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Effect of climate change and environmental factors in the morphology and capacity of *Drosophila melanogaster* in the local condition of Nepal

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Abstract
In the local condition of Chitwan Nepal the study on morphology and capacity of *Drosophila melanogaster* was not investigated. Therefore, an experiment was carried out to determine morphology and capacity of *Drosophila melanogaster* in changing climate and variable environmental factors. Laboratory and field study of *D. Melanogaster* was carried out in five sample stocks that were collected from the field and at the same time evaluation in field condition was also carried out; the research was conducted from January 2022 to December 2022. Samples of *D. Melanogaster* with similar body size and wing structure were accumulated in the laboratory condition to determine variation in morphology (wing size) with respect to different month period artificializing similar temperature in laboratory condition as that of external environment. In the field, the samples were analyzed from the same site through where the insects collected for laboratory study, however with different elevation. The variation in morphology, i.e., size of wings (length and breadth) was studied in the laboratory while for each ten minutes the flight or climbing capacity of sample *Drosophila* were counted or recorded in the field. It was found that the average length and breadth of the wings of *Drosophila* fluctuated in variable months of the year. Longest length (with average of 3mm) and width of wings (2 mm in average) observed in the sample *Drosophila* during the month of June, July, October, November, and December, while shortest length of wings (2.5 mm in average) and width (1.5 mm in average) found in the month of February, March, April, May, August, and September in the similar samples of *Drosophila.* Higher reduced flight or climbing capacity in average (81%) were observed in the *Drosophila* stock samples flying at lower elevation of 150 masl (i.e., having higher CO_2) and lower reduced flight or climbing capacity in average of 30% were observed in similar sample *Drosophila* flying at higher elevation of 850 masl (i.e., having lower CO_2). Morphology and capacity of sample *Drosophila melanogaster* was found affected by variable environmental and presumed changing climate in Chitwan district of Nepal.

Keywords: Capacity, elevation, flying, temperature, wing

Introduction
The extinction of species or organisms or its population may be caused by various pressure or stress of the environmental condition that may decrease fitness to the extent that have negative consequences in the organism or reproduction or activities (Hoffmann and Parsons, 1991) [22]. Physiological limits can have effect in the susceptibility of organisms to climate change and indicate vulnerability (Bernardo *et al.*, 2007; Williams *et al.*, 2008; Huey *et al.*, 2009) [8, 54, 23]. The population of vertebrates from lowland site may be influenced as they resemble close to their thermal threshold and are could be observed experiencing stressful condition (Bernardo and Spotila, 2006; Huey *et al.*, 2009) [9, 23].
In nature, organisms also often face small periods of stressful conditions that extend outside the range of conditions in which organisms can be cultured. Phenotypic plasticity is the cause that the organism with thermal extremes tolerating such short. At the individual level, physiological limits therefore depend on the trait being analyzed, length of exposure of the extreme conditions and plastic responses.
Adaptive evolution or transformation could be observed with environmental variables like temperature, photoperiod and food availability vary over spatial and temporal scales, driving adaptive divergence among populations, etc.

The subject of organism's life history is quite interesting due to the ecological and evolutionary impact of latitude (Roff, 1980; Mittelbach *et al.*, 2007; Kapun *et al.*, 2016; Rajpurohit *et al.*, 2017) [46, 32, 28, 39], altitude (Hawkins, & DeVries, 1996; Sørensen , 2005; Pitchers *et al.*, 2013; Klepsatel *et al.*, 2014; Ayhan *et al.*, 2016) [21, 47, 37, 29, 36, 1] and seasons (Bergland *et al.*, 2014; Dobzhansky and Ayala, 1973; Rajpurohit *et al.*, 2017; Williams *et al.* 2017*;* Machado *et al.*, 2021; Rodrigues *et at.*, 2021) [39, 54, 6, 39, 31, 44].

The seasonal consistent pattern of winter versus summer seasons in temperate regions or wet versus dry seasons in tropical regions formed by the annual pattern in photoperiod and temperature varies with latitude and seasonal changes in these environmental parameters. Therefore, seasonally varying environments can incline the selective pressures as a function of time compared to the stable non-seasonal environments. These variations in the measure of seasonality might be one of the measures where spatial clines are prepared and appear because of the seasonal phase cline models (Rhomberg and Singh, 1986) [43]. For example, we can observe the population of *Drosophila melanogaster* residing in high-latitude collected in autumn are genetically and phenotypically similar to low-latitude populations (Cogni, *et al*., 2014; Behrman *et al.*, 2015; Cogni *et al*., 2015) [13, 14, 4] The organism's evolutionary linkage to the seasonality, which is formed by a complex and fluctuating environmental pressure, ensures that existing genetic polymorphisms are preserved.

In one of the studies in North America observed that the seasonal genomic oscillations in *Drosophila* populations delivers an important insight into rapid adaptation to seasons (Bergland *et al.*, 2014) [6]. Bergland *et al.* (2014) [6] identified alleles called 'fall like' and 'spring like', which have the capacity to fluctuate rapidly within seasons, which confirms that the populations rapidly adapt to changing environments.

Clinal patterns have been observed in some scientific evidence that indicates that temperature is the primary cause for this issue. In a laboratory study it was observed that the body size of *Drosophila* evolved in concordant with latitudinal sorting under thermal adaptation (Cavicchi *et al.*, 1989; Partridge *et al.*, 1994) [11, 33]. Many other ecological and environmental parameters, such as rainfall, humidity, and UV exposure, vary along latitudes. Recent studies have attempted to identify the ecological and environmental factors that may have resulted in latitudinal changes for body size (Jones *et al.*, 2005*;* Stillwell Stillwell *et al.*, 2007; *et al.*, 2008) [25, 47-48]. Due to adaptation in polygenic traits the adaptation to new environments usually occurs and as selection alters the optimal values of life history traits in new environment. In *Drosophila* the polygenic morphological traits such as body size could be assessed (Turner *et al.*, 2011; Pitcher *et al.*, 2019) [50, 37] and previous studies showed positive correlation between size related traits such as thorax length, body weight and wing size in (Reeve, 1950; Cowley and Atchley, 1990; Partridge *et. al.*, 1994) [33, 14, 41], although wing size is the highly applied proxy of body size (Reeve and Robertson, 1953) [40]. The body size is linked with various life history characters such as lifespan (Partridge and Fowler, 1992; Rodriguez and Fanara, 1999; Norry and Loeschcke, 2002) [32, 34], mating behavior and flight ability (Ray *et al*., 2016) [39], any of the morphological traits related to body size can be used as a "model trait" to track changes in traits correlated with respect to body size.

The study on morphology and ability variation in *D. melanogaster* due to changing climate and environmental factors will be carried out for twelve months over a year in Chitwan district of Nepal.

Materials and Methods

The sample population of *D. melanogaster* were collected from the field during January 2022 to December 2022 in Chitwan district of Nepal. The sample insects were with similar body size, wing size and shape which were then accumulated in the laboratory condition. Five stocks of (each with 20 sample) were placed in the bottle with 27^0c in already prepared medium of yeast, sugar, and cornmeal.

Measurement of the wings were taken and were regularly recorded using microscope. The morphological changes (wing's size-length and width) were timely analyzed with varying environmental conditions (i.e., variable months).

The ability percentage of *D. melanogaster* climbing in different elevation were studied in the field. Each 20 samples were investigated from the same spot where five stocks of insects were assessed for field study. These samples were analyzed for 10 minutes in each stock samples of *D. melanogaster* with variable field elevation (i.e., fluctuating CO_2).

The recorded data were all tabulated and systematically arranged using MS- Excel which were subjected to Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT-0.05 level) for mean separations using Gen stat software.

Results and Discussions

Size of *D. melanogaster*'s wing in variable months

Length of the wing

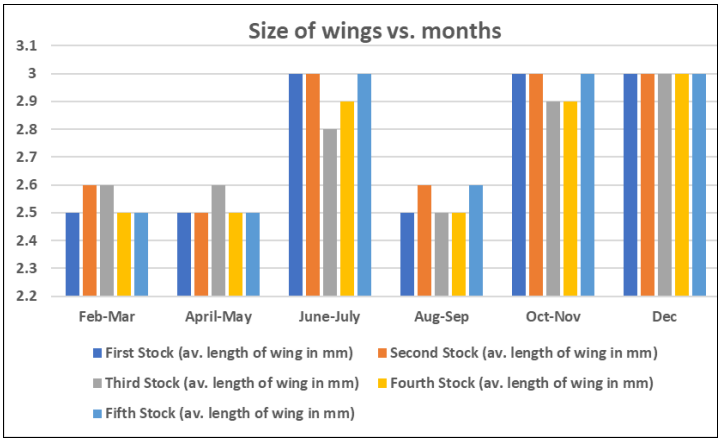

Fig 1: Variable wing size (length) with changing environment condition of each month

It was found that the length of wing of *Drosophila* samples were fluctuating in different zones of months. In each stock of insect samples, the length was with average size of 2.5mm, 2.6 mm, 2.6 mm, 2.5 mm, and 2.5 mm respectively in first, second, third, fourth and fifth stocks. Similarly, the length became consistent from April- May. They increased with average of 3 mm, 3 mm, 2.8 mm, 2.9 mm, and 3 mm in each first, second, third, fourth and fifth stock samples of *Drosophila* respectively during June-July. Again, with decrease in Aug-Sep the increment could be observed at final ends of the year (Figure 1). The temperature was maintained as that of the external environment throughout the year in laboratory.

Similar studies were caried by Bergland *et al*. (2014) [6], Machado *et al.* (2021) [31], Rodrigues *et al.*, (2021) [44] and result coincide with our investigation. Our findings were also supported by the evaluation conducted by Behrman *et al*., (2015) [4] and Behrman *et. al.*, (1870) [3].

Width of the wing

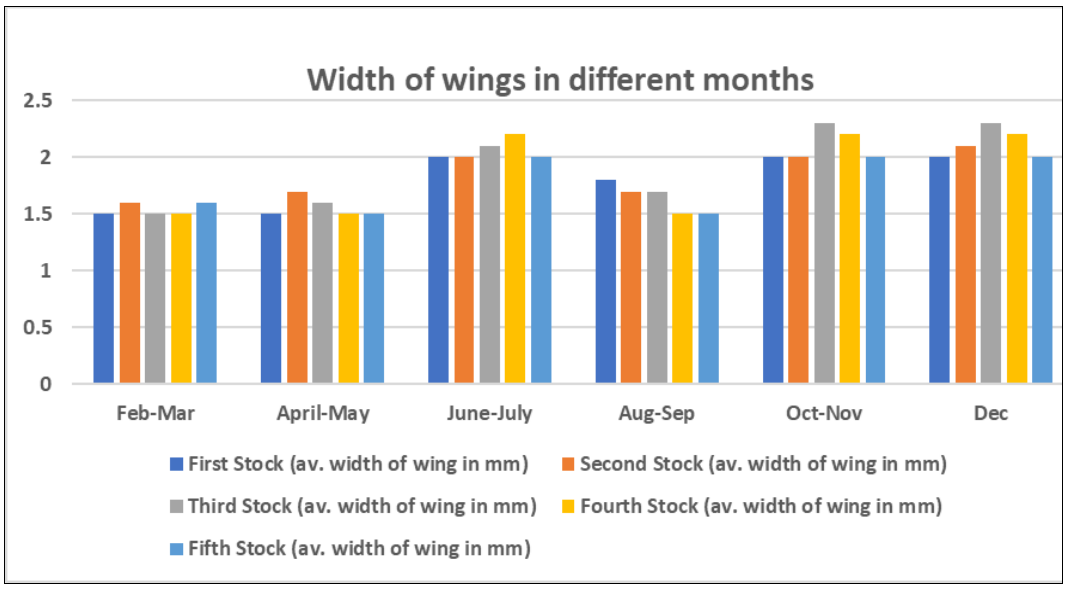

Fig 2: Variable wing (width) with changing environment condition of each month

It was analyzed that the width of wings was smaller during Feb-Mar (in av.), i.e., 1.5 mm, 1.6 mm, 1.5 mm, 1.5 mm, and 1.6 mm in first, second, third, fourth and fifth stock samples respectively in *Drosophila.* Almost similar size could be observed in the month of April-May. The width only increased in June-July and end parts of the year, and became extreme of 2 mm, 2.1 mm, 2.3 mm, 2.2 mm, and 2.0 mm first, second, third, fourth and fifth stock samples respectively during the month of Dec (Figure 2). The temperature was maintained as that of the external environment throughout the year in laboratory.

It was found that temperature plays significant role in size (width) of wings of *Drosophila*, and temperature varies with time duration (Bergmann, 1847; David *et al*., 1977; Coyne and Beecham, 1987; Capy *et al.*, 1993; Imasheva *et al*., 1994; James *et al.*, 1995; van't Land *et al.*, 1995; Gilchrist and Partridge, 1999; Flatt, 2020) [33, 19, 6, 51, 10, 18]. The accelerated temperature has irrelevant relationship with the body size and wings designs. In contrast the available temperature has significant non-linear relation with wings size of *D. melanogaster*.

Flight or climbing ability of *Drosophila* in variable carbon dioxide level

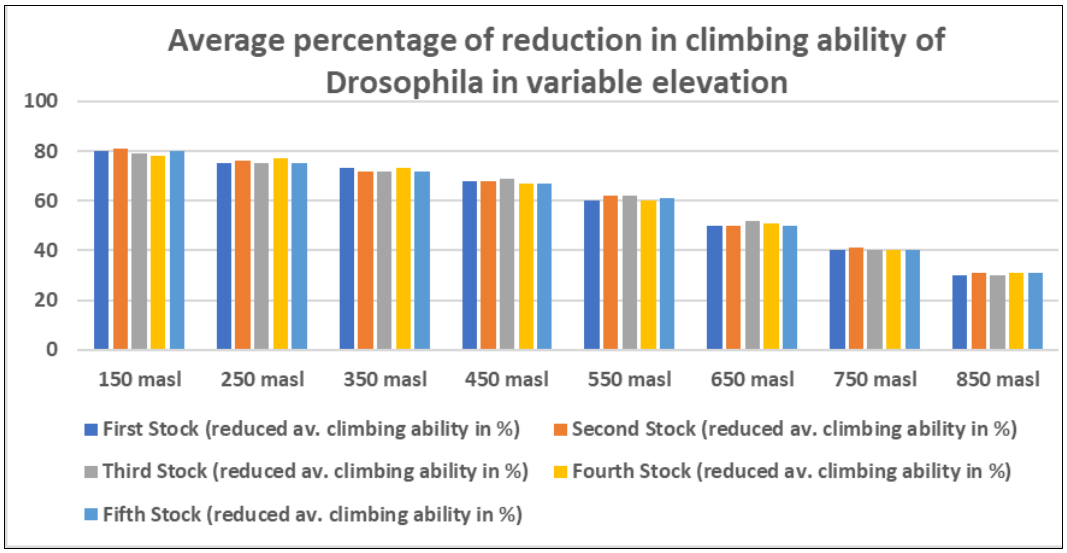

Fig 3: Climbing ability of *D. melanogaster* in different elevation or carbon dioxide level

It was found that with 150 masl (i.e., higher CO_2) the average reduction in climbing or flight were 80%, 81%, 79%, 78%, and 80% for first, second, third, fourth and fifth stock samples respectively while studying for 10 minutes for each. The climbing ability gradually increases or reduction in flight decreases with elevation (i.e., lower CO_2) and it reduced in average up to 30%, 31%, 30%, 31%, and 31% at 850 masl in first, second, third, fourth and fifth stock samples respectively while studying for 10 minutes for each (Figure 3).

This study was supported by the investigations of some similar research (Piazza *et al.*, 2009; Tinkerhess *et al.*, 2012) [35, 49]. The methods of climbing and ability of Drosophila were supported by the analysis of Tinkerhess *et al.*, 2012 [49]. Badre *et al.* (2005) [2] & Brosnan and Pham (2008) [9] explained that the CO_2 directly inhibits glutamate receptors. It works as of the mammalian nicotinic receptors on motor end plates. There could be the direct involvement in the mechanism to inhibit muscle activity in *D. melanogaster*. The probability could be minimal in the initiation of the antennal CO_2 receptor, which is made up of the Gr21a and Gr63a chemosensory receptor (Jones *et al.*, 2007) [26]. However, such receptors are likely saturated at CO_2 status much below than those that are necessary for anesthesia (Kwon *et al.*, 2007) [29]. The variation in morphology of Drosophila with each time span (within a year) and their capacity were found at different elevation of the district.

Conclusion

The sample (five stocks) *D. melanogaster* were collected from the field during January 2022 to December 2022 in Chitwan district of Nepal. Samples with similar body size and wing structure were accumulated in the laboratory condition. In field, the samples were analyzed from the same site through where the insects collected for laboratory study, but with different elevation. The variation in morphology, i.e., size of wings (length and breadth) was studied in laboratory while for each ten minutes the flight or climbing capacity of sample *Drosophila* were counted or recorded in the field. It was found that average length and breadth of the wings of *Drosophila* found fluctuating in variable months of the year, with similar kind of temperature maintained in laboratory as that of environment. Highest length and width of wings (in average) were found in the month of June, July, October, November, and December, while shortest (in average) found in February, March, April, May, August, and September in the same samples of *Drosophila.* Higher reduced flight or climbing capacity in average were observed in the *Drosophila* stock samples flying at lower elevation (i.e., having higher CO_2) and vice-versa. Morphology and capacity of sample *Drosophila melanogaster* was found effected by variable environmental and presumed changing climate in Chitwan district of Nepal.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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