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SCIENTIFIC REPORT ESF THERMADAPT EXCHANGE GRANT

Applicant

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Project title

Thermal acclimation and *Pgi* in the Glanville fritillary butterfly

Name of host and host institution

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Purpose of visit

The aim of the proposed study was to investigate larval and adult thermal acclimation and performance (chill coma recovery) and reversibility of acclimation in the Glanville fritillary (*Melitaea cinxia*) butterfly, and link this phenotypic variation to genetic variation for *Pgi* and possibly other candidate genes.

Background
Temperature is one of the key environmental factors that affect survival and growth of organisms, and population dynamics (*1*). Variation in temperature is especially critical for ectothermic (cold-blooded) animals such as insects, whose body temperature is dependent of the ambient temperature (*2*). Even though the ability to sense, cope with or even adapt to thermal variation and/or stress is essential for the survival of individuals and populations, we still know little about the variation in the possible strategies and genetic mechanisms involved in such processes in wild

populations. Acclimation to ambient temperatures can be an important mechanism for organisms to cope with short- and long-term thermal heterogeneity in the environment (e.g. *3*), and can be seen as the organism's ability to fine-tune its phenotype to the actual and potentially variable thermal conditions (*4)*. However, tests demonstrating fitness benefits of acclimation are rare, and in many cases the adaptive value is not clear. Also, very little is known about the energetic and ecological costs of acclimation (*2*). Moreover, the genetic background of acclimation and phenotypic plasticity in general is largely unknown. The metabolic gene Phosphoglucose isomerase (PGI) has been found to be highly polymorphic in a wide range of taxa, and this variation often correlates with variation in individual performance and fitness (e.g. *5,6,7*). Research has indicated PGI is particularly important for temperature adaptation (*8, 9*), and is thus an interesting candidate gene in the study of thermal acclimation. Several studies have demonstrated the importance of PGI in thermal adaptation of the Glanville fritillary, especially in relation to flight and dispersal (e.g. *7,10*). Given the importance of PGI in thermal performance in the Glanville fritillary and other species, we hypothesised that PGI may also play a role in thermal acclimation.

Work carried out during the visit

Due to problems with the breeding of Glanville fritillary larvae, the available sample size was smaller than expected and the experimental design was adjusted. However, the altered experimental set-up, as described below, still measures the influence of larval and adult thermal acclimation on adult chill coma recovery, as initially proposed.

Experimental set-up

Caterpillars were reared in two temperature treatment groups, one under cold conditions and one under optimal conditions, to test whether acclimation to temperature occurs during the larval development and whether it has an effect on the adult performance. The temperatures of the different rearing treatments represented more or less average day and night temperatures during spring (low temperature group) and summer (standard temperature group) and fall within the temperature range the larvae can experience in the field. Larval development time and weight at pupation were recorded. Upon eclosion, chill-coma recovery time was measured for individual butterflies, to assess a potential effect of thermal developmental environment on initial adult performance. Subsequently, butterflies from both larval treatment groups were kept at the same optimal temperature. After 6 days, butterfly chill-coma recovery time was again assessed to determine whether the potential differences as an effect of larval treatment were still present, or whether this difference was altered by adult acclimation to the common environment. Afterwards, butterflies were frozen for DNA extraction and sequencing to determine *Pgi* genotype.

Progress

The experimental part of the project was carried out successfully. DNA has been extracted of the butterflies used in the experiment, and soon the butterflies will be genotyped for the gene *Pgi*. In addition, I am selecting and developing primers for a set of additional candidate genes associate with thermal adaptation, and in the future the butterflies will be genotyped for these genes as well.

Preliminary results:

Because sample sizes for females were too small, we included only males in the analysis. A regression analysis between butterfly chill coma recovery on the 1st and the 6th day of adult eclosion showed a significant correlation between early and late adult thermal performance ($R^2 = 0.15$, $P < 0.001$, Figure 1). There was no significant effect of rearing temperature on pupal weight. A Kruskal-Wallis test showed a significantly lower chill coma recovery time measured one day after butterfly eclosion for the standard rearing temperature group compared to the low rearing temperature group ($P = 0.019$, Figure 1B). This indicates an effect of rearing temperature on initial adult thermal performance. When the butterflies were measured again after 6 days, there was no significant difference between treatment groups ($P = 0.17$, Figure 2B). This indicates the effect of acclimation to the common standard adult temperature, reversing the effects of the initial larval acclimation. In summary, our results indicate the influence of both larval and adult thermal acclimation on chill coma recovery in male Glanville fritillary butterflies.

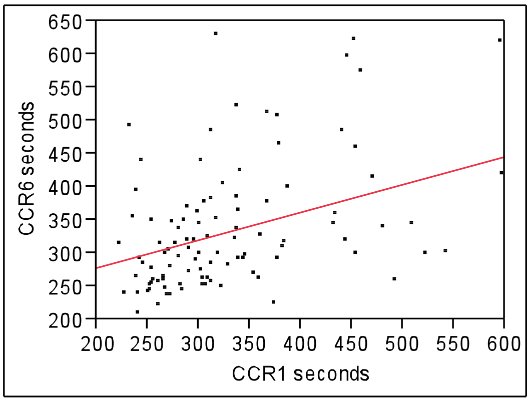

Figure 1. Significant regression of adult male chill coma recovery after one day (CCR1, x-axis) and six days (CCR2, y-axis) after butterfly eclosion.

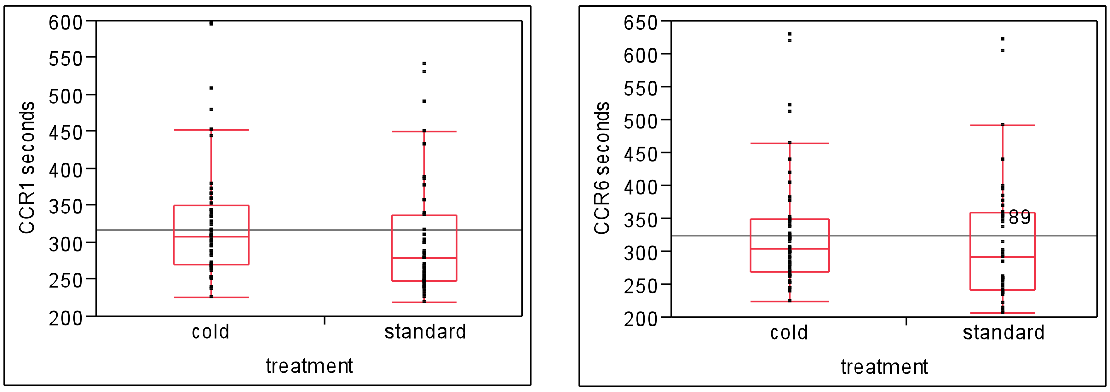

Figure 2. Box-plots of chill coma recovery time in seconds for the two larval rearing temperature treatments. 2A (left) shows a significantly lower adult recovery time for the standard larval temperature group compared to the low temperature group after 1 day of butterfly eclosion, while the difference in adult recovery time between groups is non-significant when measured after 6 days (2B, right)

Future collaboration with host institution and projected publications

The ESF ThermAdapt exchange visit has resulted in a valuable and ongoing collaboration for both sides. After my PhD defence in the Netherlands in December 2010, I was hired as a postdoc in the Metapopulation Research Group, where I will continue to do research on thermal adaptation and ecological genetics in butterflies.

We plan to publish the results from this project in a peer-reviewed journal. Furthermore, the study has provided valuable ideas and directions for future research plans on thermal adaptation in the Glanville fritillary.

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