Fly Heat and/or Cold Treatment Protocol

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Protocol references: Brown JB et al. 2014 (PMID: 24670639), Hoffman et al. 2003, Neal, S.J. et al. 2006.

Cold Treatments:

Method One: Newly eclosed flies were collected and cornmeal agar food vials containing 20 males and 20 females were kept at 25° C for 84 hours. Aged, mated flies were transferred to empty glass vials and placed in a micro-cooler water bath containing 10% glycol at 25° C. The temperature was decreased to 0° C at a rate of 0.2° C per minute and then flies were held at 0° C for 9 hours. After the cold treatment flies were transferred to fresh food vials and kept at 25° C for 2 hours for the recovery period. Following recovery flies were placed in 2 ml tubes, flash frozen in liquid nitrogen and stored at -80° C prior to RNA preparations.

Method Two: Flies were treated as above, except flies were held on food vials for four days. Aged, mated flies were transferred to empty glass vials and placed in a micro-cooler water bath containing 10% glycol at 0°C for two hours. Following the cold shock flies were transferred to fresh food vials and kept at 25°C for 30 minutes for the recovery period. Following recovery flies were placed in 2 ml tubes, flash frozen in liquid nitrogen and stored at -80°C prior to RNA preparations.

We used both methods because it is clear from the literature that acclimation and instant shock produce different transcriptional responses.

From Hoffman et al 2003: Physiologists commonly refer to a short-°©-term exposure (minutes or hours) to sublethal conditions as “hardening” (Cossins and Bowler, 1987). This treatment predominantly gives rise to reversible physiological changes, although some effects may last throughout the entire lifespan of an insect (Khazaeli et al., 1997). Long-term exposure (days or weeks) to conditions within the normal viable temperature range of an organism is usually termed “acclimation” and gives rise to both reversible and irreversible changes in physiology.

Heat treatment:

From Hoffman (pers. comm): For heat, there are many possible protocols, depending on whether one aims for the stress phase or recovery after stress phase. The latter may include acclimation responses, which will involve quite different transcription responses than the stress phase. And then it depends on how one does the stress phase, in particular whether you aim for ramping temperature stress or sudden stress. The former will produce hardening responses, but also potentially damage. What we do know about heat is that the genes affected depend critically on the details of the different stress tests.

From Tim Westwood (pers. comm.): "For flies, one can pretty much use the method we use for larvae. Note that the preferred method is to put the larvae, flies, or cells in a sealed tube and submerse them in a water bath. For cells in culture, we usually transfer about 3-°©-5 mls of cells suspension to a 15 or 50 mL screw cap centrifuge tube and submerse them is a water bath. In the Physiological Genomics paper we also used air (i.e. incubator) heat shock because for the neurophysiological preps, that what was practical. It did give a different heat shock profile though.

We have done a time course of heat treatments on Drosophila S2 cells.
The gene lists and amount of induction looks pretty similar from 10 to 30 minutes. We know from other experiments that by about 20 minutes of HS in larvae, pol II starts accumulating to a higher degree at the major hs gnee loci and starts to disappear from some of the "minor" loci. Therefore, we feel that a 20-30 min hs is a good compromise to get as many different types of heat induced transcripts as possible. A heat shock plus a recovery at room temperature will give a different transcription profile since some additional genes will be induced during the recovery."

Take note of the following table concerning % lethality of heat and cold treatments:

Treatment	Stage	% Lethality	Notes
Heat Shock	Adults	35%	1HR @36°C, Wet Heat 30 min @ 25°C
Instant Cold	Adults	~0%	2 HR @ 0°C; 30 min @ 25°C
Cold + Hardening	Adults	~0%	25°C to 0°C, 3HR 9HR @ 0°C 2HR @ 25°C