

Final report of the project:

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Project title: "Effects of food and temperature stress on adult immune function in the tropical butterfly *Bicyclus anynana*"

Host: Prof. Dr. Robby Stoks, Laboratory of Aquatic Ecology and Evolutionary Biology, Charles Deberiotstraat 32, B-3000 Leuven, Belgium.

Background and purpose of the visit:

Increasing immune defence requires resources and is therefore expected to be associated with a reduction in other fitness components, such as starvation resistance. Additionally, increasing temperature stress may also exert negative effects on immune function, either by a general reduction due to stress and / or based on resource-allocation trade-offs (assuming that both functions are costly).

The principal aim of this study was to get handle on the physiological mechanisms of the immune response and to investigate whether and how starvation and/or temperature affect the immune response in our study organism. As a first step measurement of PO (phenoloxidase) activity was optimised for this study species, and afterwards the immune response (PO activity and haemocyte number) was determined to answer the following questions:

- Does larval and/or adult food stress influence adult immune response?
- Is the immune response affected by short-time heat or cold stress?
- Do effects of temperature on immune defence depend on starvation treatments?

Therefore, larvae (reared at 27°C) have been divided into two groups on day 18, one control group (without any starvation) and one starvation group (starved for 30 h). Following adult eclosion, both groups were again randomly divided among two further treatments, adult starvation (obtaining water only) and adult control (fed with moistened banana ad libitum). Temperature stress was applied on day 3 after eclosion, with the individuals of each starvation group being treated for 24h either with 10°C (cold stress), 27°C (control) or 34°C (heat stress). Butterflies were frozen and immune function was determined.

Main results

Haemocyte number was significantly affected by larval food treatment, adult food treatment and temperature, but did not differ between sexes (see Figs 1A and 1B). While, other than expected and hard to explain, haemocyte number was higher in larval-starved groups than in non-starved groups ($7.7 \pm 0.3 > 6.5 \pm 0.3$), we found the expected patterns of reduced number of haemocytes in adult-starved individuals (6.18 ± 0.3) compared to non-starved ones (7.9 ± 0.3 , see also Fig. 1B). Further, haemocyte number decreases with increasing temperature (10°C: $8.2 \pm 0.3 > 27^\circ\text{C}$: $7.0 \pm 0.3 > 34^\circ\text{C}$: 5.9 ± 0.3). A significant sex-by-temperature interaction indicates that at 10°C females show a higher number of haemocytes than males, but not at the other temperatures.

Activity of phenoloxidase was significantly affected by all tested factors, being higher in females (46.5 ± 1.8) than in males (28.4 ± 2.1 , see Fig. 1C), in larval non-starved individuals (42.2 ± 1.7) than in starved ones (32.7 ± 2.0), in adult non-starved ones (47.4 ± 1.8) than in starved ones (27.5 ± 1.9 , see Fig. 1D) and at 10°C and 27°C than at 34°C ($41.1 \pm 2.2 = 39.6 \pm 2.3 > 31.6 \pm 2.3$). A significant interaction between adult food and temperature (see Fig. 1D) indicate that PO activity decreases with increasing temperature in the starved treatments, but was highest in the control group compared to the temperature groups under non-starved conditions. A significant adult food x sex interaction additionally shows that effects of food stress are more expressed in females than in males. Finally, significant three- and four-way interactions emphasize the complexity of the immune response.

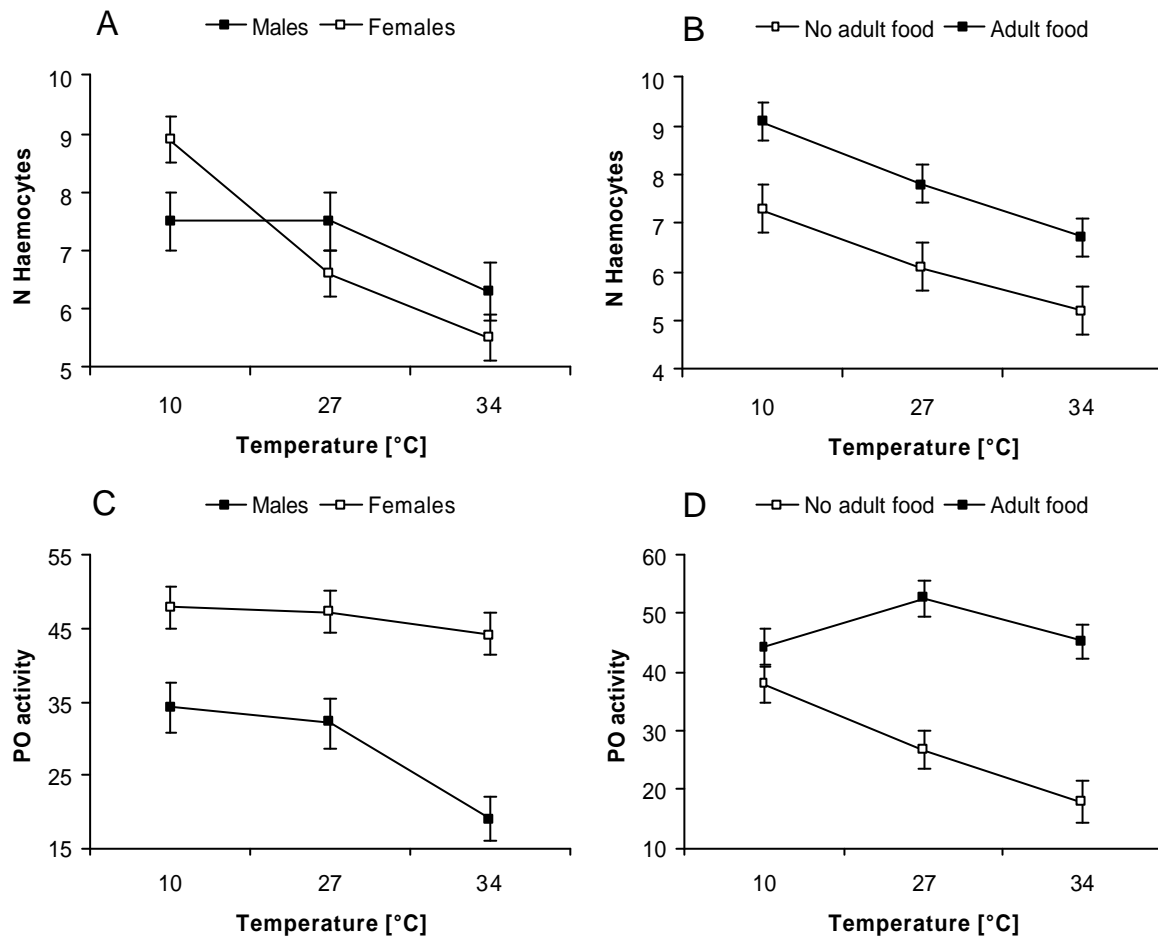


Fig. 1. Least square means \pm 1 SE across test temperatures for (A) the number of hemocytes for males and females, (B) the number of hemocytes for adult fed and non-fed individuals, (C) PO activity for males and females, and (D) PO activity for adult fed and non-fed individuals.

Expected publication resulting from the grant:

Title: "Effects of food and temperature stress on adult immune function in the tropical butterfly *Bicyclus anynana* "

Comments:

My visit in Leuven was very informative and a great scientific experience. Everything, including the supervision and organisation by Prof. Dr. Robby Stoks and Dr. Marjan De Block, the help in laboratory work by Ria Van Houdt and the overall working

atmosphere, was excellent. I was able to extend my technical skills and to do my work without any interruptions. Therefore, we are very thankful to ESF for the grant.