

## RESISTANCE AND ACCLIMATION POTENTIAL TO THERMAL STRESSES IN ASSOCIATION OF BODY COLOR PHENOTYPES OF *D. MELANOGASTER*

S. Manvender\*

Department of Biotechnology, UIET, M.D. University, Rohtak-124001(Haryana)

\*Corresponding Author Email: [msgahalaut@yahoo.com](mailto:msgahalaut@yahoo.com)

### ABSTRACT

Insects face varying temperatures daily and seasonally, so that natural populations are exposed to heat or cold stress and these thermal fluctuations impose selection pressure on organisms. Populations of *D. melanogaster* collected from three highland and lowland localities. In the present work, motility time ( $LT_{100}$ ) after heat stress was more for lowland populations as compared with flies of highland localities but the results were reversed when mortality was measured after cold stress. Further, significant trait associations between percent melanisation and thermo tolerance were evident for both sexes of different geographical populations of *D. melanogaster*.

### KEY WORDS

Thermal stress, mortality, melanization & *Drosophila melanogaster* etc.

### INTRODUCTION

For insects, differences in heat or cold resistance match the geographical distribution patterns of species that is a high level of cold resistance is linked with higher latitudes while a reverse trend occurs for heat resistance (Hoffmann, Sorensen and Loeschcke, 2003). Genetic variations for heat and cold resistance have been examined by comparing species and populations as well as on the basis of laboratory selection experiments (Gilchrist and Huey, 1999; Hoffmann et al., 2003). Clinal variations for heat and cold resistance occur in latitudinal populations of *Drosophila melanogaster* (Hoffmann, Anderson and Hallas, 2002).

The effects of heat and cold stress have been assessed using a variety of assays (based on either mortality or knockdown/recovery time). Mortality-based assays have been considered for adult as well as pre-adult stages (pupal and larval) but these were ecologically more relevant for the

latter (Hoffmann et al., 2003). By contrast, dynamic methods (critical thermal limits and knockdown/recovery time) are non-lethal and relevant for adult flies. A large data set on critical thermal limits (CT<sub>max</sub> and CT<sub>min</sub>) has shown significant differences across species in their lower thermal limits as compared with upper thermal limits (Gaston and Chown, 1999; Addo-Bediako, Chown and Gaston, 2000). Accordingly, in *D. melanogaster* (a generalist species), we expected either similar or different levels of plasticity for body melanization and thermo tolerance traits at different growth temperatures. In ectothermic organisms, simultaneous evolution of thermo regulation and thermal sensitivity forms the basis of the co-adaptation hypothesis. According to this hypothesis, morphological traits (body coloration), behavioral thermo regulation and thermal sensitivity of performance evolved together (Angilletta, 2009). If thermal physiology and body melanization co evolved, we expected

within population associations between body color phenotypes and thermo tolerance traits. A single study, at the intraspecific level, has compared  $T_{sel}$  (body temperature selected in a thermal gradient) of five distinct color morphs of pygmy grasshoppers *Tetrix subulata* and showed a link between behavioral thermo regulation and body coloration (Forsman, 2000). Thus, simultaneous analyses of these traits in dark and light phenotypes of a given population helped in ascertaining whether such changes were correlated or not. We address the following questions: (1) do genetic effects for thermal resistance traits and body melanization exhibit parallel clinal variations along altitude in *D. melanogaster*; (2) do plastic effects co vary for body melanization and thermo tolerance traits; (3) Do body color phenotypes (dark, intermediate and light) correlated with thermal resistance traits on the basis of within and between population analyses?

## MATERIALS AND METHODS

### Collections

Wild-living *D. melanogaster* individuals (n = 200-250) were collected by sweeping net over decayed organic matter from six altitudinal sites (three highland populations: Sarahan -2165m; Chail-2226m; Dalhousie -2036m; Tav -15-17° C and RH- 40%); and three lowland populations: Parwanoo-640; Renuka- 660m; Bilaspur- 673m; Tave- 28-30° C and RH- 70-75%) in a single collection trip. For each population, wild caught females were used to make 20 isofemale lines and all the cultures were grown on cornmeal-yeast agar medium for 3-4 generations before experimental analyses. The fly density was controlled by 6-8 h egg-laying period. Because wild populations were adapted to different local climatic conditions, between-population differences were assessed through a common garden experiment. Thus, geographical

populations were grown at a constant growth temperature (21° C) for assessment of genetic effects. All assays were performed separately on 7- day-old males as well as females.

### Trait analyses

For quantifying melanization, we followed two methods: (1) visual scoring under a stereo-zoom microscope; (2) Biowizard image analysis software based on video camera, image capturing and analyses of selected melanized area per fly (Dewinter Optical Inc., Delhi, India, <http://www.dewinterindia.com>). With both the methods, we scored total melanization for six abdominal segments per fly (n=200; 10 individuals each of the 20 isofemale lines per population). Melanization is a stable trait with high heritability ( $h^2=0.60$ ) and lacks variations due to age, nutrition and culture conditions (Parkash, Rajpurohit and Ramniwas, 2008). For scoring with Biowizard image analysis software, each fly's abdomen minus viscera have been mounted on a slide. Therefore, this method was a limitation for simultaneous analysis of many traits on an individual fly. For the visual scoring method, body melanization were score from a lateral view of the female abdomen, yielding values ranging from 0 (no melanization) to 10 (complete melanization) for each of the six abdominal segments (David, Capy and Gauthier, 1990). For abdominal segments second to seventh, the relative ratios of the areas of smaller segments relative to the largest fourth segment are 0.86, 0.94, 1.0, 0.88, 0.67 and 0.38, respectively. For each abdominal segment, the melanization score out of 10 was weighted with its relative area ratio. Finally, the data on per cent melanization were calculated as (Observed weighted melanization scores of abdominal segments per fly/S relative size of each abdominal segment\_10 per fly)\_100.

## RESULTS AND DISCUSSION

We found significant geographical variation for thermal stress related traits. Heat knockdown and chill coma recovery in males as well as in females of three highland and three lowland populations of *D. melanogaster* collected from western Himalayas. *D. melanogaster* from highland localities were more cold resistant as compared with flies from lowland localities. In contrast, *D. melanogaster* population from low land localities were more resistant to heat stress than high land populations. For both the traits, traits values were higher in case of females i.e. females were more resistant to heat as well as cold stress than males. We found significant correlation between percent melanisation and thermo tolerance based on isofemale line variability and population. Heat resistance was

positively correlated with body melanisation. However, cold resistance was positively correlated with percent melanisation. Thus, *D. melanogaster* populations exhibited greater genetic as well as plastic variation for adaptation to thermal stresses.

### Analyses of climatic changes

Changes in climatic variables for six altitudinal localities of origin of *D. melanogaster* population were shown in table 1. Significant changes in average temperature were observed with elevational gradient. Average temperature showed a markedly decrease in highland localities i.e. ( $T_{ave}$  - 15.8 – 17.7° C). Contrasting differences for relative humidity were also observed in different altitudinal populations. There was negative cline for average relative humidity with increase in altitude.

**Table 1. Geographical and climatic variables of origin of six latitudinal populations of *D. melanogaster*.**

Populations	Altitude (m)	Long. (°E)	$T_{ave}$ (° C)	RH (%)
a) Highland				
Sarahan	2165	76° 17'	15.23	42.12
Chail	2226	76° 15'	16.12	40.56
Dalhousie	2036	76° 47'	17.02	41.28
b) Lowland				
Parwanoo	640	76° 81'	28.56	70.21
Renuka	660	74° 65'	29.14	72.54
Bilaspur	673	77° 05'	30.26	74.26

Alt.– altitude; Long.–longitude;  $T_{ave}$ .–Average temperatures; RH (%) – percent relative humidity.

Such climatic changes are likely to impose selection pressure on thermal related traits in *D. melanogaster* collected from highland and lowland localities. Highland populations are colder and drier as compared to lowland localities.

### Geographical variations for percent mortality due to thermal stresses

We assessed whether there were geographical variation for percent mortality to heat or cold

stress as a function of different stress durations in both the sexes from one highland (Chail) and one lowland (Parwanoo) populations of *D. melanogaster* and results were illustrated in fig. 1 (A-D). For heat stress effects, lowland populations showed a higher resistance for both males as well as females ( Males -59.23; Females -74.23; fig 1 A and C) as compared with highland populations (Male -42.23; Female -49.52 ) in fig 1 (B and D) of *D. melanogaster*. Furthermore, percent mortality

for males as well as females after different duration of cold stress was lower for highland population (Males – 74.6; Females -89; fig 1 B and D) as compared with lowland population (Males – 44.57; Females -57). Thus, we found significant geographical variation for thermal stress related traits in *D. melanogaster*.

Our results suggested that highland populations could tolerate longer exposure of cold stress i.e.

could survive for longer durations than the individuals of lowland populations after cold shock ( $0^{\circ}\text{C}$ ). In contrast, *D. melanogaster* individuals from lowland localities could survive longer under heat shock as compared with highland localities. Thus, we have found significant geographical variation for percent mortality in *D. melanogaster* but in opposite directions.

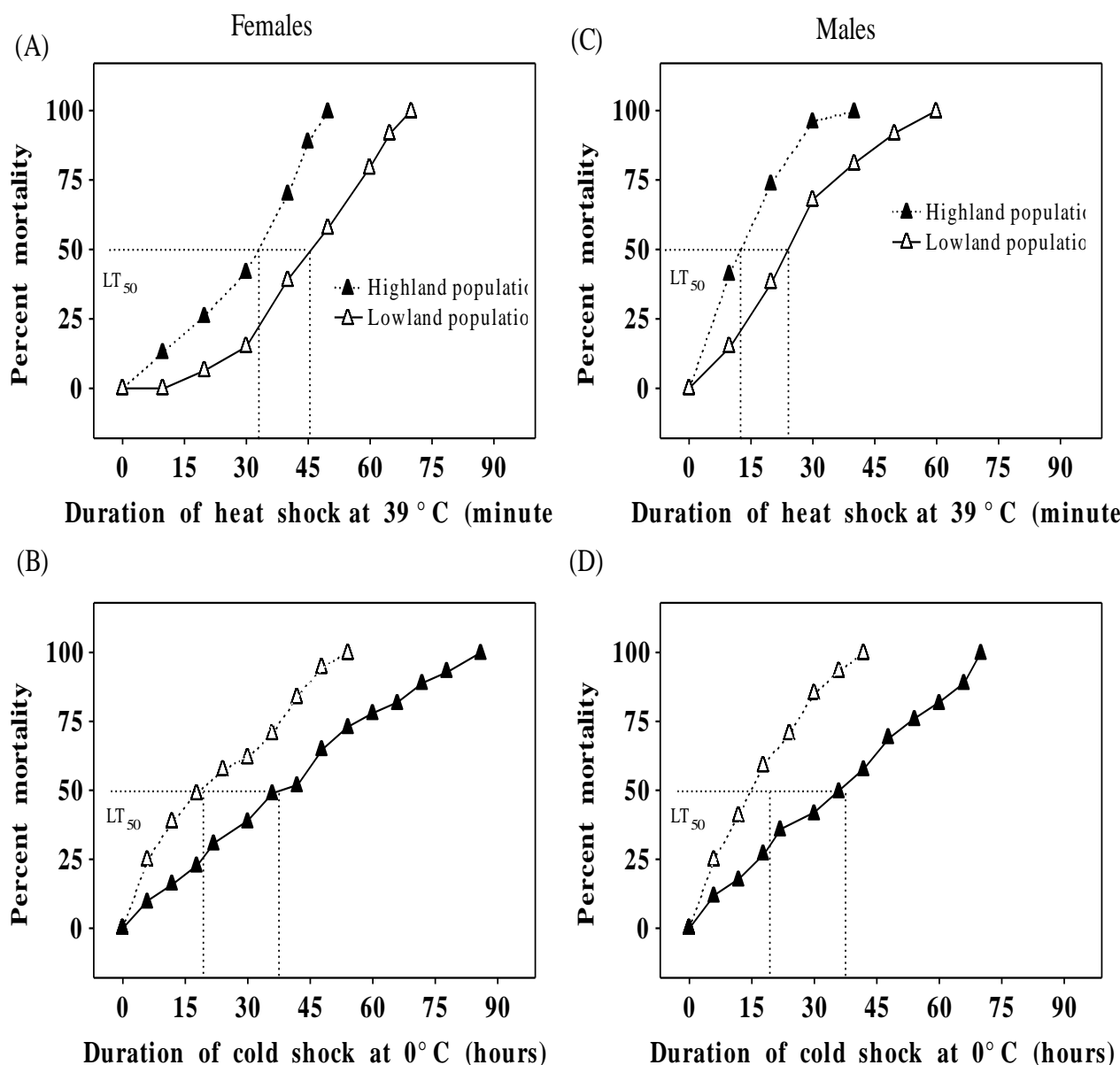
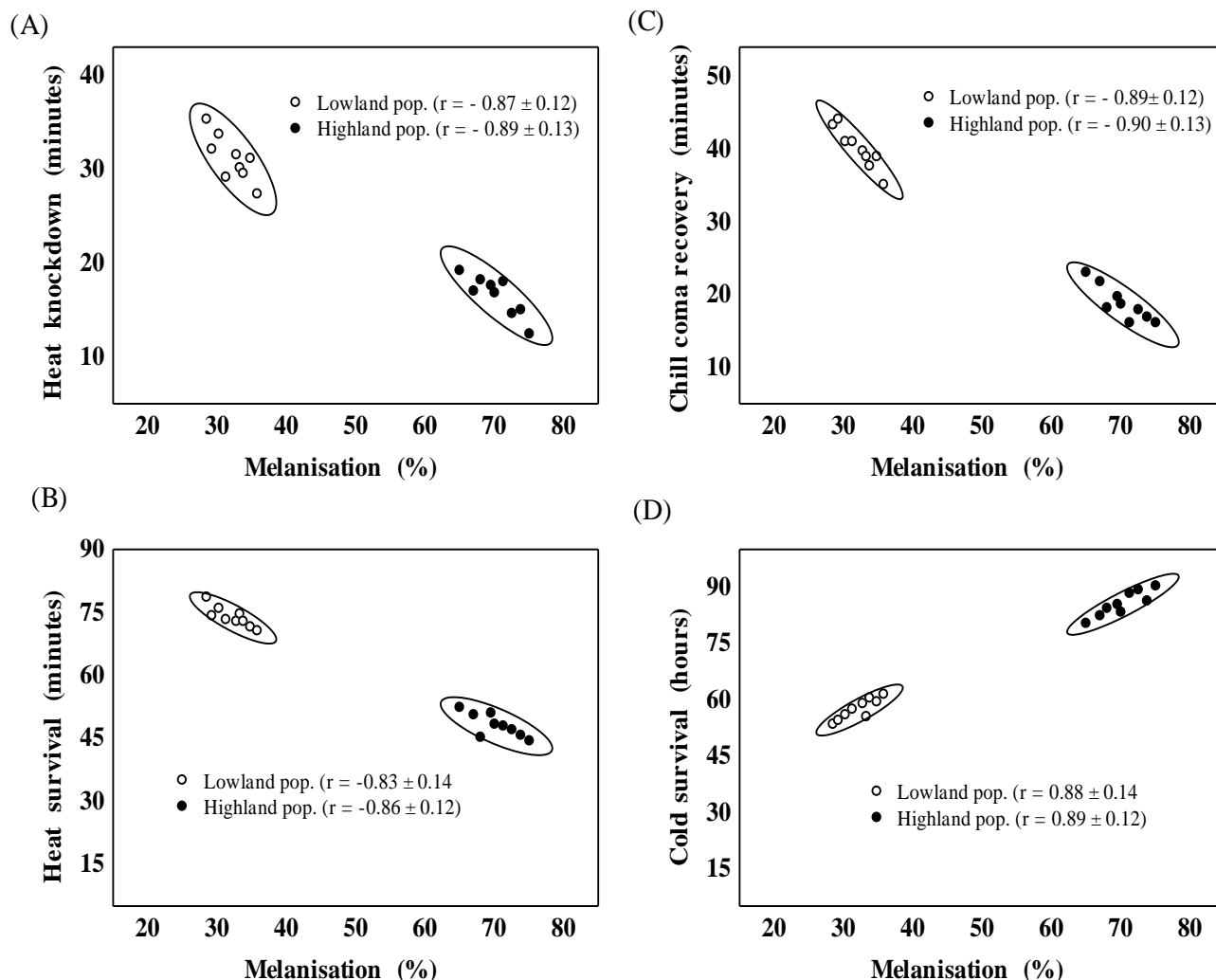


Fig. 1. Percent mortality as a function of different durations of heat stress at  $39^{\circ}\text{C}$  (A) and cold stress at  $0^{\circ}\text{C}$  (B) in one highland (Chail; HL) and one lowland (Parwanoo; LL) population of *D. melanogaster*.

### Association between percent melanisation and thermal resistance

We observed significant trait correlation between percent melanisation and thermo tolerance traits

based on isofemale line data as well as between-population analyses of one highland and one lowland population of *D. melanogaster* (figure 2).



**Figure 2. Trait correlations based on isofemale line variability between percent melanisation and thermo tolerance in one highland and one lowland population of *D. melanogaster*.**

There were significant correlations for heat and cold resistance as a function of body melanisation but in opposite directions. Heat resistance was negatively correlated with percent melanisation ( $r = -0.87 \pm 0.12$ ;  $***P < 0.001$ ) for both highland as well as lowland populations of *D. melanogaster* i.e. higher the percent body

melanisation of the fly, lesser was the resistance to heat (Fig 2A and B). In contrast, there were positive correlation between cold resistance and percent body melanisation ( $r = 0.89 \pm 0.13$ ;  $***P < 0.001$ ; Fig 2 C and D). Higher melanic individual were more resistant to heat i.e. recover faster from chill coma and could survive for longer time

after cold stress as compared with lighter individuals. However, more melanic flies were less resistant to heat and knocked down faster than the less melanic ones. Thus, trait correlations were based on isofemale lines as well as within- population variability were highly significant and suggested correlated changes for these traits.

### Conclusions

In the present work, consequently, mortality time ( $LT_{100}$ ) after heat stress was more for lowland populations as compared with flies of highland localities but the results were reversed when mortality was measured after cold stress. Further, significant trait associations between percent melanisation and thermo tolerance were evident for both sexes of different geographical populations of *D. melanogaster*. Thus, genetic as well as plastic responses for thermal tolerance traits were likely to be responsible for adaptation of *D. melanogaster* to divergent climatic conditions.

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**\*Corresponding Author:**  
S. Manvender  
E-Mail: [msgghalaut@yahoo.com](mailto:msgghalaut@yahoo.com)