

Final report of the project:

Ph.D. student, Isabell Karl, Animal Ecology I, University of Bayreuth, Universitätsstraße 30, D - 95440 Bayreuth, Germany.

Project title: "Altitudinal variation in thermal resistance and the expression of heat-shock protein in a butterfly".

Host: Prof. Dr. Volker Loeschcke, Ecology and Genetics, Department of Biological Sciences, University of Aarhus, Ny Munkegade, Buildg. 1540, DK-8000, Aarhus C, Denmark.

Background and purpose of the visit:

As for basically all terrestrial ectotherms temperature is a key environmental factor influencing virtually all aspects of their ecology and evolution (e.g. Hoffmann et al. 2003; Sinclair et al. 2003), and as evidence accumulates of man-induced global warming, we need a comprehensive understanding of how climate change will affect animals if we are to manage them appropriately. Against this background, geographic gradients are of special interest for the study of climatic adaptation.

The principal aim of my visit to Aarhus University was to get handle on the physiological and genetic mechanisms underlying thermal adaptation in *L. tityrus*, to transfer *Drosophila* expertise to this non-model organism and to answer following questions:

1. Does the expression of HSPs vary predictably across populations from different altitudes?
2. How do acclimation and induction temperatures (see below) affect HSP expression?

Therefore, Hsp/hsc70 expression patterns of replicated high- (~1800-2000 mNN) and low-altitude (~300-500 mNN) populations were measured. Butterflies were reared at two different temperatures (20 and 27°C) and treated for one hour at 1, 10, 20, 27 or 37°. Afterwards, butterflies have been back-transferred to their original temperature for another hour to allow for up-regulation of HSPs. Afterwards, they were frozen at -80°C. For analyzing Hsp/hsc70 expression, standard immunological and molecular methods (ELISA) as described by Sørensen et al. (1999) were used.

Main results and discussion

A significant interaction between altitude and rearing temperature indicates that differences in altitudes occur only when reared at the higher temperature (see Fig. 1 A), resulting in a clear increase in Hsp/hsc70 expression in butterflies from lowland populations. 27°C is a relatively high average temperature for *Lycaena tityrus* butterflies, a temperate region species. It is known that stressful conditions can amplify phenotypic differences between genotypes (Hoffmann & Merilä, 1999; Blanckenhorn & Heyland, 2004), so maybe this is also the case here. So it seems that the lowland butterflies rely more on plasticity, the highland ones rely more on fixed resistance for adaptation.

Further, Hsp/hsc70 expression varies between the different test temperatures (Fig. 1 B) and is on average higher when reared at 27°C (0.486 ± 0.005) than at 20°C (0.454 ± 0.004). Note here, that the expression is at its minimum at the respective developmental temperature (i.e. 27°C reared has lowest expression at 27°C and likewise for 20°C reared), potentially resulting from beneficial acclimation (Wilson and Franklin 2002).

Moreover, differences between the sexes were restricted to butterflies from the lowland populations (significant altitude-by-sex interaction, see Fig. 1C) and when reared at a higher temperature (significant rearing temperature-by-sex interaction, see Fig. 1 D), indicating that females generally show higher plasticity in expression patterns or faster expression patterns than males.

In conclusion, it seems that environmentally-induced plasticity (across different rearing and test temperatures) has a stronger effect on Hsp/hsc70 expression than genetic factors (across different altitudes).

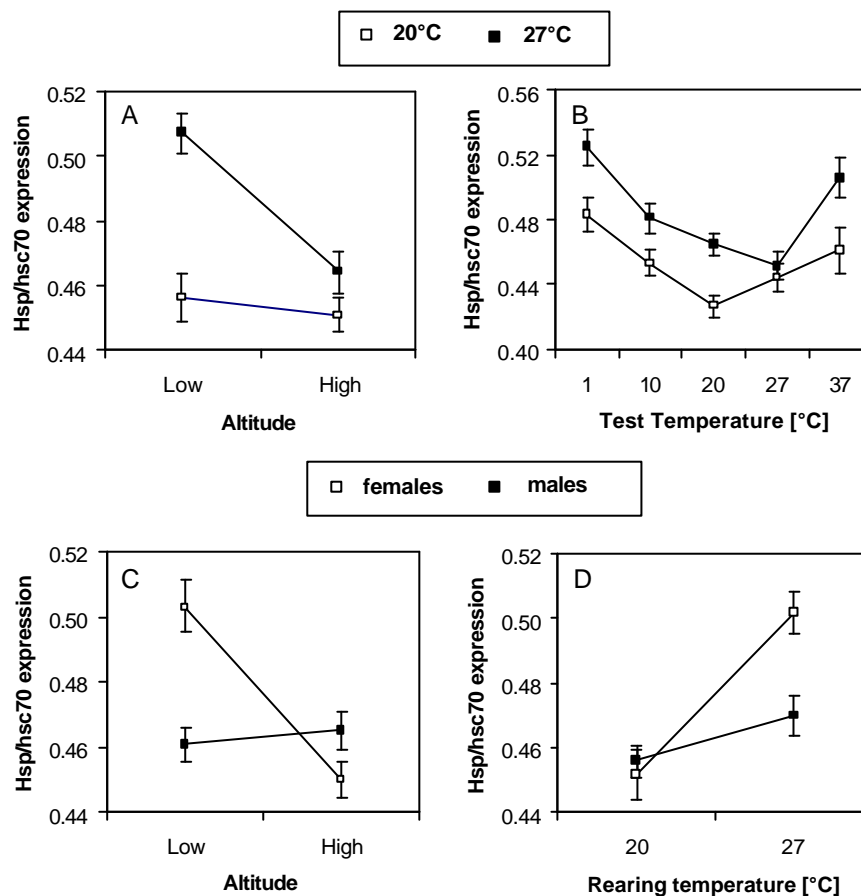


Fig 1. Means \pm 1SE for temperature and sex effects on Hsp/hsc70 expression in *Lycaena tityrus* butterflies (A) across rearing temperatures and altitudes, (B) across test temperatures and altitudes, (C) across altitudes and sexes and (D) across rearing temperatures and sexes. Data were pooled across two replicates each.

Expected publication resulting from the grant:

Title: "Altitudinal variation in thermal resistance and the expression of heat-shock protein in a butterfly".

Comments:

My visit in Aarhus was a very interesting and informative stay. The supervision and organisation by Prof. Dr. Volker Loeschcke and Dr. Jesper G. Sørensen was excellent. My work could be done without any interruptions and this stay in Aarhus gave me the opportunity to extend my technical skills, which will enhance my future career opportunities. Therefore, we are very thankful to ESF for the grant.