PHYSIOLOGICAL ECOLOGY – ORIGINAL RESEARCH

Measuring the evolutionary potential of a winter-active parasitic wasp to climate change

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Abstract

In temperate climates, as a consequence of warming winters, an increasing number of ectothermic species are remaining active throughout winter months instead of diapausing, rendering them increasingly vulnerable to unpredictable cold events. One species displaying a shift in overwintering strategy is the parasitoid wasp and biological control agent *Aphidius avenae*. The current study aimed to better understand the consequence of a changing overwintering strategy on the evolutionary potential of an insect population to adapt to the cold stress events, set to increase in frequency, even during milder winters. Using a parental half-sibling breeding design, narrow-sense heritability of the cold tolerance, morphology and longevity of *A. avenae* was estimated. The heritability of cold tolerance was estimated at 0.07 ($CI_{95\%} = [0.00; 0.25]$) for the Critical Thermal Minima (CT_{min}) and 0.11 ($CI_{95\%} = [0.00; 0.34]$) for chill coma temperature; estimates much lower than those obtained for morphological traits (tibia length 0.20 ($CI_{95\%} = [0.03; 0.37]$); head width 0.23 ($CI_{95\%} = [0.09; 0.39]$); wing surface area 0.28 ($CI_{95\%} = [0.11; 0.47]$)), although comparable with the heritability estimate of 0.12 obtained for longevity ($CI_{95\%} = [0.00; 0.25]$). The heritability estimates obtained thus suggest that *A. avenae* possesses low adaptive potential against cold stress. If such estimates are indicative of the evolutionary potential of *A. avenae* cold tolerance, more emphasis may be placed on adaptive phenotypic plasticity at the individual level to persist in a changing climate, with potential implications for the biological control function they provide.

Keywords Cereal aphids · Heritability · Parasitoid wasp · Thermotolerance · Biological control

Introduction

Climate change exposes species to a range of new selection pressures. In addition to warming temperatures, climate change will bring about an increased incidence of extreme cold and heat events (Diffenbaugh and Field 2013; Easterling et al. 2000; IPCC 2013; Kunkel et al. 1999). Insects, in particular, may be especially vulnerable to such modification of their thermal environment; as ectotherms, they possess a limited ability to regulate body temperature above and below ambient temperature (Bale and Hayward 2010). In temperate climates, winter represents a challenging season where many insects rely on diapause to persist in a state of enhanced cold tolerance (Bale and Hayward 2010; Denlinger 2002). However, with diapause induction under the control of temperature and photoperiodic cues, warming autumn and winter temperatures have profound consequences for diapause induction and success of insect overwintering. Indeed, warmer temperatures during the photoperiodic induction phase of diapause will reduce the incidence and duration of diapause, leading to an increase in winter activity (Bale and Hayward 2010; Stuhldreher et al. 2014). This increased winter activity during warming winters may bring about associated benefits including increased winter survival, earlier reproductive output and population growth, as well as enabling more generations per year (Bentz and Powell 2014). However, the enhanced activity will have costly implications for insect metabolism

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and energy consumption. Higher mean temperatures occurring during winters will raise the metabolic rate of insects and other ectotherms, increasing the use of stored energy reserves and potentially exposing species to energetic stress (Williams et al. 2015). As such, any apparent gain of enhanced winter activity may be counterbalanced by the costs of expression at the level of the individual and the population.

As temperate insects become increasingly winter active, they will also be subjected to new thermal stressors. Indeed, they will become increasingly exposed and vulnerable to unpredictable and extreme cold events associated with climate change, enhancing winter mortality should stressful conditions become more severe later in the season (Bale and Hayward 2010). When faced with such a strong environmental stress, natural populations may respond via adaptive phenotypic plasticity and genetic adaptation. The former, phenotypic plasticity, represents short-term changes to the physiology and/or behaviour of an individual, enabling a rapid response to temperature change through thermal tolerance (e.g. May 1979; Overgaard and MacMillan 2017). These changes may include physiological alterations to homeostatic balance, lipid membrane composition, cryoprotective osmolytes and heat shock protein expression (Overgaard and MacMillan 2017). It may also involve behavioural changes including alterations to the choice of habitat, foraging behaviour and level of social complexity to name a few (e.g. Alford et al. 2017; Andrew et al. 2013; Schurch et al. 2016). At a larger temporal scale, populations may also respond to cold events associated with climate change via genetic adaptation (Franks and Hoffmann 2012; Gerken et al. 2015). Here, natural selection may act on particular traits, leading to micro-evolutionary changes if the trait in question is heritable (Roff 2003). Indeed, a number of functional genes have been linked to variation in thermal tolerance (Frydenberg et al. 2003; de Jong et al. 2013; de Jong and Saastamoinen 2018; Morgan and Mackay 2006; Rolandi et al. 2018; Sørensen et al. 2003), offering mechanisms by which climate change may drive the evolution of thermal tolerance through shifts in allele frequencies (Franks and Hoffmann 2012).

Although much research has focused on the plasticity of thermal tolerance with a view of understanding how insects may respond to climate change (e.g. Gunderson and Stillman 2015; Sgrò et al. 2016), the genetic adaptation of thermal tolerance has received comparatively less research attention (cold adaptation: e.g. Gerken et al. 2015; Ma et al. 2014; Zhoa et al. 2018; heat adaptation e.g. Gilchrist and Huey 1999; Ma et al. 2014). It is, however, a key issue to quantify the ability of populations to evolve traits linked to the individual's ability to avoid winter cold stress under warming conditions, even if these traits are not expressed or triggered during milder winters.

One group of temperate insects experiencing increased winter activity is the parasitoid wasps of the genus *Aphidius* (Hymenoptera: Braconidae: Aphidiinae). Common throughout temperate Europe, *Aphidius* wasps, as all Hymenoptera, display a haplodiploid sex-determination system, whereby individual wasps are male when hemizygous at sex-determining loci, or female when homozygous (Salin et al. 2004). Females lay their eggs within aphid hosts, with the parasitoid young killing the aphid as it grows and matures (Godfray 1994). For this reason, *Aphidius* wasps play an integral role in the natural biological control of many aphid pest species including important pests of cereal crops such as the English grain aphid *Sitobion avenae* (Fabricius), the rose grain aphid *Metopolophium dirhodum* (Walker) and the bird cherry-oat aphid *Rhopalosiphum padi* (Linneaus). Following emergence from the aphid host, the adult wasp of the new generation is free to move throughout the environment in search of a mate, with the mated females then seeking out new aphid hosts to complete the lifecycle.

Most species of *Aphidius* wasps overwinter in a state of diapause as a final-stage larva inside the aphid host with photoperiod and temperature acting as important cues in diapause induction (Christiansen-Weniger and Hardie 1997, 1999). However, in areas with mild winters such as in the Brittany region of western France, parasitoid species from the *Aphidius* genus are remaining reproductively active during milder winters, along with their anholocyclic aphid hosts. Indeed, over the past four decades, field monitoring of *Aphidius* parasitoids in this region has indicated that *Aphidius* parasitoids are becoming increasingly present during winter months (Andrade et al. 2015), with positive impacts on the biological control function they perform (Damien et al. 2017). This has led to the suggestion that *Aphidius* parasitoids are shifting their overwintering strategy in response to warming winters (Andrade et al. 2016), with *A. avenae* and *A. ervi* showing a marked reduction in diapause induction, and *A. rhopalosiphi* and *A. matricariae* appearing to have lost the capacity to enter diapause entirely (Tougeron et al. 2017). Tougeron et al. (2017) report an increase of approximately 1.4 °C in maximum winter temperatures since 1976, hypothesizing that this has led to a decrease in the selection pressures maintaining diapause in *Aphidius* wasps.

Employing *A. avenae* as a study insect, the current study aimed to investigate the consequences of increased winter activity on the evolutionary potential of an insect population to resist winter cold stress. Narrow-sense heritability (h^2), that is, the proportion of trait variance that is due to additive genetic factors, was estimated utilizing a parent half-sibling breeding design for traits associated with cold tolerance. Indeed, when subjected to increasingly low temperatures, a series of behavioural and physiological responses occur before a point of temperature-induced lethality is reached (Hazell and Bale 2011). Many temperate insects are deemed chill-susceptible,

incurring chill-related injuries at temperatures far above the point of freezing (Bale 1996). Furthermore, insect fitness is detrimentally impacted as soon as temperature impedes movement, rendering the insect unable to escape unfavourable thermal conditions, predation or move in search of food or a mate (Harrington and Taylor 1990; Hughes et al. 2010). It is thus argued that non-lethal behavioural thresholds provide greater ecological relevance in the study of insect cold tolerance (Overgaard and MacMillan 2017). In the present study, we utilize behavioural thresholds of the chill-coma spectrum as defined by Hazell and Bale (2011), namely (1) the temperature at which motor coordination is lost; a point commonly referred to as the critical thermal minima (CT_{min}), and (2) the temperature at which a temperature-induced coma is reached. Commonly referred to as the chill coma temperature, it is defined by the absence of electrophysiological activity and movement (Hazell and Bale 2011). Numerous inter-related factors have been shown to impact the thermal tolerance of *Aphidius* wasps including aphid host (Andrade et al. 2013; Eoche-Bosy et al. 2016), sex (Roux et al. 2010; Le Lann et al. 2011; Tougeron et al. 2016) and size (Le Lann et al. 2011; Ismail et al. 2012). For this reason, the heritability of cold tolerance was evaluated depending on factors such as aphid host species, parasitoid sex and size. Based on estimations of the heritability of cold tolerance, we discuss the implications of winter activity on the vulnerability of *A. avenae* to winter cold stress.

Materials and methods

Parasitoid collection and rearing

In Western France, the cereal aphids *S. avenae* and *M. dirhodum* represent the main host species for *A. avenae*. A third species, *R. padi* is also present, although is not a viable host for *A. avenae*. Aphid mummies of *S. avenae* and *M. dirhodum* were collected in wheat fields near Rennes (Brittany, France) in the spring of 2013 and 2014. More than 500 mummies were collected and isolated individually in gelatine capsules. Mummies were maintained in the laboratory at 20 ± 1 °C until parasitoid emergence. All parasitoids, including subsequent generations, were maintained at 20 ± 1 °C and L16:D8 on individuals of the aphid *S. avenae* (clonal type SA1), unless otherwise stated, and fed on a solution of honey and water.

Obtaining parasitoids for experimentation using a half-sib design

First (field) generation

Following the emergence of individuals collected from the field (first generation), one virgin female (1–2d old) was placed in a vial with 5 males (1–2d old) to allow for mating. Parasitoids were observed continuously until mating occurred. Only females for which mating was detected were selected. Once mated, the female was removed, fed on honey, and allowed to oviposit in individuals of the aphid *S. avenae* (clonal type SA1). For this, microcages were created comprising winter wheat (*Triticum aestivum*) grown in vermiculite. Approximately 30–40 *S. avenae* nymphs (L3 and L4 instars) were placed within each microcage and a single mated parasitoid female allowed to oviposit for 24 h. Following oviposition, the female parasitoid was removed and the parasitized aphids left to develop into mummies within the microcages at 20 ± 1 °C and L16:D8. Resultant mummies were subsequently isolated in gelatine capsules to ensure virgin adults of the second generation.

All-female parasitoids were frozen after oviposition to enable identification to species level. Identification was performed post-mating since techniques used for the temporary immobilisation of parasitoids (exposure to CO_2 and sub-zero temperatures) can detrimentally impact parasitoid mating (P. Louâpre, personal observation). Only the offspring of *A. avenae* were used for subsequent experimentation (microcages containing aphid mummies of parasitoids found not to be *A. avenae* were discarded).

Second and third generation

Following the emergence of the second generation of *A. avenae*, one virgin male was sequentially mated with 4 non-sister virgin females in vials. Once mated, each female was placed in a microcage with 20 *S. avenae* (clonal type SA1) and 20 *Metopolophium dirhodum* aphids and allowed to oviposit. Resultant mummies were isolated in gelatine capsules until the emergence of the third generation of parasitoids, which were used in all subsequent experiments.

For each emerged parasitoid of the third generation, cold tolerance (CT_{min} and chill coma), longevity, tibia length, wing surface and head width were measured as detailed below. A total of 633 parasitoids were measured, originating from 23 sires which mated between 1 and 4 females. Among them, 19 sires had mated with at least 2 females.

Determination of cold tolerance (CT_{min} and Chill Coma)

The cold tolerance of all third-generation parasitoids was determined via measurement of the Critical Thermal Minima (CT_{min}) and the temperature of chill coma (Hazell and Bale 2011). Measurements were made using a glass column, as described by Powell and Bale (2006); a modified design from apparatus previously used by Weber and Diggins (1990) and Huey et al. (1992). The glass column (35×5 cm), similar in design to a condenser column, was connected to a programmable alcohol bath (Haake F3,

Thermo Electron Corp., Karlsruhe, Baden-Württemberg, Germany), enabling the circulation of alcohol fluid around the outer chamber and thus fine control over the air temperature experienced within the inner column. Previous work has shown that air temperature is consistent along the length of the column (Powell and Bale 2006). In addition, due to the relatively small body size of the test insects, the air temperature of the column approximates the body temperature of the insects (Huey et al. 1992). Consequently, a single thermocouple was placed against the glass surface of the inner column to monitor air temperature during experimentation.

In all experiments, a single parasitoid wasp (of known parentage) was inserted into the bottom of the column preset to the culture temperature of 20 °C. The column was subsequently closed with a sponge stopper to reduce air flow and maintain a stable thermal environment within the inner column. Following a 5 min acclimatization period, the programmable alcohol bath was set to decrease the temperature of the column from 20 °C to − 10 °C at a rate of 0.75 °C min^{-1}. The rate of 0.75 °C min^{-1} was chosen since it is fast enough not to induce a rapid cold hardening response in the test insects (Powell and Bale 2004, 2005), whilst being slow enough to not shock the insects. Furthermore, the use of faster cooling rates is recommended by some authors when measuring the evolutionary potential of thermal tolerance (Rezende et al. 2011).

During the cooling regime, the CT_{min} and the chill coma temperature were determined for each parasitoid. The CT_{min} represents the temperature at which the parasitoid is no longer able to walk in a coordinated manner and corresponds to the loss of coordinated muscle function. The chill coma temperature corresponds to the point of paralysis and was determined as the temperature at which the parasitoid wasp was unable to cling to the vertical column and subsequently fell to the bottom of the column (Le Lann et al. 2011). The temperatures of CT_{min} and chill coma were recorded manually to an accuracy of 0.1 °C. Following recovery from chill coma, parasitoids were retained for the determination of longevity and morphological measurements.

Longevity and morphological measurements

Parasitoids were checked twice daily until death and longevity (d) was determined. Following death, tibia length, wing surface area and head width were measured for all third-generation parasitoids using a camera (Zeiss AxioCam ERc5s® HD) mounted on an × 9 binocular microscope. Photographs were taken using Intelcam software and morphological measurements made using the image processing software Image J® software (v. 1.48).

Statistical analyses

The explanatory effects of parasitoid sex and aphid host species on the measured traits (tibia length, head width, wing surface, longevity, CT_{min}, chill coma) were determined. For this, linear mixed-effect models (lmer package of R) were built with the identity of offspring parents included as a random variable, and sex and aphid host species included as fixed explanatory variables. The experimenters having performed the measures were also included as a fixed factor to control for the possibility that the samples experienced different and processing environmental conditions across the two sampling years, obscuring the true phenotypic variance. This was not done for CT_{min} and chill coma as these measures were performed by one operator in a single sampling year.

Narrow-sense heritability estimates of the measured traits were performed using an animal model implemented in MCMCglmm R package (R Development Core Team, 2013). The model takes into account complex pedigrees in a mixed model and computes Bayesian inference from a Markov Chain Monte Carlo (MCMC) algorithm. Due to the haplodiploid sex-determination of *A. avenae*, only females were used in the heritability estimations. Three random effects and one fixed effect were included in the model. Experimenter was included as a fixed effect. Cold tolerance data were obtained by only one person and thus this fixed effect was not incorporated into the model for cold tolerance. The random-effects comprise: an additive effect (based on the kinship coefficient between individuals), a dominance effect (based on the probability that two individuals share the two same alleles, identical by descent, at a given locus) and a residual effect comprising all other non-additive effects. The kinship and dominance matrices were estimated using nadiv R package. Since *A. avenae* is a haplodiploid species, genetic components are transmitted in a similar way as genes carried by sex chromosomes are transmitted in a diploid species. We, therefore, indicated to the nadiv R package that our data were from sex chromosomes in a species where males are heterogametic. The heritability was calculated as the ratio between additive variance estimate and the sum of all variances estimates (additive, dominance, residual and experimenter). The analysis was performed considering progeny from a grandmother as full sister-brothers. All traits were modelled as Gaussian except longevity which was modelled as an exponential trait.

Results

Phenotypic variance analysis

There was no significant effect of aphid host species on morphological traits of *A. avenae* (Fig. 1) either for tibia

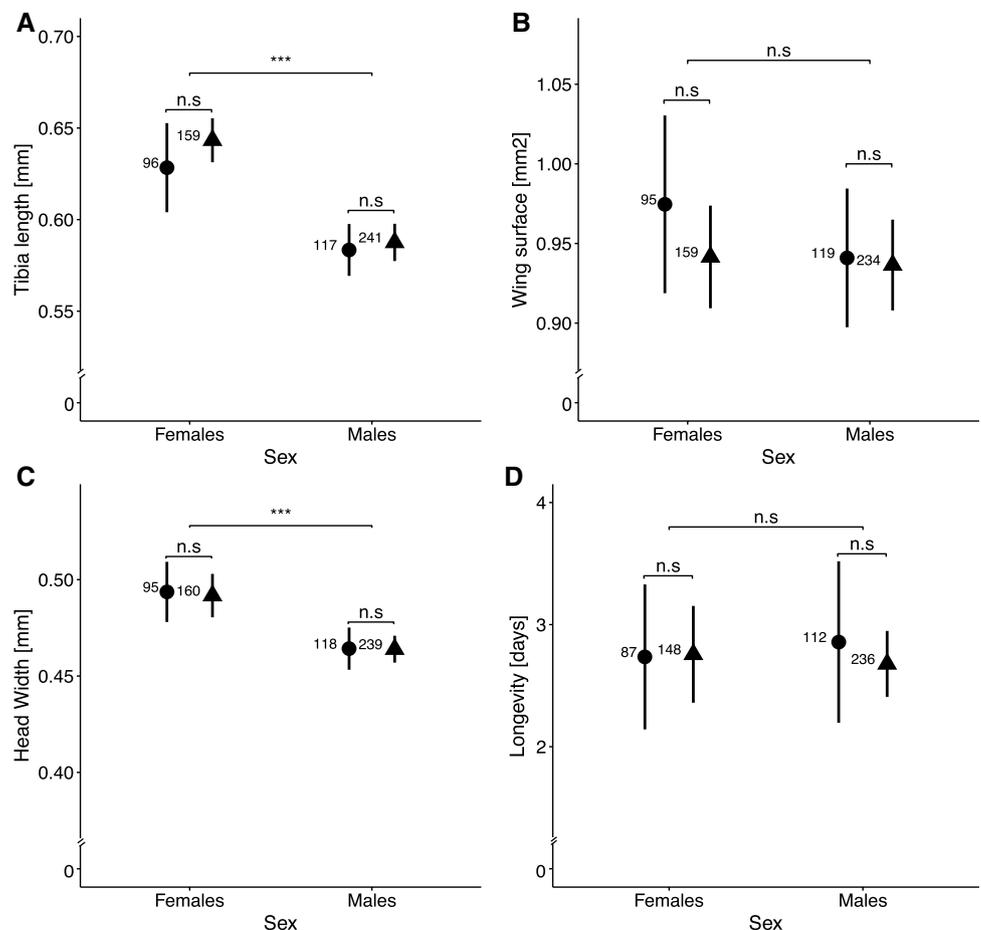**Table 1** Mean ± SE of measured traits for males and females of *Aphidius avenae*

	Male mean + SE	n	Female mean + SE	n
CT_{min} (°C)	1.77 ± 0.045	150	1.63 ± 0.049	108
Chill coma (°C)	1.18 ± 0.047	168	1.15 ± 0.042	140
Tibia length (mm)	0.59 ± 0.004	358	0.64 ± 0.006	255
Head width (mm)	0.46 ± 0.003	357	0.49 ± 0.005	255
Wing surface area (mm^2)	0.94 ± 0.012	353	0.95 ± 0.015	254
Longevity (d)	2.74 ± 0.142	348	2.75 ± 0.168	234

Due to the lack of a significant effect of aphid host species on the measured traits, data from wasps emerging from *Sitobion avenae* and *Metopolophium dirhodum* have been combined

length ($\chi^2_1 = 2.146$, $p = 0.143$), wing surface ($\chi^2_1 = 0.481$, $p = 0.488$), head width ($\chi^2_1 = 0.063$, $p = 0.802$), or longevity ($\chi^2_1 = 0.174$, $p = 0.676$), as well as on traits associated with thermal tolerance (Fig. 2), either for CT_{min} ($\chi^2_1 = 0.158$, $p = 0.691$) and chill coma temperature ($\chi^2_1 = 0.0001$, $p = 0.976$). The sex of the emergent individual influenced the tibia length ($\chi^2_1 = 47.092$, $p < 0.001$), head width ($\chi^2_1 = 23.336$, $p < 0.001$) and CT_{min} ($\chi^2_1 = 4.611$, $p = 0.032$): In comparison to males,

females have longer tibias (resp. 0.637 mm $CI_{95\%} = [0.625; 0.649]$ and 0.586 mm $CI_{95\%} = [0.574; 0.598]$), wider heads (resp. 0.492 mm $CI_{95\%} = [0.483; 0.502]$ and 0.464 mm $CI_{95\%} = [0.458; 0.469]$) and a lower temperature of CT_{min} (resp. 1.629 °C $CI_{95\%} = [1.533; 1.725]$ and 1.774 °C $CI_{95\%} = [1.686; 1.862]$) (Figs. 1 and 2). However, sex had no influence on wing surface area ($\chi^2_1 = 0.032$, $p = 0.859$), longevity ($\chi^2_1 = 0.123$, $p = 0.726$), and chill coma temperature ($\chi^2_1 = 0.011$, $p = 0.915$) (Table 1).

Fig. 1 Mean ± 95% confidence interval of morphological traits of *Aphidius avenae* depending on the sex and the aphid host species from which they emerged (Filled circle: *Metopolophium dirhodum*, Filled triangle: *Sitobion avenae*): **a** Tibia length, **b** Wing surface area, **c** Head width, **d** Longevity. Asterisks highlight significant differences (***P < 0.001, *n.s.* non-significant) and numbers refer to sample sizes
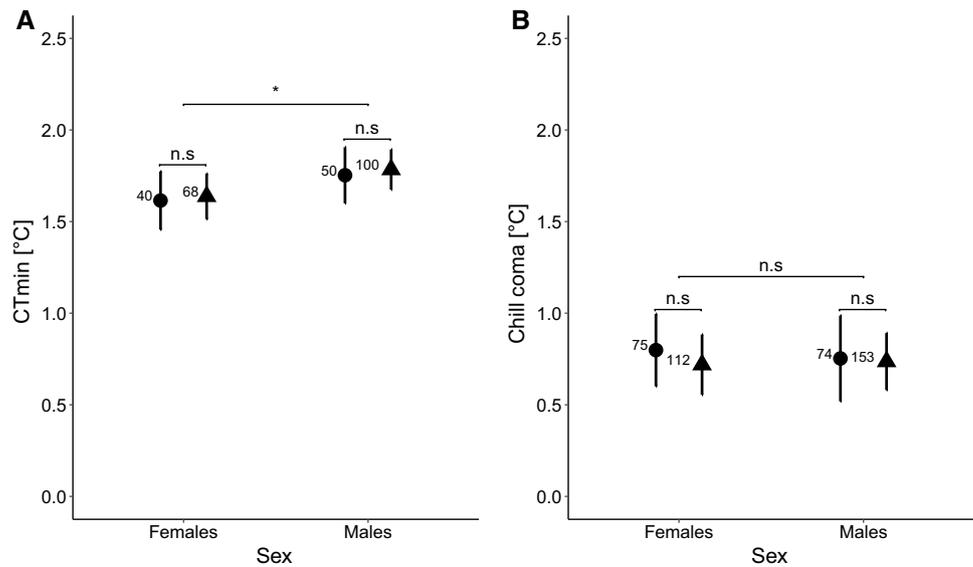
Heritability of thermal tolerance and morphological traits

Narrow-sense heritability estimates of *A. avenae* cold tolerance based on a parental half-sibling breeding design were calculated at 0.07 ($CI_{95\%} = [0.00; 0.25]$) and 0.11 ($CI_{95\%} = [0.00; 0.34]$) for CT_{min} and chill coma temperature, respectively (Table 2). A comparatively low narrow-sense heritability estimate was likewise obtained for parasitoid longevity (0.12 $CI_{95\%} = [0.00; 0.25]$). In contrast, comparatively higher values of narrow-sense heritability were obtained for morphological traits including 0.20 ($CI_{95\%} = [0.03; 0.37]$) for tibia length, 0.23 ($CI_{95\%} = [0.09; 0.39]$) for head width and 0.28 ($CI_{95\%} = [0.11; 0.47]$) for wing surface area (Table 2).

Discussion

Over the last four decades, parasitoid wasps of the genus *Aphidius* have displayed a shift in overwintering strategy. Believed to be the consequence of climate change and warming winters, these wasps are becoming increasingly active during winter months (Andrade et al. 2016; Tougeron et al.

2017). With increased winter activity rendering *Aphidius* wasps increasingly susceptible to unpredictable cold spells, the current study set out to (1) measure the cold tolerance of *A. avenae* via calculation of the CT_{min} and chill coma temperature in relation to factors known to influence thermal tolerance, and (2) quantify the evolutionary potential of *A. avenae* cold tolerance. Inter-sex differences in CT_{min} were revealed, with females being more cold tolerant than males. Furthermore, low narrow-sense heritability estimates were obtained for cold tolerance compared to estimates obtained for morphological measures. The discussion is divided into two sections: The first section details *A. avenae* cold tolerance, the factors affecting cold tolerance, and places the results within the context of climatic data for the study region. The second section explores the heritability of cold tolerance in *A. avenae* and the potential for the species to respond to climate change at the population level via evolutionary adaptation.

A. avenae cold tolerance

Among traits related to thermal tolerance, the Critical Thermal minima (CT_{min}) differed between female and male *A.*

Table 2 Mean narrow-sense heritability (h^2) estimates of cold tolerance, morphology and longevity of the parasitoid *Aphidius avenae* based on a parental half-sibling breeding design

	Mean	Median	Lower	Upper	n	Prior	Model
CT_{min} (°C)	0.07	0.04	0.00	0.25	258	Parameters expanded	Gaussian
Chill coma (°C)	0.11	0.07	0.00	0.34	308	Parameters expanded	Gaussian
Tibia length (mm)	0.20	0.19	0.03	0.37	613	Parameters expanded	Gaussian
Head width (mm)	0.23	0.23	0.09	0.39	612	Parameters expanded	Gaussian
Wing surface area (mm^2)	0.28	0.27	0.11	0.47	607	Parameters expanded	Gaussian
Longevity (*d*)	0.12	0.12	0.00	0.25	582	Parameters expanded	Poisson

Lower and upper boundaries of the 95% confidence interval are provided

avenae (1.629 and 1.774 °C, respectively). The temperature of chill coma did not differ between the two sexes and was determined as 0.746 °C, whatever host they emerged from. In a study conducted in the winter of 2013/14, meteorological data were recorded in cereal fields of Brittany, France, revealing the mean temperature for the season to be 7.43 °C ± 0.06, with minimum and maximum temperatures of − 2.08 °C and 24.90 °C, respectively (Alford et al. 2018). Temperatures below the CT_{min} and chill coma temperatures of *A. avenae* may thus be experienced during winter, indicating that winter temperature is likely a selection pressure acting on *A. avenae* in Western France.

It is worth noting that the cooling rate employed in the current study, and indeed those employed in the majority of laboratory-based thermal tolerance estimates, are faster than rates of cooling that would occur in nature (Sinclair 2003; Terblanche et al. 2011). Studies into the effects of ramping rates on critical thermal limits have found that slower rates of temperature change resulted in reduced thermal tolerance estimates in the tsetse fly, *Glossina pallidipes* (Terblanche et al. 2007) and the Argentine ant, *Linepithema humile* (Chown et al. 2009), although the reverse was true for *Drosophila melanogaster* with a small positive relationship reported between cooling rate and thermal tolerance (Chown et al. 2009). Terblanche and colleagues concluded that thermal tolerance ranges of wild populations may actually be narrower than those estimated under laboratory conditions (Terblanche et al. 2007). Consequently, the thermal tolerance indices calculated in the current study may thus overestimate the true thermal tolerance of *A. avenae* under natural conditions. As such, winter temperatures may impose a greater selection pressure than implied by the current study.

The current study further investigated effects of aphid host and parasitoid sex on the cold tolerance of *A. avenae*, finding no effect of aphid host on the cold tolerance of the emerging parasitoid, but a significant effect of parasitoid sex. Here, females were found to be more cold tolerant than males, displaying a CT_{min} of approximately 0.1 °C lower. It is commonly accepted that *Aphidius* wasps display sexual dimorphism, with the females being larger in size than the males (Roux et al. 2010; Le Lann et al. 2011; Tougeron et al. 2016). One hypothesis in support of the increased cold tolerance exhibited by the larger females is the relative efficiency hypothesis (RE) which predicts that larger-bodied individuals are more efficient at utilising reserves, conferring an advantage under stressful conditions (Cushman et al. 1993; Arnett and Gotelli 2003; Blanckenhorn et al. 2007). Although an increased female cold tolerance in a winter-active insect would be beneficial at the population level, increasing the likelihood of female winter survival and aiding the persistence of the population, whether the observed difference of 0.1 ºC would impact the ecology of the species is debatable.

The heritability of *A. avenae* cold tolerance

Narrow-sense heritability (h^2) estimates of *A. avenae* cold tolerance were calculated as 0.07 and 0.11 for CT_{min} and chill coma, respectively; estimates far lower than those obtained for morphological measures in the current study. Whilst studies into the genetic variance of insect thermal tolerance have received considerably less research attention than the phenotypic plasticity of thermal tolerance, a number of studies have addressed this deficit although with a primary focus on the genetic variance of heat tolerance (e.g. Huey et al. 1992; McColl et al. 1996; Mitchell and Hoffmann 2010; Rolandi et al. 2018). Few studies have examined the genetic variance of cold tolerance (e.g. Chown et al. 2009; Kellermann et al. 2009; Ma et al. 2014; Zhoa et al. 2018), although such studies are integral if we are to better understand how insects may respond to unpredictable cold stress events associated with climate change. For the few studies that have calculated narrow-sense heritability of insect cold tolerance, heritability estimates were greater than those obtained in the current study. For the silverleaf whitefly *Bemisia tabaci*, h^2 of chill coma recovery time was estimated in the region of 0.33–0.36 (Ma et al. 2014). Likewise, h^2 of chill coma recovery time for the ragweed leaf beetle *Ophraella communa* was estimated in the region of 0.39–0.53 (Zhao et al. 2018). In both studies, the index of cold tolerance under investigation was chill coma recovery time, defined in Andersen et al. 2015 as the time taken for an insect to spontaneously recover coordinated neuromuscular function following removal of a cold stress. In contrast, the current study investigated two indices of the chill coma spectrum: the temperature at which motor coordination is lost (CT_{min}) and the temperature at which electrophysiological activity and movement ceases (chill coma) (Hazell and Bale 2011). In their study into the heritability of *O. communa* cold tolerance, Zhao et al. further studied h^2 of the super-cooling point (h^2 0.14–0.17) and glycerol content (h^2 0.05–0.07), obtaining heritability estimates far lower than those obtained for chill coma recovery (Zhao et al. 2018). As such, the heritability of insect cold tolerance may be highly dependent on the chosen index of study. Interestingly, following a comprehensive investigation into the relative merits of commonly employed indices of insect cold tolerance, Andersen and colleagues deduced that the temperature of chill coma and the lethal temperature provide the most ecologically relevant estimates of cold tolerance in drosophilid species (Andersen et al. 2015). If correct for other insect species, heritability estimates of these indices may thus offer more ecologically relevant estimates of the evolutionary potential of insect cold tolerance in response to climate change.

Given adequate genetic variability of thermal tolerance, species will be able to respond to climate change

via evolutionary adjustments. Based on the results of this study, a small proportion of the total phenotypic variation in the cold tolerance of *A. avenae* was caused by additive genetic variation. Whether such low-level genetic variation will be sufficient to enable *A. avenae* to respond to a changing climate at the population level is unknown, although may place increasing emphasis on adaptive phenotypic plasticity (physiological and behavioural) at the individual level. However, a recent study by Gunderson and Stillman (2015) has suggested that ectotherms may possess a limited potential for physiological plasticity to buffer extreme temperatures. Through employing a broad-scale analysis of thermal tolerance plasticity across terrestrial and aquatic ectotherms, authors revealed a low overall plasticity, with terrestrial taxa, including the insects, possessing half the plasticity of aquatic taxa (Gunderson and Stillman 2015). Interestingly, greater physiological plasticity was exhibited in environments where the potential for behavioural thermoregulation was low, leading authors to conclude that a reduced potential for behavioural plasticity may favour the evolution of greater plasticity in physiological traits (Gunderson and Stillman 2015). Whilst a trade-off in behavioural and physiological plasticity may exist, warming winters in temperate climates may hinder seasonal acclimatory responses and undermine potential physiological changes (Bale and Hayward 2010). This could render insects susceptible to unpredictable cold events and place further emphasis on behavioural mechanisms to buffer extreme temperatures.

As a final note, parasitoid wasps of the genus *Aphidius* are third trophic level organisms, dependent on their aphid host for lifecycle completion. As such, any differential effect of climate change to the phenology, distribution, behaviour, and relative abundance of the parasitoid and its host may disrupt this finely-tuned interaction. Multi-species experiments are thus integral to enhancing our understanding of how climate change will impact population dynamics and community interactions within ecological systems (Paull and Johnson 2014).

Compliance with ethical standards

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