Available online at www.sciencedirect.com
ScienceDirect
Current Opinion in Insect Science

Evolutionary responses of mutualistic insect–bacterial symbioses in a world of fluctuating temperatures

François Renoz[1], Inès Pons[1] and Thierry Hance

Climate change is altering the abundance and distribution of millions of insect species around the world and is a major contributor to the decline of numerous species. Many insect species may be indirectly affected through their nutritional dependence on mutualistic bacteria. Indeed, these bacterial partners generally have a highly reduced and static genome, resulting from millions of years of coevolution and isolation in insect cells, and have limited adaptive capacity. The dependence of insects on bacterial partners with narrow environmental tolerance also restricts their ability to adapt, potentially increasing the risk of their extinction, particularly in a world characterized by increasing and fluctuating temperatures. In this review, we examine how climate change can affect the evolutionary trajectories of bacterial mutualism in insects by considering the possible alternatives that may compensate for the dependence on bacterial partners that have become 'Achilles' heels'. We also discuss the beneficial and compensatory effects, as well as the antagonistic effects associated with so-called facultative symbionts in the context of an increased incidence of transient extreme temperatures.

Address
Université catholique de Louvain, Earth and Life Institute, Biodiversity Research Center, Croix de Sud 4-5, bte L7.07.04, 1348 Louvain-la-Neuve, Belgium

Corresponding author: Renoz, François (francois.renoz@uclouvain.be)
[1] These authors contributed equally to this work.

Current Opinion in Insect Science 2019, **35**:20–26

This review comes from a themed issue on **Global change biology**

Edited by **Arnaud Sentis** and **Nicolas Desneux**

https://doi.org/10.1016/j.cois.2019.06.006

Introduction

Ongoing global climate change is altering all aspects of biological systems, from genes to ecosystems and is expected to significantly affect the abundance and distribution of many living species over the next decades [1,2]. Current scenarios predict not only a global increase in mean temperature, but also an increased variance around that mean and an increased incidence of transient extreme temperatures [3–5]. These environmental changes exert biological impacts on metazoan organisms not only directly, but also indirectly through the microorganisms with which they interact [6–11]. Disruption of partnerships between reef-building corals and their photosynthetic bacterial mutualists is now well documented [12,13•], as well as alteration of gut microbiota diversity of vertebrates [14] or disruptions in the complex bacterial communities in forest soils [15,16]. Mutualistic associations involving microorganisms play a pivotal role in the functioning of ecosystems, notably because they facilitate access to certain resources of many metazoan species [17]. However, since these interactions bind multiple species to a common destiny, they also represent a weak link that is particularly sensitive to environmental fluctuations, and their breakdown because of global change may dramatically amplify and/or accelerate biodiversity loss and ecosystem disruption [18].

Insects are ectotherms, which means they do not maintain their body temperature through homeostatic processes, which depends to varying degrees on the environment [19]. This makes these organisms particularly vulnerable to extreme temperatures that can alter cellular and physiological function, and hence the higher levels of organization [20,21]. By playing a central role in food webs, insects are crucial links in all ecosystems and changes of their biodiversity could have unpredictable consequences [22••]. Another feature that makes certain insect species particularly vulnerable to extreme temperatures is their propensity to establish mutualistic relationships with bacteria that can deeply influence their evolutionary ecology [23–25]. Recent studies suggest that climate change could severely impair these mutualistic associations, especially obligate mutualisms in which both parties are highly interdependent and cannot live one without the other [7,23,26–28]. The goal of this review is to highlight some insights and further questions about how climate change may affect the evolutionary trajectories of bacterial mutualisms in insects. We propose several evolutionary scenarios based on the general conceptual framework previously established by Sachs and Simms [29] and Toby Kiers *et al.* [18] concerning the evolution of mutualisms and their possible breakdown under altered climatic conditions. Reviewing the recent literature, we examine several alternatives regarding the evolutionary responses of symbiont-dependent insects in the current context of rising temperatures.

Obligate mutualists: the Achilles' heel of insects in fluctuating thermal conditions

Bacterial mutualisms in insects are generally classified into two categories, mainly based on the degree of codependency and co-evolution between the host insect and its bacterial partner [30]. Obligate mutualisms are generally described as evolutionarily ancient and stable associations (with bacteria that have sometimes cospeciated with host insects for tens of millions of years), and involve bacterial partners required for normal function and development of host insects, providing them with essential nutrients lacking in their diets (e.g., amino acids and vitamins). They are particularly prevalent in insect groups specialized on nutritionally unbalanced foods, such as plant sap or blood [31,25]. Facultative mutualisms result from associations with bacteria that are not necessary for host insect survival and reproduction, but which nonetheless provide host benefits under specific ecological conditions [30].

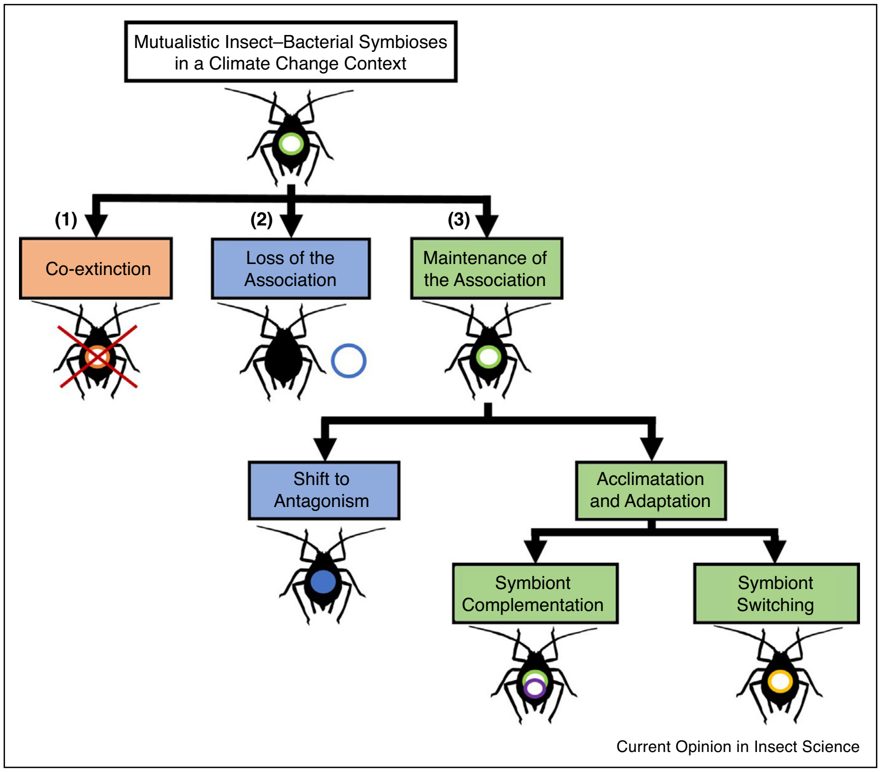
A striking feature of many obligate symbionts is that their confinement within specific tissue and cell structures (e.g. bacteriocytes) and their strict vertical transmission severely limit the possibilities for gene exchange and favor the process known as Muller's Ratchet, whereby the genomes of asexual populations accumulate deleterious mutations in an irreversible manner [28,31]. This accumulation results in a reduction of the stability of the encoded proteins. In addition, the genomes of obligate symbionts undergo a degenerative evolution that results in the purging of genes not absolutely essential, preserving mostly functionally important one's (e.g. genes encoding bacterial replication, basic cellular processes, or nutrient biosynthesis) [28]. This irreversible genes loss constrains the evolutionary potential of the symbiont and its relationships with its host. Despite compensatory mechanisms such as overexpression of heat-shock proteins for optimal protein folding [31], mutualistic associations involving obligate bacterial symbionts have little potential to cope with a changing environment, including heat stress, such as highlighted by several studies [23,26,28]. However, other sources of ecological innovation may exist in nature and provide new traits that compensate for the fragility of obligate mutualism and allow symbiont-dependent insects to adapt to a fluctuating environment. In the following sections, we examine different scenarios of how bacterial mutualism in insects could evolve in an altered thermal environment, highlighting possible compensatory mechanisms (Figure 1).

'You are the weakest link, goodbye' – the extinction risk

In obligate mutualisms, the reduced genome function of the bacterial symbiont tends to drive the insect partner into an evolutionary spiral that constrains its ecological range and makes the entire mutualistic system more vulnerable to environmental stresses [26]. One scenario currently being debated is that the increase in average temperature and temperature fluctuations due to global change may lead to the breakdown of host–endosymbiont partnerships and the possible extinction of both partners (Figure 1) [23,26,28]. Indeed, recent studies have shown that obligate bacterial mutualisms in insects are generally heat-sensitive [32,33,34•,35,36•]. For example, aphids exposed for a few hours at temperatures up to 38°C have a reduced density of the bacteriocyte-associated symbiont *Buchnera aphidicola*, which can even be completely lost [32,37]. Although the heat sensitivity of *B. aphidicola* cells is not fully understood, it is hypothesized that this may depend in particular on the presence of a mutation in the *ibpA* gene that encodes a heat-shock protein ensuring optimal protein folding under heat stress conditions [35,37,38]. Aphids whose *B. aphidicola* strain exhibiting the mutation has been experimentally replaced by an unmutated strain regain some thermal tolerance [35]. Some authors also speculate that *B. aphidicola*'s low tolerance to heat may partly explain why aphids are so rare in the tropics [39]. Another study has shown that after being exposed to a continuous heat stress at 35°C, the obligate symbiont *Portiera* and the facultative symbiont *Hamiltonella defensa* that both reside in bacteriocytes are almost completely depleted in the whitefly *Bemisia tabaci*, while facultative symbionts residing outside bacteriocytes tend to resist heat stress [36•]. These observations suggest that heat vulnerability of obligate mutualists may be due not only to the genomic feature of symbionts (i.e. a degenerate genome in which deleterious mutations accumulate), but also to the thermal susceptibility of the host cells in which they are confined (i.e. the primary bacteriocytes). Although the mechanisms underlying the thermal sensitivity of obligate bacterial mutualism in insects remain poorly understood, they are probably the result of complex interactions between host bacteriocytes and the genotype of bacterial symbionts.

Most of the studies that have focused on the impact of increasing temperatures on symbiotic systems in insects were carried out on thermal stresses involving temperatures between 35°C and 38°C. However, such experimental conditions do not necessarily reflect the gradual rise in temperatures due to global warming. A recent study examined the impact of increasing temperatures on the obligate gut symbiont of a stinkbug species, *Nezara viridula*, by simulating global warming in semi-natural conditions [7]. Interestingly, they showed that a slight increase in mean temperature severely depresses obligate symbiont titers and has adverse effects on insect development comparable to the use of antibiotics. The innovative (and rather alarming) aspect of this study is that the slight increase in temperatures imposed by climate change could already affect the obligate bacterial mutualism in which many species of insects are involved, and severely impair the normal development of symbionts-dependent insects. If there is now evidence that high temperatures can rapidly and severely affect the integrity of obligate bacterial symbioses in insects, there is currently a lack of data to generalize the impact of global change on these mutualisms. This is especially true for insects living in tropical and equatorial regions, for which there is a critical lack of data, while their bacterial

Figure 1



Scenarios drawing potential evolutionary trajectories of mutualistic symbiosis in insects in a climate change context. **(1)** One of the scenarios is that the heat stress may cause to the breakdown of host–endosymbiont associations, leading to the extinction of both partners. **(2)** Another evolutionary scenario is that the heat stress leads to the breakdown of the mutualist association without the death of one or more partners. For instance, horizontal gene transfers (HGT) from a bacterial partner to the host genome may maintain the needed functions, continuing its own evolutionary path without its microbial partner. **(3)** The last scenario is that the mutualist association is maintained facing to a heat stress through different strategies. The symbiotic bacteria may shift from mutualism to antagonism if they no longer confer a selective advantage in the prevailing environmental conditions. Otherwise, the mutualist association may acclimate and adapt to the heat stress conditions, evolving towards relationships involving a new less degenerate and vulnerable bacterial partner to compensate or replace the one that has become too weak. Orange color corresponds to co-extinction, blue color corresponds to negative consequences for mutualisms persistence and green color corresponds to positive consequences for mutualisms persistence. The circles correspond to the symbionts, where the hollows correspond to mutualists and the filled correspond to parasites. The other colored circles (yellow and purple) correspond to other mutualists.

partners may be less heat-sensitive compared to strains present in insects living in areas with a more temperate climate.

Alternative resource acquisition strategies: symbiont complementation and switching

Although obligate symbionts tend to be vulnerable to heat stress and have little room for adaptation, they are rarely the only mutualistic partners for insects that can also host facultative symbionts or additional obligate partners that are sources of ecological innovation [40]. Thereby, to avoid falling into the spiral of extinction of both partners, obligate bacterial mutualism could follow an alternative evolutionary scenario: evolve towards relationships with a less degenerate and vulnerable bacterial partner to compensate or replace the one that has become too weak. There are many situations in nature, particularly in specialized mutualisms, where environmental changes drive shifting allegiances [18]. For example, in altered environmental conditions, native symbionts of certain species of corals and sponges are replaced by new more thermally resistant bacterial partners [13•]. There are many reports of mutualistic systems in which insect species can rely on these bacterial partners to compensate for their old friend's deficits and, in some cases, which may become obligate themselves [28]. This is the case of many sap-feeding insect species that can host additional nutritional obligate symbionts (often called co-obligate symbionts) whose metabolic abilities complement those of the native but more degenerate obligate partner [41]. Aphids of the subfamily Lachninae harbor highly deteriorate *B. aphidicola* strains generally supplemented by *Serratia symbiotica* strains that fulfill the nutritional functions lost by the native obligate symbiont

[41]. It is interesting to note that, in certain aphid species, these co-obligate *S. symbiotica* strains have been replaced many times by other Enterobacteriaceae probably picked up from their diet, which demonstrates the great flexibility of the mechanisms of acquisition and replacement of symbiotic partners in insects [41–43]. Cases of metabolic complementation have been found in other clades of insects including mealybugs and cicadas, which may harbor more than three obligate symbionts working together to synthesize certain metabolites [40,44,45]. There are also examples of bacterial mutualisms in which the obligate symbiont has been completely replaced by a new microbial partner [41]. For example, in aphids of the genus *Geopemphigus*, *B. aphidicola* has been fully supplanted by a new partner from the bacterial phylum Bacteroidetes which perform the nutritional role previously fulfilled by the native symbiont [46]. It is now increasingly evident that shifts in symbiotic associations, and in particular the replacement of ancient and defective obligate symbionts by more functional microbial partners, are frequent in many insect groups [40,45,47]. And these replacements do not always involve the recruitment of new bacterial partners since in aphids and cicadas, for example, several cases of replacement of the degenerate essential bacterial symbionts by yeast-like fungal associates have been reported recently [48••,49].

Obligate symbiont complementation and/or replacement by more efficient and less vulnerable microbial partners are mechanisms providing host insects with a means of overcoming dependence on partners with limited adaptive capacity [28]. In this evolutionary context, climate change may result in switching combinations with microbial partners allowing acclimation and rapid adaptation of the host insect in a world of fluctuating temperatures, as has been demonstrated in the case of corals and sponges [13•]. However, although a growing body of literature suggests that obligate symbiont supplementation and/or switching may be more widespread in insects than previously appreciated, these events probably occur gradually over an evolutionary time-scale [31]. Given the rapid pace of environmental change imposed by global warming, it is not certain that these alternative pathways can occur quickly enough to ensure the long-term survival of insect populations and species.

Let your bacterial partner down (it's not worth it)

Another evolutionary scenario would be the integration of metabolic skills of the obligate symbiont into the host genome, which would pursue its own evolutionary path without its cumbersome microbial partner (Figure 1). This scenario requires horizontal gene transfer (HGT) from the obligate bacterial partner or other bacteria to the host genome to maintain the needed functions. Although HGT are recurrent among bacteria, their frequency and importance in the context of animal-bacterial interactions are currently less clear because of technical artifacts that lead to misinterpretation of some genomic data and because of the small number of sequenced insect genomes [50••]. However, numerous cases of HGT events between bacteria and invertebrates have recently been documented [45,51,52], and even if the bacterial transferred sequences are not necessarily functional in the recipient eukaryote, it is now clear that HGT can participate in the shaping of the genome of insects and other invertebrates. Interestingly, Luan *et al.* have shown that metabolism genes coded in the *B. tabaci* genome have been acquired from exogenous bacteria by HGT, but not from nutritional obligate symbionts [52]. In another study, Husnik *et al.* have shown that several expressed horizontally transferred genes present in the mealybug *Planococcus citri* genome come from diverse bacteria and likely complement missing genes of the degenerate obligate symbiont *Tremblaya* [53]. Finally, there is also evidence of horizontal transfers of B-vitamin biosynthetic genes between symbionts in multi-symbiotic systems in *Cinara* aphids [42]. These few examples demonstrate that HGT events are not so rare and can compensate for the loss of functionality of the native obligate symbiont.

Effects of other players: roles of facultative mutualists on insect host thermal tolerance

Facultative bacterial mutualism occurs commonly in many insect species, including species that do not harbor obligate symbionts [54,55]. Some strains of these facultative bacterial partners have been reported to confer resistance to heat stress [25]. For example, certain strains of *Wolbachia* have the ability to increase the heat stress tolerance of their host *Drosophila melanogaster* by intensifying dopamine metabolism [56•]. In aphids, certain facultative strains of *S. symbiotica* increase host survival and/or reproduction after a heat shock by releasing metabolites as a result of cell lysis, thereby preserving the integrity of the primary bacteriocytes in which *B. aphidicola* resides [32]. In addition, studies suggest that facultative symbionts could be less affected by heat stress than obligate ones as is the case with *B. tabaci* where the populations of obligate symbionts localized in bacteriocytes decrease strongly under heat stress conditions, while facultative symbionts are more able to resist [36•]. In contrast, it has been shown that high temperatures can severely impair the transmission of facultative symbionts, suggesting that climate stress could have a significant impact on the maintenance and dynamics of these symbioses in natural insect populations [23,57]. Thus, even if some laboratory studies suggest that facultative symbioses may positively affect the response of insects to rising temperatures, responses probably vary depending on the strains involved and the nature of the association.

Besides their associated benefits, the facultative symbionts described so far impose costs on their host in the absence of stress [58]. Balancing selection plays a major role in facultative symbioses maintenance: the balance of the costs and benefits they confer determines the persistence and stability of these associations in insect populations. For example, the defensive symbiont *H. defensa* that provides protective effects to infected aphids against parasitoids may be associated with detrimental effects in the absence of selection pressure (i.e. the presence parasitoids in the direct environment) [59,60]. Costs associated with the presence of facultative partners may appear more intensely when the host is under physiological stress [23] and could thus increase the sensitivity of infected insects to environmental stresses. In their study, Skaljac *et al.* have shown that the presence of the facultative symbiont *S. symbiotica* in *A. pisum* aphids decreases the tolerance of these insects to different insecticides [61•]. Costs of climate stresses could thus be added to the costs associated with facultative symbionts, and these symbiotic bacteria could shift from mutualism to antagonism if they no longer confer a selective advantage in the prevailing environmental conditions.

The pressure of a thermophilic lifestyle on bacterial genome

One aspect of the genomic evolution of symbiotic bacteria rarely mentioned in the context of bacterial mutualism in insects concerns the effects that high temperatures may have on genome reduction. Indeed, it has been shown that growth temperature and genome size in bacteria are negatively correlated [62•,63]. For example, *Escherichia coli* cells growing under a regime of increasing temperature tend to accumulate mutations and lose certain genes [64]. At high temperatures, bacteria tend to experience selective pressures favoring more compact and more efficient genomes, as well as tiny cell size. In other words, small genomes are adaptive at high temperatures [62•]. Although these aspects have mainly been studied in the context of thermophilic lifestyles, they remain poorly addressed in the context of the evolution of bacterial mutualism of insects. We will not speculate here on the role of the increase of the temperatures on the reductive evolution that undergoes the genome of bacterial symbionts. However, with an increased incidence of transient extreme temperatures expected to exacerbate the genomic reduction of bacteria, we suggest here that the repetition of thermal stresses could have direct consequences on the evolution of symbiont genomes by accelerating their deterioration.

Conclusions and future directions

Many insect species harbor nutritional obligate symbionts, sometimes embedded in complex multi-symbiotic systems involving co-obligate and facultative bacterial partners. Understanding how thermal stresses affect the susceptibility of these mutualistic relationships is of enormous interest in light of our rapidly changing climate. Many studies now suggest that thermal tolerance of certain insect species is not only governed by the host genes, but also by the bacterial partners they host. Indeed, the weak capacity of obligate symbionts to 'reinvent themselves genetically' (because of their isolation and reduced genome) severely limits their ability to adapt to rapid environmental changes. Thus, in the context of climate change, the pressure exerted on obligate bacterial mutualisms does not result so much from high temperatures, but rather from a sudden change for associations that have shaped in a relatively stable environment, sometimes millions years ago. In this review, we propose putative evolutionary scenarios that may contribute to the acclimatization and adaption of insect species in a rapidly changing climate. Unfortunately, even though there is evidence that such scenarios exist in other invertebrates (e.g. corals and sponges), there is still not enough evidence that symbiotic plasticity can allow for rapid adaptation of insects in the context of current global change. Furthermore, there is clearly a lack of data regarding the thermal tolerance of bacterial mutualism in insects living in tropical areas. Finally, insects evolve in a context of accumulation of anthropogenic environmental pressures, in particular habitat loss, pesticides applications and pollution. Future studies taking into account the multiple environmental constraints insects face are needed to more accurately predict the impact of global changes on bacterial mutualism.

Conflict of interest statement

Nothing declared.

Acknowledgements

We are very grateful to Florence Hecq and Guillaume Le Goff for their helpful comments and corrections on the manuscript.

This work was supported by the Fonds de la Recherche Scientifique - FNRS through a Fonds pour la Formation à la Recherche dans l'Industrie et dans l'Agriculture (FRIA). This paper is publication BRC 434 of the Biodiversity Research Center (Université catholique de Louvain).

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Scheffers BR, Meester LD, Bridge TCL, Hoffmann AA, Pandolfi JM, Corlett RT, Butchart SHM, Pearce-Kelly P, Kovacs KM, Dudgeon D *et al.*: **The broad footprint of climate change from genes to biomes to people**. *Science* 2016, **354** aaf7671.
2. Urban MC: **Accelerating extinction risk from climate change**. *Science* 2015, **348**:571-573.
3. Colinet H, Sinclair BJ, Vernon P, Renault D: **Insects in fluctuating thermal environments**. *Annu Rev Entomol* 2015, **60**:123-140.
4. Lelieveld J, Proestos Y, Hadjinicolaou P, Tanarhte M, Tyrlis E, Zittis G: **Strongly increasing heat extremes in the Middle East and North Africa (MENA) in the 21st century**. *Clim Change* 2016, **137**:245-260.
5. Vasseur DA, DeLong JP, Gilbert B, Greig HS, Harley CDG, McCann KS, Savage V, Tunney TD, O'Connor MI: **Increased temperature variation poses a greater risk to species than climate warming**. *Proc R Soc B Biol Sci* 2014, **281** 20132612.

6. Baker DM, Freeman CJ, Wong JCY, Fogel ML, Knowlton N: **Climate change promotes parasitism in a coral symbiosis**. *ISME J* 2018, **12**:921.

7. Kikuchi Y, Tada A, Musolin DL, Hari N, Hosokawa T, Fujisaki K, Fukatsu T: **Collapse of insect gut symbiosis under simulated climate change**. *mBio* 2016, **7** e01578-16.

8. Morales-Castilla I, Matias MG, Gravel D, Araújo MB: **Inferring biotic interactions from proxies**. *Trends Ecol Evol* 2015, **30**:347-356.

9. van der Putten WH, Bradford MA, Brinkman EP, van de Voorde TFJ, Veen GF: **Where, when and how plant–soil feedback matters in a changing world**. *Funct Ecol* 2016, **30**:1109-1121.

10. Stoks R, Verheyen J, Van Dievel M, Tüzün N: **Daily temperature variation and extreme high temperatures drive performance and biotic interactions in a warming world**. *Curr Opin Insect Sci* 2017, **23**:35-42.

11. Torda G, Donelson JM, Aranda M, Barshis DJ, Bay L, Berumen ML, Bourne DG, Cantin N, Foret S, Matz M *et al.*: **Rapid adaptive responses to climate change in corals**. *Nat Clim Change* 2017, **7**:627-636.

12. Kwiatkowski L, Cox P, Halloran PR, Mumby PJ, Wiltshire AJ: **Coral bleaching under unconventional scenarios of climate warming and ocean acidification**. *Nat Clim Change* 2015, **5**:777-781.

13. • Webster NS, Reusch TBH: **Microbial contributions to the persistence of coral reefs**. *ISME J* 2017, **11**:2167-2174.

This review offers a conceptual overview of the microbial mechanisms facilitating transgenerational acclimatization of coral reef organisms. In this article, the authors highlight the bacterial dynamism in the context of mutualistic relationships and explore various evolutionary scenarios (symbiont shuffling and switching, HGT, etc.) regarding the persistence of coral reefs.

14. Bestion E, Jacob S, Zinger L, Di Gesu L, Richard M, White J, Cote J: **Climate warming reduces gut microbiota diversity in a vertebrate ectotherm**. *Nat Ecol Evol* 2017, **1** 0161.

15. Crowther TW, Thomas SM, Maynard DS, Baldrian P, Covey K, Frey SD, van Diepen LTA, Bradford MA: **Biotic interactions mediate soil microbial feedbacks to climate change**. *Proc Natl Acad Sci* 2015, **112**:7033-7038.

16. Lladó S, López-Mondéjar R, Baldrian P: **Forest soil bacteria: diversity, involvement in ecosystem processes, and response to global change**. *Microbiol Mol Biol Rev* 2017, **81** e00063-16.

17. Fisher RM, Henry LM, Cornwallis CK, Kiers ET, West SA: **The evolution of host-symbiont dependence**. *Nat Commun* 2017, **8**:15973.

18. Toby Kiers E, Palmer TM, Ives AR, Bruno JF, Bronstein JL: **Mutualisms in a changing world: an evolutionary perspective**. *Ecol Lett* 2010, **13**:1459-1474.

19. Abram PK, Boivin G, Moiroux J, Brodeur J: **Behavioural effects of temperature on ectothermic animals: unifying thermal physiology and behavioural plasticity**. *Biol Rev* 2017, **92**:1859-1876.

20. Sgrò CM, Terblanche JS, Hoffmann AA: **What can plasticity contribute to insect responses to climate change?** *Annu Rev Entomol* 2016, **61**:433-451.

21. Sinclair BJ, Marshall KE, Sewell MA, Levesque DL, Willett CS, Slotsbo S, Dong Y, Harley CDG, Marshall DJ, Helmuth BS *et al.*: **Can we predict ectotherm responses to climate change using thermal performance curves and body temperatures?** *Ecol Lett* 2016, **19**:1372-1385.

22. •• Sánchez-Bayo F, Wyckhuys KAG: **Worldwide decline of the entomofauna: a review of its drivers**. *Biol Conserv* 2019, **232**:8-27.

A very interesting and comprehensive review providing evidence for the insect decline, which are crucial components of ecosystems and presenting the main drivers of this disappearance. The study argues that habitat loss by conversion to intensive agriculture is the main driver of this decline and that climate change, pollutants and invasive species are supplementary causes.

23. Corbin C, Heyworth ER, Ferrari J, Hurst GDD: **Heritable symbionts in a world of varying temperature**. *Heredity* 2017, **118**:10-20.

24. Hosokawa T, Ishii Y, Nikoh N, Fujie M, Satoh N, Fukatsu T: **Obligate bacterial mutualists evolving from environmental bacteria in natural insect populations**. *Nat Microbiol* 2016, **1**:15011.

25. McLean AHC, Parker BJ, Hrček J, Henry LM, Godfray HCJ: **Insect symbionts in food webs**. *Philos Trans R Soc B Biol Sci* 2016, **371** 20150325.

26. Moran NA: **When obligate partners melt down**. *mBio* 2016, **7** e01904-16.

27. Wernegreen JJ: **Mutualism meltdown in insects: bacteria constrain thermal adaptation**. *Curr Opin Microbiol* 2012, **15**:255-262.

28. Wernegreen JJ: **In it for the long haul: evolutionary consequences of persistent endosymbiosis**. *Curr Opin Genet Dev* 2017, **47**:83-90.

29. Sachs JL, Simms EL: **Pathways to mutualism breakdown**. *Trends Ecol Evol* 2006, **21**:585-592.

30. Lo W-S, Huang Y-Y, Kuo C-H: **Winding paths to simplicity: genome evolution in facultative insect symbionts**. *FEMS Microbiol Rev* 2016, **40**:855-874.

31. Bennett GM, Moran NA: **Heritable symbiosis: the advantages and perils of an evolutionary rabbit hole**. *Proc Natl Acad Sci U S A* 2015, **112**:10169-10176.

32. Burke G, Fiehn O, Moran N: **Effects of facultative symbionts and heat stress on the metabolome of pea aphids**. *ISME J* 2010, **4**:242-252.

33. Fan Y, Wernegreen JJ: **Can't take the heat: high temperature depletes bacterial endosymbionts of ants**. *Microb Ecol* 2013, **66**:727-733.

34. • Hussain M, Akutse KS, Ravindran K, Lin Y, Bamisile BS, Qasim M, Dash CK, Wang L: **Effects of different temperature regimes on survival of *Diaphorina citri* and its endosymbiotic bacterial communities**. *Environ Microbiol* 2017, **19**:3439-3449.

Among emerging work showing the thermal sensitivity of insect endosymbionts. This study found that two Asian citrus psyllid populations exposed to high temperatures resulted in an increase in host mortality and a decrease in relative densities of the obligate endosymbiont *Candidatus Carsonella ruddii* and of the facultative endosymbiont *Wobachia* surface protein (WSP).

35. Moran NA, Yun Y: **Experimental replacement of an obligate insect symbiont**. *Proc Natl Acad Sci* 2015, **112**:2093-2096.

36. • Shan H-W, Deng W-H, Luan J-B, Zhang M-J, Zhang Z, Liu S-S, Liu Y-Q: **Thermal sensitivity of bacteriocytes constrains the persistence of intracellular bacteria in whitefly symbiosis under heat stress**. *Environ Microbiol Rep* 2017, **9**:706-716.

This study demonstrated that the localization of endosymbionts in insects can affect their adaptation to temperatures. The results showed that under heat stress, the obligate symbionts *Portiera* and *Hamiltonella defensa* residing in bacteriocytes are almost totally depleted in the *Bemisia tabaci* whitefly, while the facultative symbiont residing outside bacteriocytes tend to resist heat stress, suggesting that bacteriocytes are thermosensitive.

37. Dunbar HE, Wilson ACC, Ferguson NR, Moran NA: **Aphid thermal tolerance is governed by a point mutation in bacterial symbionts**. *PLoS Biol* 2007, **5**:e96.

38. Burke GR, McLaughlin HJ, Simon J-C, Moran NA: **Dynamics of a recurrent *Buchnera* mutation that affects thermal tolerance of pea aphid hosts**. *Genetics* 2010, **186**:367-372.

39. Perkovsky E, Wegierek P: **Aphid–*Buchnera*–Ant symbiosis; or why are aphids rare in the tropics and very rare further south?** *Earth Environ Sci Trans R Soc Edinb* 2018, **107**:297-310.

40. Sudakaran S, Kost C, Kaltenpoth M: **Symbiont acquisition and replacement as a source of ecological innovation**. *Trends Microbiol* 2017, **25**:375-390.

41. Latorre A, Manzano-Marín A: **Dissecting genome reduction and trait loss in insect endosymbionts**. *Ann N Y Acad Sci* 2017, **1389**:52-75.

42. Manzano-Marín A, D'acier AC, Clamens A-L, Orvain C, Cruaud C, Barbe V, Jousselin E: **Serial horizontal transfer of vitamin-biosynthetic genes enables the establishment of new**

nutritional symbionts in aphids' di-symbiotic systems. *bioRxiv* 2019:556274.

43. Manzano-Marín A, Szabó G, Simon J-C, Horn M, Latorre A: **Happens in the best of subfamilies: establishment and repeated replacements of co-obligate secondary endosymbionts within Lachninae aphids**. *Environ Microbiol* 2017, **19**:393-408.

44. Bennett GM, Moran NA: **Small, smaller, smallest: the origins and evolution of ancient dual symbioses in a phloem-feeding insect**. *Genome Biol Evol* 2013, **5**:1675-1688.

45. Husnik F, McCutcheon JP: **Repeated replacement of an intrabacterial symbiont in the tripartite nested mealybug symbiosis**. *Proc Natl Acad Sci U S A* 2016, **113**:E5416-E5424.

46. Chong RA, Moran NA: **Evolutionary loss and replacement of *Buchnera*, the obligate endosymbiont of aphids**. *ISME J* 2018, **12**:898.

47. Koga R, Moran NA: **Swapping symbionts in spittlebugs: evolutionary replacement of a reduced genome symbiont**. *ISME J* 2014, **8**:1237-1246.

48. Matsuura Y, Moriyama M, Łukasik P, Vanderpool D, Tanahashi M,
•• Meng X-Y, McCutcheon JP, Fukatsu T: **Recurrent symbiont recruitment from fungal parasites in cicadas**. *Proc Natl Acad Sci U S A* 2018, **115**:E5970-E5979.
In this outstanding study, the authors show that in some cicada lineages, one of the obligate bacterial symbionts with an extremely streamlined genome (*Hodgkinia*) was recurrently replaced by entomopathogenic fungi. This work sheds light on the underlying mechanisms of the parasitism-symbiosis evolutionary continuum and the mechanisms that compensate for the loss of degenerate obligate symbionts.

49. Vogel KJ, Moran NA: **Functional and evolutionary analysis of the genome of an obligate fungal symbiont**. *Genome Biol Evol* 2013, **5**:891-904.

50. Husnik F, McCutcheon JP: **Functional horizontal gene transfer**
•• **from bacteria to eukaryotes**. *Nat Rev Microbiol* 2018, **16**:67-79.
A very interesting and comprehensive review that focuses on horizontal gene transfers (HGT) from bacteria to eukaryotes and gives examples where transferred genes have become functional and provide adaptations to the recipient host. In addition to the evolutionary aspects, the article develops very interesting elements from a technical point of view.

51. Leclercq S, Thézé J, Chebbi MA, Giraud I, Moumen B, Ernenwein L, Grève P, Gilbert C, Cordaux R: **Birth of a W sex chromosome by horizontal transfer of *Wolbachia* bacterial symbiont genome**. *Proc Natl Acad Sci U S A* 2016, **113**:15036-15041.

52. Luan J-B, Chen W, Hasegawa DK, Simmons AM, Wintermantel WM, Ling K-S, Fei Z, Liu S-S, Douglas AE: **Metabolic Coevolution in the bacterial symbiosis of whiteflies and related plant sap-feeding insects**. *Genome Biol Evol* 2015, **7**:2635-2647.

53. Husnik F, Nikoh N, Koga R, Ross L, Duncan RP, Fujie M, Tanaka M, Satoh N, Bachtrog D, Wilson ACC *et al.*: **Horizontal gene transfer from diverse bacteria to an insect genome enables a tripartite nested mealybug symbiosis**. *Cell* 2013, **153**:1567-1578.

54. Ballinger MJ, Moore LD, Perlman SJ: **Evolution and diversity of inherited *Spiroplasma* symbionts in *Myrmica* ants**. *Appl Environ Microbiol* 2018, **84** e02299-17.

55. Pan X, Pike A, Joshi D, Bian G, McFadden MJ, Lu P, Liang X, Zhang F, Raikhel AS, Xi Z: **The bacterium *Wolbachia* exploits host innate immunity to establish a symbiotic relationship with the dengue vector mosquito *Aedes aegypti***. *ISME J* 2018, **12**:277-288.

56. Gruntenko NE, Ilinsky YY, Adonyeva NV, Burdina EV, Bykov RA,
• Menshanov PN, Rauschenbach IY: **Various *Wolbachia* genotypes differently influence host *Drosophila* dopamine metabolism and survival under heat stress conditions**. *BMC Evol Biol* 2017, **17**:252.
This laboratory study showed that some strains of the *Wolbachia* symbiotic bacterium have the ability to increase a heat stress tolerance of their host *Drosophila* by intensifying dopamine metabolism. The results suggest that the symbiotic bacterium can increase its host survival under heat stress but that the protection depend on the genotype of the strain.

57. Ross PA, Wiwatanaratanabutr I, Axford JK, White VL, Endersby-Harshman NM, Hoffmann AA: ***Wolbachia* infections in *Aedes aegypti* differ markedly in their response to cyclical heat stress**. *PLoS Pathog* 2017, **13**:e1006006.

58. Zytynska SE, Weisser WW: **The natural occurrence of secondary bacterial symbionts in aphids**. *Ecol Entomol* 2016, **41**:13-26.

59. Oliver KM, Higashi CH: **Variations on a protective theme: *Hamiltonella defensa* infections in aphids variably impact parasitoid success**. *Curr Opin Insect Sci* 2019, **32**:1-7.

60. Leclair M, Pons I, Mahéo F, Morlière S, Simon J-C, Outreman Y: **Diversity in symbiont consortia in the pea aphid complex is associated with large phenotypic variation in the insect host**. *Evol Ecol* 2016, **30**:925-941.

61. Skaljac M, Kirfel P, Grotmann J, Vilcinskas A: **Fitness costs of**
• **infection with *Serratia symbiotica* are associated with greater susceptibility to insecticides in the pea aphid *Acyrthosiphon pisum***. *Pest Manag Sci* 2018, **74**:1829-1836.
In this study, the authors show that a facultative symbiont increases the susceptibility of infected aphids to insecticides. By showing that the costs associated with facultative bacteria make insects more vulnerable to environmental change, the study points to the antagonistic aspects of facultative symbiosis.

62. Nicks T, Rahn-Lee L: **Inside out: archaeal ectosymbionts**
• **suggest a second model of reduced-genome evolution**. *Front Microbiol* 2017, **8**:384.
The authors propose two pathways of symbiosis-driven genome reduction: thermal-symbiosis experienced by thermophilic microorganisms, and isolation-symbiosis experienced by insect endosymbionts. The comparative analysis of genome reduction in both situations takes a fresh look at the evolution of bacterial genomes.

63. Sabath N, Ferrada E, Barve A, Wagner A: **Growth temperature and genome size in bacteria are negatively correlated, suggesting genomic streamlining during thermal adaptation**. *Genome Biol Evol* 2013, **5**:966-977.

64. Blaby IK, Lyons BJ, Wroclawska-Hughes E, Phillips GCF, Pyle TP, Chamberlin SG, Benner SA, Lyons TJ, de Crécy-Lagard V, de Crécy E: **Experimental evolution of a facultative thermophile from a mesophilic ancestor**. *Appl Environ Microbiol* 2012, **78**:144-155.