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ORIGINAL ARTICLE

Different adaptive strategies in Sibling species under stressful environments: A case study in *D. simulans* and *D. melanogaster.*

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ABSTRACT

In the Indian subcontinent, flies typically display greater resilience to environmental stress compared to other regions. This research investigates the altitudinal variations in stress-related traits and body melanization in two closely related species, D. melanogaster and D. simulans. Given their well-documented history, these species share a common gene pool and exhibit significant morphological similarities. This study showed that Increased plasticity was observed in the (2nd + 3rd + 4th) segments of D. simulans at lower temperatures compared to its sibling species. D. simulans also showed greater plasticity in body melanization under lower temperatures, which is crucial for survival in drier habitats. This suggests that melanization may play a key role in adapting to extreme conditions. An increase in the range of cuticular lipids was recorded from higher to lower temperatures, with greater genetic variability in D. simulans across different temperature ranges. Clines were identified for all physiological traits except for heat knockdown. D. simulans exhibits a weak linear cline across all eco-physiological traits, whereas D. melanogaster shows a strong altitudinal cline.
Keywords*: D. simulans and D. melanogaster, Environmental Stress, cuticular lipids*

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INTRODUCTION

As we are well aware of the history of *D. simulans* and *D. melanogaster.* Both these species share a common gene pool and show strong morphological similarities [35]. Both species are native to tropical Africa and diverged from a common ancestor approximately 2 million years ago [28]. Nowadays, both species are widely distributed in both tropical and temperate regions. Both sibling species exhibit distinct behaviours in ecological and behavioural aspects. *D. simulans* loves to live in temperate regions, while *D. melanogaster* likes both temperate & tropical habitats. However, in the temperate region, *D. simulans* appears to be more dominant over its sibling, *D. melanogaster* [7].

Both species have been taken together for comparison of numerous behavioural and ecological significances. Recently, diversification forces reviewers to compare life-history and various ecophysiological parameters for a more comprehensive investigation. Many authors found valuable results while working on these species [32, 4, 28]; physiological traits [32, 11]; behavioural traits [8-10] and morphological traits [36, 32].

Environmental stress has played a significant role in the life of *Drosophila*. The capacity to adapt or tolerate environmental stresses is crucial for the survival of populations [23, 2].

In diverse insect taxa, phenotypic plasticity has played a significant role in survival in particular environments. Phenotypic plasticity is the capacity of a single genotype to produce different phenotypes in different environments. Body pigmentation of various insects shows a broad range of variation. In general, darker body colour is observed at low temperature [4, 5, 14, 15]. Being darker at a lower temperature will favour the absorption of light radiation. Darker body colours heat up and cool down more quickly compared with lighter ones, and the reverse is true for higher temperatures [16]. Phenotypic plasticity of body pigmentation was investigated for the last three abdominal segments as a function of growth temperature in *D. melanogaster* and *D. simulans* [6]. Abdominal pigmentation in *D.*

melanogaster and *D. simulans* is highly variable due to phenotypic plasticity in relation to developmental temperature. Temperature may be the major environmental factor responsible for the cline in various ecophysiological traits. Increased body melanization at higher altitudes as well as latitudes is generally considered to be adaptive for thermoregulation [27]. An increase of trait value at higher latitudes is observed for ovariole number, bristle number, thoracic pigmentation and thorax length [17]. However, stress-related traits along altitude have received less attention in *D. simulans.*

Stress-related traits contribute to the ecological success of ectothermic insects under different climatic conditions [23]. Several studies have shown that maintenance of water balance is a major challenge for ectothermic insects living under drier and cooler climatic conditions [1-14]. In drier habitats, body water loss primarily occurs through excretion, respiration, and cuticular transpiration. Excretory and respiratory water losses are less than 15% and cuticular transpiration contributes to water loss in insects more than 80% [20]. So cuticular transpiration may occur through either cuticular lipids [21, 23-28] or body melanization [29]. In ectothermic insects, desiccation resistance is negatively correlated with cuticular water loss [18]. Clines of water balance measures associated with the amount of cuticular lipid have been reported in *Melanoplus sanguinipes* [30] and also in latitudinal population of *Z. indianus* [32]. The amount of surface lipid does not differ between northern and southern Indian populations of *D. melanogaster* [31]. However, a comparative account of both the sibling species for desiccation resistance and cuticular lipid has not been studied.

In many investigators' minds, there is some conflict about *D. simulans*. Some reviewers found that *D. simulans* is better adapted to warm environments, while others claim that it performs better in colder environments. Therefore, in the literature, there is some confusion about its behaviour range and which environment suits it better. An attempt has been made to explain its nature across the whole thermal range.

Primarily, our work focuses on *D. simulans*, but *D. melanogaster* serves as a window species for genetic investigations, facilitating better comparison [5]. *D. melanogaster* is the only species that is fit for all aspects because of its wide distribution. In the present investigation, the hypothesis is tested to determine whether these two sibling species employ different adaptive strategies in response to different environmental stresses. Both species of altitudinal populations were investigated for various stress-related traits. It was also investigated the reaction norms for abdominal melanization along a range of temperatures (12 to 31° C).

MATERIAL AND METHODS

Field collection: *D.simulans* and *D. melanogaster* were collected in winter months of 2016 from four altitudinal sites of western Himalaya. A collection of both species simultaneously provides a more accurate comparison along the altitudinal gradients from Rohtak to Shimla. The entire collection was done by the net-sweeping method. Flies were cultured on cornmeal-yeast agar medium in the laboratory at 21 °C for 3 to 4 generations before the experiments. The same conditions were provided to both species to minimise the environmental effects. Cultures were initiated with 40 to 50 isofemale lines. For experimental purpose, flies were allowed to lay eggs at 21° C. Subsequently, serial egg laying of 20 isofemale line of both the species were transferred to 12, 14, 17, 25 & 28° C. Flies were analyzed for the traits, % body menanization, body size, Desiccation resistance, Cold recovery, heat knockdown, epicuticular lipids and cuticular water loss. For all assays, sexes were isolated in separate vials after mild anaesthesia with ether, followed by a day recovery period. Climatic data for the sites of origin of populations were obtained from the Indian Institute of Tropical Meteorology (IITM; www.trpmet.res.in). Data on geographical and climatic variables were given in Table 1.

Table 1. Data on geographical and climatic variables over the last 30 years for the sites of origin of *D. melanogaster* and *D. simulans* populations

Population	Altitude (m)	T_{max}	T_{min}	$T_{average}$	T_{cv}	RH (%)	H_{cv}
Rohtak	219	31.70	18.80	25.24	28.08	60-75	19.48
Kalka	600	27.80	15.80	23.75	30.25	55-65	24.69
Solan	1440	23.60	13.50	19.55	33.40	40-55	29.30
Shimla	2202	17.10	10.10	13.60	38.10	35-45	34.80

% Body melanization: Percent body melanization was estimated with visual scoring with using an Olympus stereo-zoom microscope (www.olympus.com). Body melanization was estimated from dorsal as well as lateral aspects of the female abdomen, giving values ranging from 0 (no melanization) to 10 (complete melanization) for each of the six abdominal segments (2^{nd} to 7^{th}). Since the abdominal

segments differ in size (i.e. 0.86, 0.94, 1.0, 0.88, 0.67 & 0.38 for 2nd to 7th segments respectively). Their relative size was multiplied by the segment-wise melanization score. Data on percent melanization were calculated as [30].

Body size (wing length): For measuring wing length, 10 individuals of each 20 isofemale line of each altitudinal population of both the species were analyzed. Wing length was measured from the thorax articulation to the tip of the third longitudinal vein under an Olympus stereo-zoom microscope SZ-11 fitted with a micrometer.

Desiccation resistance: To measure desiccation resistance, 200 flies/population were isolated separately. Ten flies from each of the 20 isofemale lines were analyzed (20 flies x 20 IF). Flies were placed in a dry plastic vial containing 2g of silica gel at the bottom and covered with a disc of a form piece. Such vials were placed in a desiccator chamber (secador electronic desiccator cabinet; www.tarson.com), which maintains a relative humidity of ~5%. These vials were inspected hourly, and the number of dead flies (completely immobile) was recorded.

Cuticular water loss: The rate of water loss was analyzed by giving short-term desiccation (8 hours) in a group of ten flies. Flies were weighed on a Sartorius microbalance both before and after desiccation, and the rate of water loss was calculated as mgh^{-1} (initial body weight – body weight after eight hours of desiccation stress) / initial body weight x 8.

Epicuticular lipids: For estimation of cuticular lipid, individual flies in 10 replicates per IF line in an Eppendorf tube were dried overnight at 60° C in an oven to obtain a constant dry body weight. Add 2 mL of hexane to each Eppendorf tube. Thereafter, the flies were removed from the solvent and dried again at 60 °C, and finally reweighed. (The Sartorius microbalance CPA26P with precision up to 0.001 mg ensured accuracy: www.sartorius.com). For each fly, cuticular lipid mass in mg was estimated per unit surface area (surface area scales to 2/3power of the wet body mass) as: difference between initial dry weight and dry weight after solvent treatment / initial dry weight × surface area (where area was expressed in cm^2 and wet body mass in mg).

Cuticular water loss by organic solvent: The cuticular water loss was analyzed in individual flies in a group of ten replicates from each of 20 isofemale lines per population. Flies were weighed on a Sartorius microbalance and then treated with either 1 mL of hexane or chloroform:methanol (2:1). They were gently vortexed five times each for 30 seconds. Flies were then blotted on tissue paper for 10 minutes to allow for dryness, reweighed, and placed in a desiccator chamber (Secador electronic desiccator cabinet; www.tarson.com). Organic solvents differ in their solubility properties of surface lipid, i.e. slower with hexane and faster with chloroform: methanol (2:1). So, the effects of hexane on body water loss were monitored hourly, but for chloroform: methanol (2:1) after every 15 min. However, changes in body water were recorded every 2 hr.

Thermoresistance assays

Cold recovery: To check the resistivity of 20 flies of each IF line, they were transferred without anaesthesia into an empty 5ml glass vial. These vials were set in a thermocoal box containing ice flakes (made with an ice flaking machine AICIL: Aicil lab instruments Pyt. Lt., Ambala, India) which were kept at 0° C in a refrigerator. Such vials were removed from the box after 8hr, 12hr, 16hr, 20hr and 24hr. To check the recovery, the flies were transferred to a petriplate and checked at room temperature. (21° C) with the help of a stopwatch. Flies were inspected for 1 hour.

Heat knockdown: Heat knockdown was measured on 10 individuals from each isofemale line (n = 20) of each locality on both species. Individuals were placed into 5 ml glass vials submerged in a water bath at a constant temperature (39° C). Flies were scored for the time (in minutes) taken to be knocked down.

Statistical analysis:

For analysis of all ecophysiological traits (% melanization, wing length, desiccation resistance, heat knock down, cold recovery, cuticular water loss and cuticular lipid), isofemale lines means (n = 20) along with S.E. were used for illustration and tabular data. Trait value was compared between both species based on a t-test. For all the traits, only female data for both species are given in the table and figures. Altitudinal patterns were analyzed using linear regression. For this analysis, the association between altitude and the mean value of each trait was tested. Statistical (stat soft Inc. Release 5.0, Tulsa, OK, USA) was used for all calculations as well as illustrations.

RESULTS

Body melanization

Quite a different pattern was observed in *D. melanogaster* and *D. simulans*. At lower temperature *D. simulans* is more superior to *D. melanogaster*. compared (2+3+4), (5+6+7) and total % melanization at

different temperature range. *D. simulans* shows higher plasticity in (2+3+4), and *D. melanogaster* shows in (5+6+7) as shown in fig.1.

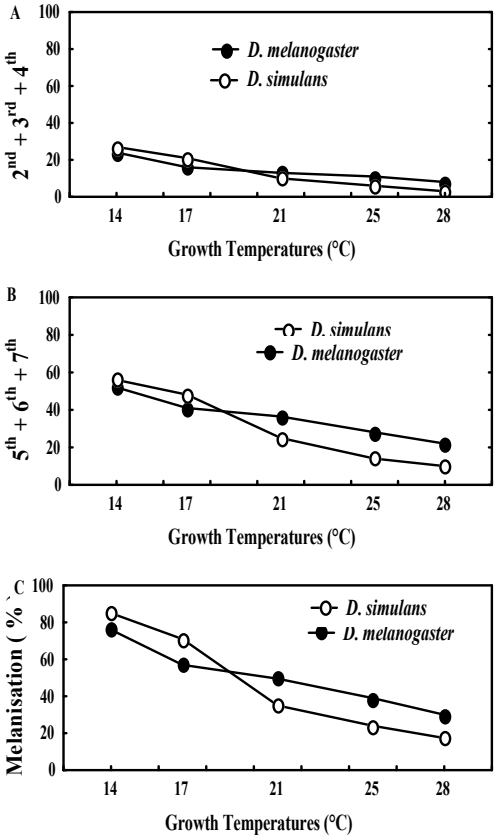

Fig.1. (A-C) comparison of reaction norms (across six growth temperatures) of (2^{nd} +3^{rd} +4^{th}), (5^{th} +6^{th} +7^{th}) and % melanization in *D.melanogaster and D. simulans*

Plastic effects show that *D. simulans* is superior at lower growth temperatures and D. melanogaster at higher temperatures. Both laboratory and wild data on body segments support our result. As (2+3+4), shows superiority in both wild and condition data, as shown in Table 2. Data were compared across a temperature range with the help of student t-test in both the species. Table 3 shows that in *D. simulans* (2+3+4), the segments exhibit a significant t-test value and p-level, whereas the reverse is observed in *D. melanogaster* (5+6+7). Fold increase from higher to lower temperature on segment-wise data was also given.

Table 2. Data on percent melanization (m ± S.E.) of (2^{nd} +3^{rd} +4^{th}) and (5^{th} +6^{th}+7^{th}) segments in wild and laboratory populations grown at 17 and 25°C of *D.melanogaster and D. simulans*

Population (Altitude ; m)		Wild			Lab. (17°C)			Lab. (25°C)	
		2+3+4	5+6+7		2+3+4	5+6+7		2+3+4	5+6+7
Rohtak (219)	Dm	14.26±0.13	27.04±0.48		14.83±0.11	30.56±0.45		11.50±0.06	16.98±0.11
	Ds	15.63±2.62	14.82±6.77		16.37±3.73	44.64±3.54		8.60±1.60	15.70±1.59
Kalka (600)	Dm	14.83±0.15	29.38±0.43		15.52±0.13	32.04±0.43		12.20±0.05	19.56±0.10
	Ds	17.20±1.27	18.52±9.25		19.22±2.36	44.56±2.35		10.86±2.36	16.53±1.25
Solan (1440)	Dm	15.84±0.17	39.14±0.29		15.29±0.16	36.27±0.40		13.99±0.09	24.90±0.08
	Ds	20.61±5.31	32.76±10.63		21.25±2.35	45.4±2.36		12.05±1.98	17.25±1.36
Shimla(2202)	Dm	16.54±0.24	45.12±0.49		16.76±0.15	43.54±0.48		14.50±0.10	27.22±0.13
	Ds	22.25±2.14	45.38±3.54		23.15±2.36	46.38±5.22		13.02±1.25	17.89±2.36

Table 3. Data on mean value for segment wise variability (2^{nd} to 7^{th}) as well as (2^{nd} + 3^{rd} + 4^{th}) and (5^{th} + 6^{th} + 7^{th}) and sum of total melanization separately. Comparison of abdominal segment variability at two extreme temperatures (12 °C & 25 °C) of both species. Fold increase also shown in table. t-test shows significant value for (2^{nd} + 3^{rd} + 4^{th}) segment in *D. simulans*, however in *D. melanogaster* t-test value significant for (5^{th} + 6^{th} + 7^{th}) segment.

	D. simulans					***D. melanogaster***			
	12°C	**25°C**	**Fold inc.**	**t- test**		**12°C**	**25°C**	**Fold inc.**	**t- test**
2 nd	12.00	3.00	4.00	8.86***		5.00	3.10	1.61	1.94*
3 rd	13.00	4.60	2.82	7.57***		7.00	4.30	1.62	1.64*
4 th	17.00	4.10	4.14	9.71***		8.80	5.20	1.69	2.11*
5 th	16.00	5.80	2.75	3.60***		15.20	6.00	2.53	4.28***
6 th	20.00	7.90	2.53	5.24**		20.00	9.40	2.12	5.98***
7 th	20.00	4.00	5.00	2.32**		20.00	6.60	3.03	3.82***
2+3+4	42.00	11.70	3.58	10.13***		20.80	12.60	1.65	4.78*
5+6+7	56.00	17.70	3.16	3.67***		55.20	22.00	2.50	6.51***
Sum	98.00	25.40	3.85	8.69***		76.00	31.60	2.41	6.68***

Trait variability

Data on mean (±SD) of body melanization, desiccation stress, heat knockdown, cold recovery, cuticular rate of water loss and cuticular lipid of highland and lowland localities are given in table (4 & 5). Laboratory populations provided large samples for analysis and each value is an average of 30 individuals of both the species in twenty isofemale line.

Table 4. Data (mean ± SD) on % melanization, Desiccation stress(hr), heat knockdown(min.), cold recovery (min.), CWL (µg/hr), cuticular lipid (mg/fly/cm) in Highland (HL) and Lowland (LL) population of *D. simulans* at different temperature.

Temperature / Traits		12° C	15° C	17° C	21° C	25° C	28° C
(%)Melanization	HL	98.00±4.44	87.12±2.32	62.96±2.37	43.10±2.11	21.40±3.45	20.18±2.46
	LL	92.00±3.59	82.50±2.233	55.41±2.34	37.20±2.30	19.12±3.12	19.06±2.53
Desiccation stress (hr)	HL	93.25±2.40	86.10±5.96	45.40±1.83	37.12±1.42	12.44±0.52	12.00±0.58
	LL	91.36±2.31	79.17±4.91	40.25±1.40	33.12±1.26	11.50±1.01	11.00±0.49
Heat knockdown (min.)	HL	3.19±2.31	3.50±2.52	4.79±2.32	8.00±2.02	8.86±1.56	12.26±1.25
	LL	3.25±1.25	5.23±1.56	6.64±2.11	8.42±1.09	10.26±1.32	18.93±1.22
Cold recovery (min.)	HL	2.50±1.11	11.21±2.32	24.56±345	28.21±3.69	40.28±4.37	63.30±4.56
	LL	3.00±0.90	12.05±2.20	27.48±3.49	30.25±4.58	43.25±4.48	79.00±4.51
CWL (mg/hr)	HL	10.00±1.25	10.15±1.56	12.29±1.98	14.25±1.36	33.05±2.32	38.20±2.46
	LL	10.15±1.32	10.23±1.23	15.23±1.56	16.43±1.20	34.14±1.56	43.00±2.32
Cuticular lipids (mg/fly/cm)	HL	0.358±0.072	0.321±0.082	0.250±0.045	0.239±0.032	0.113±0.021	0.113±0.019
	LL	0.348±0.029	0.291±0.034	0.210±0.031	0.187±0.017	0.110±0.023	0.111±0.014

Table 5. Data (mean ± SD) on % melanization, Dessication stress(hr), heat knockdown(min.), cold recovery (min.), CWL (µg/hr), cuticular lipid (mg/fly/cm) in Highland (HL) and Lowland (LL) populations of *D. melanogaster* at different temperatures.

Temperature / Traits		12° C	15° C	17° C	21° C	25° C	28° C
		♀	♀	♀	♀	♀	♀
% Melanization	HL	86.00±2.21	75.20±2.97	65.20±2.52	56.32±5.68	33.12±3.89	26.48±2.36
	LL	82.00±2.56	69.20±3.21	60.45±1.21	41.98±2.34	27.12±1.42	22.20±2.20
D. stress (hr)	HL	68.29±2.22	59.15±2.35	45.40±2.78	32.12±3.78	25.20±2.11	18.36±1.56
	LL	65.37±1.59	54.12±3.14	43.00±2.16	24.63±2.04	22.12±2.00	16.00±1.44
Heat stress (min.)	HL	4.75±2.12	8.46±2.30	10.22±2.32	16.25±1.65	23.51±3.05	55.27±2.21
	LL	5.25±1.98	10.23±2.05	12.25±2.13	19.43±2.30	30.55±1.69	69.27±2.04
Cold stress(min.)	HL	8.11±2.03	14.22±1.98	16.29±2.23	19.25±2.36	25.25±2.36	30.32±2.45
	LL	8.32±1.78	16.25±2.32	20.78±2.45	23.36±2.14	32.34±3.25	35.36±3.48
CWL(µg/hr)	HL	11.27±1.19	12.21±1.25	16.12±1.29	17.01±1.32	23.40±1.23	25.58±1.11
	LL	11.50±1.56	14.21±2.32	19.56±2.22	22.12±1.15	27.12±2.32	30.14±2.58
C. lipid (mg/fly/cm)	HL	0.134±0.012	0.134±0.012	0.133±0.014	0.132±0.014	0.126±0.017	0.126±0.017
	LL	0.134±0.011	0.134±0.011	0.133±0.013	0.132±0.013	0.126±0.019	0.126±0.019

Analysis with climatic variables

Four altitudinal sites were taken for comparison of data. Linear regression analysis was conducted to examine various traits as a function of altitude. It was found that the slope value of % body melanization is more significantly increased as a function of altitude in *D. melanogaster*, while in *D. simulans*, the slope for cuticular lipid increases more than ten times. % melanizaton., wing length, Dessication stress and cuticular lipid show a positive correlation with altitude. However, cold recovery time, heat knockdown time and cuticular rate of water loss show a negative correlation. Data are given in Table 6.

Table 6. Linear regression analysis of various traits as a function of Altitude in laboratory populations of both species (a = intercept, b = slope)

	D. melanogaster			*D. simulans*		
Traits	a ± SE	b ± SE	r	a ± SE	b ± SE	r
% melanization	32.14±3.72	0.012±0.002	0.943	44.83±1.189	0.0054±0.007	0.969
Wing length	2.50±0.036	0.0001±0.0002	0.973	2.49±0.006	0.00001±0.000004	0.824
Desiccation stress	20.69±1.47	0.0078±0.009	0.977	24.06±1.21	0.007±0.0008	0.985
Cold stress	25.32±1.23	-0.005±0.0008	-0.97	43.43±2.86	-0.016±0.001	-0.980
Heat stress	32.84±0.819	-0.011±0.005	-1.0	10.16±0.264	-0.003±0.001	-0.996
Culticular water loss	28.97±1.57	-0.007±0.001	-0.97	30.67±5.41	-0.010±0.003	-0.857
Cuticular lipid	0.131±0.0004	0.000001±0.0000	0.921	0.124±0.034	0.0001±0.0002	0.930

Wing length is non-correlated with altitude in the case of *D. simulans*., whereas % mel. and desiccation stress were increasing with altitude. In the case of water loss, D. melanogaster exhibits a negative correlation compared to *D. simulans.* A relatively high value of cuticular lipid in *D. simulans* was found along an altitude range. In contrast, *D. melanogaster* cuticular lipids are not correlated with the altitudinal pattern. For cuticular lipid, a quite different pattern was observed.

Analysis for cold

Histogram showing (fig. 2) cold recovery in both species. It was found that *D. simulans* has higher resistance to cold only at lower temperatures. However, it is cold-sensitive at higher temperatures. Quite a different pattern was observed in both species when compared at different temperatures.

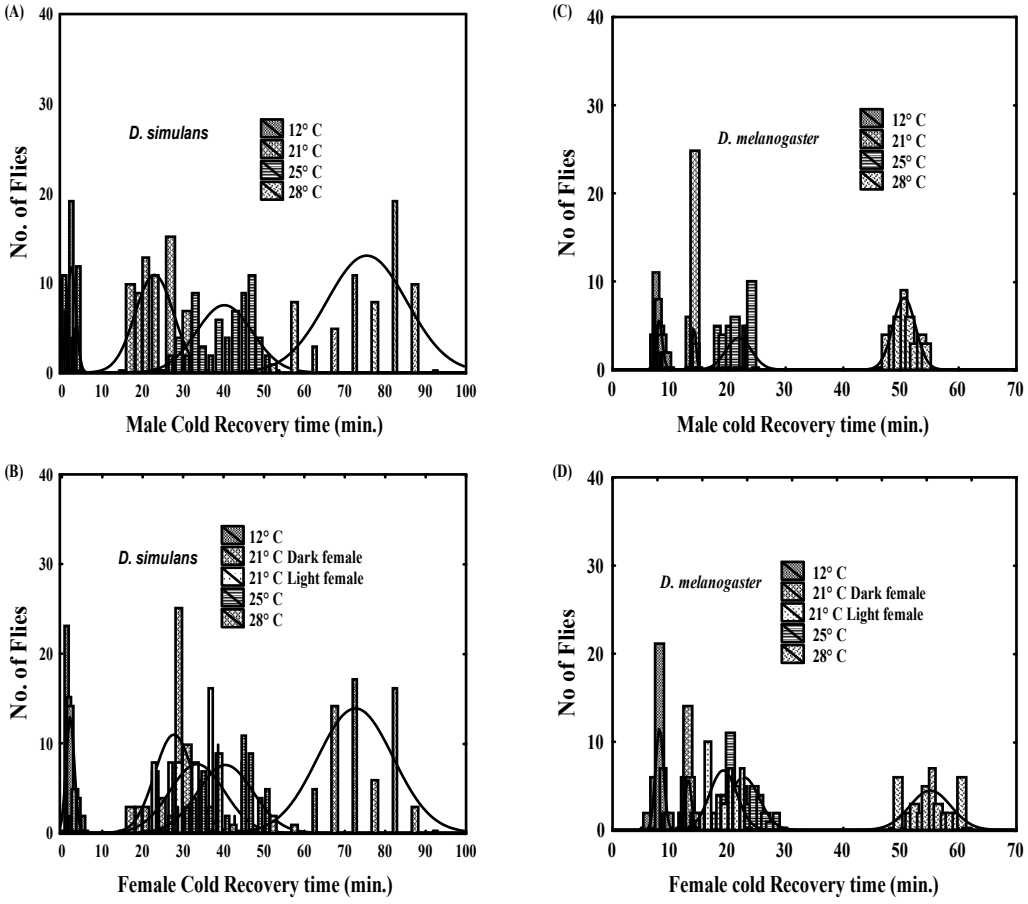

Fig.2. Histogram showing cold recovery time in (min.) of *D. simulans* and *D. melanogaster* of both sexes (male and female) across a temperature range(12, 21, 25 & 28° C).

Analysis of wild populations

No correlation of wing length was observed with % body melanization in both species. In the wild condition segment (2^{nd} +3^{rd} +4^{th}) of *D. simulans* shows higher melanization and the (5^{th} +6^{th} +7^{th}) segment shows lower melanization as compared with *D. melanogaster*. It was also found that % mel. is positively correlated with desiccation stress. Lighter flies of both species are less desiccant resistant than the darker ones shown in fig. 3.

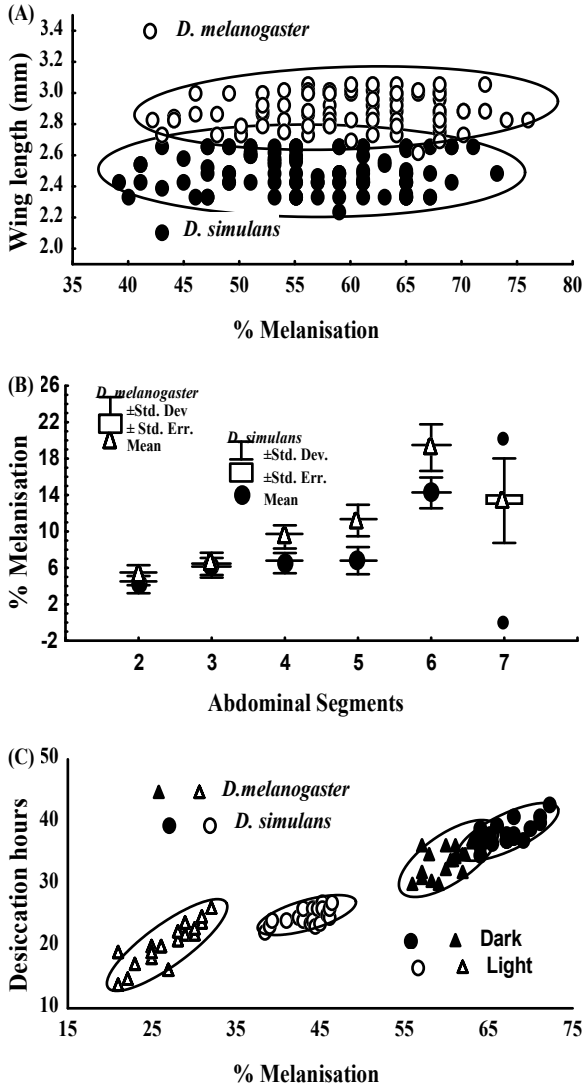

Fig 3. Analysis of wild populations. (A) No correlation of body melanization with body size in both species (B) Comparison of between species variability of abdominal melanization (no difference found for (2^{nd} + 3^{rd}) segment, clear differences shows for (4^{th} + 5^{th} + 6^{th}) segments (C) positive correlation of melanization with desiccation resistance in assorted light and dark flies of *D. simulans* and *D. melanogaster*.

Plasticity for ecophysiological traits

Reaction norms according to growth temperature for ecophysiological traits (% melanization, cold recovery, desiccation resistance, and heat knockdown) are illustrated in fig. 2. All the traits showed ~ 3-4 fold decrease in value from 12 to 28° C for *D. simulans*, whereas, for *D. melanogaster*, this difference is nearly 2 fold. So, in the case of D. simulans, there was quite high plasticity in all the traits except heat knockdown.

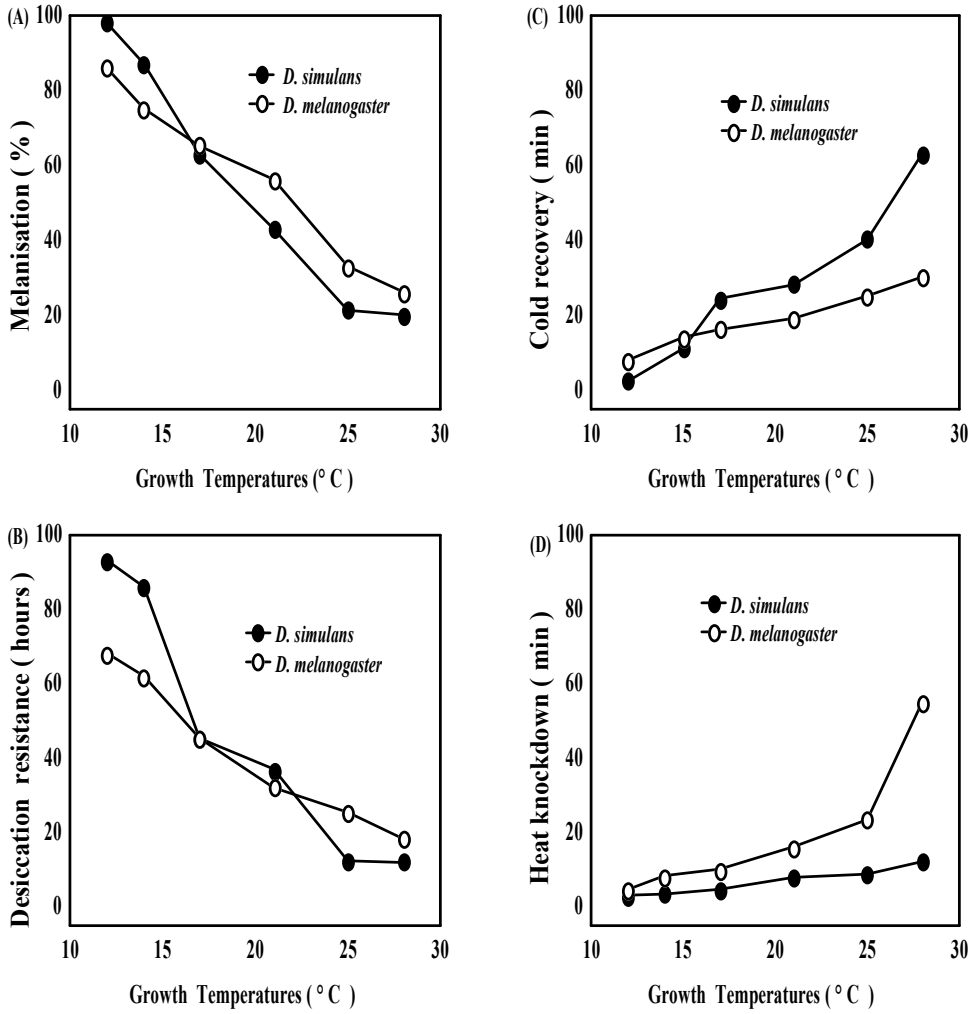

Fig. 4. (A-D) Comparison of reaction norms (across six growth temperatures) of % Melanization, Desiccation resistance, and Cold recovery and Heat knockdown traits of *D. simulans* and *D. melanogaster*

Effects of organic solvent on cuticular permeability

Data on cuticular lipid in control as well as organic solvent (hexane or chloroform: methanol) treated flies of *D. simulans* and *D. melanogaster* are shown in Table 7. Treatment with chloroform: methanol (2:1) increased the rate of cuticular water loss in *D. simulans*, while the effect in *D. melanogaster* was negligible. Thus, it was found that the impact of organic solvents was highly significant for *D. simulans* but not for *D. melanogaster*.

Table 7. Role of organic solvent (chloroform + methanol) on cuticular surface in sibling species *D. melanogaster* and *D. simulans*

Species treated with Solvent / Sites		*D. simulans* C:M (2:1)		*D. melanogaster* C:M (2:1)	
		Role of % mel.	Role of cuticle	Role of % mel.	Role of cuticle
Rohtak	♂	3.34	96.49	79.64	20.34
	♀	7.68	92.30	80.21	19.78
Kasauli	♂	9.43	90.72	78.83	21.16
	♀	6.35	93.64	82.25	17.74

DISCUSSION

This study mainly emphasises examining the altitudinal pattern for stress-related traits and body melanization in two sibling species, *D. melanogaster* and *D. simulans*. In the Indian subcontinent, flies are generally more tolerant of environmental stress than in any other habitat.

Body melanization

Melanism is common in insect taxa, but its evolutionary causes are quite diverse [30-38]. Genetic variability between isofemale lines was not affected by growth temperature, but on average, *D. simulans* was lighter than *D. melanogaster*. However, differences were mainly expressed at higher temperatures [18]. On average, *D. simulans* is lighter than *D. melanogaster* at lower temperatures. [19]. Greater plasticity was found in (2^{nd} + 3^{rd} + 4^{th}) segments in *D. simulans* at a lower temperature when compared to its sibling. A greater plasticity has been seen in *D. simulans* for body melanization at lower temperatures. Melanization is the key to survival in drier habitat. So flies living in northern habitats have higher melanization power to cope with stressful conditions. Wild data also supported our result in relation to laboratory data. It may suggest that the adaptive significance of melanization is to survive in extreme conditions.

Clinal variation for Ecophysiological traits

The northern subcontinents of India are cold and drier in habitat. A non-linear cline was found for chill coma recovery, desiccation stress, heat knockdown time, and DOD [3] along the latitudinal gradient. *D. melanogaster* and *D. serrata* found along a similar latitudinal gradient [22, 23]. The lack of clinal variation for desiccation resistance in *D. simulans* is consistent with finding in *D. simulans*, *D. melanogaster, D. serrata* from the Australian continent [13] found no variation in the geographic area due to drought resistance in the population of *D. simulans* collected from northern and southern Australia, and populations of *D. melanogaster* [24] and *D. serrata* [22]. In the Indian region, desiccation resistance increases with latitude [26]. We found a cline for all physiological traits except for heat knockdown. *D. simualns* shows a weak linear cline in all ecophysiological traits, but D. m shows a strong cline with an altitudinal gradient. At low altitude, wild flies of *D. simulans* show themselves to be an environmentally sensitive species.

Assessment of cuticular lipid.

For adaptations to drier habitats, the function of cuticular lipids is well understood in various insect taxa from deserts [21]. However, some studies have shown the role of melanization in reducing cuticular loss in different *Drosophila* species [30]. The role of cuticular lipids was found to contribute to the water-holding capacity of *D. simulans*, a sibling species of *D. melanogaster*. Data on organic solvent also support our result in the role of cuticular lipid in *D. simulans*. Both of these species are melanic, but changes in water loss are related with surface cuticular lipid. Thus, a cline for cuticular lipid was obtained in *D. simulans* along the altitudinal range. At 21° C *D. simulans* have lower melanization than its sibling and become lighter inappearence. However, when the experiment of desiccation stress was conducted, it was found that *D. simulans* is a more desiccation-tolerant species. This observation prompts us to investigate the cuticular lipids. It may be thought that there is some other mechanism rather than melanization working in stressful conditions. The cuticular lipid across the temperature range in highland and lowland flies was checked. A significant increase was observed in the range of cuticular lipids from higher to lower temperatures. A greater genetic variability was found for cuticular lipid in *D. simulans* at different temperature ranges.

Effects of organic solvent

In insects, resistance to desiccation can evolve more adaptive mechanisms, i.e. reduction in water loss [18], epicuticular lipid [34] or changes in body water content [12]. Another experiment was conducted to see the role of cuticular lipid. The flies were treated with an organic solvent, either chloroform:methanol (2:1) or hexane. A drastic difference was observed in the level of cuticular lipid, with a quite high amount observed for cuticular lipid in *D. simulans*, but *D. melanogaster* shows a non-linear trend. It may be found that *D. simulans* is more tolerant of stressful conditions at lower temperatures due to its higher cuticular lipid content. In this present study, it was found that the role of cuticular lipid in *D. simulans* provides support for coping with stressful conditions.

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