The aphid *Acyrthosiphon pisum* exhibits a greater survival after a heat shock when parasitized by the wasp *Aphidius ervi*

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ABSTRACT

The pea aphid *Acyrthosiphon pisum* is a common pest of many species of legumes and its parasitoid *Aphidius ervi* is regarded as a successful biocontrol agent. In this study, we report a greater survival rate of parasitized aphids compared with unparasitized ones, after exposure to a very high temperature (39 °C for 30 min). After the heat shock, the survival of unparasitized aphids decreases according to their age at the heat shock treatment, suggesting a different adaptation of the aphid life stage to the different microclimatic conditions they experience. Survival of parasitized aphids does not change according to the time of the heat shock treatment, but it is always significantly higher compared with the unparasitized ones. Parasitized aphids are very quickly subjected to a wide range of physiological modifications and the observed increased survival could be a consequence of these modifications before the heat shock treatment. The possible explanations as well as the possible adaptive nature of the observed phenomenon are discussed.

1. Introduction

An increase in the frequency of extreme thermal events is expected as a result of global warming (Ballester et al., 2009; Diffenbaugh and Field, 2013; Kunkel et al., 1999; Marengo et al., 2009; Meehl et al., 2000; Miyan, 2015; Vose et al., 2005). The extreme thermal events, together with the mean global warming, may produce important effects on biodiversity since they affect species distributions, life histories, community composition, ecosystem function and biotic interactions (Bale et al., 2002; Bozinovic et al., 2016a; Estay et al., 2014; Folguera et al., 2009; Hoffmann and Parsons, 1991; Johnston and Bennett, 2008; Miyan, 2015). Predictions on the ability of an organism to respond to climate change and to extreme environmental conditions are considered important topics (Gilchrist et al., 2004; Gunderson and Stillman, 2015; Huey et al., 2012; Pörtner et al., 2006).

The development and survival of ectotherms are linked to environmental temperatures and the higher frequencies of extreme conditions constitute a challenge for these organisms (Bozinovic et al., 2016b, 2013; Folguera et al., 2009). The development under sub-optimal conditions and/or the exposure to short severe stress negatively affect the life history traits of insects (Cui et al., 2008; Hance et al., 2007; Hoffmann and Hewa-Kapuge, 2000; Huey and Berrigan, 2001; Jeffs and Leather, 2014; Loeschcke and Hoffmann, 2007; Roux et al., 2010; Trotta et al., 2006). Ideally, resistance measures should be linked

to the kind of stress, defined as a condition that decreases fitness (Hoffmann and Parsons, 1991), the organisms will experience in the field. For some studies, measuring this can be important but difficult when there is incomplete information about the stress levels experienced by different species interacting in nature, including occasional stress exposures. Some laboratory procedures, like exposure to different rearing temperatures or to different heat stresses, can reflect measures of the stress experienced in nature (Hoffmann et al., 2003). One way of assessing heat resistance is by measuring survival following exposure to potentially lethal temperatures across a definite exposure time (Cui et al., 2008; Hazell et al., 2010; Hoffmann et al., 2003; Mironidis and Savopoulou-Soultani, 2010; Roux et al., 2010).

The biological aspects of organism vulnerability will also depend on the various stages of development (Bowler and Terblanche, 2008; Feder and Hofmann, 1999) and on how heat stress alters the interactions with competitors, predators, parasites, diseases and mutualists (Gilman et al., 2010; Harley, 2011; Huey et al., 2012; Lagos et al., 2001; Pincebourde and Casas, 2006). The impact of stressful temperatures is likely even more important in higher trophic levels that depend on the capacity of the lower trophic levels to adapt to these changes.

As parasitoids develop in or on hosts, a severe impact of climatic changes is expected for these organisms (Jeffs and Lewis, 2013), since they represent the third and the fourth trophic levels (Godfray, 1994). For example, koinobiont parasitoids, which maintain a strict

physiological relationship with their arthropod hosts in their early larval life, must protect themselves not only against the immune system of their host, but at the same time they should avoid lethal conditions by manipulating their hosts using behavioural and/or physiological adjustments (Hance et al., 2007; Lagos et al., 2001). Exposure to extreme temperatures can then have important effects on the outcome of host-parasite interactions (Cayetano and Vorburger, 2013; Hance et al., 2007) or, more generally, can have important effects on higher trophic levels (Harrington et al., 2001).

The pea aphid *Acyrthosiphon pisum* (Homoptera: Aphididae) is common on peas, alfalfa, and other species of legumes, reaching densities in some places high enough to become a significant pest (van Emden and Harrington, 2003). The parasitoid *Aphidius ervi* (Hymenoptera: Braconidae) is a parasitoid of the pea aphid and is regarded as a successful biocontrol agent (Starý, 1974). *Aphidius ervi* and *A. pisum* are broadly used as model systems in studies on multitrophic interactions due to the high number of relevant information about their genetics, ecology and physiology (Powell et al., 1998).

The wasps can parasitize both the adult and the four aphid instars, even though they are not equivalent for parasitoid fitness, by laying a single egg. The larva develops inside the aphid through 3 larval stages until the formation of a mummy (the skeletonised aphid).

Acyrthosiphon pisum displays a number of antipredator behaviours in response to predators and parasitoids, such as releasing an alarm pheromone (Bowers et al., 1972) and/or dropping or walking away from the threatened feeding site (Chau and Mackauer, 1997). It is also known that the antipredator behaviour of an aphid changes as a function of internal stress (Villagra et al., 2002) or of increased temperatures (Ma and Ma, 2012a, 2012b).

In general, when an endoparasitoid like *A. ervi* parasitizes a living host, factors of maternal origin that facilitate successful parasitism are also injected at oviposition, such as venom and ovarian proteins (Beckage, 1997; Digilio et al., 2000; Webb, 1998). All the parasitoid female secretions interfere both with the immune system and endocrine balance of the host (Digilio et al., 1998, 2000; Webb, 1998), hindering the encapsulation of the parasitoid egg, causing oxidative stress in ovarian cells and leading to the degeneration of the host germinal cells and of young sub-apical aphid embryos (Digilio et al., 2000; Falabella et al., 2007; Pennacchio and Strand, 2005). Other host regulation factors are of embryonic origin, derived from the serosal membrane or from other embryo-associated tissues (teratocytes) and are strictly linked to larval parasitoid survival by processing nutriments extracted from internal tissues of the host (Falabella et al., 2009, 2000; Grossi et al., 2016; Quicke, 1997). At the same time, the fight against parasitization begins with the activation of the host immune system or through microbial symbiont-based defenses (Martinez et al., 2016; Oliver et al., 2009; Oliver and Martinez, 2014; Strand and Pech, 1995).

In this study, we investigate some aspects related to the survival of aphids after exposure to a very high temperature in a trophic model system consisting of the parasitoid *A. ervi* and its host *A. pisum*.

Aphids at different developmental stages vary in many traits, including size and physiology, and may experience different microhabitats. The effects of the temporal pattern and duration of temperature exposure (the "time-dependent effects", Kingsolver and Woods, 2016) as well as the long-lasting effects of thermal stress (Roitberg and Mangel, 2016) on insect performance could be very important. Incorporating these effects on the different developmental stages of an organism are essential for making more realistic predictions on ecological responses of organisms to climate change (Kingsolver et al., 2011; Kingsolver and Woods, 2016; Roitberg and Mangel, 2016). The response of insects with complex life cycles to heat stress depends on the thermal sensitivity of all stages, reflecting differences in thermal environments they experience (Gilchrist et al., 1997; Zhao et al., 2017). The first aim of this study was then to understand if aphids at various stages of development show different levels of survival after exposure to an extreme temperature.

Based on some preliminary experiments on the thermal tolerance of *A. pisum*, we observed that parasitized adult aphids were more resistant to a severe heat shock (exposure at 39 °C for 30 min) than unparasitized aphids of the same age. As stated above, successful parasitism depends on parasitoid factors of maternal origin injected at oviposition and on factors of embryonic origin. All these factors interfere with the host physiology at different times and in different ways, necessarily causing aphid internal stress. We therefore examined whether parasitized aphids of different ages, that is, harboring parasitoids at different developmental stages and consequently subjected to different physiological modifications, showed a differential heat stress resistance compared with the unparasitized ones.

2. Materials and methods

2.1. Insect rearing

Acyrthosiphon pisum colony was started in 1985 from a few hundred specimens collected in the field from alfalfa (*Medicago sativa*) near Salerno, Italy (40°37′ N; 15°3′ E) and laboratory reared on broad beans plants (*Vicia fada L*). *Aphidius ervi* parasitoids were obtained from Koppert Italia and were laboratory-reared on *A. pisum*. Aphid and parasitoid cultures were maintained in two separate climatic chambers Binder KBF at 22 ± 1 °C and 75 ± 5% relative humidity (mean values ± accuracy), under an LD 18:6 h photoperiod. Broad bean plants (*Vicia fada L*) of the Moroccan cultivar "Aguadulce" were grown in pots (10 cm diameter) containing commercial soil (COMPO SANA® Universal Potting Soil) in a greenhouse.

Since all the experiments required same-aged aphids, approximately 120 adult virginoparae females were isolated from the mass rearing colony and put on a fresh potted broad bean plant kept in a plastic box (22 × 15 cm × 40 cm height) for 6 h at 22 ± 1 °C, 75 ± 5% RH (mean values ± accuracy) and 18:6 LD photoperiod. Adult females were then removed and discarded. The newborn nymphs were maintained as a synchronous colony on a broad bean plant for 72 h, roughly corresponding, at this rearing temperature, to the beginning of the third nymphal instar. Nevertheless, before their use in the experimental trials, aphids were inspected under a stereo-microscope and all nymphs that were not in the appropriate stage, according to the morphological features as in Digilio (1995) were discarded. Twenty-five independent synchronous colonies were generated.

The parasitoid females used in the experiment were between 24 and 48 h old. Before the experiment, each newborn female was left for 24 h with two males and fed on water and honey.

Different aphid instars, even though all accepted as hosts, are not equivalent in terms of parasitoid successful development. Aphids at the beginning of the third nymphal instar were used in this experiment since they allow a successful elevated parasitoid development (Trotta et al., 2014) and their experimental handling implies a lower mortality than in younger instars.

Each synchronous colony of three day old aphids was subsequently split into two groups and maintained on two separate plants (about 50 aphids per plant): at different times, the aphids of one group were parasitized by *A. ervi* whereas the other group was composed of unparasitized aphids used as controls.

To avoid possible effects of the plant on the growth of aphids (Guldemond et al., 1998), young vegetative plants (3 weeks after sowing seeds) were used in all the experiments.

The time of the aphid transition from the first nymphal instar to adult (I, II, III, IV, adult) was independently recorded for control and parasitized aphids. Aphids were visually inspected twice a day and the time of nymphal instar transition was recorded.

2.2. Parasitization experimental procedure

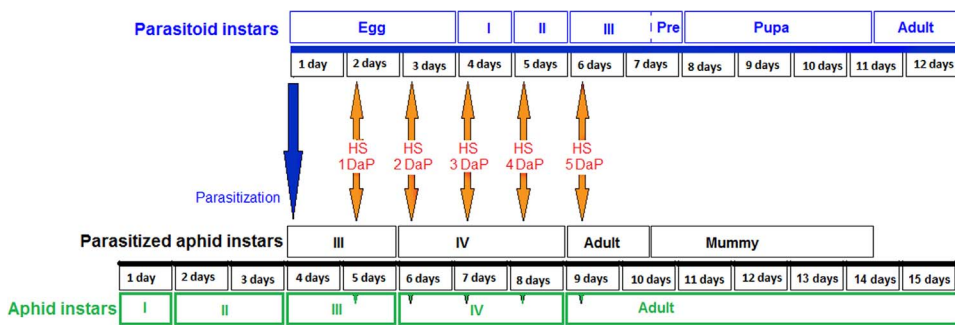The parasitized aphids used in these experiments were observed to

Fig. 1. Schematic representation of the experimental procedure. Timing of the experimental procedure in relation to the developmental stages of the aphids (parasitized and unparasitized) and the parasitoids at 22 °C. DaP is the day after parasitization in which the heat shock treatment was applied (yellow arrows). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

be stung once by a parasitoid female. In *A. ervi* the decision to accept or reject a host follows the insertion of the ovipositor (Larocca et al., 2007; Pennacchio et al., 1994). In a small number of instances there could be no insertion of egg in the host, and there is no way of knowing for sure if an aphid hosts a parasitoid egg without dissecting it. For this reason, we estimated the parasitization rate using a control group with 15 replicates of about 20 parasitized aphids that were dissected after five days by using stereomicroscope.

The parasitized aphids, as well as the control ones, were maintained on plants in an environmental chamber at 22 ± 1 °C, 75 ± 5% RH and 18:6 LD to continue their development until they were used in the heat shock treatments. Preliminary experiments showed these manipulations caused negligible aphid mortality (less than 2%).

Five experimental treatments of parasitized and unparasitized aphids were successively established according to which day after parasitization the heat shock treatment was applied (DaP), that is, aphids heat shocked one, two, three, four or five days after parasitization (Fig. 1). The control of each treatment consists of aphids of the same age but unparasitized. As a result of this experimental design, five experimental (1-DaP, 2-DaP, 3-DaP, 4-DaP, 5-DaP) and five control treatments were obtained. Each treatment consists of five replicates (five independent aphid synchronous colonies) and a replicate is composed of three (or four, when available) separate groups each one of about ten aphids, for a total of 150–160 aphids per treatment.

The parasitized aphids from each of the five treatments were dissected 24 h after heat shock to record the time of the parasitoid egg/nymphal instar transition as in Pennacchio et al. (1999)

2.3. Heat shock experiments on parasitized and control aphids

The ten aphids belonging to each experimental group were gently moved from the plant and placed in a 50 ml Falcon® tube with a mesh covered ventilation hole in the screw-top within a water bath at 39 ± 0.1 °C. Heat resistance has been assessed by measuring survival of unparasitized (control) and parasitized aphids following exposure to the potentially lethal temperature of 39 ± 0.1 °C for an exposure time of 30 min, a technical method commonly used in *Drosophila* (Hoffmann et al., 2003). The choice of the stress temperature is based on preliminary results on heat shock experiments on unparasitized adult aphids at different temperatures, since at 38.5 ± 0.1 °C the survival rate was about 80% whereas at 39.5 ± 0.1 °C the survival rate was close to 0%. Lethality is, however, a function of both temperature and time (Hoffmann et al., 2003). An exposure of 30 min was chosen since this time interval, compared with shorter exposure times, caused significant changes in the survival of aphids (Trotta et al., unpublished). In addition, this time interval minimised the chance of any heat hardening response during the heat shock (Hazell et al., 2010) and, in general, did not produce a significant impact of starvation and/or desiccation on the experimental individuals, effects that should be considered when longer exposure times are used (Terblanche et al., 2011).

The aphids were transferred after the heat shock treatment on a cut broad bean leaf, with the stalk inside an Eppendorf® tube filled with

water and sealed with parafilm® to prevent desiccation, placed in a plastic cylinder (150 ml) with a mesh covered ventilation hole in the screw-top. The plastic cylinders were placed in an environmental chamber at 22 ± 1 °C (mean values ± accuracy) and the survival was recorded after 24 h. Survival was considered to occur if the aphids were able to walk or if they moved when gently touched by a brush. This response was checked 24 h after heat exposure, as many insects are immobilized for some time following a heat stress (Hazell et al., 2010; Sørensen et al., 2001). Survival of the parasitized aphids from each treatment was then compared with the appropriate control.

2.4. Statistical analysis

A linear mixed model has been considered as a possible model for analysis of the percentage and of the percentage after a square root arcsine transformation of aphid survival after heat shock given that the sample size is quite similar for all the experimental groups. However, the raw data have a discrete probability distribution (alive/dead) and a binomial generalized linear model with a logit link function has also been considered for the identification of the best model.

We finally chose the linear model performed on the percentage of aphid survival after a square root arcsine transformation because it has the lowest Akaike Information Criterion (AIC). Therefore, this model best approximates the process that generated the observed data, minimizing the lack of model fit to the observed data (Johnson and Omland, 2004). The arcsine transformation also improved the error distribution and amended the heterogeneity of variances (Levene's tests: $F_{9,145}$ = 1.54, P = 0.14).

Data on aphid survival after heat shock were therefore analyzed using a mixed linear model ANOVA in which "treatment" (two levels: parasitized and unparasitized aphids) and "DaP" (five levels: 1-DaP, 2-DaP, 3-DaP, 4-DaP, 5-DaP) were the main fixed effects; "replicate" (five levels) was nested within treatment and DaP. This analysis was used to test for treatment and DaP differences, as well as for their interaction, using the variation among replicates as the error term.

In order to detect significant differences between parasitized and unparasitized aphids, a Tukey *post-hoc* test for multiple comparisons of means was also performed.

All the analyses in this study were carried out using R.3.2.4 revisited software (R Core Team, 2016).

3. Results

3.1. Parasitoids and aphid nymphal instars

In the present experiment, parasitized and unparasitized aphids from the third nymphal instar to adult and parasitoids from the egg stage to the fourth instar larvae were heat shocked. Under our experimental conditions, the parasitization rate (assessed by dissecting 15 replicates of about 20 parasitized aphids) was 86.7% ± 0.14 (mean ± S.E.).

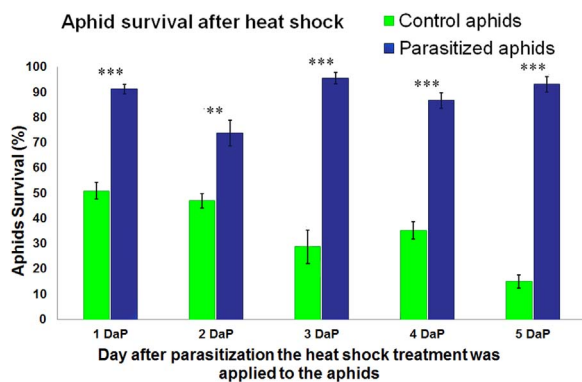The mean duration of each of the nymphal instars of the aphids

Fig. 2. Survival differences. Mean values (± standard errors) of survival of control and parasitized aphids after a heat shock at 39 ± 0.1 °C for 30 min. DaPs are the days after parasitization the heat shock treatment was applied to the aphids. Asterisks on bars indicate significant differences between control and parasitized aphids (Tukey's test, **: $P < 0.01$ and ***: $P < 0.001$).

(parasitized and unparasitized) and parasitoids are shown in Fig. 1. A specific nymphal stage was considered completed when 90% of the specimens showed the morphological traits of the next one. No differences were observed between parasitized and unparasitized aphids in the mean duration of each of the nymphal instars.

3.2. Aphid survival after heat shock

The survival of parasitized and unparasitized aphids after a heat shock at 39 ± 0.1 °C for 30 min was significantly different (Fig. 2). The linear mixed model ANOVA performed on the survival data after arcsine transformation (Table 1) shows significant differences among replicates nested within DaP and treatment, indicating a certain level of heterogeneity among aphids in response to heat shock treatment, independently of the experimental groups identity. This heterogeneity is particularly evident for the control treatment at 3-DaP and for the parasitized treatment at 2-DaP. The effects of treatment, DaP and their interaction were then compared with the replicates effect. The DaP effect was not significant, while the effects of treatment (parasitized *vs* unparasitized aphids) and the interaction "DaP X treatment" were found highly significant. Aphid survival after heat shock was significantly higher for the parasitized individuals than for the unparasitized ones. This result is independent of the number of days elapsing between the parasitization and the application of the heat shock treatment and therefore, independent of the aphid age.

It is also interesting to note that the survival of parasitized aphids is basically constant in the different DaPs.

Survival of control (unparasitized) aphids after heat shock decreases with the increase of the aphid age; this trend is particularly evident for

Table 1
Results of the mixed model ANOVA[a] on the survival of aphids after heat shock. "Treatment" (two levels: parasitized and unparasitized aphids) and "DaP" (day after parasitization aphids were heat shocked; five levels: 1-DaP, 2-DaP, 3-DaP, 4-DaP, 5-DaP) are fixed effects; "replicate" (five levels) is nested within "treatment" and "DaP". The variation among replicates was used as error term in the *F* tests for "treatment", "DaP" and their interaction.

Source of variation	df	MS	F
DaP	4	0.149	1.53 n.s.
Treatment	1	19.75	202.7***
DaP X treatment	4	0.720	7.39***
Replicate within DaP and treatment	40	0.097	3.47***
Residuals	105	0.028	

n.s., not significant; df, degrees of freedom; MS, mean square; F, variance ratio.

[a] Model: $X_{ijke} = \mu + D_i + T_j + DT_{ij} + R(D(T))_{k(i(j))} + E_{e(ijk)}$.

*** $P < 0.001$.

adult aphids (5-DaP control) that significantly differ from the other groups ($P < 0.001$) except for 3-DaP control. These trends implicate that survival differences between parasitized and unparasitized aphids increase when older aphids were heat shocked (significant interaction "DaP X treatment", Table 1).

We should also underline that the higher thermal resistance in the parasitized groups is probably slightly underestimated because the real "parasitization rate" was about 86% (see Section 3.1).

4. Discussion

Climate change could affect population dynamics of insects producing important impacts in trophic interactions since thermal fluctuation may be an important selective factor in nature (Bozinovic et al., 2016a, 2016b, 2013; Folguera et al., 2009; Jeffs and Leather, 2014). This study considers the consequences of a severe heat shock for a short exposure time in a trophic model system consisting of the parasitoid *A. ervi* and its host *A. pisum*. We found that, after a thermal stress, parasitized aphids show a significantly greater survival than unparasitized ones.

As stated in the Introduction, successful parasitism of *A. ervi* strongly depends on factors of maternal origin such as venom and ovarian proteins injected at oviposition as well as on host regulation factors of embryonic origin (Beckage, 1997; Digilio et al., 2000; Falabella et al., 2007, 2000; Pennacchio and Strand, 2005; Quicke, 1997; Webb, 1998).

In the aphid *Macrosiphum euphorbiae*, it has been shown that the resistance to *A. ervi* parasitism is mediated, among other things, by an upregulation of several aphid and symbiont proteins (Nguyen et al., 2008), including the early increase of the Hsp60 chaperone. When the ectoparasitoid *Bracon hebetor* envenomizes its host *Plodia interpunctella*, small hsp and the heat shock cognate 70 genes are upregulated 48–96 h after envenomation (Shim et al., 2008). Other heat shock genes (*hsp23* and *hsp70*) expression are also upregulated in the flesh fly *Sarcophaga crassipalpis* 13 h after envenomation by the ectoparasitic wasp *Nasonia vitripennis* (Rinehart et al., 2002).

Our results clearly show that the increased survival after the heat shock of parasitized aphids occurs already at an early stage of parasitization (1-DaP, 24 h after parasitization), that is, before the hatching of the parasitoid egg. On this basis, we can exclude that the aphid heat resistance is linked to the presence of the parasitoid larva or to the appearance of factors of embryonic origin such as the teratocytes. The increased survival displayed by parasitized aphids could be linked in a direct way to the parasitoid egg insertion and to the action of venom and ovarian proteins; all these factors cause host stress (Digilio et al., 2000; Falabella et al., 2007; Pennacchio and Strand, 2005). The results of Shim et al. (2008) and Rinehart et al. (2002) suggest a link between the parasitoid venom and HSPs.

Another explanation can be an indirect effect through the activation of the aphid immune system (Martinez et al., 2016; Oliver et al., 2009; Oliver and Martinez, 2014; Strand, 2008; Strand and Pech, 1995). It is also interesting to note that the increased survival displayed by parasitized aphids after heat shock seems to be independent of the infection with the facultative bacterial symbionts (Heyworth and Ferrari, 2016). Other kinds of experiments are needed to assess the mechanisms behind the increased survival of the parasitized aphids.

Aphids at various stages of development vary in size, morphology, physiology, behaviour and, based on the results of this experiment, they also exhibit different survival after exposure to an extreme temperature. Specifically, aphid survival decreases with the age at which the heat shock treatment was applied. The lowest survival was displayed by the unparasitized adult aphids. Similar results were observed in the aphids *Sitobion avenae* (Fabricius), where a lower basal tolerance was found in younger nymphs, increasing in older nymphs (3rd and 4th-instars) and then decreasing again in adults (Zhao et al., 2017). It is known that apterous aphids are able to move in response to extrinsic perturbations (Irwin et al., 2007) and that apterous *A. pisum* adults move, on average,

farther and cover longer distances than nymphs (Ben-Ari et al., 2015). The greater survival found in the younger nymphs could be a consequence of an adaptation to the microclimatic conditions of these aphid life stages they experience since nymphal instars of *A. pisum* are more sedentary and then more exposed to extreme temperatures than adults. The lower survival found in the adult stage might also reflect a trade-off between heat tolerance and reproductive output (Zhao et al., 2017). When aphids are parasitized by *A. ervi*, all the survival differences after heat shock among the different stages of development disappear as a consequence of the strong internal stress caused directly or indirectly by the parasitization.

5. Conclusion

Lagos et al. (2001) observed a different thermal sensitivity in *A. pisum* parasitized by *A. ervi* mediated by a different behaviour response, that is, a different walking activity. In the present study, we show evidence that parasitism in the same system can also affect the host thermal sensitivity through a physiological mechanism whose exact nature needs to be further investigated. To our knowledge, this is the one of the first papers that has reported a greater survival after a heat shock due to a metazoan parasite.

High temperatures and drought are known to induce many changes in hosts and parasitoids and can generate a disjunction between their population growth rates, affecting the role of parasitoids in pest control (Romo and Tylianakis, 2013). To avoid high temperatures, aphids may also drop off host plants, reducing the efficiency of parasitoid attacks (Ma and Ma, 2012a, 2012b). Taking this into account, it could be interesting to investigate, from an ecological perspective, if the phenomenon we observed could affect the host-parasitoid population dynamics when extreme thermal events are likely to occur.

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Conflict of interest

The authors declare that they have no conflict of interest.

References

Bale, J.S., Masters, G.J., Hodkinson, I.D., Awmack, C., Bezemer, T.M., Brown, V.K., Butterfield, J., Buse, A., Coulson, J.C., Farrar, J., Good, J.E.G., Harrington, R., Hartley, S., Jones, T.H., Lindroth, R.L., Press, M.C., Symrnioudis, I., Watt, A.D., Whittaker, J.B., 2002. Herbivory in global climate change research: direct effects of rising temperature on insect herbivores. Glob. Change Biol. 8, 1–16. http://dx.doi.org/10.1046/j.1365-2486.2002.00451.x.

Ballester, J., Giorgi, F., Rodò, X., 2009. Changes in European temperature extremes can be predicted from changes in PDF central statistics. Clim. Change 98, 277–284. http://dx.doi.org/10.1007/s10584-009-9758-0.

Beckage, N.E., 1997. The parasitic wasp's secret weapon. Sci. Am. 277, 82–87. http://dx.doi.org/10.1038/scientificamerican1197-82.

Ben-Ari, M., Gish, M., Inbar, M., 2015. Walking aphids can partake in within-field dispersal to distant plants. Basic Appl. Ecol. 16, 162–171. http://dx.doi.org/10.1016/j.baae.2014.11.007.

Bowers, W.S., Nault, L.R., Webb, R.E., Dutky, S.R., 1972. Aphid alarm pheromone: isolation, identification, synthesis. Science 177, 1121–1122.

Bowler, K., Terblanche, J.S., 2008. Insect thermal tolerance: what is the role of ontogeny, ageing and senescence? Biol. Rev. 83, 339–355. http://dx.doi.org/10.1111/j.1469-185X.2008.00046.x.

Bozinovic, F., Catalan, T.P., Estay, S.A., Sabat, P., 2013. Acclimation to daily thermal variability drives the metabolic performance curve. Evol. Ecol. Res. 15, 579–587.

Bozinovic, F., Medina, N.R., Alruiz, J.M., Cavieres, G., Sabat, P., 2016a. Thermal tolerance and survival responses to scenarios of experimental climatic change: changing thermal variability reduces the heat and cold tolerance in a fly. J. Comp. Physiol. B Biochem. Syst. Environ. Physiol. 186, 581–587. http://dx.doi.org/10.1007/s00360-016-0980-6.

Bozinovic, F., Sabat, P., Rezende, E.L., Canals, M., 2016b. Temperature variability and

thermal performance in ectotherms: acclimation, behaviour, and experimental considerations. Evol. Ecol. Res. 17, 111–124.

Cayetano, L., Vorburger, C., 2013. Effects of heat shock on resistance to parasitoids and on life history traits in an aphid/endosymbiont system. PLoS One 8 (10), e75966. http://dx.doi.org/10.1371/journal.pone.0075966.

Chau, A., Mackauer, M., 1997. Dropping of pea aphids from feeding site: a consequence of parasitism by the wasp, *Monoctonus paulensis*. Entomol. Exp. Appl. 83, 247–252. http://dx.doi.org/10.1023/A:1002935230395.

Cui, X., Wan, F., Xie, M., Liu, T., 2008. Effects of heat shock on survival and reproduction of two whitefly species, *Trialeurodes vaporariorum* and *Bemisia tabaci* biotype B. J. Insect Sci. 8, 1–10. http://dx.doi.org/10.1673/031.008.2401.

Diffenbaugh, N.S., Field, C.B., 2013. Changes in ecologically critical terrestrial climate conditions. Science 341, 486–492. http://dx.doi.org/10.1126/science.1237123.

Digilio, M., Pennacchio, F., Tremblay, E., 1998. Host regulation effects of ovary fluid and venom of *Aphidius ervi* (Hymenoptera: braconidae). J. Insect Physiol. 44, 779–784. http://dx.doi.org/10.1016/S0022-1910(98)00010-9.

Digilio, M.C., 1995. Identificazione rapida degli stadi giovanili di *Acyrthosiphon pisum* (Harris) (Homoptera Aphidoidea). Boll. di Zool. Agrar. e di Bachic. 27, 111–116.

Digilio, M.C., Isidoro, N., Tremblay, E., Pennacchio, F., 2000. Host castration by *Aphidius ervi* venom proteins. J. Insect Physiol. 46, 1041–1050. http://dx.doi.org/10.1016/S0022-1910(99)00216-4.

Estay, S.A., Lima, M., Bozinovic, F., 2014. The role of temperature variability on insect performance and population dynamics in a warming world. Oikos 123, 131–140. http://dx.doi.org/10.1111/j.1600-0706.2013.00607.x.

Falabella, P., Riviello, L., Caccialupi, P., Rossodivita, T., Teresa Valente, M., Luisa De Stradis, M., Tranfaglia, A., Varricchio, P., Gigliotti, S., Graziani, F., 2007. A γ-glutamyl transpeptidase of *Aphidius ervi* venom induces apoptosis in the ovaries of host aphids. Insect Biochem. Mol. Biol. 37, 453–465. http://dx.doi.org/10.1016/j.ibmb.2007.02.005.

Falabella, P., Riviello, L., De Stradis, M.L., Stigliano, C., Varricchio, P., Grimaldi, A., de Eguileor, M., Graziani, F., Gigliotti, S., Pennacchio, F., 2009. *Aphidius ervi* teratocytes release an extracellular enolase. Insect Biochem. Mol. Biol. 39, 801–813. http://dx.doi.org/10.1016/j.ibmb.2009.09.005.

Falabella, P., Tremblay, E., Pennacchio, F., 2000. Host regulation by the aphid parasitoid *Aphidius ervi*: the role of teratocytes. Entomol. Exp. Appl. 97, 1–9. http://dx.doi.org/10.1046/j.1570-7458.2000.00710.x.

Feder, M.E., Hofmann, G.E., 1999. Heat-shock proteins, molecular chaperones, and the stress response: evolutionary and ecological physiology. Annu. Rev. Physiol. 61, 243–282. http://dx.doi.org/10.1146/annurev.physiol.61.1.243.

Folguera, G., Bastías, D.A., Bozinovic, F., 2009. Impact of experimental thermal amplitude on ectotherm performance: adaptation to climate change variability? Comp. Biochem. Physiol. – A Mol. Integr. Physiol. 154, 389–393. http://dx.doi.org/10.1016/j.cbpa.2009.07.008.

Gilchrist, G.W., Huey, R.B., Balanya, J., Pascual, M., Serra, L., 2004. A time series of evolution in action: a latitudinal cline in wing size in south american *Drosophila subobscura*. Evolution 58, 768–780. http://dx.doi.org/10.1111/j.0014-3820.2004.tb00410.x.

Gilchrist, G.W., Huey, R.B., Partridge, L., 1997. Thermal sensitivity of *Drosophila melanogaster*: evolutionary responses of adults and eggs to laboratory natural selection at different temperatures. Physiol. Zool. 70, 403–414. http://dx.doi.org/10.1086/515853.

Gilman, S.E., Urban, M.C., Tewksbury, J., Gilchrist, G.W., Holt, R.D., 2010. A framework for community interactions under climate change. Trends Ecol. Evol. 25, 325–331. http://dx.doi.org/10.1016/j.tree.2010.03.002.

Godfray, H.C.J., 1994. Parasitoids: Behavioral and Evolutionary Ecology, Monographs in Behavior and Ecology. Princeton University Press, Princeton, NJ.

Grossi, G., Grimaldi, A., Cardone, R.A., Monné, M., Reshkin, S.J., Girardello, R., Greco, M.R., Coviello, E., Laurino, S., Falabella, P., 2016. Extracellular matrix degradation via enolase/plasminogen interaction: evidence for a mechanism conserved in Metazoa. Biol. Cell 108, 161–178. http://dx.doi.org/10.1111/boc.201500095.

Guldemond, J.A., Brink, W.J., Belder, E., 1998. Methods of assessing population increase in aphids and the effect of growth stage of the host plant on population growth rates. Entomol. Exp. Appl. 86, 163–173. http://dx.doi.org/10.1046/j.1570-7458.1998.00277.x.

Gunderson, A.R., Stillman, J.H., 2015. Plasticity in thermal tolerance has limited potential to buffer ectotherms from global warming (20150401-20150401). Proc. R. Soc. B Biol. Sci. 282. http://dx.doi.org/10.1098/rspb.2015.0401.

Hance, T., van Baaren, J., Vernon, P., Boivin, G., 2007. Impact of extreme temperatures on parasitoids in a climate change perspective. Annu. Rev. Entomol. 52, 107–126. http://dx.doi.org/10.1146/annurev.ento.52.110405.091333.

Harley, C.D.G., 2011. Climate change, keystone predation, and biodiversity loss. Science 334, 1124–1127.

Harrington, R., Fleming, R.A., Woiwod, I.P., 2001. Climate change impacts on insect management and conservation in temperate regions: can they be predicted? Agric. For. Entomol. 3, 233–240. http://dx.doi.org/10.1046/j.1461-9555.2001.00120.x.

Hazell, S.P., Neve, B.P., Groutides, C., Douglas, A.E., Blackburn, T.M., Bale, J.S., 2010. Hyperthermic aphids: insights into behaviour and mortality. J. Insect Physiol. 56, 123–131. http://dx.doi.org/10.1016/j.jinsphys.2009.08.022.

Heyworth, E.R., Ferrari, J., 2016. Heat stress affects facultative symbiont-mediated protection from a parasitoid wasp. PLoS One 11, 1–11. http://dx.doi.org/10.1371/journal.pone.0167180.

Hoffmann, A.A., Hewa-Kapuge, S., 2000. Acclimation for heat resistance in *Trichogramma* nr. *brassicae*: can it occur without costs? Funct. Ecol. 14, 55–60.

Hoffmann, A.A., Parsons, P.A., 1991. Evolutionary Genetics and Environmental Stress. Oxford Science Publications, Oxford University Press, Oxford.

Hoffmann, A.A., Sørensen, J.G., Loeschcke, V., 2003. Adaptation of *Drosophila* to

temperature extremes: bringing together quantitative and molecular approaches. J. Therm. Biol. 28, 175–216. http://dx.doi.org/10.1016/S0306-4565(02)00057-8.

Huey, R.B., Berrigan, D., 2001. Temperature, demography, and ectotherm fitness. Am. Nat. 158, 204–210. http://dx.doi.org/10.1086/321314.

Huey, R.B., Kearney, M.R., Krockenberger, A., Holtum, J.A.M., Jess, M., Williams, S.E., 2012. Predicting organismal vulnerability to climate warming: roles of behaviour, physiology and adaptation. Philos. Trans. R. Soc. B Biol. Sci. 367, 1665–1679. http://dx.doi.org/10.1098/rstb.2012.0005.

Irwin, M., Kampmeier, G., Wolfgang, W., 2007. Aphid movement: process and consequences. In: van Hemden, H.F., Harrington, R. (Eds.), Aphids as Crop Pests. CAB International, Wallingford, pp. 153–186.

Jeffs, C.T., Leather, S.R., 2014. Effects of extreme, fluctuating temperature events on life history traits of the grain aphid, *Sitobion avenae*. Entomol. Exp. Appl. 150, 240–249. http://dx.doi.org/10.1111/eea.12160.

Jeffs, C.T., Lewis, O.T., 2013. Effects of climate warming on host-parasitoid interactions. Ecol. Entomol. http://dx.doi.org/10.1111/een.12026.

Johnson, J.B., Omland, K.S., 2004. Model selection in ecology and evolution. Trends Ecol. Evol. 19, 101–108. http://dx.doi.org/10.1016/j.tree.2003.10.013.

Johnston, I.A., Bennett, A.F., 2008. Animals and Temperature. Phenotypic and Evolutionary Adaptation, Society for Experimental Biology Seminar Series. Cambridge University Press.

Kingsolver, J.G., Arthur Woods, H., Buckley, L.B., Potter, K.A., MacLean, H.J., Higgins, J.K., 2011. Complex life cycles and the responses of insects to climate change. Integr. Comp. Biol. 51, 719–732. http://dx.doi.org/10.1093/icb/icr015.

Kingsolver, J.G., Woods, H.A., 2016. Beyond thermal performance curves: modeling time-dependent effects of thermal stress on ectotherm growth rates. Am. Nat. 187, 283–294. http://dx.doi.org/10.1086/684786.

Kunkel, K.E., Pielke, R.A., Changnon, S.A., 1999. Temporal fluctuations in weather and climate extremes that cause economic and human health impacts: a review. Bull. Am. Meteorol. Soc. 80, 1077–1098. http://dx.doi.org/10.1175/1520-0477(1999)080<1077:TFIWAC>2.0.CO;2.

Lagos, N.A., Fuentes-Contreras, E., Bozinovic, F., Niemeyer, H.M., 2001. Behavioural thermoregulation in *Acyrthosiphon pisum* (Homoptera: aphididae): The effect of parasitism by *Aphidius ervi* (Hymenoptera: braconidae). J. Therm. Biol. 26, 133–137. http://dx.doi.org/10.1016/S0306-4565(00)00033-4.

Larocca, A., Fanti, P., Romano, V.A., Marsicovetere, E., Isidoro, N., Romani, R., Ruschioni, S., Pennacchio, F., Battaglia, D., 2007. Functional bases of host-acceptance behaviour in the aphid parasitoid *Aphidius ervi*. Physiol. Entomol. 32, 305–312. http://dx.doi.org/10.1111/j.1365-3032.2007.00578.x.

Loeschcke, V., Hoffmann, A.A., 2007. Consequences of heat hardening on a field fitness component in *Drosophila* depend on environmental temperature. Am. Nat. 169, 175–183. http://dx.doi.org/10.1086/510632.

Ma, G., Ma, C.-S., 2012a. Effect of acclimation on heat-escape temperatures of two aphid species: implications for estimating behavioral response of insects to climate warming. J. Insect Physiol. 58, 303–309. http://dx.doi.org/10.1016/j.jinsphys.2011.09.003.

Ma, G., Ma, C.-S., 2012b. Climate warming may increase aphids' dropping probabilities in response to high temperatures. J. Insect Physiol. 58, 1456–1462. http://dx.doi.org/10.1016/j.jinsphys.2012.08.012.

Marengo, J.A., Jones, R., Alves, L.M., Valverde, M.C., 2009. Future change of temperature and precipitation extremes in South America as derived from the PRECIS regional climate modeling system. Int. J. Climatol. 29, 2241–2255. http://dx.doi.org/10.1002/joc.

Martinez, A.J., Kim, K.L., Harmon, J.P., Oliver, K.M., 2016. Specificity of multi-modal aphid defenses against two rival parasitoids. PLoS One 11, 1–17. http://dx.doi.org/10.1371/journal.pone.0154670.

Meehl, G.A., Karl, T., Easterling, D.R., Changnon, S., Pielke, R., Changnon, D., Evans, J., Groisman, P.Y., Knutson, T.R., Kunkel, K.E., Mearns, L.O., Parmesan, C., Pulwarty, R., Root, T., Sylves, R.T., Whetton, P., Zwiers, F., 2000. An introduction to trends in extreme weather and climate events: observations, socioeconomic impacts, terrestrial ecological impacts, and model projections. Bull. Am. Meteorol. Soc. 81, 413–416. http://dx.doi.org/10.1175/1520-0477(2000)081<0413:AITTIE>2.3.CO;2.

Mironidis, G.K., Savopoulou-Soultani, M., 2010. Effects of heat shock on survival and reproduction of *Helicoverpa armigera* (Lepidoptera: noctuidae) adults. J. Therm. Biol. 35, 59–69. http://dx.doi.org/10.1016/j.jtherbio.2009.11.001.

Miyan, M.A., 2015. Droughts in Asian least developed countries: vulnerability and sustainability. Weather Clim. Extrem. 7, 8–23. http://dx.doi.org/10.1016/j.wace.2014.06.003.

Nguyen, T.T.A., Boudreault, S., Michaud, D., Cloutier, C., 2008. Proteomes of the aphid *Macrosiphum euphorbiae* in its resistance and susceptibility responses to differently compatible parasitoids. Insect Biochem. Mol. Biol. 38, 730–739. http://dx.doi.org/10.1016/j.ibmb.2008.04.005.

Oliver, K.M., Degnan, P.H., Hunter, M.S., Moran, N.A., 2009. Bacteriophages encode factors required for protection in a symbiotic mutualism. Science 325, 992–994. http://dx.doi.org/10.1126/science.1174463.

Oliver, K.M., Martinez, A.J., 2014. How resident microbes modulate ecologically-important traits of insects. Curr. Opin. Insect Sci. 4, 1–7. http://dx.doi.org/10.1016/j.

cois.2014.08.001.

Pennacchio, F., Digilio, M.C., Tremblay, E., Tranfaglia, A., 1994. Host recognition and acceptance behaviour in two aphid parasitoid species: *Aphidius ervi* and *Aphidius microlophii* (Hymenoptera: braconidae). Bull. Entomol. Res. 84, 57. http://dx.doi.org/10.1017/S0007485300032235.

Pennacchio, F., Fanti, P., Falabella, P., Digilio, M.C., Bisaccia, F., Tremblay, E., 1999. Development and nutrition of the braconid wasp, *Aphidius ervi* in aposymbiotic host aphids. Arch. Insect Biochem. Physiol. 40, 53–63. http://dx.doi.org/10.1002/(SICI)1520-6327(1999)40:1<53::AID-ARCH6>3.3.CO;2-A.

Pennacchio, F., Strand, M.R., 2005. Evolution of developmental strategies in parasitic Hymenoptera. Annu. Rev. Entomol. 51, 233–258. http://dx.doi.org/10.1146/annurev.ento.51.110104.151029.

Pincebourde, S., Casas, J., 2006. Multitrophic biophysical budgets: thermal ecology of an intimate herbivore insect-plant interaction. Ecol. Monogr. 76, 175–194. http://dx.doi.org/10.1890/0012-9615(2006)076[0175:MBBTEO]2.0.CO;2.

Pörtner, H.O., Bennett, A.F., Bozinovic, F., Clarke, A., Lardies, M.A., Lucassen, M., Pelster, B., Schiemer, F., Stillman, J.H., 2006. Trade-offs in thermal adaptation: the need for a molecular to ecological integration. Physiol. Biochem. Zool. 79, 295–313. http://dx.doi.org/10.1086/499986.

Powell, W., Pennacchio, F., Poppy, G.M., Tremblay, E., 1998. Strategies involved in the location of hosts by the parasitoid *Aphidius ervi* Haliday (Hymenoptera: Aphidiinae). Biol. Control 11, 104–112. http://dx.doi.org/10.1006/bcon.1997.0584.

Quicke, D.L.J., 1997. Parasitic Wasps. Chapman & Hall Ltd., London.

R Core Team, 2016. R: A Language and Environment for Statistical Computing.

Rinehart, J.P., Denlinger, D.L., Rivers, D.B., 2002. Upregulation of transcripts encoding select heat shock proteins in the flesh fly *Sarcophaga crassipalpis* in response to venom from the ectoparasitoid wasp *Nasonia vitripennis*. J. Invertebr. Pathol. 79, 62–63. http://dx.doi.org/10.1016/S0022-2011(02)00002-2.

Roitberg, B.D., Mangel, M., 2016. Cold snaps, heatwaves, and arthropod growth. Ecol. Entomol. 41, 653–659. http://dx.doi.org/10.1111/een.12324.

Romo, C.M., Tylianakis, J.M., 2013. Elevated temperature and drought interact to reduce parasitoid effectiveness in suppressing hosts. PLoS One 8. http://dx.doi.org/10.1371/journal.pone.0058134.

Roux, O., Le Lann, C., van Alphen, J.J.M., van Baaren, J., 2010. How does heat shock affect the life history traits of adults and progeny of the aphid parasitoid *Aphidius avenae* (Hymenoptera: aphidiidae)? Bull. Entomol. Res. 100, 543–549. http://dx.doi.org/10.1017/S0007485309990575.

Shim, J.K., Ha, D.M., Nho, S.K., Song, K.S., Lee, K.Y., 2008. Upregulation of heat shock protein genes by envenomation of ectoparasitoid *Bracon hebetor* in larval host of Indian meal moth *Plodia interpunctella*. J. Invertebr. Pathol. 97, 306–309. http://dx.doi.org/10.1016/j.jip.2007.10.001.

Sørensen, J.G., Dahlgaard, J., Loeschcke, V., 2001. Genetic variation in thermal tolerance among natural populations of *Drosophila buzzatii*: down regulation of Hsp70 expression and variation in heat stress resistance traits. Funct. Ecol. 15, 289–296. http://dx.doi.org/10.1046/j.1365-2435.2001.00525.x.

Starý, P., 1974. Population dynamics, parasitization, control and prognosis of the pea aphid (*Acyrthosiphon pisum* Harris) in Czechoslovakia. Trans. Czechoslov. Acad. Sci. Ser. Math. Nat. Sci. 84, 1–123.

Strand, M.R., 2008. The insect cellular immune response. Insect Sci. 15, 1–14. http://dx.doi.org/10.1111/j.1744-7917.2008.00183.x.

Strand, M.R., Pech, L.L., 1995. Immunological basis for compatibility in parasitoid-host relationships. Annu. Rev. Entomol. 40, 31–56. http://dx.doi.org/10.1146/annurev.en.40.010195.000335.

Terblanche, J.S., Hoffmann, A.A., Mitchell, K.A., Rako, L., le Roux, P.C., Chown, S.L., 2011. Ecologically relevant measures of tolerance to potentially lethal temperatures. J. Exp. Biol. 214, 3713–3725. http://dx.doi.org/10.1242/jeb.061283.

Trotta, V., Calboli, F.C.F., Ziosi, M., Guerra, D., Pezzoli, M.C., David, J.R., Cavicchi, S., 2006. Thermal plasticity in *Drosophila melanogaster*: a comparison of geographic populations. BMC Evol. Biol. 6, 67. http://dx.doi.org/10.1186/1471-2148-6-67.

Trotta, V., Duran Prieto, J., Battaglia, D., Fanti, P., 2014. Plastic responses of some life history traits and cellular components of body size in *Aphidius ervi* as related to the age of its host *Acyrthosiphon pisum*. Biol. J. Linn. Soc. 113, 439–454. http://dx.doi.org/10.1111/bij.12354.

van Emden, H.F., Harrington, R., 2003. Aphids as crop pests. Q. Rev. Biol. 83, 717. http://dx.doi.org/10.1086/596270.

Villagra, C.A., Ramírez, C.C., Niemeyer, H.M., 2002. Antipredator responses of aphids to parasitoids change as a function of aphid physiological state. Anim. Behav. 64, 677–683. http://dx.doi.org/10.1006/anbe.2002.4015.

Vose, R.S., Easterling, D.R., Gleason, B., 2005. Maximum and minimum temperature trends for the globe: an update through 2004. Geophys. Res. Lett. 32, 1–5. http://dx.doi.org/10.1029/2005GL024379.

Webb, B.A., 1998. Polydnavirus biology, genome structure, and evolution. In: Miller, L.K., Ball, L.A. (Eds.), The Insect Viruses. Springer, Boston, MA, USA, pp. 105–139. http://dx.doi.org/10.1007/978-1-4615-5341-0_5.

Zhao, F., Hoffmann, A.a., Xing, K., Ma, C.-S., 2017. Life stages of an aphid living under similar thermal conditions differ in thermal performance. J. Insect Physiol. 99, 1–7. http://dx.doi.org/10.1016/j.jinsphys.2017.03.003.