Effects of temperature on growth date, weight, and length of mutants of *Drosophila melanogaster*

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Abstract

The fruit fly, *Drosophila melanogaster*, was chosen due to its small size, short life cycle, significant genetic variability, and relatively inexpensive to propagate. Little is known about the effects of temperature on the development of fruit fly mutants, including scarlet eye, vestigial wing, white eye, yellow body, and wild-type strains. This study aimed to explore the effects on dry weight and body length among those five strains at two different temperatures, 20°C and 25°C. The data was analyzed using the t-test, ANOVA, and Tukey HSD test. The observation records showed that temperature did affect the number of days in each growth period for all five strains. The high temperature (25°C) shortened those periods. The low temperature (20°C) prolonged those periods. The ANOVA and Tukey DSH test found the difference between the dry weight and body length pairs among those five strains at 20ºC and 25ºC. The t-test results demonstrate the difference in dry weight and body length between the two temperature settings in females and males of each strain.

Introduction

Drosophila melanogaster, known as the fruit fly, is a model organism because of the abundance of genetic variations. Scarlet eyes were bright vermilion, darkening with age. It was a reliable trait for classification (Beadle & Ephrussi, 1936). The vestigial locus seems to be mainly involved in developing the wing margin. The mutants are recessive viable (with or without a visible phenotype), recessive lethal, or dominant (with a visible phenotype over the wild type or a *vg* allele); some alleles complement each other; others show pleiotropic effects or homeosis (Bownes & Roberts, 1981). The white locus is involved in the production and distribution of ommochrome (brown) and pteridine (red) pigments found in the compound eyes and ocelli of adult flies, as well as the pigments in adult testis sheaths and larval Malpighian tubules (Sullivan & Sullivan, 1975). The yellow locus controlled the melanotic pigment pattern of the cuticle of the adult fly. It showed a mosaic pigment pattern, some regions of the cuticle being wild-type and others yellow (Nash & Yarkin, 1974).

Temperature is an influential environmental factor for fostering *Drosophila melanogaster*. Kim et al. (2020) demonstrated how environmental temperature and macronutrient balance combine to affect key life-history fitness traits and mediate trade-offs among these traits. Rocco et al. (2022) found that temperature had mixed impacts on weight and length in females and males of the wild-type strain, apterous and bar eye mutants. The interactions between temperature and strain were preliminarily detected.

Little is known about the impacts of temperature on the development of different fly mutants. Wild type strain and four mutants, i.e., scarlet eyes, vestigial wing, white eye, and yellow body, were chosen in the study. The objectives were (1) to identify differences in growth dates at the stages of the first egg, larva, pupa, and adult fly at two different temperatures and (2) to examine the effects of temperature on the weight and length of adult flies among those mutants.

Materials and Methods

Fruit fly strain

The wild-type and mutant strains, including scarlet eye, vestigial wing, white eye, and yellow body, were purchased from Carolina Biological Supply Company (Carolina 2022).

Fruit fly handling

Flies were maintained in spongecaped plastic vials containing roughly one inch of culture media and yeast cells. The end of a wand was soaked with FlyNap (an anesthesia agent) and inserted into the vials containing the flies without allowing any to escape. The flies were monitored to determine when the FlyNap should be removed from the vial once fully anesthetized. The process of anesthetizing the flies took around 2 minutes. Caution was taken to avoid overexposing flies to FlyNap, which is lethal in excessive dosage.

Propagation of Flies

After the flies were fully anesthetized, the vial cap was removed, and the flies were transferred onto a white card. They were then

placed under a dissecting microscope to identify sexual features. Once the sex of each fly was determined, five males and five females were placed into a vial containing culture media. The vial was placed on its side to ensure the flies were not stuck to the culture medium. After the flies recuperated from the FlyNap, the vials were turned upright. In 4 days, the parental generation of flies were removed from the vial. The larvae developed into mature fruit flies within 10-20 days. Upon the emergence of the next generation, adult fruit flies were ready to be counted and measured under a dissecting microscope.

The first emergency of pupa and adult fly

The first pupa and adult fly emergence dates were recorded for the three strains.

Adult fly collection

The adult flies were placed on a standard commercial diet in a vial and allowed to mate and oviposit. After eggs were observed, parental flies were discarded. Adult flies were collected from each vial at the defined time.

Temperature treatments

Two different temperature settings in the experiment were low temperature (20°C) and medium temperature (25°C). In each treatment, there were three repeats for all measurements.

Adult fly size measurements

Pupa were collected, and size measurements were taken at the indicated time. Subsequently, each emerged adult specimen was measured using a dissection microscope camera image. The length and width of each specimen were measured using Motic Images Plus 3.0 software. Three biologically independent repeat measurements were taken for three flies per vial.

Wet and dry weight measurements for adult fly

The wet weight was determined by transferring the adult flies into pre-weighed 1.5 mL tubes and weighing them on an analytical scale. The flies were dried at 60°C with the tube lids open in an oven. After 24 hours, measurements were taken to determine the weight (dry weight). We performed three biologically independent repeats and measured all flies per vial. Single fly dry weight was calculated by dividing the flies' total weight by the number of flies in a vial.

Statistical analysis

The variance analysis (ANOVA) was performed using Data Analysis in Excel (Brase, 2023). The posthoc Tukey Honestly Significant Difference (HSD), an online test calculator, conducted the test to compare multiple treatments (Vasavada, 2016).

Results

The stain phenotypes

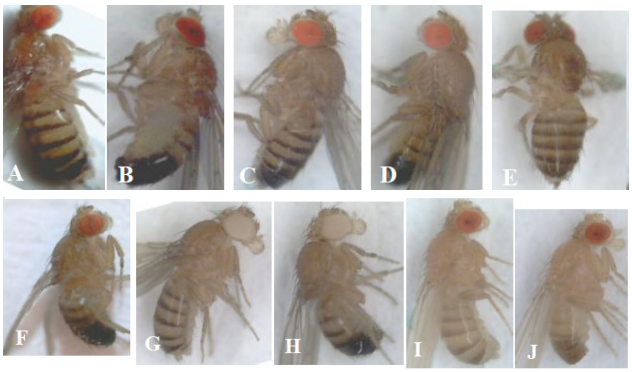

Figure 1 Phenotypes of the tested strains. A wild type female, B wild type male, C scarlet eye female, D scarlet eye male, E vestigial wing female, F vestigial wing male, G white eye female, H white eye male, I yellow body female, J yellow body male.

In Figure 1, we can observe all the phenotypes of those strains in this study.

The difference in the growth days for the strains at two temperatures

Table 1 The growth days recorded from the mating dates at two temperatures

Temperature	Strain	Egg	Larva	Pupa	Adult
20ºC	Wild type	2	6	8	21
	Scarlet eye	3	6	7	10
	Vestigial wing	2	6	8	11
	White-eye	4	8	9	14
	Yellow body	3	7	9	14
25ºC	Wild type	1	3	6	9
	Scarlet eye	1	2	5	12
	Vestigial wing	1	2	6	13
	White-eye	2	3	6	7
	Yellow body	1	2	9	10

Table 1 shows that a growth period at 25ºC is shorter than that at 20ºC for almost all strains. For the egg stage, all strains laid eggs at 25**ºC** faster than that at 20**ºC**. A shorter period at 25ºC was observed for other stages than at 20**ºC**. In summary, temperature obviously impacted the number of days at each stage.

One-way ANOVA for two traits at two temperature

Table 2 One-way ANOVA for weight and length at two temperatures

Temperature	Sex	Strain	Dry weight per fly (μg)	F	Length (μm)	F
20ºC	Female	Wild type	275.6	1.99	2251	11.67*
		Scarlet eye	319.7		2258	
		Vestigial wing	253.3		2195	
		White-eye	266.7		2395	
		Yellow body	306.8		2419	
	Male	Wild type	215.5	11.10*	1948	1.37
		Scarlet eye	260.4		1983	
		Vestigial wing	121.7		2016	
		White-eye	141.7		2048	
		Yellow body	165.6		1913	
25ºC	Female	Wild type	268.5	11.17*	2054	8.63*
		Scarlet eye	203.3		2405	
		Vestigial wing	145.0		2098	
		White-eye	188.0		2238	
		Yellow body	160.0		2247	
	Male	Wild type	169.5	30.38*	1791	9.85*
		Scarlet eye	136.4		2103	
		Vestigial wing	68.4		1832	
		White-eye	67.8		1760	
		Yellow body	111.6		1941	

Note: F critical value for both traits was 3.48 at a 5% significant level.

According to F tests in ANOVA (Table 2), there was a significant difference in dry weight among those strains for females at 25ºC and males at both temperatures. However, it was found that a significant difference in length for females at both temperatures and for males at 20**ºC** was present among those strains. This suggested that one or more strains for both traits were significantly different. The Tukey HSD test for multiple comparisons followed.

Table 3 Tukey HSD tests for weight and length at two temperatures

Temperature	Sex	Strain's pair	Q for dry weight per fly	Q for length
20ºC	Female	Wild/Scarlet	2.23	0.24
		Wild/Vestigial	1.13	1.97
		Wild/White	0.45	5.02*
		Wild/Yellow	1.58	5.86*
		Scarlet/Vestigial	3.36	2.21
		Scarlet/White	2.68	4.77*
		Scarlet/Yellow	0.66	5.62*
		Vestigial/White	0.67	6.99**
		Vestigial/Yellow	2.70	7.83**
		White/Yellow	2.03	0.84
	Male	Wild/Scarlet	2.65	0.78
		Wild/Vestigial	5.52*	1.49
		Wild/White	4.35	2.19
		Wild/Yellow	2.94	0.77
		Scarlet/Vestigial	8.17**	0.71
		Scarlet/White	6.99**	1.42
		Scarlet/Yellow	5.58*	1.54
		Vestigial/White	1.18	0.70
		Vestigial/Yellow	2.59	2.25
		White/Yellow	1.41	2.96
25ºC	Female	Wild/Scarlet	4.54	7.43**
		Wild/Vestigial	8.59**	0.93
		Wild/White	5.60*	3.90
		Wild/Yellow	7.55**	4.09
		Scarlet/Vestigial	4.06	6.49**
		Scarlet/White	1.07	3.53
		Scarlet/Yellow	3.02	3.33
		Vestigial/White	2.99	2.97
		Vestigial/Yellow	1.04	3.16
		White/Yellow	1.95	0.19
	Male	Wild/Scarlet	4.15	7.01**
		Wild/Vestigial	12.66**	0.91
		Wild/White	12.74**	0.70
		Wild/Yellow	7.25**	3.36
		Scarlet/Vestigial	8.51**	6.10**
		Scarlet/White	8.59**	7.71**
		Scarlet/Yellow	3.11	3.65
		Vestigial/White	0.08	1.61
		Vestigial/Yellow	5.41*	2.46
		White/Yellow	5.49*	4.07

Note: * 5% significant level, ** 1% significant level.

In Table 3, we can see a difference in a variety of pairs at a 5% significant level based on the results of the Tukey HSD test. For dry weight at 20ºC in males, the pairs were wild/vestigial, scarlet/vestigial, and scarlet/white. For length at 20ºC in females, there were six pairs. For dry weight at 25ºC in females, wild/vestigial, wild/white, and wild/yellow were found. For length at 25ºC in females, they were wild/scarlet and scarlet/vestigial. For dry weight at 25ºC in males, seven pairs were present. For length at 25ºC in males, they were wild/scarlet, scarlet/vestigial, and scarlet/white.

The t-test for two traits at two temperature

Table 4 The t-tests for weight and length between 20ºC and 25ºC

Sex	Strain	t for dry weight per fly	t for length
Female	Wild type	0.22	2.53
	Scarlet eye	6.75**	-3.73*
	Vestigial wing	3.94*	2.99*
	White-eye	10.88**	5.11**
	Yellow body	5.26**	2.29
Male	Wild type	2.58	1.92
	Scarlet eye	4.24*	-1.52
	Vestigial wing	4.38*	4.31**
	White-eye	6.48**	5.73**
	Yellow body	3.08*	-0.49

Note: t critical value of two-tail for both traits was 2.78 and df=4. *: significant at 5%. **: significant at 1%.

In Table 4, the results of the t-tests revealed that the dry weight at 20ºC was higher than that at 25ºC for both females and males in five strains at a 5% significant level. It is almost the same in length except that the length at 20ºC for scarlet eye strain was shorter than that at 25ºC. It seemed that the more weight and length gained at low temperatures.

Discussion

Temperature impacted the number of days in each growth period for all five strains. The high temperature shortened those periods. The ANOVA and Tukey DSH test results led to finding the difference between the dry weight and body length pairs among those five strains at 20ºC and 25ºC. The t-test results detected the difference in dry weight and body length between the two temperature settings in females and males of each strain. Future studies for other environmental factors such as pH, disinfectant, humidity, photoperiod, and microbes on fruit flies would expand the knowledge in this regard.

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