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1 Heritable symbionts in a world of varying temperature

2

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13

14 Heritable microbes represent an important component of the biology, ecology and evolution of
15 many plants, animals and fungi, acting as both parasites and partners. In this review, we examine
16 how heritable symbiont-host interactions may alter host thermal tolerance, and how the dynamics
17 of these interactions may more generally be altered by thermal environment. Obligate symbionts,
18 those required by their host, are considered to represent a thermally sensitive weak point for their
19 host, associated with accumulation of deleterious mutations. As such, these symbionts may
20 represent an important determinant of host thermal envelope and spatial distribution. We then
21 examine the varied relationship between thermal environment and the frequency of facultative
22 symbionts, which provide ecologically contingent benefits or act as parasites. We note some
23 facultative symbionts directly alter host thermotolerance. We outline how thermal environment will
24 alter the benefits/costs of infection more widely, and additionally modulate vertical transmission
25 efficiency. Multiple patterns are observed, with symbionts being cold sensitive in some species, heat
26 sensitive in others, with varying and non-co-incident thresholds at which phenotype and
27 transmission are ablated. Nevertheless, it is clear that studies aiming to predict ecological and
28 evolutionary dynamics of symbiont-host interactions need to examine the interaction across a range
29 of thermal environments. Finally, we discuss the importance of thermal sensitivity in predicting the
30 success/failure of symbionts to spread into novel species following natural/engineered introduction.
31
32
33

34 Introduction

35

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

50

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

61

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

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

78

79 **Obligate heritable microbes commonly represent a thermal ‘weak link’ for their hosts**

80

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

90

91 Removal of the obligate symbiont typically results in the death or sterilization of its host. Many
92 examples of this come from insects, where the obligate symbionts reside in specialized cells known
93 as bacteriocytes (Sacchi *et al.*, 1993; Montllor *et al.*, 2002). Thermal stress commonly causes the
94 death of bacteriocytes, which once killed do not regenerate. A model for symbiont studies, the
95 aphid-*Buchnera aphidicola* symbiosis, can be disrupted through exposing the insects to both high
96 (Wilcox *et al.*, 2003; Dunbar *et al.*, 2007) or low temperatures (Parish and Bale, 1991) as the
97 symbiont populations decrease. Indeed, inter-clonal variation in the thermal sensitivity of aphids is
98 associated with variation in *Buchnera*, with a single nucleotide deletion in the heat shock promoter
99 region of the heat shock gene *ibpA* being associated with reduced tolerance to thermal stress, but
100 improved fitness at normal environmental temperatures (Dunbar *et al.*, 2007; Moran and Yun,
101 2015). In field cages, aphid clones carrying the reduced heat tolerance strain of *Buchnera*

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

107

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

120

121 The process of mutational decay has a major impact upon thermal tolerance. For instance, extensive
122 genome reduction in *Buchnera* is reflected in this symbiont producing just five heat shock proteins, a
123 substantial decrease compared to the seventy-five produced by its free-living and more
124 thermotolerant relative *Escherichia coli* (Bronikowski *et al.*, 2001; Wilcox *et al.*, 2003; Pérez-Brocal *et*
125 *al.*, 2006; Liu *et al.*, 2012). More widely, accumulation of deleterious mutations in remaining genes
126 (Moran, 1996) is reflected in weaker secondary and tertiary structure of proteins in *Buchnera* (van
127 Ham *et al.*, 2003), with the result that the function of proteins in obligate symbionts is
128 disproportionately impaired at elevated temperatures compared to proteins encoded in the host
129 genome. It is notable also that chaperonin genes – which stabilize protein structure under stress –
130 are highly expressed in obligate symbionts at normal temperature. GroEL, for instance, comprises c.
131 10% and 6% of the proteome of *Buchnera* in aphids and *Blochmannia* in ants respectively in normal
132 thermal environments (Baumann *et al.*, 1996; Fan *et al.*, 2013). More widely, chaperonins represent
133 22% of protein abundance in *Buchnera* and 15% in *Blochmannia*. This high level of chaperonin
134 expression is hypothesized to represent a means to cosset proteins that are structurally weak, which
135 then fail at elevated temperatures where no further failsafe is possible (Moran, 1996).

136

137 The inability of symbionts to cope with temperature stress makes many obligate symbionts into a
138 'weak link' in host thermal tolerance. While the services provided by heritable microbes have been
139 credited with allowing early host range expansion by permitting the exploitation of widespread but
140 nutritionally-poor resources (Feldhaar and Gross, 2009; Hansen and Moran, 2011), their narrow
141 temperature requirements have been implicated in restricting host spread. Insects such as aphids
142 may be limited to temperate regions by their intracellular symbionts (Dixon *et al.*, 1987) while
143 fungus-cultivating ants are restricted to tropical environments by the temperature requirements of
144 their obligate cold-susceptible fungal symbiont (Mueller *et al.*, 2011). To date, there has been no
145 formal comparative test of this hypothesis, in which thermal niche breadth of hosts with and
146 without symbionts are compared. What is clear, however, is that as global temperatures rise (Cox *et al.*,
147 2000), plants and animals may be required to move ranges to maintain their ideal environment,
148 or to adapt to higher temperatures (Walther *et al.*, 2002; Parmesan and Yohe, 2003). The small
149 genomes and lack of horizontal gene transfer in obligate symbionts (O'Fallon, 2008) may mean that
150 the latter process of adaptation is likely to be barred, thus requiring the host to move range rather
151 than adapting *in situ*.

152

153

154 **The interaction between thermal environment and facultative heritable symbionts.**

155

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

163

164 a) The effects the symbiont has upon host fecundity, survival or sex ratio.

165 b) The vertical transmission efficiency of the symbiont (separated into paternal and maternal
166 components for biparentally inherited agents).

167

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

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

174
175 Symbiont-mediated phenotypes that enable facultative heritable microbes to invade populations are
176 very diverse. Some symbionts are reproductive parasites that spread through biasing sex allocation
177 to the production of daughters or inducing incompatibility in uninfected zygotes (Werren *et al.*,
178 2008). Other interactions are mutualistic and involve benefits to their host which are ecologically
179 contingent– they exist only under particular circumstances, with hosts retaining full function in the
180 absence of symbionts outside these conditions. Symbionts can provide protection from natural
181 enemies (Kellner, 2002; Oliver *et al.*, 2005; Scarborough *et al.*, 2005; Xie *et al.*, 2010; Nakabachi *et*
182 *al.*, 2013) and disease (Caragata *et al.*, 2013), enhance immune response (Márquez *et al.*, 2007; de
183 Souza *et al.*, 2009) or determine plant host range. They may also be used in offence, as is the case
184 for *Photorhabdus* released from entomopathogenic nematodes into insects on infection, and which
185 then kill the insect (Poinar, 1975). Biparentally inherited agents may also be mutualists, but a
186 positive effect on their host is not necessary for them to invade a population (L’Heritier, 1970; Fine,
187 1975).

188
189 What then are the likely impacts of thermal environment on the population biology of heritable
190 microbes in natural populations? Associative studies, linking seasonal and spatial variation in
191 symbiont frequency, are limited in power to detect thermal impacts by the presence of multiple
192 covarying factors in natural populations (e.g. thermal environment and desiccation) and the
193 presence of spatially varying coevolution. Clinal variation in symbiont prevalence is a more powerful
194 indicator of thermal environment driving symbiont dynamics, and does support temperature-
195 symbiont interactions in a number of cases (Table 1). However, this data has multiple potential
196 sources for the association. Thus, a more precise view can be gained through defined experimental
197 study. At its most powerful, this may involve varying thermal environment within laboratory or
198 caged populations over a number of generations and examining its impact on symbiont dynamics.
199 For instance, Versace *et al.* (2014) noted that the *Wolbachia* strain that spread in passage through
200 *Drosophila melanogaster* population cages depended upon the temperature at which the population
201 was maintained (Versace *et al.*, 2014). However, studies such as this are logistically complex for
202 many species. More common are single generation studies that examine one or more aspects of the
203 host-symbiont interaction under different temperatures. Below we summarise these studies. We

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

210

211 *i) Direct effects of symbiont presence on host thermal tolerance.* Laboratory study indicates that
212 facultative heritable bacteria can affect host thermal tolerance in a number of cases. In aphids, at
213 least three different facultative symbionts increase insect survival or reproduction after heat shock
214 (Chen *et al.*, 2000; Russell and Moran, 2006; Heyworth and Ferrari, 2015). *Hamiltonella* infections in
215 whitefly confer a similar protection (Brumin *et al.*, 2011). The mechanisms behind symbiont-
216 conferred increase in thermal tolerance are not always known, although there are several
217 hypotheses. *Serratia symbiotica*'s ability to permit pea aphids to survive at high temperatures was
218 hypothesised to be due to *Serratia* replacing the amino acid biosynthesis function of the obligate
219 symbiont *Buchnera* (Koga *et al.*, 2003, 2007), but Burke and Moran noted *Serratia symbiotica* is
220 incapable of this, due to deletion or degradation of amino acid biosynthesis pathways, and indeed it
221 may itself be dependent on *Buchnera* (Burke and Moran, 2011). Instead, it seems that *Serratia*
222 protects *Buchnera*, possibly by lysing to release metabolites (Montllor *et al.*, 2002; Burke *et al.*,
223 2010). Meanwhile in whitefly, the presence of the facultative symbiont increases host-produced
224 stress genes, inadvertently preparing it for thermal stress (Brumin *et al.*, 2011).

225

226 Heritable fungal endophytes also impact upon plant heat stress adaptation (Rodriguez and Redman,
227 2008; Rodriguez *et al.*, 2009). Most notably, endophytes of panic grass permit plant growth on
228 geothermal soils in Yellowstone National Park (Redman *et al.*, 2002; Rodriguez *et al.*, 2008). This is a
229 mutualistic relationship, as in some cases neither plant nor fungus can survive the high temperature
230 without the other (Redman *et al.*, 2002; Márquez *et al.*, 2007). Fascinatingly, the heat tolerance
231 property is determined by a viral heritable symbiont of the endophyte fungus, with the presence of
232 the virus enabling both endophyte and plant persistence. Further to this, endophytes may increase
233 seed germination under thermal stress (Hubbard *et al.*, 2012).

234

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

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

247

248 *ii) Impact of temperature on ecologically contingent benefits.* We found two studies relating the
249 impact of temperature on protective phenotype in natural infections of insects. In the European
250 beewolf *Philanthus triangulum*, *Streptomyces* heritable symbionts secrete antibiotics that protect
251 the host cocoon from pathogen attack during diapause in the soil. Koehler & Kaltenpoth (2013)
252 found thermal environment (from 15°C to 25°C including diurnal variation) had no impact on the
253 quantity of antibiotic produced (Koehler and Kaltenpoth, 2013). In contrast to this, pea aphids
254 carrying *H. defensa* were nearly completely resistant to attack by *Aphidius ervi* parasitic wasps at
255 20°C, but were susceptible at 25°C and 30°C, postulated to represent thermal sensitivity of symbiont
256 mediated protection (Bensadia *et al.*, 2006; Guay *et al.*, 2009). Further work confirmed this result,
257 but additionally showed protection was insensitive to temperature in clones where *H. defensa* co-
258 occurred with PAXS (Guay *et al.*, 2009). Whilst this would have an impact upon symbiont dynamics,
259 the role of host and symbiont factors in establishing this pattern were not ascertained.

260

261 Outside of heritable microbe interactions with insects, temperature modulates the effect of
262 heritable virus infection in the chestnut blight fungus *Cryphonectria parasitica*. In this interaction,
263 viral presence commonly alters fungal growth and sporulation *in vitro*, and produces a hypovirulent
264 phenotype when the fungus is introduced to the chestnut tree. The hypovirulent phenotype
265 associated with virus presence is temperature sensitive, commonly greatest at 24°C, compared to
266 12°C, 18°C and 30°C. The authors also noted a fungal and viral genotype dependence of the
267 virulence phenotype, and conclude that the coevolutionary dynamics of the system would thus be
268 determined by a complex GxGxE interaction (Bryner and Rigling, 2011).

269

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

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

281

282 *iii) Impact of temperature on reproductive parasitic phenotypes.* Many studies examine the impact of
283 thermal environment on the expression of reproductive parasitic phenotypes in insects (Table 2).
284 Most commonly, *Wolbachia*-induced male-killing, parthenogenesis induction and cytoplasmic
285 incompatibility are ablated at high temperatures. However, the temperature required for the
286 phenotype to be affected varies – in the temperate species *Drosophila bifasciata*, male-killing
287 becomes incomplete above 23.5°C (Hurst *et al.*, 2000, 2001). Cytoplasmic incompatibility (CI) is
288 commonly less strongly expressed at high temperatures, becoming incomplete in *D. simulans* at
289 28°C, and at temperatures >30°C in other species (Wright and Wang, 1980; Trpis *et al.*, 1981;
290 Stevens, 1989; Clancy and Hoffmann, 1998; Johanowicz and Hoy, 1998; van Opijnen and Breeuwer,
291 1999). However, there are a number of cases where phenotype is only impacted following
292 multigenerational passage at elevated temperatures. There is also evidence that heat shock
293 (exposure to temperatures exceeding 35°C for between 30 minutes and 2 hours) alters the
294 expression of CI (Feder *et al.*, 1999). Currently, it is unclear why thermal sensitivity of these traits is
295 so variable, and whether it is associated with host or microbial factors. In contrast to *Wolbachia*-
296 induced phenotypes, *Spiroplasma*-induced male-killing is ablated at lower temperatures
297 (Williamson, 1965; Counce and Poulson, 1966; Anbutsu *et al.*, 2008).

298

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

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

315

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

327 Study of *Wolbachia*-infected *D. melanogaster* also indicates thermal impacts on the cost of carrying
328 a symbiont. *D. melanogaster* were established in field cages in tropical and temperate areas of
329 Australia during winter. *Wolbachia*'s effect on the host, relative to uninfected flies, depended on
330 whether the fruit fly nuclear background was tropical or temperate. In tropical cages, infected flies
331 of both backgrounds had lower fecundity than their uninfected counterparts. In contrast, in the
332 temperate cage, the effects of *Wolbachia* depended on the nuclear background, with temperate-
333 background flies experiencing higher fecundity when infected. This example demonstrates that a
334 previously-beneficial symbiont might become a liability when local climate is unfavourable (Olsen *et al.*,
335 2001). More recently, Kriesner *et al.* (2016) have demonstrated that *Wolbachia* has a particular
336 negative impact upon fecundity in flies that survive through winter. Flies with *Wolbachia* post
337 dormancy have a lower fecundity than flies without the infection (Kriesner *et al.*, 2016).

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

344

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

352

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

360

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

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

378

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

392

393 Variation in vertical transmission efficiency is thought to be an important driver of endophyte
394 dynamics and equilibrium prevalence, as the ‘benefit’ from endophyte infection is relatively weak
395 (Afkhami and Rudgers, 2008; Gundel et al., 2008). However, whilst loss in seed storage argues for a
396 role of temperature in endophyte dynamics, exploration of the whole transmission cycle under
397 natural conditions is required to determine the sensitivity of endophyte dynamics to thermal
398 environment: loss of endophyte infection can occur at any of three stages – from tiller to seed, seed
399 to seedling, and during tiller growth (Afkhami and Rudgers, 2008). These authors conclude that
400 vertical transmission variation may be important in determining intra-specific spatial and inter-
401 species differences in endophyte prevalence, and the role of the environment in generating vertical
402 transmission variation warranted investigation. However, they note that variation in transmission
403 and prevalence of infection may be additionally associated with the frequency with which the
404 drought tolerance phenotype is induced (Davitt et al., 2011), or may derive from coevolutionary
405 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,

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

442

443

444 The centrality of titre in expression of phenotype and vertical transmission further suggests that
445 thermal sensitivity of host-symbiont interactions may affect the success/failure of heritable
446 symbionts in novel host species. Facultative symbiont incidence in host communities is partly a
447 function of their movement into, and subsequent propagation through, new host species (Zug *et al.*,
448 2012; Longdon *et al.*, 2014). Further, *Wolbachia* transinfected into novel host species is in applied
449 usage as a means to interrupt vector competence of focal species. It is notable that when symbionts
450 are placed into novel hosts they may attain a different titre from the native host (Kageyama *et al.*,
451 2006), and this is likely to be reflected in changes to the thermal sensitivity of the host-symbiont
452 interaction. Thermal sensitivity of phenotype in novel hosts has been investigated in two mosquito
453 species transinfected with *Wolbachia* from *D. melanogaster* as a means of altering vector
454 competence. Studies show that the impact of wMel on reducing *Aedes aegypti* competence for
455 dengue virus transmission is insensitive to environmental temperature (Ye *et al.*, 2016). In contrast,
456 the impact of *Wolbachia* strain wAlbB on *Plasmodium* proliferation in *An. stephensi* is temperature
457 sensitive (Murdock *et al.*, 2014). wAlbB reduced mosquito potential to transmit *Plasmodium* at
458 28°C but had no effect at either 20°C or 24°C. Thus, whilst focal traits can be robust to thermal
459 variation on transinfection, this characteristic must be determined on a case-by-case basis, and
460 this is an important biosafety and efficacy consideration with respect to releases. It also indicates
461 that temperature may affect the ability of an infection to propagate through a novel host species

462

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

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

486

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

501

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

508 selection for adopting temperatures that reduce the impact and transmission of the symbiont.
509 Secondly, are the patterns of thermal impact on symbionts that we observe ever adaptive for the
510 symbiont? Certain phenotypes (e.g. natural enemy resistance) are only beneficial at particular times
511 of year (when the natural enemy is active). If the expression of high titre to gain the phenotype is
512 associated with a physiological cost, then titre may be expected to evolve as a thermally plastic trait
513 of the symbiont, elevating only when the enemy is active. Microbial pathogens are well known to
514 alter behaviour with temperature; for example, *Listeria* pathogenicity determinants are expressed at
515 37°C in association with ingestion by a mammal (Leimeister-Wächter *et al.*, 1992). Thus, the
516 machinery for microbial adaptive thermal plasticity clearly exists. Whether it is employed by
517 heritable symbionts is an interesting question.

518

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

530

531

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538

539

540 **References**

541

542 Afkhami ME, Rudgers JA (2008). Symbiosis lost: imperfect vertical transmission of fungal endophytes
543 in grasses. *Am Nat* **172**: 405–416.

544 Ahmed MZ, Araujo-Jnr E V, Welch JJ, Kawahara AY (2015). Wolbachia in butterflies and moths:
545 geographic structure in infection frequency. *Front Zool* **12**: 16.

546 Akman L, Yamashita A, Watanabe H, Oshima K, Shiba T, Hattori M, *et al.* (2002). Genome sequence
547 of the endocellular obligate symbiont of tsetse flies, *Wigglesworthia glossinidia*. *Nat Genet* **32**:
548 402–7.

549 Anbutsu H, Goto S, Fukatsu T (2008). High and low temperatures differently affect infection density
550 and vertical transmission of male-killing *Spiroplasma* symbionts in *Drosophila* hosts. *Appl*
551 *Environ Microbiol* **74**: 6053–9.

552 Anderson RD, Blanford S, Thomas MB (2013). House flies delay fungal infection by fevering: At a
553 cost. *Ecol Entomol* **38**: 1–10.

554 Aschehoug ET, Metlen KL, Callaway RM, Newcombe G (2012). Fungal endophytes directly increase
555 the competitive effects of an invasive forb. *Ecology* **93**: 3–8.

556 Bandi C, Anderson TJ, Genchi C, Blaxter ML (1998). Phylogeny of Wolbachia in filarial nematodes.
557 *Proc Biol Sci* **265**: 2407–2413.

558 Baumann P (2005). Biology of Bacteriocyte-Associated Endosymbionts of Plant Sap-Sucking Insects.
559 *Annu Rev Microbiol* **59**: 155–189.

560 Baumann P, Baumann L, Clark MA (1996). Levels of *Buchnera aphidicola* chaperonin GroEL during
561 growth of the aphid *Schizaphis graminum*. *Curr Microbiol* **32**: 279–285.

562 Bensadia F, Boudreault S, Guay J-F, Michaud D, Cloutier C (2006). Aphid clonal resistance to a
563 parasitoid fails under heat stress. *J Insect Physiol* **52**: 146–57.

564 Bordenstein SR, Bordenstein SR (2011). Temperature affects the tripartite interactions between
565 bacteriophage WO, Wolbachia, and cytoplasmic incompatibility. *PLoS One* **6**: e29106.

566 Bronikowski AM, Bennett AF, Lenski RE (2001). Evolutionary Adaptation to Temperature. Viii. Effects
567 of Temperature on Growth Rate in Natural Isolates of *Escherichia Coli* and *Salmonella Enterica*
568 from Different Thermal Environments. *Evolution (N Y)* **55**: 33–40.

569 Brumin M, Kontsedalov S, Ghanim M (2011). *Rickettsia* influences thermotolerance in the whitefly
570 *Bemisia tabaci* B biotype. *Insect Sci* **18**: 57–66.

571 Bryner SF, Rigling D (2011). Temperature Dependent Genotype-by-Genotype Interaction between a
572 Pathogenic Fungus and Its Hyperparasitic Virus. *Am Nat* **177**: 65–74.

573 Burke G, Fiehn O, Moran N (2010). Effects of facultative symbionts and heat stress on the

574 metabolome of pea aphids. *ISME J* **4**: 242–252.

575 Burke GR, Moran NA (2011). Massive genomic decay in *Serratia symbiotica*, a recently evolved
576 symbiont of aphids. *Genome Biol Evol* **3**: 195–208.

577 Caragata EP, Rancès E, Hedges LM, Gofton AW, Johnson KN, O’Neill SL, *et al.* (2013). Dietary
578 cholesterol modulates pathogen blocking by *Wolbachia*. *PLoS Pathog* **9**: e1003459.

579 Chen DQ, Montllor CB, Purcell AH (2000). Fitness effects of two facultative endosymbiotic bacteria
580 on the pea aphid, *Acyrtosiphon pisum*, and the blue alfalfa aphid, *A. kondoi*. *Entomol Exp Appl*
581 **95**: 315–323.

582 Clancy DJ, Hoffmann AA (1998). Environmental effects on cytoplasmic incompatibility and bacterial
583 load in *Wolbachia*-infected *Drosophila simulans*. *Entomol Exp Appl* **86**: 13–24.

584 Clay K (1990). Fungal Endophytes of Grasses. *Annu Rev Ecol Syst* **21**: 275–297.

585 Clay K, Holah J (1999). Fungal Endophyte Symbiosis and Plant Diversity in Successional Fields. *Science*
586 **285**: 1742–1744.

587 Clay K, Holah J, Rudgers JA (2005). Herbivores cause a rapid increase in hereditary symbiosis and
588 alter plant community composition. *Proc Natl Acad Sci U S A* **102**: 12465–12470.

589 Clay K, Marks S, Cheplick GP (1993). Effects of Insect Herbivory and Fungal Endophyte Infection on
590 Competitive Interactions among Grasses. *Ecology* **74**: 1767–1777.

591 Counce SJ, Poulson DF (1966). The expression of maternally-transmitted sex ratio condition (SR) in
592 two strains of *Drosophila melanogaster*. *Genetica* **37**: 364–390.

593 Cowles HC (1915). Hereditary Symbiosis. *Bot Gaz* **59**: 61–63.

594 Cox P, Betts R, Jones C, Spall S, Totterdell I (2000). Acceleration of global warming due to carbon-
595 cycle feedbacks in a coupled climate model. *Nature* **408**: 184–187.

596 Darby AC, Armstrong SD, Bah GS, Kaur G, Hughes MA, Kay SM, *et al.* (2012). Analysis of gene
597 expression from the *Wolbachia* genome of a filarial nematode supports both metabolic and
598 defensive roles within the symbiosis. *Genome Res* **22**: 2467–2477.

599 Davitt AJ, Chen C, Rudgers JA (2011). Understanding context-dependency in plant-microbe
600 symbiosis: The influence of abiotic and biotic contexts on host fitness and the rate of symbiont
601 transmission. *Environ Exp Bot* **71**: 137–145.

602 Dedeine F, Vavre F, Fleury F, Loppin B, Hochberg ME, Bouletreau M (2001). Removing symbiotic
603 *Wolbachia* bacteria specifically inhibits oogenesis in a parasitic wasp. *Proc Natl Acad Sci U S A*
604 **98**: 6247–6252.

605 Dixon AFG, Kindlmann P, Leps J, Holman J (1987). Why There are So Few Species of Aphids,
606 Especially in the Tropics. *Am Nat* **129**: 580–592.

607 Douglas AE (2009). The microbial dimension in insect nutritional ecology. *Funct Ecol* **23**: 38–47.

608 Dunbar HE, Wilson ACC, Ferguson NR, Moran NA (2007). Aphid thermal tolerance is governed by a
609 point mutation in bacterial symbionts. *PLoS Biol* **5**: 1006–1015.

610 Duron O, Bouchon D, Boutin S, Bellamy L, Zhou L, Engelstädter J, *et al.* (2008). The diversity of
611 reproductive parasites among arthropods: Wolbachia do not walk alone. *BMC Biol* **6**: 27.

612 Ebbert MA, Nault LR (1994). Improved Overwintering Ability in *Dalbulus maidis* (Homoptera:
613 Cicadellidae) Vectors Infected with *Spiroplasma kunkelii* (Mycoplasmatales:
614 Spiroplasmataceae). *Environ Entomol* **23**: 634–644.

615 Faeth SH, Fagan WF (2002). Fungal endophytes: common host plant symbionts but uncommon
616 mutualists. *Integr Comp Biol* **42**: 360–368.

617 Fan Y, Thompson JW, Dubois LG, Moseley MA, Wernegreen JJ (2013). Proteomic analysis of an
618 unculturable bacterial endosymbiont (*Blochmannia*) reveals high abundance of chaperonins
619 and biosynthetic enzymes. *J Proteome Res* **12**: 704–718.

620 Feder ME, Blair N, Figueras H (1997). Oviposition site selection: unresponsiveness of *Drosophila* to
621 cues of potential thermal stress. *Anim Behav* **53**: 585–588.

622 Feder ME, Karr TL, Yang W, Hoekstra JM, James AC (1999). Interaction of *Drosophila* and its
623 endosymbiont *Wolbachia*: Natural heat shock and the overcoming of sexual incompatibility.
624 *Am Zool* **39**: 363–373.

625 Feldhaar H, Gross R (2009). Insects as hosts for mutualistic bacteria. *Int J Med Microbiol* **299**: 1–8.

626 Fenton A, Johnson KN, Brownlie JC, Hurst GDD (2011). Solving the *Wolbachia* Paradox: Modeling the
627 Tripartite Interaction between Host, *Wolbachia*, and a Natural Enemy. *Am Nat* **178**: 333–342.

628 Fine PE (1975). Vectors and vertical transmission: an epidemiologic perspective. *Ann N Y Acad Sci*
629 **266**: 173–194.

630 Gibson CM, Hunter MS (2010). Extraordinarily widespread and fantastically complex: comparative
631 biology of endosymbiotic bacterial and fungal mutualists of insects. *Ecol Lett* **13**: 223–234.

632 Girin C, Boulétreau M (1995). Microorganism-associated variation in host infestation efficiency in a
633 parasitoid wasp, *Trichogramma bourarachae* (Hymenoptera: Trichogrammatidae). *Experientia*
634 **51**: 398–401.

635 Grill LK, Garger SJ (1981). Identification and characterization of double-stranded RNA associated with
636 cytoplasmic male sterility in *Vicia faba*. *Proc Natl Acad Sci U S A* **78**: 7043–7046.

637 Guay J-F, Boudreault S, Michaud D, Cloutier C (2009). Impact of environmental stress on aphid clonal
638 resistance to parasitoids: Role of *Hamiltonella defensa* bacterial symbiosis in association with a
639 new facultative symbiont of the pea aphid. *J Insect Physiol* **55**: 919–926.

640 Gundel PE, Batista WB, Texeira M, Martínez-Ghersa MA, Omacini M, Ghersa CM (2008).
641 *Neotyphodium* endophyte infection frequency in annual grass populations: relative importance

642 of mutualism and transmission efficiency. *Proc Biol Sci* **275**: 897–905.

643 Gundel PE, Rudgers JA, Ghersa CM (2011). Incorporating the process of vertical transmission into
644 understanding of host-symbiont dynamics. *Oikos* **120**: 1121–1128.

645 van Ham RCHJ, Kamerbeek J, Palacios C, Rausell C, Abascal F, Bastolla U, *et al.* (2003). Reductive
646 genome evolution in *Buchnera aphidicola*. *Proc Natl Acad Sci U S A* **100**: 581–586.

647 Hansen AK, Moran NA (2011). Aphid genome expression reveals host-symbiont cooperation in the
648 production of amino acids. *Proc Natl Acad Sci U S A* **108**: 2849–54.

649 Harmon JP, Moran NA, Ives AR (2009). Species response to environmental change: impacts of food
650 web interactions and evolution. *Science* **323**: 1347–1350.

651 Haselkorn TS (2010). Understanding the distribution of the *Spiroplasma* heritable bacterial symbiont
652 in *Drosophila*. University of California, San Diego.

653 Heddi A, Grenier A-M, Khatchadourian C, Charles H, Nardon P (1999). Four intracellular genomes
654 direct weevil biology: Nuclear, mitochondrial, principal endosymbiont, and *Wolbachia*. *Proc
655 Natl Acad Sci* **96**: 6814–6819.

656 Heyworth ER, Ferrari J (2015). A facultative endosymbiont in aphids can provide diverse ecological
657 benefits. *J Evol Biol* **28**: 1753–1760.

658 Hoffmann AA, Turelli M, Simmons GM (1986). Unidirectional Incompatibility between Populations of
659 *Drosophila simulans*. *Evolution (N Y)* **40**: 692–701.

660 Hosokawa T, Hironaka M, Mukai H, Inadomi K, Suzuki N, Fukatsu T (2012). Mothers never miss the
661 moment: a fine-tuned mechanism for vertical symbiont transmission in a subsocial insect. *Anim
662 Behav* **83**: 293–300.

663 Hosokawa T, Kikuchi Y, Nikoh N, Shimada M, Fukatsu T (2006). Strict Host-Symbiont Cospeciation
664 and Reductive Genome Evolution in Insect Gut Bacteria. *PLoS Biol* **4**: e337.

665 Hubbard M, Germida J, Vujanovic V (2012). Fungal endophytes improve wheat seed germination
666 under heat and drought stress. *Botany* **90**: 137–149.

667 Hurst GDD, Frost CL (2015). Reproductive Parasitism: Maternally Inherited Symbionts in a Biparental
668 World. *Cold Spring Harb Perspect Biol* **7**: a017699.

669 Hurst GDD, Jiggins FM, Robinson SJW (2001). What causes inefficient transmission of male-killing
670 *Wolbachia* in *Drosophila*? *Heredity (Edinb)* **87**: 220–226.

671 Hurst GDD, Johnson AP, Fuyama Y (2000). Male-Killing *Wolbachia* in *Drosophila*: A Temperature-
672 Sensitive Trait With a Threshold Bacterial Density. *Genetics* **156**: 699–709.

673 Hyder R, Pennanen T, Hamberg L, Vainio EJ, Piri T, Hantula J (2013). Two viruses of *Heterobasidion*
674 confer beneficial, cryptic or detrimental effects to their hosts in different situations. *Fungal Ecol*
675 **6**: 387–396.

676 Jaenike J (2009). Coupled population dynamics of endosymbionts within and between hosts. *Oikos*
677 **118**: 353–362.

678 Jia F-X, Yang M-S, Yang W-J, Wang J-J (2009). Influence of continuous high temperature conditions
679 on Wolbachia infection frequency and the fitness of *Liposcelis tricolor* (Psocoptera:
680 Liposcelididae). *Environ Entomol* **38**: 1365–72.

681 Johanowicz DL, Hoy MA (1998). Experimental induction and termination of non-reciprocal
682 reproductive incompatibilities in a parahaploid mite. *Entomol Exp Appl* **87**: 51–58.

683 Kageyama D, Anbutsu H, Watada M, Hosokawa T, Shimada M, Fukatsu T (2006). Prevalence of a non-
684 male-killing spiroplasma in natural populations of *Drosophila hydei*. *Appl Environ Microbiol* **72**:
685 6667–73.

686 Kellner RLL (2002). Molecular identification of an endosymbiotic bacterium associated with pederin
687 biosynthesis in *Paederus sabaeus* (Coleoptera: Staphylinidae). *Insect Biochem Mol Biol* **32**: 389–
688 95.

689 Koehler S, Kaltenpoth M (2013). Maternal and Environmental Effects on Symbiont-Mediated
690 Antimicrobial Defense. *J Chem Ecol* **39**: 978–988.

691 Koga R, Tsuchida T, Fukatsu T (2003). Changing partners in an obligate symbiosis: a facultative
692 endosymbiont can compensate for loss of the essential endosymbiont *Buchnera* in an aphid.
693 *Proc R Soc B Biol Sci* **270**: 2543–2550.

694 Koga R, Tsuchida T, Sakurai M, Fukatsu T (2007). Selective elimination of aphid endosymbionts:
695 Effects of antibiotic dose and host genotype, and fitness consequences. *FEMS Microbiol Ecol*
696 **60**: 229–239.

697 Kriesner P, Conner WR, Weeks AR, Turelli M, Hoffmann AA (2016). Persistence of a Wolbachia
698 infection frequency cline in *Drosophila melanogaster* and the possible role of reproductive
699 dormancy. *Evolution (N Y)*. in press doi: 10.1111/evo.12923

700 L’Heritier PH (1970). *Drosophila* viruses and their role as evolutionary factors. *Evol Biol* **4**: 185–209.

701 Leimeister-Wächter M, Domann E, Chakraborty T (1992). The expression of virulence genes in
702 *Listeria monocytogenes* is thermoregulated. *J Bacteriol* **174**: 947–952.

703 Liu S, Chougule NP, Vijayendran D, Bonning BC (2012). Deep Sequencing of the Transcriptomes of
704 Soybean Aphid and Associated Endosymbionts. *PLoS One* **7**: e45161.

705 Longdon B, Brockhurst MA, Russell CA, Welch JJ, Jiggins FM (2014). The Evolution and Genetics of
706 Virus Host Shifts. *PLoS Pathog* **10**: e1004395.

707 Longdon B, Wilfert L, Jiggins FM (2012). The Sigma Viruses of *Drosophila*. In: *Rhabdoviruses:*
708 *Molecular Taxonomy, Evolution, Genomics, Ecology, Cytopathology and Control*, pp 117–132.

709 Łukasik P, Hancock EL, Ferrari J, Godfray HCJ (2011). Grain aphid clones vary in frost resistance, but

710 this trait is not influenced by facultative endosymbionts. *Ecol Entomol* **36**: 790–793.

711 Malogolowkin C (1959). Temperature Effects on Maternally Inherited ‘ Sex-Ratio ’ Conditions in
712 *Drosophila willistoni* and *Drosophila equinoxialis*. *Am Nat* **93**: 365–368.

713 Márquez LM, Redman RS, Rodriguez RJ, Roossinck MJ (2007). A Virus in a Fungus in a Plant: Three-
714 Way Symbiosis Required for Thermal Tolerance. *Science* **315**: 513–515.

715 McMeniman CJ, Lane R V, Cass BN, Fong AW, Sidhu M, Wang Y-F, *et al.* (2012). Stable introduction of
716 a life-shortening *Wolbachia* infection into the mosquito *Aedes aegypti*. *Science* **323**: 141–144.

717 Min KT, Benzer S (1997). *Wolbachia*, normally a symbiont of *Drosophila*, can be virulent, causing
718 degeneration and early death. *Proc Natl Acad Sci U S A* **94**: 10792–10796.

719 Montenegro H, Hatadani LM, Medeiros HF, Klaczko LB (2006). Male killing in three species of the
720 tripunctata radiation of *Drosophila* (Diptera: Drosophilidae). *J Zool Syst Evol Res* **44**: 130–135.

721 Montenegro H, Klaczko LB (2004). Low temperature cure of a male killing agent in *Drosophila*
722 *melanogaster*. *J Invertebr Pathol* **86**: 50–1.

723 Montenegro H, Solferini VN, Klaczko LB, Hurst GDD (2005). Male-killing *Spiroplasma* naturally
724 infecting *Drosophila melanogaster*. *Insect Mol Biol* **14**: 281–287.

725 Montllor CB, Maxmen A, Purcell AH (2002). Facultative bacterial endosymbionts benefit pea aphids
726 *Acyrtosiphon pisum* under heat stress. *Ecol Entomol* **27**: 189–195.

727 Morag N, Klement E, Saroya Y, Lensky I, Gottlieb Y (2012). Prevalence of the symbiont *Cardinium* in
728 *Culicoides* (Diptera: Ceratopogonidae) vector species is associated with land surface
729 temperature. *FASEB J* **26**: 4025–4034.

730 Moran NA (1996). Accelerated evolution and Muller’s ratchet in endosymbiotic bacteria. *Proc Natl*
731 *Acad Sci U S A* **93**: 2873–2878.

732 Moran NA, Dunbar HE (2006). Sexual acquisition of beneficial symbionts in aphids. *Proc Natl Acad Sci*
733 *U S A* **103**: 12803–6.

734 Moran NA, Yun Y (2015). Experimental replacement of an obligate insect symbiont. *Proc Natl Acad*
735 *Sci U S A* **112**: 2093–6.

736 Mueller UG, Mikheyev AS, Hong E, Sen R, Warren DL, Solomon SE, *et al.* (2011). Evolution of cold-
737 tolerant fungal symbionts permits winter fungiculture by leafcutter ants at the northern
738 frontier of a tropical ant-fungus symbiosis. *Proc Natl Acad Sci U S A* **108**: 4053–4056.

739 Murdock CC, Blanford S, Hughes GL, Rasgon JL, Thomas MB (2014). Temperature alters *Plasmodium*
740 blocking by *Wolbachia*. *Sci Rep* **4**: 3932.

741 Nakabachi A, Ueoka R, Oshima K, Teta R, Mangoni A, Gurgui M, *et al.* (2013). Defensive bacteriome
742 symbiont with a drastically reduced genome. *Curr Biol* **23**: 1478–1484.

743 Neelakanta G, Sultana H, Fish D, Anderson JF, Fikrig E (2010). *Anaplasma phagocytophilum* induces

744 Ixodes scapularis ticks to express an antifreeze glycoprotein gene that enhances their survival
745 in the cold. *J Clin Invest* **120**: 3179–3190.

746 Nikoh N, Hosokawa T, Oshima K, Hattori M, Fukatsu T (2011). Reductive evolution of bacterial
747 genome in insect gut environment. *Genome Biol Evol* **3**: 702–714.

748 O’Fallon B (2008). Population Structure, Levels of Selection, and the Evolution of Intracellular
749 Symbionts. *Evolution (N Y)* **62**: 361–373.

750 Oldrup E, McLain-Romero J, Padilla A, Moya A, Gardner D, Creamer R (2010). Localization of
751 endophytic fungi in locoweed seed and influence of environmental parameters on a locoweed
752 in vitro culture system. *Botany* **88**: 512–521.

753 Oliver KM, Moran NA, Hunter MS (2005). Variation in resistance to parasitism in aphids is due to
754 symbionts not host genotype. *Proc Natl Acad Sci U S A* **102**: 12795–800.

755 Olsen K, Reynolds KT, Hoffmann AA (2001). A field cage test of the effects of the endosymbiont
756 Wolbachia on *Drosophila melanogaster*. *Heredity (Edinb)* **86**: 731–737.

757 van Opijnen T, Breeuwer JA (1999). High temperatures eliminate Wolbachia, a cytoplasmic
758 incompatibility inducing endosymbiont, from the two-spotted spider mite. *Exp Appl Acarol* **23**:
759 871–81.

760 Osaka R, Nomura M, Watada M, Kageyama D (2008). Negative effects of low temperatures on the
761 vertical transmission and infection density of a spiroplasma endosymbiont in *Drosophila hydei*.
762 *Curr Microbiol* **57**: 335–9.

763 Parish WEG, Bale JS (1991). Effect of low temperatures on the intracellular symbionts of the grain
764 aphid *Sitobion avenae* (F.) (Hem., Aphididae). *J Insect Physiol* **37**: 339–345.

765 Parkinson JF, Gobin B, Hughes WOH (2014). Short-term heat stress results in diminution of bacterial
766 symbionts but has little effect on life history in adult female citrus mealybugs. *Entomol Exp*
767 *Appl* **153**: 1–9.

768 Parmesan C, Yohe G (2003). A globally coherent fingerprint of climate change impacts across natural
769 systems. *Nature* **421**: 37–42.

770 Pastok D (2015). Causes of spatial variation in parasite and pathogen pressure in insects. University
771 of Liverpool.

772 Patiño-Navarrete R, Moya A, Latorre A, Peretó J (2013). Comparative genomics of *Blattabacterium*
773 *cuenoti*: the frozen legacy of an ancient endosymbiont genome. *Genome Biol Evol* **5**: 351–361.

774 Pérez-Brocail V, Gil R, Ramos S, Lamelas A, Postigo M, Michelena JM, *et al.* (2006). A small microbial
775 genome: the end of a long symbiotic relationship? *Science* **314**: 312–313.

776 Perrot-Minnot MJ, Guo LR, Werren JH (1996). Single and double infections with Wolbachia in the
777 parasitic wasp *Nasonia vitripennis*: Effects on compatibility. *Genetics* **143**: 961–972.

778 Pintureau B, Chapelle L, Delobel B (1999). Effects of repeated thermic and antibiotic treatments on a
779 Trichogramma (Hym., Trichogrammatidae) symbiont. *J Appl Entomol* **123**: 473–483.

780 Poinar GO (1975). Description and biology of a new insect parasitic rhabditoid, Heterorhabditis
781 bacteriophora n. gen., n. sp. (Rhabditida: Heterorhabditidae n. fam.). *Nematologica* **21**: 463–
782 470.

783 Polin S, Simon J-C, Outreman Y (2014). An ecological cost associated with protective symbionts of
784 aphids. *Ecol Evol* **4**: 836–840.

785 Pool JE, Wong A, Aquadro CF (2006). Finding of male-killing Spiroplasma infecting Drosophila
786 melanogaster in Africa implies transatlantic migration of this endosymbiont. *Heredity (Edinb)*
787 **97**: 27–32.

788 Redman RS, Sheehan KB, Stout RG, Rodriguez RJ, Henson JM (2002). Thermotolerance generated by
789 plant/fungal symbiosis. *Science* **298**: 1581.

790 Reynolds KT, Thomson LJ, Hoffmann AA (2003). The effects of host age, host nuclear background and
791 temperature on phenotypic effects of the virulent wolbachia strain popcorn in Drosophila
792 melanogaster. *Genetics* **164**: 1027–1034.

793 Rodriguez RJ, Henson J, Van Volkenburgh E, Hoy M, Wright L, Beckwith F, *et al.* (2008). Stress
794 tolerance in plants via habitat-adapted symbiosis. *ISME J* **2**: 404–416.

795 Rodriguez R, Redman R (2008). More than 400 million years of evolution and some plants still can't
796 make it on their own: Plant stress tolerance via fungal symbiosis. *J Exp Bot* **59**: 1109–1114.

797 Rodriguez RJ, White JF, Arnold AE, Redman RS (2009). Fungal endophytes: diversity and functional
798 roles. *New Phytol* **182**: 314–330.

799 Roossinck MJ (2015). Move over bacteria! Viruses make their mark as mutualistic microbial
800 symbionts. *J Virol* **89**: 6532–6535.

801 Russell JA, Moran NA (2006). Costs and benefits of symbiont infection in aphids: variation among
802 symbionts and across temperatures. *Proc R Soc B Biol Sci* **273**: 603–610.

803 Sacchi L, Grigolo A, Biscaldi G, Laudani U (1993). Effects of heat treatment on the symbiotic system
804 of Blattodea: morphofunctional alterations of bacteriocytes. *Ital J Zool* **60**: 271–279.

805 Sakamoto H, Kageyama D, Hoshizaki S, Ishikawa Y (2008). Heat treatment of the Adzuki bean borer,
806 Ostrinia scapularis infected with wolbachia gives rise to sexually mosaic offspring. *J Insect Sci* **8**:
807 1–5.

808 Scarborough CL, Ferrari J, Godfray HCJ (2005). Aphid protected from pathogen by endosymbiont.
809 *Science* **310**: 1781.

810 Sheeley SL, McAllister BF (2009). Mobile male-killer: similar Wolbachia strains kill males of divergent
811 Drosophila hosts. *Heredity (Edinb)* **102**: 286–292.

812 Shigenobu S, Stern DL (2013). Aphids evolved novel secreted proteins for symbiosis with bacterial
813 endosymbiont. *Proc Biol Sci* **280**: 20121952.

814 Slatko BE, Taylor MJ, Foster JM (2010). The Wolbachia endosymbiont as an anti-filarial nematode
815 target. *Symbiosis* **51**: 55–65.

816 de Souza DJ, Bézier A, Depoix D, Drezen J-M, Lenoir A (2009). *Blochmannia* endosymbionts improve
817 colony growth and immune defence in the ant *Camponotus fellah*. *BMC Microbiol* **9**: 29.

818 Stevens L (1989). Environmental factors affecting reproductive incompatibility in flour beetles, genus
819 *Tribolium*. *J Invertebr Pathol* **53**: 78–84.

820 Stouthamer R, Luck RF, Hamilton WD (1990). Antibiotics cause parthenogenetic Trichogramma
821 (Hymenoptera/Trichogrammatidae) to revert to sex. *Proc Natl Acad Sci U S A* **87**: 2424–2427.

822 Sugimoto TN, Kayukawa T, Matsuo T, Tsuchida T, Ishikawa Y (2015). A short, high-temperature
823 treatment of host larvae to analyze Wolbachia–host interactions in the moth *Ostrinia*
824 *scapularis*. *J Insect Physiol* **81**: 48–51.

825 Thomas MB, Blanford S (2003). Thermal biology in insect-parasite interactions. *Trends Ecol Evol* **18**:
826 344–350.

827 Tinsley MC (2003). The ecology and evolution of male-killing bacteria in ladybirds. University of
828 Cambridge.

829 Toju H, Fukatsu T (2011). Diversity and infection prevalence of endosymbionts in natural populations
830 of the chestnut weevil: relevance of local climate and host plants. *Mol Ecol* **20**: 853–868.

831 Trpis M, Perrone JB, Reissig M (1981). Control of Cytoplasmic incompatibility in the *Aedes scutellaris*
832 complex. *J Hered* **72**: 313–317.

833 Tsuchida T, Koga R, Shibao H, Matsumoto T, Fukatsu T (2002). Diversity and geographic distribution
834 of secondary endosymbiotic bacteria in natural populations of the pea aphid, *Acyrtosiphon*
835 *pisum*. *Mol Ecol* **11**: 2123–2135.

836 Unckless RL, Jaenike J (2012). Maintenance of a male-killing Wolbachia in *Drosophila innubila* by
837 male-killing dependent and male-killing independent mechanisms. *Evolution (N Y)* **66**: 678–689.

838 do Valle Ribeiro MAM (1993). Transmission and survival of *Acremonium* and the implications for
839 grass breeding. *Agric Ecosyst Environ* **44**: 195–213.

840 Versace E, Nolte V, Pandey RV, Tobler R, Schlötterer C (2014). Experimental evolution reveals
841 habitat-specific fitness dynamics among Wolbachia clades in *Drosophila melanogaster*. *Mol*
842 *Ecol* **23**: 802–814.

843 Vorburger C, Ganesanandamoorthy P, Kwiatkowski M (2013). Comparing constitutive and induced
844 costs of symbiont-conferred resistance to parasitoids in aphids. *Ecol Evol* **3**: 706–13.

845 Walther G, Post E, Convey P, Menzel A, Parmesan C, Beebee TJC, *et al.* (2002). Ecological responses

846 to recent climate change. *Nature* **416**: 389–395.

847 Watanabe K, Yukuhiro F, Matsuura Y, Fukatsu T, Noda H (2014). Intrasperm vertical symbiont
848 transmission. *Proc Natl Acad Sci U S A* **111**: 7433–7.

849 Wernegreen JJ (2002). Genome evolution in bacterial endosymbionts of insects. *Nat Rev Genet* **3**:
850 850–861.

851 Werren JH, Baldo L, Clark ME (2008). Wolbachia: master manipulators of invertebrate biology. *Nat*
852 *Rev Microbiol* **6**: 741–751.

853 Wilcox JL, Dunbar HE, Wolfinger RD, Moran NA (2003). Consequences of reductive evolution for
854 gene expression in an obligate endosymbiont. *Mol Microbiol* **48**: 1491–1500.

855 Williamson DL (1965). Kinetic studies of ‘sex ratio’ spirochetes in *Drosophila melanogaster* Meigen
856 females. *J Invertebr Pathol* **7**: 493–501.

857 Williamson DL, Poulson DF (1979). Sex ratio organisms (spiroplasmas) of *Drosophila*. In: *The*
858 *mycoplasmas*, Vol 3, pp 175–208.

859 Wright JD, Wang BT (1980). Observations on wolbachiae in mosquitoes. *J Invertebr Pathol* **35**: 200–
860 208.

861 Xie J, Vilchez I, Mateos M (2010). *Spiroplasma* bacteria enhance survival of *Drosophila hydei* attacked
862 by the parasitic wasp *Leptopilina heterotoma*. *PLoS One* **5**: e12149.

863 Ye YH, Carrasco AM, Dong Y, Sgro CM, McGraw EA (2016). The Effect of Temperature on Wolbachia-
864 Mediated Dengue Virus Blocking in *Aedes aegypti*. *Am J Trop Med Hyg.* 94:812-9

865 Zientz E, Dandekar T, Gross R (2004). Metabolic interdependence of obligate intracellular bacteria
866 and their insect hosts. *Microbiol Mol Biol Rev* **68**: 745–770.

867 Zug R, Koehncke A, Hammerstein P (2012). Epidemiology in evolutionary time: The case of
868 Wolbachia horizontal transmission between arthropod host species. *J Evol Biol* **25**: 2149–2160.

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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
<i>Acyrtosiphon pisum</i>	<i>Regiella insecticola</i>	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 <i>Serratia</i> , <i>Rickettsia</i> , or <i>Spiroplasma</i> , though the latter two are found only in the southwest at low frequency.	(Tsuchida <i>et al.</i> , 2002)
<i>Adalia bipunctata</i>	<i>Spiroplasma</i>	Sweden	<i>Spiroplasma</i> absent north of 63°N in 2011-2013. The northernmost limit was 61°N in 2000-2002.	(Tinsley, 2003; Pastok, 2015)
<i>Culicoides imicola</i>	<i>Cardinium</i>	Israel	Prevalence declines with increasing maximum daytime temperature in locality and increases with increasing minimum night-time temperature.	(Morag <i>et al.</i> , 2012)
<i>Curculio sikkimensis</i>	<i>Sodalis</i> , <i>Rickettsia</i> and <i>Wolbachia</i>	Japan	Higher prevalence of three symbionts in warmer areas to the south-west. Significant correlation with temperature. No correlation for <i>Spiroplasma</i> .	(Toju and Fukatsu, 2011)
<i>Drosophila melanogaster</i>	<i>Wolbachia</i>	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 <i>et al.</i> , 1986; Kriesner <i>et al.</i> , 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
<i>Aedes polynesiensis</i>	<i>Wolbachia</i>	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)
<i>Drosophila equinoxialis</i>	ESRO <i>Spiroplasma</i>	MK	Phenotype	MK reduced by embryonic heat-treatment with various temperatures and durations between 34°C and 40°C.	(Malogolowkin, 1959)
<i>D. nebulosa</i>	NSRO <i>Spiroplasma</i>	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 <i>et al.</i> , 2008)
<i>D. willistoni</i>	WSRO <i>Spiroplasma</i>	MK	Phenotype	No effect of embryonic heat-treatment, at various temperatures and durations between 34°C and 40°C.	(Malogolowkin, 1959)
<i>D. bifasciata</i>	A-group <i>Wolbachia</i>	MK	Phenotype, cytology	Phenotype lost between 23.5°C and 25°C.	(Hurst <i>et al.</i> , 2000, 2001)
<i>D. melanogaster</i>	wMelPop <i>Wolbachia</i> (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 <i>et al.</i> , 2003)
<i>D. simulans</i>	wRi <i>Wolbachia</i>	CI	Phenotype, cytology	Ageing and rearing males at elevated temperature (27°C) reduces incompatibility; larval thermal environment critical.	(Clancy and Hoffmann, 1998)

<i>D. simulans</i>	<i>Wolbachia</i>	CI	Phenotype	CI suppressed in crosses between two unidirectionally-incompatible fly strains exposed to 28°C in early life.	(Hoffmann <i>et al.</i> , 1986)
<i>D. simulans</i>	<i>Wolbachia</i>	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 <i>et al.</i> , 1999)
<i>Nasonia vitripennis</i>	<i>Wolbachia</i> 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)
<i>Ostrinia scapularis</i> , adzuki bean borer moth	<i>Wolbachia</i>	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 <i>et al.</i> , 2008; Sugimoto <i>et al.</i> , 2015)
<i>Tribolium confusum</i>	<i>Wolbachia</i>	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)
<i>Trichogramma cordubensis</i>	<i>Wolbachia</i>	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 <i>et al.</i> , 1999)
<i>Tetranychus urticae</i>	<i>Wolbachia</i>	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
<i>Acyrtosiphon pisum</i>	<i>Regiella insecticola</i>	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)
<i>Aedes kesseli</i> males crossed with <i>Ae. polynesiensis</i> females	<i>Wolbachia</i>	CI (<i>Ae. polynesiensis</i> females have <i>Wolbachia</i>)	Cytology	Loss from ovaries with a heat treatment of 32.5°C (versus 27°C). This also killed the host.	(Trpis <i>et al.</i> , 1981)
<i>Drosophila hydei</i>	hy1 <i>Spiroplasma</i>	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 <i>et al.</i> , 2008)
<i>D. melanogaster</i>	MSRO <i>Spiroplasma</i>	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)
<i>D. nebulosa</i>	NSRO <i>Spiroplasma</i>	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 <i>et al.</i> , 2008)
<i>D. bifasciata</i>	A-group <i>Wolbachia</i>	MK	Phenotype, cytology	Estimated at 92.9% at 25°C, compared to c. 100% at 18°C.	(Hurst <i>et al.</i> , 2000, 2001)

<i>Liposcelis tricolor</i>	<i>Wolbachia</i>	Increases fertility and fecundity	PCR	Complete elimination of <i>Wolbachia</i> over 6 generations at 33°C. Base population had 100% infection.	(Jia <i>et al.</i> , 2009)
<i>Metaseiulus occidentalis</i>	<i>Wolbachia</i>	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)
<i>Nasonia vitripennis</i>	<i>Wolbachia</i> (2 strains)	CI, various	Phenotype, PCR, cytology, Southern hybridisation	AB Double-infected wasps lose strains A and/or B in diapause.	(Perrot-Minnot <i>et al.</i> , 1996)
<i>Ostrinia scapulalis</i>	<i>Wolbachia</i>	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 <i>et al.</i> , 2008; Sugimoto <i>et al.</i> , 2015)
<i>Tetranychus urticae</i>	<i>Wolbachia</i>	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)