An association test for survival to heat stress in larvae and adults in *Drosophila melanogaster*.

Arias, Leticia N., Federico H. Gomez, and Pablo Sambucetti. Departamento de Ecología, Genética y Evolución, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires. (C-1428-EHA) Buenos Aires, Argentina.

Introduction

In insects, survival to heat-stress (SHS) is a trait related to thermal adaptation (Hoffmann *et al*., 2003). SHS can increase by acclimation, as heat acclimation is the increase in thermotolerance due to either repeated or long-term exposures to heat stress (Hoffmann *et al*., 2003). In *Drosophila melanogaster,* previous work showed that both SHS and another trait of thermal adaptation, knockdown resistance to heat stress (KRHT), is influenced by several Quantitative Trait Loci (QTL) including one large-effect QTL in the middle of chromosome 2 (Norry *et al*., 2004, 2008, 2009; Morgan and Mackay 2006). Here we used a subset of recombinant inbred lines (RIL), which segregate different alleles of the above mentioned QTL (Norry *et al*., 2009), to explore for possible associations between SHS in larvae and adults as well as between SHS and a trait of resistance to a stress by UV radiation (UV-C resistance) in adult flies.

Material and Methods

Recombinant inbred lines: The lines in this study were described elsewhere (Norry *et al*., 2008, 2009). Briefly, parental lines used to construct RIL were previously selected for knockdown resistance to heat stress. Here we used nearly the same subset of RIL used by Norry *et al.* (2009) as these lines differ in QTL-alleles affecting thermotolerance in adult flies: D48-4, D48-31, D48-35, D48-39, D48-57, D48-78, and D48-110 (all of them described in Norry *et al*., 2008, 2009).

*Phenotypes measured***:** In adult flies, SHS was measured as the proportion of survivors 1 day after exposing 20-30 flies of 4-5 days of age at 39°C for 35 min, using small vials within a water bath. These measurements were performed in both acclimated and non-acclimated flies with two averaged replicates per RIL. Acclimation treatment in adults consisted in exposing flies at 32°C (water bath) for 4 h (12:00 to 16:00) at the ages of 1 to 4 days. Flies received no anesthesia treatment throughout the experiment. Survival was scored only in females, 24 h after the heat-shock exposure, as the number of females that were able to stand up on their legs.

In the pre-adult stage of the life cycle, survival to heat stress was measured as the proportion of newly-emerged flies after a heat stress (33°C for 3 h) in third instar larvae, with and without an acclimation pre-treatment. To acclimate larvae, culture vials (at 25°C) were transferred to 29°C for 3 h per day, for 3 consecutive days, starting two days after transferring 40 eggs in each experimental vial per RIL, with 8 replicated vials per RIL. The mean value of the 7 replicates per RIL was used for analysis. All cultures were maintained at 25°C otherwise.

To measure adult survival to UV-C, flies were exposed directly to UV-C radiation at 25°C until the last fly was dead. Mortality was scored every day in females, when flies were transferred to new vials with fresh medium. Experimental vials were plastic vials (2 × 10 cm) with culture medium, using a thin net as lid to allow UV-C radiation and 20 flies per vial. These vials were

placed within a box containing an UV-C lamp at the top. About 20 females of 1 day of age were placed within each vial, with two replicates per RIL.

Results and Discussion

In adults, survival to heat-stress was highly variable across RIL without acclimation (Figure 1). Acclimation consistently increased SHS in adults (Figure 1; Table 1, the difference between acclimated and non-acclimated flies being significant in ANOVA using "acclimation status" as fixed factor, $P < 0.001$). SHS was negatively correlated between acclimated and non-acclimated flies across RIL in adults (Table 2). There was no correlation between SHS in adults and UV-C resistance (Table 2). In contrast to adults, SHS was positively correlated between acclimated and non-acclimated larvae (Table 2).

The only across-RIL correlation that was significant between life stages in the present study was a positive association between UV-C resistance in adults and SHS in larvae, but this correlation disappeared after acclimation in larvae (Table 2). This result suggests that heat acclimation can reduce the phenotypic correlation between different traits of resistance to environmental stress. The overall lack of SHS associations between larvae and adults suggests that phenotypic variation in survival to heat stress is, at least partially, influenced by different loci in larvae as compared to adult flies. The subset of RIL lines used in the present study strongly segregates major QTL for thermotolerance in adult flies

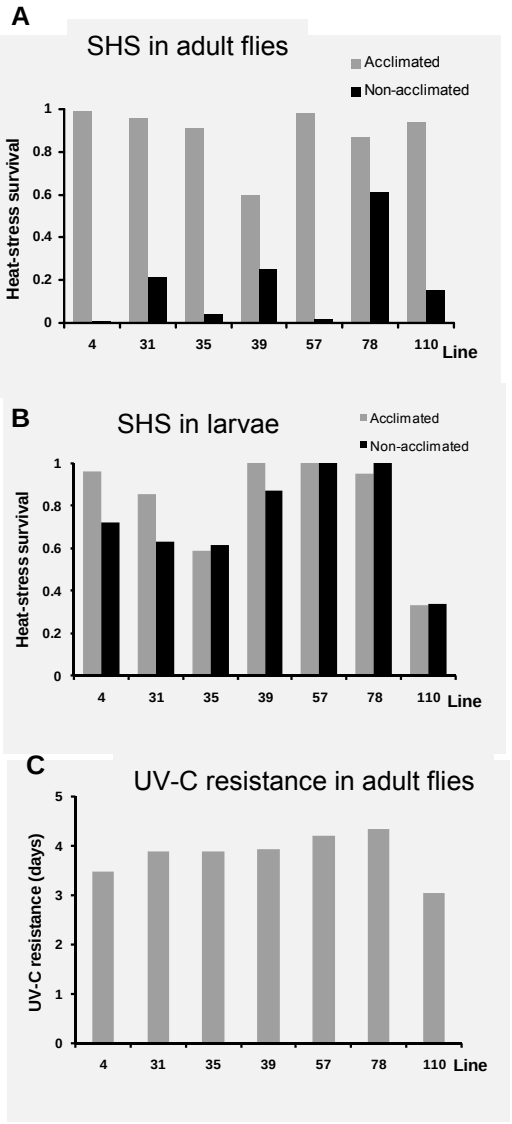

Figure 1. Heat-stress survival (SHS) in seven RIL lines is shown for both acclimated and non-acclimated adults (A) and larvae (B). Survival to UV-C is also shown for adult flies (C). Numbers on the X-axis correspond to arbitrary labels used for each RIL.

Table 1. Mean value (±SD) is shown for each trait averaged across RIL. Abbreviations for traits are: SHS-A-NA for survival to heat stress in non-acclimated adults, SHS-A-AC for survival to heat stress in acclimated adults, SHS-L-NA for survival to heat stress in non-acclimated larvae, SHS-L-AC for survival to heat stress in acclimated larvae, and UV-C for survival to UV-C in adults.

SHS-A-NA	SHS-A-AC	SHS-L-NA	SHS-L-AC	UV-C (days)
0.18 ± 0.20	0.89 ± 0.19	0.80 ± 0.32	0.83 ± 0.28	3.83 ± 0.44

Table 2. Kendall´s rank correlations between SHS phenotypes and UV-C resistance across RIL. Trait abbreviations are as in Table 1.

SHS-A-AC - SHS-A-NA	-0.62*
SHS-A-AC - UV-C	-0.33
SHS-A-AC - SHS-L-AC	-0.04
SHS-A-AC - SHS-L-NA	-0.05
SHS-A-NA - UV-C	0.33
SHS-A-NA - SHS-L-AC	0.05
SHS-A-NA - SHS-L-NA	0.24
SHS-L-AC - SHS-L-NA	0.62*
SHS-L-AC - UV-C	0.33
SHS-L-NA - UV-C	0.71*

*$P < 0.05$.

(Norry *et al*. 2009), and the present results suggest that larvae may not share these QTL as no genetic correlation was significant for SHS between larvae and adults (Table 2). A QTL mapping for SHS in acclimated and non-acclimated larvae and adults is in progress to determine what thermotolerance QTL are specific for each life stage in this set of RIL.

Acknowledgments: We thank Fabian Norry and Volker Loeschke for letting us use a subset of RIL. This exploratory test was supported by a grant from ANPCyT to FN.

References: Hoffmann, A.A., J.G.. Sørensen, V. Loeschcke 2003, Journal of Thermal Biology 28: 175-216; Morgan, T.J., and T.F.C. Mackay 2006, Heredity 96: 232-242; Norry, F.M., J. Dahlgaard, V. Loeschcke 2004, Molecular Ecology 13: 3585-3594; Norry F.M., P. Larsen, Y Liu, V. Loeschcke 2009, Journal of Insect Physiology 55: 1050-1057; Norry, F.M., A.C. Scannapieco, P. Sambucetti, C.I. Bertoli, V. Loeschcke 2008, Molecular Ecology 17: 4570-4581.

Drosophilid species collected from Nainital and Almora district, Kumaon region, India.

Kandpal, Mukul, and B.K. Singh. Cytogenetics Laboratory, Department of Zoology, Kumaon University, Nainital-263002, India; Emil-ID mukul83.ntl@Gmail.Com

The Drosophilidae is a large family of world-wide distribution. From the literature it seems that the Drosophilidae are fairly widely distributed throughout the subcontinent of India (Gupta, 1981, 1985). Studies on Indian Drosophilidae were started as early in 1920, but still a large part of sub continent awaits exploration. In recent years our studies in Kumaon region (Singh and Bahtt, 1988; Singh and Negi, 1989, 1992; Singh and Das, 1993, 1994, 1998; Singh and Fartyal, 1997, 1998; Fartyal and Singh, 2000, 2001, 2002; Fartyal, Singh, and Toda, 2005; Upadhyay and Singh, 2006), which is located at an elevation of just below 2000 m altitude on the north east periphery of the state of Uttarakhand. This region includes six border districts of the state, *viz*, Nainital, Almora, Pithoragarh, Bageshwar, Champawat, and Udham Singh Nagar. The present collection was made from Kilburry, Kailakhan, Khrishnapur, Sariatal, Anyarpata, Pines, Lariakanta, Hanuman Gari, Pangote, Bhatelia, Sheetla, Lesal, and Mukteshwar in Nainital district and Chaubatia forest (Ranikhet) in Almora district.

Several traps, usually small tin containers containing fermenting banana, guava, and some other local fruits were placed at different places under cool and shady areas. Flies were obtained by net sweeping over these traps as well as by sweeping over natural habitat. In order to procure the maximum number of flies, collection were made several times during the day. The collected flies were then transferred to culture vials containing *Drosophila* food medium for raising their progeny. The rest were preserved in 70% alcohol for further study.