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Differential stressor-induced adaptations in *Drosophila melanogaster*

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Abstract
Adaptation is a key feature of evolution, enabling organisms to better suit to their environments to thrive. To observe adaptive changes, this study established some *Drosophila melanogaster* cultures in glass containers under different environmental conditions. Fifteen flies were placed in each of four containers: one in complete darkness, one in natural conditions, one subjected to daily heat shock, and one to daily cold shock. We hypothesize that *Drosophila melanogaster* will develop distinct morphological features in response to these varied conditions. The main goal is to determine how environmental factors drive evolutionary adaptations by contrasting these traits with those of flies reared in their native environments. The objective of this research is to clarify adaptive evolutionary tactics by examining the morphological expressions of *Drosophila melanogaster*. The consequences of the research can be used to comprehend how living things are affected by climate change on a global scale. *Drosophila melanogaster* is a great research model that may be used as an environmental indicator.

Keywords: Adaptation, *Drosophila melanogaster*, cold shock, heat shock, environmental factors

Introduction

Questions about changes in the morphological adaptive behavior of organisms are best answered through experimentation, which can often be straightforward and illustrative. Nature provides ongoing experiments that offer insights into evolutionary or adaptive changes. For meaningful results in such studies, it is crucial to observe several generations and include a large number of individuals to ensure reliable conclusions. Organisms that breed rapidly, are easy to raise and produce many offspring are ideal for these experiments.

A popular model organism for these kinds of experiments is the fruit fly, *Drosophila melanogaster*. In about ten to fifteen days, a single couple of *Drosophila* flies may generate several hundred offspring, marking the completion of a generation. These flies are ideal for adaptation research because they may be reared on a variety of basic culture media. Researchers may successfully explore morphological changes in response to different settings by using *Drosophila*. Additionally, by enabling the study of behavioral alterations in response to various dietary circumstances, this model contributes to a thorough comprehension of adaptation processes. *Drosophila* larval populations selected on nutrient-poor medium exhibit various behavioral and morphological changes (Kolss *et al*. 2009). These changes include altered foraging behavior, accelerated development, reduced body weight, and smaller body size (Vijendravarma *et al*. 2011). It is important to mention that under prolonged malnutrition it can be hypothesized that fly nutrient-sensing pathways might be under selective pressure.

In this study, we investigate the morphological, physiological, and behavioral alterations that arise under diverse environmental stress circumstances in order to better understand the differential stressor-induced adaptations in *Drosophila melanogaster*.

Materials and Method

Collection: The samples were collected with the help of a big jar keeping decaying fruits like banana, and papaya in natural condition. After 4-5 days there were a lot numbers of the species developing inside the jar.

Identification: Collected specimens are observed under a microscope randomly and identification was done using keys provided by Markow and O'grady, 2005; Yuzuki and Tidon, 2020.

Procedure

To observe adaptive evolutionary features, four glass containers of uniform volume (250ml), marked as A, B, C and D were taken. Banana was used as the food source. The food was prepared by dipping ripened banana in a suspension of yeast in water subsequently placing it in each glass container.

15 flies form the initial stock were introduced to each of this 4 glass containers. The openings of these containers were blocked with cotton plugs to avoid the escape of the insects and ensuring sufficient movement of air into the containers.

The prepared containers were then placed in various artificial (laboratory) conditions as followed

a. Glass container A was kept in natural conditions with proper sunlight, wind and natural darkness.
b. Glass container B was wrapped with a black cloth and placed it in darker area within the laboratory where the light is constantly absent.
c. Glass container C was subjected to heat shock with the help of hot water with temperature around 75ºC. The container was dipped twice daily in hot water to provide such condition.
d. Glass container D was subjected to cold shock with the help of cold water with temperature around 4ºC. The container was dipped twice daily in cold water to provide such condition.

The insect was allowed to grow inside the containers subjected to the different environmental conditions. Observations were made and recorded on daily basis to look for eggs laid, dead individuals and other morphological features. In each generation, the changes observed were noted for further statistical analysis.

Data were gathered for each glass container up until the conclusion of the third generation of *Drosophila*. For counting the number of eggs and individuals a simple magnifying glass was used. For morphological observations, five specimens were randomly extracted from each bottle and refrigerated for 3-4 minutes to immobilize them. The specimens were then placed on graph paper to measure size, length, etc.

The following features were noted in each case for further statistical analysis

a. Time required for the hatching of eggs.
b. Death rate or Mortality Rate
c. Life cycle duration
d. Body Length

Results

During the experimental study, rearing of *Drosophila* was done up to 3rd generation. The following results were observed during the period:

Time taken for hatching eggs

The number of eggs hatched was recorded at different intervals of times (in hours) for all the experimental conditions. It is represented in the following Table 1.

Table 1: No. of egg hatched at different time interval

Time (hours)	Conditions.											
	Natural			Dark			Heat Shock			Cold Shock		
	1G	2G	3G	1G	2G	3G	1G	2G	3G	1G	2G	3G
0-10	0	0	0	0	0	0	15	30	46	0	0	0
10-20	10	30	40	5	9	20	22	52	37	0	0	0
20-30	21	45	75	10	20	32	10	20	30	10	25	34
30-40	18	40	60	22	35	40	0	0	0	18	30	32
40-50	0	0	0	10	18	30	0	0	0	15	31	48
Total	49	115	175	47	82	122	47	102	113	43	86	114

(G-Generation)

Death Rate or Mortality Rate

To calculate the mortality rate the number of dead individual were counted after each generation and divided by the total number of eggs observed and then multiplying it by 100. The mortality rate recorded at different conditions has been tabulated in Table 2.

Table 2: Mortality Rate

Conditions	Natural			Dark			Heat shock			Cold shock		
Generations	1G	2G	3G	1G	2G	3G	1G	2G	3G	1G	2G	3G
Total Eggs	49	115	175	47	82	122	47	102	113	43	86	114
No. Dead Individual	3	14	21	12	18	22	12	19	21	15	21	26
Mortality Rate (%)	6.12	12.17	12	25.53	21.95	18.03	25.53	18.62	18.58	34.88	24.41	22.80

(G-Generation)

Duration of life cycle

To estimate the life cycle, the number of individual at particular time interval was counted. The observations recorded are represented in Table 3.

Table 3: Duration of life cycle

Day required	Conditions											
	Natural			Dark			Heatshock			Cold shock		
	1G	2G	3G	1G	2G	3G	1G	2G	3G	1G	2G	3G
5-7	0	0	0	0	0	0	5	32	67	0	0	0
7-9	5	10	13	3	2	5	25	36	12	0	0	0
9-11	27	75	132	5	3	13	5	15	13	0	3	5
11-13	13	15	9	19	52	61	0	0	0	18	35	50
13-15	1	1	0	8	7	21	0	0	0	10	27	33

(G-Generation)

Body Size

For calculation of the body sizes, five random individuals from the each sample were extracted and the size was measured placing them on graph paper. The result is represented in the Table 4.

Table 4: Body size at different stage (in mm)

Observation Individual	Conditions											
	Natural			Dark			Heatshock			Cold shock		
	1G	2G	3G	1G	2G	3G	1G	2G	3G	1G	2G	3G
1	6	7	7.2	6.2	6	6	7	7.2	7.1	6	5.5	5.2
2	6.3	7.2	7.3	6	6	5.8	7.2	7.2	7	5.5	5.2	5.3
3	6.2	6.3	7.5	5.8	5.5	5.7	6.9	7	6.9	5.6	5.6	5.3
4	7	6.5	6.8	5.8	5.8	5.7	7	6.9	6.5	5.4	5.4	5.4
5	6.8	6.6	6.9	5.9	5.8	5.5	7.1	6.7	6.7	5.5	5.5	5.5

(G-Generation)

Analysis of the Result and Discussion

Statistical techniques were used to examine a variety of data that were collected following attentive observation. The results of these analyses are presented below.

For every generation of *Drosophila* grown in every glass container, means, variances, coefficients of variation, and standard errors were computed. The tables that follow display the outcomes. The following are the abbreviations that were used f – Function; M – Mean; SE– Standard Error; S^2 – Phenotypical Variances;

CV – Coefficient of variation.

N – Natural Condition; D – Dark Condition; H – Heat shock Condition; C – Cold shock Condition.

The results of the calculations are represented in tabular form in table 5.

Table 5: Statistical Parameters

	1st Generations			2nd Generations			3rd Generations			
F	M± SE	S2	CV±SE	M± SE	S2	CV±SE	M± SE	S2	CV±SE	Hatching Time
N	26.63± 0.64	20.67	17.07±0.67	25.86± 0.27	8.72	11.41± 0.27	26.14± 0.18	5.75	9.17± 0.18	
D	32.87± 0.76	27.86	16.05± 0.76	31.34 ±0.42	14.64	12.20 ±0.42	31.55 ±0.28	9.95	9.99 ±0.28	
H	13.93 ±0.84	34.31	42±0.84	14.01 ±0.39	15.72	28±0.39	13.58 ±0.35	14.62	28.15 ±0.35	
C	36.16 ±0.93	37.73	16.70 ±0.93	35.69 ±0.46	18.27	11.97 ±0.46	36.22 ±0.35	14.29	10.43 ±0.35	
f	M± SE	S2	CV±SE	M± SE	S2	CV±SE	M± SE	S2	CV±SE	Duration of Life Cycle
N	10.43±0.13	0.88	8.99±0.13	10.13 ±0.06	0.39	6.16±0.06	9.94 ±0.04	0.26	5.12 ±0.04	
D	11.82±0.21	1.62	10.77 ±0.21	12±0.27	0.94	8.08±0.27	11.96 ±0.07	0.59	6 ± 0.07	
H	8 ± 0.22	1.71	16.34 ±0.22	7.59±0.11	0.83	12.12 ±0.11	6.82 ±0.11	0.98	14.36 ±0.11	
C	12.71±0.31	2.74	13.02 ±0.31	12.73 ±0.13	1.18	8.53±0.13	12.63 ±0.09	0.84	7.25 ±0.09	
f	M± SE	S2	CV±SE	M± SE	S2	CV±SE	M± SE	S2	CV±SE	Body Size
N	6.46 ±1.66	13.92	57.75 ±1.66	8.02 ±2.32	27.21	65.04±2.32	7.14 ±1.95	19.13	61.25 ±1.95	
D	5.94 ±1.55	10.64	54.92 ±1.45	5.82± 1.4	9.95	54.19± 1.4	5.74 ±1.37	9.51	53.65 ±1.37	
H	7.04 ±1.91	18.32	60.79 ±1.91	7 ± 1.89	18	60.61±1.89	6.84 ±1.83	16.74	59.81 ±1.83	
C	5.6 ±1.32	8.76	52.85 ±1.32	5.44 ±1.25	7.95	51±1.25	5.28 ±1.19	7.19	50.75 1.19	

Time taken for hatching eggs

The mean hatching time under natural conditions was 26.21 hours, as can be seen from the table (). The longest duration in the first generation (26.63 hours) was followed by a decline (25.86 hours) in the second generation and a slight rise (26.14 hours) in the third. Because newly produced *Drosophila* was less adaptive, variation was highest in the first generation (20.67) and dropped as adaption improved in subsequent generations. From the first to the third generation, the coefficient of variation (CV) likewise dropped.

In dark conditions, the average hatching time was 31.92 hours. In the first generation, it was 32.87 hours; in the second, it was 31.34 hours; in the third, it increased slightly to 31.55 hours. Because of its heat association, it is likely that hatching took longer in the dark than in normal settings, demonstrating the significance of light. Within the first 20 hours, there was no notable hatching. In line with greater adaptability, variance and coefficient of variation (CV) reduced across generations (from 16.05 to 9.99). Table 1 shows that the third generation's standard error was 0.28, confirming accurate readings.

Under heat shock circumstances, the average hatching time was 13.84 hours. With no eggs remaining to hatch after 30 hours and the largest number of eggs hatching between 10 and 20 hours, heat dramatically expedited the hatching process. Because of increased adaptability in succeeding generations, the coefficient of variation (CV) fell from its greatest value of 42% in the first generation, which indicated wider dispersion around the mean. The CV and variance values fell from the first to the last generation, as previously mentioned. Many eggs hatched even earlier in the third generation, in 0–10 hours which is evident from Table 5.

Due to the lack of heat, the mean hatching period under cold shock settings was 36.02 hours, which was noticeably longer than in other situations. Subsequent generations hatched faster, demonstrating adaptability, even though the first generation took longer. As the dispersion around the mean shrank, the coefficient of variation dropped from 16.70 in the first generation to 10.43 in the third. From the first to the third generation, variance also decreased, suggesting that the environment had stabilised. In the third generation, the standard error was 0.35, confirming accurate measurements referring to Table 5.

Death Rate

The mortality rates of three generations of *Drosophila melanogaster* under natural, dark, heat shock, and cold shock conditions were closely observed in this study. Under all circumstances, the first generation's high death rate was first noted with an exception in the case of natural condition; by the third generation, however, this rate had dramatically dropped, suggesting adaptability.

The lowest mortality rate was recorded in case of natural condition, with an average of 10.09%. In case of dark condition, the mortality decreased from 25.53% in the first generation to 18.03% in the third generation suggesting an improved adaptation to the absence of light.

The highest mortality rate was observed in cold shock condition with an average rate of 27.36%, followed by heat shock condition with an average of 20.91%. The mortality rate in both of this condition dropped from first to third generation, attributed to the adaptation of the insect to the temperature variation.

Even though the death rate in the dark condition (21.83%) was higher than in natural settings (10.09%), it was still lower than in heat- and cold-shock conditions. This indicates that environmental stress substantially prevents *Drosophila melanogaster* from developing normally, with the greatest stress being caused by drastic temperature changes.

Duration of life cycle

In our investigation, we found that *Drosophila melanogaster's* life cycle time varied considerably depending on the type of laboratory setting. The life cycle in the natural environment averaged 10.20 days, which is quite similar to the 10 days needed in an open setting. The life cycle under dark conditions lasted 11.92 days because the lack of light delayed hatching. At 30–40°C, heat shock conditions quickened up development, resulting in an early hatch and a shortened egg-larval period of roughly 4 days which ultimately contributed to the shortest life cycle of 7.47 days. On the other hand, cold shock circumstances resulted in a longer life cycle, averaging 12.63 days with a standard error of 0.09. This was due to a delayed hatching and an increased egg-larval period of approximately 8 days.

The life cycle duration significantly lowered under all conditions and across generations, suggesting adaptive responses. For example, the average life cycle in heat shock conditions decreased from 8 days in the first generation to 6-7 days in the third generation, highlighting the need of adaptability to cope with environmental stressors.

Body Size

The *Drosophila melanogaster* study shows significant differences in body size between generations and environmental variables. The average body length under each condition showed clear trends: under normal conditions, it was 7.20 mm; under dark settings, it was 5.83 mm; under heat shock, it was 6.96 mm; and under cold shock, it was 5.44 mm.

Over the course of several generations, variation showed a rise across specific environmental variables, suggesting genetic alterations. Variance was initially low, ranging from 8mm to 18mm under all circumstances, but it increased significantly in the second and third generations, ranging from 7mm to 27mm. The observed rise in variance and coefficients of variation from the first to the third generation highlights the varying pace of adaptation among individual specimens.

Smaller *Drosophila* sizes were regularly produced under cold shock circumstances, averaging 5 mm across generations. This was explained by adaptive processes meant to reduce heat loss. On the other hand, larger sizes, around 7mm, were made possible by heat shock circumstances. These specimens showed the greatest variance (CV of 60), indicating a strong degree of adaptation. Natural circumstances, free from outside influences, preserved natural body sizes. Furthermore, under all circumstances, a noticeable drop in body size was seen from the first to the third generation, indicating adaptation to imposed environmental stressors. All things considered, body size differences in *Drosophila* highlight the organism's adaptive plasticity in reaction to various environmental stimuli.

Conclusion

In conclusion, it is firmly stated that the morphology of an organism is modified to better suit environmental conditions. Such modifications affect the genetic makeup, leading to hereditary genetic changes that can be passed from generation to generation. These changes ultimately contribute to the evolution of the species. From an ecological perspective, the experiment serves as an important model for studying animal reactions to changing environments. As *Drosophila* is well suited for various scientific studies, the conclusions drawn from our experiment can be applied to other animals as well. The behaviour of animals in response to sudden environmental changes can be studied using the same model discussed.

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