Population Dietary Variation of *Drosophila Melanogaster* Associated with Different Yeasts

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**ABSTRACT**

Objective: To analyze the importance of the quality of the growth medium as to the development of *Drosophila melanogaster* grown in the laboratory, in order to assess changes in physical fitness and in the survival and fertility rates of the studied populations, both of the White and wild strains, according to the yeast provided as a food resource. Method: To do this, different yeasts were used as food resources (*Saccharomyces cerevisiae*, *Dekkera bruxelensis* and *Meyerozyma caribbica*) in the standard medium and in order to evaluate the variables, the RING test and analysis of survival and fertility rates were performed. Results: Through the data, the growth medium with *Saccharomyces cerevisiae* was that with all analyzed rates were within the expected value and that the individuals were able to complete their life cycle, while the other resources did not obtain the expected values. Conclusion: The efficiency of *Saccharomyces cerevisiae* for maintenance medium is confirmed, in addition to this fact, with the intriguing result of the other two yeasts, it is necessary to add different evaluation methods so that the results become increasingly robust.

**Keywords**: *Drosophila melanogaster*; growth medium; yeast; resource.
INTRODUCTION

Insects have a great potential for use in biology, both for teaching and for research (MATTHEWS et al., 1997; BREWER, 2002), having interesting resources for experiments in ecology such as their short life cycle, large quantity and ease of handling and maintenance (MATTHEWS et al., 1997).

The order Diptera is represented by about 10,000 genera, 150 families with an estimated 150,000 described species (PAPE et al., 2009) known as flies, mosquitoes and horseflies. Drosophila flies are highly spatially differentiated (DAVID, CAPY 1988, HUEY et al. 2000, HOFFMANN, SHIRRIFFS 2002, GILCHRIST, HUEY 2004, SAMBUCETTI et al. 2006) and remain limited to environmental conditions (TIDON 2006, MATA et al 2010, WILLI, HOFFMANN 2012). In addition, its use in research has several advantages, such as the use of its sequenced genome, easy use, simple anatomy and clarification and a short life cycle. It is also highlighted the fact that children are relatively affected, being able to originate in a week several times descendants (GOMES, 2001).

First used for research in genetics by Thomas Hunt Morgan, as Drosophila melanogaster (popularly known as the fruit fly) it is still used freely worldwide for this purpose. It is an excellent biological model for carrying out genetic analyzes (GOMES, 2001).

A variation in the environment is an extremely important factor in an individual's phenotypic development process, since the variation may be responsible for a unique model that shows a range of phenotypes. Such a phenomenon is defined as phenotypic plasticity, addressing chemical or environmental changes perceived by the organism that can cause inheritable phenotypic changes over a natural age of selection (PIGLIUCCI 2001; WEST-EBERHARD 2003).

When used in laboratories, there are different means of cultivation that can be used for food as fruit flies, among the means, it is possible to use yeast as a resource. In fields such as yeasts and drosophilia formed a mutual association, where microorganisms are sources of nutrients for adults and fly larvae, and have an important role in the detoxification of substrates used as food reproduction and reproduction; and the flies disperse like yeast between the different microhabitats (MORAIS et al., 2000).

Drosophila species have specific associations with certain yeasts, that is, they have different degrees of food resource preferences, a fact that allows the inference about the type of substrate colonized by flies in the environment and aspects of the evolutionary history of the genus, according to metabolic abilities and physiological characteristics of each yeast species (STARMER et al. 1988, MORAIS et al. 2000).

The objective of this work was to analyze the importance of the quality of the environment as to the development of Drosophila melanogaster grown in the laboratory, in order to evaluate changes in physical fitness and in the survival and fertility rates of the studied populations, both of the White and wild strains, according to with the yeast provided as a food resource.

MATERIAL AND METHODS

Obtaining yeasts

Three yeast strains obtained from the Laboratory of Genetics of Microorganisms of the Federal University of Pernambuco were used, Dekkera bruxelensis (GDB) e Meyerozyma caribbica (53T) from the inoculum in liquid medium (YPD). The standard yeast, Saccharomyces cerevisiae (SMC), was conventionally obtained through the purchase of a package of biological yeast (500 g of organic yeast brand Fermipan ©). S. cerevisiae was selected because it is already used in the laboratory (LEONI, 2011, BERNARDES, 2018), D. bruxelensis, due to its characteristics similar to S. cerevisiae, in other words, they are yeasts known in the production of beer, capable of replace or accompany S. cerevisiae in specific
industrial fermentations (STEENSELS et al., 2015), and M. caribbica, which also has fermentation capacity (glucose, galactose, maltose, sucrose, trehalose and raffinose) (MARTINI et al., 2004).

Production of the culture medium:

Table 1: Quantities indicated for the culture medium manufactured in the Experimentation Laboratory in Drosophila – UFPE

| Ingredients                  | Quantity (g) |
|------------------------------|--------------|
| Banana                       | 50           |
| Corn meal                    | 75           |
| Oatmeal                      | 25           |
| Nippagin                     | 1,5          |
| Sugar                        | 40           |

| Ingredients                  | Quantity (g) |
|------------------------------|--------------|
| Water                        | 800 mL       |
| Alcohol                      | 15 mL        |
| Phosphoric acid              | 3 mL         |
| Yeasts (GDB, 53T or SMC)     | 6,5 mL       |

Three culture media adapted from Leoni (2011) were produced, which differed only in the yeast strain used, totalizing 12 growing medium: GDB 1, GDB 2, 53T 1, 53T 2 e SMC 1, SMC 2.

The culture medium was prepared in 4 stages according to the adapted methodology of Leoni (2011); Preparing:

1st step: All sources of carbohydrates (banana, cotton, oats and sugar) were mixed in the plastic container.

2nd step: The mix was put in a microwave oven, adding small amounts of water during the homogenized cooking process - every 2 minutes for 15 minutes;

3rd step: The yeast was added at the end of the process.

4th step: 1.5g of Nippagin was diluted in 15mL of alcohol in a separate glass container. The dilution was added to the medium with 3 ml of phosphoric acid when the cook was completed. After shaking with a glass stick, the medium was poured into separate glass jars and sealed with sponges.

NOTE: Before the introduction of the flies into the medium, a wet filter paper was inserted inside the glass jars to supply the water requirement of the specimens.

*Drosophila melanogaster* and growing medium

The flies used in this study were obtained from the stock of D. melanogaster of the Laboratory of Experimentation in Drosophila (LED) of the Federal University of Pernambuco (UFPE). The strains used were OREGON-C (wild phenotype) and w67c23 (White phenotype). The Diptera were distributed in three different media, each one of them containing one type of yeast: *S. cerevisiae* (SMC); *D. bruxelensis* (GDB) and *M. caribbica* (53T). Flies (n = 10) of each phenotype were introduced in each medium, they were: 5 virgin females and 5 males not necessarily virgin, in each medium. The experiment was
carried out in duplicate, obtaining, in total, 12 cultures with 10 flies in each, totaling 120 flies, being 60 of the OREGON-C strain and 60 w67c23. After 12 days of cultivation of the flies in their specific environments, the parental (P) and daughters (f1) generations were removed and the survival and birth rates of both were calculated and analyzed using the ANOVA AND Tukey tests. The RING physical fitness test (GARGANO et al., 2005) was performed posteriorly.

RESULTS AND DISCUSSION

Parental Survival Rate

At the end of 12 days, the period from the birth of the individual to the moment when the individual breeds (GOMES, 2001), the survival rate of the parents was observed. (Table 2) (Figure 1)

The results analyzed in regarding the culture medium with SMC were already expected, once S. cerevisiae yeast is already used in various types of fly maintenance culture medium for scientific research, such as banana, cornmeal, mashed potatoes and enriched mashed potatoes (LEONI, 2011, BERNARDES).

Regarding the data obtained with GDB, it was observed that the females of the White lineage were more adapted to this yeast, showing a survival rate greater than or equal to males. In the wild lineage, the results in both replicates were controversial, once GBD1 had a higher survival rate of females compared to males, while in GBD2 the highest survival rate was of males.

In the rates obtained in the 53T culture medium, only the 53T1 of the White lineage presented surviving parental, in an equal value for males and females, while in the wild lineage, in 53T1 and 53T2, females presented a higher survival rate.

The higher survival rate of females in most populations is probably due to the fact that in D. melanogaster, repression of the insulin / IGF-1 and RD pathways gives a greater increase in female longevity (MAGWERE et al., 2004). In the opposite event, as in GBD2, where the male survival rate was higher, the difference in the response between the sexes may be explained by a higher energy requirement for females due to the reproduction process (MAGWERE et al., 2004).

Birth Test (F1)

The results showed that flies, both White and wild, showed fertility rate only in the SMC culture medium, with no F1 in the GDB and 53T culture medium. Regarding the White lineage, 10 males and 10 females were counted in SMC1, and 24 males and 29 females in SMC2. As for the wild ones, 14 males and 30 females emerged in SMC1, and in SMC2 20 males and 28 females (Table 3) (Figure 2).

A possible explanation for the success in the reproduction process of only one of the three culture mediums is due to the fact that there is an inverse correlation between fertility and longevity due to the dietary change (BASS et al., 2007; PIPER, PATRIDGE, 2007). Thus, the dietary restriction would cause an energy reallocation for a longer life time at the expense of reproduction, that is, reducing fertility.

The results elucidate the LEONI, 2011, BERNARDES, 2018). In both White and wild lineages, the number of females was greater than or equal to the number of males, however the numbers of males and females were significantly different between replicates. Still, the wild lineage showed a higher fertility rate compared to the White lineage.

This data is possibly due to the w + gene, which characterizes the White lineage (white eyes), directly controls copulation success, once it has a role in the organism's learning and memory. Thus, White flies may have their ability to learn or develop their normal sexual performance impaired (XIAO et al., 2016). These authors comment that sexual performance is influenced by weight loss, which causes delayed sexual development and maturation.

Parental fitness test
From the tests carried out to evaluate the physical conditions of the flies, the White strain showed in SMC1 3 able males and 3 able females, from a total of 5 individuals for each sex, and in SMC2 it presented 1 able male and 2 able females, from a total of 5 individuals for each sex.

The population of GDB1 did not present able individuals in the White strain, whereas GDB2 had only 1 able male of the White strain.

In the 53T medium, the 53T1 group shows just 1 able female of the White strain. The individuals in the 53T2 group did not have survivors, so it was not possible to perform the capacity test.

As for the wild ones, both SMC1 and SMC2 had 2 able males and 2 able females, while the other culture mediums were not identified as adequate.

The results are probably related to phenotypic plasticity events, however, parameters such as wing morphometry, thorax size and wing/thorax ratio were not analyzed. Currently, there are related discussions on how the environment can generate phenotypic variations, mainly with the advancement of epigenetics (Pigliucci & Müller, 2010; Schoener, 2011; Danchin, 2013; Müller, 2013) (Jablonka & Lamb, 2010), with a validation of the meeting of contributions from ecology and population genetics studies (Pennisi, 2008; Pigliucci & Müller, 2010) without a Lamarckist approach.

From an aerodynamic view, flight depends crucially on the weight that a pair of wings can support, a fact that is related to variants such as wing area, flight speed, air density and angle of the wings in relation to the direction of flight (Tennekes, 2009). Thus, the wing load, the wing area supporting a given body weight, is an important factor for the development of the flight, concluding that the individual's nutritional issue is directly linked to the capacity and quality of the flight.

The difference in physical fitness between the culture mediums, in terms of percentage, was greater in the culture medium with SMC, which reflects the interaction of individuals with the different yeasts, showing a better relationship with S. cerevisiae. (Table 4) (Figure 3)

**Table 2. Parental Survival**

| Survival (total=5) | rate | White male (%) | White female (%) | Savage male (%) | Savage female (%) |
|--------------------|------|----------------|-----------------|-----------------|------------------|
| SMC1               | 100  | 100            | 100             | 100             | 100              |
| SMC2               | 100  | 100            | 100             | 100             | 100              |
| GDB1               | 60   | 60             | 20              | 100             | 100              |
| GDB2               | 20   | 60             | 60              | 20              | 20               |
| 53T1               | 60   | 60             | 20              | 40              | 40               |
| 53T2               | 0    | 0              | 40              | 40              |                  |

**Figure 1. Parental Survival**
Table 3. Birth Test (F1)

| Birth Test (F1) | White macho | White fêmea | Selvagem macho | Selvagem fêmea |
|-----------------|-------------|-------------|----------------|----------------|
| SMC1            | 10          | 10          | 14             | 30             |
| SMC2            | 24          | 29          | 20             | 28             |
| GDB1            | 0           | 0           | 0              | 0              |
| GDB2            | 0           | 0           | 0              | 0              |
| 53T1            | 0           | 0           | 0              | 0              |
| 53T2            | 0           | 0           | 0              | 0              |

Figure 2. Natalidade (F1)

Table 4. Physical fitness test

| Physical fitness test P (total=5) | White male(%) | White female (%) | Savage male (%) | Savage female (%) |
|-----------------------------------|---------------|------------------|-----------------|-------------------|
| SMC1                              | 60            | 60               | 40              | 40                |
| SMC2                              | 20            | 40               | 40              | 40                |
| GDB1                              | 0             | 0                | 0               | 0                 |
| GDB2                              | 20            | 0                | 0               | 0                 |
| 53T1                              | 0             | 20               | 0               | 0                 |
| 53T2                              | -             | -                | 0               | 0                 |

Figure 3. Physical fitness test
CONCLUSION
In view of the above, the efficiency of the use of Saccharomyces cerevisiae for use in maintaining the stocks of Drosophila melanogaster is confirmed, both for wild and carriers of the White mutation in the laboratory that use them in different experiments. Important fact is that it has great cost benefit, because they are sold in small and large businesses, at affordable prices.

It is also worth noting the intriguing result with the low efficiency of the other yeasts used for experimentation, mainly the GDB (Dekkera bruxelensis), which is also a fermenter widely used in the food and beverage industry because it has large amounts of volatile compounds (STEENSELS et al., 2015). Thus, further studies are necessary to better evaluate the optimal conditions for the development of these in the culture medium, as well as the addition of different evaluation methods so that the data exposed here will become increasingly robust.

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