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Growth of water adaptive strategy in *Drosophila nepalensis* in Himalayan region of Nepal

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Abstract

The growth of water strategy in *Drosophila* species has not been studied clearly in Nepal. So, an investigation was carried out to understand the growth mechanisms of water adaptive strategy in *D. nepalensis*. Samples of *D. nepalensis* (100-150) collected from different parts of high Himalayas during November 2018-January 2019. The study was carried out in the Entomology laboratory of Agriculture and Forestry University, Chitwan, Nepal. The cultures of the insects were based at low density (60-70 eggs per vial; egg vials were 40 × 100 mm in size) with the cornmeal-yeast-agar medium at 15°, 21°, and 25 °C and 65±1% relative humidity in a temperature-and humidity-controlled incubator for five generations before experimental analysis. Required data for climatic variation were obtained from the meteorological department, Nepal government. Study revealed that the relative abundance of *D. nepalensis* has a transformation in relative humidity along an altitudinal gradient in the Western Himalayas. Thus, dehydration tolerance evolved as a common physiological mechanism to support survival under desiccation stress in *D. nepalensis*.

Keywords: Dehydration, growth, relative humidity

Introduction

We observe physiological scarcity when the abiotic conditions of an organism's environment become pressurized, reducing fitness to survive, potentially resulting to the loss of populations and even species (Hoffmann and Parsons, 1991) ^[13]. Due to the changing climate the physiological limits can be seen influencing susceptibility of organisms. Climate change have created numerous insect species to move from one place to another that may ranges to higher latitudes and altitudes (Parmesan *et al.*, 1999; Parmesan and Yohe, 2003; Hill *et al.*, 2011) ^[19, 20, 12]. The insects having specific limit of thermal tolerances (Addo-Bediako *et al.*, 2000; Deutsch *et al.*, 2008) ^[1, 6] are seen specifically sensitive to temperature variation. There is a significant loss in the number of *Drosophila nepalensis* from lower ranges, and climate warming results its range transfer in the Western Himalayas (Singh, 2012; Parkash *et al.*, 2013) ^[16, 23]. Most of the *Drosophila* species including *D. nepalensis* could be observe in the western high Himalayas.

D. nepalensis was initially investigated from the collections made from Nepal by Okada (Okada, 1955) ^[26]. Subsequently, Parshad and Paika (1964) ^[21] informed that the diversity of *D. nepalensis* (70-80%) could be observed with lower temperature at cooler environment of 16-20°C and humidity ~43-45% at Manali, India (2050 m). The species of *Drosophila* found in this region have desiccation-linked traits with variable quantitative traits resulting in adaptation to harsh climatic conditions in the high Himalayas. Therefore, the contrast that *D. nepalensis* induces its physiological mechanisms affecting the evolving of adaptive capacity in drier climatic conditions was investigated. *D. nepalensis* might employ phenotypic plasticity as a strategy to cope with colder and drier conditions in temperate regions, but this aspect has not been tested so far. Species distribution and diversity pattern is the reason why insect maintains water balance in their body (Willmer *et al.*, 2000) ^[25]. Staying and growing in different habitats the *e* of *Drosophila* has reflected substantial variation desiccation resistance (Gibbs and Matzkin, 2001; Gibbs *et al.*, 2003) ^[10, 9]. It has been described that insect can incline their desiccation resistance through three different methods of water balance:

- 1) Higher bulk water
- 2) Reduced rate of water loss, and
- 3) Greater dehydration tolerance (Hadley, 1994; Gibbs *et al.*, 1997)^[8, 11]

Laboratory-identified desiccation-resistant strains of *D. melanogaster* has resulted a 300% growth in hemolymph water content compared with control strains (Folk *et al.*, 2001)^[7]. If laboratory selection responses result in increases in the bulk water mass, then it would be potential to compare the water budget of *Drosophila* species differing in their desiccation resistance levels. Further, water balance related traits have been investigated in Indian populations of *D. nepalensis* reared at 21°C, but not at 15 and 25°C (Parkash *et al.*, 2012)^[18]. Thus, the water balance for *D. nepalensis* is still unclear with the plasticity for evolving physiological mechanisms.

The study in acclimation to desiccation stress has found lesser attention (Hoffmann, 1990, 1991; Bazinet *et al.*, 2010)^[13, 14, 2]. It was decided to investigate the acclimation potentiality of *D. nepalensis* abundance in Western Himalayas. This study was concerned with the relative abundance of *D. nepalensis* as a measure of transformation in relative humidity along an altitudinal gradient in the Western Himalayas. The desiccation related traits were evaluated that effect the development of phenotypic plasticity (15° versus 25°C growth temperatures) on desiccation-related traits.

Materials and Methods

Samples of *D. nepalensis* (100-150) were collected from different parts of high Himalayas during November 2018-January 2019. The study was carried out in the Entomology laboratory of Agriculture and Forestry University, Chitwan, Nepal. The cultures of the insects were based at low density (60-70 eggs per vial; egg vials were 40 × 100 mm in size) with the cornmeal-yeast-agar medium at 15°, 21°, and 25°C and 65±1% relative humidity in a temperature-and humidity-controlled incubator for five generations before experimental analysis. All assays were experimented on 7-day-old female flies, as the trait values remain similar as a measure of age between 6 and 21 days (Gibbs and Matzkin, 2001; Parkash *et al.*, 2008)^[10]. Required data for climatic variation were obtained from meteorological department, Nepal government.

The resistance of desiccation was analyzed as the time to lethal dehydration (LT100) effect at dry air. A week-old flies were placed out and kept individually in dry plastic vials (40 × 100 mm) in which the open end was covered with muslin cloth. These vials were kept on top of another vial containing 2 g of silica gel at the bottom the experiment was maintained at 0-5% relative humidity. The mass of immobile flies was recorded after every one-hour interval, and LT100 values in dry air were analyzed.

The total body water content and dehydration tolerance (%) was calculated from 10 flies of each ISO female line (20 I.F lines × 10 replicates each) were used. Firstly, the individual flies were weighed on Sartorius microbalance (Model-CPA26P; 0.001 mg precision) and then reweighed after drying overnight at 60°C. Until death the mild anesthesia (one minute) with solvent ether after flies were weighed on a Sartorius microbalance both before and after desiccation stress. Total body water content (m) was calculated as the

difference between wet (f) and dry mass (d), i.e., $m = f - d$. Individual flies were weighed and placed at 0-5% relative humidity for a specified time at one-hour intervals (1 to 8 h) and reweighed. The rate of water loss was determined from the slope of regression line on a plot of $\ln(m_t / m_0)$ against time according to Wharton's exponential equation (Wharton, 1985)^[24] $m_t = m_0 e^{-kt}$, where m_t is the water mass at time t , and m_0 is the initial water content. Rate (kt) is the slope of the regression line and was expressed as % per hour.

Results and Discussion

D. nepalensis was found abundant (~43.5%) in highland localities but occurs less frequently in lowland localities (~21%). The highland localities observed with average colder and drier (Tave = 15.2°C; RH = 43.5%), while lowland localities are warm and less desiccating (Tave = 27.6°C; RH = 62.8%). Therefore, significant reduction in Tave (~2°C per 200 m) as well as relative humidity (~3.9% per 200 m) along an elevational gradient may act as selection factors for affecting its relative abundance. Thus, *D. nepalensis* was regarded appropriately adapted under colder and drier conditions in highland localities.

The raise in body melanisation from ~2-fold at 17°C and ~16-fold at 24°C from mid thermal range temperature and we observed constant change in epicuticular mass due to thermal plastic effects (melanisation: $p < 0.001$; epicuticular lipids: 15°C = 22.10±0.37 µg cm⁻²; 25°C = 22.00±0.27 µg cm⁻²) in *D. nepalensis*. We observed ~1.5-fold higher desiccation resistance at 16°C and ~2.5-fold lower desiccation resistance at 26°C from mid thermal range temperature, i.e., 21°. So, we found the plastic responses for desiccation related traits changes in *D. nepalensis*.

Later the application of ten sets of independent experiments to detect the variation in the rate of body water loss in control versus flies exposed to different durations (1 to 9 h) of desiccation stress in *D. nepalensis* reared at 17°, 22°, and 26°C. We found relative growth of water loss in adult flies reared at 26°C, 22°, and 17°C I adult flies. These findings on rate of water loss provide the evidence of greater desiccation resistance of *D. nepalensis* when grown at 17°C.

In a comparative analysis of body mass it was evaluated consistent variation in alter content and body weight studied at multiple measures in *D. nepalensis* (wet mass: F2, 18 = 253.63; $p < 0.001$; dry mass: F2, 18 = 195.50; $p < 0.001$; body water content: F2, 18 = 229.47; $p < 0.001$) when reared at three variable temperatures, i.e., 17°, 22°, and 26°C. Similarly, the content of hemolymph including hemolymph water content have shown ~2.5-fold rise and decrease at 15°C than 25°C, respectively, as compared to 21°C.

It was found that there was rise in ~2 fold in tissue water, it was because of the change in temperature that was increased (F2, 18 = 265.94, $p < 0.001$). Even the temperature varied at 170c and 260c the dehydration tolerance did not change significantly as compared with 21°C (F2, 18 = 1.86, ns)

It was found that longer survival under the dehydration condition supported by hemolymph, which is a major source for changes in higher level of body in insects (Hadley, 1994; Folk *et al.*, 2001; Chown and Nicolson, 2004; Folk and Bradley, 2005)^[11, 7, 4]. In contrast, numerous studies on wild populations of various *Drosophila* species have not sensitively studied through the changes in hemolymph

content to enhance survival under desiccation stress (Gibbs and Matzkin, 2001; Gibbs *et al.*, 2003; Parkash *et al.*, 2010)^[10, 9, 17]. In our study we observed the changes in hemolymph content due to the result of developmental plastic effects (i.e., 17°, 22°, and 26°C). *D. nepalensis* has shown changes in hemolymph content consistent with their different levels of desiccation resistance potential. Further, most arthropods can tolerate ~30-50% loss of body water, but some taxa adapted to drier habitats have evidenced higher dehydration tolerance (Hadley, 1994; Willmer *et al.*, 2000; Benoit *et al.*, 2005)^[11, 25, 3]. In the present study, we found increased dehydration tolerance at 17°C as compared with 22°C, which is consistent with differences in desiccation resistance. Thus, the evolving of dehydration tolerance regarded as a common physiological mechanism to support survival under desiccation stress in *D. nepalensis*.

Conclusion

Samples of *D. nepalensis* (100-150) were collected from different parts of high Himalayas during November 2018-January 2019. The cultures of the insects were based at low density (60-70 eggs per vial; egg vials were 40 × 100 mm in size) with the cornmeal-yeast-agar medium at 15°, 21°, and 25 °C and 65±1% relative humidity in a temperature-and humidity-controlled incubator for five generations before experimental analysis. Required data for climatic variation were obtained from the meteorological department, Nepal government. It was found that dehydration tolerance evolved as a common physiological mechanism to support survival under desiccation stress in *D. nepalensis*.

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