Quest Journals Journal of Research in Agriculture and Animal Science Volume 4 ~ Issue 2 (2016) pp: 11-19 ISSN(Online) : 2321-9459 www.questjournals.org

**Research Paper** 



# Fecundity and the Development Time of *D. melanogaster* of *wild-type, white,* and *ebony* strains for Several Generations in High Temperature Environment

Ika Sukmawati<sup>1</sup>, Aloysius Duran Corebima<sup>2\*</sup>, Siti Zubaidah<sup>2</sup>

<sup>1)</sup> Graduate Student Of Biology Education, State University Of Malang <sup>2)</sup> Department Of Biology, State University Of Malang

**R**eceived 09 August, 2016; **A**ccepted 13 August, 2016 © The author(s) 2014. **P**ublished with open access at <u>www.questjournals.org</u>

**ABSTRACT :** Exposure to high temperatures in a short or long term has been reported having potency to affect various aspects of the life of D. melanogaster, including the fecundity and the development time. In order to survive in environments with changing temperatures, insects perform thermoregulatory mechanisms which are essential to ensure their survival and reproduction. This research aims to reveal the effect of high temperature exposure (30° C) on the fecundity and the development time of D. melanogaster of wild-type, white, and ebony strains for several generations. The results of the research showed that only wild-type strains could grow for five generations, whereas the two mutants had lower success. High temperatures had a significant effect on the fecundity and the development time. Higher fecundity and more rapid development time occur in normal temperature environments. Meanwhile, the types of strains do not have significant effect on the fecundity, neither does the interaction between the environment temperature and the types of strain. Further description related to the results of the research indicate that generally the endurance of the wild-type strain in the extreme heat temperatures was better than the white and ebony mutants. Therefore, it is recommended that the breeding of D. melanogaster related to the mutant strains pays attention of the temperature settings of the breeding environment.

Keywords: Drosophila melanogaster, fecundity, environment temperature, strain, development time

### I. INTRODUCTION

The environment temperature is one of the abiotic factors having a major role in the life of insects, including *D. melanogaster*. The environment temperature is associated with the species abundance and the distribution of insects, as well as also plays a role as a selecting factor of genetic variation in its natural population [1-2]. Related to this, a diversity hotspot of insects had been found in tropical regions, and the closer it got to the polar region, the fewer number of the insect species was [3].

*D. melanogaster* has certain temperature preferences to survive. The highest temperature tolerance level of *D. melanogaster* constantly exposed is reported at  $30^{\circ}$  C [4]. The other research results showed that exposure to a temperature of  $29^{\circ}$  C is already stressful for *D. melanogaster* [5-9]. However, exposure to more extreme temperatures can give a more negative effect on the affected characters [10]. Thus, exposure to a temperature of  $30^{\circ}$  C will show a more significant effect.

The high temperatures continuously given can cause various effects on the characteristics of the *D*. *melanogaster*. Some of the characteristics are associated with the life history and the fitness determinants. These characteristics include the number of offspring, the development time, and the patterns of activity [3].

Fecundity is one of the main determinants of female fitness of *D. melanogaster* [11-12]. Fecundity is interpreted by some researchers as the number of eggs produced by female individuals that survive to hatch in the adult phase [13-15]. Fecundity of *D. melanogaster* can be determined by internal and external factors. Internal factors determining the fecundity include genetic factors that regulate fecundity [12], the presence of mutations [14], the age of the females [16], body size [17], and infections of microorganisms [13]. Meanwhile, external factors affecting fecundity include long copulation and the environment temperature [13, 18].

D. melanogaster develops through four stages, which include stage of egg, larva, pupa, and adult [4]. The time required to complete the all four stages of the life cycle is called as the development time. The

E-mail: durancorebima@gmail.com

development time could vary up to a few days because of the differences in environment temperature [19]. In an environment at  $25^{\circ}$  C, the development time of *D. melanogaster* is about 10 days. Meanwhile, at the high temperature environment, the development time may occur more rapidly, for about 9 days [10]

Fecundity and development time can be observed for several generations to know the trend of adaptation to constant extreme environmental temperature. Previous studies reported that there was an indication of adaptation to temperature [6,10,20]. However, even at a constant simple temperature environment, the patterns of adaptation is very complex.

Thermal adaptation is a very interesting topic among some groups of living organisms especially those of exotherm one whose body temperature strictly follows the temperature of the surrounding environment [21]. Thermal adaptation of *D. melanogaster* is important to ensure the survival and reproductive success [22]. A population is more suitable within their environment by hereditary changes that pass from generation to generation. Thus, a research that reveals a declining number of offspring and the development time of several generations of *D. melanogaster* in high temperature environment can be conducted.

As one of the genetic model organisms, *D. melanogaster* has many mutant strains in addition to the *wild-type* strain. Two examples of mutant *D. melanogaster* are *white* and *ebony* strains. *white* strain of *D. melanogaster* has mutations on the X chromosome at locus 1.5 that affects the formation of pigment in the eye, so that morphologically it has a white eye. *ebony* strain of *D. melanogaster* has mutations on chromosome 3 at locus 70.7. Morphologically, ebony strain cuticle has pigmentation darker than that of the *wild-type*. The morphological conditions of these strains are stable, not easily changed by the influence of environmental temperature [19].

Although the mutations that occur in both of these strains are associated with pigmentation, a number of reports reveal that there are other characteristics that are affected by the mutation. The characteristics are also associated with fecundity and development time. The survival of embryos and larvae of *ebony* strain was significantly different and lower than that of the *wild-type* [23]. The ability to survive of embryos and larvae may affect the number of offspring that develop into adulthood. Furthermore, a report stated that wild-type (Oregon), white, ebony and sepia strains of D. melanogaster had different mating capabilities [24]. The difference in mating ability can lead to differences in the number of offspring produced. Based on these facts, the trend of fecundity value and development time of *wild-type*, white, and ebony strains of D. melanogaster can be compared among several generations, especially when exposed to high environment temperatures.

The information obtained from this research is expected to be useful for the care of *D. melanogaster*. So far, information about high temperature tolerance in Drosophila related to its care is still general and not specific to each strain. These study results related to the tolerance level of *wild-type*, *white*, and *ebony* strains in high temperature environment at several generations regarding to the fecundity and development time can be useful information to determine the most appropriate environment temperature for each strain.

### II. RESEARCH METHODS

One group of *D. melanogaster* crossbred was exposed continuously to a constant temperature of 30° C for five generations in an incubator. Meanwhile, one group of *D. melanogaster* was crossbred at normal (dayly) temperatures fluctuating naturally following the condition of the surrounding temperature (25-30° C) as a control. The type of crossbreeding performed was similar to the crossbreeding in the treatment group. The data measured consisted of fecundity and development time. The fecundity was measured by counting the number of offspring on each crossbreeding. The development time was determined by the time required by *D. melanogaster* to develop from egg to adult phase expressed in days.

Based on the number of the independent variables involved in this research, the suitable data analysis technique was the analysis of variants of three ways. The first variable was the environment temperature, the second variable was the type of *D. melanogaster* strain, and the third variable was the generation of the crossbredding. However, because the data related to the generation successfully obtained from each strain was not similar, so the data analysis performed was the analysis of variance of two ways including the environment temperature and the strains. The analysis of variance of two ways was done by the program IBM SPSS Statistics Version 22:00 with a significance level of 0.05. The fecundity and development time of every generation of the crossbreeding were analyzed descriptively. The description was done by describing the success of every strain crossbreeding and its ability to thrive in the next generation.

### III. RESEARCH RESULTS

### 3.1 Description Of The Results Of The Crossbreeding

Based on the crossbreeding that had been done at high temperature environment, only the crossbreeding of the *wild-type* strain could survive up to five generations. Among the three strains crossbred, the crossbred of the *wild-type* showed a higher success; six from 10 crossbreeding could produce offspring up to second generation (the success of a crossbreeding to the second generation was 60%). The success of the crossbreeding decreased to 50% in the third generation. Meanwhile, only 30% of the total crossbreeding survived and could develop until five generations.

The crossbreeding of *white* as well as of *ebony* strain had a lower success than that of the *wild-type*. Based on the 19 crossbreeding carried out related to the *white* 3 > 9 *white*, only three crossbreeding could produce offspring. Thus, the success rate of the *white* strain crossbreeding was 16%. Only one crossbreeding of the three crossbreeding of the first generation could produce offspring (second generation). In other words, the success of the second generation crossbreeding declined to 5.2% of the original crossbreeding. However, the crossbreeding did not successfully develop in the third generation. When compared to the *wild-type*, the decrease of the crossbreeding success of *white* strain had occurred since the second generation, and it could not be sustained into the third generation.

In the crossbreeding of  $\delta ebony < \varphi ebony$ , only three out of the 21 crossbreedings could produce offspring; so only 14% of the crossbreedings could develop. Among the three crossbreedings only one crossbreeding could produce offspring. Thus, the crossbreeding success in the second generation decreased to 4.7%. Like the *white* strain, the *ebony* crossbreeding did not successfully develop into the third generation. If compared with the *white* and *wild-type* strain, the crossbreeding of the *ebony* strain at high temperature environment (30°C) had the lowest success. Fig. 1 shows the success percentage of the crosses performed related to all the three strains.

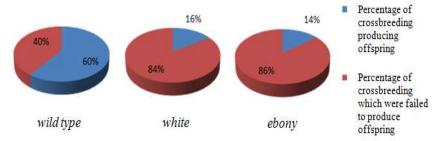


Figure 1. Results of D. melanogaster crossbreeding on high-temperature environment

Some crossbreeding which were failed to produce offspring produced eggs, but some others did not produce eggs. Related to the crossbreedings that have produced the eggs, the eggs did not hatch and failed to develop into larvae. The failure of the eggs to hatch producing larvae made this cross not able to produce mature offspring that could be used to continue the next crossbreeding. On the crossbreedings that successfully produced offspring, the calculation of the number of the offspring which reflect the fecundity and the development time measurement were carried out. Fig. 2 and 3 shows the comparison of the offspring number and the development time of *wild-type*, *white* and *ebony* strains in the normal environment temperature and in 30 ° C.

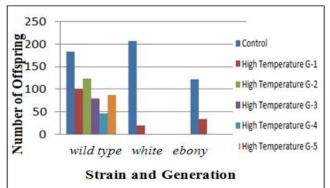


Figure 2. The offspring number mean of several generations of D. melanogaster in normal and high temperature

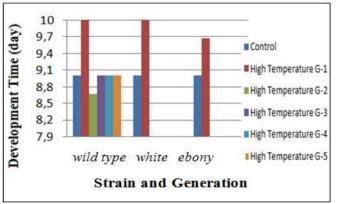


Figure 3. The development time mean of several generations of *D. melanogaster* in normal and high temperature

Related to the crossbreeding results of the *wild-type* from the first until the fifth generation, it appears that at  $30^{\circ}$  C environment temperature the offspring mean number of each generation tends to decrease. The greatest decrease of the offspring number occurred on the fourth generation, with a mean of 45.33 individuals. In the fifth generation, the number of the offspring began to increase with the mean number of 86 individuals. These results show a pattern, that is, the *D. melanogaster* began to adapt to the environment temperature of  $30^{\circ}$  C. The development time data show a pattern that appears to be in line with data of the offspring number. In the first generation, the development time mean needed was quite long (10 days mean), but in the second generation it became faster (8.6 days mean), and in the third up to the fifth generation, it was likely to be similar to that of the control time (9 days mean).

### 3.2 Total Number of *D. melanogaster* Offspring, Strains of *wild-type*, *white*, and *ebony* in different Environment Temperatures

The effect of the environmental temperature and the kinds of strain on the number of the *D*. *melanogaster* offspring of the first generation had been analyzed using two-way anova. The results of the analysis are presented in Table 1. Based on the results of the analysis, the kinds of strains of *D*. *melanogaster* crossbreeding did not have any significant effect on the number of offspring (p = 0.067 > 0.05). The environment temperature had a very significant effect on the number of offspring of *D*. *melanogaster* crossbreeding, (p=0.000 < 0.05). The mean number of offspring of *D*. *melanogaster* crossbreeding at normal temperature environments was 169.89, while the mean number of offspring at high temperatures was 50.67. Based on the mean comparison, the offspring number of *D*. *melanogaster* at high temperatures decreased by 70.17%. Meanwhile, the interaction between the kinds of strain and environment temperature did not have a significant effect on the offspring number of *D*. *melanogaster* (p = 0.095 > 0.05).

Although the results of the data analysis show that the interaction between the kinds of strains and the environment temperature is not significant, based on the results of the LSD post hoc test on the treatment combination it is seen that in the environments with normal temperature, the *white* strain has the highest mean number of offspring which is significantly different from that of *ebony* strain, but it is not significantly different from that of *white* strain offspring at high environment temperature was the lowest of all, and it was significantly different from that of the *wild-type* strain, but it was not significantly different from that of *ebony* strain the of the *wild-type* strain, but it was not significantly different from that of *ebony* strain. The results of LSD test related to the interaction between the types of strain and the environment temperature can be seen in Table 2.

source	Type III Sum of df		mean Square	F	Sig.
	Squares		1		U
corrected Model	86534.278ª	5	17306.856	9664	.001
intercept	218901.389	1	218901.389	122 227	.000
strain	12219.111	2	6109.556	3,411	.067
Temperature	63962.722	1	63962.722	35,715	.000
Strain * Temperature	10352.444	2	5176.222	2,890	.095
Error	21491.333	12	1790.944		
Total	326927.000	18			
corrected Total	108025.611	17			
a.R Squared =, 801 (Adjusted)	R Squared =, 718)				

**Table 1.** Results of the two Way Anova on the Data of the Offspring Number

and E	invironment Temperature on the	ie Offspring Number of t	he First Generation
No.	Combination Groups	Mean	Notation of LSD
1	white 30°C	19.333	a
2	ebony 30°C	33,333	a b
3	wild-type 30°C	99.333	b c
4	ebony control	121.000	c d
5	wild-type control	182.333	d e
6	white control	206.333	e

**Table 2.** The Results of LSD Test related to the Interaction Effect between the Types of *D. melanogaster* Strain and Environment Temperature on the Offspring Number of the First Generation

## 3.3 The Development Time of *D. melanogaster* Strains of *wild-type*, *white*, and *ebony* in different Environment Temperatures

The effect of environment temperature and the types of strain on the *D. melanogaster* development time of the first generation based on the results of two-way anova can be seen in Table 3. Based on the results of the analysis, the kinds of strain did not have significant effect on the development time of *D. melanogaster* (p = 0.397 > 0.05). The environment temperature had a very significant effect on the development time (p = 0.000 < 0.05). The mean of the development time in the normal temperature environment was for 9 days, while the mean of the development time in the hot temperature environments was for 9.889 days. Based on the comparison of the mean, there was retardation of development time in hot temperature environments as much as 8.98%. Meanwhile, the interaction between the kinds of strain and environment temperature did not have any significant effect on the development time of *D. melanogaster* (p = 0.397 > 0.05).

Based on the LSD test (Table 3), at the environment temperature of  $30^{\circ}$  C, the development time of all the three *D. melanogaster* strains was longer and significantly different from that of those strains in the normal environment temperature. Both at normal as well as at high environment temperature, the development time of the three strains was not significantly different.

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	3.778 <sup>a</sup>	5	.756	13.600	.000
Intercept	1605.556	1	1605.556	2.890E4	.000
Strain	.111	2	.056	1,000	.397
Temperature	3.556	1	3.556	64,000	.000
Strain * Temperature	.111	2	.056	1,000	.397
Error	.667	12	.056		
Total	1610.000	18			
Corrected Total	4.444	17			
a.R Squared =, 850 (Adjusted R Squared =, 788)					

Table 3. The Results of the Data Analysis of two-way Anova related to the Development Time

**Table 3.** The Results of LSD Test related to the Interaction of the Type of *D. melanogaster* Strain and the Environment Temperature on the Development Time of the First Generation

r	a di di a			
No	Combination Groups	Mean	LSD notation	
1	white control	9.00	а	
2	ebony control	9.00	а	
3	wild-type control	9.00	а	
4	ebony 30 ° C	9.67	b	
5	wild-type 30 ° C	10,00	b	
6	white 30 ° C	10,00	b	

### IV. DISCUSSION

The results of this research showed that not all the crossbreeding carried out at the environment temperature of 30°C could develop. The most successful crossbreeding was related to *the wild-type* strain, followed by *white* and *ebony* strains. Thus, at 30°C environment temperature, *wild-type* strain have better endurance than the other two mutant strains used. The crosses performed on *wild-type* strain could survive up to five generations. The results also showed a decline in the percentage of successful crossbreeding in the third generation, which continued to decrease until the fifth generation. The decline in the percentage of successful crossbreeding along with the increasing levels of generation was also observed in *white* and *ebony* strains. However, in the second generation, the crossbreeding success of both of the two mutant strains had already undergone a decrease compared to *wild-type* strain.

The level differences of crossbreeding success in every generation showed a different response of *wild-type, white,* and *ebony* strains related to the environmental stress such as high temperatures. Different genotypes might respond differently to the same environmental stress, and the reaction was the key determining the resilience of the individual in the environment [25]. In this case, the response of the wild type strain is more likely better to survive in unfavorable environmental conditions than the other two mutant strains. Males having

a more healthy phenotype tended to have greater mating success than the other males having unhealthy phenotype. In this case, the *wild-type* strain had better and "healthier" phenotype than the *white* and *ebony* mutant strains.

In a normal environmental condition, the research conducted by Singh [26] using the method of random mating on every generation showed that the frequency of mutant genotype of *ruby* eye color totally disappeared from the population of *D. ananassae* at the ninth generation. Thus, it was found that the *wild-type* could adjust to the environment better than those mutant individuals related to the eye color. In this research, the mating or the breeding (inbreeding) was not randomly carried out, and these conditions did not seem favorable to be carried out in a high temperature environment. Sexual selection can influence the fitness of a population. This statement was supported by Pedersen et al. [9] saying that inbreeding carried out under conditions of stress exposed to high temperatures could cause a decrease in the fitness components (inbreeding depression), including the sterility in males.

The crossbreeding success of *wild-type*, *white*, and *ebony* strains gradually declining in every generation indicates that the mating is not always successful in the next generation although it was successful in previous generations. Thus, although *D. melanogaster* has successfully adapted in their previous generations and successfully produced offspring, it did not seem to guarantee the same mating success in the next generation. This is consistent with the statement of Correia et al. [27] saying that local adaptation of *D. melanogaster* strain at low temperatures or high temperatures was not always able to predict a high success of mating; local adaptation and mating success did not show a significant correlation.

The success decline of crossbreeding at a high temperature environment was probably correlated with the survival cost of reproduction, namely trade-off or selecting between longevity and reproduction rates. The study conducted by Flatt [28] on a large number of researches related to *D. melanogaster* and *C. elegans* showed that there was a negative correlation between mating success and lengevity of life when an individual was exposed to stress conditions, including environment temperature. Survival and longevity of life in high temperature environment is correlated with the decrease in the reproductive aspects such as fertility and mating ability.

The results of the research showed that the ebony strain had the lowest crossbreeding success compared to the *wild-type* and *white* strains. The lowest crossbreeding success of the *ebony* strain is correlated with the mutations experienced. Beside it affect the formation of cuticle pigment [19], the mutation related also affect the formation of neurotransmitter and the regulation of the dopamine and N- $\beta$ -alanyldopamine levels (NBAD) in the body [23]. This is caused by the formation of *ebony* protein (NBAD-synthase) due to the mutation that occur [29].

Furthermore, it is stated that any disturbance on the formation of these components can cause higher mortality rates in embryos and larvae, or failure of eclosion [23]. The statement may answer the question of the existence of eggs that did not hatch found in 86% of crossbreeding related to *ebony* strain. The intensity of pigmentation in Drosophila is also correlated with thermoregulation [30]. Furthermore, it is stated that some populations of flies having darker colored body is found in the area with cooler temperature.

In addition, the inability of the eggs to hatch is also concerned with the effect of temperature stress exposed. The research conducted by Deshmukh et al. [31] showed that exposure of *D. melanogaster* embryo to high temperatures led to an increase in cytosine methylation. Although the real role of DNA methylation in Drosophila is quite unclear, if there is a change of chromatin structure, physiological changes may occur as a result of the epigenetic modification. Meanwhile, related to the crossbreeding that did not produce eggs, it was probably because the mating between Drosophila did not actually occur. A mutant with low levels of neurotransmitters such as *ebony* had low fertility and mating ability, as well as impaired development and viability [32].

The results of two-way Anova on the number of offspring show that kinds of strain do not have any effect on the number of offspring of *D.melanogaster*. Based on the analysis result, it is clearly seen that the numbers of offspring produced by the *wild-type*, *white*, and *ebony* strains are not different to each other.

The environment temperature had a very significant effect on the number of offspring that could be produced. Several issues related to the high temperature exposed may explain these results. One of the issues was the disturbance of maturation of eggs at high temperature environment. The maturation of insect eggs had a dependency on temperature, so did the laying of eggs by females which concerned with a certain temperature range [17]. On a hot temperature environment, damage can occur on a growing individual, associated with the protein denaturation, changes in phase transitions, as well as changes in membrane potential [33]. The damage can stop the development of eggs, and die before it can develop into adults.

A decrease of fecundity in females as well as of the mating ability in males could also explain the decrease in the number of offspring of *D. melanogaster* in high temperature environment. Short-term exposure to high temperatures could only have an effect on *fitness* component of *D. melanogaster*, which included a decrease in the frequency of mating and female fecundity [34]. In this research, *D. melanogaster* of the

treatment group was kept in hot temperature environment from egg to adult phase; mating activities was carried out to in the hot temperature environment. In other words, the exposure to thermal stress was applied in the long term. Huey et al. [11] suggested that the level of fecundity of female Drosophila can be affected by temperature since the beginning of its development, from eggs laid by the parent. Females growing in extreme temperatures usually have lower fecundity because of the low level of oogenesis. The low levels of oogenesis are affected by *heat shock* gene activity related to reproduction and fecundity, namely *Hsp83* playing an important role in the process of oogenesis and spermatogenesis. *Hsp83* gene organizes the oogenesis molecular pathways through the proteins it produces. In addition, *Hsp83* RNA is a component of posterior polar plasm. The decrease in the levels of spermatogenesis may also result in reduced ability of mating or even sterility in male [4,9].

However, based on data obtained from the five generations of *wild-type* strain on the environment with the temperature of  $30^{\circ}$  C, the adjustments or adaptations was observable indicated by the increase in the number of offspring in the fifth generation. Thermal adaptation related to the number of offspring was conducted by *D. melanogaster* in a less favorable environment, as proposed by Dillon et al. [10]. While, Correia et al. [27] used *D. melanogaster* strain which had been successfully adapted to survive in extreme temperatures for about two years in their research. Due to the time limitation of the research, the adaptation related to the number of offspring that occurs in *D. melanogaster* of *wild-type* strain could be observed in the fifth generation, but unfortunately the following patterns could not be predicted in successive generations.

The results of the analysis also showed that the interaction between the kinds of strain and temperature did not have any significant effect on the number of offspring. Nevertheless, the results of LSD test in order to uncover the position of combination group related showed that at the environment with temperature of  $30^{\circ}$ C, the *wild-type* strain had the highest number of offspring, and it was significantly different from that of the *white* strain, even though the mean was not significantly different from that of the *ebony* strain.

The analysis of the development time showed a similar result with the number of offspring, that was, the kinds of strain did not have any significant effect on the development time of *D. melanogaster*. Thus, each strain crossbred, *wild-type, white*, and *ebony* strain, generally requires the same time to develop since the lying of eggs until the hatching of the first adult individuals. The mean of the development time of the *D. melanogaster* strain was about 10 days. The embryonic stage lasted for 1 day, and larval stages of instar 1 and 2 lasted for 1 day each. Larval stage of instar 3 lasted for 3 days. Pupa stage lasted for 4 days, and then the last was the adult stage [19]. However, those development times occured at room temperature of  $25^{\circ}$ C. The development time may change due to the environmental temperature changes.

The results of the analysis showed that the environment temperature had a very significant effect on the development time. Thus, the environment temperature can affect the pre-programmed time by individuals of *D. melanogaster*. As a character expressed, developmental time surely has a basic genetic control. The controlling of development time has taken place since the stage of embryonic development [35], as well as related to how quickly the aging comes and the longevity of an individual [36]. However, in line with the statement of Allis et al. [37], the suitable environmental stimuli can reprogram the development of organisms.

In this research, a temperature of 30°C exposed to *D. melanogaster* caused the development to become 8.89% slower, compared with that of *D. melanogaster* in the control group. These results appeared to be contradictive to the results of several researches, such as those conducted by Dillon et al. [10] and Paaby and Schmidt [36]. Generally, slower development will occur in *D. melanogaster* exposed to cold temperatures [4]. The different results were probably due to the limited number of repetitions, the incomplete generation data so that the tendency of the development time was not observed, or due to the recording of the development time which was expressed in units of days, so that it was not quite accurate.

Related to *wild-type* strain that could to produce the fifth-generation offspring at high temperatures, it appeared that the mean of the development time of the first generation became 1 day longer compared to that of the *wild-type* in normal temperature. However, on the next generation until the fifth generation, there was an acceleration of the development time, which was equal to that under normal conditions. The acceleration can occur as an adaptation mechanism and thermal regulation in an unfavorable environment [6,10,20].

Similar to the number of offspring, the interaction between types of strain and environment temperature did not have any significant effect on the development time. However, the results of LSD test related to combination of these two factors revealed more information. At high temperature environment, the highest mean of the development time was of the *wild-type* and *white* (10 days) strains, and the lowest was of the *ebony* strain (9.67 days). At 30°C temperature environment, the development time of the three strains was longer and significantly different from the development time of all three strains at normal temperature, which was 9 days.

### V. CONCLUSION

The environment temperature had a very significant effect on the number of offspring and developmental time of *D. melanogaster*. The mean number of offspring of *D. melanogaster* at high temperature  $(30^{\circ} \text{ C})$  was 50.67 individuals. It experienced a decrease of 70.17% from the mean number of offspring in the

normal temperature environment as much as 169.89 individuals. The mean of the development time at high temperature environment was 9.889 days. It slowed 8.98% compared to the mean of the development time at normal temperature environment, 9 days. The types of strain and the interaction between the types of strain and environment temperature did not have a significant effect on the number of offspring and the development time of *D. melanogaster* crossed.

A pattern indicating an adaptation related to the number of offspring and the development time of wild type strain of *D. melanogaster* has been detected in the environment with temperature of  $30^{\circ}$ C. The decrease in the number of offspring of *wild-type* strain had occurred since the first generation up to the fourth generation, but it increased back in the fifth generation. The development time of the first generation became slower compared to the normal temperature environment, but the adaptation had been occured since the second generation up to five, similar to the development time of *D. melanogaster* under normal conditions.

Related to the crossbreeding success between generations, a decrease of the *wild-type* crossbreeding began to occur in the third generation, while related to the *white* and *ebony* strains, it had occurred since the second generation. The better durability of *wild-type* compared to that of the *white* and *ebony* strains at the high temperature appeared from the crossbreeding of *wild-type* strain that was capable of growing up to the fifth generation. Meanwhile, the success of crossbreeding of *ebony* strain was the lowest among all the strains investigated. Based on these results, it is suggested that the breeding of *white* and *ebony* strains. At normal temperature environment (25-30 °C), both the mutant strains can produce more offspring with a more rapid development.

#### REFERENCES

- J. Bouletreau-Merle and D. Sillans, Effects of Interaction Between Temperature and CO<sub>2</sub> on Life-History Traits of Two Drosophila Species (Diptera: Drosophilidae). Eur. J. Entomol. 93, 1996, 451-459.
- [2]. A. Ayrinhac, V. Debat, P. Gilbert, A. G. Kister, H. Legout, B. Moreteau, R. Vergilino, and J. R. David, Cold Adaptation in Geographical Populations of *Drosophila melanogaster*: Phenotypic Plasticity is More Important than Genetic Variability. Functional Ecology 18, 2004, 700-706.
- [3]. B. V. Feldmeyer, The Effect of Temperature on Sex Determination, PhD Thesis, University of Groningen, The Netherlands, 2009.
- [4]. M. Demerec and B. P. Kaufman, Drosophila Guide: Introduction to The Genetics and Cytology of Drosophila melanogaster, Tenth Edition (Washington D. C: Carnegie Institution of Washington, 1996)
- [5]. M. F. D. D'Avila, R. N. Garcia, E. L. Loreto, and V. L. da S. Valente, Analysis of Phenotypes Altered by Temperature Stress and Hypermutability in *Drosophila willistoni*. Iheringia, Sér. Zool., Porto Alegre, 98(3), 2008, 345-354.
- [6]. G. W. Gilchrist and R. B. Huey, Parental and Developmental Temperature Effects on The Thermal Dependence of Fitness in Drosophila melanogaster. Evolution 55 (1), 2010, 209-214.
- [7]. P. Sambucetti, A. C. Scannapieco, V. Loeschcke, and F. M. Norry, Heat-Stress Survival in The Pre-Adult Stage of The Life Cycle in an Intercontinental Set of Recombinant Inbred Lines of *Drosophila melanogaster*. The Journal of Experimental Biology 216, 2013, 2953-2959.
- [8]. J. Chen, V. Nolte, and C. Schlotterer, Temperature Related Reaction Norms of Gene Expression: Regulatory Architecture and Functional Implications, 2015, Online, http://mbe.oxfordjournals.org/, accessed on July 23<sup>rd</sup>, 2015.
- [9]. L. D. Pedersen, A. R. Pedersen, R. Bijlsma, and J. Bundgaard, The Effects of Inbreeding and Heat Stress on Male Strerility in Drosophila melanogaster. Biological Journal of the Linnean Society 104, 2011, 432-442.
- [10]. M. E. Dillon, L. R. Y. Cahn, and R. B. Huey, Life History Consequences of Temperature Transients in *Drosophila melanogaster*. The Journal of Experimental Biology 210, 2007, 2897-2904.
- [11]. R. B. Huey, T. Wakefield, W. D. Crill, and G. W. Gilchrist, Within-and Between-Generation Effects of Temperature on Early Fecundity of *Drosophila melanogaster*. Heredity 74,1995, 216-223.
- [12]. M. R. Rose and B. Charlesworth, Genetics of Life History in Drosophila melanogaster, I. Sib Analysis of Adult Females. Genetics 97, 1980, 173-186.
- [13]. B. P. Lazzaro, H. A. Flores, J. G. Lorigan, and C. P. Yourth, Genotype-by-Environment Interactions and Adaptation to Local Temperature Affect Immunity and Fecundity in *Drosophila melanogaster*. PLoS Pathog 4(3), 2008, e1000025.
- [14]. B. Chen and A. Wagner, Hsp90 is Important For Fecundity, Longevity, and Buffering of Cryptic Deleterious Variation in Wild Fly Populations. Evolutionary Biology 12, 2012, 12-25.
- [15]. J. F. S. Barker, Adult Population Density, Fecundity, and Productivity in Drosophila melanogaster and Drosophila simulans. Oecologia (Berl.) 11, 1972, 83-92.
- [16]. J. Leips, P. Gilligan, and T. F. C. Mackay, Quantitative Trait Loci with Age-Specific Effects on Fecundity in *Drosophila melanogaster*. Genetics, 2005, 105.048520.
- [17]. D. Berger, R. Walters, and K. Gotthard, What Limits Insect Fecundity? Body Size and Temperature-Dependent Egg Maturation and Oviposition in a Butterfly. Functional Ecology 22, 2008, 523-529.
- [18]. A. Lefranck and J. Bundgaard, The Influence of Male and Female Body Size on Copulation Duration and Fecundity in *Drosophila melanogaster*. Hereditas 132, 2000, 243-247.
- [19]. S. Chyb and N. Gompel, Atlas of Drosophila Morphology: Wild-type and Classical Mutants (London: Elsevier Inc., 2013).
- [20]. L. B. Huang, B. Chen, and L. Kang, Impact of Mild Temperature Hardening on Thermotolerance, Fecundity, and Hsp Gene Expression in Liriomyza huidobrensis. Journal of Insect Physiology 53, 2007, 1199–1205.
- [21]. B. J. Sinclair, Field Ecology of Freeze Tolerance: Inter Annual Variation in Cooling Rates, Freeze-thaw and Thermal Stress in the Microhabitat of the Alpine Cockroach *Celatoblatta quinquemaculata*. Oikos 93, 2001, 286–293.
- [22]. C. J. Austin and A. J. Moehring, Optimal Temperature Range of a Plastic Species: Drosophila simulans. Journal of Animal Ecology, 82, 2013, 663-672.
- [23]. G. Sabio, L. A. Quesada-Allue, and M. M. Perez, Embryo and Larval Survival in *Drosophila melanogaster* Pigmentation Mutants *tan* and *ebony*. Drosophila Information Service 93, 2010, 175-178.
- [24]. S. Cakir, and A. Kence, Lack of Minority Advantage in Drosophila melanogaster Mutants. Tr. J. of Biology 23, 1999, 433–443.

- [25]. G. A. Clark and C. D. Fucito, Stress Tolerance and Metabolic Response to Stress in *Drosophila melanogaster*. Heredity 81, 1998, 514-527.
- [26]. A. K. Singh, Elimination of Mutant Types in Selection Experiment Between Wild Type and Mutant Eye Colour in *Drosophila ananassae*. Journal of Scientific Research Banaras Hindu University, Varanasi 56, 2012, 73-79.
- [27]. L. Correia, S. Yeaman, and C. Whitlock, Local Adaptation Does Not Always Predict High Mating Success. J. Evol. Biol 23, 2010, 875-878.
- [28]. T. Flatt, Survival Costs of Reproduction in Drosophila. Experimental Gerontology 46, 2011, 369-375.
- [29]. G. Sabio, L. A. Quesada-Allue, and M. M. Perez, NBAD-hydrolase processing in brain and epidermis of *Drosophila melanogaster*. Drosophila Information Service 94, 2011, 100-104.
- [30]. P. Gibert, B. Moreteau, A. Munjal, and J. R. David, Phenotypic Plasticity Of Abdominal Pigmentation In *Drosophila kikkawai*: Multiple Interactions Between A Major Gene, Sex, Abdomen Segment And Growth Temperature. Genetica 105, 1999, 165–176.
- [31]. S. Desmukh, C. Paniker, and D. D. Deobagkar, Exposure to Heat Stress Modulates DNA Methyltransferase Activity in The Embrionic S2 Cell Line of *Drosophila melanogaster*. Drosophila Information Service 95, 2012, 89-91.
- [32]. W. Neckameyer, Multiple roles for dopamine in Drosophila development. Dev Biol 176, 1996, 209-219.
- [33]. K. E. Marshall and B. J. Sinclair, Repeated Stress Exposure Results in a Survival-Reproduction Trade-Off in Drosophila melanogaster. Proceedings of The Royal Society B, 277, 2009, 963-969.
- [34]. R. A. Krebs and V. Loeschcke, Effects of Exposure to Short-Term Heat Stress on Fitness Components in *Drosophila melanogaster*. J. Evol. Biol 7, 1994, 39-49.
- [35]. J. C. do Nascimento, I. B. M. da Cruz, L. A. Monjelo, and A. K. Oliveira, Genetic Components Affecting Embryonic Developmental Time of *Drosophila melanogaster*, Genetics and Molecular Biology 25(2), 2002, 157-160.
- [36]. A. B. Paaby and P. S. Scmidt, Dissecting the Genetics of Longevity in *Drosophila melanogaster*, 2009, Online, http://www.landesbioscience.com/journals/fly/article/7771, accessed on November 8th, 2015.
- [37]. C.D. Allis, T. Jenuwein, and D. Reinberg, Overview and Concepts, Epigenetics, Cold Spring Harbor Laboratory Press, 2007, 23-62.