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Seasonal changes in humidity impact drought resistance in tropical *Drosophila leontia*: Testing developmental effects of thermal versus humidity changes

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A B S T R A C T

Drosophila leontia is native to highly humid equatorial tropical habitats but its desiccation sensitivity (~10 h) is not consistent with its abundance during the drier autumn season in the subtropical regions. We have tested the effects of developmental acclimation on desiccation resistance and water balance related traits of *D. leontia* collected during rainy and autumn seasons. The isofemale lines of seasonal populations were reared under ecologically relevant growth temperatures (18 or 26 °C) or humidity conditions (35 or 85% RH) but tested at different times under identical experimental conditions. The larvae as well as flies reared under two thermal conditions (18 or 26 °C) showed no effect on desiccation related traits as well as storage and utilization of energy metabolites. In contrast, for *D. leontia* reared under low humidity (35% RH), significant changes at larval as well adult stages include increase in the desiccation resistance as well as cuticular lipid quantity, reduced levels of rate of body water loss, higher storage of carbohydrates but lower rate of utilization of carbohydrates as compared with flies reared at high humidity (85% RH). *D. leontia* has responded to rearing under low humidity conditions by increasing its desiccation resistance but not due to changes in the growth temperatures. These laboratory observations on seasonal populations highlight differences due to rearing conditions but not due to seasons. Further, direct analysis of wild-caught seasonal populations has shown trends similar to developmental acclimation effects. For wild caught flies, there are significant seasonal differences i.e. higher desiccation resistance as well as cuticular lipid quantity but reduced rate of water loss for autumn than rainy season flies. Thus, our laboratory observations are relevant for understanding seasonal adaptations of natural populations of tropical *D. leontia* to wet-dry conditions in the wild.

1. Introduction

Physiological traits such as desiccation resistance and thermotolerance are associated with the distribution patterns of many species of insects (Parsons, 1982; Kimura and Beppu, 1993; Kellermann et al., 2009, 2012; Calosi et al., 2010). Widespread *Drosophila* species have higher levels of genetic variation for desiccation and cold resistance consistent with their ability to evolve rapidly (Overgaard et al., 2011; Rajpurohit et al., 2013). In contrast, five *Drosophila* species restricted to tropics have low levels of additive genetic variation or narrow sense heritability for desiccation and cold resistance traits (Kellermann et al., 2009). However, some tropical species are able to extend their distribution range to subtropical habitats but their underlying physiological mechanisms remain to be assessed. Tropical species such as *Drosophila leontia*, *Drosophila kikkawai and Drosophila malerkotliana* have colonized tropical and subtropical localities on different continents including Africa, India and South America (Markow and O'Grady, 2006). It is likely that these *Drosophila* species may have used different strategies to increase their physiological tolerance to different climatic stressors because there are species-specific differences in the range of distribution patterns along spatial and temporal dimensions. However, previous studies have mainly focused on geographical variation in stress resistance of different *Drosophila* species (Hoffmann and Weeks, 2007; Parkash et al., 2010) while little attention has been paid to seasonal variation in drought resistance. Thus, it would be interesting to examine seasonal adaptive changes in stress resistance traits in tropical *Drosophila* species.

Insects can improve their stress resistance through irreversible developmental acclimation effects as well as by reversible adult acclimation responses (Hoffmann, 1990; Hadley, 1994; Angilletta, 2009). For thermal tolerance related traits, several studies have shown developmental as well as adult acclimation effects which can alter resistance level to different types of cold or heat stresses (Hoffmann and Watson, 1993; Rako and Hoffmann, 2006; Sgro et al., 2010; Cooper et al., 2012). In contrast, few studies have considered the effects of developmental and adult acclimation on desiccation related traits (Hoffmann, 1990; Gibbs et al., 1998; Hoffmann et al., 2005). Some studies have shown lack of changes in desiccation related traits due to adult acclimation at different temperatures i.e. in the desert dwelling—*D. mojavensis*, high temperature acclimation did not alter desiccation resistance (Gibbs

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et al., 1998). Adults acclimated to different temperatures showed no changes in the rates of water loss in the chrysomelid beetle—*Chirodica chalcoptera* (Terblanche et al., 2005). In contrast, in several insect taxa, water loss rates respond to rearing temperature acclimation i.e. thermal acclimation of pupae or adult of *Glossina pallidipes* to higher temperature (29 °C) has evidenced increase in desiccation resistance (Terblanche and Chown, 2006). *D. melanogaster* reared under simulated summer-like thermal conditions showed increased desiccation resistance (Hoffmann et al., 2005). Thus, the effects of developmental acclimation at different rearing temperatures seem complex and vary among diverse insect taxa. Further studies are needed to find generality of such developmental acclimation effects on various stress related traits.

For seasonally varying populations of *Drosophila* species, mechanisms that increase resistance to climatic stresses need to be identified along with environmental cues/variables that change resistance patterns. This can help in identifying associations of stress-related traits with specific environmental variables. Since climatic conditions (variation in the ambient temperature as well as relative humidity) exert selection pressure on stress related traits, it is important to disentangle the effects due to each climatic variable. For desiccation related traits, changes in the humidity conditions across seasons may be more important than the thermal changes for tropical species. If climatic variables vary in a seasonal manner, *Drosophila* species are likely to differ in the physiological tolerance level to stressors across seasons. The assessment of seasonal adaptations in insects including *Drosophila* is difficult due to many reasons. For meaningful comparisons of stress related traits in seasonally varying populations, isofemale lines need to be tested at the same time to ensure that the observed differences are not the consequences of differences in the experimental procedure. However, isofemale lines derived from seasonally varying wild-caught flies might show laboratory adaptation for desiccation related traits if grown in the laboratory for several generations before testing at the same time. There are evidences that desiccation resistance of *D. melanogaster* can change due to laboratory adaptations for cultures differing in their years of collections and duration of rearing in the laboratory (Hoffmann et al., 2001). Alternative approaches include undertaking experiments at different times or seasons across multiple years or to test the effects of seasonally varying abiotic factors through developmental acclimation. The latter approach allows testing seasonal changes at different times (after rearing for two-three generations) but this makes comparisons across rearing conditions valid but not across seasons. However, laboratory observations on developmental acclimation effects can be compared with direct trait analysis of wild-caught seasonally varying populations. We have followed the last approach in the present work. For developmental acclimation effects of seasonally relevant thermal or humidity conditions, the seasonal populations become two different replicates and not proxy of two different seasons. Thus, there are limitations in the assessment of seasonal adaptations in the *Drosophila* species.

Drosophila leontia belongs to montium species subgroup of subgenus Sophophora (Bock, 1980). This species is abundantly available in the equatorial humid habitats in Southeast Asia and is a desiccation sensitive species. *D. leontia* occurs during the rainy season all along the latitudinal transect on the Indian subcontinent. However, mechanistic basis of adaptations of this species to drier autumn season in the northern subtropical localities has not been investigated. *D. leontia* might involve developmental plasticity for growth conditions. In the present work, we tested the hypothesis whether developmental acclimation of *D. leontia* under high vs. low levels of thermal or humidity conditions can account for seasonal adaptations to wet–dry conditions. We assessed changes due to developmental acclimation under different rearing conditions i.e. under low versus high temperatures (18 or 26 °C) or humidity conditions (35 or 85% RH) for two generations. The resulting flies were investigated for desiccation related traits, water balance traits and storage of energy metabolites. Further, we compared body water loss in dead flies with and without treatment of cuticle with organic solvents. We also assessed rate of utilization of energy metabolites in flies reared under different thermal or humidity conditions. Finally, we directly examined desiccation related traits in wild-caught flies collected during rainy and autumn seasons of two different years.

2. Materials and methods

2.1. Collections

Wild living individuals of *D. leontia* ($n = 500$–650 flies) were collected from Pathankot (Lat: 32°40′ N; Long: 75.64 °E; Alt: 332 m) in two different seasons (rainy and autumn) of two consecutive years—2010 and 2011. During the collection times, the thermal and humidity conditions of this locality were measured with a thermo-cum-hygrometer. Variations in average temperature (T_{ave}) and relative humidity (RH_{ave}) was observed in two seasons i.e. rainy season ($T_{ave} = 25.98 \pm 1.35$ °C; $RH = 83.9 \pm 2.71$ %) versus autumn season ($T_{ave} = 18.02 \pm 1.98$ °C; $RH_{ave} = 42.5 \pm 2.21$ %). Climatic data for the last fifty years on thermal variables of origin of populations were obtained from Indian Institute of Tropical Meteorology (IITM; www.tropmet.res.in) but fifty years data on relative humidity for monsoon and autumn season were obtained from 'Climatological Tables' published by the Indian Meteorological Department, Govt. of India, New Delhi.

2.2. Experimental procedures

2.2.1. Seasonal variations in wild-caught flies

Wild-caught flies of *D. leontia* ($n = {\sim}600$) were obtained during rainy as well as autumn seasons of 2010 and 2011 from Pathankot and investigated directly for seasonal changes in desiccation related traits. All assays were made under identical laboratory controlled conditions (22 °C and 65% RH) but at different times for the two seasons as well as years.

2.2.2. Developmental acclimation effects

For investigating the rearing acclimation effects, wild-caught females collected in year 2010 during rainy and autumn season were used to initiate isofemale lines (20 lines per seasonal population). The isofemale lines were reared under different conditions but at different times for the two seasons. Mated female flies of each isofemale line ($n = 20$ IF lines per seasonal population) of rainy or autumn season were considered as two independent replicate populations for laboratory analysis) and were allowed to lay eggs for a limited time period (12 h) at 22 °C and 65% RH. Thereafter, several batches of the eggs of each isofemale line were transferred for rearing under different thermal or humidity conditions in the BOD incubators—(i) under different humidities (35 or 85% RH) but at a common growth temperature (22 °C) and; (ii) different thermal conditions (18 or 26 °C) but at a common humidity condition (65% RH). The different developmental acclimation groups (for each rearing condition) for a seasonal population were set up at separate time points so that the emergence of adult flies occurred simultaneously. All assays for developmental thermal as well as humidity acclimation were done at the same time for all the isofemale lines of a season. Thus, isofemale lines were reared under different conditions but at different times for the two seasons. The egg-to-adult developmental time was 23 ± 1.23 and 9 ± 0.56 days at 18 and 26 °C growth temperatures; and 16 ± 1.12 and 14.5 ± 0.74 days for 35 and 85% RH reared flies respectively. Seven days post eclosion, adult females were used for desiccation related assays.

2.3. Analysis of body melanisation

Body melanisation of individual female flies ($n = 10$ replicates $\times$ 20 I.F. lines) was visually scored with Olympus stereo-zoom microscope