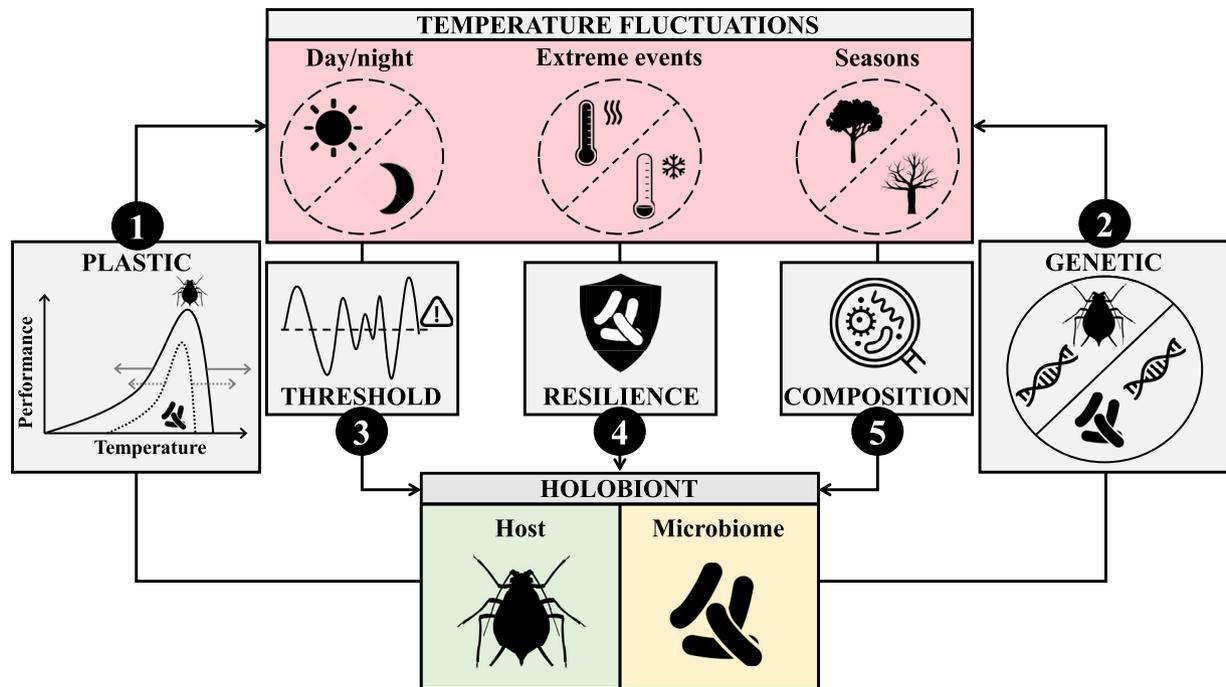
Fig. 1. Responses of insect–microbe holobionts to different patterns of temperature fluctuations, and impacts of thermal variability on the outcomes of these partnerships. (1) Plastic responses can operate over short timescales (e.g. within a generation) to enable rapid mitigation of thermal stress through behavioural (thermoregulation) and physiological (acclimation) processes. They are usually described through the lens of thermal performance curves (the relationship between a fitness metric and temperature), which can differ between insect and microbial players. The ecological and molecular mechanisms underlying these holobiont plastic responses need to be thoroughly elucidated, as well as their adaptive significance (fitness consequences) for partners. (2) Genetic (evolutionary) responses consist of long-term modifications of the host and/or the microbiome genomes in response to selective forces driven by temperature fluctuations. The relative contribution of each interacting species to the holobiont genetic adaptation to fluctuating temperatures remains to be examined, with careful considerations of the genetic diversity apparent at the microbiome level (species, strains, patterns of gene expression). (3) Daily thermal range can impact the holobiont through threshold effects, whereby daytime and/or night-time temperatures are stress-inducing when crossing a set of minimal and/or maximal values. The holobiont ability to express protective mechanisms against altered patterns of daily thermal range, in a manner similar to a single thermal shock, should be evaluated. (4) Extreme thermal events, like heat waves and cold spells, should be profoundly disruptive to insect–microbe associations. Future studies could be conducted to test for the resilience of these systems when facing different patterns of thermal extremes (e.g. heat or cold shock, number, duration and temporal sequence of stressful and recovery periods, timing of application during symbiogenesis). (5) Seasonal fluctuations of temperature can alter the composition of microbiome community. Much less is known about their consequences for host physiology and performance, and their potential indirect impacts on microbial dynamics and host vital functions through induction and termination of insect diapause. [Color figure can be viewed at wileyonlinelibrary.com]

when integrating the behavioural and physiological interactions between insects and microbiomes. First, the reliance of many insect hosts – especially sap-feeding taxa like aphids, whiteflies and stinkbugs – upon heat susceptible obligate symbionts constitutes a weak link for the interaction (see subsection on physiological acclimation below) (Wernegreen, 2012; Kikuchi *et al.*, 2016; Moran, 2016; Renoz *et al.*, 2019). This dependency likely reinforced the selective pressure exerted by high temperatures in fluctuating environments on the evolution of host buffering traits like thermoregulatory behaviour, which confines the partners within a range of favourable temperatures. Second, the involvement of certain microbial players (e.g. heat-protective facultative symbionts in aphids and whiteflies) might affect the general profile of holobiont thermal performance curve and reshape heat susceptibility (shift of thermal optimum), thermal tolerance

(narrowing or widening of thermal breadth) or sensitivity to thermal fluctuations (curvature around the optimum). This may potentially reverse the conclusions of Jensen's inequality by comparison with symbiont-free hosts (or infected with a different symbiont genotype) due to complex interactions between host genotype, symbiont genotype and thermal environment.

Only a handful of experimental works have examined the effects of thermal fluctuations (by standardizing the thermal budget received in constant and fluctuating treatments) on phenotype modifications undergone by symbiont-infected insects, reporting either negative or neutral effects at the holobiont level (Guay *et al.*, 2009; Ye *et al.*, 2016). However, the comparisons were performed at only one mean temperature, limiting the broadening of the conclusions drawn and their accordance with Jensen's inequality. We tend to believe that thermal

variability should have much of an impact on these associations considering the non-linearity of the relationship linking symbiont density to temperature on the one hand (e.g. Doremus *et al*., 2018), and the greater thermal sensitivity of symbiont-bearing insects – by comparison with symbiont-free ones – highlighted under fluctuating temperatures on the other (Higashi *et al*., 2020). We think efforts could be invested in order to test for the generality of the predictions of Jensen's inequality (at different mean temperatures) when applied to the interplay between insects and microbiomes, and to decipher the different mechanisms underpinning holobiont thermal sensitivity, as described in the section below.

Which mechanisms might be involved in the responses of insect–microbe holobionts to fluctuating temperatures?

Role of behavioural thermoregulation

Behavioural flexibility through thermoregulation may help insects to avoid negative effects of exposure to stressful thermal conditions by exploiting suitable thermal niches in heterogeneous environments (Kearney *et al*., 2009; Beever *et al*., 2017). Just as many other aspects of insect behaviour, thermoregulation can be modulated by hosted endosymbionts to promote their own replication and transmission – not necessarily at the expense of host fitness – as recently postulated in the system involving *Drosophila* hosts and their residing *Wolbachia* bacteria (Arnold *et al*., 2019; Truitt *et al*., 2019; Hague *et al*., 2020). When exposed to a spatial gradient of temperatures in laboratory conditions, *Wolbachia*-infected flies tend to select cooler microclimates relative to symbiont-free individuals, although conclusions are not generalizable to all symbiont genetic lines. This symbiont-related modification of insect thermal preference may have evolved as a behaviour actively expressed by the host flies to limit proliferation of bacterial cells and associated fitness costs, or alternatively as a behavioural manipulation induced by the symbiont to enhance its probability of vertical transmission onto the next generation (Corbin *et al*., 2017; Arnold *et al*., 2019; Truitt *et al*., 2019; Hague *et al*., 2020). Additional data are to be collected through fitness assays to unveil the adaptive significance of thermoregulatory behaviour for both components of the holobiont (insect life history, symbiont titres and transmission efficiency), and identify potential molecular signatures witnessing symbiont manipulation of insect behaviour.

Future studies should also incorporate climate change consideration, because environments could become warmer and more homogeneous in terms of microclimatic habitats, thereby influencing the future opportunities for thermoregulation (Caillon *et al*., 2014). This should be of great relevance to the study of insect–microbe associations, since insects can seek out warmer places to elevate the heat budget received as a way to combat parasitic microbial infection (Shang *et al*., 2015; Lovett *et al*., 2020). Whether similar behavioural mechanisms can be expressed in altered thermal habitats would have clear consequences for insect ability to control symbiont densities and holobiont evolution under climate change. One could expect the symbionts to be selected toward gaining higher heat tolerance to counter such host reactions, especially if there remains a misalignment between the fitness interests of the two participants. The evolution of a thermophilic lifestyle related with host thermoregulatory behaviour has already been suggested for *Snodgrassella* gut symbionts of honeybees and bumblebees, able to thrive in particularly warm microenvironments selected or artificially generated by their insect hosts (Hammer *et al*., 2021). It is thus likely that interacting partners of the holobiont may impact each other thermal niches with reciprocal influence on fitness, providing opportunities for coevolution in a changing climate.

From a more ecological perspective, thermoregulatory behaviour may affect the spatial and temporal dynamics of host–symbiont interactions. The interacting responses of the two partners can constrain their spatial distribution, possibly explaining the differences in thermal preference observed between spatially segregated populations of *D. melanogaster* (Rajpurohit and Schmidt, 2016; Truitt *et al*., 2019). One could therefore speculate that fine-scale temperature fluctuations contribute to spatially structuring insect populations and communities through their action on host–symbiont associations. There is still to study how microclimatic variations – at the scale of a forest plot, a crop field, or even a single plant – can affect insect–microbe associations and be exploited (or even reshaped) to buffer larger-scale temperature fluctuations (Pincebourde and Woods, 2020; Porras *et al*., 2020). Thermoregulatory behaviour may also be at the origin of variations of symbiont titres occurring alongside temporal thermal fluctuations and could relate with known examples of natural variations in microbiome composition occurring with seasonality, as observed in the whitefly *Bemisia tabaci* for example (Zhao *et al*., 2021).

Role of physiological acclimation

Thermal variations and extreme temperatures affect the metabolic and physiological homeostasis of all living organisms, triggering physiological adjustments that rely on the synthesis of stress-lowering compounds (e.g. heat shock proteins, cryoprotectants) (Feder and Hofmann, 1999; Bale *et al*., 2002). The global physiological response observed at the holobiont level will depend on the interacting

physiological capacities of insect and microbial associates under thermal stress.

On the one hand, the vulnerability of many obligate endosymbionts to extreme temperatures is supposed to result from their intracellular lifestyle and ancient coevolutionary history with their hosts, leading to an irreversible genome-wide accumulation of deleterious mutations and loss of genes that impact protein stability and transcriptional response to stress (Wernegreen, 2012). They act as the 'Achilles' heel' of the interaction by making the two interdependent components of the holobiont particularly vulnerable to stressful thermal conditions, as documented in aphids (Dunbar et al., 2007), mealybugs (Parkinson et al., 2014), stinkbugs (Kikuchi et al., 2016), psyllids (Hussain et al., 2017), whiteflies (Shan et al., 2014), bed bugs (Chang, 1974), planthoppers (de la Fuente et al., 2019) and weevils (Heddi et al., 1999). However, the obligate symbiont might display genetic variations underlying different transcriptional responses and subsequent holobiont susceptibilities to thermal stress, as witnessed by symbiont heat-tolerant variants in the nutritional mutualism between aphids and their obligate bacterial partner *Buchnera aphidicola* (Dunbar et al., 2007; Moran and Yun, 2015; Zhang et al., 2019).

On the other hand, some facultative symbionts are able to alleviate the fitness consequences of extreme temperatures on the holobiont. The most common mechanism implies an increase in the concentration of protective metabolites produced or triggered by the symbionts, and granting either direct (for examples in flies, see Gruntenko et al., 2017; Burdina et al., 2021) or indirect protection to the host through shielding populations of obligate endosymbiont from heat-related depletion (for examples in aphids, see Burke et al., 2010; Heyworth et al., 2020). Other protective mechanisms include the overexpression of host cytoskeleton genes induced by the bacteria *Rickettsia* sp. in the whitefly *B. tabaci* (Brumin et al., 2011) or the potential role of the gut microbiota in preventing loss of ion homeostasis under cold event in *D. melanogaster* (Henry and Colinet, 2018). The democratization and diversification of 'Omic' methods, such as dual RNA-sequencing, will probably improve our understanding of these molecular mechanisms and disentangle the relative contribution of each partner of the holobiont as well as their sensitivity to thermal conditions (Wolf et al., 2018). Indeed, we could expect that the timescale, frequency, magnitude and stochasticity of thermal fluctuations might favour differentially plastic mechanisms involved in holobiont physiological tolerance to temperature stress.

Aside from the benefits provided under heat stress, it should be noted that heat-tolerant genotypes of obligate symbionts and heat-protective facultative symbionts might be physiologically costly for their host in the absence of stressful temperatures (Dunbar et al., 2007;

Corbin et al., 2017). There is, therefore, a need to expand on the costs/benefits balance of carrying a particular symbiont genotype under different kinds of stress arising from patterns of thermal fluctuations. Furthermore, thermal acclimation, that is, the effect of historical temperatures on current thermal tolerance, is a crucial determinant of the response of poikilotherms to fluctuating temperatures (Bowler and Terblanche, 2008). Up to date, *Wolbachia* is the unique symbiont known to affect the host production of heat shock proteins as part of the acclimation process (Feder and Hofmann, 1999; Xi et al., 2008). Thus, the effect of symbionts on the acclimation capacities of the holobiont should be investigated and analysed at different timescales of temperature fluctuations, from rapid hardening to developmental acclimation and even transgenerational effect, provided that gut microbiota has been recently associated with transgenerational inheritance of cold response in *D. melanogaster* (Zare et al., 2018).

Role of genetic adaptation

Another process by which the holobiont might withstand temperature fluctuations is genetic adaptation: the modification of the genetic information of the host and/or the microbiome (Fig. 1). The holobiont evolutionary responses to environmental dynamics could be primarily driven by the microbiome, through diverse processes (Ye and Siemann, 2020; Perreau and Moran, 2021; Rosenberg, 2021). First, frequent genetic changes (e.g. mutation, recombination, horizontal gene transfer) occurring in these microorganisms due to their large population size and short generation time may represent a significant source of ecological innovations for the holobiont (Sudakaran et al., 2017; Renoz et al., 2019). Second, the community composition of the microbiome (species and strains) is flexible and modulated by environmental fluctuations over different timescales, providing natural variations in the microbial genetic pool upon which selective pressures may act (Ferguson et al., 2018; Moghadam et al., 2018). In the ongoing climatic context, symbionts can thus be viewed as a highly dynamic, flexible pool of genes that would enable the holobiont to match the fast pace of environmental change through evolutionary responses.

The contribution of symbiont evolution to holobiont thermal adaptation could, however, be nuanced across biological systems, because coevolution requires that symbiont genetic modifications are heritable between host generations and impact holobiont fitness (Moran and Sloan, 2015). This should be the case for obligate mutualisms, but the genomic features of many obligate endosymbionts shaped by strict intracellular lifestyle (small gene repertoire, fixation of deleterious mutations,

reduced recombination) could severely limit their evolutionary potential (Moran *et al*., 2008; Wernegreen, 2012; Renoz *et al*., 2019). Still, local thermal adaptation of obligate endosymbionts (e.g. *B. aphidicola* in aphids) has already been reported, underlying the thermal tolerance of their hosts (Dunbar *et al*., 2007; Zhang *et al*., 2019). It is challenging to assess the importance of mean temperatures and thermal variability in driving the evolution of these symbiont haplotypes, because of multiple covarying environmental factors and local coevolutionary dynamics. Much information could definitely be gained from experimental selection enabling to decouple the environmental pressures exerted on the two partners, in order to evaluate their respective contribution to the holobiont thermal adaptation. Promising models to address these questions could involve strains of facultative symbionts that can be cultured outside of their host body (e.g. free-living strains of *Serratia symbiotica* in aphids), or obligate symbionts acquired from environmental sources postnatally every generation, which experience their own thermal history prior to host colonization (e.g. gut symbionts of some heteropteran species) (Kikuchi *et al*., 2007; Perreau *et al*., 2021).

Microbial genetic diversity can also be considered at the microbiome level through restructuration of communities living inside the host (e.g. number and relative abundance of species and strains, patterns of gene expression). There is ample experimental support showing that the composition of insect microbiota is dynamic and influenced by temperatures varying during a brief bout of thermal stress (Jaramillo and Castañeda, 2021), over one or several developmental stages (Moghadam *et al*., 2018), and even across seasons and generations (Ayyasamy *et al*., 2021; Zhao *et al*., 2021). While these examples conclusively demonstrate that insect microbiome is flexible and responsive to temperature dynamics, the significance of these temperature-driven modifications of microbial communities for holobiont fitness remains to be ascertained (e.g. Moghadam *et al*., 2018), as well as the ecological mechanisms generating these variations. They could be caused by environmental filtering (successive rearrangements of microbial communities when thermal conditions are shifting), community plasticity (adaptive acclimation to stress, potentially involving adjustments in gene expression), or evolutionary changes (rapid genetic selection on species and strains).

How could different patterns of thermal fluctuations drive the outcomes and the dynamic of insect–microbe mutualisms in a climate change context?

Potential impacts of daily thermal range

Daily thermal range, defined as the thermal contrast between daytime and night-time periods, is identified as a key thermal parameter driving many interspecific relationships (Speights *et al*., 2017; Stoks *et al*., 2017), but its significance for mutualistic interactions remains fairly undocumented (Stoks *et al*., 2017; Higashi *et al*., 2020). Altered daily thermal range – as forecasted under climate change scenario – might constitute a major selective force influencing the dynamic of symbiont prevalence in field conditions (Fig. 1). It can induce increased incidence of high temperatures, if the magnitude of the fluctuations widens, or warmer night-time conditions exploited as cooler temporal refuges allowing recovery from daytime heat loads, if it contracts (Speights *et al*., 2017; Higashi *et al*., 2020). Thus, synergetic effects between the different facets of climate change could be expected, as altered patterns of daily thermal range could exacerbate the disruptive impacts caused by an overall warmer environment on the most heat vulnerable insect–microbe partnerships.

Only one study has hitherto investigated the combined effects of changing mean temperature and daily thermal range on the interaction between an insect (the pea aphid *Acyrthosiphon pisum*) and its microbial ally (the facultative defensive symbiont *Hamiltonella defensa*) (Higashi *et al*., 2020). These authors concluded that the symbiont-mediated protection toward parasitoids faded because of warming, regardless of daily thermal range and associated threshold effects (whereby stress occurs when maximal and/or minimal daily temperatures cross a given set of values). In this vein, future studies should incorporate full factorial designs to disentangle the relative impacts of mean temperature, daily thermal range and their potential interaction on the association. Such investigations should hold promise for developing a mechanistic understanding of the effects involved in thermal modulation of the outcomes of the interaction (symbiont prevalence, expression of symbiont-mediated ecological traits). They should also allow to investigate the biotic responses of the holobiont to different scenarios of climate change, as elegantly done by Higashi *et al*. (2020).

Besides, the attention paid to daily thermal fluctuations should expand to other ecologically relevant holobionts, as those involving insect hosts, obligate symbionts and heat-protective facultative symbionts. Indeed, the literature provides little information about the ability of these tripartite systems to tolerate altered patterns of daily thermal range. Mutualistic association between the insect and its nutrient-provisioning obligate partner should be sensitive to daily thermal range, provided that modifications of this thermal parameter impact the performance of strictly sap- (aphids) and blood-feeding insects (triatomines) relying on the well-being of their obligate symbiont (Zhao *et al*., 2014; Ma *et al*., 2015; Rolandi and Schilman, 2018). Much less is known, however, about

the ability of heat-protective facultative symbionts to increase the resilience of these holobionts in such specific fluctuating thermal environments. Aphids and whiteflies could be valuable study models to tackle this question, because the facultative symbionts involved in heat protection and the underlying molecular mechanisms have been previously identified (Burke *et al*., 2010; Brumin *et al*., 2011; Yang *et al*., 2021). It would thus be possible to test whether such heat responses could be triggered by recurrent exposure to stressful daytime or night-time temperatures in a manner comparable to a single heat shock, as previously documented.

Potential impacts of extreme thermal events

Extreme thermal events like heat waves have recently received increased recognition because they are expected to become a major component of future climates while being highly disruptive to organisms and their interactions (Stoks *et al*., 2017; Ma *et al*., 2021). Though very informative data were obtained from previous studies using heat shock procedures, the potential for extrapolation of these experimental setups to field dynamics during natural heat waves can be deemed somewhat uncertain (Fig. 1).

Above all, heatwaves involve complex successions of hot days and milder periods that can allow recovery from heat loads (Ma *et al*., 2021). The resilience of the holobiont when facing complex environmental dynamics driven by different parameters during heatwave episodes (number, duration and temporal sequence of stressful and recovery periods) should be more carefully examined. One interesting model to probe into these biological responses could be the association between aphids and *S. symbiotica*, because the heat protection here involved likely relies on the lysis and population decline of the bacteria (Burke *et al*., 2010). Hence, it remains to be explored how the balance of stress/recovery phases determined by heat wave characteristics could affect the opportunities to replenish symbiont densities and host vital functions. The timing of exposure to heat shock during insect development and symbiont establishment may also impact the levels of heat protection bestowed by the microorganism (Montllor *et al*., 2002; Russell and Moran, 2006). This parameter would therefore warrant further experimental investigations considering the impacts of transient heat event during ontogeny, at the onset of symbiogenesis. This could involve transgenerational effects modulating the efficiency of symbiont vertical transmission, or impacts of heat stress before and after symbiont acquisition in case of environmental infection (see above), that is, on the two organisms independently or in interaction.

The responses of insect–microbe holobionts to extreme temperatures can also pertain to extremely low temperatures (cold spells). In a climate change context, many insects thriving in tropical or sub-tropical regions could gradually migrate to temperate, more thermally variable environments, where they will experience an increased incidence of low temperatures (Osland *et al*., 2021). Because they previously evolved in relatively buffered and warm thermal environments, their ability to resist or tolerate such harsh climatic conditions could be largely influenced by the acquisition of locally adapted microbes. Yet, the impacts of cold shocks on insect–microbe holobionts have been quite understudied so far. Some studies reported no detectable effects of the presence of facultative symbionts on the cold resistance of their aphid host (Łukasik *et al*., 2011), while other outlined a symbiont-modulated upregulation of host synthesis of antifreeze compounds in other arthropod taxa (e.g. ticks and flies), resulting in an enhanced host ability to survive a cold shock (Neelakanta *et al*., 2010; Raza *et al*., 2020). Considering the insufficiently detailed body of knowledge, we think that additional data on diverse biological models need to be gathered before concluding about the generality of cold tolerance and underlying molecular mechanisms in insect–microbe holobionts.

Potential impacts of seasonal thermal range

A number of studies have examined seasonal changes in the microbiome of different insect models, including bees, crickets, aphids, whiteflies and butterflies (Fig. 1) (Ferguson *et al*., 2018; Liu *et al*., 2019; Kešnerová *et al*., 2020; Zhao *et al*., 2021). For example, Kešnerová *et al*. (2020) recently demonstrated that the gut microbial community of bees radically differs between winter and summer morphs, both in terms of abundance and diversity. Similarly, Liu *et al*. (2019) showed strong seasonal dynamics in the incidence of several bacterial endosymbionts (*Regiella*, *Hamiltonella*, *Rickettsia*, *Serratia*) in populations of grain aphid *Sitobion avenae*. Difficulties arise in isolating specifically the importance of temperature in shaping seasonal variations of microbiome observed under natural settings, because multiple factors are at play (e.g. photoperiod, nutrition, seasonal polyphenism, strength of trophic interactions) (Smith *et al*., 2021). This would require further experimental studies based on mesocosm approaches to track the long-term evolution of the microbiome community following a realistic seasonal gradient of temperatures (Ferguson *et al*., 2018). Another interesting question would be to connect these seasonal dynamics of microbiome to host physiology (thermal tolerance) to ascertain their adaptive value (by facilitating the anticipation of the

Table 1. Summary of the state of the art, outstanding issues, proposed research avenues with methods and biological models (when relevant) about the ecology and evolution of insect–microbe holobionts in fluctuating thermal environments.

State of the art	Outstanding issues	Proposed methods and models
Do temperature fluctuations specifically impact the holobiont? Fluctuations elicit neutral/detrimental effects on holobiont traits[1,2]. Symbiotic infection increases host thermal sensitivity in fluctuating conditions[3]	Each protagonist might reshape the thermal performance curve of the holobiont The relevance of Jensen's inequality has to be evaluated for insect–microbe associations	Modelling thermal performance curves of hosts uninfected or infected with heat-protective facultative symbionts, through fitness assays (aphids, whiteflies) Comparisons between constant and fluctuating treatments at different points of the thermal performance curve
How behavioural thermoregulation helps the holobiont cope with thermal fluctuations? Host thermoregulation is affected by some strains of facultative symbionts[4,5,6] and can determine symbiont thermal niche[7]	Fitness consequences of thermoregulation might unravel the host-symbiont bidirectional influence (symbionts affect host thermal preference, host controls symbiotic community by selecting temperature) Mosaic of microclimatic conditions can structure and be exploited by host–symbiont interactions	Combination of behavioural tests with fitness assays on both partners, manipulating infection status (occurrence of facultative symbionts and strains) and spatial heterogeneity of temperatures (fruit flies) Field samplings to capture micro-scale variations of symbiont communities in relation with microclimates
How physiological acclimation helps the holobiont cope with thermal fluctuations? Host stress tolerance is improved by symbiont-mediated physiological processes[8,9,10], in a potentially inheritable way[11]	These mechanisms can be more or less resilient to different patterns of temperature fluctuations (temporal dynamic, frequency, stochasticity) The partners might contribute to physiological acclimation at different timescales (rapid hardening, developmental acclimation, transgenerational effects)	Characterization of the holobiont physiological profile and fitness assays under different patterns of thermal fluctuations Evaluation of acclimation capacities of uninfected and infected hosts with well-documented physiological response to heat (aphids, whiteflies, fruit flies)
How genetic adaptation helps the holobiont cope with thermal fluctuations? Holobiont thermal biology is shaped by genetic diversity of obligate symbionts[12,13]. Microbiome composition responds to temperature dynamics[14,15,16]	The evolutionary potential of holobionts facing altered patterns of thermal variability and the adaptive value of the mechanisms causing variations in microbiota (community plasticity, evolutionary changes) remain unknown	Experimentally applying selective pressures related with temperature fluctuations on each or both partner(s) to follow the evolution of organism traits (thermobiology, physiology) over generations (aphids and heteropterans housing symbionts acquired from the environment)
What could be the impacts of daily thermal range? Daily thermal range does not alter defensive mutualism, which is only affected by changes in mean temperature[3]	The potentially interactive impacts of daily thermal range and mean temperature might affect the adaptive value of heat-protective mutualism	Full factorial designs (controlling for mean temperature, daily thermal range and host infection status) combining fitness assays with molecular measurements (symbiont abundance, holobiont physiological profile) to assess the efficiency of symbiont-mediated responses to daytime or night-time thermal stress (aphids, whiteflies)
What could be the impacts of extreme thermal events (heat waves, cold spells)? Facultative symbionts can improve the host ability to withstand heat shock[9,17,18], while few data are available for cold shock tolerance[10,19]	The holobiont resilience might be challenged by more complex patterns of extreme thermal events, while the stress tolerance of some biological models (holobionts from tropical origins) is understudied	Experimental designs controlling for timing of stress application, frequency, number and temporal sequence of stress and recovery on various models involving heat-protective facultative symbionts or symbionts with free-living capacities (aphids, whiteflies, heteropterans) Field samplings to track natural variations of symbiotic communities following an extreme thermal event
What could be the impacts of seasonal fluctuations? The composition of insect microbiome is highly dynamic across the seasons[20,21,22]	Seasonal temperature fluctuations might directly or indirectly (through diapause induction and termination) impact microbiome and holobiont physiology, the latter being poorly understood	Experimental approaches involving thermal gradient reflecting seasonality, linking microbiome dynamics to variations in host/holobiont traits (behaviour, physiology, performance) in species with well-marked seasonal strategies (e.g. winter diapause)

[1]Guay *et al*. (2009).
[2]Ye *et al*. (2016).
[3]Higashi *et al*. (2020).
[4]Arnold *et al*. (2019).
[5]Truitt *et al*. (2019).
[6]Hague *et al*. (2020).
[7]Hammer *et al*. (2021).
[8]Burke *et al*. (2010).
[9]Brumin *et al*. (2011).
[10]Raza *et al*. (2020).
[11]Zare *et al*. (2018).
[12]Dunbar *et al*. (2007).
[13]Zhang *et al*. (2019).
[14]Moghadam *et al*. (2018).
[15]Ayyasamy *et al*. (2021).
[16]Jaramillo and Castañeda (2021).
[17]Montllor *et al*. (2002).
[18]Heyworth *et al*. (2020).
[19]Łukasik *et al*. (2011).
[20]Ferguson *et al*. (2018).
[21]Liu *et al*. (2019).
[22]Zhao *et al*. (2021).

upcoming environment), particularly since the regulation of the microbial community might be to a large extent under host command, via action of immune system (Ferguson *et al.*, 2018).

Seasonal fluctuations of temperature may also indirectly impact microbiome composition through induction, maintenance and termination of insect diapause. Insect diapause has evolved as a response to recurrent seasonal variations in temperature, but climate change modifies the selective pressures exerted on biological cycle and overwintering strategies (Bale and Hayward, 2010). Microorganisms affect many of insect vital functions when undergoing diapause, including nutrition, immunity, toxin evacuation, or preservation of hydric balance (for one example, see Kashima *et al.*, 2006). Temperature fluctuations during insect diapause may have both short-term (provision of microbe-associated vital functions) and long-lasting implications for the maintenance of interacting partners (effects on the microbial community after diapause termination). Hence, it is now time to understand whether insects can safely reassemble microbiome and maintain the continuity of the microbial community after diapause if they have been confronted to different thermal conditions before, during and after the diapause process, which may become a crucial determinant of host survival in a world of increasingly varying temperatures (Mushegian *et al.*, 2018; Mushegian and Tougeron, 2019).

Concluding remarks

We here discussed the current corpus of knowledge about the responses of insect–microbe holobionts to temperature fluctuations in a climate change context. We conclude that thermal variability should play a key role in the ecology and evolution of insect–symbiont interactions. However, we still know relatively little about the ability of these associations to withstand temperature dynamics through plastic and genetic mechanisms, and many questions are to be answered (Table 1). This is a daunting but timely challenge, because the view of the host and the microbiome as two parties of a single entity subjected to environmental pressures is a relatively recent concept that elicits intellectually stimulating debates in the modern evolutionary framework (Moran and Sloan, 2015; Theis *et al.*, 2016; Rosenberg, 2021). Genetic polymorphism among natural insect populations has been largely investigated since the pioneering works of Dobzhansky (1955), but the composite nature of the holobiont could magnify the amount of genetic variance upon which selection may act, because host and microbiome represent two sources of heritable variability at the population level, each experiencing its own evolutionary dynamic with various processes involved. However, selection may not necessarily operate at the holobiont

level, depending on the convergence of transmission routes of host and symbiont genomes, and the adaptive value of their mutual dependence in a given environmental context (Moran and Sloan, 2015). These lines of reasoning suggest that conclusions about the resistance/resilience of insect–microbe holobionts to climate change are likely to be species-specific. Another challenge lies in the design of experimental thermal treatments, and we recommend a more systematic integration of ecologically realistic patterns of thermal fluctuations through controlled laboratory approaches based on climate projections (Higashi *et al.*, 2020) or warming in semi-natural and natural conditions (Harmon *et al.*, 2009; Kikuchi *et al.*, 2016). These approaches should be combined with a thorough elucidation of the mechanistic basis – including both molecular and ecological processes – to draw a more integrative picture about the holobiont abilities to cope with different patterns of temperature fluctuations.

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