Direct and correlated responses to laboratory selection for body melanisation in *D. melanogaster*: support for melanism- desiccation resistance hypothesis.

Seema Ramniwas*, Babita Kajla, Kapil Dev and Ravi Parkash

Department of Genetics, Lab No. 13, Maharshi Dayanand University, Rohtak-124001, India.
Tel. no. +91- 8930787521

**Author for correspondence* (seema.ramniwas@gmail.com)

Running title: Melanism- Desiccation hypothesis revisited

SUMMARY

For *Drosophila melanogaster*, cuticular melanisation is a quantitative trait, varying from no melanin to complete dark. Variation in melanisation has been linked with stress resistance, especially desiccation, in *D. melanogaster* and other species. As melanism has a genetic component, we selected melanic and non- melanic phenotypes of *D. melanogaster*, in order to confirm the association of desiccation resistance and rate of water loss with cuticular melanisation previously reported for this species. A bidirectional selection experiment for dark (D1- D4) and light (L1- L4) body color in *D. melanogaster* was conducted for 60 generations. In 60 generations of selection for pigmentation, an increase of 1.6 fold in selected dark strain and decrease of 14 folds in selected light strain was observed as compared to control populations. Desiccation resistance increased significantly in the dark selected morphs as compared with control. The observed increase in desiccation resistance appeared as a consequence of decrease in cuticular permeability. Our results show that water balance related traits were significantly correlated with abdominal melanisation and were simultaneously selected bidirectionally along with melanisation.

Keywords: Artificial selection experiment, abdominal melanisation, correlated responses, desiccation resistance, *Drosophila melanogaster*

INTRODUCTION

Several studies have shown evidences for the role of natural selection in maintaining phenotypic variation in body melanisation of diverse insect taxa (Majerus, 1998; True, 2003). In *D. melanogaster*, body melanisation is a quantitative trait and shows significant levels of both within and between population variations (Parkash et al., 2008). Geographical populations of *D. melanogaster* from Africa (Pool and Aquadro, 2007), India (Parkash et al., 2008) and Australia (Telonis- Scott et al., 2011) have shown clinal variation in abdominal or thoracic melanisation which suggest adaptations to local climatic conditions. In several other studies, clinal variation is evident for many stress related traits which covary strongly with latitude or altitude (Parkash et al., 2008). However, it is not clear whether various ecologically relevant quantitative traits show independent or correlated selection responses. For example, associations between body melanisation and desiccation related traits have been observed in latitudinal as well as altitudinal populations of *D. melanogaster* from India but these studies provide indirect evidence (Parkash et al., 2008a). If desiccation resistance evolves through changes in cuticular permeability in *D. melanogaster*, the target of selection might be cuticular components (cuticular melanisation and/ or cuticular lipids). A possible link between body melanisation and desiccation resistance can be shown if laboratory selected strains for higher melanisation evidence increased desiccation but this hypothesis has not been tested so far in *D. melanogaster* as well as other *Drosophila* species.

For wild populations, ecophysiological and morphological traits might coevolve according to their combined influence on fitness (Angilletta, 2009). For example, tropical habitats on the Indian subcontinent select lighter body color phenotype as well as starvation

tolerance (Parkash and Munjal, 2000) but it is not clear whether these traits are under independent selection or coevolve. Further, there are evidences in favor of coadaption hypothesis (Angilletta, 2009) e.g. behavioral thermoregulation and body coloration are coadapted traits in pygmy grasshopper (Tetrix subulata; Forsman, 2000). Coadapted traits are also represented by associations between body melanisation and desiccation related traits (Parkash et al., 2008a, 2010a) and thermo-resistance traits in *D. melanogaster* (Parkash et al., 2010b). Cuticular hydrocarbons are also subject to natural selection, being important in providing desiccation resistance for many insect species (Gibbs & Rajpurohit, 2010). Several insect taxa have shown variable cuticular permeability due to changes in the composition or amount of cuticular lipids (Edney, 1977; Toolson, 1984; Hadley, 1994; Rourke, 2000). There are clines of water balance measures correlated with the amount of cuticular lipids (Rourke, 2000; Parkash et al., 2008a). Seasonal changes in the composition or amount of cuticular lipids also affect water loss in scorpions and tenebrionid beetles (Hadley, 1977; Toolson and Hadley, 1979; Hadley and Schultz, 1987). In contrast, analysis of water balance mechanisms in diverse *Drosophila* species have shown lack of changes in cuticular traits for reduced body water loss (Gibbs et al., 1998, 2003; Gibbs and Matzkin, 2001; Parkash et al., 2008a) Further, there is lack of differences in Tm (melting temperature), composition and/or amount of surface lipids in laboratory selected desiccation-resistant and sensitive strains of *D. melanogaster* (Gibbs et al., 1997). Within species, Indian populations of *D. immigrans* differ in water-loss rate, but not in surface lipid amounts (Parkash et al., 2008c). In a laboratory selection experiment, populations selected for desiccation resistance lost water ~50% less rapidly than unselected controls, but the two groups exhibited minor differences in lipid composition and Tm (Gibbs et al ., 1997). Thermal acclimation of the desert fly, *D. mojavensis*, results in substantial changes in HC composition,

but relatively little change in water-loss rates (Gibbs et al., 1998). It must be noted that not all studies result in negative findings (e.g. Toolson, 1982; Toolson and Kuper-Simbrón, 1989). If ecophysiological traits and cuticular traits (body melanisation and/ or cuticular lipids) coevolve, we may expect correlated responses between body color phenotypes and stress-related traits in *D. melanogaster* but such traits associations have not been analyzed so far.

Several studies have focused on laboratory selection of desiccation resistance (Hoffmann and Parson, 1989a, b; 1993; Gibbs et al., 1997; Chippindale et al., 1998; Djawdan et al., 1998; Telonis- Scott et al., 2006); thermal sensitivity (Huey et al., 1991; Gilchrist and Huey 1999; Anderson et al., 2005) and starvation resistance (Chippindale et al., 1996; Harshman et al., 1999; Bubliy and Loeschcke, 2005). In contrast, a single study on laboratory selection of abdominal spot number in *Drosophila falleni* has shown that selection exerted by nematode parasites may influence pigmentation patterns (Dombeck and Jaenike, 2004). In most of the laboratory selection studies, replicate lines at the end of selection protocol were investigated for changes in the trait of interest as well as correlated selection responses but time course of evolutionary response in the selected lines has not been investigated. A time course analysis is although time consuming but can be helpful in better understanding the selected as well as correlated traits.

Experiments of artificial selection in *Drosophila* have recently been used as an experimental evolutionary tool to identify the relevant traits that are most likely to be involved in adaptation to environmental temperature in ectotherms (Hoffmann et al., 2003; Bowler andTerblanche, 2008). One of the main advantages of artificial selection experiments is the possibility to evaluate not only direct, but also the correlated responses to selection (Harshman and Hoffmann, 2000). Artificial selection experiments have revealed moderate to relatively high levels of heritability for resistance to high-temperature stress in *D. melanogaster* (Hoffmann et

al., 2003; Reusch and Wood, 2007). Remarkably, most artificial selection programs were mainly performed in *D. melanogaster*, though recent studies have also addressed the question whether or not results in *D. melanogaster* are consistent across species (Hoffmann and Willi, 2008). Further, artificial selection on thermal-stress traits was generally performed in only a single direction (mainly, for increased resistance), but studies have shown that selection for decreased resistance to heat stress can also be informative as the selection response can often be asymmetrical for thermal-stress traits (Gilchrist and Huey, 1999; Folk et al., 2006; Norry et al., 2007; Mori and Kimura, 2008; Gomez et al., 2009; Bertoli et al., 2009).

Body melanisation is one of the most common types of phenotypic variations in insects (Majerus, 1998). Phenotypic variation of body melanisation in some lepidopterans and coelopterans are represented by discrete morphs (melanic and non-melanic) consistent with a major locus (da Cunha, 1949; Napp Martinez and Cordeiro, 1970). Several studies have shown changes in the frequencies of two or more allelic variants in response to temporally or spatially variable climatic conditions (Umina et al., 2005; Parkash et al., 2009; 2012). In contrast, variation in body melanisation in a *D. melanogaster* population follows a bell shaped curve and such a quantitative trait is expected to respond fast to laboratory as well as field selection. Such pigmentation differences are polygenic and interact with abiotic factors of the environment (Wittkopp et al., 2003). In *D. melanogaster*, melanisation varies continuously across geographical gradients on different continents and such clines linked with body melanisation reflect adaptations to local climatic conditions (David et al., 1985; Capy et al., 1988; Munjal et al., 1997; Pool and Aquadro, 2007, Clusella Trullas et al., 2007; Telonis-Scott et al., 2011). Several studies have considered plastic change in melanisation scores at different growth

136 temperatures in the laboratory populations of *D. melanogaster* (Das et al., 1994; Ottenheim et al.,
137 1999; Gibert et al., 1996, 2000; DeWitt and Scheiner, 2004).

138 Melanin patterns are involved in diverse aspects of insect ecology (Majerus, 1998, True
139 2003). For example, increased melanisation has been associated with higher fitness under
140 thermal as well as aridity stresses in *D. melanogaster* i.e. a darker cuticle may improve
141 thermoregulation as well as may reduce cuticular water loss (Parkash et al., 2008). Changes in
142 body melanisation are associated with thermal and/ or water stress related traits but the target of
143 selection is not clear. Laboratory selection of desiccation and starvation resistance show parallel
144 responses in *D. melanogaster* while field population show opposite clines. Such a mismatch
145 between field vs. laboratory selection may be due to selection acting on some other associated
146 trait which may impact resistance to starvation and desiccation in different ways. There is ample
147 empirical support for the thermal melanism hypothesis (Watt, 1969, David et al., 1985, 1990; de
148 Jong et al., 1996, Majerus, 1998, Rajpurohit et al., 2008). Several studies have demonstrated a
149 direct influence of melanization on body temperature, by increasing solar absorption under cool
150 conditions (de Jong et al., 1996; Ottenheim *et al.*, 1999; Ellers and Boggs, 2004). Furthermore,
151 the higher body temperature in more melanized females also increases egg maturation rate
152 (Ellers and Boggs, 2004) and, in ladybird beetles, dark-coloured individuals benefit from
153 increased mating success and earlier emergence in spring (Ellers & Boggs, 2004). In copper
154 butterflies, pupal and wing melanization increased with increasing altitude (Karl et al., 2009).
155 Conversely, lighter individuals may be better protected against overheating in warm
156 environments (Munjal *et al.*, 1997; Ellers and Boggs, 2002; Pereboom and Biesmeijer, 2003).
157 Accordingly, selection is expected to favour darker phenotypes in colder environments.
158 However, a longitudinal cline for body color in *D. americana* is not associated with desiccation

resistance (Wittkopp et al., 2011). Thus, it is not clear whether change in melanisation can play a role in multiple abiotic stressors. If body melanisation is the target of selection, we may expect changes in correlated traits consistent. Laboratory selected darker and lighter lines may clarify whether such traits may coevolve or not.

The primary aim of the present study is to establish laboratory selected replicate lines with high and low abdominal melanisation and to analyze change after every five generation in abdominal melanisation due to direct selection response as well as correlated selection responses in physiological traits related to water stress. We assessed traits related to water conservation i.e. body water content, rate of water loss, hemolymph content and dehydration tolerance level in dark as well as light selected replicate lines of *D. melanogaster*. We further tested whether desiccation resistance show covariation with selected darker and lighter body color lines. We also provide data on realized heritability based on laboratory selected lines. This paper presents the results of a long-term artificial selection experiment having three major strengths: 1) selection was maintained for 60 generations, a greater length than most experiments of this type; 2) the trait under selection varies clinally in the field, and a suite of correlated responses were also measured, giving the work a good grounding in the biology of the organism in the field; and 3) the genetic variation in the trait was measured every 5 generations, a rarely implemented addition to a selection experiment.

MATERIALS AND METHODS

Selection of lines for dark and light body color

Wild living flies of *Drosophila* species (n = 150- 200 flies per site) were collected from six localities, two lowland: 500-600m (Kalka and Parwanoo); two midland: 1200- 1400m (Kandaghat and Solan); and two highland localities: 2000- 2200m (Kasauli and Shimla) along an altitudinal transect in the western Himalayas. On an average, about 40 % were *D. melanogaster* out of each sample of wild caught flies. From each population, thirty pairs of wild caught flies were pooled to make a mass bred population which was grown for seven generation (two week cycle on standard *Drosophila* media) at a constant growth temperature of 21±0.05 °C and 65 ± 1 % relative humidity in a thermo as well as humidity controlled incubator. This mass-bred population was maintained in a population cage (n = 5000- 6000 flies) in the laboratory for seven generations before onset of selection protocol. Four stocks (P_1- P) each with randomly chosen 500- 600 flies were derived from this mass-bred population. For each stock, two replicate lines (control and selected) were established. Thus, we had four control and their respective four selected (S_1- S_4) lines, each with about 300- 350 pairs of flies.

For the selection regime, from each of the four selected (S_1- S_4) lines, forty dark and forty light female flies were selected to initiate the next generation while remaining flies were discarded. Each generation, about 300 emergent female flies per replicate were aged for 7 days prior to establishment of the next generation. It was observed that melanisation of flies did not change after two days. The flies with > 45 % and < 30 % body melanisation were sorted as darker and lighter flies for the first generation of selection. In the selection regime, each generation forty dark and forty light female flies were selected to initiate the next generation while remaining flies were discarded. These selected female flies correspond to selection intensity of about 1.40 (Falconer, 1981), this selection intensity depends only on the proportion

of the population included in the selected group and, provided the distribution of phenotypic values is normal. This selection procedure was followed independently for each of the mass bred five stocks. Selection protocol was followed for 60 generations resulting in dark selected line (D_1, D_2, D_3 and D_4) and light selected line (L_1, L_2, L_3 and L_4).

A replicate of each selected line (D_1, D_2, D_3, D_4 and L_1, L_2, L_3, L_4) was maintained without further selection after 60^{th} generation i.e. from 61^{st} through 65^{th} generation. These replicate lines were maintained with the same number of flies without any further selection regime. For relaxed lines i.e. 61^{st} through 65^{th} generation, a sample of 100- 150 flies of each dark and light selected line was again scored for changes in abdominal melanisation, if any.

Quantification of melanisation: direct response to selection

(a) General scoring method

Body melanisation was estimated under a stereo zoom microscope (Olympus, www.olympus.com) from a lateral view of the female and male abdomen giving values ranging from 0 (no melanisation) to 10 (complete melanisation) for each of the six visible (2^{nd} to 7^{th}) abdominal segments (David et al., 1990). Since the abdominal segments differ in size, relative sizes (i.e. 0.86, 0.94, 1.0, 0.88, 0.67 and 0.38 for 2^{nd} to 7^{th} segments respectively) were multiplied with segment wise melanisation scores. Abdominal melanisation scores were weighted to the relative sizes of the respective segments. The abdomen of each fly minus viscera was mounted on a slide and total body melanisation per fly was also estimated through Biowizard image analysis software, - Dewinter Optical Inc. - (www.dewinterindia.com). Data on

percent melanisation were calculated as (Σ observed weighted melanisation scores of six abdominal segments per fly/ Σ relative size of each abdominal segment x 10 per fly) x100.

(b) Response to selection

The selection response was measured at each fifth generation. Selection of abdominal melanisation was done on female flies only, but in the time course of selection males were also analyzed. For both sexes and each replicate line of control as well as selected dark and light flies 60 flies were scored for abdominal melanisation at each 5^{th} generation.

Correlated responses to selection: analysis of stress resistance/assay

We analyzed correlated changes in body water content, hemolymph content, desiccation resistance, rate of water loss and dehydration tolerance in selected darker and lighter replicate lines of *D. melanogaster*. We further analyzed changes in epicuticular lipids in control and selected replicates, if any. For analysis of stress resistance and epicuticular lipids, we analyzed ten replicates from each of the control (C_1 to C_4), dark selected (D_1 to D_4) and light selected (L_1 to L_4) lines of *D. melanogaster*.

a) Desiccation resistance

Desiccation resistance was measured as time to lethal dehydration effect under dry air in ten replicates of female and male individuals of each of the control (C_1 to C_4), dark selected (D_1 to D_4) and light selected (L_1 to L_4) lines. 10 male and female individuals were isolated in dry

plastic vials which contained 2 g of silica gel at the bottom of each vial and was covered with a disc of plastic foam piece. Finally, such vials with foam plugs were placed in a desiccator chamber (Secador electronic desiccators cabinet; www.tarson.com) which maintains 6-8% relative humidity. Number of immobile flies was counted at every 1h interval. The time period to lethal desiccation (LT_{100}) effect in dry air was recorded.

b) Basic measures of water balance

In order to estimate total body water content, rate of water loss and dehydration tolerance (%), 10 flies of each of the C, D and L (10 replicate each) were used. First, the flies were weighed on Sartorious microbalance (Model- CPA26P; with precision 0.001 mg) and then reweighed after drying at 60 °C overnight. Total body water content was estimated as the difference between masses before and after desiccation stress of 8h at 6- 8% relative humidity, and water loss was calculated as: (initial body mass- body mass after 8h desiccation stress)/ initial body mass x 8; and the values were given in $\mu g\ h^{-1}$. Dehydration tolerance was estimated as the percentage of total body water lost at death due to desiccation (until death) and was calculated by the formula (wet body mass – mass at death) / (wet body mass – dry body mass) x 100.

To measure hemolymph volume, blotting assays were conducted. Ten replicates of 7 days old male and female individual were anesthetized and weighed as a group. The abdomen of each was gently torn with surgical forceps, and hemolymph was blotted from the opening with a piece of Kimwipe® slightly moistened with isotonic saline. Within a maximum of 10 min, 10 blotted flies were reweighed as a group and dried for 1h at 60 °C and weighed a third time. Hemolymph volume was estimated from the reduction in mass following blotting.

c) Response of cuticular lipids to selection

For estimation of cuticular lipid mass per fly, individual flies in ten replicates per replicate line were dried overnight at 60 °C to get constant dry mass i.e. devoid of body water. Such dried flies were kept in HPLC-grade hexane for 1h; thereafter the flies were removed from the solvent and were again dried at room temperature and finally reweighed. The sartorius microbalance (CPA26P, www.sartorius.com) with precision upto 0.001mg ensured accuracy. For each individual fly, cuticular lipid mass in mg was estimated per unit surface area (surface area scales to 2/3 power 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).

Statistical analyses

The response to selection was analyzed by computing realized heritability over every fifth generation of selection in both darker and lighter selected replicate lines. The realized heritability for cuticular melanisation was calculated for each line by plotting the mean melanisation score for each generation against the cumulative selection differential. The expected selection differential was calculated as the deviation of the mean cuticular melanisation score of the selected individuals in each generation from the population mean before selection. This was then summed each generation to give the cumulative selection differential. The realized heritability (h^2) was then calculated from the slope of the regression of mean color score (R) against the cumulative selection differential (S), as $h^2 = R/S$ (Falconer and Mackay, 1996). To

compare control and selected dark and light replicate lines analysis of variance (ANOVA) was used, with replicate line (4 replicate each control, dark and light group x 10 replicate each). Mean ± s.d values of replicates were used for tabular and figure illustrations. Statistica (Statsoft Inc., Release 5.0, Tulsa, OK, USA) was used for calculations as well as illustrations.

RESULTS

Response to selection: changes in abdominal melanisation in dark and light selected lines

Selection was initiated from mass-bred populations having high variability for abdominal melanisation (m ± s.d. = 41.39±13.12, Fig. 1A). Selection for abdominal melanisation in *D. melanogaster* for 60 generations resulted in a decrease of around 14 fold (ANOVA; females: $p < 0.0001$; males: $p < 0.0001$; Table 1) in light selected strains; and 1.6 fold increase in D selected strains (ANOVA; females: $p < 0.0001$; males: $p < 0.0001$; Table 1). A plot of the response to selection as a function of cumulative selection differential (Fig. 1B) indicated that the response was asymmetric: divergence from unselected controls was faster and greater in lighter selected lines than dark selected lines.

Realized heritabilities were estimated from the regression of selection response on cumulative selection differential. The regression of cumulative selection differential on response was highly significant for each of the dark and light selected lines giving heritability estimates ($h^2 \pm$ SE) for cuticular melanisation 0.46±0.03 for dark selected lines (mean value of five

replicates) and 0.39±0.02 for light selected lines (Fig 1B). For abdominal melanisation, females responded quickly to selection. The laboratory selection was done on females only but males were also found to respond to selection for changes in abdominal melanisation (Fig 2A). Dark selected females showed ~87 % melanisation, while males exhibited ~88 % melanisation (Fig. 2A). However, in light selected strains, females and males were very light (about 3% melanisation only, Fig 2A). The replicate selected lines reached significantly different final % melanisation, and the complete lack of response to relaxed selection. Further, the variability in each D and L selected strains was quite low (dark: m ± s.d = 87.12 ± 0.79; light = 3 ± 0.21).

Physiological assays

Male and female flies were tested for changes in body water content (Table 1) and the analysis showed a significant line term in the ANOVA ($p < 0.001$). Darker selected lines have around 10.0 % more water content, whereas in light selected replicates body water content reduced by ~13.0% as compared with their unselected control. Figure 2 (B-D) illustrates a comparison of desiccation-related traits in selected darker and lighter body color strains as compared with unselected control lines. For control and selected strains of *D. melanogaster*, grown at 21º C, there is lack of differences in cuticular lipid mass. In contrast, we found significant difference in the desiccation resistance of these two selected darker and lighter strains (Table 2, Fig. 2B) and significant difference in their rate of water loss i.e. 0.3% / hr (1.5% / hr in darker versus 1.8% / hr in lighter strains; Fig. 2C). However, the level of dehydration tolerance is

much higher in darker (~82.32%) than lighter strains (~22%) as compared with control lines (~50%; Table 2; Fig. 3D).

Correlated responses to selection

For any trait, consistent bidirectional changes in selected lines relative to their unselected control lines are due to the effect of selection (Lynch, 1980) and indicate that abdominal melanisation and the co-responding traits are influenced by some of the same genes (i.e. show genetic covariance; Falconer 1981). 60 generations of artificial selection on abdominal melanisation produced consistent bidirectional selection, correlated response in body water content, desiccation resistance, rate of water loss, dehydration tolerance (Table 1; Fig 2 B-D) and mean trait values increased significantly for body water content, desiccation resistance and dehydration tolerance in dark selected replicate lines, whereas rate of water loss showed a negative correlated response. However, in light selected replicate lines body water content, desiccation resistance and dehydration tolerance decreased significantly; and rate of water loss showed a positive correlated response (Table 1; Fig. 2 B-D).

Direct selection of cuticular melanisation impact desiccation resistance significantly (Fig. 2). Comparison of direct and indirect selection responses provide a clearer view of melanism – desiccation correlation hypothesis. Replicates selected for higher melanisation showed higher desiccation resistance and reduced rate of water loss, whereas desiccation survival decreased and rate of water loss increased significantly in replicates selected for low cuticular melanisation as compared with control (Table 2). Selection for cuticular melanisation had a significant effect on

water balance and desiccation resistance. Darker lines had significantly higher desiccation resistance ($p < 0.0001$) than control flies, whereas lighter ones have 2 fold decreases in desiccation survival. Similar effects were observed for rate of water loss ($p < 0.0001$). For body water content, there were non significant changes in selected vs. control replicates ($p < 0.23$). Further, we observed no changes in wing length (as a measure of body size) due to selection ($F_{7, 72} = 5.01$ ns).

Flies were analyzed for cuticular lipids in control as well as selected replicates to examine their response towards selection and relation with desiccation resistance. Cuticular lipids do not change in response to selection (Fig. 3D, Table 2). There was a non significant difference for cuticular lipids between the control lines ($p < 0.07$) and also between the selected lines ($p < 0.20$) when tested by ANOVA. Changes in desiccation and changes in water loss rate are significantly correlated with selection in cuticular melanisation (Fig. 4, Table 3). Cuticular lipids do not respond to selection and were not correlated with increasing desiccation resistance and water balance (Table 3).

DISCUSSION

D. melanogaster exhibits considerably high variation in abdominal melanisation in natural populations. Laboratory selected dark and light body color strains of *D. melanogaster* differ significantly in abdominal melanisation. Our study shows that there is a great deal of

genetic variation in abdominal melanisation in *D. melanogaster*, as evidenced by consistent, rapid and substantial response to selection for high and low melanisation phenotypes. For body melanisation, we found a rapid response to laboratory selection. Interestingly, selection produced both light females as well as light males, in contradiction with the well known sexual dimorphism of body melanisation in *D. melanogaster*. The replicate selected lines reached significantly different final % melanisation, and the complete lack of response to relaxed selection suggests that different combinations of alleles affecting abdominal melanisation may have become fixed in the different selected lines. Although, the external appearance of our selected strains is different from typical wild populations, we detected no statistically significant effects of melanisation on basic morphometric traits such as wing length, thorax length and wing width ($p<0.12$ns). However, dark and light selected strains differ significantly in wet body mass ($p<0.001$).

We found an asymmetric response to selection, in lines selected for dark and light abdominal melanisation in *D. melanogaster* (Fig 1B). Asymmetric responses to selection are a common finding in selection experiments. The reasons given by Falconer and Mackay (1996) involve experimental artifacts (random drift, inbreeding depression and unmeasured natural or sexual selection acting during the experiment) as well as a multitude of potential genetic causes (genetic asymmetry, presence of major genes, scalar asymmetry). Random drift is unlikely to explain the present results because of the consistency of response between replicates. Inbreeding depression is also unlikely because the mean of the unselected control line did not decline. However, we can not at this point rule out the genetic causes (major genes, directional dominance or genetic asymmetry); it is possible that the asymmetric response is explained by scalar asymmetry: high phenotypic values might be particularly subject to environmental

influence (e.g condition-dependency, rearing conditions) and the extreme values an artifact of laboratory rearing. One interesting possibility is that the asymmetry results from the previous action of selection in the base population. Favorable alleles are expected to have frequencies above their symmetrical points (Falconer and Mackay, 1996).

Artificial selection experiments are longer in duration and correlations are limited to the trait being selected, but they have the advantage of directly revealing patterns of responses and co- responses to a specified selection regimen. Thus, they can provide an independent test for the presence of significant patterns of genetic variation and co- variation in a given environment. Due to the physiological relationship between melanisation and desiccation resistance, we predicted that selection for melanisation would result in a correlated increase and decrease in desiccation resistance of *D. melanogaster*. We found that desiccation resistance was higher in dark selected strains and was significantly low in light selected strains (Fig 1C). For adaptations to drier habitats, the function of cuticular lipids in reducing cuticular water loss is well known in different insect taxa from deserts (Hadley, 1994). However, some studies have shown the role of melanisation in reducing cuticular water loss in *Drosophila* species (Parkash et al., 2008b,c). Associations between pigmentation and desiccation resistance was initially proposed by Kalmus (1941). Fraenkel and Rudall (1940) and Pryor (1940) concluded that the darkening and hardening of the cuticle are due to the same biochemical processes, which may involve cross-linking of proteins with melanin. In the present work, we have investigated effects of bidirectional selection of body melanisation selection on correlated traits. For *D. melanogaster*, rapid response to direct selection of melanisation confirms the existence of substantial additive genetic variation for this trait. Populations of *D. melanogaster*, therefore, have the potential to undergo rapid genetic changes when they are exposed to one or the other selective environment.

The correlated responses to selection indicate that at least some of the genes which contribute to variation in abdominal melanisation have pleiotropic effects on other traits. Specifically, body melanisation shares positive additive genetic covariance with body water content, hemolymph content desiccation resistance and dehydration tolerance and it shares negative genetic covariance with rate of water loss. Epicuticular lipids, however, do not respond to selection.

Another problem is to differentiate water proofing role of cuticular melanisation versus cuticular tanning (hardening) in *D. melanogaster*. Different cuticular components may affect cuticular transpiration in insects (Chapman, 1998). Darker cuticle achieves its color due to deposition of melanin granules (polymers of dopa and other tyrosine derivatives; True, 2003). Like cuticular lipids, melanin is also hydrophobic and therefore may reduce cuticular permeability. It has been suggested that the darkening and hardening of cuticle result due to cross-linking of cuticular proteins with melanin (Pryor, 1940; Fraenkel and Rudall, 1940; Hopkins and Kramer, 1992). Thus, melanisation and sclerotization pathways (tanning) could be related because in the insects, harder body parts are generally darker. In the present study, we have not considered the role of sclerotization. However, further studies are needed to differentiate the effects of cuticular melanisation and cuticular tanning for waterproofing function in *Drosophila* species and in insects in general.

Insect cuticle is a complex structure and its components vary greatly between populations and species (Rajpurohit et al., 2008). Changes in body melanisation have been shown to affect cuticular permeability in some *Drosophila* species (Parkash et al., 2008b,c). According to melanisation–desiccation hypothesis, darker flies of *D. melanogaster* are abundant in cooler uplands while lighter flies are predominant in foothills (Parkash et al., 2008c). The laboratory selected desiccationresistant and sensitive strains of D. melanogaster have shown similar

amounts of cuticular lipid mass (Gibbs et al., 1997); and there is also lack of differences in the amount of cuticular lipid mass in northern versus southern population of *D. melanogaster* (Parkash et al., 2008a). However, in two cases (*Melanoplus sanguinipes* and *Z. indianus*), there are significant intrapopulation differences in the amount of cuticular lipid mass per cm^2 (Rourke, 2000; Parkash et al., 2008a; Parkash et al., 2011)

The present work provides good evidence that the associations between both desiccation resistance and the rate of water loss with cuticular melanisation in *D. melanogaster* populations from India have a genetic basis, which has been suggested by population comparisons in field and lab populations. This is the first experiment to explore these associations with a long term artificial selection experiment. However, pigmentation clines have been reported in *Drosophila* (Parkash et al., 2008), and desiccation resistance is often proposed as an adaptive benefit of increased melanization. We tested the association between pigmentation and desiccation in *D. melanogaster* using selection and found that there was a causative relationship between melanisation and rate of water loss, as selection for both high and low melanin resulted in a correlated response to selection in both desiccation resistance and water balance (however, note that Wittkopp et al., 2011. performed a similar experiment using *D. americana* and *D. novamexicana*, and found no effect of pigmentation genotype on desiccation resistance.

We observed a substantial increase in both male and female survival to desiccation stress following selection for abdominal melanisation. There was a sexual dimorphism for abdominal melanisation as well as desiccation resistance at the onset of our selection regime, but at the 60^{th} generation of selection these differences vanishes. The presence of similar resistant levels in males and females implies that there is a common genetic basis between the sexes underlying the selection responses. Figure 2 represents a genetic association between traits involved in

correlated responses to selection. Selection was associated with an increase in wet weight. This observation is consistent with lines directly selected for desiccation resistance (Gibbs et al., 1997) and in lines selected indirectly in response to very mild desiccation stress (Kennington et al., 2003), but not in other direct selected lines (Bubliy and Loeschcke, 2005; Hoffmann and Parson, 1989). The changes in correlated trait such as desiccation survival with melanisation of selected replicates were independent of body size. The absence of changes in body size suggests that changes in the surface to volume ratio were not involved in increased desiccation tolerance. There were also correlated responses in the weight loss of flies, suggesting that selected strains had a different rate of water loss through the cuticle.

Selection experiments are vital tools of evolutionary biology and the results of nearly a century's worth of selection experiments have helped establish the genetic component of evolutionary theory (Provine, 1971; Falconer, 1992). In addition, selection experiments have provided stocks that have been useful for many other topics, from estimating mutation rates to understanding the molecular, biochemical, and physiological foundations of trait variation (Hill and Caballero, 1992; Mackay, 2001; Conner, 2003; Garland, 2003). The association between melanisation and other correlated traits has been well documented in natural populations of *D. melanogaster* (Parkash et al., 2008), but such associations have not been analyzed following artificial selection experiment of abdominal melanisation. Selection experiments in other drosophilids and insect have detected patterns of genetic covariance between traits (Pitnick and Miller, 2000, Nunney, 1996, Beldade et al., 2002). The results should be especially informative, since the application of quantitative- genetic theory to the evolution of phenotypic differences between populations assumes the pattern of genetic variances and covariances remains relatively constant across evolving populations (Lande, 1982; Arnold, 1981).

CONCLUSIONS

We detected through the analysis of correlated responses to direct selection on abdominal melanisation, a pattern of genetic co-variation among desiccation related traits. A direct selection for cuticular melanisation has selected correlated traits (water balance related traits) in *D. melanogaster*. The evidence for correlated traits is based on trait correlation analysis i.e. darker flies have lower rate of water loss which confers greater desiccation resistance. In contrast, higher rate of water loss in lighter flies sustains lower desiccation tolerance. Results of this study therefore indicate the potential for abdominal melanisation evolution to facilitate or constrain the evolution of desiccation resistance in *Drosophila melanogaster*. Still, the generality of these results deserves further investigations.

ACKNOWLEDGEMENTS

We are indebted to the reviewers and the editor for several helpful comments that improved the manuscript.

FUNDING

S.R. (INSA/ INSPIRE Faculty Fellow) and B.K. (Senior Research Fellow) are grateful to Department of Science and Technology, New Delhi for supporting their research work through DST- INSA/INSPIRE faculty fellow Project (IFA- 11LSBM-08). Financial assistance as University Research Fellowship to K.D. is acknowledged.

REFERENCES

Anderson, A. R., Hoffmann, A. A. and McKechnie, S. W. (2005). Response to selection for rapid chill-coma recovery in *Drosophila melanogaster*: physiology and life-history traits. *Genet. Res.* **85**: 15–22.

Angilletta, M. J. (2009). *Thermal Adaptation: A Theoretical and Empirical Synthesis*. Oxford University Press, Oxford, UK.

Arnold, S. J. (1981). Behavioral variation in natural populations. I. Phenotypic, genetic, and environmental correlations between chemoreceptive responses to prey in the garter snake, *Thamnophis elegans*. *Evolution* **35**: 489-509.

Beldade, P., Koops, K. and Brakefield, P. M. (2002). Developmental constraints versus flexibility in morphological evolution. *Nature* **416**: 844-847

Bertoli, C. I., Scannapieco, A. C., Sambucetti, P. and Norry, F. M. (2009). Direct and correlated responses to chill-coma recovery selection in *Drosophila buzzatii*. *Entomol. Exp. Appl.* **134**: 154–159.

Bowler, K. and Terblanche, J. S. (2008). Insect thermal tolerance: what is the role of ontogeny, ageing and senescence? *Biol. Rev.* **83**: 339–355.

Bubliy, O. A., and Loeschcke, V. (2005). Correlated responses to selection for stress resistance and longevity in a laboratory population of *Drosophila melanogaster*. *J. Evol. Biol.* **18**: 789-803.

Capy, P., David, J. R. and Robertson, A. (1988). Thoracic trident pigmentation in natural populations of *Drosophila simulans*: a comparison with *D. melanogaster*. Heredity, **61(2)**: 263–268.

Chapman, R. F. (1998). *The Insects: Structure and Function*. Cambridge University Press, Cambridge, United Kingdom.

Chippindale, A. K., Chu, T. J. F. and Rose, M. R. (1996). Complex trade-offs and the evolution of starvation resistance in *Drosophila melanogaster*. *Evolution* **50**: 753–766.

Chippindale, A. K., Gibbs, A. G., Sheik, M., Yee, K. J., Djawdan, M., Bradley, T. J. and Rose, M. R. (1998). Resource acquisition and the evolution of stress resistance in *Drosophila melanogaster*. *Evolution* **52**: 1342–1352.

Clusella Trullas, S., van Wyk, J. H. and Spotila, J. R. (2007). Thermal melanism in ectotherms. *J. Therm. Biol.* **32(5):** 235–245.

Conner, J. K. (2003). Artificial selection: A powerful tool for ecologists. *Ecology* **84**: 1650-1660.

da Cunha, A.B. (1949). Genetic analysis of the polymorphism of color pattem in *Drosophila polymorpha. Evolution* **3:** 239-251.

Das, A., Mohanty, S. and Parida, B. B. (1994). Abdominal pigmentation and growth temperature in Indian *Drosophila melanogaster*: evidence for genotype–environment interaction. *J. Biosci.* **19(2):** 267–275.

David, J. R., Capy, P. and Gauthier, J. P. (1990). Abdominal pigmentation and growth temperature in *Drosophila melanogaster*: similarities and differences in the norms of reaction of successive segments. *J. Evol. Biol.* **3(5-6):** 429–445.

David, J. R., Capy, P., Payant, V. and Tsakas, S. (1985). Thoracic trident pigmentation in *Drosophila* melanogaster: differentiation of geographic populations. *Genet. Sel. Evol.* **17(2):** 211–222.

De Jong, P. W., Gussekloo, S. W. S. and Brakefield, P. M. (1996). Differences in thermal balance, body temperature and activity between non-melanic and melanic two-spot ladybird beetles (*Adalia bibunctata*) under controlled conditions. *J. Exp. Biol.* **199**: 2655-2666.

DeWitt, T. J. and Scheiner, S. M. (Ed). 2004. *Phenotypic plasticity: functional and conceptual approaches.* Oxford University Press, New York.

Djawdan, M., Chippendale, A. K., Rose, M. R. and Bradley, T. J. (1998). Metabolic reserves and evolved stress resistance in *Drosophila melanogaster*. *Physiol Zool*, **71**: 584–594. |

Dombeck I. and Jaenike, J. (2004). Ecological genetics of abdominal pigmentation in *Drosophila falleni*: a pleiotropic link to nematode parasitism. *Evolution* **58**: 587-96.

Edney, E. B. (1977). *Water balance in land arthropods*. Springer, Berlin

Ellers, J. and Boggs, C. L. (2002). The evolution of wing color in *Colias* butterflies: heritability, sex-linkage, and population divergence. *Evolution* **56**: 836-540.

Ellers, J. and Boggs, C. L. (2004). Functional ecological implications of intra specific differences in wing melanism in *Colias* butterflies. *Biol. J. Linn. Soc.*, **82**, 79-87.

Falconer, D. S. (1981). *Introduction to Quantitative Genetics,* Longman, London, UK.

Falconer, D. S. and Mackay, T. F. C. (1996). *Introduction to quantitative genetics,* 4th edition. Longman, Harlow, UK.

Falconer, D. S. (1992). Early selection experiments. *Ann. Rev. Genet.* **26**: 1-14.

582 **Folk, D. G., Zwollo, P., Rand, D. M. and Gilchrist, G. W.** (2006). Selection on knockdown
583 performance in *Drosophila melanogaster* impacts thermotolerance and heat–shock
584 response differently in females and males. *J. Exp. Biol.* **209:** 3964–3973
585 **Forsman, A.** (2000). Some like it hot: intra-population variation in behavioral thermoregulation
586 in color-polymorphic pygmy grasshoppers. *Evol. Ecol.* **14(1):** 25–38.
587 **Fraenkel, G. and Rudall, K. M.** (1940). A study of the physical and chemical properties of the
588 insect cuticle. *Proc. R. Soc. Lond. Ser. B* **129**: 1–35.
589 **Garland, T.** (2003). Selection experiments: An underutilized tool in biomechanics and
590 organismal biology. *In* V. L. Bels, J.-P. Gasc, and A. Casinos (eds.), *Vertebrate*
591 *biomechanics and evolution*, pp. 23–56. Scientific Publishers Ltd, Oxford.
592 **Gibbs, A. G. and Matzkin, L. M.** (2001). Evolution of water balance in the genus *Drosophila*.
593 *J. Exp. Biol.* **204**, 2331-2338.
594 **Gibbs, A. G. and Rajpurohit, S.** (2010). *Water-proofing properties of cuticular lipids*. In:
595 Insect Lipids; Biology, Biochemistry and Chemical Biology G.J. Blomquist and A.G.
596 Bagneres, eds. pp. 100-120.
597 **Gibbs, A. G., Chippindale, A. K. and Rose, M. R.** (1997). Physiological mechanisms of
598 evolved desiccation resistance in *Drosophila melanogaster. J. Exp. Biol.* **200**: 1821-1832.
599 **Gibbs, A. G., Fukuzato, F. and Matzkin, L. M.** (2003). Evolution of water conservation
600 mechanisms in desert *Drosophila. J. Exp. Biol*. **206**, 1183-1192.
601 **Gibbs, A. G., Louie, A. K. and Ayala, J. A.** (1998). Effects of temperature on cuticular lipids
602 and water balance in a desert *Drosophila*: is thermal acclimation beneficial? *J. Exp. Biol.*
603 **201**: 71–80

Gibbs, A. G., Louie, A. K. and Ayala, J. A. (1998). Effects of temperature on cuticular lipids and water balance in a desert *Drosophila*: Is thermal acclimation beneficial? *J. Exp. Biol.* **201:** 71-80.

Gilchrist, G. W. and Huey, R. B. (1999). The direct response of *Drosophila melanogaster* to selection on knockdown temperature. *Heredity* **83**: 15-29

Gomez, F. H., Bertoli, C. I., Sambucetti, P., Scannapieco, A. C. and Norry, F. M. (2009). Heat induced hormesis in longevity as correlated response to thermal-stress selection in *Drosophila buzzatii*. *J. Therm. Biol.* **34:** 17–22.

Hadley, N. F. (1977). Epicuticular lipids of the desert tenebrionid beetle, *Eleodes armatus*: seasonal and acclimatory effects on chemical composition. *Insect Biochem.* **7:** 227–283

Hadley, N. F. (1994). *Water Relations of Terrestrial Arthropods.* San Diego, CA: Academic Press.

Hadley, N. F. and Schultz, T. D. (1987). Water loss in three species of tiger beetles (*Cicindela*): correlations with epicuticular hydrocarbons. *J. Insect Physiol.* **33:** 677–682.

Harshman, L. G. and Hoffmann, A. A. (2000). Laboratory selection experiments using *Drosophila*: what do they really tell us? *Trends Ecol. Evol.* **15:** 32–36.

Harshman, L. G., Hoffmann, A. A. and Clark, A. G. (1999). Selection for starvation resistance in *Drosophila melanogaster*: physiological correlates, enzyme activities and multiple stress responses. *J Evol Biol*, **12**: 370–379.

Hill, W. G., and Caballero, A. (1992). Artificial selection experiments. *Ann. Rev. Ecol. Syst*, **23**: 287-310.

Hoffmann, A. A. and Parsons, P. A. (1989a). An integrated approach to environmental stress tolerance and life-history variation: desiccation tolerance in *Drosophila*. *Biol. J. Linn. Soc.* **37**: 117–135.

Hoffmann, A. A. and Parsons, P. A. (1989b). Selection for increased desiccation resistance in *Drosophila melanogaster*: additive genetic control and correlated responses for other stresses. *Genetics* **122**: 837–845.

Hoffmann, A. A. and Parsons, P. A. (1993). Direct and correlated responses to selection for desiccation resistance – a comparison of *Drosophila melanogaster* and *D. simulans*. *J. Evol. Biol.* **6**: 643–657.

Hoffmann, A. A. and Willi, Y. (2008). Detecting genetic responses to environmental change. *Nat. Rev. Genet.* **9:** 421–432.

Hoffmann, A. A., Sørensen, J. G. and Loeschcke, V. (2003). Adaptation of *Drosophila* to temperature extremes: bringing together quantitative and molecular approaches. *J. Thermal Biol.* **28**, 175-216.

Hopkins, T. L. and Kramer, K. L. (1992). Insects cuticle sclerotization. *Annu. Rev. Entomol.* **37:** 273–302.

Huey, R. B., Partridge, L. and Fowler, K. (1991). Thermal sensitivity of *Drosophila melanogaster* responds rapidly to laboratory natural selection. *Evolution* **45:** 751–756.

Kalmus, H. (1941). The resistance to desiccation of *Drosophila* mutants affecting body colour. *Proc. R. Soc. Biol. Sci. B.* **130**: 185–201.

Karl, I., Sørensen, J. G., Loeschcke, V. and Fischer, K. (2009). HSP70 expression in the copper butterfly *Lycaena tityrus* across altitudes and temperatures. *J. Evol. Biol.* **22:** 172–178

Kennington, W. J., Killeen, J. R., Goldstein, D. B., and Partridge, L. (2003). Rapid laboratory evolution of adult wing area in *Drosophila melanogaster* in response to humidity. *Evolution* **57**: 932-936.

Lande, R. (1982). A quantitative genetic theory of life history evolution. *Ecology* **63**: 607–615.

Lynch, C. B. (1980). Response to divergent selection for nesting behavior in *Mus musculus*. *Genetics* **96:** 757–765

Mackay, T. F. C. (2001). Quantitative trait loci in *Drosophila*. *Nature Rev. Genet.* **2**: 11-20.

Majerus, M. E. N. (1998). Melanism: Evolution in Action. Oxford, United Kingdom: Oxford University Press.

Mori, N. and Kimura, M. N. (2008). Selection for rapid and slow recovery from chill- and heat-coma in *Drosophila melanogaster*. *Biol. J. Linn. Soc.* **95:** 72–80.

Munjal, A. K., Karan, D., Gibert, P., Moreteau, B., Parkash, R.,and David, J. R. (1997). Thoracic trident pigmentation in *Drosophila melanogaster*: latitudinal and altitudinal clines in Indian populations. *Genet. Sel. Evol.* **29(5):** 601–610.

Napp Martinez, M., and Cordeiro, A. R. (1970). Modifiers of color pattern genes in *Drosophila polymorpha*. *Genetics* **64**: 573-587.

Norry, F. M, Gomez, F. H. and Loeschcke, V. (2007). Knockdown resistance to heat stress and slow recovery from chill coma are genetically associated in a quantitative trait locus region of chromosome 2 in *Drosophila melanogaster*. *Mol. Ecol.* **16:** 3274–3284.

Nunney, L. (1996). The response to selection for fast larval development in*Drosophila melanogaster* and its effect on adult weight: An example of a fitness trade-off. *Evolution* **50**: 1193-1204.

Ottenheim, M. M., Wertheim, B., Holloway, G. J. and Brakefield, P. M. (1999). Survival of colour-polymorphic *Eristalis arbustorum* hoverflies in semi-field conditions. *Funct. Ecol*. **13(1):** 72–77.

Parkash, R. and Munjal, A. K. (2000). Evidence of independent climatic selection for desiccation and starvation tolerance in Indian tropical populations of *D. melanogaster*. *Evol. Ecol. Res.* **2:** 685–699

Parkash, R., Chahal, J., Sharma, V. and Dev, K. (2012). Adaptive associations between total body color dimorphism and climatic stress related traits in a stenothermal circumtropical *Drosophila* species. *Insect Sci.* **19(2):** 247−262.

Parkash, R., Chahal, J., Sharma, V., and Dev, K. (2011). Adaptive associations between total body color dimorphism and climatic stress related traits in a stenothermal circumtropical *Drosophila* species. *Insect Sci.* **18**: doi: 10.111/j.1744-7917.2011.01426.x

Parkash, R., Kalra, B. and Sharma, V. (2008a). Changes in cuticular lipids, water loss and desiccation resistance in a tropical drosophilid: analysis of within population variation. *Fly* **2:**189–197

Parkash, R., Kalra, B. and Sharma, V. (2010a). Impact of body melanisation on contrasting levels of desiccation resistance in a circumtropical and a generalist *Drosophila* species. *Evol. Ecol.* **24:** 207–225

Parkash, R., Rajpurohit, S. and Ramniwas, S. (2008). Changes in body melanisation and desiccation resistance in highland vs. lowland populations of *D. melanogaster*. *J. Insect Physiol.* **54**: 1050-1056

Parkash, R., Rajpurohit, S. and Ramniwas, S. (2008b). Changes in body melanisation and desiccation resistance in highland vs. lowland populations of *Drosophila melanogaster*. *J. Insect Physiol.* **54:** 1050–1056

Parkash, R., Ramniwas, S., Rajpurohit, S. and Sharma, V. (2008c). Variations in body melanisation impact desiccation resistance in *Drosophila immigrans* in Western Himalayas. *J. Zool.* **276:** 219–227

Parkash, R., Sharma, V. and Kalra, B. (2010b), Correlated changes in thermotolerance traits and body color phenotypes in montane populations of *Drosophila melanogaster*: analysis of within- and between-population variations. *J. Zool.* **280:** 49–59.

Parkash, R., Singh, S. and Ramniwas, S. (2009). Seasonal changes in humidity level in the tropics impact body color polymorphism and desiccation resistance in *Drosophila jambulina*– Evidence for melanism–desiccation hypothesis. *J. Insect Physiol.* **55**: 358–368.

Pereboom, J. J. M. and Biesmeijer, J. C. (2003). Thermal constraints for stingless bee foragers: the importance of body size and coloration. *Oecologia* **137:** 42–50.

Pitnick, S., and Miller, G. T. (2000). Correlated response in reproductive and life history traits to selection on testis length in *Drosophila hydei*. *Heredity* **84**: 416-426.

Pool, J. E., and Aquadro, C. F. (2007). The genetic basis of adaptive pigmentation variation in *Drosophila melanogaster*. *Mol. Ecol.* **16**(14): 2844-2851.

Provine, W. B. (1971). *The origins of theoretical population genetics*. Univ. of Chicago Press, Chicago

Pryor, M. G. M. (1940). On the hardening of the cuticle of insects. *Proc. R. Soc. Biol. Sci. B.* **128**: 393–407.

Rajpurohit, S., Parkash, R. and Ramniwas, S. (2008). Body melanisation and its adaptive role in thermoregulation and tolerance against desiccating conditions in drosophilids. *Entomol. Res.* 38:49–60

Reusch, T. B. H. and Wood, T. E. (2007). Molecular ecology of global change. *Mol. Ecol.* **16:** 3973–3992.

Rourke, B. C. (2000). Geographical and altitudinal variation in water balance and metabolic rate in a California Grasshopper, *Melanoplus sanguinipes*. *J. Exp. Biol.* **203:** 2699–2712

Telonis-Scott, M., Guthridge, K. M. and Hoffmann, A. A. (2006). A new set of laboratory-selected *Drosophila melanogaster* lines for the analysis of desiccation resistance: response to selection, physiology and correlated responses. *J. Exp. Biol.* **209**: 1837–1847.

Telonis-Scott, M., Hoffmann, A. A. and Sgrò, C. M. (2011). The molecular genetics of clinal variation: a case study of *ebony* and thoracic trident pigmentation *in Drosophila melanogaster* from eastern Australia. *Mol. Ecol.* **20**: 2100–2110.

Toolson, E. C. (1984). Interindivididual variations in epicuticular hydrocarbon composition and water loss rates of the cicada, *Tibicen dealbatus* (Homoptera: Cicadidae). *Physiol. Zool.* **57:** 5505–5556.

Toolson, E. C. and Hadley, N. F. (1979). Seasonal effects on cuticular permeability and epicuticular lipid composition in *Centruroides sculpturatus* Ewing 1928 (Scorpiones: Buthidae). *J. Comp. Physiol.* **129:** 319–325

Toolson, E. C. and Kuper-Simbro´n, R. (1989). Laboratory evolution of epicuticular hydrocarbon composition and cuticular permeability in *Drosophila pseudoobscura*: effects of sexual dimorphism and thermal-acclimation ability. *Evolution* **43:** 468–473

Toolson, E.C. (1982). Effects of rearing temperature on cuticle permeability and epicuticular lipid composition in *Drosophila pseudoobscura*. *J. Exp. Zool.* **22:** 249–253.

True, J. R. (2003). Insect melanism: the molecules matter. *Trends Ecol. Evol.* **18**: 640-647.

Umina, P. A., Weeks, A. R., Kearney, M. R., McKechnie, S. W. and Hoffmann, A. A. (2005). A rapid shift in a classic clinal pattern in *Drosophila* reflecting climate change. *Science* **308:** 691–693.

Watt, W B. (1969). Adaptive significance of pigment polymorphisms in *Colias* butterflies. II Thermorégulation and photoperiodically controlled melanin variation in *Colias furytheme. Proc. Nat. Aca. Sci.* U.S., **63:** 767 -774.

Wittkopp, P. J., Caroll, S. B. and Kopp, A. (2003). Evolution in black and white: genetic control of pigment patterns in *Drosophila*. *Trends Genet.* **19**: 495-504.

Wittkopp, P. J., Smith-Winberry, G., Arnold, L. L., Thompson, E. M., Cooley, A. M., Yuan, D., Song, Q. and McAllister, B. F. (2011). Local adaptation for body color in *Drosophila americana*. *Heredity* **106**: 592–602

Legend to figures

Fig. 1 (A) Variation (m ± s.d.) of abdominal melanisation in a sample of flies (n= 1236) after mass breeding for seven generations in the laboratory at 21 °C and before onset of laboratory selection. (B) data on regression of R (response to selection) on ∑S (cumulative selection differential) are shown for estimation of realized heritability of laboratory selected dark and light lines of *D. melanogaster*.

Fig 2 .(A) Results of laboratory selection upto 60^{th} generation for changes in total body melanisation per fly in control and selected dark and lighter lines of *D. melanogaster*. (B - D) correlated changes in desiccation resistance, cuticular water loss and dehydration tolerance are shown after every five generations of laboratory selection in darker and lighter lines alongwith their respective control lines. For each trait, data are shown as means of five replicate lines. Selection was made on the melanisation of female flies but corresponding changes in males are also shown.

Figure 3. (A) Changes (mean ± s.d.) in cuticular melanisation; (B) desiccation resistance hours (C) cuticular water loss; and (D) cutitcular lipid content in dark and light selected lines of *D. melanogaster* as compared with control lines. Trait values represent data on 60^{th} generation of selection. Each value is based on analysis of ten replicates of ten individual flies each of five replicate lines of control as well as selected dark and light lines of *D. melanogaster*.

Fig. 4 (A) Desiccation survival curves of control and laboratory selected darker and lighter body color lines of *D. melanogaster* (n = 5 each; values are shown as average percentage survival per hour) when stressed in groups of ten female flies of 60^{th} generation. Correlated changes in desiccation resistance, cuticular water loss and dehydration tolerance in control and five selected (dark and light) lines. Between line variability (mean ± s.d.) are shown for desiccation related traits as a function of changes in body melanisation of control and selected lines of dark and light body color.

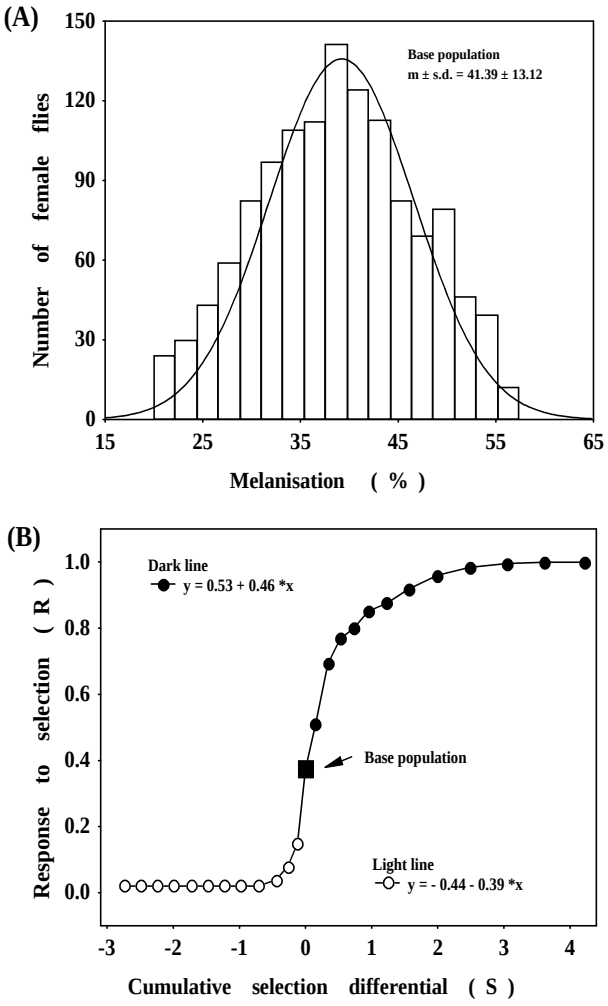


Fig. 1 (A) Variation (m ± s.d.) of abdominal melanisation in a sample of flies (n= 1236) after mass breeding for seven generations in the laboratory at 21 °C and before onset of laboratory selection. (B) data on regression of R (response to selection) on ∑S (cumulative selection differential) are shown for estimation of realized heritability of laboratory selected dark and light lines of *D. melanogaster*.

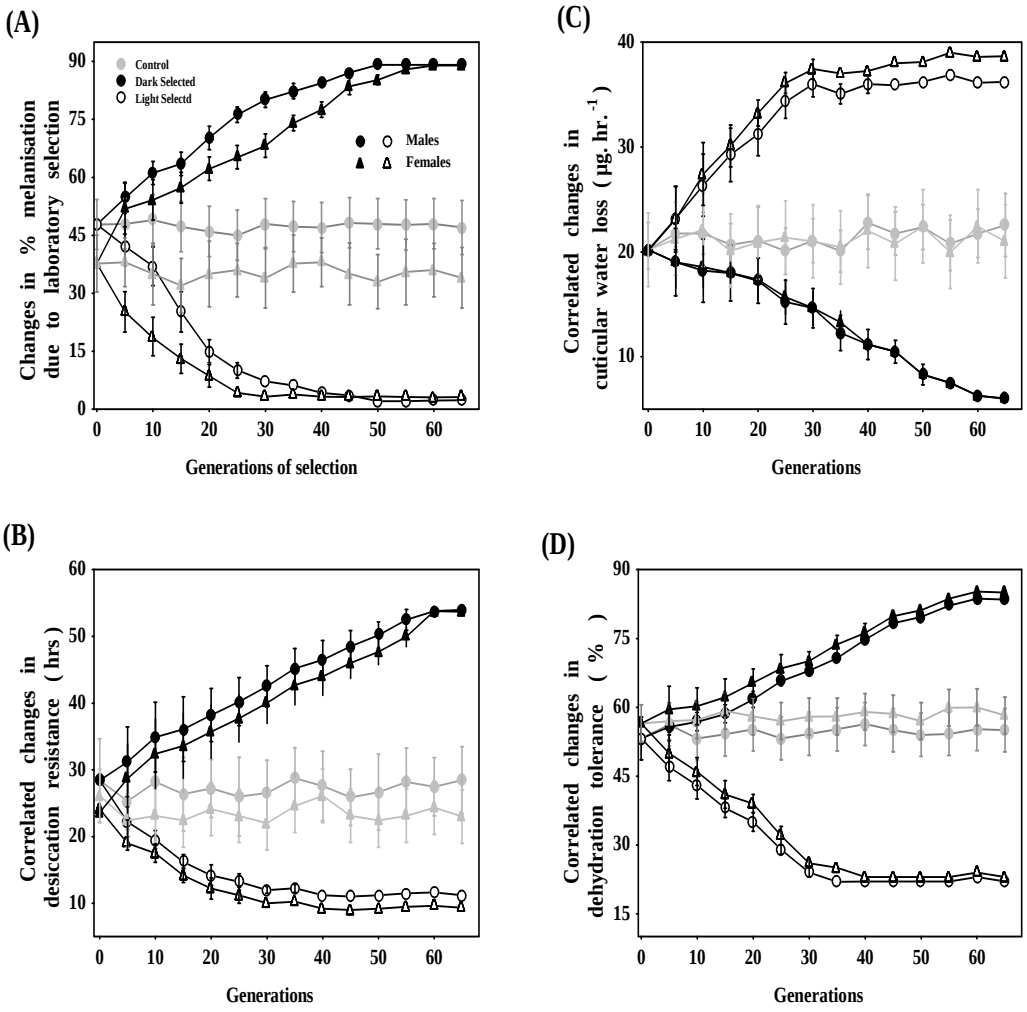
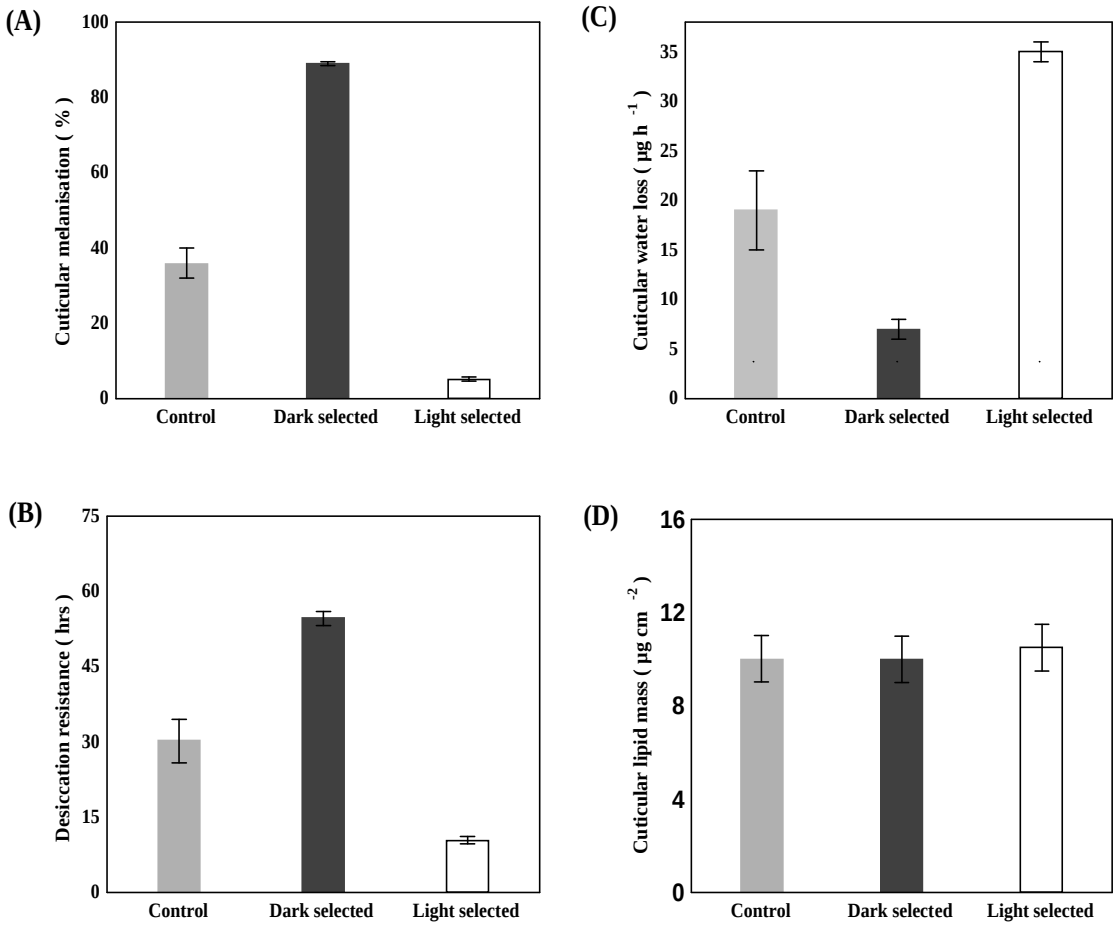


Fig 2 .(A) Results of laboratory selection upto 60th generation for changes in total body melanisation per fly in control and selected dark and lighter lines of *D. melanogaster*. (B - D) correlated changes in desiccation resistance, cuticular water loss and dehydration tolerance are shown after every five generations of laboratory selection in darker and lighter lines alongwith their respective control lines. For each trait, data are shown as means of four replicate lines. Selection was made on the melanisation of female flies but corresponding changes in males are also shown.



832

833 Figure 3. (A) Changes (mean ± s.d.) in cuticular melanisation; (B) desiccation resistance
834 hours (C) cuticular water loss; and (D) cutitcular lipid content in dark and light selected
835 lines of *D. melanogaster* as compared with control lines. Trait values represent data on
836 60^{th} generation of selection. Each value is based on analysis of ten replicates of ten
837 individual flies each of four replicate lines of control as well as selected dark and light
838 lines of *D. melanogaster*.
839
840
841
842

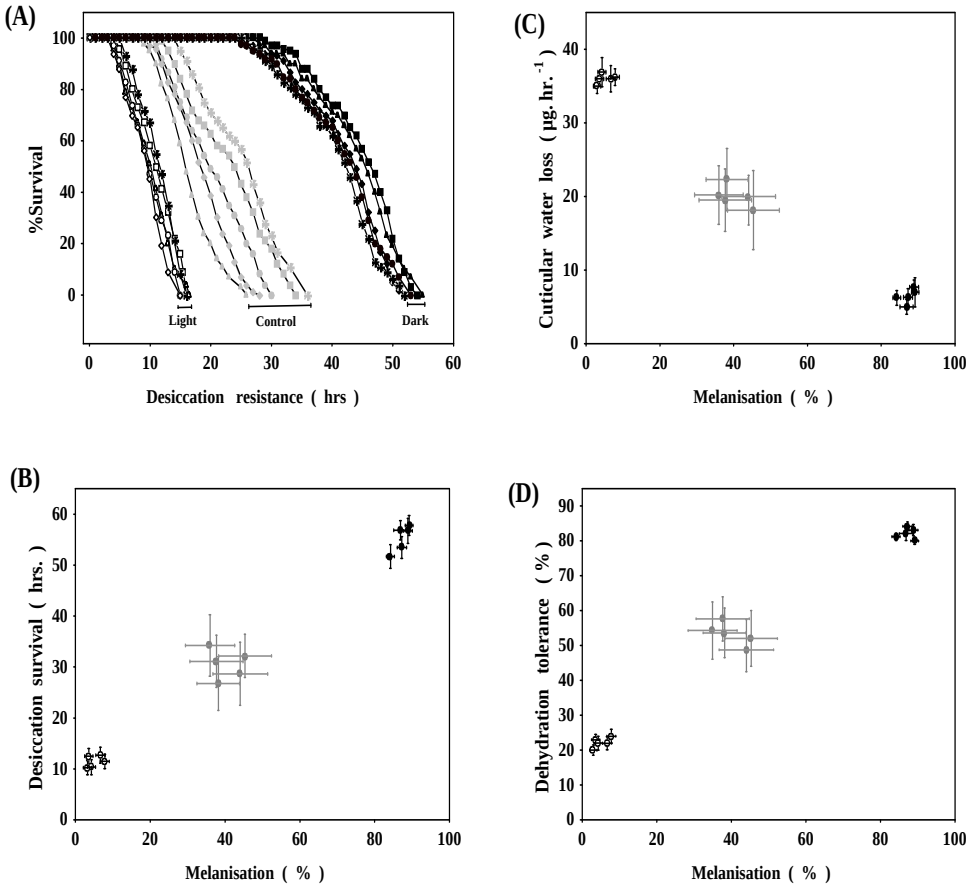


Fig. 4 (A) Desiccation survival curves of control and laboratory selected darker and lighter body color lines of *D. melanogaster* (n = 5 each; values are shown as average percentage survival per hour) when stressed in groups of ten female flies of 60th generation. Correlated changes in desiccation resistance, cuticular water loss and dehydration tolerance in control and four selected (dark and light) lines. Between line variability (mean ± s.d.) are shown for desiccation related traits as a function of changes in body melanisation of control and selected lines of dark and light body color.

Table 1. *Data (mean ± s.e.) for changes in body melanisation, desiccation resistance and rate of water loss in four replicate lines of dark and light selected as compared with control lines (n = 4). For each trait, ANOVA values represent statistical differences between selected (dark- D or light- L) and control lines. Each value is based on analysis of ten replicates of ten individual flies of each sex.*

	Replicate lines	Melanisation (%)		Desiccation resistance (hrs)		RWL (µg fly $^{-1}$)	
		♂	♀	♂	♀	♂	♀
Control (C)	C 1	47.21±5.21	43.50±4.25	26.01±3.21	29.1±3.10	19.51±4.25	21.23±5.33
	C 2	48.32±7.02	38.21±4.98	29.53±4.29	34.4±4.02	20.21±3.98	23.21±4.22
	C 3	47.25±3.25	42.57±5.65	25.55±4.71	32.8±4.21	18.14±5.36	21.50±3.65
	C 4	46.36±4.98	39.57±6.21	21.22±3.89	26.5±3.29	20.30±4.22	22.11±4.78
Dark selected (D)	D 1	90.00±0.65	87.25±0.33	52.21±1.22	54.4±1.12	6.30±1.20	7.21±1.00
	D 2	86.58±0.36	88.26±0.18	53.11±2.00	56.7±2.45	7.12±0.89	7.56±0.89
	D 3	89.14±0.12	88.56±0.14	50.98±1.89	51.6±1.30	6.23±1.01	6.39±1.77
	D 4	87.98±0.14	86.00±0.54	54.22±1.25	56.8±1.89	6.89±1.00	7.14±0.77
Light selected (L)	L 1	3.60±0.21	4.22±0.54	14.00±1.70	16.5±0.56	36.21±1.13	37.54±1.32
	L 2	1.10±0.13	3.23±0.36	13.74±0.99	16.5±0.49	35.98±1.02	36.87±1.00
	L 3	2.25±0.19	3.21±0.25	16.04±1.74	18.0±0.51	36.89±0.98	37.00±1.89
	L 4	2.32±0.17	5.00±0.52	12.36±1.12	16.0±0.61	36.11±1.00	36.98±1.00
Mean squares for ANOVAs (df)							
C vs. D	Lines (7)	17512.7***	19854.3***	3264.47***	2963.52***	546.61***	547.85***
	Error (72)	4.141	3.012	0.723	0.954	0.519	0.405
C vs. L	Lines (7)	12242.5***	10254.1***	3065.28***	2732.96***	629.46***	759.78***
	Error(72)	5.323	4.901	0.712	0.623	0.730	0.619

df – degree of freedom; MS – means square ***p< 0.001; ns = non-significant. *ANOVAs on melanisation were carried out on arcsine- transformed data.*

Table 2. *Data (mean ± s.e.) for changes in body water content, hemolymph volume and dehydration tolerance in four replicate lines of dark and light selected as compared with control lines. For each trait, ANOVA values represent statistical differences between selected (dark- D or light- L) and control lines. Each value is based on ten replicates of ten individual flies.*

	Replicate lines	Water content (mg fly $^{-1}$) ♀	Water content (mg fly $^{-1}$) ♂	Hemolymph (%) ♀	Hemolymph (%) ♂	Dehydration tolerance (%) ♀	Dehydration tolerance (%) ♂
Control (C)	C 1	1.25±0.04	1.23±0.04	15.25±2.21	13.43±3.21	56.66±2.32	50.01±3.21
	C 2	1.22±0.05	1.19±0.04	13.54±3.21	12.00±2.21	54.32±3.21	49.53±4.29
	C 3	1.19±0.04	1.18±0.03	14.12±2.36	12.12±2.98	52.01±2.98	50.55±4.71
	C 4	1.21±0.03	1.20±0.04	14.00±2.07	12.56±2.13	53.65±3.14	51.22±3.89
Dark selected (D)	D 1	1.32±0.01	1.29±0.02	20.21±0.89	18.19±1.01	84.20±1.21	82.21±1.22
	D 2	1.34±0.02	1.30±0.01	21.32±1.11	19.30±0.98	83.11±1.58	81.11±2.00
	D 3	1.33±0.02	1.31±0.01	22.32±0.85	20.11±1.00	81.21±1.05	81.98±1.89
	D 4	1.33±0.01	1.28±0.02	21.27±0.99	20.21±0.88	82.11±0.97	82.22±1.25
Light selected (L)	L 1	1.06±0.01	1.03±0.02	11.67±0.21	10.11±0.87	22.23±1.23	21.00±1.70
	L 2	1.09±0.01	1.05±0.02	10.99±0.80	10.00±1.03	23.13±2.51	21.74±0.99
	L 3	1.05±0.02	1.02±0.01	9.78±0.74	11.21±0.56	22.98±1.58	22.04±1.74
	L 4	1.06±0.02	1.03±0.02	10.23±0.81	10.88±0.87	22.19±1.21	21.36±1.12
Mean squares for ANOVAs (df)							
C vs. D	Lines (7)	0.038**	0.406**	0.015***	0.019***	0.3013***	0.2843***
	Error (72)	0.003	0.002	0.00001	0.00004	0.0004	0.0007
C vs. L	Lines (7)	0.291**	0.191**	0.0026***	0.0061	0.7427***	0.6943***
	Error (72)	0.002	0.001	0.0001	0.0002	0.001	0.002

df – degree of freedom; MS – means square ***p< 0.001; ns = non-significant. *ANOVAs on percent data were carried out on arcsine- transformed data*.

Table 3. *Correlation values (r ± s.e.) for each of different ecophysiological traits with changes in cuticular melanisation and with cuticular lipid mass in selected lines for dark and light body color of D. melanogster.*

Traits	Trait correlation (r ± s.e.)			
	% Melanisation		Cuticular lipids	
	Dark	Light	Dark	Light
1. Melanisation (%)	--	--	-0.24±0.28 ns	0.33±0.27 ns
2. Cuticular lipid mass (μg cm $^{-2}$)	-0.24±0.28 ns	0.18±0.31 ns	--	--
3. Body water content	0.89±0.07**	0.85±0.08**	-0.21±0.21 ns	0.28±0.31 ns
4. Hemolymph (%)	0.91±0.03***	0.89±0.07***	-0.35±0.19 ns	0.31±0.27 ns
5. Desiccation (hrs)	0.98±0.04***	0.99±0.03***	-0.25±0.27 ns	0.32±0.27 ns
6. Cuticular water loss (μg h $^{-1}$)	-0.96±0.07***	-0.97±0.05***	0.19±0.23 ns	-0.30±0.27 ns
7. Dehydration tolerance	0.96±0.05***	0.90±0.12***	-0.21±0.23 ns	0.15±0.28 ns

ns = nonsignificant, $^{**}P < 0.01$; $^{***}P < 0.001$