How environmental conditions affect the acquisition, establishment, and persistence of microbial endosymbiosis in insects

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Abstract

Long-term interactions between insect hosts and their internal microbial symbionts are ubiquitous. As these interactions support many aspects of the insect host biology, restrictions to their establishment and maintenance could have important consequences to the survival of insects and functioning of ecosystems. The current literature provides extensive evidence that rapidly changing environmental conditions can challenge the four steps to the successful establishment of novel symbioses — namely (i) the horizontal transfer of the microbe between hosts, (ii) the internalization of the microbe within the naive host, (iii) the persistence of the symbiosis over generations in the host lineage, and (iv) the spread of the symbiont across the host population. We provide here a timely synthesis of this field and show that despite the richness of case studies conducted over the last decades, the majority of these studies test, under laboratory conditions, the effects of variation in temperatures on symbiotic partnerships between bacteria and Hemiptera. These studies cover a small fraction of taxa in which, and environmental conditions under which, endosymbiosis occur.

We discuss biotic and abiotic uncertainties in the acquisition, the establishment, and the persistence of symbioses in insects under changing environmental conditions.

Keywords: host shifts, horizontal transfer, vertical transmission, abiotic factors, climate change, symbiont, *Wolbachia*, ecological interaction

Contents

I. Introduction

Endosymbiosis is the long-term relationship between a microbial organism, such as a bacterium or fungus, within the cells, tissues or body cavities of a host (Douglas, A. E. and Smith, D. C., 1989; Wernegreen, 2004; Martin & Schwab, 2012). The ubiquity of such relationships has been attributed to the ability of the symbionts to both colonize a wide array of hosts and to manipulate the hosts' biology towards their own benefit, which in turn support their spread in the host population over generations (Moran & Baumann, 2000; Wernegreen, 2004). Symbionts benefit by manipulating their hosts' reproduction systems (Wernegreen, 2012b) or other life history traits, such as fecundity (Himler *et al.*, 2011), nutrition (Douglas, 1998; Skidmore & Hansen, 2017), and defence against parasites and pathogens (Hedges *et al.*, 2008; Pimentel *et al.*, 2020; Cogni *et al.*, 2021). Association with endosymbionts has led to evolutionary innovations by their insect hosts, contributing to the great taxonomic diversity and success of insects (Stork, 2018). Endosymbionts are crucial to the central roles of insects in terrestrial ecosystem functions, such as pollination, pest control and nutrient cycling (Losey & Vaughan, 2006; Noriega *et al.*, 2018; Elizalde *et al.*, 2020).

The colonization of an insect host by symbiotic partners mostly occurs through vertical transmission (i.e., inheritance from a parent) (Table 1), via either the germline (Moran & Telang, 1998; Moran, McCutcheon & Nakabachi, 2008), or extracellular mechanisms, such as ingestion of symbiont-infected parental secretions (Hosokawa *et al.*, 2005; Salem *et al.*, 2015). However, phylogenetic studies have demonstrated that many endosymbionts are not restricted to vertical transmission. They also transfer horizontally between unrelated individuals of the same species and between individuals of different species (Table 1) (Bright & Bulgheresi, 2010; Turelli *et al.*, 2018). As an example, the widespread endosymbiotic bacterium, *Wolbachia,* which is primarily transmitted vertically from mother to offspring (Bailly-Bechet *et al.*, 2017), is known to transfer horizontally among species occasionally (Haine, Pickup & Cook, 2005; Werren, Baldo & Clark, 2008; Stahlhut *et al.*, 2010), thus exhibiting a mixed-mode transmission. Successful colonization and long-term establishment of an endosymbiont in a naïve host lineage, or species, requires four main steps (Bright & Bulgheresi, 2010; Chrostek *et al.*, 2017; McCutcheon, Boyd & Dale, 2019). These include (*i*) a novel acquisition of the endosymbiont from the environment or through a 'jump' of the endosymbiont between a donor and a naïve host, (*ii*) the proliferation within the naïve host's body, (*iii*) the persistence through generations via maternal vertical transmission, and (*iv*) the spread within the host population across generations (Sanaei, Charlat & Engelstädter, 2021). These last two steps are characteristic of vertical transgenerational transmission of the symbiont and long-term persistence of the interaction in the host population.

Current anthropogenic environmental changes are profoundly modifying the Earth (Calvin *et al.*, 2023). They affect ecosystems stability (Hautier *et al.*, 2015), as well as ecological and evolutionary processes underpinning species biodiversity (Jordano, 2016), including at the macro- but also at the micro-community levels (Lovejoy, T. E. & Hannah, L., 2006; Toby Kiers *et al.*, 2010; Barlow *et al.*, 2016; Renoz, Pons & Hance, 2019; Vidal *et al.*, 2021). In this context, a large body of work has addressed how changes in environmental factors can re-shape the physiology (Yang *et al.*, 2021), persistence and establishment of endosymbiosis, and/or the prevalence of insect-endosymbiont interactions (Gange *et al.*, 2011; Wernegreen, 2012a; Hector *et al.*, 2022). For instance, Martins and collaborators (2023) showed that temperature correlates positively with endosymbionts' titer within a host, and negatively with other endosymbionts' traits, such as maternal transmission rate and protection against natural enemies. This exemplifies the complexity of the effects of abiotic factors on host-endosymbionts systems.

In this review, we synthetise the current knowledge on how environmental conditions affect endosymbiotic host shifts in insects. We discuss the literature from two perspectives: (i) what biological systems the field is currently built on, and (ii) how abiotic factors influence the acquisition, establishment, and persistence of microbial endosymbiosis in insects. Specifically, we first present a systematic literature review of studies on the effects of environmental factors on host-symbiont associations. Based on a list of 50 studies of endosymbionts, insect hosts, and abiotic factors, we discuss findings and highlight biases and gaps in the literature. We consider only non-pathogenic endosymbionts (Table 1) and abiotic factors known to affect terrestrial insects (Wagner, 2020), which include atmospheric gases (e.g., O_3 concentration), environmental toxin (e.g., pesticide, antibiotics), inorganic nutrient (e.g., nitrogen), precipitation, relative humidity, seasonality, temperature, and water stress (e.g., drought) (S1 Text). Our analysis demonstrates a disproportionate exploration of temperature effects on beneficial symbionts of aphids, which may limit our view of how host shifts operate in nature. Secondly, we discuss how the four steps necessary for endosymbiotic host shifts in insects may be affected by distinct environmental factors, focusing on how changing environments could interfere on the host shift success. Overall, the findings of these studies vary, yielding no clear consensus on the effects of environmental change on insect-endosymbiont interactions (Six *et al.*, 2011; Wernegreen, 2012a; Hom & Penn, 2021; Hector *et al.*, 2022). Bailly-Bechet and coauthors (2017) estimated that the acquisition rate (gain minus loss) of *Wolbachia* by a new host is once every few million years. The work summarized in this review suggests that anthropogenetic environmental change is likely to both hinder and facilitate different aspects of the complex process of endosymbiont acquisition.

Table 1. Glossary

Term	Definition
Symbiosis	Relationship of a host with a microbial symbiont (e.g., bacterium, virus, fungus). Symbiotic associations range along a continuum from parasitic to mutualistic (de Bary, 1878; Douglas, A. E. and Smith, D. C., 1989; Martin & Schwab, 2012).
Endosymbiosis	Symbiotic system in which the microorganism lives within the host rather than outside or on it. Endosymbionts may live in the somatic or germ cells or extracellularly in the host body cavities (Martin & Schwab, 2012). Although there is debate about whether pathogens should be considered symbionts (Pérez-Brocal, Latorre & Moya, 2013; Rosenberg & Zilber-Rosenberg, 2013), in this review we focus on non-pathogenic endosymbionts. Endosymbionts have evolved mechanisms to persist long-term in association with their hosts, whereas pathogens are strongly depending on regular transfer among hosts.
Host shift	In the context of this review, a host shift is when a symbiont colonises and associates over the long term with a new host species or a new lineage in a host population. A large body of literature on host shifts targets pathogens spreading infectiously through transfer between hosts (Longdon *et al.*, 2014).

Transfer	The horizontal (not trans-generational) 'jump' of a microbial symbiont from a donor host to a naïve receiving host within or between host species.
Transmission	The trans-generation, vertical, or maternal transmission of microbial symbionts within a host lineage.

II. How is the field of symbiotic host shifts in changing environments structured?

We searched the Institute for Scientific Information Web of Science database for literature investigating the effect of abiotic conditions on microbial host shifts in insects. This was done by a searching for variations of the key words 'abiotic factor', 'host shift', 'symbiont,' and 'insect' (S1 Text). We obtained a final curated list of 50 research articles published between 1994 and 2022, which included 177 unique host-symbiont-abiotic factor combinations (S1 Text, S1 Figure). We statistically compared frequencies of different species-factor combinations in the literature using a χ^2 test (Pearson, 1900) with the "stats" package (R Core Team, 2018). The studies were treated as statistically independent. Data visualisation was done using the package ggalluvial0.12.5 (Brunson, 2020) in ggplot2 3.4.4 (Wickham, 2011) and edited using BioRender.

With a 46% increase in the number of articles over the last five years (S4 Figure), the field of insect symbiosis host shifts in changing environments is clearly active and growing. However, biases in the study of the effects of abiotic changes on endosymbiosis in insects persist, and we present the three most significant ones in details below.

(1) Patterns and biases related to symbionts

Our results revealed that symbiotic systems involving bacteria were significantly more abundant than those with fungi or viruses (χ^2 = 157.560; df = 1; $p < 0.0001$). Bacteria were often present as single endosymbionts (e.g., different *Wolbachia* strains, *Buchnera*), and sometimes as whole bacterial communities (i.e., microbiota) (Figure 1). The bacteria *Spiroplasma*, *Hamiltonella*, and *Wolbachia* were investigated more frequently than other bacterial taxa (*Spiroplasma*: χ^2 = 80.006; df = 1; $p < 0.0001$; *Hamiltonella*: χ^2 = 91.124; df = 1; $p < 0.0001$; *Wolbachia*: χ^2 = 96.955; df = 1; $p < 0.0001$). However, these three symbionts were all equally prevalent in the literature ($p > 0.05$) (Table S3).

In the field of non-pathogenetic insect symbioses, there is a historical emphasis toward studies involving bacteria (Gurung, Wertheim & Falcao Salles, 2019). Just like bacteria, fungal and viral non-pathogenic endosymbiosis are extremely common and are affected by environmental conditions (Martin, 1992; Rosa *et al.*, 2019; Biedermann & Vega, 2020). However, they may not respond in the same way to environmental conditions as do bacteria. For instance, Rosa and coauthors (2019) demonstrated that the fungal and bacterial communities in *Melitaea cinxia* caterpillars responded differently to their hosts feeding on water-stressed plants compared to well-watered plants. The fungal community composition shifted, while the bacterial communities increased in heterogeneity among host individuals. Fungi and viruses have been studied extensively as pathogens. However, we cannot simply generalize to non-pathogenic systems because they have such different life histories. Thus, there is still much to be learned about environmental effects on endosymbiotic associations and their establishment by studying the microbial symbionts that are not bacteria.

(2) Patterns and biases related to hosts

Comparing insect Orders, Hemiptera was the most studied host system, comprising 42% of the recorded host-symbiont combinations ($\chi2$ = 10.446; df = 1; p = 0.0012). This was followed by Diptera, which were in 16% of the combinations ($\chi2$ = 62.288; df = 1; p < 0.0001) (Figure 1, Table S3). Among Hemiptera, 78% of the combinations included aphid species ($\chi2$ = 28.509; df = 1; p < 0.0001), while *Drosophila* species were in 83 % of the Diptera combinations ($\chi2$ = 16.000; df = 1; p < 0.0001). Many studies involved *Wolbachia* in *Drosophila* (Detcharoen *et al.*, 2020; Hague, Caldwell & Cooper, 2020; Hague *et al.*, 2022; Radousky *et al.*, 2023), and nutritional symbiosis within aphids, which have unveiled fascinating insights into microbial coevolution, nutrient acquisition, host fitness, and ecological dynamics (Douglas, 1998; Sandström, Telang & Moran, 2000). This particular bias is not surprising, as these insects are easy to rear and well-characterized laboratory systems, for which a wealth of relevant contextual information is available. However, while these species of Hemiptera and Diptera serve as excellent model organisms for studying symbiosis and host shift between host lineages (Koch & McFall-Ngai, 2018), findings using them are not all applicable to other host species and their associated microbes. In a meta-analysis, Malacrinò (2022) pointed out the importance of hosts identity in shaping hosts-symbionts interactions by observing that insect taxonomy was the primary determinant of microbiota diversity, surpassing factors such as sex, life stage, sample origin, and treatment (Malacrinò, 2022; Maritan *et al.*, 2024). Research on diverse symbionts in non-model insect species is poised to uncover new insights and patterns in the establishment of insect-symbiont relationships, and to offer a more accurate estimate of the true diversity and complexity of these relationships under various environmental conditions.

(3) Patterns and biases related to abiotic factors

Temperature, as warming or cooling of the air, or as heat shocks (S1 Text), was investigated in 74% of the combinations, dominating the list of abiotic factors addressed by the literature in the field ($\chi2 = 12.480$; df = 1; $p = 0.0004$) (Figure 1). The next most frequent abiotic factors were climate and precipitation (Table S3). The predominance of temperature among the combinations can be explained by its role as a major determinant of life functioning, influencing systems across all levels of biological organization and timescales. Temperature is particularly important for ectothermic animals, such as insects, as it directly affects their development and growth (Gilbert & Raworth, 1996; Sunday *et al.*, 2014). Additionally, temperature plays a crucial role in cellular physiology and metabolism, including that of microbes, which rely on optimal temperature conditions for proper functioning (Knapp & Huang, 2022). However, other largely unexplored environmental factors, such as humidity and pollution, are changing rapidly and have the potential to drastically reshape ecosystems (Wagner, 2020). Studying a broader range of abiotic factors will balance our understanding of how environmental change impact symbiotic host shifts dynamics. In addition, research combining different abiotic factors will elucidate their mixed effects on systems in ways that studying individual factors alone cannot predict.

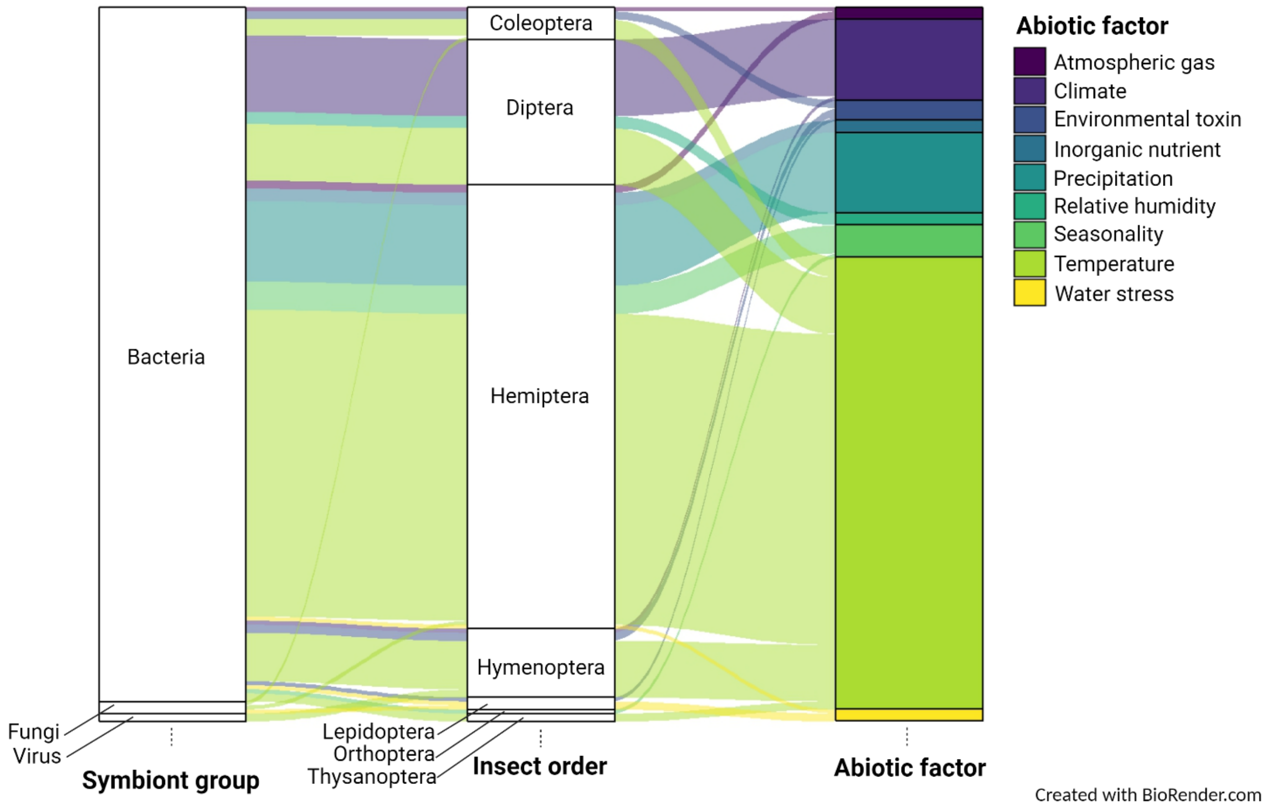

Figure 1. Frequency of distinct “endosymbiont-insect host-abiotic factor” combinations present in the literature (as of 14/03/2022, Web of Science). Full description of sorting criteria (S1 Text) and raw data (S2 Dataset) can be found in the Supporting Information.

The systematic literature search presented here validates common perceptions about the field (e.g., temperature is a widely tested abiotic factor). While this finding may not be novel, it provides a valuable overview of how the field is currently structured. A clear understanding of the field’s existing structure is essential for driving meaningful advancements, as identifying biases and gaps enables future studies to build upon underexplored areas. Drawing upon the insights from the existing literature, and despite the biases in the field, we proceeded to review the studies that evaluate the impacts of environmental factors on endosymbiotic host shifts in insects, while also proposing systems and methodologies for further investigation.

III. Foundation steps of novel endosymbiosis under abiotic changes

A symbiont can increase its prevalence beyond the limits of its own host lineage through host shifts. A novel endosymbiosis via a host shift can be established through a complex four steps process (Figure 2): (1) the acquisition by a naïve host species or lineage, (2) the survival and proliferation in the hosting cells and tissues, (3) the vertical transmission to the next generation, and (4) the successful spread in the host population through reproductive success of the host lineage via advantage to or manipulation of the host. As we review below, each of these four steps depends on successive events that can be either hindered or facilitated by environmental conditions. Pressures at each event might lead to failure of new symbioses to establish, or to selection of resilient symbiotic strains capable of overcoming the environmental stresses, and of maintaining the endosymbiotic association.

(1) Acquisition of an endosymbiont by a naïve host

(a) Shared space and time

Although direct physical contact between the donor and recipient hosts is not always necessary for an endosymbiont to transfer to a new host, the microbe and hosts must share the same space and time within the limits of the symbiont and recipient host lifespans (Kümmerer, 1996; Chrostek *et al.*, 2017) (Figure 2a). The horizontal acquisition of an endosymbiont by a naïve host has been shown to have occurred through various ecological routes, such as shared resources (Haine *et al.*, 2005; Caspi-Fluger *et al.*, 2012; Gonella *et al.*, 2015; Li *et al.*, 2017; Miraldo & Duplouy, 2019; Cardoso & Gómez-Zurita, 2020). For example, Stahlhut and colleagues (2010) identified mushrooms as potential platforms for the transfer of the bacterium *Wolbachia* across different *Drosophila* species. Other routes include shared prey or parasites (Shaikevich & Romanov, 2022), hybridization (Figure 2h), or traumatic events (Rigaud & Juchault, 1995; Jiggins, 2003; Raychoudhury *et al.*, 2009; Salem *et al.*, 2015; Cooper *et al.*, 2019). While these specific ecological routes have been observed and explored, and although they are presumably not mutually exclusive, the relative frequency, with which each route is used for the transfer of endosymbionts between hosts is unknown.

The probability of a naïve host encountering a symbiont-infected host, and the colonization of the naïve host by the symbiont, is changing as the environment changes. Encounter rate also changes as human movement of goods across the globe leads to shifting geographical insect species ranges (Bell & Collins, 2008; Nogués-Bravo *et al.*, 2018; Larson, Tinghitella & Taylor, 2019; Detcharoen *et al.*, 2020), and to changes in behaviour, phenology, and/or population size of local species (Gornish & Tylianakis, 2013; Carbonell *et al.*, 2017; Yang *et al.*, 2021; Fenn-Moltu *et al.*, 2022). For instance, as species shift their home ranges to follow optimal niches and avoid local environmental changes or extinction, we observe the remodelling of local insect host communities, which alters the probability of encounter between potential hosts and symbionts (Bell & Collins, 2008; Nogués-Bravo *et al.*, 2018; Detcharoen *et al.*, 2020). To our knowledge, however, no study yet investigated how the remodelling of insect communities under environmental changes — whether it is through species invasions, hybridization, geographical range shift, or phenological mismatch — might have already affected the colonization of naïve species by endosymbiotic microbes.

(b) Endosymbiont survival outside the host

Because endosymbionts' lifestyle is adapted to their hosts' cells, tissues, or body cavities, it is generally presumed that endosymbionts lack mechanisms for surviving outside of the host. An endosymbiotic microbe going through host shift (Vavre *et al.*, 1999; Ahmed, Breinholt &

Kawahara, 2016) is however, likely to experience an extra-organismal phase at the time of transfer between the donor and recipient hosts (Figure 2c-d). While never observed in the wild, some endosymbionts have been shown to survive outside their host under laboratory conditions. For example, the bacteria *Arsenophonus* and *Spiroplasma*, have been cultured on brain heart infusion medium (BHI; Oxoid) (Nadal-Jimenez *et al.*, 2022) and Barbour-Stoenner-Kelly H medium (BSK-H) (Masson *et al.*, 2018), respectively, while, Rasgon and colleagues (2006) found that *Wolbachia* could survive for over a week in suspension in culture media. Similarly, Li and coauthors (2017) observed live *Wolbachia* cells over several days in the phloem and in globular regions along the phloem sieve tube of cotton plants. These findings indicate that even endosymbionts can survive outside of a host, which raises the question of how abiotic conditions may affect the suitability of shared resources for exchange of symbionts between donor and naïve hosts. It has not yet been tested whether, and how, *Arsenophonus* and *Spiroplasma* would survive in situations where laboratory conditions change.

Notably, some *Wolbachia* strains have been described as “super spreader” strains that seem to rapidly shift among host species. In particular, the *Wolbachia* strain *w*Ri transferred between eight *Drosophila* species in less than 30,000 years (Turelli *et al.*, 2018). While studies suggest that the establishment in novel hosts is facilitated by the fact that *w*Ri tends to be less deleterious than other strains to novel hosts (Turelli *et al.*, 2018), other mechanisms could be involved, including higher tolerance to non-host environments by the super spreader strains.

The survival of endosymbionts outside the host body may also vary with the changes in the quality and availability of the supporting non-host platform. In their study, Stahlhut and coauthors (2010) identified mushrooms as potential platforms facilitating *Wolbachia* transfer among *Drosophila* species. Most fungi species produce fruiting bodies that last only a few days and it has been shown that global environmental change has shifted mushroom fruiting time in the past decades (Kauserud *et al.*, 2008). Such phenological shifts in the shared resource could lead to the death of the symbiont when outside the host body, simply because the platform is no longer available at the required timing.

(c) Endosymbiont titer and tropism

Titer and tropism, which are, respectively, the concentration or density of a symbiont in the host, and where in the host body the endosymbiont resides, vary greatly among symbioses. These variations reflect different needs for accessing nutrients, and/or for regulating the host bioenergetics and immunity (Uchiyama, 2012; Pietri, DeBruhl & Sullivan, 2016; Li *et al.*, 2020). Environmental conditions affect symbiont titers, and thus the quantity of symbiotic cells available for transfer, and the probability of their transfer to naïve hosts (Figure 2b, 2e) (López-Madrigal & Duarte, 2019). For instance, the titers of the *Wolbachia* strain *w*Mel in *Drosophila melanogaster* (Hague *et al.*, 2022), and of *Spiroplasma* in *Drosophila hydei* (Osaka *et al.*, 2008), were lower in hosts exposed to cool temperatures (20 °C for *w*Mel, and 15 °C or 18 °C for *Spiroplasma*) than in hosts under warmer conditions (28 °C for *w*Mel, and 25 °C or 28 °C for *Spiroplasma*). In contrast, exposures to heat treatments lead to reduced titers of the symbiotic bacterium *Arsenophonus* in *Aphis gossypii* (above 30 °C) (Chang *et al.*, 2022), and of the symbiotic bacteria *Rickettsia*, *Arsenophonus*, and *Wolbachia* in *Bemisia tabaci* (above 44 °C) (Barman *et al.*, 2022). Beyond temperature, environmental contaminants, including veterinary antibiotics used on livestock, and now commonly found in soil and water (Daghrir & Drogui, 2013; Roy, Naidu & Bagchi, 2023), can make their way into insect hosts (Goutte & Molbert, 2022). Nian and collaborators (2022) showed that after ten generations of sublethal tetracycline treatment, the parasitoid wasp *Trichogramma pretiosum* exhibited a significantly lower *Wolbachia* titer, with negative consequences for the female offspring across generations.

Endosymbionts may reside in distinct microhabitats within the host germline and in somatic cells, including specialized bacteriocytes (Frydman *et al.*, 2006; Toomey *et al.*, 2013; Pietri *et al.*, 2016; Luan, 2024). Environmental conditions are again likely to affect the probability of transfer of symbionts to naïve hosts by affecting endosymbionts' tropism, inhibiting or promoting symbionts replication in a certain tissue or their movements inside the host's body (Anbutsu, Goto & Fukatsu, 2008) (Figure 2b, 2e). Parasitoids might offer nice systems to assess this hypothesis. They can acquire and spread symbionts via their ovipositor when pricking and laying their eggs into other insects (Heath *et al.*, 1999; Ahmed *et al.*, 2015b), however, any change in the symbiont tropism within the donor hosts can affect the availability of symbiotic cells and the transfer of the cells to naïve hosts. Tropism also represents a mechanism that enhances the survival of the symbiont within the host, thereby potentially increasing the likelihood of a host shift. In the aphid *Bemisia tabaci*, the tropism of *Portiera* within bacteriocytes reduces the symbiont's vulnerability to temperature fluctuations (Su *et al.*, 2014). Although bacteriocytes are not transferred among hosts, some of the endosymbionts they harbour, such as *Wolbachia* (Michalik *et al.*, 2023), may transfer horizontally. Those symbionts residing temporarily in bacteriocytes can shield themselves against changes in the environment and increase their potential for host switching.

(2) Establishment of an endosymbiont within the new host individual

To persist within the host organism, a newly acquired endosymbiont must navigate a series of physiological challenges, including overcoming the host's immune response, to survive and proliferate in the new host individual (Figure 2f-g).

(a) Host immunity

Insects have a diverse set of immune defences, ranging from physical barriers and behavioural mechanisms, to cellular and humoral responses against non-self-entities (de Roode & Lefèvre, 2012; Rosales, 2017) (Figure 2g). These strategies are profoundly influenced by environmental factors (Shaurub, 2003; Wojda, 2017; Schoenle, Downs & Martin, 2018). The exoskeleton and cell walls are the first lines of physical defences against infection in insects. However, even the thickest cuticles can be weakened or breached, for example through piercing by a parasitoid during oviposition, or during other traumatic events leaving open wounds. Kiefer and collaborators (2021) found that in the sawtoothed grain beetle *Oryzaephilus surinamensis*, a bacterial symbiont that produces the amino acid tyrosine, essential to the host's cuticle, is lost in presence of glyphosate herbicide. Once the symbiont's pathway for tyrosine production is interrupted, there is a reduction in the quality of the beetle's cuticle. In the context of symbiosis, it remains unclear whether beetles with a more vulnerable cuticle would also be more susceptible to the acquisition of new symbiotic associations.

Once an invader, such as a new endosymbiont, enters the naïve host body, behavioural (de Roode & Lefèvre, 2012), humoral (e.g., antimicrobial peptides, melanization, and Toll, IMD, JAK/STAT pathways) and cellular immune mechanisms (e.g., hemocytes) should activate to support the host response to the new infection (Welchman *et al.*, 2009; Rosales, 2017). However, diverse environmental stresses modify the components of insects' immune responses. In the cricket *Acheta domesticus*, specimens infected with *Rickettsiella grylli* exhibited behavioural fever (Wojda, 2017). The crickets moved to warmer places, raising their body temperatures by 6 °C, which was harmful to the bacterium (Adamo, 1998). The rise of global temperatures is likely to increase opportunities for insects to engage in behavioural fever, potentially enhancing such escape strategies, and challenging the maintenance or spread of *Rickettsiella* and any other endosymbiont in their host populations. At the cellular level, Takano and coauthors (2021) observed that an increase in rearing temperatures caused a reduction in haemocyte concentration in the moth *Chrysodeixis eriosoma*. However, the haemocyte phagocytic activity was simultaneously enhanced, suggesting compensation for the lower haemocyte numbers in the moth larvae. Finally, in relation to humoral defense, larvae of the beetle *Tenebrio molitor* had higher haemolymph phenoloxidase activity and antibacterial activity at 30 °C than at 10 °C or 20 °C (Catalán *et al.*, 2012), indicating a stronger humoral immune response in elevated temperatures.

The host's immune response to newly acquired microbial infection can also be supplemented by their resident microbial partners, either single endosymbionts or complex microbial communities (i.e., gut microbiota) (Engel & Moran, 2013; Douglas, 2015; Duplouy, Minard & Saastamoinen, 2020). In the cereal weevil *Sitophilus oryzae,* bacteriocytes are colonized by endosymbiotic bacteria that actively boost the host immune response by inducing the production of antimicrobial peptides (Ferrarini *et al.*, 2022). Changing abiotic conditions may disrupt the functioning of established endosymbionts and gut microbiota, consequently altering host immunity (Mason & Shikano, 2023) and possibly hindering or allowing colonization by new symbiotic species.

Sudden changes in the environment can trigger a heat shock response in the host. For example, under osmotic and oxidative stress, hypoxia, cold and heat shocks, or exposure to novel infections, insects can synthesize heat shock proteins (HSPs) (Wojda, 2017). In the greater wax moth *Galleria mellonella*, immunologically challenged specimens had more of heat shock proteins in the fat body and hemolymph when exposed to higher temperatures (Wojda & Jakubowicz, 2007; Wojda & Kowalski, 2013; Wojda, 2017). Since HSPs prevent the denaturation of other proteins and act as alarmins, they activate insects' defence mechanisms and protect insects' nervous system and gut (González-Tokman *et al.*, 2020; Mason & Shikano, 2023). Whether the host is infected before or after exposure to abiotic stress could affect the outcome of potential endosymbiotic host shifts.

(b) Host metabolism

Exposure to elevated temperatures raises an insects metabolic rate, alters hormone synthesis, and can induce oxidative stress (González-Tokman *et al.*, 2020; Mason & Shikano, 2023). High levels of reactive oxygen species (ROS molecules) can cause oxidative damage to proteins, lipids, and nucleic acids in the symbiont, impairing its function and integrity and reducing its viability (Douglas, 2014; Zug & Hammerstein, 2015). Additionally, as symbionts often rely on metabolites provided by their host (i.e., amino acids, lipids, or iron) (Brownlie *et al.*, 2009; Gill, Darby & Makepeace, 2014; Jiménez *et al.*, 2019; Currin-Ross *et al.*, 2021), the depletion of host nutritional reserves under high temperatures affects both the host and symbionts' metabolisms, compromises symbiont survival (Fisher *et al.*, 2017), and the persistence of their interactions (Figure 2f) (Brownlie *et al.*, 2009; Řezáč *et al.*, 2023). For instance, under nutrient-limiting conditions (i.e., low lipids), the titer of *Spiroplasma* in *Drosophila melanogaster* was found to decline (Herren *et al.*, 2014), which may jeopardize symbiont persistence in the host and subsequent transfer to the next generation.

(3) Transmission to next host generation

(a) Extracellular transmission

In some insects, the maternal transmission of symbionts is extracellular. For instance, stinkbugs offspring acquire their obligate symbiont by ingesting the content of capsules deposited by the mother on the underside of her egg mass after oviposition (Fukatsu & Hosokawa, 2002) (Figure 2i), while adult females of the Hemiptera species, *Pyrrhocoris apterus,* spread *Coriobacterium glomerans* bacteria on the surface of their eggs (Kaltenpoth, Winter & Kleinhammer, 2009). In another example, three species of bumble bees acquire endosymbionts through trophallaxis, or the mouth-to-mouth transfer of nutritious fluids (Wilson, 1971; Koch & Schmid-Hempel, 2011) (reviewed in (Salem *et al.*, 2015)) (Figure 2i-j). In such systems, the quality of the capsule or of the fluid secretion, and its resistance to degradation due to temperature, humidity, or UV light, could determine the survival of the symbionts and their successful acquisition by the new generation of host upon hatching. In a recent study, Fisher and collaborators (2021) found that low humidity reduces the survival of early instar stinkbug nymphs. Although not tested in their study, a possible mechanism for reduced survival is failure of the nymphs to acquire the beneficial symbionts due to desiccation of the capsule or secretion under low humidity (Figure 2j).

(b) Cell-to-cell transmission to the germline

For intracellular endosymbionts, effective transmission requires that the endosymbionts reach specialized cells of the germline or somatic organs, for passage of the microbe to the host zygote (Goto, Anbutsu & Fukatsu, 2006; Pietri *et al.*, 2016; Russell, Chappell & Sullivan, 2019; Kaur *et al.*, 2021; Radousky *et al.*, 2023). The cell-to-cell transmission may be disrupted if specific circumstances change (Figure 2k). For instance, at high temperatures *Spiroplasma* strains can lose their helical morphology which decreases their mobility (Konai *et al.*, 1996). Under such conditions, the less effective movements of the symbiont between cells hinder their vertical transmission to the next host generation (Corbin *et al.*, 2017). Similar mechanism could be taking place in *Drosophila bifasciata*, where *Wolbachia* is perfectly vertically transmitted at 18, 21 and 23.5 °C, but not at 25 °C (Hurst, Jiggins & Robinson, 2001). Microtubules and motor proteins, such as dynein and kinesin, facilitate the localization of *Wolbachia* in the posterior side of *Drosophila* eggs. More precisely, host dynein is hijacked by *Wolbachia* to move within the host cells. Dynein is however directly affected by temperature, as its velocity accelerates with temperature (Hong *et al.*, 2016). Thus, low temperatures may delay or inhibit the ability of *Wolbachia* to reach its optimal titer and colonize the eggs, hampering its transmission to the next generation.

(4) Long-term maintenance of endosymbiosis in the host population

For a symbiont host shift to be successful it must spread beyond the original host individual in the naïve host population. Endosymbionts are inherited microorganisms that gain in fitness as they spread toward fixation within their host population (Wernegreen, 2004). This can be through increasing the host fitness, or inflicting a cost to competing uninfected conspecific host lineages (Figure 2m) (Gerardo & Hurst, 2017). Symbiont-induced phenotypes are diverse. For instance, *Spiroplasma* improves the resistance of *D. hydei* against the parasitoid wasp *Leptopilina heterotoma* (Corbin *et al.*, 2021), while the endosymbiont *Hamiltonella defensa* protects its aphid host, *Acyrthosiphon pisum,* against the parasitoid *Aphidius ervi* (Bensadia *et al.*, 2006). Many of these symbiont-induced phenotypes are, however, sensitive to temperatures. *Spiroplasma* is only beneficial to *D. hydei* at temperatures below 18 °C (Corbin *et al.*, 2021), while *H. defensa* does not protect its host under heat stress (30 °C) (Bensadia *et al.*, 2006). With climate change, endosymbionts such as these might become costly to their host, potentially shifting toward parasitism, or leading to the purging of the endosymbiont from the host population (Hoffmann & Cooper, 2024). Symbionts are not powerless partners, limited by the environmental conditions experienced by their hosts. *Drosophila melanogaster* individuals infected with the *Wolbachia* strain *w*MelCS were shown to occupy sites with cooler mean temperatures (25.06 ± 0.25 °C) than uninfected flies (25.78 ± 0.24 °C) (Arnold *et al.*, 2019), providing an advantage to the *Wolbachia*. This illustrates that endosymbionts have the ability to manipulate their host behaviour toward their thermal optimal (Arnold *et al.*, 2019), leading to increased replication and transmission in the host population (Truitt *et al.*, 2019).

If symbionts exhibit different thermal optima, variations in the prevalence of endosymbionts in the host population should then occur along natural latitudinal and altitudinal climatic gradients (Kriesner *et al.*, 2016; Corbin *et al.*, 2017, 2021; Charlesworth *et al.*, 2019; Hague *et al.*, 2022) (Figure 2l-m). A 20 years survey of *Wolbachia* in Australian populations of *D. melanogaster* has indeed shown that infection frequencies are inversely proportional to latitude along the east coast, i.e., rising towards the Equator, where the average temperature is high (Kriesner *et al.*, 2016; Hague *et al.*, 2022). In Lepidoptera, another study showed that *Wolbachia* frequencies were higher in populations inhabiting lower latitude and altitude (Ahmed *et al.*, 2015a), a pattern also observed for the symbiont *Cardinium* in diverse insects (Charlesworth *et al.*, 2019). However, in a study considering several insect species distributed globally, Charlesworth and collaborators (2019) showed that *Wolbachia* frequency increases with temperature within the temperate climate zone but not in the tropics. These contrasting results again illustrate the geographical mosaic pattern (Thompson, 2005; Parfrey, Moreau & Russell, 2018) of the outcome of environmental factors on the host-endosymbiont interaction, and the importance of the interaction of different genotypes (G_{host} x $G_{endosymbiont}$ x Environment) (Lazzaro & Little, 2009; Hague *et al.*, 2020, 2022).

Long-term symbioses are often characterized by symbionts that have undergone significant genomic reductions, along with the evolution of specialized roles within their insect hosts. For instance, "*Candidatus* Tremblaya princeps" in the mealybug *Planococcus* spp. (Thao, Gullan & Baumann, 2002) and *Buchnera aphidicola* in pea aphid *Acyrthosiphon pisum* (Shigenobu *et al.*, 2000) showcase extreme cases of gene loss and decreased GC contents (Wernegreen, 2002, 2012a, 2015; McCutcheon & Moran, 2012; Moran, 2016). Some of the genes lost are involved in DNA repair and temperature related functional plasticity (Wernegreen, 2012a; Moran, 2016; Corbin *et al.*, 2017; Renoz *et al.*, 2019), while heat shock proteins are conserved, probably buffering the effect of deleterious mutations (McCutcheon & Moran, 2012). Although the exact mechanisms behind the AT-biased reduced genomes of endosymbionts remain unclear (McCutcheon & Moran, 2012; McCutcheon *et al.*, 2024), evidence suggests that high AT content impacts the amino acid composition in prokaryotic proteins, resulting in proteins with reduced thermostability (McCutcheon *et al.*, 2024). This reduces the ability of endosymbionts adapt to temperature fluctuations, likely making insect-endosymbiont interactions vulnerable in the face of the ongoing climate change (Renoz *et al.*, 2019).

IV. Future directions

It is an exciting time to study the effects of abiotic factors for the acquisition, establishment, and persistence of terrestrial insect-symbiont interactions. The literature we have compiled demonstrates the diversity of investigation within this field, highlighting a handful of research systems and approaches that are well represented and noting those that are understudied, or even unstudied.

Shifts between host lineages and between host species is essential for the persistence, spread and evolutionary diversification of endosymbiotic associations. Successful shifts are rare (Bailly-

Bechet *et al.*, 2017), which is understandable when we consider the complexity of the four necessary steps presented by Sanaei *et al.* (2021), each of which involves many biotic processes. These biotic processes depend on abiotic conditions. We have elucidated ways in which the changing environment can affect relevant conditions of host shifts, but how these affects the pace of evolutionary acquisition of symbionts (Bailly-Bechet *et al.*, 2017) remains unknown. Perhaps the investigation of the *Wolbachia* "super spreader" strain (*w*Ri) from *Drosophila* species, which has a very high transition rate between hosts (Turelli *et al.*, 2018), will be useful for us to study mechanisms behind the rates of host shift and factors affecting them.

Temperature variation plays an obvious crucial role in the interactions of bacterial symbionts with their hosts, and researchers have made notable progress in understanding how symbionts respond to thermal changes. However, there is still a significant gap in our field, particularly regarding symbionts other than bacteria, and systems in extreme environments such as deserts and polar regions. The current literature covers only a fraction of the diverse natural systems and abiotic factors that influence symbiotic host shifts, leading us to question, for instance, whether temperature — the most studied abiotic factor — is truly the most important in driving host shifts, or if our knowledge is simply more extensive on the topic because it has been the most studied variable.

Moreover, abiotic factors in natural systems do not act in isolation; and hosts generally have multiple different symbionts. Nonetheless, many studies examine only one abiotic factor and one symbiont at a time, leading to oversimplified conclusions (Wootton, 1994). For instance, Heyworth and collaborators (2020) exposed four clonal lines of pea aphids *Acyrthosiphon pisum*, which carry *Buchnera*, to heat shock treatments. Three lines were infected with one facultative endosymbiont – *Regiella inseticola*, *Fukatsuia symbiotica* and *Hamiltonella defensa* – and one line was uninfected. After the heat shock, *Buchnera* density reduced, but recovered in the aphids infected with *Regiella* or *Fukatsuia*, and not recover in *Hamiltonella*-infected and uninfected aphids. Similarly, Burke and coauthors (2010) described that the *Buchnera* symbiont of pea aphids was only affected by heat shock when the other symbiont *S. symbiotica* was absent. Future studies that parse the effects of various abiotic conditions on complex insect-symbiont relationships hold promise for revealing the full range of species responses to environmental changes.

Research on terrestrial insects, or the terrestrial stages of many insects with complex life-cycles, such as mosquitos (Leggewie *et al.*, 2018; Bamou *et al.*, 2021; Wijegunawardana *et al.*, 2024), dominate the field. Given the physical properties and connectivity of water, however, mechanisms and rates of host shifts may be different than on land (Harvey *et al.*, 2023). Afterall, aquatic environments tend to have lower oxygen capacity and more stable thermal conditions due to water's higher heat capacity compared to air, and variation in oxygen availability can restrict the distribution and survival of both hosts (Silberbush, Abramsky & Tsurim, 2015; Harvey *et al.*, 2023) and their symbionts, and success of host shifts. The thermal stability of aquatic environments can also contribute to the evolution of narrow thermal breadths in aquatic insects, which are then more susceptible to rapid and acute environmental changes (Shah *et al.*, 2017; Birrell *et al.*, 2020; Harvey *et al.*, 2023). Because of the drastic differences between these environments, and despite recent progress made with some aquatic systems (Sazama *et al.*, 2017; Men *et al.*, 2022). Overall, symbioses in aquatic insects and their responses to environmental changes would merit greater attention in future research (Harvey *et al.*, 2023).

Initially, endosymbiotic host shifts were studied to investigate how endosymbionts, such as *Wolbachia*, could colonize phylogenetically distant hosts (Haine *et al.*, 2005; Stahlhut *et al.*, 2010). Despite substantial recent research into various steps necessary for host shift, from survival outside host cells (Rasgon *et al.*, 2006; Nadal-Jimenez *et al.*, 2022) to cellular integration (Goto *et al.*, 2006; White *et al.*, 2017; Russell *et al.*, 2019; Radousky *et al.*, 2023), our understanding of the mechanisms underlying how endosymbionts switch hosts remains fragmented. Although the field of pathogen host shifts has different objectives, often focused on infectious spread in a host population or the forecast of emerging diseases (Longdon *et al.*, 2014), it is related, and perhaps can be applied to endosymbiont research: how does environmental change influence the factors underlying variation in host susceptibilities to novel infections? What role do environmental changes play in making some endosymbionts more likely to successfully switch hosts compared to others? How are different transmission modes and routes affected by abiotic conditions? Drawing on mechanistic insights from neighbouring fields offers a promising strategy for advancing the study of endosymbiotic host shifts in changing environments.

The field of endosymbiosis host shifts presents biases and inherent limitations, which leads us to conclude, so far, that the abiotic effects on this process are likely case-dependent. The intricate nature of endosymbiotic host shifts, involving multiple species-specific, and even tissue-specific events, underscores the vulnerability of symbiotic systems to abiotic conditions, both known and yet to be discovered. There is still much to learn about the complex mechanisms underlying host shifts, and especially how they may be affected by current environmental changes.

V. Conclusions

1. Our review discusses the fact that most of the research to date has focused on the effects of temperature on symbioses involving bacteria in Hemiptera and Diptera hosts.
2. The literature presents host shifts as events often vulnerable to abiotic stresses at various points in the process. However, examples showing how abiotic changes may also create opportunities for the formation of novel symbiotic associations also exist, leading us to conclude that abiotic effects on this process are case-dependent.
3. We emphasize that integrating insights from other disciplines could help us better comprehend the mechanisms underlying how environmental conditions influence endosymbiotic host shifts in insects.

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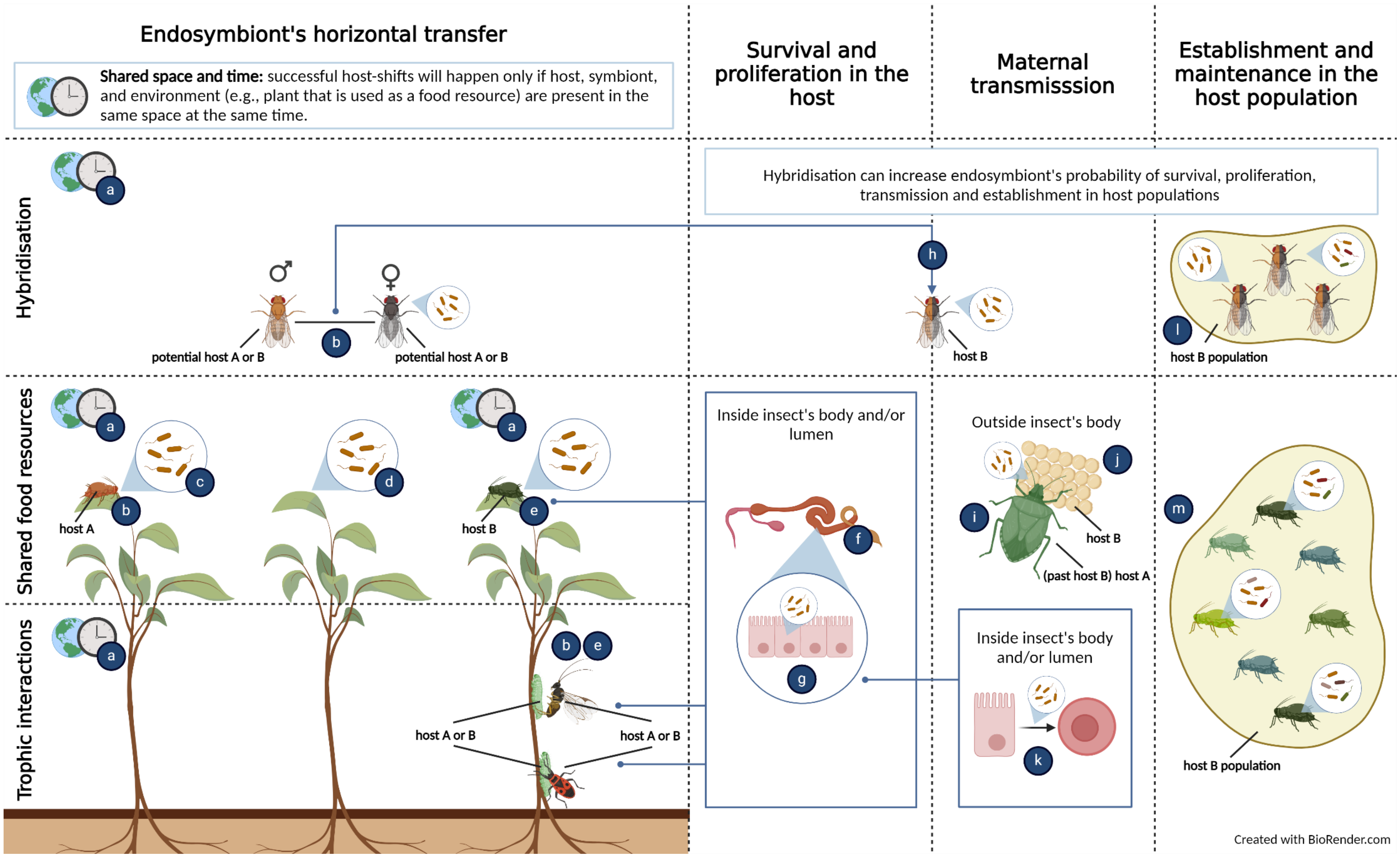

Endosymbiont's horizontal transfer
Shared space and time: successful host-shifts will happen only if host, symbiont, and environment (e.g., plant that is used as a food resource) are present in the same space at the same time.
Survival and proliferation in the host
Maternal transmisssion
Establishment and maintenance in the host population
Hybridisation
Hybridisation can increase endosymbiont's probability of survival, proliferation, transmission and establishment in host populations
potential host A or B
potential host A or B
host B
host B population
Shared food resources
host A
host B
Inside insect's body and/or lumen
Outside insect's body
host B
(past host B) host A
Inside insect's body and/or lumen
Trophic interactions
host A or B
host A or B
host B population
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Figure 2. Conceptual framework of the four main steps required for the establishment of a naïve endosymbiosis. The A hosts are donors, and the B hosts are recipients of endosymbionts. Lower case letters indicate events within the main steps that can be affected by environmental conditions: (a) host shifts occur if hosts (donor A or recipient B), endosymbiont, and environment (e.g., shared resource) share the same space at the same time; (b) endosymbiont's titer and tropism in the donor host A; (c) immediate (short-term) survival of the endosymbiont outside host A's cells or body; (d) long-term (permanence for a period, season) survival of the endosymbiont outside host A's cells or body; (e) endosymbiont's titer and tropism in the recipient host B; (f) endosymbiont's ability to bear host's metabolic changes; (g) endosymbiont's ability to overcome host's immunity; (h) hybridisation as a shortcut for promoting survival and proliferation in the novel host; (i) mechanism that maintains extracellular vertical transmission in host A; (j) endosymbiont's survival to environmental exposure and acquisition by the offspring (host B); (k) endosymbiont's survival to the cell-to-cell transmission until reaching the germline; (l) hybridisation as a shortcut for promoting establishment in the novel host population; (m) fitness outcome of the interaction endosymbiont-host B promote endosymbiont's establishment in host B's population.

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Author contributions

C.S.B, S.vN. and A.D. defined the scope of the review and sorted the data during the systematic literature search. C.S.B. analysed the data. All authors contributed to writing the original manuscript and editing the subsequent versions of the manuscript.

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Conflicts of interest

The authors declare no conflict of interest.

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