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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

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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