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HABITAT SELECTION OF PUPATION HEIGHT AND CORRELATED CHANGES IN BODY MELANIZATION AND DESICCATION RESISTANCE IN *DROSOPHILA MELANOGASTER*: ADAPTATIONS TO ALTITUDINALLY VARYING ENVIRONMENTS.

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ABSTARCT:

In heterogeneous environment behavior related with habitat selection of pupation height may be of great importance in determining the genetic structure of populations. In the present study wild collected nine Indian altitudinal populations of cosmopolitan species, *D. melanogaster*, from low to high altitude locality (219 m to 2202 m) were investigated for abdominal melanization and desiccation resistance. Their laboratory populations reared at two different growth temperatures (17 & 25 ºC) were analyzed for pupation height, abdominal melanization and desiccation resistance. Genetic correlations, based on family means, were significantly high for both abdominal melanization and desiccation resistance, as a function of altitude. Present investigation evidence that habitat selection of pupation height and correlated changes in body melanization confers desiccation resistance in altitudinal populations of *Drosophila melanogaster*.

Keywords: *Habitat selection, abdominal melanization, desiccation resistance, pupation height.*

1. INTRODUCTION:

In all living organisms, physiological traits play a major role in conferring adaptations under varying environmental conditions [Hoffmann & Parsons (1991); Bijlsma & Loeschecke (1997); Parkash *et. al.* (2005)]. Behaviour related with habitat selection may be of great importance in determining the genetic structure of populations [Toylor (1976)]. Insect cuticle is an important interface between physiological systems and the varying environmental conditions [Neville (1975). In *Drosophila*, third instar larvae show a well defined tendency to leave the humid food, searching for a suitable pupation site [Grossfield (1978)]. The choice of a place to pupate could be of adaptive value because pupae remain immobile for several days and exposed to biotic and climatic conditions of the elected site, such as predation, diseases, desiccation etc. Thus, habitat selection of pupation height is considered to be the primary factor for controlling the genetic variability in heterogeneous environment.

The role of body melanisation has been reported for thermoregulation in beetles and butterflies [de Jong *et al.* (1996); Ellers and Boggs (2004)]; and for conferring desiccation resistance in altitudinal as well as latitudinal populations of two *Drosophila* species [Parkash *et al.* (2008a, b)]. However, it is not clear whether species specific differences in body melanisation can impact their stress resistance levels. It is generally assumed that populations have abundant genetic variations in quantitative traits for adaptation [Hoffmann and Weeks, 2007)]. By contrast, there are limited data on the evolutionary responses of stress related traits in tropical *Drosophila* species from humid habitats [Hoffmann *et al.* (2003)].

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In the present investigation, an attempt was made to analyse the habitat selection of pupation height and correlated changes in abdominal melanization and desiccation resistance in nine altitudinal populations of *Drosophila melanogaster*.

2. MATERIALS AND METHODS:

Wild living adults of *Drosophila melanogaster* were collected from nine Indian geographical sites which were quite distant from each other and included three low altitudinal sites (219 to 600 m); three mild (761 to 1440m) and three high altitudinal localities (1770 to 2202m). From each collection site, about 30 to 75 wild-caught individuals were obtained which were used for measuring segment-wise abdominal melanization. The individuals scored for abdominal melanization, were used to initiate the mass culture and ten isofemale lines per population. Five replicates of isofemale lines from each population were maintained at both 17 ° & 25 °C growth temperature.

For scoring pupation height, 30 pairs were allowed for egg laying in long measuring cylinders at both 17 & 25 °C growth temperature for maximum time period of four hours to avoid experimental error due to age difference. After egg laying the parents were discarded and the cylinders with eggs were put in BOD incubators at their respective growth temperatures. At the end of pupation time, the height of each pupa was measured and recorded. These measurements were made when all the larvae had pupated but prior to the eclosion of the adults. Pupation height was determined as the distance in centimeter of each pupa from the surface of food. The pupation height was considered to be zero when larvae pupated on the food surface. Five different classes were considered for scoring pupation height. Figure 1 depicts the variability of pupation height in three (low, mid and high) altitudinal populations of *D. melanogaster*.

Mass cultures of different populations were used to standardize experimental protocols for abdominal pigmentation and desiccation resistance. These preliminary experiments helped in ensuring accuracy and repeatability for all subsequent experiments done simultaneously for various traits in replicated isofemale lines of each population. For all cultures, transfers were made with randomly selected forty pairs each generation. For each population, isofemale lines were maintained as 3 to 5 replicates because experiments required simultaneous analysis of pigmentation and mortality based desiccation resistance. All experiments were initiated soon after collections and were performed with successive generations.

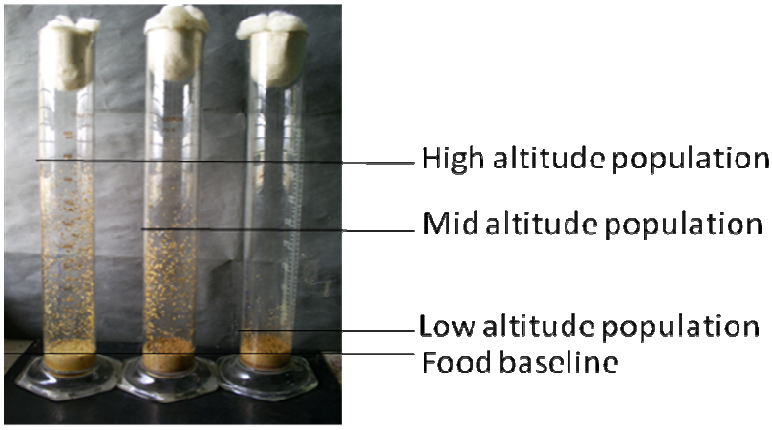

Figure 1: Comparison of pupation height in three (low, mid and high) altitudinal populations of *D. melanogaster*.

The newly emerged adults were fed on *Drosophila* nutrient media, that provides sufficient nourishment during development, for a period of six days and then their segment-wise abdominal melanization was scored. The dark pigmentation on the abdominal segments (5th to 7th) was estimated visually, and their sum was also calculated. For each segment, 11 cases ranging from 0 (no dark pigment) to 10 (segment completely dark) were used.

For measuring desiccation resistance, after scoring segment-wise as well as sum of pigmentation for each isofemale line, ten individuals were isolated in a dry plastic vial closed with a plastic cap. These vials

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contained 4 gm of silica gel at the bottom of each vial and covered with a disc of plastic foam piece. Four such replicates were run for each isofemale line (n=40 for males and females). The vials were inspected every hour and the numbers of dead flies (completely immobile) were recorded. As the numbers of dead approached half, vials were inspected after every 30 minutes intervals till all the flies died. Such experiments were run for all the altitudinal populations of *D. melanogaster*. The survival curves due to desiccation were based on pooled data for isofemale lines per sex per population and LT_{50} values were extrapolated from such graphics.

For establishing relationship among pupation height, abdominal melanization and desiccation resistance, a small experiment was designed in which three classes of pupation height were selected. The pupae were removed carefully with the help of dissecting needles from each class separately in different vials holding food and the individuals emerged from these pupae were subjected to pigmentation and desiccation assay.

Statistical analyses:

For all the traits, isofemale line means (n=10) and population means (n=10x10) along with S.E. were used for illustrations and tabular data. Genetic basis of two physiological traits (abdominal melanization and desiccation resistance) was analyzed on the basis of repeatability across generations. Climatic data for each collection sites was obtained from climatological data book published by Indian Meteorological Department, New Delhi (Table 1). In order to find a possible link between altitudinal trait variability of physiological trait with climatic conditions (T_{max}, T_{min}, $T_{average}$, T_{cv}, RH and RH_{cv}) we attempted simple regression analysis. The usual correlations with family means, and simple regression analysis as well as all other statistical and graphical operations were done with the help of Statistica software.

Table 1: Data on geographical and climatic variables, as a function of altitude for the sites of origin of *Drosophila melanogaster*.

Population	Altitude (meters)	Latitude (°N)	T_{max} (° C)	T_{ave} (° C)	T_{cv} (%)	RH (%)	RH_{CV} (%)
Rohtak	219	28.50	31.40	25.12	15.00	68.50	3.00
Chandigarh	347	30.44	30.40	23.45	17.44	65.00	3.67
Kalka	600	30.51	22.00	15.00	18.35	57.55	6.86
Mandi	761	31.43	21.20	14.70	19.72	55.50	6.75
Dharamshala	1219	32.00	17.10	12.10	24.14	52.20	10.14
Solan	1440	30.58	16.20	11.10	27.34	52.16	12.61
Kariaghat	1770	32.20	15.00	9.80	28.09	51.00	13.00
Dalhousie	1959	32.32	14.40	9.35	29.55	48.50	13.90
Shimla	2202	31.06	11.65	7.82	31.24	46.00	15.10

T_{max} and T_{ave} represent overall average of mean monthly maximum and average values of ambient temperature respectively. T_{cv} and RH_{cv} refer to mean monthly coefficient of variation of temperature and relative humidity respectively.

3. RESULTS:

Basic data on population mean values (±SD) for abdominal pigmentation of 5th, 6th and 7th segments of *D. melanogaster* females from wild collected and laboratory reared at 17 and 25°C are given in Table 2. Regression analysis of pigmentation score is given in Fig. 2 & 3, which are showing a linear increase along with altitude. Segment wise comparison of pigmentation score for wild versus laboratory populations showed that the score was low at 25ºC in comparison with 17ºC and wild population. Slop values for wild and 17ºC were steeper in comparison with 25ºC. In wild and 17ºC, low altitudinal populations were having high pigmentation score at 17ºC as compare to wild whereas high altitude populations were showing high pigmentation score for wild in comparison of 17ºC for all the three segments scored. Slope values for segment 5, 6 and 7 were similar for wild

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collected laboratory reared populations at 17ºC whereas the populations reared at 25ºC were having lower slope values for pigmentation score.

Table 2: Data on female abdominal segment specific pigmentation scores and their sum (mean ± standard deviations) in wild collected samples (n=30-75) and their laboratory progeny [based on ten isofemale lines (n=10*10=100)] for nine altitudinally varying populations of *D. melanogaster*.

		Rohtak	Chandigarh	Kalka	Mandi	Dharamshala	Solan	Kariaghat	Dalhousie	Shimla
Wild	Seg. 5	2.30± 1.08	2.80± 1.22	3.05± 1.28	3.10± 1.33	4.30± 1.52	4.65± 1.68	4.90± 1.75	5.50± 1.79	5.60± 1.88
	Seg. 6	5.80± 1.72	6.20± 1.82	6.35± 1.85	6.90± 1.99	7.35± 1.99	7.80± 2.18	8.40± 2.29	9.10± 2.30	9.40± 2.43
	Seg. 7	3.10± 1.26	3.70± 1.41	3.90± 1.45	4.55± 1.61	5.45± 1.72	5.95± 1.90	6.20± 1.96	6.90± 2.01	7.00± 2.10
	Sum	11.20± 2.40	12.70± 2.62	13.30± 2.68	14.55±2 .90	17.10±3 .05	18.40±3. 35	19.60± 3.50	21.50± 3.55	22.00± 3.73
	N	58	75	63	37	34	72	30	48	55
17ºC	Seg. 5	2.50± 0.78	2.90± 0.86	3.00± 0.88	3.50± 0.96	3.90± 1.04	4.40± 1.14	4.70± 1.18	5.30± 1.28	5.50± 1.35
	Seg. 6	6.00± 1.20	6.40± 1.28	6.60± 1.30	7.05± 1.37	7.50± 1.45	8.00± 1.54	8.60± 1.60	8.70± 1.64	9.10± 1.73
	Seg. 7	3.60± 0.93	4.00± 1.01	4.10± 1.01	4.70± 1.12	5.00± 1.18	5.60± 1.29	5.90± 1.33	6.50± 1.42	6.70± 1.49
	Sum	12.10± 1.72	13.30±1 .85	13.80±1 .89	15.25±2 .02	16.40±2 .15	18.00±2. 32	19.20± 2.40	20.50± 2.53	21.30±2 .66
25ºC	Seg. 5	1.78± 0.73	1.81± 0.75	1.96± 0.82	2.20± 0.86	2.50± 0.94	2.55± 0.96	2.93± 0.99	2.95± 1.04	3.10± 1.07
	Seg. 6	4.00± 1.10	4.30± 1.15	4.40± 1.24	4.90± 1.28	5.20± 1.37	5.67± 1.39	6.20± 1.44	6.62± 1.57	6.68± 1.58
	Seg. 7	2.10± 0.79	2.28± 0.84	2.35± 0.90	2.70± 0.95	2.95± 1.03	3.41± 1.08	3.70± 1.11	3.75± 1.18	4.00± 1.22
	Sum	7.88± 1.55	8.39± 1.62	8.71± 1.75	9.80± 1.82	10.65±1 .97	11.63±2. 00	12.83± 2.08	13.32± 2.23	13.78±2 .27

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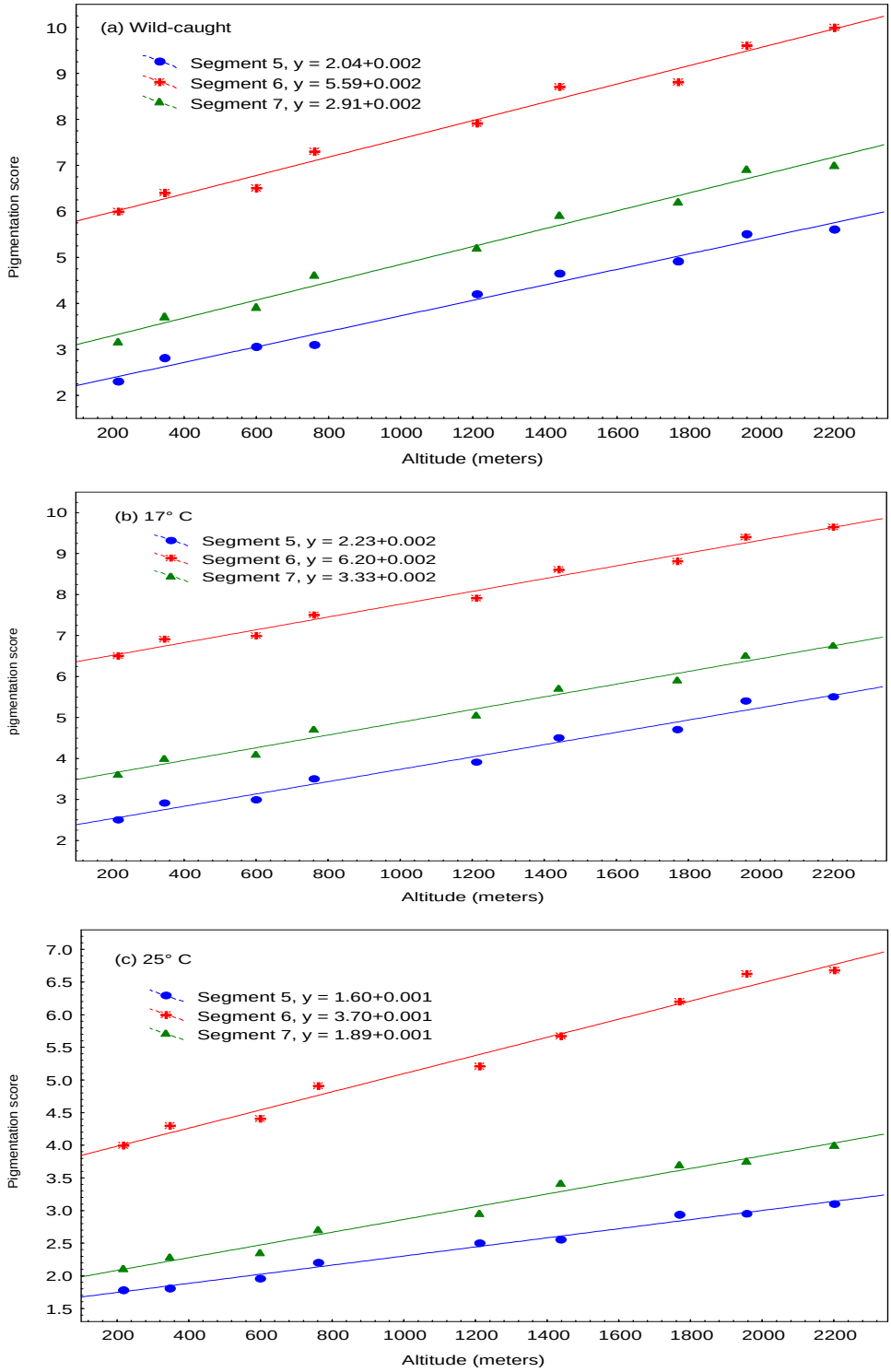

Figure 2: Regression analysis of population mean values for pigmentation score of 5th, 6th and 7th segments of wild and laboratory populations of *D. melanogaster* as a function of altitude of origin.

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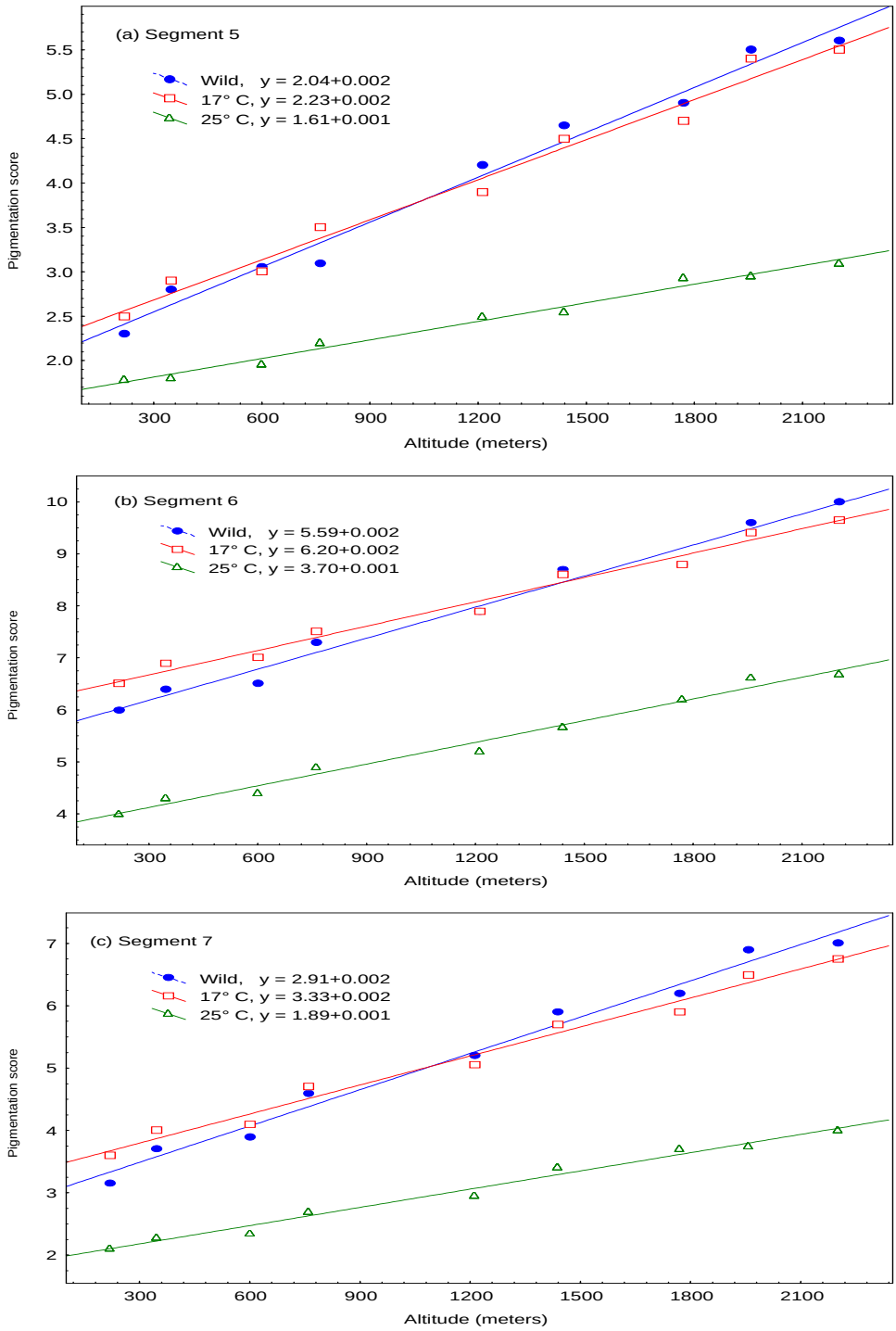

Figure 3: Segment-specific regression analysis of population mean values for pigmentation score of wild and laboratory populations of *D. melanogaster* as a function of altitude of origin.

Regression analysis and positive correlation of sum of pigmentation score and desiccation resistance with altitude is given in figure 4 (a) & (b) respectively. Genetic repeatability for both the traits was further

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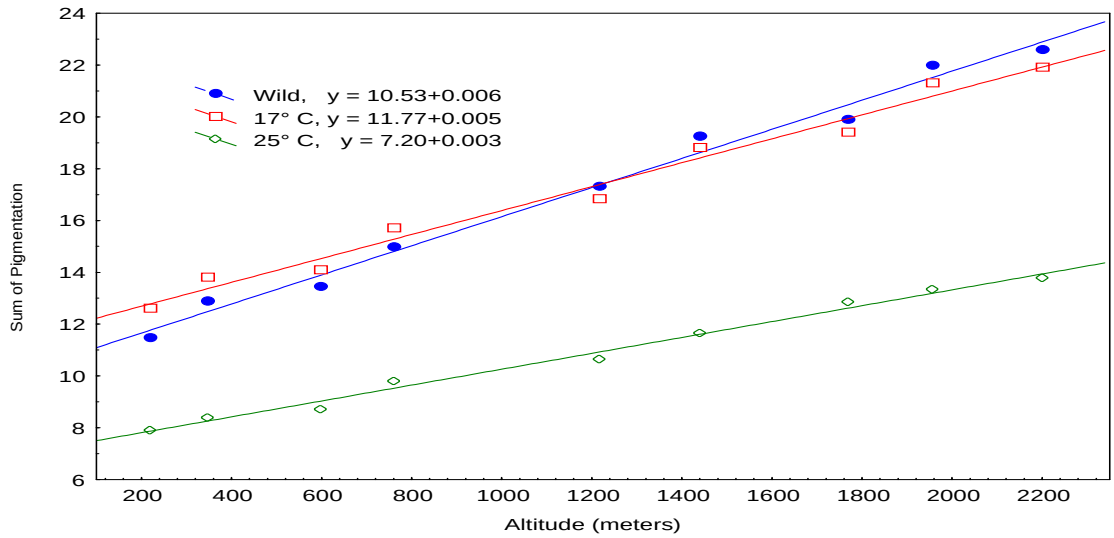
analysed in four more generation (G2, G4, G6 and G8) and the data on correlation across five generation showed significant values at both 17 and 25° C growth temperature (Table 3).

(a)



(b)

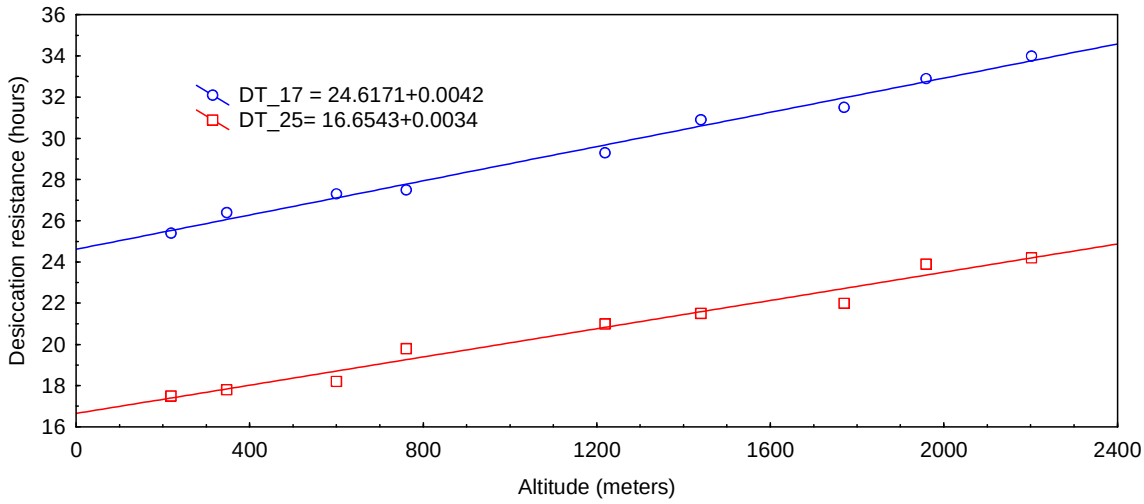


Figure 4: Regression analysis and positive correlation of sum of abdominal pigmentation (a) and desiccation resistance (b) with origin of nine altitudinal populations of *Drosophila melanogaster.*

Table 3: Data on correlation between family means for abdominal pigmentation and desiccation resistance across five generations (G_1 to G_5) in nine altitudinal populations of *D. melanogaster.*

Trait	Temp.	G_{1-2}	G_{1-3}	G_{1-4}	G_{1-5}	G_{2-3}	G_{2-4}	G_{2-5}	G_{3-4}	G_{3-5}	G_{4-5}
Abdominal pigmentation	17	0.90	0.91	0.88	0.86	0.92	0.93	0.90	0.89	0.86	0.88

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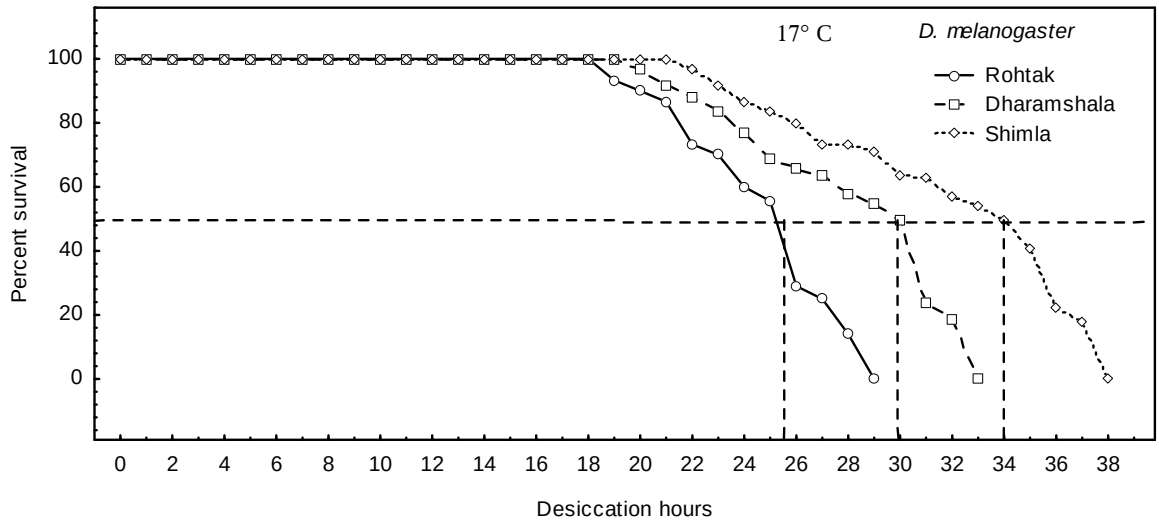
	25	0.94	0.92	0.87	0.83	0.90	0.87	0.88	0.90	0.85	0.87
Desiccation resistance	17	0.84	0.86	0.85	0.83	0.88	0.82	0.84	0.88	0.82	0.80
	25	0.82	0.84	0.88	0.89	0.83	0.81	0.86	0.85	0.81	0.83

Comparison of percent survival due to desiccation resistance observed at hourly intervals in three altitudinal populations of *D. melanogaster* at 17 & 25° C growth temperatures is depicted in figure 5 (a & b respectively).

(a)



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(b)

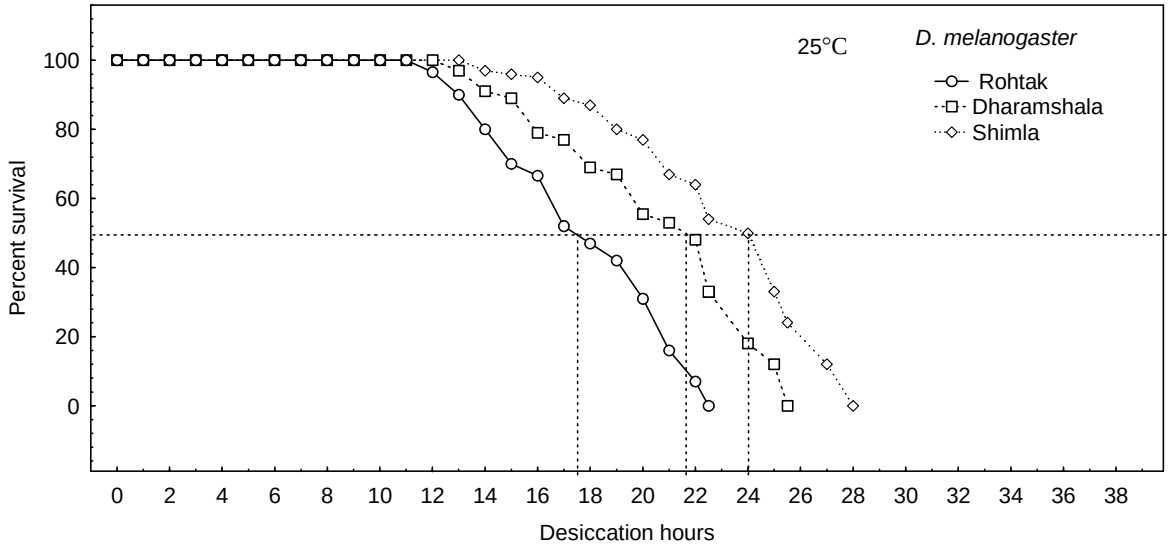
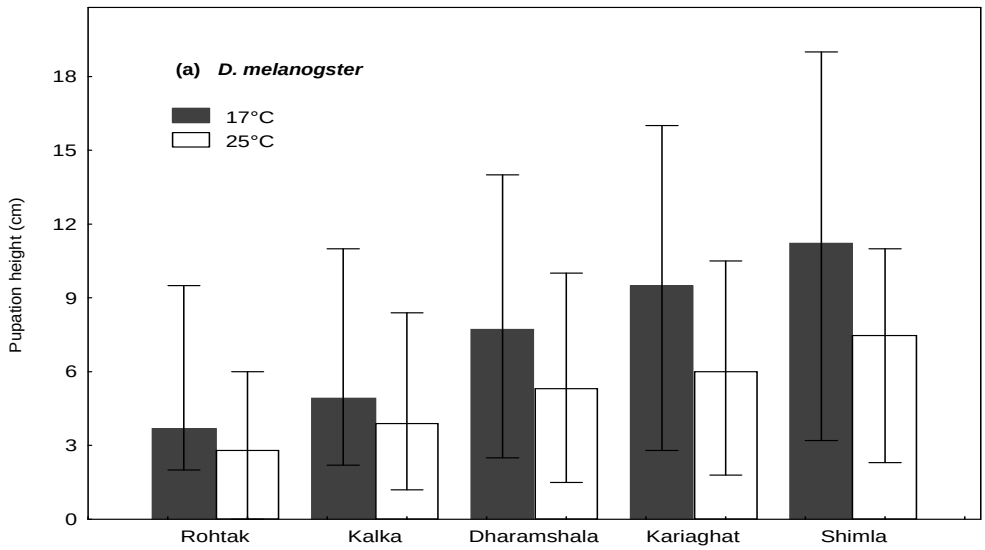


Figure 5: Comparison of percent survival due to desiccation resistance observed at hourly intervals in three altitudinal populations of *D. melanogaster* at 17 & 25° C growth temperatures (b & c). Vertical lines show LT_{50} values.

Mean pupation height of five altitudinal populations of *D. melanogaster* reared at 17 and 25° C growth temperature is depicted in figure 6 (a). Range of pupation height is depicted by vertical bars. For mid altitudinal population, Dharamshala, percentage of pupae at each of the five height classes of the culture vial of *D. melanogaster* reared at 17 and 25° C growth temperature are depicted in Figure 6 (b & c respectively).



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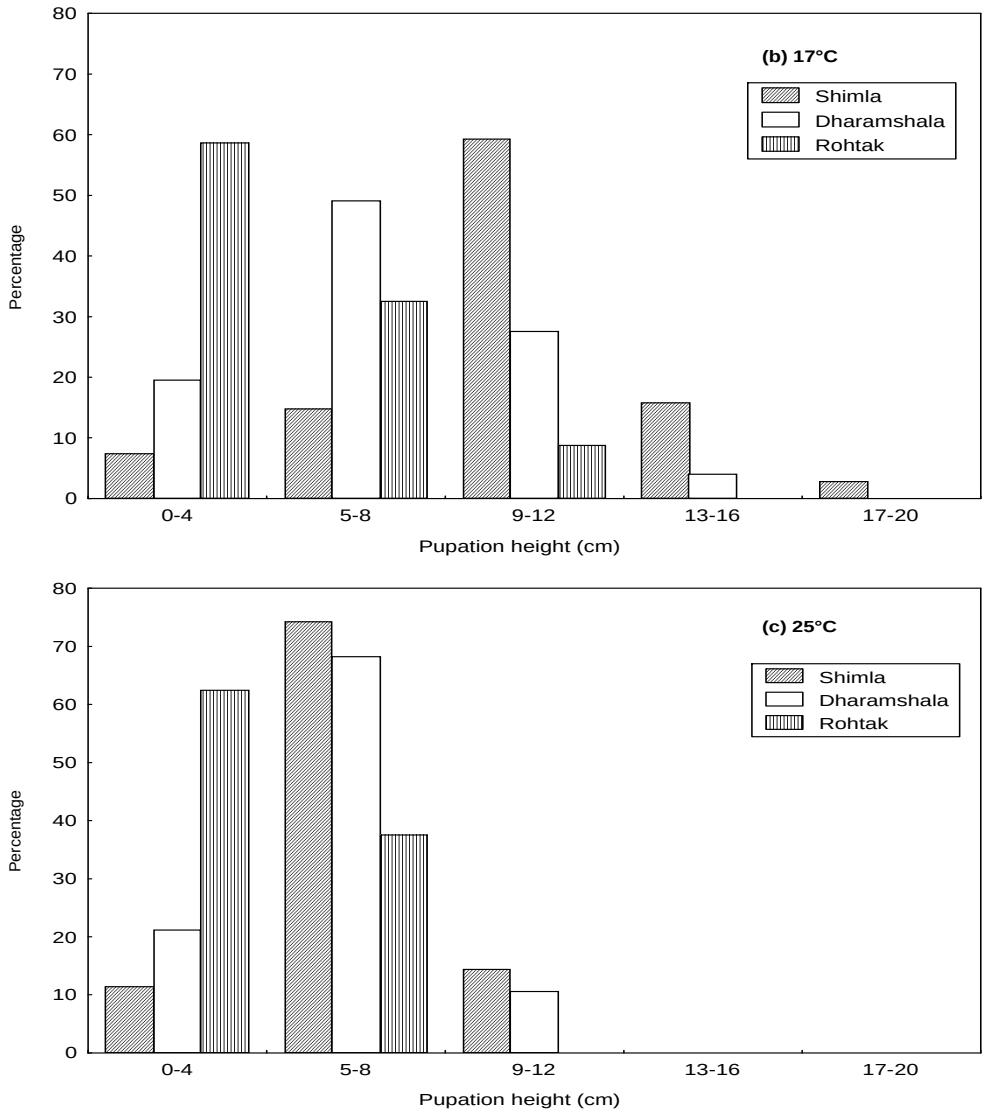

Figure 6 : Percentage of pupae of *D. melanogaster* at each of the five height classes of the culture vial reared at 17 and 25° C growth temperature(a & b); mean pupation height of five altitudinal populations of D. melanogaster reared at 17 and 25° C growth temperature. Vertical bars show the range of pupation height. (c).

Data on mean values (±SE) on pupation height, pigmentation score and desiccation resistance in control and selection lines (low and high) derived from mid-altitude populations of *D. melanogaster* from Dharamshala (1959m) is given in Table 4.

Trait variability for Pigmentation score, desiccation resistance and pupation height as a simultaneous function of T_{cv} (mean monthly coefficient of variation of temperature) and RH_{cv} (mean monthly coefficient of variation of relative humidity) in different altitudinal populations of *D. melanogaster* is depicted in three dimensional scatterplot (Figure 7 a,b, and c respectively).

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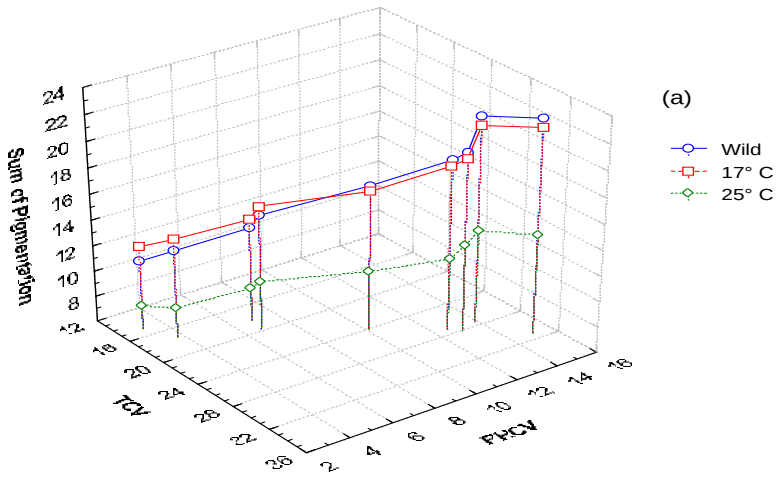
Table 4: Data on mean values (±SE) on pupation height, abdominal pigmentation score and desiccation resistance in control and selection lines (low and high) derived from mid-altitude populations of *D. melanogaster* from Dharamshala (1959m).

Class	Pupation height (cm)		Abdominal pigmentation score		Desiccation resistance (hr)	
	17° C	**25° C**	**17° C**	**25° C**	**17° C**	**25° C**
Control	7.70±0.25	5.30±0.16	17.30±0.52	10.65±0.32	30.00±1.10	21.80±0.67
Low	3.70±0.15	2.80±0.08	16.00±0.50	8.00±0.25	27.10±0.85	20.00±0.61
High	11.00±0.30	7.50±0.23	18.50±0.56	12.00±0.37	32.90±1.25	23.30±0.72



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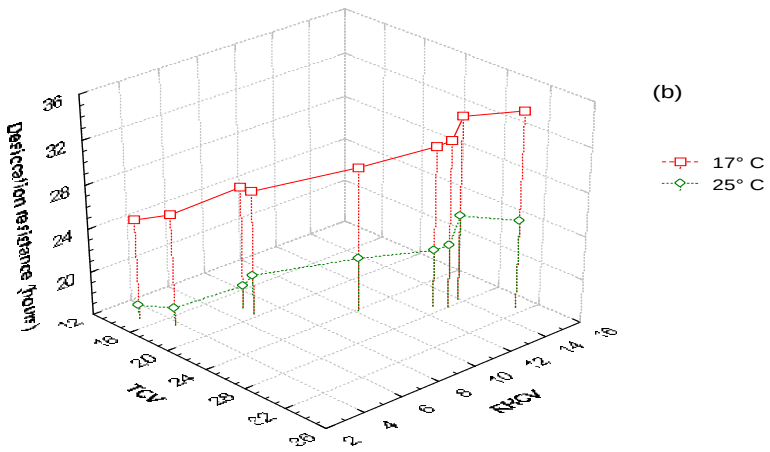

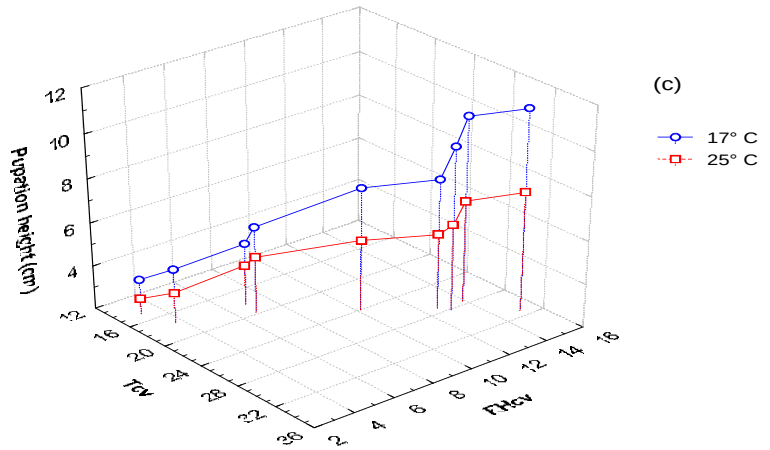

Figure 7: Three dimensional scatterplot illustrating trait variability [Pigmentation score (a); desiccation resistance (b) and pupation height ()] as a simultaneous function of T_{cv} (mean monthly coefficient of variation of temperature) and RH_{cv} (mean monthly coefficient of variation of relative humidity) in different altitudinal populations of *D. melanogaster*.

4. DISCUSSION:

In the present studies, altitudinal populations of *D. melanogaster* demonstrate significant phenotypic variation in the pupation height, sum of abdominal pigmentation scores and desiccation resistance between individuals within a population and also between populations. There is great deal of genetic variability for all

the three physiological traits in *D. melanogaster*. Even wild caught samples showed considerable variations for abdominal pigmentation (40 to 50% higher S.D. values) that could be due to having experienced variable climatic conditions during development under field conditions.

Present study reveals that altitudinal populations of *D. melanogaster* from high altitude locations with colder and drier habitat select for high pupation height and survive desiccating conditions significantly longer than the low altitude populations with warmer and humid habitat which select low pupation height.

In conclusion, analysis of *D. melanogaster* populations from low to high altitude localities for various physiological traits demonstrate that pupation height has substantial genetic variability which is subject to natural selection pressures under colder and drier conditions and has shown correlated selection responses on abdominal pigmentation and desiccation resistance. The analysis of climatic factors has shown that seasonal variations in temperature and humidity (T_{cv} and RH_{cv}) can be responsible for maintaining genetic heterogeneity in the three physiological traits related to thermal and water balance. Further investigation are needed in several species and populations of drosophilids and other insect taxa in order to establish such pleiotropic effects of melanism other than thermoregulation.

5. ACKNOWLEDGEMENTS:

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