



Alford, L., Kishani Farahani, H., Pierre, J.-S., Burel, F. and van Baaren, J. (2017) Why is there no impact of the host species on the cold tolerance of a generalist parasitoid? *Journal of Insect Physiology*, 103, pp. 71-77. (doi:[10.1016/j.jinsphys.2017.10.008](https://doi.org/10.1016/j.jinsphys.2017.10.008))

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26 revealed no effect of host species on the cold tolerance of the emerging parasitoid, as
27 determined by CT_{min} and Chill Coma, for all parasitoid species. Host species significantly
28 affected the size of the emerging parasitoid for *A. rhopalosiphi* only, with individuals
29 emerging from *R. padi* being significantly larger than those emerging from *S. avenae*,
30 although this did not correspond to a difference in thermal tolerance. Furthermore, a
31 significant difference in the size of male and female parasitoids was observed for *A. avenae*
32 and *A. matricariae*, although, once again this did not correspond to a difference in cold
33 tolerance. It is suggested that potential behavioural thermoregulation via host manipulation
34 may act to influence the thermal environment experienced by the wasp and thus wasp thermal
35 tolerance and, in doing so, may negate physiological thermal tolerance or any impact of the
36 aphid host.

37

38 **Keywords** Aphidius, biological control, cereal aphids, host quality, host manipulation,
39 behavioural thermotolerance.

40

41 **Introduction**

42

43 Ectothermic organisms such as insects have a limited ability to control their internal
44 temperature above and below ambient. What ability they do possess to withstand and survive
45 unfavourable temperatures, referred to as thermal tolerance, is conferred via a variety of
46 biochemical and physiological mechanisms (Parsell and Lindquist, 1993; Bale, 2002). These
47 may include the synthesis of cryoprotectants e.g. antifreeze proteins (AFPs), polyols and
48 sugars (Duman, 1982, 2001; Zachariassen and Husby, 1982; Zeng et al., 2008) and ice
49 nucleating agents (INAs) (Zachariassen and Hammel, 1976; Duman and Patterson, 1978), the
50 restructuring of cell membranes (Los and Murata, 2004; Lee et al., 2006; Michaud and
51 Denlinger, 2006; Tomčala et al., 2006), and the upregulation of heat proteins (Parsell and
52 Lindquist, 1993; Denlinger, 2002; Rinehart et al., 2000; 2007).

53

54 The thermal tolerance of an individual is not a fixed trait and numerous internal and external
55 factors are known to impact the physiological thermal tolerance of an individual. These can
56 include 1) characteristics of the temperature stress itself e.g. the rate of temperature change
57 and the duration of exposure, in addition to any prior exposure eliciting an acclimation or
58 hardening response (e.g. Kelty and Lee, 1999, 2001; Powell and Bale 2006, 2008; Lachenicht
59 et al., 2010; Chidawanyika and Terblanche, 2011; Allen et al., 2012), 2) characteristics of the
60 individual exposed to the stress e.g. age (Bowler and Terblanche, 2008), size, which in turn
61 may be linked to sex in insects displaying sexual dimorphism (Renault et al., 2003; Roux et
62 al., 2010; Le Lann et al., 2011), the level of inbreeding (Dierks et al., 2012), developmental
63 plasticity (Colinet and Hoffmann, 2012; Foray et al., 2013), or infection by symbiotic bacteria
64 (Montllor et al., 2002; Russell and Moran, 2006; Dunbar et al., 2007), and 3) aspects of the
65 physical environment e.g. photoperiod (Fischer et al., 2012), landscape intensification

66 (Tougeron et al., 2016; Alford et al., 2017) and 4) aspects of the biological environment e.g.
67 available diet (Andersen et al., 2010; Verdu et al., 2010), population overcrowding (Sørensen
68 and Loeschcke, 2001), or contact with the host plant for phytophagous insects (Butts et al.,
69 1997; Alford et al., 2016).

70

71 For generalist species, be they phytophagous organisms exploiting multiple plant species,
72 predators exploiting multiple prey species, or parasites and parasitoids exploiting multiple
73 host species, the choice of plant, prey or host can greatly affect the fitness of the generalist
74 concerned (e.g. Salt, 1941; Awmack and Leather, 2002; Gnanvossou et al., 2003). Since
75 thermal tolerance is not a fixed trait, such choices are likely to have implications for the
76 physiological thermal tolerance of an organism. However, for the insect parasitoids, the
77 choice of host has perhaps more far-reaching consequences because the parasitoid is confined
78 to the insect host during immature development. Here, the insect host represents the sole
79 source of nutrients for the developing parasitoid and subsequently, the quality of the host can
80 greatly affect development and resultant fitness of the emerging parasitoid (Godfray 1994).
81 Host quality is known to impact an array of life history traits including development time,
82 adult size, fecundity, longevity and offspring sex ratio (e.g. Harvey et al., 2004; Ris et al.,
83 2004; Colinet et al., 2005; Silva et al., 2008; Xu et al., 2008; Sidney et al., 2010; Kishani
84 Farahani et al., 2016). However, how the host affects the physiological thermal tolerance of a
85 generalist parasitoid is unknown.

86

87 Much research has focused on the thermal biology and thermal tolerance of insect parasitoids
88 to better understand and predict their efficacy as biological control agents (Wanderley et al.,
89 2007; Hughes et al., 2010, 2011; Iranipour et al., 2010; Le Lann et al., 2011) and their
90 potential for establishment following release (Hughes et al., 2010, 2011; Hanson et al., 2013).

91 This is especially pertinent in the face of global climate change, where any disruption to the
92 temporal or spatial synchronization of the host-parasitoid system could increase the risk of
93 host outbreaks (Hance et al., 2007). However, whilst research has focused on the thermal
94 requirements and thermal tolerance of parasitoid insects, research on the impact of the host
95 has not been considered.

96

97 The current study aims to elucidate the effect of host species on the thermal tolerance,
98 specifically the low temperature tolerance, of a generalist parasitoid using parasitoid wasps
99 belonging to the genus *Aphidius* (Hymenoptera: Braconidae) and their cereal aphid hosts
100 (Hemiptera: Aphididae) as a study system. In winter months, three cereal aphid species are
101 present in the cereal fields of Northern France: The English grain aphid *Sitobion avenae*
102 (Fabricius), the bird cherry oat aphid *Rhopalosiphum padi* (L.) and the rose grain aphid
103 *Metopolophium dirhodum* (Walker) (Andrade et al., 2013; Alford et al., 2014, Andrade et al.,
104 2016). During this period, the female parasitoids must make a choice of which species to
105 parasitize at a time when thermal tolerance is vital to the survival of their offspring. The
106 literature suggests that these species of cereal aphids may differ in their nutritional value for
107 aphid predators and parasitoids, impacting life-history traits such as size, wing area,
108 developmental time, and survival rate during development (Andrade et al., 2013; Eoche-Bosy
109 et al., 2016). For the generalist spider, *Erigone atra*, a diet of *M. dirhodum* resulted in
110 increased juvenile size and survival when compared to a diet of *R. padi* and *S. avenae*, leading
111 authors to conclude that *M. dirhodum* was of greater nutritional value (Bilde and Toft, 2001).
112 However, for the parasitoid wasps, *Aphidius rhopalosiphi* and *Aphidius matricariae*, females
113 produced more offspring when parasitizing *R. padi*, although they produced larger offspring
114 when parasitizing *S. avenae*, (Andrade et al., 2013) suggesting the aphid host can
115 differentially impact varying aspects of parasitoid fitness. As such, the choice of host is vital

116 for parasitoids of the genus *Aphidius* when faced with multiple host species. This would be
117 especially true in harsh winters where thermal tolerance is integral to survival should host
118 species impact the thermal tolerance of the emerging parasitoid. Furthermore, in the studied
119 area (Brittany), it has been shown that parasitoid species have ceased to enter diapause (since
120 2010) (Andrade et al., 2016, Tougeron et al., 2017), making thermal tolerance all the more
121 crucial. In addition, such information concerning the impact of the aphid host on parasitoid
122 wasp thermal tolerance may contribute to the mass rearing of parasitoid wasps to enhance
123 their efficacy as biological control agents.

124

125 Here we report on laboratory experiments designed to investigate the effect of cereal aphid
126 host on the cold tolerance of three species of parasitoid wasps (*Aphidius avenae*, *A.*
127 *matricariae* and *A. rhopalosiphi*). The thermal tolerance of parasitoids was determined using
128 measures of Critical Thermal Minima (CT_{min}) and Chill Coma (Hazell and Bale, 2011).
129 However, for *Aphidius* parasitoids, and indeed all insects, size is an important factor
130 influencing thermal tolerance, with smaller wasps commonly displaying enhanced cold
131 tolerance (Le Lann et al., 2011; Ismail et al., 2012). This finding is in line with the “absolute
132 energy demand hypothesis” (AED), which predicts that larger individuals require more
133 energy to sustain body functions (Blanckenhorn et al., 1995, Reim et al., 2006), most likely
134 the result of possessing a proportionately higher metabolic rate (Calder 1984, Gillooly et al.,
135 2001). As such, these larger individuals are at a disadvantage in stressful environments. In
136 addition, *Aphidius* parasitoids are sexually dimorphic, with males smaller in size than females
137 (Roux et al., 2010; Le Lann et al., 2011; Tougeron et al., 2016). Aphid host is known to
138 impact both the size and sex of the emerging parasitoid (Andrade et al., 2013; Eoche-Bosy et
139 al., 2016). For this reason, morphological measures of the host and emerging male and female
140 parasitoids will further be measured to elucidate any possible size, sex or interaction effect

141 with thermal tolerance. The following hypotheses were subsequently tested 1) aphid host
142 species will significantly affect the thermal tolerance of the emerging parasitoid; 2) for a
143 given parasitoid species, larger aphids, whatever the species, will produce parasitoids of
144 greater size, but a reduced cold tolerance. This will follow the AED theory which proposes
145 that larger individuals are less resistant to stress, 3) female wasps, which are generally larger
146 than male wasps, will display a reduced cold tolerance.

147

148 **Materials and methods**

149 *Aphid collection and rearing*

150 Cultures of anholocyclic *Metopolophium dirhodum*, *Rhopalosiphum padi* and *Sitobion avenae*
151 were established using aphids collected in the Long Term Ecological Research (LTER) site
152 Armorique (<http://osur.univ-rennes1.fr/za-armorique/>) located in Brittany, North-western
153 France. Aphids were reared in the laboratory on winter wheat, *Triticum aestivum*, ‘Renan’
154 cultivar grown in vermiculite within Plexiglas cages (50 x 50 x 50 cm) and housed in a
155 controlled environmental room at 20±1°C and LD 16:8 h photoperiod.

156

157 *Parasitoid collection and rearing*

158 Aphid mummies were originally collected in wheat, triticale and clover fields near Rennes
159 (Brittany, France) in October and November 2014 and used to establish laboratory cultures of
160 *Aphidius avenae*, *Aphidius matricariae* and *Aphidius rhopalosiphi*. Additional mummy
161 collection occurred throughout December and January to maintain genetic diversity of the
162 laboratory culture. Parasitoid wasp cultures were maintained within Plexiglas cages (50 x 50 x
163 50 cm) at 20±1°C and L16:D8 on the aphid *Sitobion avenae*, unless otherwise stated, and fed
164 on a solution of honey and water. Pots of winter wheat infested with *S. avenae* were added to

165 the cages containing the parasitoid wasps on a weekly basis to provide the wasps with a
166 continuous supply of hosts.

167

168 ***Obtaining parasitoids for experimentation***

169 To obtain parasitoids for experiments, aphid mummies were collected from the stock
170 parasitoid cultures and isolated in gelatin capsules. Isolating mummies in this way enabled
171 tight control over access to food and the aging of parasitoids to within a day. The capsules
172 were checked twice daily for emergence. Following emergence, parasitoids of each species
173 were pooled in a plastic vial containing honey and water for 24 h to allow for feeding and
174 mating.

175

176 To provide hosts for the mated parasitoids, aphids of a single species were put in microcages
177 (L = 16 cm, Ø = 4 cm) comprising *T. aestivum* grown in vermiculite. Aphids were selected at
178 the L3 and L4 stage, the preferred instar for all the species, and placed at densities of
179 approximately 10. Microcages were created in this way containing *M. dirhodum*, *R. padi* or *S.*
180 *avenae*. Mated female parasitoids were subsequently placed within microcages at densities of
181 one per microcage and allowed to lay eggs within the aphids during a 24 h period. Each
182 parasitoid was tested on the host species that represent the natural host range in Brittany
183 (Andrade et al., 2015; Eoche-Bosy et al., 2016), allowing successful parasitoid host
184 acceptance, oviposition and the production of viable offspring under laboratory conditions.
185 This included *A. avenae* on *M. dirhodum* and *S. avenae*, a parasitoid-host combination
186 common in spring months (Eoche-Bosy et al., 2016), and both *A. matricariae* and *A.*
187 *rhopalosiph* on *R. padi* and *S. avenae*, combinations common in winter months (Eoche-Bosy
188 et al., 2016). Following the 24 h period, the female parasitoid was removed and the aphids left
189 to develop into mummies. One mummy was selected from each microcage to avoid selecting

190 mummies containing sibling larvae. Resultant mummies were isolated in gelatin capsules and
191 checked twice daily for emergence. After emergence, parasitoids were isolated individually in
192 plastic vials containing honey and water for a period of 24 h. Following the 24 h, parasitoids
193 were used in cold tolerance experiments. As a result, all parasitoids used in experiments were
194 no more than 48h old, but older than 24h. This procedure was repeated to produce test
195 parasitoids for every viable parasitoid species x host species combination. All aphid mummies
196 were retained for morphological measurements.

197

198 *Determination of thermal tolerance (CT_{min})*

199 Thermal tolerance was determined by measuring distinct stages of the Chill Coma process
200 (Hazell and Bale, 2011) using an experimental design described by Powell and Bale (2006).
201 The method employed a glass column (35 x 5cm) which was connected to a programmable
202 alcohol bath (Haake F3, Thermo Electron Corp., Karlsruhe, Baden-Württemberg, Germany),
203 enabling the circulation of alcohol fluid around the outer chamber and thus fine control over
204 the air temperature experienced within the inner column. Previous work has shown that air
205 temperature is consistent along the length of the column (Powell and Bale, 2006). In addition,
206 due to the relatively small body size of the test insects, it is concluded that the air temperature
207 of the column approximates the body temperature of the insects (Huey *et al.*, 1992).
208 Consequently, a single thermocouple was placed against the glass surface of the inner column
209 to monitor air temperature during experimentation.

210

211 In all experiments, a single parasitoid wasp was inserted into the bottom of the column pre-set
212 to the culture temperature of 20°C. The column was subsequently closed with a sponge
213 stopper to reduce air flow and maintain a stable thermal environment within the inner column.
214 Following a 5 min acclimatization period, the programmable alcohol bath was set to decrease

215 the temperature of the column from 20°C to -10°C at a rate of 0.75°C min⁻¹. The rate of
216 0.75°C min⁻¹ was chosen since it is fast enough not to induce a rapid cold hardening response
217 in the test insects (Powell and Bale, 2004, 2005), whilst being slow enough to not shock the
218 insects.

219

220 Two distinct stages of the Chill Coma process (Hazell and Bale, 2011) were measured, as
221 previously described for *Aphidius* wasps in Le Lann et al. 2011. The first, referred to as the
222 Critical Thermal Minima (CT_{min}), represents the temperature at which the parasitoid is no
223 longer able to walk in a coordinated manner and corresponds to the loss of coordinated
224 muscle function. The second stage is determined as the temperature at which the parasitoid
225 wasp is unable to cling to the vertical column and subsequently falls to the bottom of the
226 column in a comatose state (Le Lann et al., 2011). This is synonymous with the point of cold-
227 induced paralysis and will be referred to as the temperature of Chill Coma, as defined by
228 Hazell and Bale (2011).

229

230 The temperatures of CT_{min} and Chill Coma were recorded manually to an accuracy of 0.1°C.
231 The procedure was repeated to obtain CT_{min} and Chill Coma values for approximately 25-30
232 individuals, of approximately equal gender, for each parasitoid species x host species
233 combination. Following determination of the CT_{min} and Chill Coma, all parasitoids were
234 preserved in 70% ethanol for morphological measurements.

235

236 ***Morphological measurements***

237 The hind tibia of parasitoids and of the corresponding aphid mummy (which gives a measure
238 of the host size at the moment of its death) were measured using a camera (Zeiss AxioCam
239 ERc5s® HD) mounted on an x9 binocular microscope. Photographs were taken using

240 Intelcam software and morphological measurements made using the image processing
241 software Image J® software (v. 1.48). Tibia length was chosen since it is a commonly used
242 indicator of body size in insects and correlates strongly to other measures such as dry mass
243 (Godfray 1994; Cohen et al., 2005).

244

245 *Statistical analyses*

246 Statistical analyses were performed using R Software (R Development Core Team, 2013).
247 Statistical differences in thermal tolerance measures and size (as determined by hind tibia
248 length) of parasitoids emerging from different cereal aphid hosts were tested using a standard
249 repeated-measures ANOVA. Aphid host species, parasitoid sex and host size were treated as
250 predictors, qualitative factors for the first two, quantitative covariate for the third, and
251 possible interactions were tested.

252

253 **Results**

254

255 *Aphid host species effects*

256 For all parasitoid species tested, there was no significant effect of aphid host species on the
257 CT_{min} or Chill Coma temperature of the resultant parasitoid (Table 1), nor were there
258 significant interactions between aphid host, host size and parasitoid sex (Supplementary Table
259 1). There was no significant effect of aphid host species on the size of the resultant parasitoid
260 for *A. avenae* and *A. matricariae*, although a significant effect was observed for *A.*
261 *rhopalosiphi* with parasitoids emerging from *R. padi* being significantly larger than those
262 emerging from *S. avenae* (Figure 1) (Table 1), but without impacting thermotolerance.

263

264 *Aphid host size effects*

265 As there is no effect of the aphid species, all the data (aphids and parasitoids species) were
266 pooled to analyse separately the effect of the host size, as determined by hind tibia length
267 (mm) of the aphid mummy. Host size did not significantly affect the size of the emerging
268 parasitoid for *A. avenae* ($F = 0.01$, $df = 1$, $p = 0.924$), *A. matricariae* ($F = 0.004$, $df = 1$, $p =$
269 0.948) and *A. rhopalosiphi* ($F = 2.39$, $df = 1$, $p = 0.129$), nor were there significant
270 interactions between aphid host, host size and parasitoid sex (Supplementary Table 1).
271 Furthermore, aphid host size did not significantly affect the CT_{min} or the Chill Coma
272 temperature of the emerging parasitoid for any of the tested species (Supplementary Table 1).

273

274 *Parasitoid sex effects*

275 There was no significant effect of sex on parasitoid CT_{min} or Chill Coma temperature (Table
276 2), nor were there significant interactions between aphid host, host size and parasitoid sex
277 (Supplementary Table 1). However, there was a significant effect of sex on parasitoid size, as
278 determined by hind tibia length (mm), for *A. avenae* and *A. matricariae* (Figure 2) (Table 2).
279 No significant effect of sex on parasitoid size was observed for *A. rhopalosiphi*.

280

281 **Discussion**

282 In the current study, host species did not significantly affect parasitoid cold tolerance, as
283 determined by CT_{min} and Chill Coma temperature, for any of the tested parasitoid species.
284 This disproves hypothesis 1 which stated that the aphid host species would significantly affect
285 the thermal tolerance of the emerging parasitoid. In addition, aphid host size, as determined
286 by hind tibia length, did not significantly affect the size nor the thermal tolerance of the
287 emerging parasitoid. This disproves hypothesis 2 which stated that, for a given parasitoid
288 species, larger aphids, whatever the species, will produce parasitoids of a greater size, but a
289 reduced cold tolerance. Furthermore, whilst females of *A. avenae* and *A. matricariae* were

290 found to be significantly larger than males, this increase in size did not correspond to a
291 difference in thermal tolerance. This disproves hypothesis 3 which stated that the larger size
292 of female wasps would correspond to a reduction in cold tolerance.

293

294 During development, the immature parasitoid is confined to the insect host and, as such, the
295 host represents the sole source of nutrients for the developing parasitoid. For this reason, the
296 choice of host has profound implications for the emerging parasitoid and has been shown to
297 impact an array of life history traits such as development time, emergence rate, longevity, size
298 and sex ratio (e.g. Harvey et al., 2004; Colinet et al., 2005; Silva et al., 2008; Xu et al., 2008;
299 Sidney et al., 2010; Kishani Farahani et al., 2016). However, the impact of the host on adult
300 parasitoid thermal tolerance was unknown until this study. Host species is regarded as an
301 indicator of host quality for the ovipositing parasitoid (Brodeur and Boivin, 2004). For the
302 cereal aphids *R. padi* and *S. avenae*, Andrade et al. (2013) revealed that aphid host species
303 significantly impacted *A. rhopalosiphi* fitness. Here, parasitoid females produced more
304 offspring when parasitizing *R. padi* compared to *S. avenae*, although they produced larger
305 offspring when parasitizing *S. avenae*. Furthermore, female *A. avenae*, preferentially oviposit
306 in *S. avenae* suggesting that *S. avenae* is a better quality host for offspring development than
307 *M. dirhodum* (Eoche-Bosy et al., 2016) . For these reasons, it is surprising that aphid host
308 species may impact parasitoid fitness via wasp fecundity or development rate, but not
309 significantly affect thermal tolerance. One possible explanation is that certain traits may be
310 more susceptible to variation in host species and associated quality. For example, in the study
311 of Eoche-Bosy (2016), some of the studied traits did not vary with host species, as for
312 example the size of the emerging parasitoid. The same result was obtained in another family
313 of parasitoids, the wasp *Diaeretiella rapae*, where the aphid host species was found to impact
314 parasitoid developmental time, although host species had no significant effect on emergence

315 rate, sex ratio and longevity (Silva et al., 2011). This may explain the absence of an effect of
316 aphid host on the thermal tolerance of the emerging parasitoid.

317

318 The absence of host size and parasitoid size (even mediated by sex) effects is also surprising
319 because, it is widely accepted that adult body size of many parasitoid species is determined by
320 host size (Daza-Bustamante et al., 2003; Henry et al., 2006; De Conti et al., 2008; Sampaio et
321 al., 2008), with larger hosts containing more resources for the developing parasitoid and thus
322 acting to enhance adult fitness. In line with this, female wasps are commonly larger than
323 males (Godfray 1994; Le Lann et al., 2011; Rehman and Powell, 2010) and this size
324 difference is believed to be the consequence of sex-specific allocation of offspring to hosts of
325 varying quality (Charnov et al., 1981; Mackauer, 1996; Rehman and Powell, 2010). However,
326 whilst larger aphids may represent hosts of greater quality, resulting in a female bias and
327 individual parasitoids of increased size, the literature suggests that a larger size may
328 negatively impact the thermal tolerance of parasitoids in line with the Absolute Energy
329 Demand hypothesis (Blanckenhorn et al., 1995, Reim et al., 2006). The Absolute Energy
330 Demand hypothesis states that larger individuals are at a disadvantage when faced with
331 conditions of stress since a larger body requires greater energy to sustain bodily functions
332 (Blanckenhorn et al., 1995; Reim et al., 2006). Le Lann et al. (2011) and Ismail et al. (2012)
333 have shown that this theory could match with the lowest cold resistance observed in the larger
334 *Aphidius* individuals. However, in this study, firstly, we did not observe an effect of size of
335 host on the size of the emerging parasitoids, and secondly, we did not observe an effect of size
336 on parasitoid thermotolerance. For the first point, it could be that we only used 3rd and 4th
337 instars (the preferred instars), which may not present enough variation in size to influence the
338 size of the emerging parasitoids. Moreover, Sequeira and Mackauer (1992a,b) showed that
339 parasitoid adult size was not a linear function of host size at parasitization for *A. ervi* on the

340 aphid *Acyrtosiphon pisum*. For the second point, it seems that physiological thermotolerance
341 is not affected by the host, whatever the species or its size, meaning that thermotolerance is
342 probably achieved by other means, as detailed below.

343

344 The current study investigated the effect of the aphid host on the thermal tolerance of the
345 emerging parasitoid. However, a parasitoid, and indeed any living organism, may also survive
346 unfavourable thermal conditions employing behavioural thermoregulation (e.g. Larsen and
347 Lee, 1994; Barton et al., 2014; Ward and Seely, 1996), instead of physiological
348 thermoregulation. For the parasitoid wasp, confined to the aphid host during development,
349 behavioural thermoregulation may occur via host manipulation. Such behavioural
350 thermoregulation could provide an explanation to why parasitoid thermal tolerance is not
351 significantly affected by aphid host. In a recent study investigating the potential for host
352 manipulation by *A. avenae* to aid thermal tolerance, behavioural differences between
353 parasitized and non-parasitized aphid hosts were observed, in addition to inter-host species
354 differences (Alford et al., 2016). Here, differences in the behaviours of parasitized aphids at
355 unfavourable temperatures were observed for the less cold tolerant aphid *M. dirhodum*, but
356 not for the more cold tolerant *S. avenae*. As such, immature parasitoid survival at
357 unfavourable temperatures may represent a trade-off between the physiological thermal
358 tolerance of the aphid host and the need for additional behavioural thermoregulation via host
359 manipulation. It may be possible, therefore, that the parasitoid can manipulate the behaviour
360 of the host in order to influence its thermal environment and thus thermal tolerance, and, as
361 such, may utilise behavioural thermoregulation over physiological thermotolerance.

362

363 In conclusion, the present study showed with three different parasitoid species, each of them
364 tested on two different aphid hosts species, that there is no effect of the host on the

365 physiological thermotolerance of the three parasitoids. With recent work suggesting that at
366 least one of the studied species of parasitoid, *A. avenae*, may be capable of behavioural
367 thermoregulation via host manipulation, any ability to influence the thermal environment and
368 thus thermal tolerance may negate physiological thermal tolerance or any impact of the aphid
369 host.

370

371 **Acknowledgements**

372 The authors gratefully acknowledge Stephanie Llopis, Jean-Pierre Caudal, Herve Amat, Jean-
373 Luc Foulon, Thierry Fontaine and Fouad Nassur for technical assistance, and to Charlotte
374 Alford for morphological measures. Thanks also to Valérie Briand for bibliographic
375 assistance and to Sandra Rigaud for handling the administrative aspects of the grant. A special
376 thanks to Saurav Ghimire for assistance with the graphical abstract.

377

378 Funding: This work was funded by a Marie Curie Intra-European Fellowship for the project
379 ‘Climland’ (FP7-PEOPLE-2012-IEF-326943) awarded to L Alford, F Burel and J van Baaren.

380

381 **References**

382 Alford, L., Burel, F., van Baaren J. 2016. Improving methods to measure critical thermal
383 limits in phloem-feeding pest insects. *Entomol. Exp. Appl.* 159, 61-69

384

385 Alford, L., Tougeron, K., Pierre, J-S, Burel, F., van Baaren J. 2017. The effect of landscape
386 complexity and microclimate on the thermal tolerance of a pest insect. *Insect Sci.*
387 doi:10.1111/1744-7917.12460.

388

389 Allen, J.L., Clusella-Trullas, S., Chown, S.L. 2012. The effects of acclimation and rates of
390 temperature change on critical thermal limits in *Tenebrio molitor* (Tenebrionidae) and
391 *Cyrtobagous salviniae* (Curculionidae). *J. Insect. Physiol.* 58, 669-678.

392

393 Andersen, L.H., Kristensen, T.N., Loeschcke, V., Toft, S., Mayntz, D. 2010. Protein and
394 carbohydrate composition of larval food affects tolerance to thermal stress and desiccation in
395 adult *Drosophila melanogaster*. *J. Insect. Physiol.* 56, 336-340.

396

397 Andrade, T.O., Herve, M., Outreman, Y., Krespi, L., van Baaren, J. 2013. Winter host
398 exploitation influences fitness traits in a parasitoid. *Entomol. Exp. Appl.* 147, 167-174.

399

400 Andrade, T.O., Outreman, Y., Krespi, L., Plantegenest, M., Vialatte, A., Gauffre, B., van
401 Baaren, J. 2015. Spatiotemporal variations in aphid-parasitoid relative abundance patterns and
402 food webs in agricultural ecosystems. *Ecosphere.* 6, 113.

403

404 Andrade T.O., Krespi L., Bonnardot V., van Baaren J., Outreman Y. 2016. Impact of change
405 in winter strategy of one parasitoid species on the diversity and function of a guild of
406 parasitoids. *Oecologia*. 180, 877-888
407

408 Awmack, C.S., Leather, S.R. 2002. Host plant quality and fecundity in herbivorous insects.
409 *Annu. Rev. Entomol.* 47, 817-844.
410

411 Bale, J.S. 2002. Insects and low temperatures: from molecular biology to distributions and
412 abundance. *Philos. T. Roy. Soc. B.* 357, 849-861.
413

414 Barton, M., Porter, W., Kearney, M. 2014. Behavioural thermoregulation and the relative
415 roles of convection and radiation in a basking butterfly. *J. Therm. Biol.* 41, 65-71.
416

417 Bilde, T., Toft, S. 2001. The value of three cereal aphid species as food for a generalist
418 predator. *Physiol. Entomol.* 26, 58-68.
419

420 Blanckenhorn, W.U., Preziosi, R.F., Fairbairn, D.J. 1995. Time and energy constraints and the
421 evolution of sexual size dimorphism- to eat or to mate? *Evol. Ecol.* 9, 369-381.
422

423 Bowler, K. Terblanche, J.S. 2008. Insect thermal tolerance: what is the role of ontogeny,
424 ageing and senescence? *Biol. Rev.* 83, 339-355.
425

426 Brodeur, J., Boivin, G. 2004. Functional ecology of immature parasitoids. *Annu. Rev.*
427 *Entomol.* 49, 27-49.
428

429 Butts, R.A., Howling, G.G., Bone, W., Bale, J.S., Harrington, R. 1997. Contact with the host
430 plant enhances aphid survival at low temperatures. *Ecol. Entomol.* 22, 26-31.
431

432 Calder, W. A. 1984. Size, function and life history. Harvard University Press.
433

434 Charnov, E.L., los-den Hartogh, R.L., Jones, W.T., van den Assem, J. 1981. Sex ratio
435 evolution in a variable environment. *Nature*, 289, 27-33.
436

437 Chidawanyika, F., Terblanche, J.S. 2011. Rapid thermal responses and thermal tolerance in
438 adult codling moth *Cydia pomonella* (Lepidoptera: Tortricidae). *J. Insect. Physiol.* 57, 108-
439 117.
440

441 Cohen, J.E., Jonsson, T., Müller, C.B., Godfray, H.C.J., Savage, V.M., 2005. Body sizes of
442 hosts and parasitoids in individual feeding relationships. *P. Natl. Acad. Sci. USA.* 102, 684-
443 689.
444

445 Colinet, H., Hoffmann, AA, 2012. Comparing phenotypic effects and molecular correlates of
446 developmental, gradual and rapid cold acclimation responses in *Drosophila melanogaster*.
447 *Funct. Ecol.* 26, 84-93.
448

449 Colinet, H., Salin, C., Boivin, G., Hance, T. 2005. Host age and fitness-related traits in a
450 koinobiont aphid parasitoid. *Ecol. Entomol.* 30, 473-479.
451

452 Daza-Bustamante, P., Fuentes-Contreras, E., Niemeyer, H.M. 2003. Acceptance and
453 suitability of *Acyrtosiphon pisum* and *Sitobion avenae* as hosts of the aphid parasitoid
454 *Aphidius ervi* (Hymenoptera: Braconidae). Eur. J. Entomol. 100, 49-53.
455

456 De Conti, B.F.D, Bueno, V.H.P., Sampaio, M.V. 2008. The parasitoid *Praon volucre*
457 (Hymenoptera: Braconidae: Aphidiinae) as a potential biological control agent of the aphid
458 *Uroleucon ambrosiae* (Hemiptera: Aphididae) on lettuce in Brazil. Eur J Entomol 105, 485-
459 487.
460

461 Denlinger, D. L. 2002. Regulation of diapause. Annu. Rev. Entomol. 47, 93- 122.
462

463 Dierks, A., Hoffmann, B., Bauerfeind, S.S., Fischer, K. 2012. Effects of inbreeding on life
464 history and thermal performance in the tropical butterfly *Bicyclus anynana*. Popul. Ecol. 54,
465 83-90.
466

467 Duman, J.G. 1982. Insect antifreezes and ice-nucleating agents. Cryobiology. 19, 613- 627.
468

469 Duman, J.G. 2001. Antifreeze and ice nucleator proteins in terrestrial arthropods. Annu. Rev.
470 Entomol. 63, 327-357.
471

472 Duman, J.G., Patterson, J.L. 1978. Role of ice nucleators in frost tolerance of overwintering
473 queens of the bald faced hornet. Comp. Biochem. Phys. A. 59, 69-72.
474

475 Dunbar, H.E., Wilson, A.C.C., Ferguson, N.R., Moran, N.A. 2007. Aphid thermal tolerance is
476 governed by a point mutation in bacterial symbionts. PLoS. Biol. 5, 1006-1015.

477

478 Eoche-Bosy, D., Outreman, Y., Andrade, T.O., Krespi, L., van Baaren, J. 2016. Seasonal
479 variations in the availability of host resources influence foraging strategy in parasitoids.
480 Entomol. Exp. Appl. 16, 11-19.

481

482 Fischer, K., Liniek, S., Bauer, M., Baumann, B., Richter, S. & Dierks, A. 2012. Phenotypic
483 plasticity in temperature stress resistance is triggered by photoperiod in a fly. *Evol. Ecol.* 26,
484 1067-1083.

485

486 Foray, V., Desouhant, E., Voituren, Y., Larvor, V., Renault, D., Colinet, H., Gibert P., 2013.
487 Does cold tolerance plasticity correlate with the thermal environment and metabolic profiles
488 of a parasitoid wasp? *Comp. Biochem. Phys. A.* 1, 77-83.

489

490 Gillooly, J.F., Brown, J.H., West, G.B., Savage, V.M., Charnov, E.L. 2001. Effects of size
491 and temperature on metabolic rate. *Science.* 293, 2248-2251.

492

493 Gnanvossou, D., Yaninek, J. S., Hanna, R., Dicke, M. 2003. Effects of prey mite species on
494 life history of the phytoseiid predators *Typhlodromalus manihoti* and *Typhlodromalus aripo*.
495 *Exp. Appl. Acarol.* 30, 265-278.

496

497 Godfray, H.C.J. 1994. *Parasitoids: Behavioural and Evolutionary Ecology.* Princeton.
498 University Press, Princeton, New Jersey.

499

500 Hance, T., van Baaren, J., Vernon, P., Boivin, G. 2007. Impact of extreme temperatures on
501 parasitoids in a climate change perspective. *Annu. Rev. Entomol.* 52, 107–126.

502

503 Hanson, A.A., Venette, R.C., Lelito, J.P. 2013. Cold tolerance of Chinese emerald ash borer
504 parasitoids: *Spathius agrili* Yang (Hymenoptera: Braconidae), *Tetrastichus planipennis* Yang
505 (Hymenoptera: Eulophidae), and *Oobius agrili* Zhang and Huang (Hymenoptera: Encyrtidae).
506 Biol. Control. 67, 516-529.

507

508 Harvey, J.A., Bezemer, T.M., Elzinga, J.A, Strand, M.R. 2004. Development of the solitary
509 endoparasitoid *Microplitis demolitor*: host quality does not increase with host age and size.
510 Ecol. Entomol. 29: 35-43.

511

512 Hazell, S.P., Bale, J.S. 2011. Low temperature thresholds: are chill coma and CT_{min}
513 synonymous? J. Insect. Physiol. 57, 1085-1089.

514

515 Henry, L.M., Roitberg, B.D., Gillepsie, D.R. 2006. Covariance of phenotypically plastic traits
516 induces an adaptive shift in host selection behaviour. P. Roy. Soc. Lond. B. Bio. 273, 2893-
517 2899.

518

519 Huey, R.B., Crill, W.D., Kingsolver, J.G., Weber, K.E. 1992. A method for rapid
520 measurement of heat or cold resistance of small insects. Funct. Ecol. 6, 489-494.

521

522 Hughes, G.E., Owen, E., Sterk, G., Bale, J.S. 2010. Thermal activity thresholds of the
523 parasitic wasp *Lysiphlebus testaceipes* and its aphid prey: implications for the efficacy of
524 biological control. Physiol. Entomol. 35, 373-378.

525

526 Hughes, G.E., Sterk, G., Bale, J.S. 2011. Thermal biology and establishment potential in
527 temperate climates of the aphid parasitoid, *Lysiphlebus testaceipes*. *Biocontrol*. 56, 19-33.
528

529 Iranipour, S., Nozadbonab, Z., Michaud, J. P. 2010. Thermal requirements of *Trissolcus*
530 *grandis* (Hymenoptera: Scelionidae), an egg parasitoid of sunn pest. *Eur. J. Entomol.* 107, 47-
531 53.
532

533 Ismail, M., Vernon, P., Hance, T., Pierre, J.-S., van Baaren, J. 2012. What are the possible
534 benefits of small size for energy constrained ectotherms in cold stress conditions? *Oikos*. 121,
535 2072–2080.
536

537 Kelty, J.D., Lee, R.E. 1999. Induction of rapid cold hardening by cooling at ecologically
538 relevant rates in *Drosophila melanogaster*. *J. Insect. Physiol.* 45, 719-726.
539

540 Kelty, J.D., Lee, R.E. 2001. Rapid cold-hardening of *Drosophila melanogaster* (Diptera :
541 Drosophilidae) during ecologically based thermoperiodic cycles. *J. Exp. Biol.* 204, 1659-
542 1666.
543

544 Kishani Farahani, H., Ashouri, A., Zibae, A., Abroon, P. Alford, L. 2016. The effect of host
545 nutritional quality on multiple components of *Trichogramma brassicae* fitness. *B. Entomol.*
546 *Res.* 106, 633-641.
547

548 Lachenicht, M.W., Clusella-Trullas, S., Boardman, L., Le Roux, C., Terblanche, J.S. 2010.
549 Effects of acclimation temperature on thermal tolerance, locomotion performance and

550 respiratory metabolism in *Acheta domesticus* L. (Orthoptera: Gryllidae). J. Insect. Physiol. 56,
551 822-830.

552

553 Larsen, K.J., Lee, R.E. 1994. Cold tolerance including rapid cold-hardening and inoculative
554 freezing of fall migrant monarch butterflies in Ohio. J. Insect. Physiol. 40, 859-864.

555

556 Lee, R.E., Damodaran, K., Yi, S. X., Lorigan, G.A. 2006. Rapid cold-hardening increases
557 membrane fluidity and cold tolerance of insect cells. Cryobiology. 52, 459-463.

558

559 Le Lann, C., Roux, O., Serain, N., van Alphen, J.J.M., Vernon, P., van Baaren, J. 2011.
560 Thermal tolerance of sympatric hymenopteran parasitoid species: does it match seasonal
561 activity? Physiol. Entomol. 36, 21-28.

562

563 Los, D.A., Murata, N. 2004. Membrane fluidity and its roles in the perception of
564 environmental signals. BBA. Biomembranes. 1666, 142- 157.

565

566 Michaud, M.R., Denlinger, D.L. 2006. Oleic acid is elevated in cell membranes during rapid
567 cold-hardening and pupal diapause in the flesh fly, *Sarcophaga crassipalpis*. J. Insect.
568 Physiol. 52, 1073-1082.

569

570 Montllor, C.B., Maxmen, A., Purcell, A.H. 2002. Facultative bacterial endosymbionts benefit
571 pea aphids *Acyrtosiphon pisum* under heat stress. Ecol. Entomol. 27, 189-195.

572

573 Parsell, D.A., Lindquist, S. 1993. The function of heat-shock proteins in stress tolerance -
574 degradation and reactivation of damaged proteins. Annu. Rev. Genet. 27, 437-496.

575

576 Powell, S.J., Bale, J.S. 2006. Effect of long-term and rapid cold hardening on the cold torpor
577 temperature of an aphid. *Physiol. Entomol.* 31, 348-352.

578

579 Powell, S.J., Bale, J.S. 2008. Intergenerational acclimation in aphid overwintering. *Ecol.*
580 *Entomol.* 33, 95-100.

581

582 Rehman A., Powell W. 2010. Host selection behaviour of aphid parasitoids (Aphidiidae:
583 Hymenoptera). *J. Plant. Breed. Crop. Sci.* 2, 299-311.

584

585 Reim, C. Teuschl Y., Blanckenhorn, W.U. 2006.. Size-dependent effects of larval and adult
586 food availability on reproductive energy allocation in the yellow dung fly. *Funct. Ecol.* 20,
587 1012-1021.

588

589 Renault, D., Hance, T., Vannier, G., Vernon, P. 2003 Is body size an influential parameter in
590 determining the duration of survival at low temperatures in *Alphitobius diaperinus* Panzer
591 (Coleoptera : Tenebrionidae)? *J. Zool.* 259, 381-388.

592

593 Rinehart, J.P., Yocum, G.D., Denlinger, D.L., 2000. Developmental upregulation of inducible
594 hsp70 transcripts, but not the cognate form, during pupal diapause in the flesh fly, *Sarcophaga*
595 *crassipalpis*. *Insect. Biochem. Mol. Biol.* 30, 515-521.

596

597 Rinehart, J.P., Li, A., Yocum, G.D., Robich, R.M., Hayward, S.A.L., Denlinger, D.L., 2007.
598 Up-regulation of heat shock proteins is essential for cold survival during insect diapause. *P.*
599 *Natl. Acad. Sci. USA.* 104, 1130-11137.

600

601 Ris, N., Allemand, R., Fouillet, P., Fluery, F., 2004. The joint effect of temperature and host
602 species induce complex genotype-by-environment interactions in the larval parasitoid of
603 *Drosophila*, *Leptopilina heterotoma* (Hymenoptera: Figitidae). *Oikos*. 106, 451-456.

604

605 Roux, O., Le Lann, C., van Alphen, J. J. M., van Baaren, J. 2010. How does heat shock affect
606 the life history traits of adults and progeny of the aphid parasitoid *Aphidius avenae*
607 (Hymenoptera: Aphidiidae)? *B. Entomol. Res.* 100, 543-549.

608

609 Russell, J.A., Moran, N.A. 2006. Costs and benefits of symbiont infection in aphids: variation
610 among symbionts and across temperatures. *P. Roy. Soc. Lond. B. Bio.* 273, 603-610.

611

612 Sampaio, M.V., Bueno, V.H.P., De Conti, B.F. 2008. The effect of the quality and size of host
613 aphid species on the biological characteristics of *Aphidius colemani* (Hymenoptera:
614 Braconidae: Aphidiinae). *Eur. J. Entomol.* 105, 489-494.

615

616 Salt, G. 1941. The effects of hosts upon their insect parasites. *Biol. Rev.* 16, 239-264.

617

618 Sequeira, R., Mackauer, M. 1992a. Covariance of adult size and development time in the
619 parasitoid wasp *Aphidius ervi* in relation to the size of its host, *Acyrtosiphon pisum*. *Evol.*
620 *Ecol.* 6, 34-44.

621

622 Sequeira, R., Mackauer, M. 1992b, Nutritional ecology of an insect host parasitoid association
623 - the pea aphid *Aphidius-ervi* system. *Ecology.* 73, 183-189.

624

625 Sidney, L.A., Bueno, V.H.P., Lins, J.C., Silva, D.B., Sampaio, M.V. 2010. Quality of
626 different aphids species as hosts for the parasitoid *Aphidius ervi* Haliday (Hymenoptera:
627 Braconidae: Aphidiinae). Neotrop. Entomol. 39, 709-713.
628

629 Silva, R.J., Bueno, V.H.P., Sampaio, M.V. 2008. Quality of different aphids as hosts of the
630 parasitoid *Lysiphlebus lestaceipes* (Cresson) (Hymenoptera : Braconidae, Aphidiinae).
631 Neotrop. Entomol. 37, 173-179.
632

633 Silva, R.J., Cividanes, F.J., Pedroso, E.C., Sala, S.R.D. 2011. Host quality of different aphid
634 species for rearing *Diaeretiella rapae* (McIntosh) (Hymenoptera: Braconidae). Neotrop.
635 Entomol. 40, 477-482.
636

637 Sørensen, J.G., Loeschcke, V. 2001. Larval crowding in *Drosophila melanogaster* induces
638 Hsp70 expression, and leads to increased adult longevity and adult thermal stress resistance. J.
639 Insect. Physiol. 47, 1301-1307.
640

641 Tomčala, A., Tollarova, M., Overgaard, J., Simek, P., Kostal, V. 2006. Seasonal acquisition
642 of chill tolerance and restructuring of membrane glycerophospholipids in an overwintering
643 insect: triggering by low temperature, desiccation and diapause progression. J. Exp. Biol. 209,
644 4102- 4114.
645

646 Tougeron K., van Baaren J., Burel F., Alford L. 2016. Comparing thermal tolerance across
647 contrasting landscapes: first steps towards understanding how landscape management could
648 modify ectotherm thermal tolerance. Insect. Conserv. Divers. 9, 171-180.
649

650 Tougeron K., Le Lann C., Brodeur J., van Baaren J. 2017. Are aphid parasitoids from mild
651 winter climates losing their winter diapause? *Oecologia*. 183, 619-629.
652

653 Verdu, J.R., Casas, J.L., Lobo, J.M., Numa, C. 2010. Dung beetles eat acorns to increase their
654 ovarian development and thermal tolerance. *PLoS. One*. 5, e10114.
655

656 Wanderley, P.A., Ramalho, F.S., Zanuncio, J.C., Leite, G.L.D. 2007. Thermal requirements
657 and development of *Bracon vulgaris*, a parasitoid of the cotton boll weevil. *Phytoparasitica*.
658 35, 336-345.
659

660 Ward, D., Seely, M.K. 1996. Behavioral thermoregulation of six Namib Desert tenebrionid
661 beetle species (Coleoptera). *Ann. Entomol. Soc. Am.* 89, 442-451.
662

663 Xu, Q.H., Meng, L., Li, B., Mills, N. 2008. Influence of host-size variation on the
664 development of a koinobiont aphid parasitoid, *Lysiphlebus ambiguus* Haliday (Braconidae,
665 Hymenoptera). *B. Entomol. Res.* 98, 389-39
666

667 Zachariassen, K.E., Hammel, H.T. 1976. Nucleating-agents in hemolymph of insects tolerant
668 to freezing. *Nature*. 262, 285-287.
669

670 Zachariassen, K.E., Husby, J.A. 1982. Antifreeze effect of thermal hysteresis agents protects
671 highly supercooled insects. *Nature*. 298, 865-867.
672

673 Zeng, J.P., Ge, F., Su, J.W., Wang, Y. 2008. The effect of temperature on the diapause and
674 cold hardiness of *Dendrolimus tabulaeformis* (Lepidoptera: Lasiocampidae). Eur. J. Entomol.
675 105, 599-606.

676

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681 **Figure 1** Tibia length (mm) of cereal parasitoids *Aphidius avenae* (Aa), *A. matricariae* (Am)
682 and *A. rhopalosiphi* (Ar) emerging from the aphid host species *Metopolophium dirhodum*
683 (Md), *Sitobion avenae* (Sa) and *Rhopalosiphum padi* (Rp). Box plots represent median tibia
684 length and the upper and lower quartiles, and mean tibia length is represented by X.
685 Significant differences are indicated by an asterisk.

686

687 **Figure 2** Tibia length (mm) of male (M) and female (F) cereal parasitoids *Aphidius avenae*
688 (Aa), *A. matricariae* (Am) and *A. rhopalosiphi* (Ar). Box plots represent median tibia length
689 and the upper and lower quartiles, and mean tibia length is represented by X. Significant
690 differences are indicated by an asterisk.

Figure 1

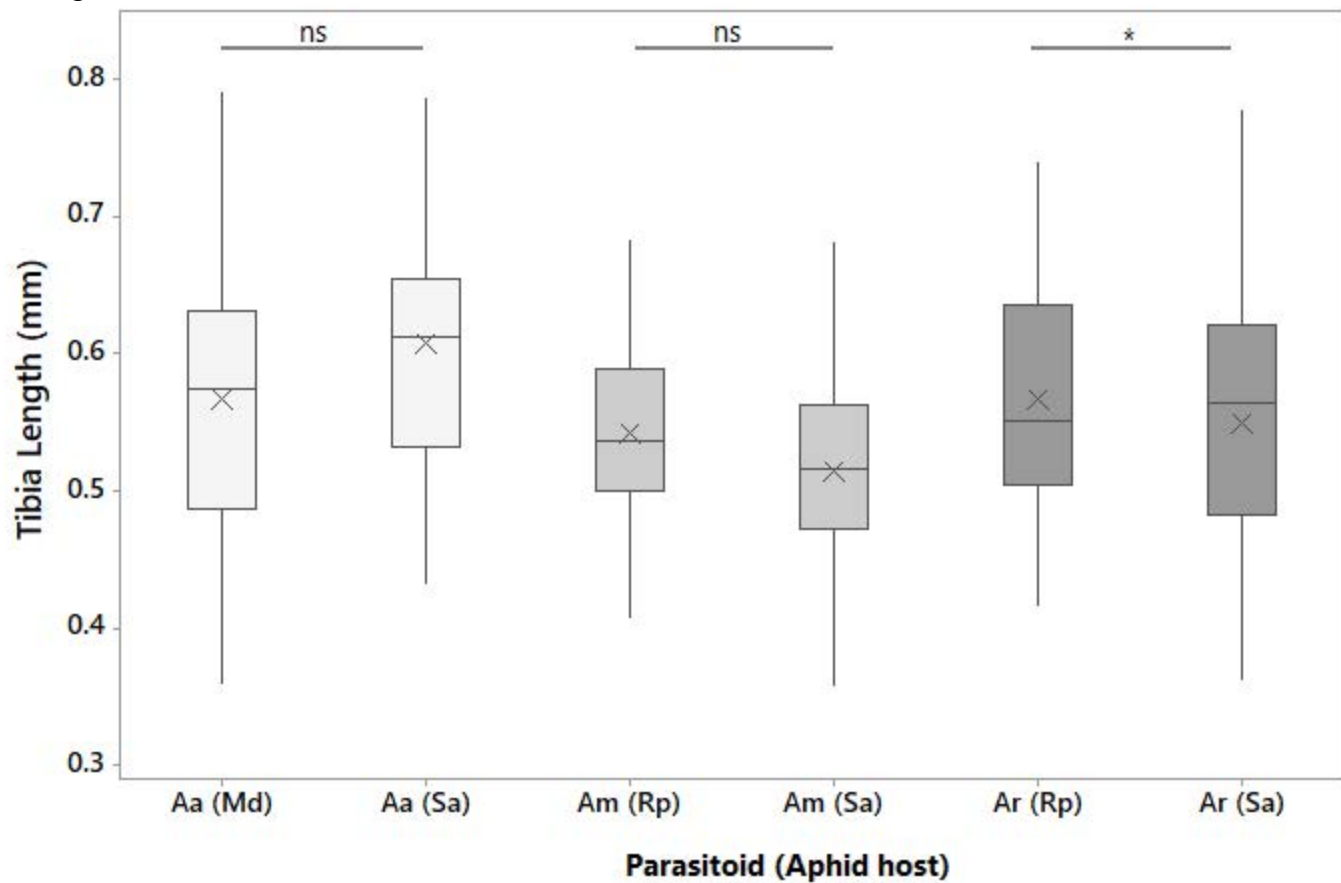


Figure 2

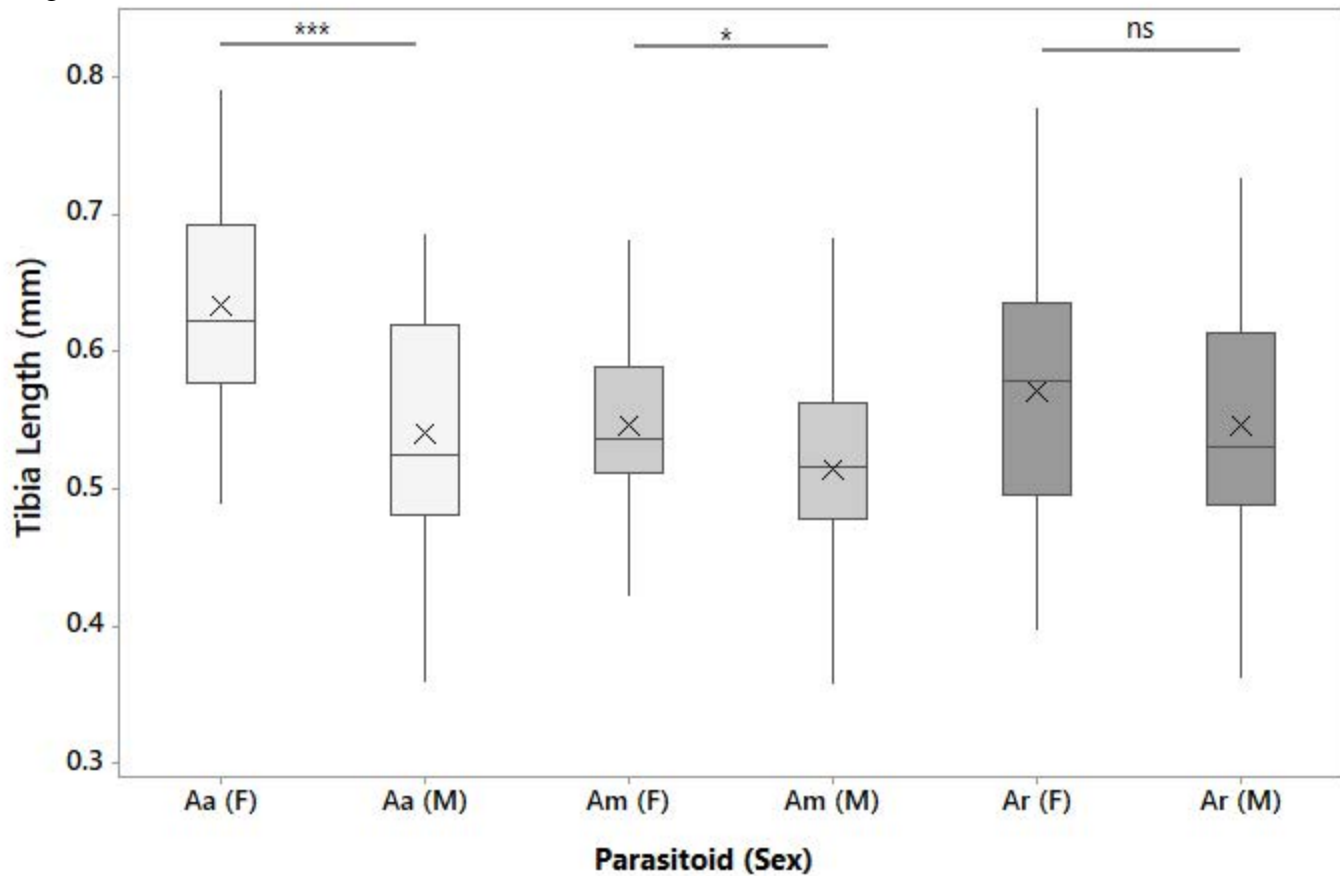


Table 1. Mean \pm SE CT_{min} and Chill Coma temperature ($^{\circ}$ C) for *Aphidius* parasitoid species (*Aphidius avenae*, *Aphidius matricariae* and *Aphidius rhopalosiphi*) emerging from different cereal aphid hosts (*Metopolophium dirhodum*, *Rhopalosiphum padi* and *Sitobion avenae*). Parasitoid and aphid host tibia length (mm) is also provided.

Parasitoid	Aphid host	CT _{min} ($^{\circ}$ C)	<i>n</i>	Chill Coma ($^{\circ}$ C)	<i>n</i>	Aphid Host Tibia (mm)	<i>n</i>	Parasitoid Tibia (mm)	<i>n</i>
<i>A. avenae</i>	<i>M. dirhodum</i>	1.52 \pm 0.15	22	0.93 \pm 0.20	23	1.04 \pm 0.04	22	0.57 \pm 0.02	24
	<i>R. padi</i>	-		-		-		-	
	<i>S. avenae</i>	1.64 \pm 0.08	25	0.83 \pm 0.08	28	0.95 \pm 0.03	27	0.61 \pm 0.01	28
	ANOVA (d.f. = 1)	$F = 0.71, P = 0.407$		$F = 0.06, P = 0.801$				$F = 1.47, P = 0.232$	
<i>A. matricariae</i>	<i>M. dirhodum</i>	-		-		-		-	
	<i>R. padi</i>	1.25 \pm 0.18	18	0.89 \pm 0.11	36	0.69 \pm 0.02	34	0.54 \pm 0.01	38
	<i>S. avenae</i>	1.30 \pm 0.16	26	0.79 \pm 0.14	30	0.88 \pm 0.02	30	0.52 \pm 0.01	33
	ANOVA (d.f. = 1)	$F = 0.92, P = 0.344$		$F = 0.02, P = 0.893$				$F = 1.70, P = 0.198$	
<i>A. rhopalosiphi</i>	<i>M. dirhodum</i>	-		-		-		-	
	<i>R. padi</i>	1.55 \pm 0.11	28	0.96 \pm 0.12	29	0.69 \pm 0.03	27	0.57 \pm 0.01	30
	<i>S. avenae</i>	1.24 \pm 0.12	28	0.76 \pm 0.13	28	1.03 \pm 0.03	28	0.55 \pm 0.02	30
	ANOVA (d.f. = 1)	$F = 1.90, P = 0.162$		$F = 0.81, P = 0.450$				$F = 4.14, P = 0.022$	

Results of repeated-measures ANOVA comparing the cold tolerance and size of parasitoids emerging from different aphid hosts.

-Denotes non-variable parasitoid x host combinations that failed to produce offspring

Table 2. Mean \pm SE CT_{min} and Chill Coma temperature ($^{\circ}$ C) and parasitoid tibia length for male and female parasitoids belonging to the genus *Aphidius*. Data were pooled across aphid host species for each parasitoid species since the effect of aphid host species on the measured parameters proved non-significant.

Parasitoid	Parasitoid sex	CT _{min} ($^{\circ}$ C)	<i>n</i>	Chill Coma ($^{\circ}$ C)	<i>n</i>	Parasitoid Tibia (mm)	<i>n</i>
<i>A. avenae</i>	Male	1.59 \pm 0.11	23	0.93 \pm 0.15	24	0.54 \pm 0.02	25
	Female	1.58 \pm 0.13	24	0.83 \pm 0.13	27	0.63 \pm 0.02	27
	ANOVA (d.f. = 1)	$F = 0.001, P = 0.974$		$F = 1.8, P = 0.670$		$F = 17.02, P < 0.001$	
<i>A. matricariae</i>	Male	1.31 \pm 0.16	24	0.78 \pm 0.13	36	0.52 \pm 0.01	37
	Female	1.24 \pm 0.19	20	0.92 \pm 0.12	30	0.55 \pm 0.01	34
	ANOVA (d.f. = 1)	$F = 0.18, P = 0.678$		$F = 0.25, P = 0.618$		$F = 4.93, P = 0.030$	
<i>A. rhopalosiphi</i>	Male	1.50 \pm 0.11	28	0.85 \pm 0.11	29	0.55 \pm 0.01	30
	Female	1.31 \pm 0.11	30	0.91 \pm 0.14	30	0.57 \pm 0.02	31
	ANOVA (d.f. = 1)	$F = 0.79, P = 0.378$		$F = 0.52, P = 0.476$		$F = 0.35, P = 0.557$	

Results of repeated-measures ANOVA comparing cold tolerance and size of different *Aphidius* parasitoids

Supplementary Table 1 Generalized linear model for thermal tolerance and size traits of *Aphidius* species emerging from different aphid host species.

Variables and factors	<i>A. avenae</i>		<i>A. matricariae</i>		<i>A. rhopalosiphi</i>	
	F value	P	F value	P	F value	P
<i>CTmin</i>						
Aphid tibia	0.96	0.335	1.40	0.246	0.71	0.403
Sex	0.001	0.974	0.18	0.678	0.79	0.378
Host	0.71	0.407	0.92	0.344	1.90	0.162
Aphid tibia x Sex	0.15	0.700	0.60	0.446	0.07	0.796
Aphid tibia x Host	2.23	0.144	1.45	0.237	0.15	0.859
Sex x Host	0.0004	0.983	0.06	0.814	0.15	0.699
Aphid tibia x Sex x Host	0.74	0.395	0.001	0.975	1.04	0.313
<i>Chill Coma</i>						
Aphid tibia	1.93	0.173	0.77	0.392	1.11	0.299
Sex	0.18	0.670	0.25	0.618	0.52	0.476
Host	0.06	0.801	0.02	0.893	0.81	0.450
Aphid tibia x Sex	0.09	0.772	1.04	0.312	0.06	0.808
Aphid tibia x Host	0.88	0.353	0.03	0.866	0.15	0.857
Sex x Host	0.07	0.788	2.86	0.097	0.03	0.874
Aphid tibia x Sex x Host	0.21	0.650	0.07	0.798	0.39	0.537
<i>Parasitoid tibia</i>						
Aphid tibia	0.01	0.924	0.004	0.948	2.39	0.129
Sex	17.02	<0.001	4.93	0.030	0.35	0.557
Host	1.47	0.232	1.70	0.198	4.14	0.022
Aphid tibia x Sex	2.84	0.100	0.02	0.889	0.71	0.403
Aphid tibia x Host	0.26	0.613	0.02	0.883	0.00	0.969
Sex x Host	0.16	0.687	0.28	0.599	0.11	0.741
Aphid tibia x Sex x Host	0.01	0.904	0.03	0.855	0.51	0.481