CHAPTER – 1

GENERAL INTRODUCTION
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Ecological genetics

Ecological genetics is the study of genetics of traits of ecological significance i.e. traits related to fitness which affect survival and reproduction of an organism. It involves a mixture of field and laboratory studies. This discipline was founded by the English biologist E.B. Ford (1901-1988) in early 20th century. Ecology and genetics together form the mechanism of evolutionary change. Ecological interactions of organisms with the biotic and abiotic environment are the context of natural selection -- they determine the phenotype that does the best in a given situation. The ecological structure of populations (size, degree of fragmentation, etc.) also determines the potential for genetic drift (non-adaptive genetic changes in the populations). Genetic variability within populations for phenotypic characters that are ecologically important determines the speed and directionality of the response of populations to the selection resulting from ecological interactions. Thus, to understand the potential for (and constraints on) evolution in contemporary populations, one must consider both ecological and genetic issues. The cornerstone of ecological genetics is the joint theoretical and empirical analysis of the two components of adaptive evolution—natural selection on the phenotypes and genetic variability in the phenotypic traits. This integration of ecology and genetics provides a window on both the causes of and constraints on evolution in contemporary populations.

Ecophysiological stress and adaptations

Ecophysiology or environmental physiology is a biological discipline which studies the adaptation of an organism’s physiology to the environmental conditions. It is closely related to comparative physiology and evolutionary physiology. The most important factors in environment that influence the physiology of insects are temperature and humidity. Insects display a remarkable range of adaptations to changing environments and maintain their internal temperature (thermoregulation) and water content within tolerable limits, despite wide fluctuations in their surroundings.

Stress is an environmental factor that causes directly or indirectly a potentially adverse change in the physiology and fitness related traits of any organism. Temperature and humidity are two most common environmental stress factors which vary geographically as well as
seasonally on the Indian subcontinent. Insects can adjust to changing environmental conditions by adapting to new conditions or shifting their geographical range and distribution. Diverse *Drosophila* species differ in their ability to changes in their environment (Angilletta, 2009). Adaptation is a complex and dynamic state that widely differs in species. Surviving under changing environment in insects depends on dispersal, habitat selection, habitat modification, relationship with ice and water, resistance to cold, heat and developmental rate, sensitivity to environmental signals. There is need to investigate the adaptive changes and evolutionary shifts in stress-related traits at within populations, among populations, and among species levels. Further, these levels are linked to understand how evolutionary processes within populations relate to adaptive divergence among species exposed to different climatic conditions.

**Geographical and seasonal climatic variations across the Indian subcontinent**

Climatic conditions vary temporally as well as spatially on the Indian subcontinent which impose selection pressure on living organisms. Environmental factors play an important role in shaping ecology and evolution of biological systems. Living systems harbor genetic variability for a given trait so as to cope with varying environmental conditions. Across the Globe, average annual temperature is negatively correlated with increasing latitude on a broader scale. In contrast, no such trends are observed for relative humidity on different continents. On the Indian subcontinent, average yearly temperature does not vary much along latitude but there is a steeper gradient for coefficient of variation of average temperature (Tcv) and relative humidity (RHcv) along the latitude i.e. there are significant seasonal variations in climatic variables which increase with latitude. India is characterised by significant temperature variations in different seasons ranging from mean temperature of about 10°C in winter to about 32 °C in summer season. Southern localities are characterized by constant high temperature and high humidity throughout the year whereas large variations in temperature as well as humidity occur across seasons in the northern region i.e. warm and humid rainy season versus colder and drier autumn season. Seasonal changes in climatic variables are significant for subtropical populations as compared with southern tropical populations of various *Drosophila* species. Such a climatic pattern does not occur on many continents including Africa and Australia. Thus, Indian subcontinent offers opportunities for the analysis of stress impact on physiology of various life forms.
Different species possess divergent strategies to survive under changing environments in the same place (Angilletta, 2009). Chromosomal inversions have been reported in *D. pseudoobscura* and *D. persimilis* (Dobzhansky, 1948) and in *D. subobscura* (Rodriguez-Trelles and Rodriguez, 1998) due to changes in thermal conditions across seasons. Further, several studies have reported seasonal changes for frequencies of melanic morphs in *Harmonia axyridis* (Tan, 1946); body size traits in *D. robusta, D. melanogaster* and *D. simulans* (Stalker and Carson, 1949; Tantawy, 1964); and for body color in *D. putrida* (Sabath et. al., 1973). The adaptive role of seasonal polyphenism due to plasticity has also been observed in several species of butterflies (Brakefield, 1987; Brakefield and Reitsma, 1991; Kemp and Jones, 2001; Stoehr and Goux, 2008). However, these studies considered the role of temperature changes (Ritland, 1986; Brakefield, 1987) but the physiological changes due to changes in seasonal relative humidity has been unexplored so far. Thus, there is need to investigate the role of temperature as well as humidity for seasonal changes in stress related traits in the field-caught *Drosophila* species. Further, evidence of climatic selection factors (temperature or humidity) for various stress-related traits in different *Drosophila* species should be explored by simultaneous laboratory experiments.

**Desiccation tolerance**

Water conservation is a most crucial issue for insects which affect their survival, distribution and abundance (Hadley, 1994; Chown and Nicolson, 2004). Tropical *Drosophila* species are less tolerant to desiccation stress as compared to temperate ones (Gibbs and Matzkin, 2001; Gibbs et al., 2003). Increased tolerance to drier conditions has been observed in wild populations of several drosophilids living under drier conditions due to evolution of genetic mechanisms to adapt to the local environments (Endler, 1986; Hoffmann and Sgro, 2011; Kellermann et al., 2012). Insects can increase their desiccation resistance by three major avenues of water balance i.e. (i) higher bulk water storage (ii) reduced rate of water loss and (iii) ability to sustain greater loss of body water under dehydration stress before succumbing to death (Hadley, 1994; Gibbs et al., 1997). Laboratory selection experiments for desiccation resistance have reported increased bulk water and higher dehydration tolerance in desiccation resistant lines as compared to control lines in *Drosophila melanogaster* (Gibbs et al., 1997; Chippindale
et al., 1998; Folk et al., 2011). In contrast, several studies did not evidence differences in the stored water content between selected versus control lines (Hoffmann and Parsons, 1989, 1991). However, reduced rate of water loss is the general mechanism evidenced for greater tolerance of xeric Drosophila species to drier habitats than mesic species (Gibbs and Matzkin, 2001); and in laboratory selected desiccation resistant strains of D. melanogaster (Gibbs et al., 1997).

Cuticular transpiration is most common route for water loss in insects (Hadley, 1977; Toolson and Hadley, 1979; Hadley and Schultz, 1987). Cuticle is the main barrier between organism physiology and environmental conditions in diverse insect taxa. Cuticular permeability varies between different species as well as within species at interpopulation and intrapopulation levels (Rourke, 2000; Parkash et al., 2008a; Parkash et al., 2008b; Parkash et al., 2011). Differences in cuticular permeability occur due to cuticular melanisation or cuticular lipids. Role of melanisation to confer desiccation stress has been evidenced in dark ebony mutants which were more desiccation resistance than yellow mutants as well as wild Drosophila flies (True, 2003). Further, several studies have also observed higher desiccation resistance in darker (more-melanic) flies as compared with lighter flies of D. polymorpha (Brisson et. al., 2005); D. melanogaster (Parkash et al., 2008); and D. ananassae (Parkash et al., 2010). However, changes in the amount of epicuticular lipids are also known to alter cuticular water loss in insects (Hadley, 1994; Gibbs, 2002). A negative correlation was observed between epicuticular lipids amount and cuticular permeability in scorpions and tenebrionid beetles (Hadley, 1977; Toolson and Hadley, 1979; Hadley and Schultz, 1987); grasshopper – Melanoplus sanguinipes (Rourke, 2000); and drosophilid - Zaprionus indianus (Parkash et al., 2008a). The removal of cuticular lipids with organic solvents can differentiate Drosophila species with surface lipids as barriers to cuticular permeability but this aspect has received lesser attention so far. Further, significant increase in cuticular permeability after removal of surface lipids with organic solvents is evident in the black widow spider – Latrodectus hesperus (Hadley and Quinlan, 1989); in the cricket – Acheta domesticus (Hadley, 1989); in the larvae of gall fly – Eurosta solidaginis (Ramløv and Lee, 2000). However, some studies on wild Drosophila species found no association between water loss and surface lipid amount at intra as well as inter-population levels (Parkash et al., 2008b; Parkash et al., 2012a).
Acquisition of greater carbohydrates as energy reserves has been observed to alleviate the desiccation stress in laboratory selected desiccation resistant strains of *D. melanogaster* (Graves et al., 1992; Gibbs et al., 1997; Chippindale et al., 1998; Djawdan et al., 1998). In contrast, storage of higher levels of lipid content under dehydration stress are evidenced for large sized insects (locusts and tse-tse flies) and a new set of laboratory selected desiccation resistant strains of *D. melanogaster* (Loveridge and Bursell, 1975; Telonis-Scott et al., 2006). Thus, storage and utilization of energy reserves (trehalose, glycogen, and body lipids) for conferring desiccation resistance in wild populations divergent *Drosophila* species need to be investigated.

**Starvation tolerance**

Starvation stress refers to the the biological condition where an organism is unable to eat due to some extrinsic limitation on food resources (McCue, 2010). It may be complete lack or shortage of food resources (Chippindale et al., 1996, 1998; Bubily and Loecheke, 2005). The period of starvation may be either due to acute or chronic weather conditions or diel or seasonal cycles or long-term ecological change. Organisms need to employ effective mechanisms to endure starvation stress (Hoffmann and Harshmann, 1999). Starvation resistance is the time taken till death under starved conditions and it can be increased by insects by one or more ways (1) sequestering greater energy reserves; (2) reducing the rate of utilization of reserves under starvation conditions (3) lowering the threshold energy budget for survival. Selection experiments have reported enhanced starvation tolerance in starvation resistant lines having greater amount of lipid reserves than control lines of *D. melanogaster* (Chippindale et al., 1996; Schwasinger- Schmidt et al., 2012; Hansen et al., 2013). However no association between starvation resistance and body lipid content was evident in natural populations of drosophilids (Hoffmann et al., 2001; Jumbo-Lucioni et al., 2000). Therefore, further studies are needed for exploring the relationship between starvation and accumulation of energy reserves and to emphasize on the alternate mechanisms to increase starvation resistance.

Clinal variation along latitude has been observed for starvation resistance in *D. melanogaster*, *D. ananassae*, *D. kikkawai* and *Zaprionus indianus* (Karan et al., 1998; Karan and Parkash, 1998) as well as for *D. bipectinata* and *D. malerkotliana* (Parkash et al., 1994) from the Indian subcontinent. Geographical variations for starvation resistance have also been found for
European and African populations of *D. melanogaster* (Da Lage et al., 1990) and among isofemale lines of the some populations of *D. melanogaster* (Hoffmann et al., 2001) and *D. serrata* (Hallas et al., 2002). However, there is no cline for starvation tolerance and fat content in South American populations of *D. melanogaster*. It may be likely due to non-occurrence of clines for humidity on other continents but there is steeper gradient for relative humidity across latitude on the Indian subcontinent (Robinson et al., 2005). In addition, large seasonal variations in relative humidity in northern Indian subtropical localities lead to higher humidity in the rainy season but drier conditions in the winter season which induce plastic responses for starvation resistance in wild populations of drosophilids. However, seasonal changes in resistance to starvation stress in various *Drosophila* species have received lesser attention. Therefore, investigation of changes in starvation resistance and correlated levels of energy metabolites in wild-caught flies may be helpful for studying seasonal adaptations in starvation stress related traits of different *Drosophila* species.

**Thermotolerance**

Temperature is a major environmental factor responsible for species abundance and geographical distribution of ectothermic organisms (Andrewartha and Birch, 1954; Cossins and Bowler, 1987; Anderson et al., 2003). Insects face varying temperatures daily and seasonally, so that natural populations are often exposed to heat or cold stress and these thermal fluctuations impose selection pressure on organisms (Angilletta, 2009). Different insect taxa vary in their response to the same thermal environment i.e. same change in temperature does not affect all ectothermic organisms equally (Angilletta, 2009). Geographical distribution of diverse insect taxa depends upon their resistance to thermal extremes i.e. high level of cold resistance is linked with the higher latitudes while a reverse trend is observed for heat resistance (Hoffmann et al., 2003; Chown and Nicolson, 2004). Addo-Bediako and coworkers found that species from higher latitudes are usually more tolerant to cold than those from lower latitudes but no correlation was observed between heat resistance of insects and their latitudes of origin (Addo-Bediako et al., 2000). Further, physiology and basal thermal tolerance of an individual are the best predictor of its latitudinal range (Calosi et al., 2010). Relationship between individual thermal physiology and latitudinal range has been studied in fourteen ecologically similar congeners of European diving beetles (Calosi et al., 2010). This study has suggested that low
temperature tolerant species are more abundant in northern localities while southern range favors high temperature tolerant species. Thus, geographical distribution of species appears to be associated with their physiological tolerances to climatic stress.

Geographical variation in thermotolerance traits have been mainly investigated in *D. melanogaster* populations from different continents such as Australia (Hoffmann et al., 2002), South America (Lindsey et al., 2012), few European populations (Guerra et al., 1997) and other regions (Hoffmann et al., 2003). In contrast, the sibling species- *D. simulans* has shown lack of clinal variation for heat or cold resistance despite its sympatric distribution with *D. melanogaster* on different continents (Arthur et al., 2008). Thus, these closely related *Drosophila* species differ in their adaptive strategies for resistance to heat or cold stress. Further, investigation of plastic responses to rearing temperatures revealed increased heat tolerance in *D. melanogaster* after development at higher temperature (Cooper et al., 2012). In contrast, lower rearing temperature increase the cold tolerance in *D. watanabei*, *D. trapeziforms*, *D. triauraria* and *D. melanogaster* (Goto and Kimura, 1998; Rako and Hoffmann, 2006). However, no effects of rearing temperatures has been observed for heat resistance of *D. watanabei*, *D. trapeziforms* and *D. triauraria* (Goto and Kimura, 1998); and a temperate *Drosophila* species – *D. curviceps* as well as two subtropical related *Drosophila* species – *D. immigrans* and *D. albomicans* (Ohtsu et al., 1999). A strong association between humidity and heat tolerance has also been observed in *Drosophila* species (Bubily et al., 2012; Kellermann et al., 2012b). Humidity conditions on different continents show no regular trend along latitude but there is a steeper gradient for relative humidity on the Indian subcontinent along the latitude as well across seasons in the northern localities. Howevevre, there are no data on the effects of rearing humidity on thermal tolerance of various drosophilids.

**Acclimation potential and climatic stress adaptation**

Global climate change leads to increase in air temperatures and decrease in humidity which is putting the *Drosophila* species around the globe in peril. The ability to tolerate these climatic conditions depends upon the species acclimation potential or plasticity (Hoffmann et al., 2003; Hoffmann, 2010). Acclimation or hardening is a phenomenon of physiological and behavioural changes occurring within an organism, which reduces the strain or enhances endurance of strain, caused by experimentally induced stressful changes in particular climatic
factors (Bowler, 2005). Short-term exposure (minutes or hours) to sublethal conditions is termed as “hardening” while long-term exposure (days or weeks) of an organism to mild stress is usually referred as acclimation (Cossins and Bowler, 1987). However, the terms hardening and acclimation are often used interchangeably to determine plastic responses to various stresses in evolutionary literature. Adaptive role of acclimation capacity has been observed after prior exposures to desiccation stress (Hoffmann, 1990, 1991) and thermal stresses (Loescheke et al., 1994; Sarup et al., 2009; Hoffmann and Watson, 1993; Kristensen et al., 2008).

Acclimation responses to climatic stresses vary at interspecific as well as intraspecific levels in drosophilids. Increased desiccation resistance has been observed for females of four Drosophila species but only in males of D. serrata and there is lack of geographical variation in acclimation response in three Drosophila species (D. melanogaster, D. simulans and D. serrata) from Australia (Hoffmann, 1991). However, temperate populations of D. melanogaster and D. simulans has more acclimation potential to cold stress than tropical populations but lack of geographical variations for acclimation to heat stress (Hoffmann and Watson, 1993). Further, A positive correlation was found between basal thermostolerance and absolute hardening capacity for eight Drosophila species i.e. more thermotolerant species (D. melanogaster, D. simulans and D. vulcana) showed greater hardening response than less thermotolerant species -D. sechelia, D. serrata, D. yakuba and D. birchii (Kellet et al., 2005). Another study described that tropical populations of D. melanogaster showed a greater heat hardening response than temperate populations (Sgro et al., 2010). Large amount of genetic variation for adaptations to spatially and temporally varying climatic conditions is evident for the generalist Drosophila species, (Powell, 1997; Hoffmann and Weeks, 2007). In contrast, lack or low acclimation potential has found for two rainforest Drosophila species- D. serrata (Hallas et al., 2002) and D. birchii (Hoffmann et al., 2003), which match with their restricted distribution patterns.

**Major objectives of research work are as under:**

1. To investigate the mechanistic basis of desiccation resistance in geographical populations of two related Drosophila species of immigrans group- D. immigrans and D. nasuta. Whether these species differ in their cuticular traits (body melanisation and cuticular lipids mass) which might affect cuticular transpiration and multiple measures
of water balance (bulk water, rate of water loss and dehydration tolerance) in conferring desiccation resistance in pre-adult (larval) as well as adult stages. Whether there are differences in the storage and utilization of energy metabolites in northern and southern populations of *D. immigrans* and *D. nasuta*? Whether these two *Drosophila* species can acclimate to drought stress and what is the physiological basis of drought acclimation?

2. To test the hypothesis whether sex specific differences in cuticular traits (melanic females and non-melanic males) of *D. kikkawai* correspond with divergence in their water balance mechanisms. To investigate the geographical variation in desiccation resistance, cuticular traits and energy metabolites in larvae and adults of both the sexes of *D. kikkawai*. To assess seasonal changes in desiccation related traits in males and females of this species and physiological basis of drought acclimation in both the sexes.

3. To investigate the associations of species-specific divergence of thermal adaptations of *D. nasuta* and *D. immigrans* with seasonal as well as geographical changes in the relative abundance. To examine genetic variation for resistance to heat or cold stress and egg-to-adult viability (a fitness related trait) between populations of these two related species.

4. To test 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.

5. To investigate the effects of developmental humidity or thermal acclimation on the thermotolerance of two sibling *Drosophila* species—*Drosophila leontia* and *D. kikkawai*. To analyse the geographical variations in acclimation responses to thermal stresses after rearing at two different developmental temperature or humidity conditions. Whether relative abundance and thermal stress related traits of these sibling species vary across seasons?

6. To assess the resource acquisition and starvation resistance of *D. melanogaster* after rearing at contrasting low (35% RH) versus high (85% RH) humidity conditions.
Whether there are changes in the duration of development, storage and utililization rate of energy metabolites (trehalose, glycogen and lipid content) under starvation stress at different humidities. To investigate the seasonal changes in starvation resistance and energy metabolites in wild-caught flies of *D. melanogaster*.

7. To analyze the seasonal variations and effects of developmental thermal acclimation in the stress resistance of two cosmopolitan species from western Himalayas- *D. busckii* and *D. melanogaster*. 