A SOURCE OF FOOD ENRICHMENT: SEARCHING ALTERNATIVES TO INCREASE THE PRODUCTIVITY OF *Drosophila melanogaster* ADULTS (DIPTERA: DROSOPHILIDAE)

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ABSTRACT

The productivity of three type strains of *Drosophila melanogaster* Meigen, 1830 in enrichment culture media with banana, orange, pear or blackberry was evaluated. An Interaction Effects Test was performed for the twelve possible strain/culture media combinations. Subsequently, 60 treatments including five repetitions of each possible strain/culture media combination were analyzed by ANOVA. Results showed a higher productivity in banana and pear culture media for the wild type and *dumpy*, *dumpy*-banana and wild type-orange combinations. Results suggest that there is a variance of nutritional requirement between the strains that can be provided by enrichment culture media of banana and pear.
Key words: *dumpy*, fruits, *sepia*, wild type.

RESUMEN

La productividad de tres cepas de *Drosophila melanogaster* Meigen, 1830 fue evaluada en medios de cultivo enriquecidos con banano, naranja, pera o mora. Una prueba de efecto inter-sujetos fue ejecutada para doce posibles combinaciones entre cepa y medio de cultivo. Posteriormente, un total de 60 tratamientos fue analizado con ANOVA. Los resultados mostraron una mayor productividad de adultos en medios de cultivo enriquecido con banana y pera para las cepas silvestre y *dumpy*, y para las combinaciones *dumpy*-banano y silvestre-naranja. Los resultados sugieren que las diferencias en los requerimientos nutricionales de individuos en distintas cepas, pueden ser proveídos en mayor medida por los medios de cultivo enriquecidos con banano y pera.
Palabras clave: *dumpy*, frutas, *sepia*, silvestre.

INTRODUCTION

Drosophila melanogaster Meigen, 1830 is an insect used as a biological model almost a century ago (Arnaiz 2005). The production of individuals in species such as *D. melanogaster* is strongly associated with culture media characteristics such as physicochemical components and percentage of nutrients (Santos 1997). These factors shape the dynamics of population density differentially because of their influence on phenotypic expression (Ushakumari & Ranganth 1985), mortality rates increase or decrease, and time of organism development (Gluecksohn-Waelsch 1953). Traditionally, it has been recognized that culture media enriched with nutrients-enriched such as sugar and yeast generate high productivity in microorganism, but it has been found that these components can produce physiological problems, obesity, reduced longevity in organisms such as *D. melanogaster* (Skorupa *et al.* 2008), and reduce the productivity rate of the strains. Taking into account that *D. melanogaster* should breed on a large scale for several types of research (e.g. behavior and genetics) and the purpose of considering alternatives for other culture media enrichment than those traditionally are used such as sugar and yeast, this study aimed to analyze the productivity of *D. melanogaster* adults in culture media with various sources of enrichment and the productivity related with the strain, the culture media and strain-culture media.

MATERIAL AND METHODS

Guideline for culture media preparation

Glass bottles, cotton cloths, and gauzes were sterilized to avoid the presence of microorganisms. Then, 23g of fruit (banana, orange, pear or blackberry) 150 ml of purified water, 33g of wheat flour, 33g of white sugar and 3g of yeast Levapan®were liquefied simultaneously (Blender Premium PB323), and 480ml of water were added while stirring until the solution reached a homogenous mixture. The mixture was heated and stirred until it began to boil. Then, 7,2g of nutrient agar DIFCO^{TM}were added and maintained at 29°C for 15 minutes. Soon after, 3ml of propionic acid ($C3H6O2$) were added, and then, 2 cm^3 of culture medium were deposited in each sterile flask.

Record of productivity

We studied a total of 60 treatments that included five repetitions out of the twelve possible strain combinations (wild, eye colour mutant *sepia* and wing mutant *dumpy*) which were enriched with banana (*Musa paradisiaca* L.), orange (*Citrus* sp.), pear (*Pyrus* sp.) or blackberry (*Rubus* sp.). In each treatment, five males and five females were introduced. In the present study, productivity was expressed as the number of adults that emerged after completing their development cycle (Bonnier & Jonsson 1957). Productivity recording started eight days after seeding and subsequent extraction of their parentally. The larvae were fed *ad libitum*, and adult headcount adult was performed twice a day (9:00 h and 16:00 h) until the culture media was finished.

Data analysis

Counts were expressed in terms of averagerages. An Interaction Effects Test (ANOVA) was conducted to observe the relation between the two kinds of strain types of factors and culture media. Then, an analysis of variance (ANOVA) with post-hoc Tukey test was performed by comparison of averages. Statistical analyzes were performed using SPSS 11.5 software (`http://www-01.ibm.com/software/analytics/spss/`).

RESULTS

The most productive culture media were banana and pear. The average productivity in the culture media was greater for enriched media with bananas (n=245 individuals), than pear (n=232), orange (n=221) or blackberry (n=102). The average productivity of strains was higher for *dumpy* (238 adults emerged), followed by wild (237 adults emerged) and *sepia* (125 adults emerged). However, no significant difference was found between average culture media ($p= 0.37$) or productivity of strains ($p= 0.99$). The Interaction Effect Test showed an existing relation ($p <0,001$, Table 1, Figure 1) between the type of strain and the culture media in which it is present. Treatments dumpy-banana (330 individuals) and wild-orange (301 individuals) showed greater productivity and significant difference compared to the remaining treatments, much higher productivity than treatments such as a *sepia*-blackberry that indicated less average productivity with 82 individuals.

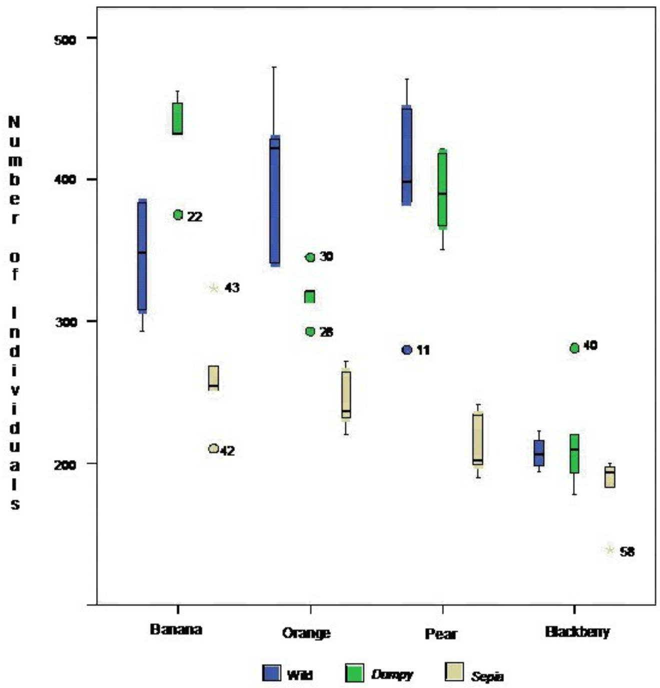

Figure 1. Boxplot of productivity expressed in number of adults emerged in the wild, dumpy and sepia strains.

Table 1. Analysis of variance for productivity expressed as number of adults. SS: Sum of squares type III, DF: Degrees of freedom, F. Probability distribution, Sg: Significance.

Inter-subjects effects Test					
Dependent variable: Number of individuals					
Fountain	**SS**	**DF**	**Quadratic mean**	**F**	**Sg**
Corrected model	441922.58(a)	11	40174.78	26.19	0.00
Intersection	2410010.41	1	2410010.41	1571.27	0.00
Strain	168846.03	2	84423.01	55.04	0.00
Culture	197780.85	3	65926.95	42.98	0.00
Strain * Culture	75295.70	6	12549.28	8.18	0.00
Error	73622.00	48	1573.79		
Total	2925555.00	60			
Corrected total	515544.58	59			
a. Squarer R= 0.85 (squarer corrected= 0.82)					

DISCUSSION

The differences in productivity of *D. melanogaster* are possibly due to differences in nutritional values and physicochemical characteristics of each culture media, which significantly influence the entire life cycle, especially the larval stages (González *et al.* 2011). During the larval stage, individuals burrow into the substrate, which allows aeration through the opening of galleries used to move across the culture media (Godoy 2001). Thus, the enrichment with banana and orange gave a soft texture for the larva that required lower energy cost in burrowing.

Food composition table for Colombia (Table 3, Anonymous 2016) shows that among the four selected fruits, bananas and pear provide higher calories and more carbohydrates. These nutrients are well assimilated and are crucial for *D. melanogaster* good diet (Skorupa *et al.*

2008), because they meet the energy requirements for larval excavation (Godoy 2001). Amounts of carbohydrates in suitable proportion (30g/150ml of media) with low protein feed ration (25g/150ml of media) may lead to increased longevity of individuals (Skorupa *et al.* 2008), which enables longer life and higher offspring. Additionally, larval development requires reaching a minimum weight to turn into pupa, which needs proteins and lipids for structure formation (Sang 1978). Banana, pear and orange have similar percentages of proteins and lipids (Table 2).

Although, the blackberry-enriched media showed a lower productivity than any other media, the daily count records indicated a faster consumption than the others, probably as a result of larvae intake to meet the requirements of carbohydrates and blackberry provides low levels of calories (Table 2). However, the fastest disappearance of blackberry juice-enriched food could be an indicator of better suitability food for the flies, and maybe the overall fitness of flies on that food might be better than other enrichment used in the study. Moreover, nutritional potential of the media influences the size of adults, males abilities to get couple and high reproductive success to lead more offspring (Partridge *et al.* 1987), while the best females nourished are better able to fertilize (Lefranc & Bundgaard 2000).

The differences between the productivity of mutant and wild strains are explained as related to characteristics of the culture media, which negatively and more intensely affect courtship, copulation, oviposition, sperm viability and development stages in mutant strains (Mora *et al.* 2000) but which do not represent a significant disadvantage for wild strains (Díaz-González *et al.* 2008). For example, brown eye males in sepia strain show pigmentation caused by a blockage of pyrimidodiazepine synthase biosynthesis (Vera 2003), which makes difficult the visual communication during courtship orientation (Wilkinson & Dodson 1997). Also, these alterations lead to neurological defects associated to impaired learning and memory (Savvateeva *et al.* 2000) that would be related to the low productivity of this strain, even in culture media with high nutritional values.

Short and blunt-tipped male wings of dumpy strain represent a difficulty for courtship, as it hampers reproductive success by the inability to produce rhythmic vibrations needed to attract females (Geer & Green 1962). This is not consistent with our results, where dumpy had the most productive strains as well as higher productivity was dumpy in the banana culture media. This suggests, as found in other studies, that the effect of a particular mutation can be modified by environmental influence (Díaz-González *et al.* 2008).

Thus, certain culture media can meet the organismal requirements, which causes a mutation to lose its effect or to be unnoticed in normal development and productivity of a population (Gluecksohn-Waelsh 1953). A media enriched with carbohydrates nutrients promotes positive results and these cases, can help individuals who have a “disadvantage”to generate lots of offspring (Pitnick 1991). Culture media enriched with banana and pear offers subtract of high nutritional value and physicochemical characteristics (texture, moisture and pH) that allows individuals sepia achieve high productivity despite the limitations of their product mutations. Therefore, for *D. melanogaster* high productivity, we recommend the use of banana and pear to enrich the culture media.

Table 2. Table of composition for each 100g of fruit used in the supplements of culture media (Anonymous 2016). Values for Protein, Lipids and Carbohydrates are reported in g/100g

Food	**Kcal**	**Protein**	**Lipids**	**Carbohydrates**
Pear	88.9	0.2	0.1	10.50
Banana	88	1.2	0.1	20.8
Orange	49	0.6	0.1	11.6
Blackberry	29	0.6	0.1	6.4

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