Comparative Biochemistry and Physiology, Part A 164 (2013) 77–83

Contents lists available at SciVerse ScienceDirect

Comparative Biochemistry and Physiology, Part A

journal homepage: www.elsevier.com/locate/cbpa

Does cold tolerance plasticity correlate with the thermal environment and metabolic profiles of a parasitoid wasp?

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ARTICLE INFO

Article history:
Received 1 August 2012
Received in revised form 12 October 2012
Accepted 15 October 2012
Available online 23 October 2012

Keywords:
Acclimation
Insect
Metabolomic
Temperature
Chill coma
Venturia canescens

ABSTRACT

Tolerance of ectotherm species to cold stress is highly plastic according to thermal conditions experienced prior to cold stress. In this study, we investigated how cold tolerance varies with developmental temperature (at 17, 25 and 30 °C) and whether developmental temperature induces different metabolic profiles. Experiments were conducted on the two populations of the parasitoid wasp, *Venturia canescens*, undergoing contrasting thermal regimes in their respective preferential habitat (thermally variable *vs.* buffered). We predicted the following: i) development at low temperatures improves the cold tolerance of parasitoid wasps, ii) the shape of the cold tolerance reaction norm differs between the two populations, and iii) these phenotypic variations are correlated with their metabolic profiles. Our results showed that habitat origin and developmental acclimation interact to determine cold tolerance and metabolic profiles of the parasitoid wasps. Cold tolerance was promoted when developmental temperatures declined and population originating from variable habitat presented a higher cold tolerance. Cold tolerance increases through the accumulation of metabolites with an assumed cryoprotective function and the depression of metabolites involved in energy metabolism. Our data provide an original example of how intraspecific cold acclimation variations correlate with metabolic response to developmental temperature.

1. Introduction

Many ectotherms including insects have to cope with cold periods during their lifetime, and this may have strong consequences on their fitness, which in turn can contribute to determine their geographical distribution (Bale, 2002; Chown and Terblanche, 2007). Given the importance of winter periods on population dynamics, cold tolerance in insects usually exhibits a high degree of phenotypic plasticity (*e.g.* Ayrinhac et al., 2004). The ability of such organisms to sustain a cold stress first depends on the basal thermal tolerance and second on their capacity to respond to thermal variations *via* plasticity (Nyamukondiwa et al., 2011). Cold tolerance can be enhanced by pre-exposure to low temperatures during larval or adult stages (Colinet and Hoffmann, 2012), the so-called adaptive thermal acclimation (Rako and Hoffmann, 2006). The amplitude of thermal acclimation that a species can reach is expected to be linked to the environmental variability and predictability of the thermal conditions of their habitats, *i.e.* species growing in highly variable habitats are expected to exhibit a higher capacity for cold acclimation compared to their relatives from more buffered environments (van Tienderen, 1991; Gabriel and Lynch, 1992; DeWitt et al., 1998; Angilletta, 2009). Although, differences in the level of cold acclimation have been reported among species from distinct geographical origins in several arthropod species (*e.g.* Bahrndorff et al., 2009; Overgaard et al., 2011; Boher et al., 2012), the possible relationship between the level of cold acclimation and the thermal characteristics of habitats has been less examined at the intra-specific level (but see Klok and Chown, 2003; Ayrinhac et al., 2004; Cooper et al., 2012; Sinclair et al., 2012).

In insects, the enhancement of the cold tolerance level involves physiological adjustments particularly on membrane composition, enzyme activity and concentration of compatible solutes (Sinclair et al., 2003; Chown and Terblanche, 2007; Clarke and Worland, 2008). This physiological remodelling prevents the accumulation of lethal chill injuries and allows a faster recovery when the environmental conditions are permissive again for the insect species. During acclimation, the progressive alteration of the concentrations of several compounds related to energetic metabolism provides useful biochemical fingerprints allowing a reliable monitoring of the acclimatory response of cold exposed insects (Bundy et al., 2009; Colinet et al., 2012a). In addition, the thermal-induced accumulation of compatible solutes

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http://dx.doi.org/10.1016/j.cbpa.2012.10.018

such as polyols, sugars and free amino acids represents an ubiquitous physiological response in cold acclimated insects (Storey and Storey, 2005; Michaud and Denlinger, 2007; Koštál et al., 2011). Metabolic fingerprinting approaches have been used to depict the cold acclimation responses of adult insects (Lalouette et al., 2007; Michaud and Denlinger, 2007; Overgaard et al., 2007; Michaud et al., 2008), however metabolomic analyses of developmental acclimation of cold tolerance have not been studied to the same extent (Koštál et al., 2011; Colinet et al., 2012a). So far, no study has simultaneously investigated amplitude of cold acclimation according to thermal habitat characteristics and physiological adjustments among insect populations using metabolic fingerprints.

The parasitoid *Venturia canescens* Gravenhorst (Hymenoptera: Ichneumonidae) is characterized by a high level of intra-specific variation, which manifests itself in distinct reproduction modes among populations that grow and develop into distinct habitats (Beukeboom et al., 1999). The parthenogenetic arrhenotokous populations (sexual strain) live exclusively in natural environments, where they are subjected to seasonal and daily thermal fluctuations. Conversely, the parthenogenetic thelytokous populations (asexual strain) thrive in anthropogenic environments (*i.e.*, granaries and mills) that confer buffered thermal conditions (Amat et al., 2006). Thelytokous wasps are unlikely to survive in natural habitats during the cold season (Amat, 2004), and anthropogenic habitats serve as a refuge during winter periods.

In this study, we used targeted gas chromatography and mass-spectrometry (GC/MS) to examine changes in metabolic profiles between thelytokous and arrhenotokous populations of *V. canescens* that were reared under controlled conditions at different temperatures. We assumed that development at low temperature would promote cold tolerance, and hypothesize that this may be associated with metabolic changes. Because the arrhenotokous population thrives in thermally variable habitats, we expected that they may be characterized by a higher capacity for cold acclimation than thelytokous individuals, and that the two populations will display specific metabolic profiles according to the developmental temperature.

2. Materials and methods

2.1. Biological material and cultures

V. canescens is a Mediterranean endoparasitoid of lepidopteran larvae, mainly of the family Pyralidae (Salt, 1976). We conducted our experiments on thelytokous and arrhenotokous populations established from a large sample of wild individuals collected in orchards and near anthropogenic habitats near Valence, France (North: 44°58′34″, East: 4°55′66″, Gotheron INRA station). Thelytokous parthenogenesis is not induced by endosymbiotic bacteria (Mateo-Leach et al., 2009; Foray et al., in press). The thelytokous and arrhenotokous wasps that we used for cold tolerance assays were collected in the summer of 2006 and 2007, respectively, and both strains were maintained under controlled conditions (25 ± 1 °C, 70 ± 5% RH and 12:12 (L:D) for 20 and 8 generations, respectively). Parasitoids used for metabolic profiling were collected during summer 2010 and maintained under controlled conditions for 8 generations. Such a short-duration stay prevents laboratory adaptation in *V. canescens* (Foray et al., 2011). *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) larvae were used as hosts for the development of the parasitoids. They were reared with wheat semolina as a substrate.

2.2. Developmental acclimation

To produce individuals for the assays, we randomly chose 40 thelytokous females and 40 arrhenotokous couples and placed them in boxes containing approximately 500 *E. kuehniella* third-instar larvae with access to food (50% water-diluted honey on a piece of cotton wool). Wasps were free to mate and to parasitize hosts during a 96-h period. This procedure was performed under controlled conditions (25 ± 1 °C, 70 ± 5% RH and 12:12 L:D). The parasitized hosts were then randomly distributed among three identical MLR-352 H incubators (SANYO Electric Biomedical Co., Ltd., Osaka, Japan) set at 17, 25 and 30 °C (± 1 °C) to continue development until adulthood. These temperatures are within the lower and the upper thermal thresholds for development of *V. canescens* (Eliopoulos and Stathas, 2003). The hosts were inspected twice a day at the onset of emergence of *V. canescens* (between 8:00 and 12:00 p.m.) to collect newly emerged wasps for assays.

2.3. Cold tolerance assay

The newly emerged wasps were placed individually, without anaesthesia, into plastic vials (Ø: 30 mm, h: 70 mm) with a piece of cotton wool soaked with 2 ml of water under controlled conditions (25 ± 1 °C, 70 ± 5% RH, 12:12 L:D). The cold tolerance of 1-day-old adult females was assessed by measuring their recovery time from chill coma following an exposure to cold stress. Cold exposure consisted in placing females individually into a dry 2 ml Eppendorf vials immersed in a glycol solution cooled to −7 °C for 7 h. Preliminary tests revealed that such conditions are non-lethal and do not induce freezing of female parasitoids (data not shown). Chill coma recovery time (CCRT) was measured by monitoring the necessary time for the females to stand on their legs after being transferred to room temperature (25 ± 1 °C). This index has been linked to adaptive patterns that match expectations based on climatic conditions (Ayrinhac et al., 2004; Hoffmann et al., 2005). The maximum observation time was 2 h; beyond this period, CCRT was considered censored.

2.4. Metabolic fingerprinting

Metabolic profiling was made using whole body extracts of thelytokous and arrhenotokous females developed at 17, 25 and 30 °C and frost at −80 °C at their emergence until analysis. The wasps were weighed (wet mass) using a microbalance accurate to within 0.01 mg (Mettler microbalance). For each modality, an analysis was performed using 8 true biological replicates ($n = 8$), each consisting of a pool of 3 wasps, except at 17 °C, where 7 and 10 replicates were used for arrhenotokous and thelytokous wasps, respectively. We used a GC-MS platform to measure metabolites from the whole insect body as described in details by Colinet et al. (2012b). Briefly, metabolites were extracted using methanol-chloroform (2:1) and then derivatized with methoxyamine HCl hydrochloride in pyridine followed by MSTFA. We completely randomized the injection order of the samples. All samples were run under the SIM mode. We therefore only screened for the 60 pure reference compounds included in our custom-made spectral database. Quantification was based on calibration curves obtained from pure reference compounds. The system consisted of a CTC CombiPal autosampler (GERSTEL GmbH & Co.KG, Germany), a Trace GC Ultra chromatograph and a Trace DSQII quadrupole MS (Thermo Fischer Inc., USA). Chromatograms were deconvoluted using XCalibur v2.0.7.

2.5. Statistical analyses

To compare the CCRT, we used parametric survival analysis assuming a non-constant hazard function following a Weibull distribution. This model allowed for the incorporation of censored data. The significance of the explanatory variables was assessed using z statistics. The instantaneous probability of wasp recovery over time was analysed by testing whether the scale parameter differed from unity (Crawley, 2007).

Metabolite profiles were analysed using a between principal component analysis (Between PCA, Dolédec and Chessel, 1991) to test a clustering effect according to the experimental modalities, *i.e.*, the population and the developmental temperature. Between PCA finds linear combinations of variables maximising the between-modalities. The inertia calculated in a Between PCA represents the part of the total