Starvation tolerance in six geographical populations of *Drosophila melanogaster*

Report by Himanshi Soni

Abstract

As the climate is changing drastically around the world knowing its effect on existing species has become one of the fundamental questions, as its ability to evolve itself for better survival and reproductive fitness will decide its existence in new stressful climatic conditions. *Drosophila* species have been chosen in a laboratory experiment to know how better they can survive in stressful conditions such as starvation tolerance. these species are found everywhere (except for extreme cold climate). It has been observed that species from different geographical locations and have better starvation tolerance than any other climatic region and females have higher starvation tolerance than males at 23^{o} C. Through ANOVA and Tukey's post-hoc test it has also been observed that there is no significant difference in starvation tolerance between sub-tropical and continental flies but these two climatic region flies are significantly different in starvation tolerance when compare with temperate region flies but for knowing how female tolerance is higher than males' further studies have to be done.

Biological and Life sciences

School of Arts and Sciences

Ahmedabad University, Ahmedabad

CERTIFICATE

This is to certify that the dissertation entitled "**Starvation tolerance in six geographical populations of *Drosophila melanogaster***" submitted by **Miss Himanshi Soni,** to the Biological and Life Sciences, School of Arts and Sciences, Ahmedabad University, Ahmedabad – 380009, for the Summer Research Fellowship Program is her original work, based on the results of experiments and investigations carried out independently by her during the period **June 2022** to **August 2022** of study under the supervision of **Dr. Subhash Rajpurohit.**

This is also to certify that the above said work has not been previously submitted for the award of any degree, diploma or fellowship in Indian or Foreign University.

Supervisor

Subhash Rajpurohit

Introduction

As the climate is drastically changing around the world it has become important to know at what rate can an organism evolve itself to cope with certain stressful conditions. *Drosophila* species is usually used in laboratory experiments as they have originated from Tropical 2 and over time they have migrated to different parts of the world where they have gone through different genetic, physiological, and behavioural changes to make themselves suitable according to the different abiotic conditions they have found themselves in for better survival and reproductive fitness. We are focusing on *Drosophila melanogaster* collected from six different geographical locations and trying to make some correlation between their adaptive strategies to tolerate seasonal variations. In both genders, Tropical 2 and Temperate 2 *Drosophila* have managed to survive for the longest hours. Though North America is having higher starvation tolerance among female than males it is Temperate 3 flies that has higher starvation tolerance in male though Temperate 2 males have survived for longer hours. It can be concluded that the *Drosophila* species from different geographical locations may have differed in their tolerance capacity due to experiencing greater seasonal variations. Starvation is a major stress for temperate population due to prolonged unfavourable cold climatic conditions although it was considered of lesser significance for tropical population. Flies from temperate regions are higher starvation tolerance when compared to tropical regions. Present investigation evidence that starvation is also a major ecological and physiological stress in the tropics.

We used model mixed ANOVA to assess the direct and interactive effects of isofemale lines, sex (male and female). Through ANOVA it has been observed that there is no significant difference in starvation tolerance among continents like Tropical 1, Temperate 2, Tropical 2, and Temperate 3for both males and females but there was a significant difference in females from Temperate 1, Temperate 2, Tropical 2 and Tropical 3 males. Starvation was significantly difference with Tropical 2, Temperate 2 and Temperate 3 males and it has also been observed that for every population there was significant difference in starvation tolerance between males and female. For males and females, the exact reason for more starvation tolerance in female than in males at $23\ ^{o}$Celsius cannot be stated as experiment done by (J.-L. da Lage et al., 1989). *Drosophila melanogaster* at 9 different temperature shows that below 21^{0}C males are more tolerant when compare to female. For knowing proper reason for starvation tolerance difference between males and females' further studies and data should be analysed on physiological level that effects starvation tolerance at different abiotic condition

Table 1- Location and temperature details of flies

Geographical sources	Min Temp ºC	Max. Temp º C	Difference between º C
Tropical 1	30^0	10^o	20^o
Tropical 2	32^o	14^o	18^o
Tropical 3	24^o	13^o	9^o
Temperate 1	29^o	19^o	10^o
Temperate 2	27^o	-30^o	57^o
Temperate 3	43^o	-5.4^o	48^o

Stocks and Maintenance:

Flies were collected from 6 different localities using fermented fruit baits. From this collection, six replicate populations were established. Captured flies were sexed in the laboratory using microscope and each caught female was isolated into a separate food vial and iso-female lines were established. The flies were maintained in standard food media (Agar-Jaggery-Yeast-Maize flour) at 23°C. Newly eclosed flies were collected and aged for 3 days in the vials containing the cornmeal- active yeast, sucrose media before being subjected to assay. A total of 40 lines of *D. melanogaster* from different populations were chosen randomly for this experiment. From this, ten males and females were taken from each line and kept in a narrow vial for the assay.

Starvation assay:

For starvation assay, replicates were made by collecting eggs from the stock population and reared them at 23ºC, 12L-12D photoperiod cycle. All the replicate population were grown and maintained on standard *Drosophila* media (corn-meal media). On emergence, fresh emerged adult flies were sexed and sorted into ten males and female each, by immobilizing them with lightCO_2 anaesthesia. After anaesthetizing flies were placed into fresh food vials containing standard food media and allowed to acclimate to the

experimental condition for at least 24 hours before subjecting to the assay. Following 24 hours of testing on standard food medium, flies were transferred to vials containing2% agar medium and starvation resistance was assessed. Starvation tolerance reading were taken three time points; in a day. 9;30 am, 1;30 pm and 6:00 pm. and assessed them until ~50 % mortality is not achieved. The time of death was manually determined and noted for each individual fly as the last bout of activity.

Data analysis

For each replicate populations different statistical analyses such as LT-50 were performed on data measurements of starvation. From data collected on starvation assay, LT-50 value was calculated through computerized system, the LT-50 value indicated lethal treatment where 50 % of initial flies are death. LT-50 for each vial is calculated. Later average of LT-50 for each population are run through ANOVA to indicate significant difference among population of male and female separately and both together.

Result and conclusion:

When starvation tolerance is plot against number of dead flies for each line for a population, it has been observed that continental region has more starvation tolerance than the temperate climate region in both gender. Males of tropical region have managed to survived more than 95 hours without food (except for tropical 1)(fig.1 and fig.2). Where as male flies from temperate region has able to survived maximum for 92 hours except for Tropical 3 flies. In female it was observed tropical 1 and temperate 1 and temperate 2 region flies have survived for longer hours than other climatic region flies. Same pattern is seen when LT-50 graph for each line of population is plot for males and females separately. Significant difference in LT-50 value among groups of males and females are seperately and together calculated through ANOVA to know the significant difference among group and Turkey's pairwise comparasion table is given below to know that mean of a population is significantly different from mean of every population . In males and females significant difference among group is $p<0.05$ but through turkeys table is can be noted that the significant difference among group doesn't mean that between all the groups, there is significant diffeerence in starvation tolerance of sub-tropical and continental flies with temperate region flies but there are no significant difference within sub-tropical and continental flies or within temperate climatic population for both genders, it has also been seen that population of Aisa share no significant difference either among continental or temperate climatic region has population of Tropical 1 were collected from temperate oceanic

climatic region. Through analytical data and statistics, it has been noted that female among all population have higher starvation tolerance than males. Average value of LT-50 for each population is plotted for males and females for each population indicating female as better starvation tolerance compare to male and there is also significant difference between males and females for each value analysed through ANOVA.

Figure 1. Starvation tolerance against number of lines for female population

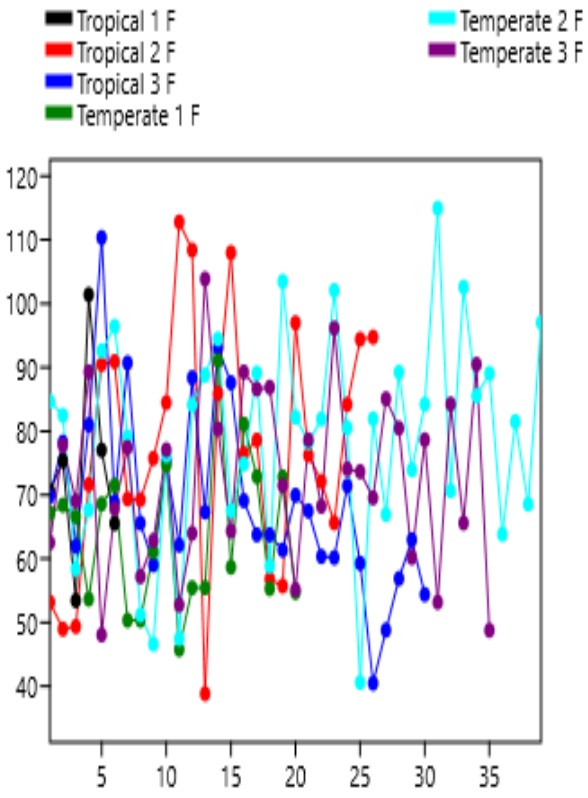
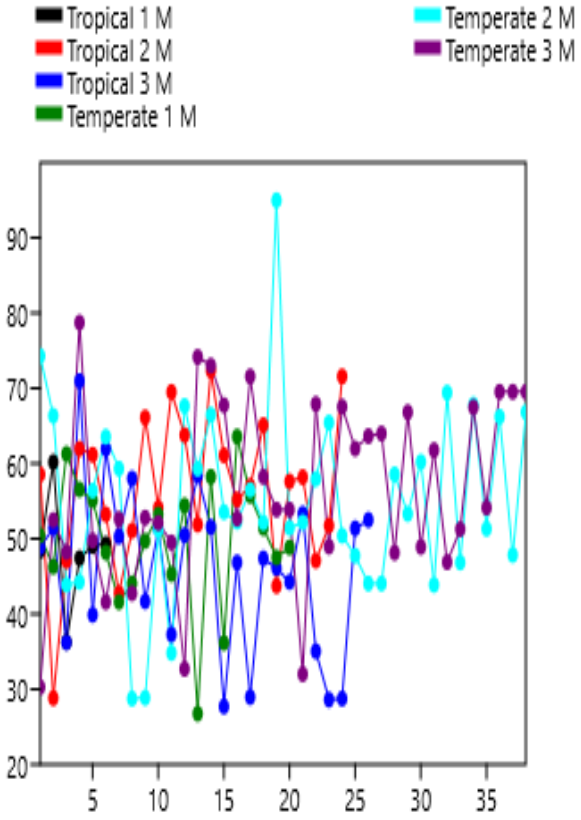
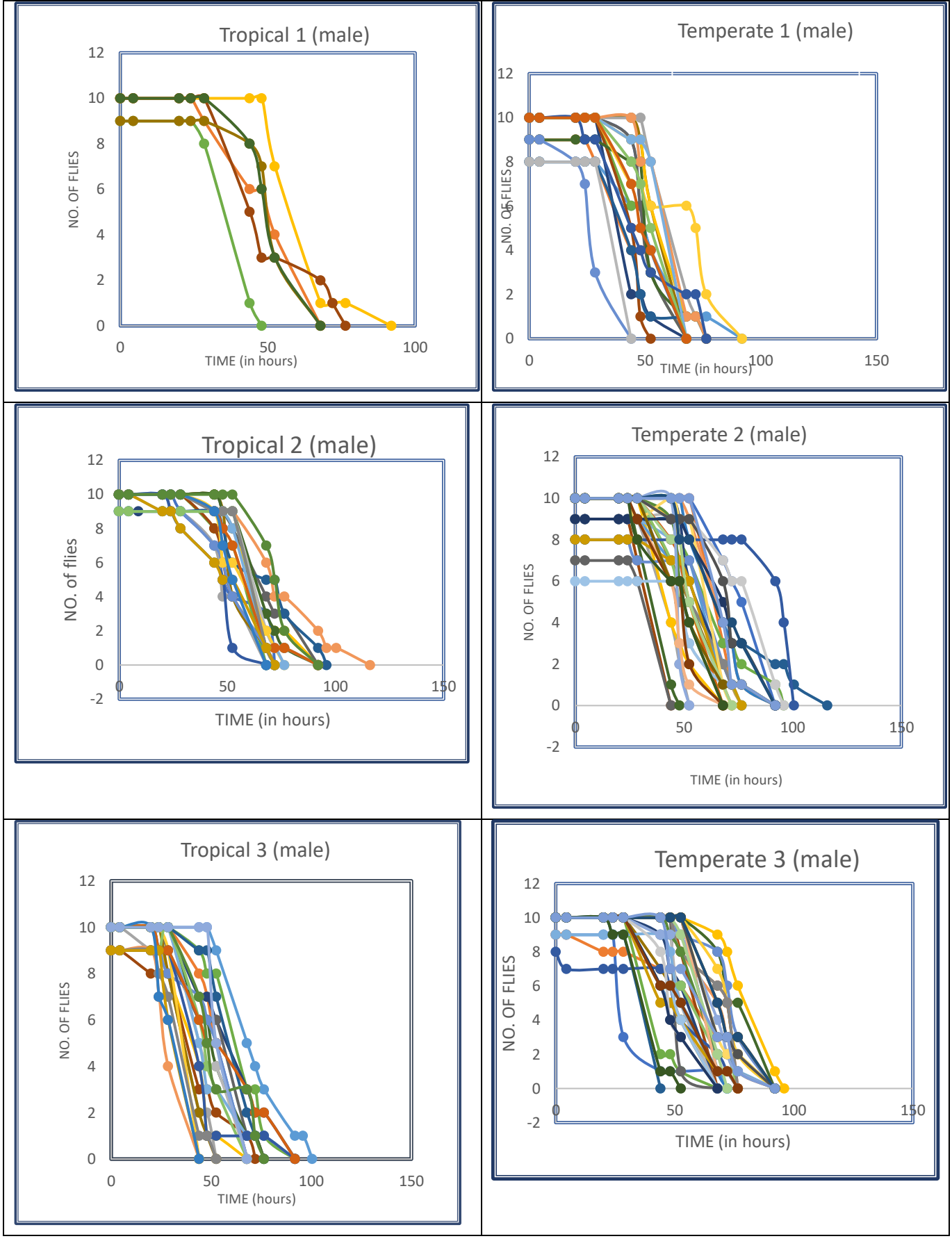
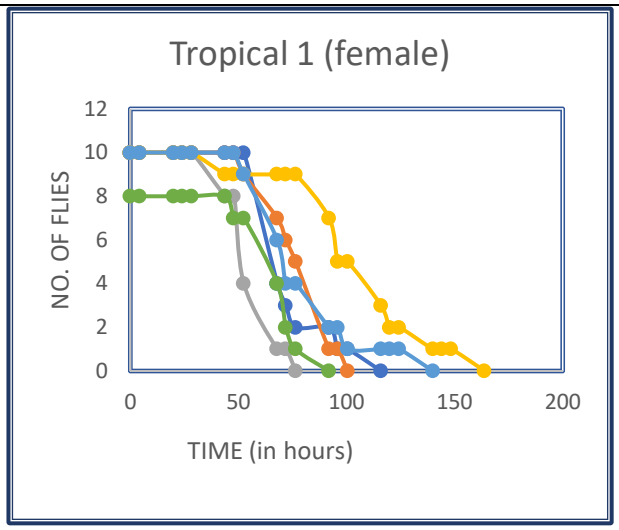


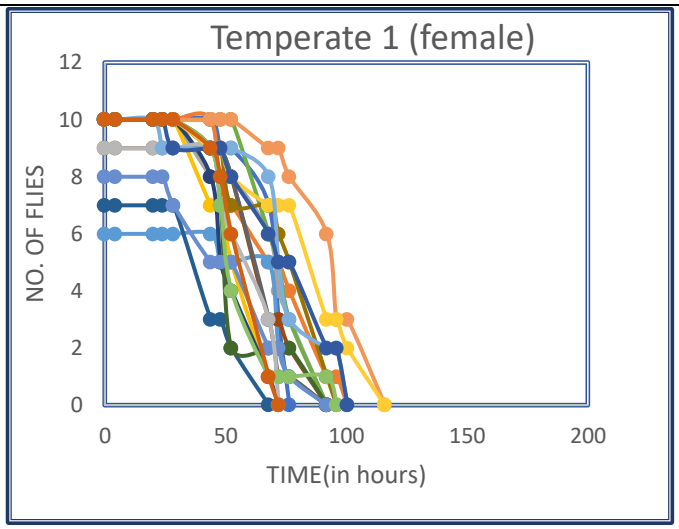
Figure 2. Starvation tolerance against number of lines for male population

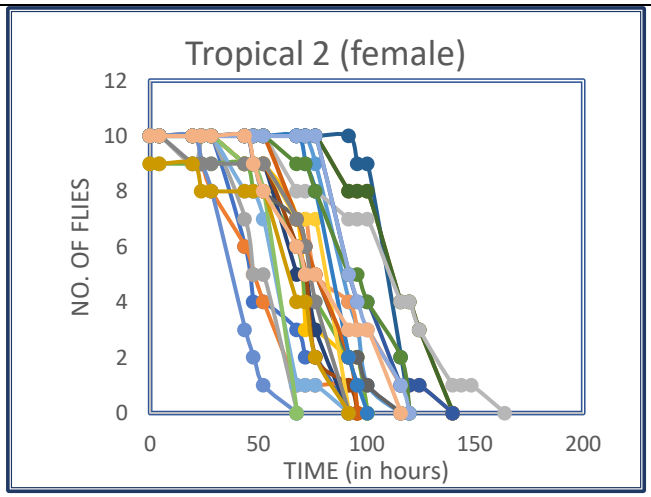


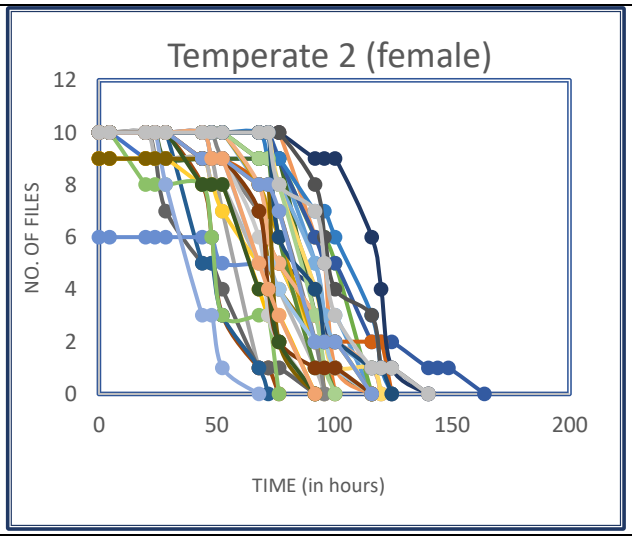


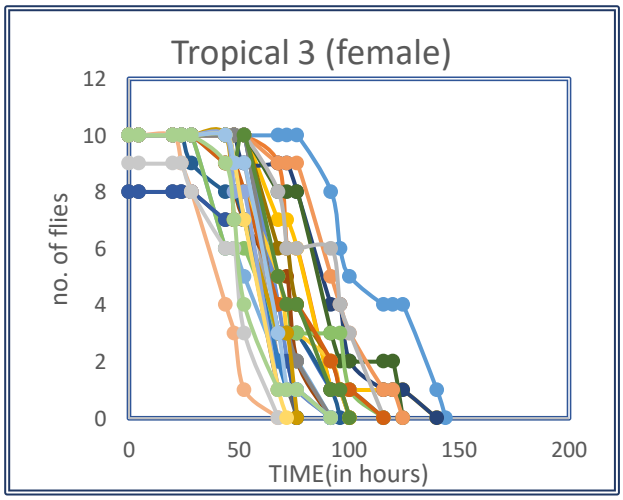
Survivorship curve of different population of *Drosophila melanogaster* (males) after subjecting to starvation. (each curve represent different iso-female line)

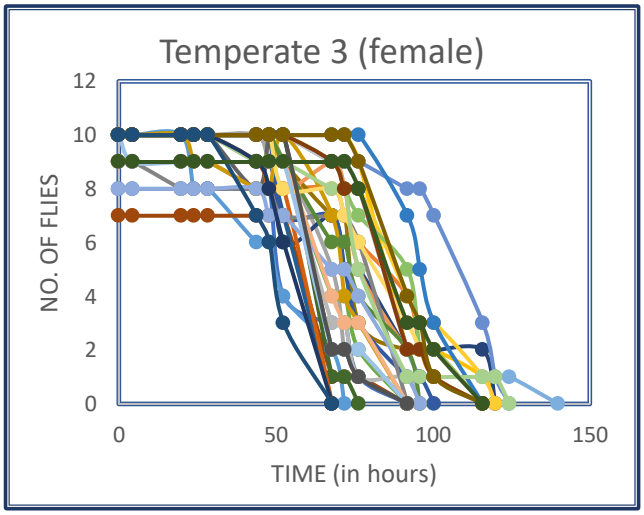

Tropical 1 (female)
NO. OF FLIES
0
2
4
6
8
10
12
0
50
100
150
200
TIME (in hours)


Temperate 1 (female)
NO. OF FLIES
0
2
4
6
8
10
12
0
50
100
150
200
TIME(in hours)


Tropical 2 (female)
NO. OF FLIES
0
2
4
6
8
10
12
0
50
100
150
200
TIME (in hours)


Temperate 2 (female)
NO. OF FILES
0
2
4
6
8
10
12
0
50
100
150
200
TIME (in hours)


Tropical 3 (female)
no. of flies
0
2
4
6
8
10
12
0
50
100
150
200
TIME(in hours)


Temperate 3 (female)
NO. OF FLIES
0
2
4
6
8
10
12
0
50
100
150
TIME (in hours)

Survivorship curve of *Drosophila melanogaster* (females) subjected to starvation assay. (each curve represent different iso-female lines of drosophila)

Tukey's Q below the diagonal, p(same) above the diagonal.
Significant comparisons are pink.

Copenhaver-Holland 1988

	Tropical 1 M	Tropical 2 M	Tropical 3 M	Temperate 1 M	Temperate 2 M	Temperate
Tropical 1 M		0.6449	0.9975	0.9999	0.7366	0.5705
Tropical 2 M	2.165		0.02077	0.3873	0.9993	1
Tropical 3 M	0.643	4.52		0.8903	0.02065	0.004859
Temperate 1 M	0.3464	2.731	1.521		0.4766	0.243
Temperate 2 M	1.957	0.4925	4.522	2.529		0.9961
Temperate 3 M	2.326	0.1284	5.159	3.115	0.7057	

Tukey's Q below the diagonal, p(same) above the diagonal.
Significant comparisons are pink.

Copenhaver-Holland 1988

	Tropical 1 F	Tropical 2 F	Tropical 3 F	Temperate 1 F	Temperate 2 F	Temperate
Tropical 1 F		0.9968	0.9811	0.73	0.9762	1
Tropical 2 F	0.6783		0.3548	0.04653	0.9981	0.8841
Tropical 3 F	0.9965	2.81		0.856	0.09455	0.9161
Temperate 1 F	1.973	4.12	1.637		0.006834	0.3017
Temperate 2 F	1.05	0.605	3.731	5.013		0.5479
Temperate 3 F	0.2092	1.544	1.42	2.946	2.374	

Turkey's Q analyses data table: columns which are highlighted in pink colour indicate population of among two different locations are significantly different from one and other with respect to starvation tolerance

Starvation tolerance assay graph of male and female comparing LT-50

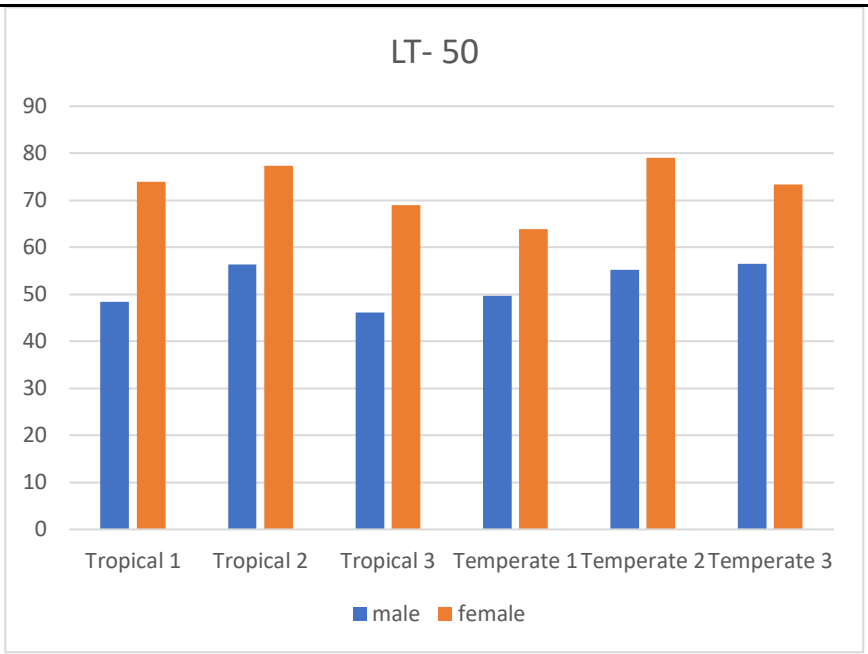


Table 2- ANOVA Test between male and female for each population

Continent	p- value	Degree of Freedom	F
Tropical 2	$\mathbf{2.103\ X\ 10^{-5}}$	1	**22.24**
Temperate 2	$\mathbf{9.154\ X\ 10^{-10}}$	1	**49.05**
Temperate 3	$4.268\ X\ 10^{-7}$	1	**30.94**
Tropical 1	0.005355	1	12.51
Temperate 1	$9.647\ X\ 10^{-5}$	1	18.99
Tropical 3	$1.692\ X\ 10^{-8}$	1	43.85

Discussion

In this experiment we evaluated starvation tolerance of *Drosophila melanogaster* at 23^{o}C for six continents. It has been observed that Temperate 2 and Tropical 2 populations have survived for longer than other continental populations. The average value of LT-50 analysis shown that in females of Temperate 2,

Temperate 3 and Tropical 2 survived longer compared to Temperate 1 and Tropical 3. Temperate 2. The same pattern observed in males of Temperate 2, Temperate 3, Tropical 2 and Tropical 1. This may suggest that starvation, as an environmental stress and is more prevalent in temperate sites than the tropical sites. In contrary Africa population from tropical region survived longer in spite of its low lipid content. Various behavioral studies shown that African population had a low locomotor activity which could reduce their metabolic activity and rate of utilization of glycogen. Thus, increase survival capacity. The flies with lower metabolic rate have more starvation tolerance as it helps in conservation of energy metabolites such as trehalose, lipids and glycogen in the haemolymph which increases longevity of flies in stressful situations (Berrigan & Partridge, 1997; Brown et al., 2019; Dillon et al., 2009).

Our ecophysiology data confirm that starvation tolerance is significant fitness parameter especially in species living in temperate populations and which are more likely to experience seasonal variation more frequently than tropical populations. This highlights the physiological changes in stress related traits such as starvation tolerance that are geographically influenced and expected to show some degree of adaptive phenotypic plasticity. According to (J. L. da Lage et al., 1990; J.-L. da Lage et al., 1989) males starvation tolerance is greater than female when done at temperature below 21^{o}C contrasting from our results which were at 23^{o}C female starvation tolerance is higher compared with males. Starvation tolerance is also extremely variable among species. In *D. melanogaster* the survival duration in the presence of water is clearly related to amount of lipids and death occurs when all lipids disappear. All these results indicate that starvation resistance is considerably phenotypically plastic and can be enhanced in response to environmental cues indicating the likelihood of food shortage. Natural selection seems to produce a diversity of adaptations using different physiological mechanisms.

Future Prospective

From above discussion we can say hypothetically that temperature role on starvation tolerance is not as simply has it seems, temperature directly does not affect starvation tolerance it effects physiological trait such as lipid metabolism determine by fly's genotype effects starvation tolerance., Over period of time it is possible that flies have evolve themselves according to environment that may affect starvation tolerance in them in negative or positive direction To understand the association of temperature and lipid metabolism we can analyse:

- The effect of lipid metabolism on starvation tolerance of flies at different temperature can be carried out for same flies belonging to same climatic region having same lipid content in their body to know

how does lipid metabolism get effected by temperature and to determine is it different for males and female.

- Greater number of species should be investigated to reach more precise conclusion.
- There are number of other ways or mechanisms through which different species could cope with these environmental stresses. A systematic and in-depth study is needed.

References

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