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No trade-off between high and low temperature tolerance in a winter acclimatized Danish *Drosophila subobscura* population

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ABSTRACT

Coping with cold winter conditions is a major challenge for many insects.

In early spring we observed newly emerged *Drosophila subobscura*, which had overwintered as larvae and pupae. As temperatures increase during spring these flies are faced with higher minimum and maximum temperatures in their natural microhabitat. Thus, there is a potential costly mismatch between winter and early spring acclimatization and the increased ambient temperatures later in adult life.

We obtained individuals from a natural Danish population of *D. subobscura* and acclimated them in the laboratory to 20 °C for one generation, and compared critical thermal maximum (CTmax) and minimum (CTmin) to that of individuals collected directly from their natural microhabitat. The two populations (laboratory and field) were subsequently both held in the laboratory at 20 °C and tested for their CTmax and CTmin every third day for 28 days.

At the first day of testing, field acclimatized *D. subobscura* had both higher heat and cold resistance compared to laboratory flies, and thereby a considerable larger thermal scope. Following transfer to the laboratory, cold and heat resistance of the field flies decreased over time relative to the laboratory flies. Despite the substantial decrease in thermal tolerances the thermal scope remained larger for field acclimatized individuals for the duration of the experiment.

We conclude that flies acclimatized to their natural microhabitat had increased cold resistance, without a loss in heat tolerance. Thus while a negative correlation between cold and heat tolerance is typically observed in laboratory studies in *Drosophila* sp., this was not observed for field acclimatized *D. subobscura* in this study. We suggest that this is an adaptation to juvenile overwintering in temperate cold environments, where developmental (winter) temperatures can be much lower than temperatures experienced by reproducing adults after emergence (spring). The ability to gain cold tolerance through acclimatization without a parallel loss of heat tolerance affects thermal scope and suggests that high and low thermal tolerance act through mechanisms with different dynamics and reversibility.

1. Introduction

In temperate environments temperatures can fluctuate widely on a daily and seasonal basis, whereby the ability to adapt to temperature variations is likely to be under strong selection (Ghalambor et al., 2006; Chown and Terblanche, 2007; Angilletta, 2009). The expression of adaptive phenotypic plasticity is one way for an organism to achieve an increased thermal tolerance, and this is expected to be pronounced in species adapted to highly fluctuating temperate environments (Moreteau et al., 1997; Ghalambor et al., 2006; Chown and Terblanche, 2007; Angilletta, 2009).

The expression of phenotypic plasticity in response to thermal challenges is collectively known as acclimation. Acclimation can be defined as physiological responses occurring within the lifespan of an organism in response to exposure to environmental conditions (but see Ferrer et al., 2013 for cross-generational acclimation). Acclimation responses are usually assumed to be adaptive, however, the frequent failure to identify an universal adaptive significance of acclimation has been suggested to rely on the complex nature of the phenomenon and failure to include the potential costs of acclimation, and the occurrence of multiple overlapping mechanisms and processes involved (see discussions in Huey

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et al., 1999; Wilson and Franklin, 2002; Woods and Harrison, 2002; Angilletta, 2009).

Acclimation has been separated into developmental acclimation (largely considered to be irreversible) and reversible acclimation in the adult stage (Angilletta, 2009), suggesting that these are separate processes. Further, in *Drosophila* decreased tolerance to both heat (Sørensen and Loeschcke, 2002; Pappas et al., 2007) and cold (Colinet et al., 2013b) with age has been shown. This decrease occurs in early adult life and is not related to senescence but has been suggested to be a carry-over effect from a previous life stage (Bowler and Terblanche, 2008) and seems to be accompanied by decreased expression of stress response proteins (Sørensen and Loeschcke, 2002; Pappas et al., 2007; Colinet et al., 2013b). The resulting phenotype of an adult insect is likely influenced by both developmental and adult acclimation and further by the interaction with changes related to age.

Effects of cold acclimation in insects tested in the laboratory typically provide evidence for strong benefits in the form of increased cold resistance later in life but not necessarily associated with cost in terms of decreased heat resistance (Hoffmann et al., 2003; Chown and Nicolson, 2004; Chown and Terblanche, 2007; Hoffmann, 2010). On the contrary the few studies investigating cold acclimation under field conditions provide evidence that cold acclimation can lead to strong benefits but also strong costs if temperatures are high and *vice versa* with heat acclimation (Angilletta et al., 2002; Loeschcke and Hoffmann, 2007; Kristensen et al., 2008, 2012; Chidawanyika and Terblanche, 2011). In these field studies however, acclimation is typically performed in the laboratory at constant temperatures and then tested in the field or under semi-natural conditions (Loeschcke and Hoffmann, 2007; Kristensen et al., 2008, 2012; Chidawanyika and Terblanche, 2011) and the temperature fluctuations of the microhabitat are often not really known. Thus, research on thermal acclimation to extreme temperatures performed in the laboratory, may not always be ecologically relevant. More information on microhabitat temperatures, the ability to acclimatize to field temperatures, costs and benefits of field acclimatization, and reversibility of acclimation responses are needed to expand our understanding of plastic responses to temperature fluctuations.

To properly understand the adaptive background and ecological consequences of a species' winter acclimatization strategy, an in depth understanding of the species' thermal physiology is necessary. However, the literature on overwintering strategies of temperate *Drosophila* species is generally scarce (Andersen et al., 2015). Most *Drosophila* species are chill sensitive, and will die at subzero temperatures above their super cooling point (Strachan et al., 2011; Storey and Storey, 2012). In the *obscura* group several cold tolerance strategies including pupal and adult diapause have been described (Goto et al., 1999) and available literature suggests that *Drosophila subobscura* has no adult diapause and that only the adult life stage survive the winter (Begon, 1976) as is assumed to be the case for most temperate *Drosophila* species (Lumme and Lakovaara, 1983).

Here we study overwintering strategies and winter field thermal acclimatization of the cold tolerant drosophilid *D. subobscura* collected from a microhabitat in Denmark. *D. subobscura* is distributed widely in temperate areas (http://www.taxodros.uzh.ch/) and is well adapted to cold temperatures with critical thermal minima (CTmin) below 0 °C (Gibert and Huey, 2001). Our results are discussed in relation to ongoing climate change and we suggest that natural selection will favor thermally plastic genotypes (high thermal scope) in a future with higher incidence of more variable and unpredictable thermal episodes (IPCC, 2014).

2. Materials and methods

2.1. Field site

During the winter and spring 2014 we performed field observations at Karensminde orchard at the Danish peninsula of Jutland (55°56′42.46″N, 10°12′45.31″E). The observations were aimed at investigating the overwintering of *D. subobscura* occurring in a 2 m * 1 m * 0.5 m heap of discarded apples. The temperatures in the air (air), 0.1 m below the surface of the pile (top of pile) and 0.4 m below the surface of the pile (bottom of pile) were concurrently measured with dataloggers (iButton Data Loggers, Maxim, Sunnyvale, California, USA) and extracted with the software OneWireViewer (Maxim, Sunnyvale, California, USA). The fermentation process kept the temperature in the top of the pile approximately 5 °C above air temperature during the early winter (December–February) and buffered daily temperature variation (Fig. 1).

2.2. Overwintering of D. subobscura

To investigate how *D. subobscura* overwinters at the field site, we collected flies for: (i) assessment of cold tolerance of flies collected in the field in early spring, (ii) assessment of the reproductive status of field caught females and (iii) the presence of newly emerged flies. We further transferred rotten apples containing active larvae and pupae from the heap to 5 °C in the laboratory to monitor emergence of *D. subobscura*. The low air temperature prevented wild flies from flying; therefore flies were collected from the surface of the heap with aspirators. Flies were transferred to vials containing standard oatmeal–sugar–yeast–agar Drosophila medium for further investigation in the laboratory. For further details see the results section.

2.3. Thermal tolerance and acclimation effects

We contrasted thermal tolerance of two populations originating from the same natural source population but sampled one generation (24 days) apart. One population was acclimated to the laboratory for one generation (laboratory population) after collection in the field and another one was freshly collected (field population). The laboratory population was based on twenty-five wild female *D. subobscura* flies, which were collected in Karensminde Orchard the 1st of April 2014. The females produced eggs in individual vials at 20 °C with a 12/12 h light/dark cycle for eight consecutive days, and were transferred to new vials every second day. Emerging offspring were collected every day during five days. These flies constitute the 20 °C laboratory-acclimation treatment. The field population consisted of *D. subobscura* males sampled the 25th of April in the field, and constitute the field-acclimatization treatment. From each of the field and laboratory populations, approximately 400 male flies (sexed after being anaesthetized with CO_2) were obtained and distributed in vials in groups of 30 and kept at 20 °C. Flies were reared on standard oatmeal–sugar–yeast–agar Drosophila medium throughout the experiment.

After one day in the laboratory, for the field population, and the day after emergence of adult flies of the laboratory population, we performed the first heat and cold tolerance assays. Flies were distributed individually into small glass vials, which were sealed with plastic lids. For the assessment of the critical thermal maximum (CTmax), flies were then submerged into a water bath at 20 °C, and the temperature was increased with a rate of 0.1 °C/min. Conversely, for the assessment of critical thermal minimum (CTmin), flies were submerged into a water bath at 20 °C