responsible for buffering the traits development against both external and internal environmental variations are interrelated (Lajus *et al.,* 2003b).

In conclusion, the present study provides the evidence for 1) phenotypic variability among populations and individuals which is caused due to genotypic and micro environmental diversity and within-individual variation is caused due to the developmental instability, 2) trait variability arises due to the evolutionary forces (sexual and natural selection) acting on them at individual level, and 3) the contribution of stochastic variance in comparison to the factorial variance to the total phenotypic variance is small, but is considerable. This is the first report on the relative contribution of stochastic and factorial components of phenotypic variance in any bilateral trait in the genus *Drosophila*. However, present study provides new avenues for further research in order to get more information regarding the contribution of factorial and stochastic variance in other *Drosophila* species with a much wider range of traits.

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Parallel trend in pigmentation and desiccation tolerance: altitudinal and latitudinal effects in *Drosophila melanogaster*.

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Abstract

Body melanization and desiccation tolerance were analyzed in ten *Drosophila melanogaster* populations collected at high and low altitudes from five latitudes along the Indian subcontinent. Significant latitudinal cline (11–31°N) and altitudinal (10–2200 m) differentiation were observed for cuticle pigmentation (8–30 points of maximum 60) and survival time of desiccation (17–25 hours). Both traits were found to be closely associated with average temperature (10–28°C) and relative humidity (40–73%) among study sites. Desiccation tolerance was linearly correlated with pigmentation ($D = 0.31.P+15.5$ hours; $r = 0.94$, $p < 0.0001$). The results from this study suggest that factors linked to temperature are likely selective forces and that genes controlling pigmentation and desiccation tolerance are good candidates for studying climatic adaptation.

Introduction

Morphological and life history traits of living organisms may vary gradually along existing environmental clines. Two of the geographical characteristics may be easily identified and correlated with organism traits: latitude (distance from equator) and altitude (height above sea level). Important environmental conditions like ambient temperature are dependent on both these clines, although the rate of change is hardly comparable. Altitudinal temperature changes are generally steep and the climate is less predictable, while latitudinal gradients are smoothly spread over long distances (Heath and Williams, 1979; Baur and Raboud, 1988). For example, mean temperature difference between the foot (200 m) of Sierra Nevada, California, and the peak (3979 m) of Mount Dana at a distance of 97 km is around 24°C. This difference is roughly equivalent to 4500 km in south–north (latitude) direction (Hopkins, 1938). On the Indian sub-continent, both latitudinal and altitudinal ranges are substantial, offering a chance for local adaptation of populations to prevailing climates. The average temperature differs approximately by 2.6°C between south (Kaniya kumari: 8.05 °N / 37 m) and north (Chandigarh: 30.44 °N / 347 m) regions, whereas from lowland Kalka (30.51 °N / 600 m) to mountain Shimla (31.06 °N / 2202 m), the average temperature differs around 10.2°C. The distance between Kaniya kumari to Chandigarh is around 2400 km and between Kalka to Shimla it is around 150 km.

Despite high thermal and other environmental differences between close localities at very different altitudes, genetic adaptation to high-altitude conditions appeared weak in two dung fly species (Blanckenhorn, 1997). The phenotypic plasticity of the populations was extensive, while genotypic differences were probably reduced by substantial gene flow across short distance over an altitudinal cline. Similarly, water loss rate showed high variation (3.7×) among populations, seasons, and acclimation treatments in *Glossina pallidipes* (Terblanche *et al.*, 2006), but most of the intraspecific variation was accounted for by within-generation phenotypic plasticity in response to temperature acclimation. Limitations of spreading and distribution of organisms in the tropics caused by high mountain ranges were reviewed by Ghalambor *et al.* (2006).

Genetics as well as ecophysiology of fruit flies have been thoroughly studied previously. Latitudinal clines in physiological tolerance to environmental stress have been documented in wild populations of *Drosophila melanogaster* in several continents (James *et al.*, 1995; James and

Partridge, 1995; Azevedo *et al.*, 1996; Hoffmann *et al.*, 2002; Watada *et al.*, 1986; Capy *et al.*, 1993; Hallas *et al.*, 2002). The wide geographical distribution range of *D. melanogaster* is exceptional, providing large opportunity for selection of strains adapted to local climate. However, fruit-flies generally tend to have wide distribution with low beta diversity among sites (Novotný *et al.*, 2007). The widespread existence of clinal variability suggests that the climate plays a key role in life history and physiology of the flies. On the other hand, altitudinal gradients in traits of *Drosophila* have been less well studied (Etges and Levitan, 2004; Sorensen *et al.*, 2001, 2005; Parkash *et al.*, 2005; Collinge *et al.*, 2006).

Heritable clines along latitude in eastern Australia have been established for quantitative traits of *D. melanogaster* including wing length/area, thorax length and cold and heat resistance. Expected clinal pattern for resistance to desiccation has not been supported (Hoffmann and Weeks, 2007). Desiccation resistance was higher under summer compared with winter simulating laboratory conditions, but this trait did not exhibit clinal variation across a latitudinal range of 27 degrees in eastern Australia (Hoffmann *et al.*, 2005). In cool, high latitude regions, *Drosophila* species are often active throughout the day, whereas in lower hot latitudes, they are most active early and late during the daylight hours. Indeed, a heritable latitudinal cline in circadian activity has been found suggesting avoidance of potential desiccation and/or thermal stress (Simunovic and Jaenike, 2006).

In this study, we examine a morphological (pigmentation) and an ecophysiological (desiccation resistance) trait in *D. melanogaster* collected from high and low altitude sites at five locations along the Indian subcontinent. We directly assess the relative importance of altitudinal and latitudinal clinal differences for the adaptations of local populations. We, therefore, test the hypothesis that populations of *D. melanogaster* are adapted to local conditions for morphological and physiological traits. In addition, we evaluate potential correlations between these traits among populations. Pigmentation was studied because previous studies (Munjal *et al.*, 2007) found it to be positively correlated with both the latitude and altitude of original populations (not original populations). Although a clinal variation of desiccation tolerance has not been previously found in other continents (Hoffmann and Weeks, 2007), our preliminary data suggested adaptive pleiotropic effects of melanisation in coping with problems related to thermal balance as well as water balance in *D. melanogaster*.

Material and Methods

From each collection site, about 80–90 wild caught individuals were obtained using banana bait traps. Isogroups were set up from field collected individuals. All collections were done in winter months (Nov–Dec, 2004) when flies are commonly available throughout India. The flies were collected from five pairs of low and high altitudinal sites at five different latitudes (Calicut/ 11°15’ and Coonoor/ 11°21’; Ratnagiri/ 16°59’ and Mahabaleshwar/ 17°56’; Deesa/ 24°12’ and Mt. Abu/ 24°36’; Bareilly/ 28°22’ and Nainital/ 29°24’; Chandigarh/ 30°44’ and Shimla/ 31°06') along the Indian sub-continent (Figure 1). Except for the Calicut (coastal region) site, the rest of the collection sites were inland. The sampling sites were characterised by their latitude, altitude, longitude, average temperature and relative humidity. The climatic data for the geographical sites were obtained from climatological tables of Meteorological Department of India which represent last 30 years averages.

Flies of each population were reared on cornmeal sugar medium inoculated with live yeast. All experiments were performed with F1 generation adults. For each population, ten randomly chosen individuals (females only) from each replicate (a total of four replicates for each isogroup) of each isogroup (a total of four isogroup) were simultaneously analyzed for pigmentation scores and desiccation tolerance.

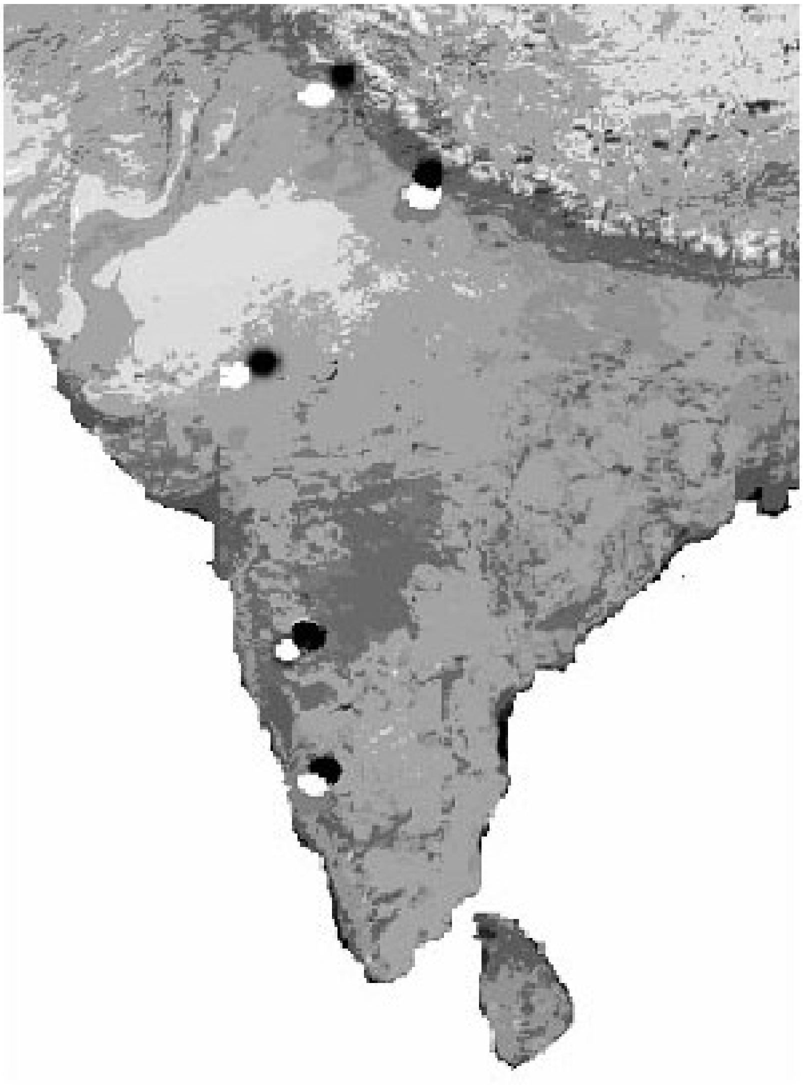

Figure 1. Map of India showing origin of populations of *Drosophila melanogaster*. Locations with black, and white circles correspond to highland and lowland sites.

For abdominal pigmentation and desiccation tolerance the methods of David *et al*. (1990) and Hoffmann and Parsons (1989) were followed. The degree of pigmentation was estimated from a lateral view of the female abdomens giving values ranging from 0 (no pigment) up to 10 (complete darkness) for each of the six visible segments. To improve the accuracy of the measurements, double blinded study (*i.e*., two persons did the pigmentation scoring and they did not know the origin of the population) was done and good repeatability observed.

For measuring desiccation tolerance (as survival time under low humidity conditions), ten individuals of each line were isolated in a dry plastic vial. These vials contained 2 g of silica gel at the bottom of each vial and were covered with a disc of plastic foam to create a <10% relative humidity environment. Four replicates were run for each isogroup to yield a total sample of n = 40. The vials were inspected hourly and the number of dead flies (completely immobile) was recorded (in order to confirm this vials was shaken twice to check the fly response). When the numbers of dead individuals approached 50%, vials were inspected every 30 minutes until all the flies had died.

ANOVA was undertaken to examine the effects of the latitude and altitude on the traits of the populations as well as the interactions between these effects. These factors were treated as fixed effects, because we deliberately selected latitude points along the Indian sub-continent and high/low altitude sites. Multiple regression analyses (Statistica 7) were carried out to examine associations between population trait means and the two macroclimatic variables: mean annual temperature and mean annual humidity and the influence of altitude and latitude. Under strong climatic selection, we might expect association between trait means and climatic variables regardless of the distance between populations.

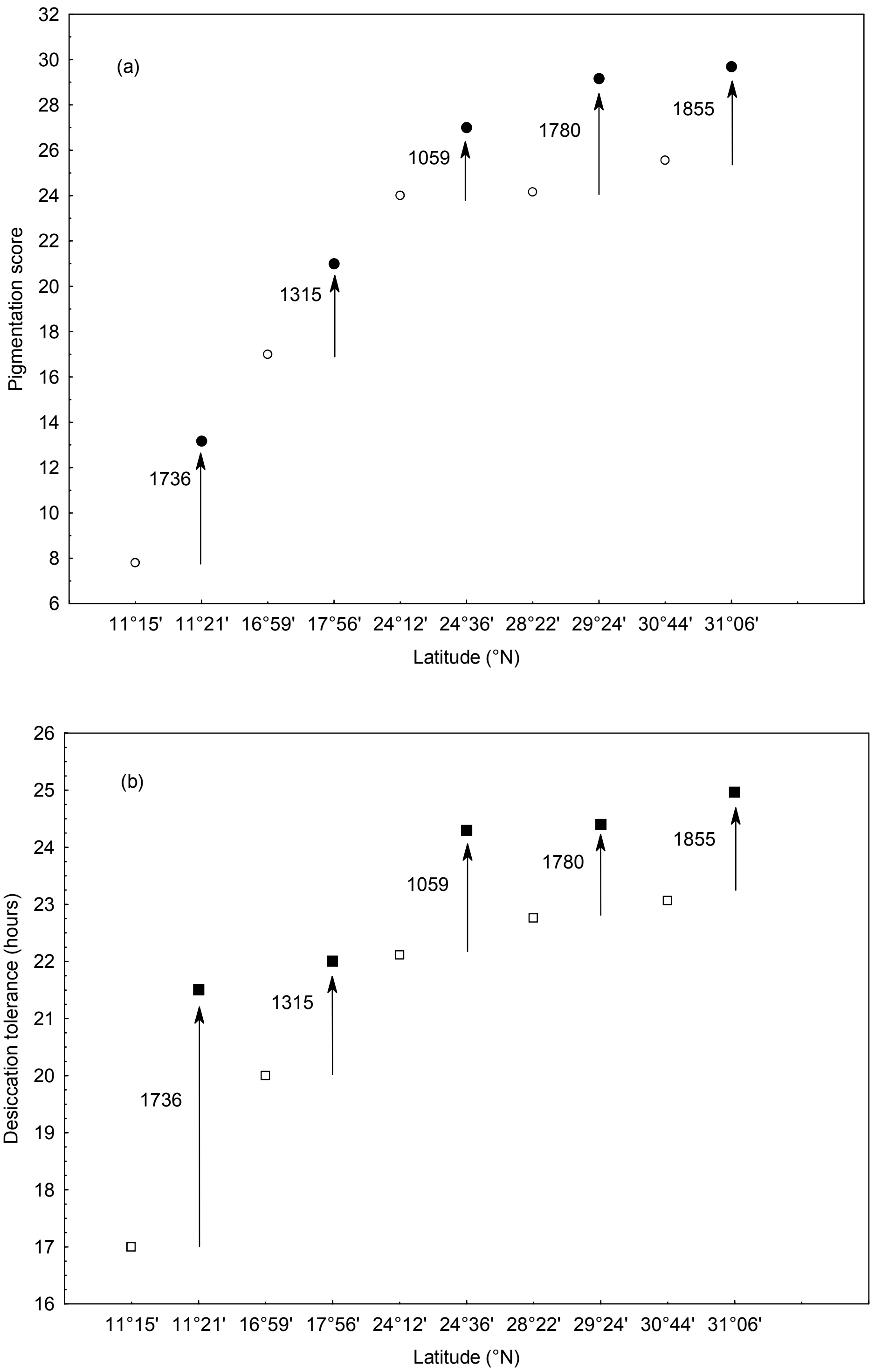

Figure 2. Trait variability along latitude for pigmentation sum (a) and desiccation tolerance (b). Numeric figures given besides arrows indicate altitudinal difference in meters between lowland and highland sites. All the lowland populations are represented in black circles or blank rectangles and highland populations in filled circles or filled rectangles.

Results

The degree of pigmentation was dependent on latitude (one-way ANOVA: $F_{x,y} = 1877$, $p < 0.001$), altitude ($F_{x,y} = 864$, $p < 0.001$), and also latitude by altitude interaction ($F_{x,y} = 8.6$, $p < 0.01$). Flies from the northernmost regions showed highest pigmentation scores (Shimla: 29.70 ± 0.86 and Chandigarh: 25.70 ± 0.71). All highland populations showed stronger pigmentation than the corresponding lowland populations (Figure 2a).

Populations from the northern India had greater desiccation tolerance (Shimla: 24.97 ± 1.21 h and Chandigarh: 23.06 ± 1.31 h) than those from southern parts (Figure 2b) of India (Calicut: 17.00 ± 0.90 h and Coonoor: 21.50 ± 0.87 h, one-way ANOVA: $F_{x,y} = 246$, $p < 0.001$). All highland populations showed better desiccation tolerance compared to lowland populations localized nearby ($F_{x,y} = 406$, $p < 0.001$, interaction between latitude and altitude: $F_{x,y} = 15.7$, $p < 0.01$).

Significant correlation coefficients were obtained when the two traits of flies were pairwise correlated with geographic (r = 0.41 for Pigmentation vs. Altitude; r = 0.61 for Desiccation vs. altitude; r = 0.93 for pigmentation vs. latitude; r = 0.81 for desiccation vs. latitude) and climatic factors (r = -0.65 for pigmentation vs. temperature; r = -0.82 for desiccation vs. temperature; r = -0.78 for pigmentation vs. relative humidity; r = -0.75 for desiccation vs. relative humidity), except for longitude ('r' less than 0.26 for both the traits). Pigmentation and desiccation tolerance increased with altitude and latitude, and in the same time decreased with average temperature and relative humidity of the site of origin. Both temperature and humidity decreased with both altitude and latitude. Both traits were strongly correlated ($R^2 = 0.88$, $p < 0.0001$).

The correlation parameters further increased when all four significant geographic and climatic parameters were used in a common multiple regression ($R^2 = 0.95$, $p < 0.002$ for pigmentation, $R^2 = 0.89$, $p < 0.003$ for desiccation). However, latitude was a single significant factor among the four ones (r = 0.74) predictive for pigmentation, the other (including altitude) contributed only slightly for the correlation (Figure 3a). For desiccation, no single factor had a significant predictive contribution to the correlation. For this reason, we used forward stepwise method of regression, resulting in two significant predictive factors for desiccation – temperature (r = –0.53) and humidity (r = –0.38) (Figure 3b).

Genetic basis of trait variability was analyzed on the basis of repeatability across generations as well as by subjecting the isofemale line data to ANOVA. For the two traits (pigmentation and desiccation), repeatability was further analyzed in two more generations (F3 and F7 added to F1). Correlation across generations (F1-F3, F3-F7 and F1-F7) showed highly significant values (0.91, 0.89, and 0.86 for pigmentation and 0.86, 0.80, and 0.83 for desiccation).

In order to explore potential associations between pigmentation and desiccation resistance, preliminary experiments were set up with wild female flies from a single mid–altitude site (Barog/1680m a.s.l.; 30°44'N, 77°01'E). The flies were separated into two groups of high and low pigmentation. The two groups differed in their desiccation tolerance (data not shown). This was further confirmed on wild females (n = 60+65) collected in January. Within this sampling location, there was, therefore, a significant positive correlation between darker flies and longer desiccation tolerance and paler flies and shorter desiccation tolerance (r = 0.79, p < 0.003).

Discussion

In the present study, populations of *D. melanogaster* sampled in a range of latitudes (11–31°N) and altitudes (0–2200 m a.s.l.) within the Indian sub-continent showed significant phenotypic differences in the degree of abdominal melanic pigmentation and desiccation tolerance (survival time). Although a clinal trend of desiccation tolerance with latitude has not been previously found in other continents (Hoffmann and Weeks, 2007), the latitudinal pattern described here (strong increase of values of both traits with latitude) is consistent with previous studies done by Parkash and Munjal (1999) and Das (1995). High latitude sites were cooler and in the same time drier – an opposite situation to subtropical–temperate climatic regions.

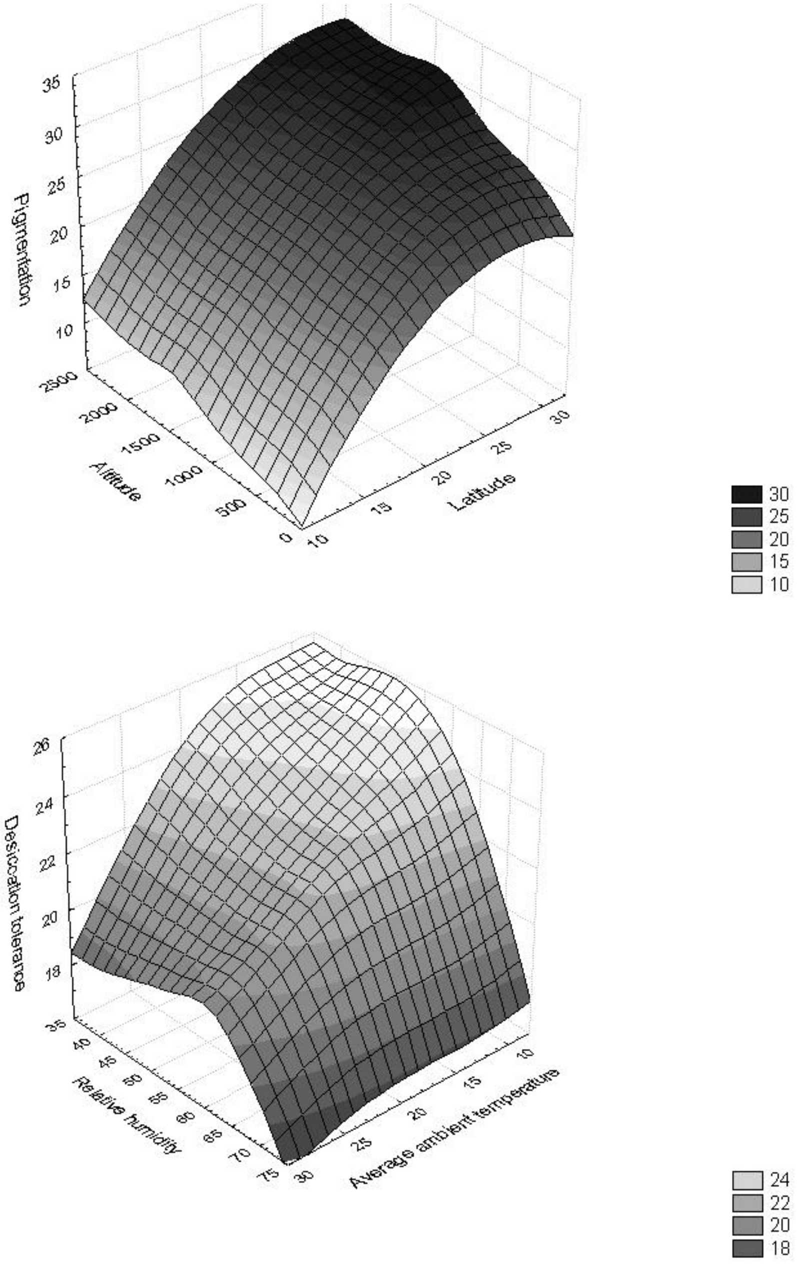

Figure 3. General trends of (a) increase of melanic pigmentation score of abdomen with latitude (°N) and altitude (m a.s.l.), and (b) increase of desiccation tolerance (hours of survival) with average ambient temperature (°C) and relative humidity (%) through Indian subcontinent in female *Drosophila melanogaster*. Surface = Distance Weighted Least Squares.

Higher altitude also hosted flies that were more resistant to desiccation and darker. This correlation of the traits with altitude was not so strong over the entire scale and when analysed by multivariate regression, but constant in pairwise comparison. Mountain sites were always cooler and also drier than correspondent lowland sites.

Because mean ambient temperature and relative air humidity are inversely correlated with both latitude and altitude, it might be possible to explain the observed variation as the result of adaptation to local environments. We tried to decide between the roles of either geographical coordinates or climatic factors by forward stepwise method of multiple regression. While the desiccation tolerance (survival time) increased with decreasing ambient temperature and air humidity, *i.e.*, with climatic factors (Figure 3b), degree of pigmentation increased significantly only with latitude and slightly with altitude of the site of origin (geographical factors). The strong correlation of desiccation tolerance with climatic variables supports a direct role for climatic selection in generating altitudinal as well as latitudinal patterns in this trait. The selection effect on pigmentation may be non-direct or more complicated. Gene flow between sites with different climate may be more effective for pigmentation and penalized by selection for desiccation tolerance. Although proximate selection factors for the two traits may be different, they were repeatedly found to be strongly correlated to each other.

The role of body melanization for thermoregulation has been explored in several ectothermic animals, *e.g.*, in ladybird beetles (*Adalia bipunctata*) and in *Colias* butterflies (de Jong and Brakefield, 1998; Ellers and Boggs, 2002, 2004). Clusella-Trullas *et al.* (2007) and Majerus (1998) have reviewed all such evidence in favor of thermal melanism. Heritable increase in melanization of abdominal segments with latitude and altitude in *D. melanogaster* agree with the hypothesis that black body surfaces better absorb solar radiation in order to maintain optimum body temperature under colder ambient temperatures. A disadvantage of being darker is that the animal may overheat more easily, but this is often compensated by behavioural mechanisms, as in the firebug (Honěk, 1986). The assumption that colour, thermal physiology and behaviour are coadapted has been supported (Clusella-Trullas *et al.*, 2007).

Direct evidence of different effects of solar radiation and thermal properties on melanics versus typical pale forms has been demonstrated in butterflies and in beetles (Roland, 1982; Guppy, 1986; Brakefield and Willmer, 1986). Significant differences in behavioural thermo regulation between dark and pale individuals appeared in females, but not in males of the grasshopper *Tetrix undulata* (Forsman *et al.*, 2002). Surprisingly, behavioural differences between individuals belonging to different colour morphs were genetically determined, rather than simply reflecting a response to different heating rates according to the actual body coloration.

In montane habitats, organisms have to cope with colder and usually wetter conditions. In all our sites throughout Indian subcontinent, the conditions at high altitudes were slightly drier. At low ambient temperature, the *absolute* water content of the ambient air is reduced even at the same *relative* humidity. Several investigations have considered interspecific differences in desiccation tolerance with mechanistic link to the problems of water balance (Zachariaseen, 1996; Gibbs *et al.*, 1997; Hoffmann and Harshman, 1999; Addo-Bediako *et al.*, 2001). However, similar studies on intraspecific level are limited (Eckstrand and Richardson, 1981). Amount of cuticular lipids varied among populations of *Glossina pallidipes* and was not correlated with prevailing temperatures, humidities, and vegetation density (Jurenka *et al.*, 2007).

The present study suggests that *D. melanogaster* females from high altitude locations survive desiccating conditions significantly longer than lowland populations, at least when geographically close pairs of localities are considered. This could be because of selection to reduced rates of water loss and an increased ability to tolerate dehydration. Numerous studies have considered the mechanistic basis of water balance under hot and dry environmental conditions in diverse taxa of insects (Gibbs *et al.*, 1997). Neither a reduction in body size (which might be selected to reduce chances of freezing at high altitudes) nor corresponding increase of water loss (and thus probable reduction of survival time in desiccating conditions) were observed. Only the Nainital locality periodically experiences subzero temperatures.

The water loss rate can be strongly influenced by body size variation, while little direct support could be found for the assumption that there is a consistent melanism-body size tradeoff (Clusella-Trullas *et al.*, 2007). This problem is reviewed by Chown and Terblanche (2007). We did not observe a consistent pattern of body size in our material.

Moreover, desiccation tolerance was not linearly dependent on the ambient relative humidity, but rather steeply increased with decreasing humidity between 75–60%, and then remained unchanged (Figure 3b). Dependence of desiccation tolerance on average temperature was gradual.

Pleiotropic effect of genes on several phenotypic traits was found in some animals. In flour moth (*Ephestia kuehniella*) the effects of genes controlling melanism resulted in significant higher flight as well as walking activities in melanics than non-melanic genotype (Verhoog *et al.*, 1998). Since our finding of strong intercorrelation and heritability of pigmentation and desiccation tolerance has no direct explanation, we consider it as a hidden pleiotropic effect of genes. These findings are consistent with the hypothesis of multiple-trait coevolution demonstrated in females of a grasshopper (Forsman *et al.*, 2002), and suggest that the different colour morphs represent alternative evolutionary strategies. Since filial (F1) generation of wild-caught flies was studied in the laboratory, the revealed differences in measured traits among populations may be mostly related to heritable (genotypic) differences, while the role of phenotypic plasticity in processes of local adaptation (Driessen *et al.*, 2007) remains relatively unclear (see Hoffmann *et al.*, 2005; Terblanche *et al.*, 2006, for discussion).

In conclusion, analysis of populations of *D. melanogaster* demonstrates that body pigmentation is genetically variable and subjected to natural selection pressure under colder and drier conditions. Parallel and correlated to pigmentation was desiccation tolerance, while selection responses might slightly differ. Melanisation is a likely candidate for cuticular impermeability for reducing water loss under increasing dehydrating conditions along longitudinal and altitudinal transects. In almost all cases, altitudinal differences match patterns evident at the latitudinal level. The analysis of climatic factors has shown that temperature average and humidity can be responsible for maintaining genetic heterogeneity in the traits related to thermal and water balance.

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Analysis of the drosophilid *Zaprionus indianus* introduction in Brazil: contribution of esterase loci polymorphisms.

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Introduction

The colonization of environmental niches by exotic species constitutes an unplanned experiment that provides historical information on ecology and evolution (Carroll and Boyd, 1992; Cox, 2004; Strauss *et al*., 2006). Such invasions allow comparing the biological responses to the new niche with those to the ancestral environments, by direct observation rather than by inference as it is usually done, as observed by Carroll (2007).

Recently, a drosophilid - *Zaprionus indianus* - was introduced in Brazil (Vilela, 1999). This is an African species (Tsacas, 1985) that had already spread over Asia several decades ago (Gupta, 1970; Amoudi, 1993; Parkash and Yadav, 1993a,b; Karan *et al*., 2000). Since then, a number of reports have indicated its occurrence in several regions of Brazil (Galinkin and Tidon, 2000; Moraes *et al*., 2000; Vilela *et al*., 2001; Toni *et al*., 2001; Tidon *et al*., 2003; Machado *et al*., 2005; Galego *et al*., 2006), in other South American countries (Goñi *et al*., 2001, 2002) and recently in the United States of America (Linde *et al*., 2006). In Brazil, *Z. indianus* was known as fig fly because of the loss of 50% of the fig crop in 1999 due to larvae infestation. The potential status of pest during the early period of its introduction stimulated Brazilian researchers to study *Z. indianus* aiming at understanding the biology, ecology, genetics, and population structure of this well succeeded invader. An adequate tool to investigate the genetic structure and the relationships between populations are the alloenzyme markers as, for instance, esterases, a multi-functional and heterogeneous group of enzymes that frequently show polymorphic loci in Drosophilidae (Brady and Richmond, 1990; Parkash and Yadav, 1993; Russell *et al.*, 1996; Dumancic *et al*., 1997; Nascimento and Bicudo, 2002; Campbell *et al*., 2003; Machado *et al*., 2005; Galego *et al.,* 2004, 2006).

In this study, populations from 22 regions of Brazil were sampled, so as to propose a model of *Z. indianus* introduction in the country, using as a reference the genetic structure of these populations by means of data on esterase polymorphisms. The polymorphic Est3 locus was used as marker,