Heritable symbionts in a world of varying temperature

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Heritable microbes represent an important component of the biology, ecology and evolution of many plants, animals and fungi, acting as both parasites and partners. In this review, we examine how heritable symbiont-host interactions may alter host thermal tolerance, and how the dynamics of these interactions may more generally be altered by thermal environment. Obligate symbionts, those required by their host, are considered to represent a thermally sensitive weak point for their host, associated with accumulation of deleterious mutations. As such, these symbionts may represent an important determinant of host thermal envelope and spatial distribution. We then examine the varied relationship between thermal environment and the frequency of facultative symbionts, which provide ecologically contingent benefits or act as parasites. We note some facultative symbionts directly alter host thermotolerance. We outline how thermal environment will alter the benefits/costs of infection more widely, and additionally modulate vertical transmission efficiency. Multiple patterns are observed, with symbionts being cold sensitive in some species, heat sensitive in others, with varying and non-co-incident thresholds at which phenotype and transmission are ablated. Nevertheless, it is clear that studies aiming to predict ecological and evolutionary dynamics of symbiont-host interactions need to examine the interaction across a range of thermal environments. Finally, we discuss the importance of thermal sensitivity in predicting the success/failure of symbionts to spread into novel species following natural/engineered introduction.

Introduction

Heritable symbionts – viruses, bacteria, protists or fungal associates which pass from parent to offspring – are found widely in multicellular fungi, plants and animals. It is currently considered that heritable bacteria infect more than half of all arthropod species (Duron *et al.*, 2008), that fungal symbionts are common in both insects and grasses (Clay, 1990; Gibson and Hunter, 2010), and that heritable viruses are widespread in fungi, plants and insects (Roossinck, 2015). Biologically, symbionts such as these represent important modulators of host phenotype and provide heritable variation upon which natural selection acts. Variously, they may provide defence against natural enemies, play a role in host nutrition (through digestive processes, anabolic processes, or as farmed symbionts, as in fungal ant gardens), or determine host plant use for insects. These microbes may also modulate the competence of their host for pathogenesis (Bryner and Rigling, 2011) or for vector capability (McMeniman *et al.*, 2012). Maternally-inherited symbionts may also act as reproductive parasites, manipulating host reproductive processes towards the production and survival of daughters (Hurst and Frost, 2015). This process is most well recognised in insects, but is also observed in the case of viral induced male sterility plants (Grill and Garger, 1981).

The effect of symbiont infection upon host individuals produces further effects at the population and community levels. Sex ratio distorting symbionts affect the reproductive ecology of their host, and may additionally affect population persistence. Those involved in contribution to anabolic function permit their host to exist in nutritional niches that would not otherwise be occupied. Protective symbionts, of course, are likely to impact upon the dynamics of the natural enemies against which they protect (Fenton *et al.*, 2011), and those which affect parasite virulence likewise alter the dynamics of parasite and host. At the community level, plant endophytes alter the pattern of competition between plant species (Clay *et al.*, 1993, 2005; Clay and Holah, 1999), facilitate invasion (Aschehoug *et al.*, 2012) and may change patterns of succession, through for example reducing herbivory.

In this paper, we examine the sensitivity of these interactions to thermal environment. Thermal environment is well recognised as altering the outcome of host-parasite interactions, both in terms of progression of infection within an individual and in terms of ecological and evolutionary dynamics in populations (Thomas and Blanford, 2003). We examine the thesis that temperature will be an important modulator of heritable symbiont/host interactions. We note that these interactions are distinct from parasite/host comparators in that they may be either beneficial or parasitic, and the

symbiont may on occasions be obligatory for survival. We first outline the evidence that obligate heritable symbionts – those required by their host – form a weak link under thermal stress, potentially limiting the geographic range of their host species. We then outline the interaction between thermal environment and facultative heritable microbes – microbes that are not required, but commonly provide ecologically contingent benefits or act as reproductive parasites, or both. We first note heritable symbiont frequency is affected by the magnitude of any benefit they bring to host biology, the physiological cost of carriage of symbionts, and the fraction of female offspring that fail to inherit them (segregational loss). We argue thermal environment affects all of these parameters, and that understanding heritable symbiont dynamics in natural populations requires detailed study across a range of thermal environments.

Obligate heritable microbes commonly represent a thermal 'weak link' for their hosts

There are many animals (and some plants) in which curing an individual of symbionts through antibiotic, heat, or other treatments results in the death or sterility of their host. Dependence upon symbionts is commonly observed in insects (Wernegreen, 2002; Zientz *et al.*, 2004), nematodes (Slatko *et al.*, 2010; Darby *et al.*, 2012), and plants (Rodriguez *et al.*, 2009). In many cases these are coadapted metabolic partnerships where the symbiont provides essential nutrients to the host, allowing the exploitation of nutrient-poor resources or habitats (Baumann, 2005; Douglas, 2009). In others the microbe gives little metabolic contribution to the host, yet the host has evolved to become dependent on the symbiont, as in the wasps *Asobara* (Dedeine *et al.*, 2001) and *Trichogramma* (Stouthamer *et al.*, 1990), and the plant *Psychotria* (Cowles, 1915).

Removal of the obligate symbiont typically results in the death or sterilization of its host. Many examples of this come from insects, where the obligate symbionts reside in specialized cells known as bacteriocytes (Sacchi *et al.*, 1993; Montllor *et al.*, 2002). Thermal stress commonly causes the death of bacteriocytes, which once killed do not regenerate. A model for symbiont studies, the aphid-*Buchnera aphidicola* symbiosis, can be disrupted through exposing the insects to both high (Wilcox *et al.*, 2003; Dunbar *et al.*, 2007) or low temperatures (Parish and Bale, 1991) as the symbiont populations decrease. Indeed, inter-clonal variation in the thermal sensitivity of aphids is associated with variation in *Buchnera*, with a single nucleotide deletion in the heat shock promoter region of the heat shock gene ibpA being associated with reduced tolerance to thermal stress, but improved fitness at normal environmental temperatures (Dunbar *et al.*, 2007; Moran and Yun, 2015). In field cages, aphid clones carrying the reduced heat tolerance strain of *Buchnera*

outcompetes clones carrying the tolerant strain at low temperatures, but these clones are outcompeted where heat shocks occur (Harmon *et al.*, 2009). Heat treatments in weevils (Heddi *et al.*, 1999) and cockroaches (Sacchi *et al.*, 1993) kill their bacteriocytes in a similar manner. Mealybug symbionts are also killed at elevated temperature, though this only has an impact on survival/fertility if it occurs during pre-adult development (Parkinson *et al.*, 2014).

There are strong evolutionary reasons to believe thermal impacts on obligate symbiont function will be general and widespread. These obligate symbionts are vertically transmitted from the parent to offspring with high fidelity (Bandi *et al.*, 1998; Faeth and Fagan, 2002; Hosokawa *et al.*, 2006, 2012). Indeed, obligate symbionts infecting hosts such as aphids (Shigenobu and Stern, 2013), tsetse flies (Akman *et al.*, 2002), cockroaches (Patiño-Navarrete *et al.*, 2013) and nematodes (Slatko *et al.*, 2010) form close partnerships which have lasted for many millions of years, with host and symbiont phylogenies showing little evidence of horizontal transmission. This long coevolution within the protective confines of a host has led to a Muller's ratchet process in which there is accumulation of mildly deleterious mutations, alongside large reductions in genome size as loss of non-essential genes occurs over time (Moran, 1996; Nikoh *et al.*, 2011). The process is likely to lead to the degradation of any systems not under strong selection, such as occasional exposure to high temperature.

The process of mutational decay has a major impact upon thermal tolerance. For instance, extensive genome reduction in *Buchnera* is reflected in this symbiont producing just five heat shock proteins, a substantial decrease compared to the seventy-five produced by its free-living and more thermotolerant relative *Escherichia coli* (Bronikowski *et al.*, 2001; Wilcox *et al.*, 2003; Pérez-Brocal *et al.*, 2006; Liu *et al.*, 2012). More widely, accumulation of deleterious mutations in remaining genes (Moran, 1996) is reflected in weaker secondary and tertiary structure of proteins in *Buchnera* (van Ham *et al.*, 2003), with the result that the function of proteins in obligate symbionts is disproportionately impaired at elevated temperatures compared to proteins encoded in the host genome. It is notable also that chaperonin genes – which stabilize protein structure under stress – are highly expressed in obligate symbionts at normal temperature. GroEL, for instance, comprises c. 10% and 6% of the proteome of *Buchnera* in aphids and *Blochmannia* in ants respectively in normal thermal environments (Baumann *et al.*, 1996; Fan *et al.*, 2013). More widely, chaperonins represent 22% of protein abundance in *Buchnera* and 15% in *Blochmannia*. This high level of chaperonin expression is hypothesized to represent a means to cosset proteins that are structurally weak, which then fail at elevated temperatures where no further failsafe is possible (Moran, 1996).

The inability of symbionts to cope with temperature stress makes many obligate symbionts into a 'weak link' in host thermal tolerance. While the services provided by heritable microbes have been credited with allowing early host range expansion by permitting the exploitation of widespread but nutritionally-poor resources (Feldhaar and Gross, 2009; Hansen and Moran, 2011), their narrow temperature requirements have been implicated in restricting host spread. Insects such as aphids may be limited to temperate regions by their intracellular symbionts (Dixon *et al.*, 1987) while fungus-cultivating ants are restricted to tropical environments by the temperature requirements of their obligate cold-susceptible fungal symbiont (Mueller *et al.*, 2011). To date, there has been no formal comparative test of this hypothesis, in which thermal niche breadth of hosts with and without symbionts are compared. What is clear, however, is that as global temperatures rise (Cox *et al.*, 2000), plants and animals may be required to move ranges to maintain their ideal environment, or to adapt to higher temperatures (Walther *et al.*, 2002; Parmesan and Yohe, 2003). The small genomes and lack of horizontal gene transfer in obligate symbionts (O'Fallon, 2008) may mean that the latter process of adaptation is likely to be barred, thus requiring the host to move range rather than adapting *in situ*.

The interaction between thermal environment and facultative heritable symbionts.

Facultative heritable symbionts are those where cured host individuals retain reproduction and fertility. Commonly, bacterial and fungal symbionts are heritable through the female line (but see (Moran and Dunbar, 2006; Watanabe *et al.*, 2014), whereas viruses are heritable through both parents, albeit commonly with higher efficiency through egg than sperm. For maternally inherited agents, their capacity to invade populations depends on their impact on the production, survival and reproduction of female hosts. Minimal models of heritable microbe dynamics thus include two parameters, whose temperature sensitivity will then determine response to thermal environment:

a) The effects the symbiont has upon host fecundity, survival or sex ratio.
b) The vertical transmission efficiency of the symbiont (separated into paternal and maternal components for biparentally inherited agents).

Under this minimal model, a maternally inherited symbiont will spread if, when rare, an infected female leaves on average more infected daughters than an uninfected female leaves daughters.

Where the magnitude of improvement in host fecundity/survival/sex ratio is low (i.e. an infected female on average leaves a few more infected daughters than an uninfected female leaves daughters), equilibrium prevalence becomes very sensitive to changes in vertical transmission efficiency (Jaenike, 2009; Gundel *et al.*, 2011).

Symbiont-mediated phenotypes that enable facultative heritable microbes to invade populations are very diverse. Some symbionts are reproductive parasites that spread through biasing sex allocation to the production of daughters or inducing incompatibility in uninfected zygotes (Werren *et al.*, 2008). Other interactions are mutualistic and involve benefits to their host which are ecologically contingent– they exist only under particular circumstances, with hosts retaining full function in the absence of symbionts outside these conditions. Symbionts can provide protection from natural enemies (Kellner, 2002; Oliver *et al.*, 2005; Scarborough *et al.*, 2005; Xie *et al.*, 2010; Nakabachi *et al.*, 2013) and disease (Caragata *et al.*, 2013), enhance immune response (Márquez *et al.*, 2007; de Souza *et al.*, 2009) or determine plant host range. They may also be used in offence, as is the case for *Photorhabdus* released from entomopathogenic nematodes into insects on infection, and which then kill the insect (Poinar, 1975). Biparentally inherited agents may also be mutualists, but a positive effect on their host is not necessary for them to invade a population (L'Heritier, 1970; Fine, 1975).

What then are the likely impacts of thermal environment on the population biology of heritable microbes in natural populations? Associative studies, linking seasonal and spatial variation in symbiont frequency, are limited in power to detect thermal impacts by the presence of multiple covarying factors in natural populations (e.g. thermal environment and desiccation) and the presence of spatially varying coevolution. Clinal variation in symbiont prevalence is a more powerful indicator of thermal environment driving symbiont dynamics, and does support temperature-symbiont interactions in a number of cases (Table 1). However, this data has multiple potential sources for the association. Thus, a more precise view can be gained through defined experimental study. At its most powerful, this may involve varying thermal environment within laboratory or caged populations over a number of generations and examining its impact on symbiont dynamics. For instance, Versace et al. (2014) noted that the *Wolbachia* strain that spread in passage through *Drosophila melanogaster* population cages depended upon the temperature at which the population was maintained (Versace *et al.*, 2014). However, studies such as this are logistically complex for many species. More common are single generation studies that examine one or more aspects of the host-symbiont interaction under different temperatures. Below we summarise these studies. We

first outline evidence that indicate heritable symbionts may directly alter host thermal tolerance. We then outline how phenotypes providing ecologically contingent benefits to their host and reproductive manipulation phenotypes are altered by thermal environment. We then examine data with respect to temperature impacts upon vertical transmission and the direct physiological cost of symbiont infection. We draw this information together to create a generalised picture of the thermal sensitivity of heritable microbe-host interactions.

i) Direct effects of symbiont presence on host thermal tolerance. Laboratory study indicates that facultative heritable bacteria can affect host thermal tolerance in a number of cases. In aphids, at least three different facultative symbionts increase insect survival or reproduction after heat shock (Chen *et al.*, 2000; Russell and Moran, 2006; Heyworth and Ferrari, 2015). *Hamiltonella* infections in whitefly confer a similar protection (Brumin *et al.*, 2011). The mechanisms behind symbiont-conferred increase in thermal tolerance are not always known, although there are several hypotheses. *Serratia symbiotica's* ability to permit pea aphids to survive at high temperatures was hypothesised to be due to *Serratia* replacing the amino acid biosynthesis function of the obligate symbiont *Buchnera* (Koga *et al.*, 2003, 2007), but Burke and Moran noted *Serratia symbiotica* is incapable of this, due to deletion or degradation of amino acid biosynthesis pathways, and indeed it may itself be dependent on *Buchnera* (Burke and Moran, 2011). Instead, it seems that *Serratia* protects *Buchnera*, possibly by lysing to release metabolites (Montllor *et al.*, 2002; Burke *et al.*, 2010). Meanwhile in whitefly, the presence of the facultative symbiont increases host-produced stress genes, inadvertently preparing it for thermal stress (Brumin *et al.*, 2011).

Heritable fungal endophytes also impact upon plant heat stress adaptation (Rodriguez and Redman, 2008; Rodriguez *et al.*, 2009). Most notably, endophytes of panic grass permit plant growth on geothermal soils in Yellowstone National Park (Redman *et al.*, 2002; Rodriguez *et al.*, 2008). This is a mutualistic relationship, as in some cases neither plant nor fungus can survive the high temperature without the other (Redman *et al.*, 2002; Márquez *et al.*, 2007). Fascinatingly, the heat tolerance property is determined by a viral heritable symbiont of the endophyte fungus, with the presence of the virus enabling both endophyte and plant persistence. Further to this, endophytes may increase seed germination under thermal stress (Hubbard *et al.*, 2012).

To date, the majority of studies of heritable symbiont impacts on thermal tolerance have investigated the impacts of elevated temperature. We found a single study examining frost resistance in relationship to heritable symbionts in insects, and this revealed no impact of symbiont

presence on frost tolerance (Łukasik *et al.*, 2011). However, the presence of non-heritable symbionts with freeze-tolerance phenotypes suggests that similar phenotypes warrant more extensive examination for heritable microbe-host interactions. *Anaplasma phagocytophilum* is acquired horizontally each generation by its tick host *Ixodes scapularis* following blood feeding. Observations and experiments indicate that *Anaplasma* infection protects its host against damage from frost and cold damage. This occurs through *Anaplasma*-induced induction of anti-freeze protein production by the host individual (Neelakanta *et al.*, 2010). Further to this, non-heritable *Spiroplasma* infections increase corn leafhopper survival during overwintering periods (Ebbert and Nault, 1994), indicating there may be impacts of symbionts on overwinter (freeze) survival.

ii) Impact of temperature on ecologically contingent benefits. We found two studies relating the impact of temperature on protective phenotype in natural infections of insects. In the European beewolf *Philanthus triangulum*, *Streptomyces* heritable symbionts secrete antibiotics that protect the host cocoon from pathogen attack during diapause in the soil. Koehler & Kaltenpoth (2013) found thermal environment (from 15°C to 25°C including diurnal variation) had no impact on the quantity of antibiotic produced (Koehler and Kaltenpoth, 2013). In contrast to this, pea aphids carrying *H. defensa* were nearly completely resistant to attack by *Aphidius ervi* parasitic wasps at 20°C, but were susceptible at 25°C and 30°C, postulated to represent thermal sensitivity of symbiont mediated protection (Bensadia *et al.*, 2006; Guay *et al.*, 2009). Further work confirmed this result, but additionally showed protection was insensitive to temperature in clones where *H. defensa* co-occurred with PAXS (Guay *et al.*, 2009). Whilst this would have an impact upon symbiont dynamics, the role of host and symbiont factors in establishing this pattern were not ascertained.

Outside of heritable microbe interactions with insects, temperature modulates the effect of heritable virus infection in the chestnut blight fungus *Cryphonectria parasitica*. In this interaction, viral presence commonly alters fungal growth and sporulation *in vitro*, and produces a hypovirulent phenotype when the fungus is introduced to the chestnut tree. The hypovirulent phenotype associated with virus presence is temperature sensitive, commonly greatest at 24°C, compared to 12°C, 18°C and 30°C. The authors also noted a fungal and viral genotype dependence of the virulence phenotype, and conclude that the coevolutionary dynamics of the system would thus be determined by a complex GxGxE interaction (Bryner and Rigling, 2011).

Studies investigating the impact of thermal environment upon heritable symbiont dynamics have largely focussed on the direct impact of temperature on the phenotype of the symbiont as outlined

above. However, the dynamics of heritable microbes may also be altered by changes in the benefit derived from a given phenotype, which may be driven by temperature driven changes in other biotic interactions. For instance, the frequency achieved by a symbiont that protects against natural enemies depends upon the rate of attack by enemies against which the symbiont defends. Thermal environment may alter both individual wasp movement patterns, the density of attackers, their ability to parasitize in the absence of protection, and indeed the community of species that do attack. In so doing, it would alter the dynamics of the symbiont even if the transmission and phenotype of the symbiont are temperature invariant. Understanding thermal impacts on this ecological context is a key area for future work.

iii) Impact of temperature on reproductive parasitic phenotypes. Many studies examine the impact of thermal environment on the expression of reproductive parasitic phenotypes in insects (Table 2). Most commonly, *Wolbachia*-induced male-killing, parthenogenesis induction and cytoplasmic incompatibility are ablated at high temperatures. However, the temperature required for the phenotype to be affected varies – in the temperate species *Drosophila bifasciata*, male-killing becomes incomplete above 23.5°C (Hurst *et al.*, 2000, 2001). Cytoplasmic incompatibility (CI) is commonly less strongly expressed at high temperatures, becoming incomplete in *D. simulans* at 28°C, and at temperatures >30°C in other species (Wright and Wang, 1980; Trpis *et al.*, 1981; Stevens, 1989; Clancy and Hoffmann, 1998; Johanowicz and Hoy, 1998; van Opijnen and Breeuwer, 1999). However, there are a number of cases where phenotype is only impacted following multigenerational passage at elevated temperatures. There is also evidence that heat shock (exposure to temperatures exceeding 35°C for between 30 minutes and 2 hours) alters the expression of CI (Feder *et al.*, 1999). Currently, it is unclear why thermal sensitivity of these traits is so variable, and whether it is associated with host or microbial factors. In contrast to *Wolbachia*-induced phenotypes, *Spiroplasma*-induced male-killing is ablated at lower temperatures (Williamson, 1965; Counce and Poulson, 1966; Anbutsu *et al.*, 2008).

As previously discussed with respect to the dynamics of protective symbionts, the impact of temperature on symbiont prevalence may also be impacted by the effect of the phenotype on host survival and fecundity. For instance, the drive associated with male-killing relates to the intensity of sibling-sibling interactions, with male host death on symbiont fitness having little impact when these interactions are weak (e.g. food excess), and are strong when siblings strongly compete (e.g. food paucity) (Hurst and Frost, 2015). Thus, external ecological characteristics that may be thermally dependent (e.g. aphid supply for ladybirds) are likely to impact upon symbiont dynamics. In contrast,

the impact of thermal ablation of phenotype on symbiont prevalence is likely to be much lower for traits like CI, where the effect is not strongly ecologically contingent, and which is under positive frequency dependent selection. Where CI causing *Wolbachia* are common, nearly all females mate to infected males. If CI strength diminishes by 50%, this remains a very high fitness loss for uninfected females, such that declines in prevalence associated with thermal ablation of phenotype will be small. In contrast, ablation of male-killing, which produces only a small (1-20%) impact on female survival will have a more profound influence, potentially making the symbiont net costly to female host (measured in terms of production/survival of daughters). Thus, theory predicts the impacts to be greater in this case (Jaenike, 2009).

iv) Physiological cost of symbionts at different temperatures. Endosymbionts, which rely on their hosts for nutrition, can impose a cost on their host. For example, the defensive symbiont *Hamiltonella defensa* can be costly to the hosts *Acyrthosiphon pisum* and *Aphis fabae* (e.g. (Vorburger *et al.*, 2013; Polin *et al.*, 2014) and references therein). Costs may manifest, or be manifested more dramatically, when the host is under physiological stress. Thus far, there have been few studies examining the physiological cost of symbionts at different temperatures. In *A. pisum,* the endosymbiont *Regiella insecticola* was found to be costly under heat stress, but not when hosts were reared in standard conditions. The cost was observed after 2-day-old nymphs were exposed to a period of heat-shock at 37.5°C. Uninfected heat-shocked aphids were 24% more likely to survive to adulthood than infected heat-shocked aphids, and infected heat-shocked aphids also suffered higher sterility rates (Russell and Moran, 2006).

Study of *Wolbachia*-infected *D. melanogaster* also indicates thermal impacts on the cost of carrying a symbiont. *D. melanogaster* were established in field cages in tropical and temperate areas of Australia during winter. *Wolbachia*'s effect on the host, relative to uninfected flies, depended on whether the fruit fly nuclear background was tropical or temperate. In tropical cages, infected flies of both backgrounds had lower fecundity than their uninfected counterparts. In contrast, in the temperate cage, the effects of *Wolbachia* depended on the nuclear background, with temperate-background flies experiencing higher fecundity when infected. This example demonstrates that a previously-beneficial symbiont might become a liability when local climate is unfavourable (Olsen *et al.*, 2001). More recently, Kriesner et al. (2016) have demonstrated that *Wolbachia* has a particular negative impact upon fecundity in flies that survive through winter. Flies with *Wolbachia* post dormancy have a lower fecundity than flies without the infection (Kriesner *et al.*, 2016).

Outside of insect-bacterium interactions, temperature dependence of heritable viral impacts on fungal growth *in vitro* has also been reported in a number of interactions (e.g. (Hyder *et al.*, 2013) and references therein) . Further, Sigma virus in *Drosophila melanogaster* causes a deleterious CO2 sensitivity which is highest at low temperatures, with reduced concentrations required to induce death (see (Longdon *et al.*, 2012) and references therein). Thus, it seems that viral, as well as bacterial symbionts, show temperature-dependent phenotypes in multiple host species.

v) Thermal environment and transmission efficiency. Studies of heritable bacteria in insects have concluded that vertical transmission efficiency is sensitive to rearing temperature (Table 3). In a manner similar to that observed for phenotype, *Wolbachia* vertical transmission efficiency has been observed to be reduced at raised temperature, and *Spiroplasma* vertical transmission efficiency reduced at cool temperatures. However, it is notable that phenotype expression is commonly more sensitive than transmission, with phenotype ablation occurring before loss of vertical transmission in a number of cases.

Few studies examine the impact of overwintering on heritable symbiont transmission. Perrot-Minnot *et al.* 1996 note that segregational loss of *Wolbachia* is increased during artificially prolonged (2-6 year) larval diapause (Perrot-Minnot *et al.*, 1996). In pea aphids, *Regiella insecticola* shows segregational loss in sexually produced eggs that persist through winter, but 100% vertical transmission in asexual summer reproduction (Moran and Dunbar, 2006). These observations raise the potential importance of overwinter phases on symbiont transmission, but this requires evaluation over natural diapause periods across a number of symbioses.

One caveat to studies of transmission efficiency is the degree to which we can accurately score infected and uninfected individuals in a standard PCR assay. This is an issue of detectability of low titre infections. For instance, van Opijnen and Breeuwer (1999) studied the impact of high temperature (32°C) passage of laboratory stocks of the red spider mite *Tetranychus urticae* upon the presence of *Wolbachia*. PCR assays were used to detect *Wolbachia* infection, and indicated that prevalence decreased over four generations of exposure to this temperature, with no individual scored as infected in generation 4. However, *Wolbachia* infection was detected in 29% of individuals two generations after restoration of these lines to 25°C, the permissive temperature. Only after six generations of exposure to 32°C was *Wolbachia* found to be lost after restoration to the permissive temperature (van Opijnen and Breeuwer, 1999). The most parsimonious explanation for these data is that the symbiont declined in titre during passage, and by generation 4 the titre was sufficiently

low that it was undetectable by the PCR methodology used. Care should thus be taken to either use a recovery period before concluding symbiont absence (see examples in Table 3) or using very stringent quality control with respect to symbiont detectability in PCR assays. Such assays could involve 'spiking' of symbiont carrying material at varying dilutions into uninfected carrier host DNA, to establish the limit to detectability, and also employ qPCR to robustly determine limits to detection.

Outside insect-heritable bacteria interactions, it is known that transmission of sigma virus in *Drosophila melanogaster* is thermally sensitive. Vertical transmission is ablated at high temperatures, with 30°C passage curing flies. In plants, fungal endophyte vertical transmission in cool season grasses is also known to be impacted by temperature. Endophyte fungi commonly transfer on the exterior of seeds. Do Valle Ribeiro (1993) reviewed the impact of seed storage conditions on the survival of the fungus and its propagation following germination. They concluded that storage time, humidity and temperature of storage affected the likelihood of plants germinating from seeds acquiring the symbiont. Overall, seeds maintained at higher temperatures, at low relative humidity and for longer periods of time were less likely to retain the infection, presumably associated with loss of fungal viability on the seed (do Valle Ribeiro, 1993). However, the impact of temperature is not universal: Oldrup et al. (2010) noted that 80% of locoweed seed maintained in uncontrolled warehouse conditions over 40 years retain *Undifilum* endophyte infection (Oldrup *et al.*, 2010).

Variation in vertical transmission efficiency is thought to be an important driver of endophyte dynamics and equilibrium prevalence, as the 'benefit' from endophyte infection is relatively weak (Afkhami and Rudgers, 2008; Gundel *et al.*, 2008). However, whilst loss in seed storage argues for a role of temperature in endophyte dynamics, exploration of the whole transmission cycle under natural conditions is required to determine the sensitivity of endophyte dynamics to thermal environment: loss of endophyte infection can occur at any of three stages – from tiller to seed, seed to seedling, and during tiller growth (Afkhami and Rudgers, 2008). These authors conclude that vertical transmission variation may be important in determining intra-specific spatial and inter-species differences in endophyte prevalence, and the role of the environment in generating vertical transmission variation warranted investigation. However, they note that variation in transmission and prevalence of infection may be additionally associated with the frequency with which the drought tolerance phenotype is induced (Davitt *et al.*, 2011), or may derive from coevolutionary interactions between host and fungus affecting transmission efficiency.

406

407 **A generalised view of thermal impacts on facultative heritable symbionts**

408

409 The above account creates a few clear messages. The first of these is that many aspects of heritable
410 symbiont phenotype and transmission are thermally sensitive. Whilst our review is biased to
411 heritable bacteria-insect interactions, thermal sensitivity was noted in a wide range of interactions
412 (bacteria-insect, fungus-plant, virus-plant, virus-insect), and is likely to be general. However, the
413 pattern of thermal sensitivity (chill vs heat; threshold for thermal impact) varies greatly across
414 interactions. Thus, it is clear that while thermal environment is very likely to affect facultative
415 symbiont dynamics in many systems, the way in which it does so will be vary greatly.

416

417 A second observation is that different aspects of the host-symbiont interaction have different
418 thermal sensitivities. One commonly measured 'linking' variable is symbiont titre – the number of
419 symbionts resident in a host. Thermal environment impacts upon titre, and phenotype ablation and
420 segregational loss during reproduction is commonly associated with low titre. Commonly, phenotype
421 ablation occurs before high levels of segregational loss, as attested by the recovery of phenotypes
422 after passage through permissive temperature regimes. Indeed, studies of paternal inheritance of
423 bacterial symbionts indicate as few as four bacterial cells are sufficient to establish infection in the
424 new generation (Watanabe *et al.*, 2014).

425

426 The underpinning of phenotype and transmission by titre is important as it indicates that the impact
427 of thermal environment is not simply associated with the current thermal regime, but will have
428 strong historical influences (e.g. (Jaenike, 2009)). Temperature previously experienced in life impacts
429 upon current titre, and thus on the expression of phenotype and vertical transmission rate. Indeed,
430 thermal impacts in a number of systems have been shown to be transgenerational, with symbioses
431 taking a number of generations to recover to maximum expression following return to the
432 permissive temperature. An important property of a symbiont host interaction, therefore, is the rate
433 at which symbiont titre is impacted by temperature, both in terms of reduction and recovery. A
434 practical consequence of this short term evolution is that laboratory passage conditions may
435 produce rather rapid changes in this aspect of host biology. For *Drosophila*, the simple act of
436 maintaining a *Spiroplasma* stock at 18°C may cure the host of heritable symbiont infection. Changing
437 thermal environment may more subtly alter symbiont titre in other cases, which may take time to
438 recover. Overall, the heritable symbiont element of a host may be inadvertently (and in the case of
439 curing) permanently altered by simply placing stocks at a different temperature during maintenance,

or during an experiment. The heritable symbiont component of an organism is much less fixed in the creation of isofemale lineages than is nuclear genetic variation.

The centrality of titre in expression of phenotype and vertical transmission further suggests that thermal sensitivity of host-symbiont interactions may affect the success/failure of heritable symbionts in novel host species. Facultative symbiont incidence in host communities is partly a function of their movement into, and subsequent propagation through, new host species (Zug *et al.*, 2012; Longdon *et al.*, 2014). Further, *Wolbachia* transinfected into novel host species is in applied usage as a means to interrupt vector competence of focal species. It is notable that when symbionts are placed into novel hosts they may attain a different titre from the native host (Kageyama *et al.*, 2006), and this is likely to be reflected in changes to the thermal sensitivity of the host-symbiont interaction. Thermal sensitivity of phenotype in novel hosts has been investigated in two mosquito species transinfected with *Wolbachia* from *D. melanogaster* as a means of altering vector competence. Studies show that the impact of wMel on reducing *Aedes aegypti* competence for dengue virus transmission is insensitive to environmental temperature (Ye *et al.*, 2016). In contrast, the impact of *Wolbachia* strain wAlbB on *Plasmodium* proliferation in *An. stephensi* is temperature sensitive (Murdock *et al.*, 2014). *w*AlbB reduced mosquito potential to transmit *Plasmodium* at 28°C but had no effect at either 20°C or 24°C. Thus, whilst focal traits can be robust to thermal variation on transinfection, this characteristic must be determined on a case-by-case basis, and this is an important biosafety and efficacy consideration with respect to releases. It also indicates that temperature may affect the ability of an infection to propagate through a novel host species

Overall, linking laboratory measures with field data remains a challenge. In part this is because (as discussed above) impacts can be historical. As noted previously, the presence of latitudinal clines in symbiont prevalence in focal species supports a link between thermal environment and symbiont dynamics in nature (Table 1). Further, broad between-species surveys indicate latitudinal patterns that indicate general patterns. For instance, *Wolbachia* is generally rare in butterflies from high latitudes, both in terms of more commonly being absent, and where present, more commonly being at low prevalence (Ahmed *et al.*, 2015). Determining the role of thermal environment in creating these patterns is complicated by temperature being one of a number of abiotic, biotic and coevolutionary factors that affect symbiont-host dynamics. There are, however, examples where the pattern is consistent with experimental data. For instance, *Wolbachia* in *D. melanogaster* is costly in the context of overwintering, and *Wolbachia* is less common in temperate populations than tropical

populations of this species. For male-killing *Spiroplasma* in *Drosophila*, experiments indicate symbiont phenotype and vertical transmission are ablated at low temperatures. Consistent with this, male-killing *Spiroplasma* are recorded commonly in drosophilids from tropical biomes (Williamson and Poulson, 1979; Montenegro *et al.*, 2005, 2006; Pool *et al.*, 2006), but not in temperate species/temperate parts of species range (see (Haselkorn, 2010)). This is unlikely to be a study bias, as male-killing *Wolbachia* have been isolated from temperate flies following observation of female biased sex ratios produced by individual females (Hurst *et al.*, 2000; Sheeley and McAllister, 2009; Unckless and Jaenike, 2012). Further, whilst male-killing *Spiroplasma* strains have been isolated from South American and Sub-Saharan African *D. melanogaster*, no records exist from *D. melanogaster* from temperate biomes. Given that the intensity of collection and study is biased towards temperate collection, it is fair to conclude that male-killing *Spiroplasma* show a tropical bias in *Drosophila*, consistent with the observed thermal sensitivity of this symbiotic interaction.

The review above also highlights a variety of areas for future study. The impact of overwintering environment on symbiont survival and reciprocally of symbionts on host survival overwinter, are both very poorly researched. There are good reasons (outlined above) to believe diapause/overwinter period may be an important contributor to symbiont dynamics, and these factors should be studied both in the field and laboratory. Further, laboratory experiments on thermal impacts should adopt greater realism, incorporating diurnal temperature cycles in addition to investigating impacts of static temperatures. These may benefit also from adding in covarying factors such as day length, in case host/symbionts thermal behaviour has photoperiodic sensitivity. Further, effects in a number of systems are known to be genotype dependent. Thus, prediction of dynamics may require a GxGxE framework. Finally, the impact of particular symbiont phenotypes of fitness (rather than their expression) is also likely to be thermally sensitive, and will require detailed examination of the wider ecological context in which the host exists. It is likely we will only get a predictive picture of thermal impacts when these aspects of natural environment complexity are incorporated.

The thermal sensitivity of heritable-microbe interactions begs two further questions. First, is host behaviour in terms of selecting thermal environments ever an adaptation to symbionts? Many organisms exhibit behavioural thermoregulation (Feder *et al.*, 1997; Anderson *et al.*, 2013). The possibility is that species carrying beneficial symbionts will be selected for temperature optima that cosset their symbionts, and may indeed be constrained in using behavioural fever as a means of curing pathogen infections. Reciprocally, presence of parasitic heritable symbionts may lead to

selection for adopting temperatures that reduce the impact and transmission of the symbiont. Secondly, are the patterns of thermal impact on symbionts that we observe ever adaptive for the symbiont? Certain phenotypes (e.g. natural enemy resistance) are only beneficial at particular times of year (when the natural enemy is active). If the expression of high titre to gain the phenotype is associated with a physiological cost, then titre may be expected to evolve as a thermally plastic trait of the symbiont, elevating only when the enemy is active. Microbial pathogens are well known to alter behaviour with temperature; for example, *Listeria* pathogenicity determinants are expressed at 37°C in association with ingestion by a mammal (Leimeister-Wächter *et al.*, 1992). Thus, the machinery for microbial adaptive thermal plasticity clearly exists. Whether it is employed by heritable symbionts is an interesting question.

In conclusion, laboratory studies have revealed that symbiont presence may in part determine host thermal tolerance, and that many aspects of host-symbiont interactions are thermally sensitive such that thermal environment will likely alter the prevalence of heritable symbionts and the strength of phenotype observed in interactions. However, there commonly remains a research disconnect between laboratory measures and field dynamics. All laboratory measures in essence create hypotheses about how phenotype and transmission may be affected in the field, as the experimental study simplifies systems for purposes of experimental control. Further, the ecological context will alter the benefits of particular phenotype in ways which are not easily predictable from the laboratory, but are likely to be thermally sensitive. These, and the degree to which thermal sensitivity is part of an adapted symbiosis, as opposed to an uncontrollable biological constraint, remain major questions for future research.

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Table 1 – Studies showing geographical variation in symbiont prevalence which may be attributable to temperature differences.

Host	Symbiont	Locality	Pattern	References
Acyrthosiphon pisum	*Regiella insecticola*	Japan	Higher prevalence in colder north and east. Significant correlation with temperature, as well as precipitation and host plant. There was no temperature correlation for *Serratia, Rickettsia,* or *Spiroplasma*, though the latter two are found only in the southwest at low frequency.	(Tsuchida *et al.*, 2002)
Adalia bipunctata	*Spiroplasma*	Sweden	*Spiroplasma* absent north of 63°N in 2011-2013. The northernmost limit was 61°N in 2000-2002.	(Tinsley, 2003; Pastok, 2015)
Culicoides imicola	*Cardinium*	Israel	Prevalence declines with increasing maximum daytime temperature in locality and increases with increasing minimum night-time temperature.	(Morag *et al.*, 2012)
Curculio sikkimensis	*Sodalis, Rickettsia* and *Wolbachia*	Japan	Higher prevalence of three symbionts in warmer areas to the south-west. Significant correlation with temperature. No correlation for *Spiroplasma*.	(Toju and Fukatsu, 2011)
Drosophila melanogaster	*Wolbachia*	Eastern Australia	Higher prevalence in tropical regions of Australia compared to subtropical and temperate regions. Pattern stable over 20 years. Similar, weaker pattern observed in North America.	(Hoffmann *et al.*, 1986; Kriesner *et al.*, 2016)

Table 2 –Thermal effects on the phenotypes of natural reproductive parasites of insects. 'Nature of symbiosis' details: MK = male-killing; CI = cytoplasmic incompatibility. 'Assay type' details: Phenotype = strength of phenotype measured; qPCR, PCR, cytology, Southern hybridization = means by which symbiont presence confirmed; permissive passage = test for symbiont presence conducted after recovering the lineage to standard thermal environment.

Host	Symbiont	Nature of symbiosis	Assay type	Impact of temperature on phenotype	Source
Aedes polynesiensis	*Wolbachia*	CI	Phenotype, cytology	CI eliminated by 32-33°C exposure as larvae for 5-7 days. 30-32°C did not eliminate CI. Larva dies above 33°C.	(Wright and Wang, 1980)
Drosophila equinoxalis	ESRO *Spiroplasma*	MK	Phenotype	MK reduced by embryonic heat-treatment with various temperatures and durations between 34°C and 40°C.	(Malogolowkin, 1959)
D. nebulosa	NSRO *Spiroplasma*	MK	Phenotype, qPCR	Highly penetrant MK at 25°C. At 18°C there is loss of fully-female broods at generation 2. At 28°C, gradual loss occurs until at generation 8, 1/8 strains show strong female-bias.	(Anbutsu *et al.*, 2008)
D. willistoni	WSRO *Spiroplasma*	MK	Phenotype	No effect of embryonic heat-treatment, at various temperatures and durations between 34°C and 40°C.	(Malogolowkin, 1959)
D. bifasciata	A-group *Wolbachia*	MK	Phenotype, cytology	Phenotype lost between 23.5°C and 25°C.	(Hurst *et al.*, 2000, 2001)
D. melanogaster	wMelPop *Wolbachia* (may not exist in wild)	Premature host death	Phenotype	No mortality effect at 19°C. At 25°C, wMelPop induces early mortality, with effect increasing at 29°C.	(Min and Benzer, 1997; Reynolds *et al.*, 2003)
D. simulans	wRi *Wolbachia*	CI	Phenotype, cytology	Ageing and rearing males at elevated temperature (27°C) reduces incompatibility; larval thermal environment critical.	(Clancy and Hoffmann, 1998)

D. simulans	*Wolbachia*	CI	Phenotype	CI suppressed in crosses between two unidirectionally-incompatible fly strains exposed to 28°C in early life.	(Hoffmann *et al.*, 1986)
D. simulans	*Wolbachia*	CI	Phenotype	Larval heat shock at 36°C (1 hour) reduced CI in adult male flies. Egg mortality was 90% rather than 45%. Heat shock didn't influence survival or fertility.	(Feder *et al.*, 1999)
Nasonia vitripennis	*Wolbachia* strain A	CI	Phenotype, qPCR	Positive correlation between density and CI penetrance within temperature groups. However, density and CI were decoupled between groups. Temperature may change the density threshold required for CI.	(Bordenstein and Bordenstein, 2011)
Ostrinia scapulalis, adzuki bean borer moth	*Wolbachia*	MK	Phenotype, PCR	Exposing larval female moths to 63°C for 20-30 minutes suppresses phenotype. 40 minutes has a greater effect but causes high lethality. 53°C not efficient at non-lethal exposure times. 34-38°C for long periods doesn't fully suppress MK.	(Sakamoto *et al.*, 2008; Sugimoto *et al.*, 2015)
Tribolium confusum	*Wolbachia*	CI	Phenotype	Suppression of CI with exposure to 37°C for 12 days in larval stage. Number of individuals lacking the phenotype increases with exposure time.	(Stevens, 1989)
Trichogramma cordubensis	*Wolbachia*	Induces thelytoky	Phenotype with 'permissive passage'	Thelytoky reduced over 4 generations at 30°C, significant during generations 2-4. Recovery with 4 generations of passage at 23°C.	(Girin and Boulétreau, 1995; Pintureau *et al.*, 1999)
Tetranychus urticae	*Wolbachia*	CI	Phenotype, PCR with 'permissive passage'	High loss of phenotype after 4 generations at 32°C (threshold at 31-32°C). Development time was reduced, and many heat-cured lines died out.	(van Opijnen and Breeuwer, 1999)

Table 3 – Thermal effects on the vertical transmission of natural bacterial symbionts of insects. **'Nature of symbiosis' details: MK = male-killing; CI = cytoplasmic incompatibility. 'Assay type' details: Phenotype = strength of phenotype measured; qPCR, PCR, cytology, Southern hybridization = means by which symbiont presence confirmed; permissive passage = test for symbiont presence conducted after recovering the lineage to standard thermal environment.**

Host	Symbiont	Nature of symbiosis	Assay type	Impact of temperature on vertical transmission	Source
Acyrthosiphon pisum	*Regiella insecticola*	Parasitoid protection	PCR	Segregational loss in sexually produced eggs that persist through winter, but 100% vertical transmission in asexual summer reproduction.	(Moran and Dunbar, 2006)
Aedes kesseli males crossed with *Ae. polynesiensis* females	*Wolbachia*	CI (*Ae. polynesiensis* females have *Wolbachia*)	Cytology	Loss from ovaries with a heat treatment of 32.5°C (versus 27°C). This also killed the host.	(Trpis *et al.*, 1981)
Drosophila hydei	hy1 *Spiroplasma*	Parasitoid protection	qPCR	Blocked at 15°C, impaired at 18°C (2/5 broods had imperfect transmission), near-perfect at 25°C and 28°C.	(Osaka *et al.*, 2008)
D. melanogaster	MSRO *Spiroplasma*	MK	Phenotype after 'permissive passage'	Transmission loss at 16.5°C between F1 and F2. No phenotype recovery in non-MK lines returned to permissive temperature.	(Montenegro and Klaczko, 2004)
D. nebulosa	NSRO *Spiroplasma*	MK	Phenotype, qPCR	Rapid loss at 18°C (by generation 2). Stable maintenance at 25°C. Gradual loss at 28°C over several generations.	(Anbutsu *et al.*, 2008)
D. bifasciata	A-group *Wolbachia*	MK	Phenotype, cytology	Estimated at 92.9% at 25°C, compared to c. 100% at 18°C.	(Hurst *et al.*, 2000, 2001)

Liposcelis tricolor	*Wolbachia*	Increases fertility and fecundity	PCR	Complete elimination of *Wolbachia* over 6 generations at 33°C. Base population had 100% infection.	(Jia *et al.*, 2009)
Metaseiulus occidentalis	*Wolbachia*	CI	Phenotype, PCR after 'permissive passage'	After passage at 33°C for at least 8 generations, 0/10 tested females were infected. At 24°C, 12/20 tested females were infected. Males were also heat-cured.	(Johanowicz and Hoy, 1998)
Nasonia vitripennis	*Wolbachia* (2 strains)	CI, various	Phenotype, PCR, cytology, Southern hybridisation	AB Double-infected wasps lose strains A and/or B in diapause.	(Perrot-Minnot *et al.*, 1996)
Ostrinia scapulalis	*Wolbachia*	MK	Phenotype, PCR	Some cured progeny (shown by PCR) were derived from the 63°C-treated females, indicating transmission loss. Males uninfected, females/sexual mosaics infected.	(Sakamoto *et al.*, 2008; Sugimoto *et al.*, 2015)
Tetranychus urticae	*Wolbachia*	CI	Phenotype, PCR after 'permissive passage'	29% of mites remain infected after 4 generations at 32°C (threshold at 31-32°C). Undetectable by PCR until passaged at 23°C for 2 generations. Complete cure with 6 generations at 32°C.	(van Opijnen and Breeuwer, 1999)