Influence of Food on Reproductive Behavior in *Drosophila melanogaster*

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Abstract

In a constantly changing environment, animals are forced to make crucial behavioral choices for survival. Food determines whether wild animals evolve, whether populations decline, or how ecological groups are structured. Animals have the ability to adapt their nutritional needs according to the availability of food, they detect and assimilate nutrients. *Drosophila melanogaster*, the fruit fly, is considered an important model for various studies. For example, in nutrition research and for understanding the mechanisms of human disease. In this study, we have provided an overview of fruit fly feeding behavior, how diet influences mating behavior and what volatiles attract flies. We show that mating behavior is very closely related to food and that egg laying is influenced by yeast, a very important source of protein.

Keywords: *Drosophila melanogaster*, fruit fly, feeding behavior, diet, mating behavior

1. Introduction

In the past, nutrition was considered to be part of the field of medicine and the field of agriculture, and not part of biology, but it coordinates all aspects of the natural world. Food determines whether wild animals evolve, whether populations decline, or how ecological groups are structured [1]. Almost all components of an organism's fitness are strongly influenced by macronutrients, carbohydrates and proteins being the most studied from the perspective of nutritional ecology [1]. These macronutrients have very interesting effects because they also work interactively, not just simultaneously.

For example, when a macronutrient is added to the diet, not only does the content increase, but the balance of macronutrients in the diet is altered, which significantly affects the actual fitness [2].

Nutritional geometry refers to how animals balance their needs against nutrient availability and what response they provide. For example, mouse and insect studies have shown that high-carbohydrate and low-protein diets have been correlated with the longest lifespans for ad libitum-fed animals, so macronutrient interactions are very important [1,2]. Animals have the ability to adapt their nutritional needs according to the food availability by detecting and assimilating nutrients [5]. Animals are constantly forced to make choices regarding their behavior in order to have optimal survival. These choices have their beginning in an impulse that comes from a need and can be influenced by sensory information [6]. Because the function and organization of chemosensory systems are similar in invertebrates and vertebrates, *D. melanogaster* is a very useful model for analyzing the feeding behavior [5].

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2. Feeding behavior in *Drosophila melanogaster*

In *D. melanogaster* feeding behavior is composed of several programs of searching and consuming food [7]. Most insects, including *D. melanogaster*, find their food source using the olfactory system [8]. Fruit fly detects food using gustatory and olfactory chemosensors [9]. To detect food and feed, the fruit fly activates its gustatory neurons on its legs and proboscis. *Drosophila* insulin-like peptides (DILPs), and neuropeptide F (dNPF) are neuromodulatory systems involved in regulating the feeding schedule that depends on the nutritional status of the fly [10].

Sugar and yeast are considered the most preferred foods by *Drosophila*. The fermentation product of yeast is acetic acid, which is perceived by Ir75a, an ionotropic odorant receptor neuron, and sugar is attractive by its sweet taste and is sensed by the taste receptor Gr64a [11]. To have optimal survival, *D. melanogaster* individuals need a yeast-based diet, and in its presence the mortality rate is low and reproduction is increased, improved survival in the presence of a yeast diet is a genetically variable trait [12]. Regarding sugar, both larval and adult *Drosophila* are attracted to several types of sugar, but their preferences are different. For example, in the case of maltose and sucrose, adult flies had almost the same responses to these two types of sugars, while larvae preferred maltose over sucrose. This difference appears to be attributable to sugar receptor molecules that are different in larvae and adults [13]. At the same time, well-fed larvae are attracted to sugar solutions, but fed adult flies no longer ingest sugar solutions and are no longer attracted to them [13].

When flies were maintained on diets of varying amounts of calories it was found that lifespan was extended when caloric intake was reduced [14]. In the laboratory, *D. melanogaster* is maintained on a diet consisting of yeast, sugar and water, but careful attention must be paid to the amount of sugar added to the culture medium because more than 50g/L of sugar negatively influences egg laying. It is likely that this effect is caused by the presence of unnatural sugar [15]. Another component found in fruit fly growth media is ripe banana which has a sugar content of about 4.5 g/L [16].

To answer a question about what were the initial sources of food used by *D. melanogaster*, Keller Andreas (2007) states that the first fruits on which fruit flies were found were raisins. The species has been reported to prefer bananas and other fruits such as mango, papaya and apple guava. The study by Zhu et al. (2003) places fly preference for bananas lower because *D. melanogaster* was observed to favor mango fruit. Flies were made to choose from a variety of ripe fruits including strawberry, mango, grape, banana, pear and plum. Because mango was the preferred fruit, the volatile compounds present in this fruit that sensitize the flies were identified. These compounds include acetic acid, amyl acetate, ethanol, 2-phenylethanol, and phenylethyl acetate [17].

Next, based on the literature, we will try to identify if these volatile compounds are found in bananas, papaya and apple guava. Acetic acid was the only compound found in two of the food sources observed (Table 1). This is consistent with Becher et al.'s (2010) study showing that this vinegar compound can attract flies by itself. Acetic acid is considered to be of ecological importance, its sensitivity being expressed in *D. melanogaster* larvae and adults [18].

Oviposition substrates or the presence of fermented fruit can be indicated by acetic acid. There are other volatile compounds that are preferred by *D. melanogaster* and are considered strong odorants, including phenylacetaldehyde, phenylacetic acid or ethanol.

Phenylacetaldehyde (PA) is found in various plant tissues and fruits, it is an aromatic compound found in overripe bananas and the cactus Opuntia ficus-indica. These fruits are oviposition sites for the fly and a vital source of food [19,20]. In the adult fruit fly, PA acts as a potent ligand for the odorant receptor (OR67a) and the ionotropic receptor (IR84a) as revealed by electrophysiological analyses [21,22]. Thus, in the wild PA is a very important olfactory cue for *D. melanogaster* adults because it has the ability to activate odorant and ionotropic receptor pathways to guide feeding and courtship behavior [23].

Phenylacetic acid (PAA) is a volatile chemical found in various fly food sources [24]. This compound is present on fruits and plants, but also in their fermentation products [25]. In plant tissues, PAA acts as a growth-regulating auxin [26] and is synthesized by plant-associated microorganisms such as yeast [27].PAA acts as a dietary aphrodisiac and enhances male courtship via the ionotropic receptor IR84a [24].

Table 1. The presence of volatile compounds in different food sources

Volatile compounds	Banana	Apple guava	Papaya	References
acetic acid	+	-	+	Zhu et al., 2018; Pino et al., 2003
amyl acetate	+	-	-	Zhu et al., 2018
ethanol	+	-	-	Zhu et al., 2018
2-phenylethanol	-	-	+	Pino et al., 2003
phenylethyl acetate	-	-	+	Pino et al., 2003

Ethanol is another chemical compound that is sensed by the fruit fly. In nature, this compound is present in low concentrations, being a secondary product of organisms [28]. *D. melanogaster* is guided by ethanol to a fermenting fruit, for example to a putative oviposition site or food source [29]. Ethanol can attract insects from long distances, and this preference can be viewed as behavior based on an olfactory cue. In *Drosophila*, odor recognition occurs at the level of odor receptors in olfactory neurons [30].

But what happens when flies face a food shortage and become malnourished? Several components of fruit fly fitness under malnutrition were investigated, such as lifespan, heat stress tolerance, locomotor activity, lipid content and weight. The negative phenotypic effects on all investigated traits were sex-dependent, with females being much more resistant to malnutrition than males [31]. In general, females have adapted to nutritional stress because they invest more in reproductive traits throughout evolution. Female insects successfully cope with environmental stressors [32].

3. Influence of diet on reproductive behavior

Food availability is known to play a very important role in the reproduction process for many species [33]. *D. melanogaster* is a species primarily associated with fruit, but these flies are not just phytophagous insects. They also feed on the bacteria and fungi responsible for fruit decay and on the fruit itself. *Drosophila* lives in an environment with a strong odor that provides chemical cues that influence reproduction [34,35].

Diet is one of the best known environmental factors influencing reproduction in *Drosophila*. When female fruit flies were maintained on different nutrient levels, mating frequency and egg production were observed to increase with food intake [33]. For female *Drosophila*, the relationship between food and reproduction is very important because their offspring will thrive on food [11].

During the year, food availability fluctuates, so many animals are reproductively active when food is plentiful [36]. A favorable period for the reproduction of these creatures is summer and autumn because the ripe fruits attract flies, and volatile fermentation products produced by yeasts that reproduce on the fruit surface provide a sexual stimulus for *D. melanogaster* [37,38].

Sugar and yeast are two of the most important nutritional mainstays for *Drosophila*, and their availability influences female reproductive behaviors. Yeast stimulates female sexual receptivity due to the interaction between proteins and the fermentation product, acetic acid, which is perceived by Ir75a [11]. Yeast becomes a more attractive food source for females after mating, probably because it is a protein and amino acid source with an effect on egg production. The fruit fly adjusts its egg production rate based on yeast availability [39]. The increased yeast preference of mated females may be due to faster depletion of protein stores because when exposed to choose between an alternative food source and yeast, protein-deprived flies prefer yeast [40]. The study by Fricke et al. (2008), suggests that yeast does not only influence the mating process in female *Drosophila*, but also influences mating in males. Thus, it was observed that males maintained on a high protein diet attracted more females, mated faster and more often than males fed low amounts of protein [41].

Regarding *Drosophila* females, it was observed that protein-deprived females show a drastic reduction in ovary size and a concomitant increase in yeast consumption compared to fully fed females

[42]. When food is insufficient, oogenesis is arrested and cell apoptosis is induced in mid-oogenesis by a mechanism that is directed by ecdysteroid hormone [42]. In fruit flies, protein-based diet not only influences egg production, but also has an effect on mating duration. In males, dietary protein negatively influenced mating duration, and in females no effect on mating duration was observed [43].

For both insects and higher organisms, peptides are important in appetite control [44] and mating activity causes major changes in female behavior in many species. In female *Drosophila* species, this activity leads to increased egg laying and decreased sexual receptivity. These changes are controlled by a family of about 80 small peptides that are produced by the male accessory glands and transferred into the seminal fluid [45]. Among these peptides 3 play an important role, namely ovulin, sex peptide and DUP99B [46].

However, sex peptide is thought to have the most important role in modulating appetite, it binds to the subesophageal ganglion [47]. This ganglion is a neural center involved in taste recognition and feeding [48]. Sex peptide also reduces male acceptance by *Drosophila* females [46,49]. It also has an important function because it stimulates the immune system of female *Drosophila* after mating activity [50], an action attributed to prostaglandins in the human species [51].

In a study by Carvalho et al. in 2006 it was shown that female *Drosophila* that were mated consumed approximately 2 times more food than virgin females of the same age. Thus, it can be stated that copulation stimulates female food intake due to the sex peptide because when male *Drosophila* lack this protein following a chromosomal deletion, female feeding was not induced. For male *Drosophila*, mating status does not influence feeding [52].

Mating status plays a very important role in female decision-making and is influenced by sex peptide receptor action in ppk + sensory interneurons. Another critical element in this decision it is the neuronal function of TOR/S6K (Target of rapamycin/S6Kinaza) that is likely to signal the nutritional status of the fly [40,53]. Although mating has been shown to reduce the lifespan of female *Drosophila* [54,55], it is possible that this shortening of lifespan in mated females is related to nutrient intake, as it is in other organisms [52]. Lifespan was greatly reduced when the protein intake in the food of the flies increased and the amount of carbohydrate decreased [56].

In a constantly changing environment, animals are forced to make crucial behavioral choices for survival. These decisions can be binary. When encountering a food source, organisms have two options, either initiate feeding or choose to avoid the food [57]. But the most difficult is when animals are subjected to environmental cues and internal drives that stimulate incompatible behaviors. For example, these behaviors may be threat avoidance or mating versus feeding [57].

Over time researchers have tried to analyze competing behaviors in *Drosophila* and what are the mechanisms that mediate these choices [58 - 65]. Feeding behavior and walking behavior are two activities that cannot be performed simultaneously and are influenced by a single pair of interneurons located in the ventral nerve cord [59]. Activating these interneurons while the fly flies eliminate feeding, and inhibiting them activates feeding to the detriment of locomotion [57].

Feeding and sleeping are other behaviors that cannot be performed at the same time and have attracted the attention of researchers [58, 61, 63]. When flies are hungry, they give up sleep and choose to feed, they will probably sleep after a meal [63].

However, when an animal is restricted from food and mating activity, one of the most interesting conflicts occurs as the animal is forced to decide which behavior to prioritize. Even if the lack of food is a threat to survival, it is not very clear whether the reproductive activity exceeds the coordinated activities of hunger [57] however, when males were deprived of food for 24h, courtship was overtaken by starvation. This is also shown in Maslow's hierarchy of needs. A key mediator of this decision is the tyramine signaling pathway [57].

4. Conclusions

The mechanism of food and oviposition site choice in *Drosophila melanogaster* is still poorly understood because this insect is driven by a number of factors in its decision-making process. However, it is certain that mating activity is influenced by food.

Mating frequency and egg production were observed to increase with food intake. Not only the presence of food is sufficient, but also its content is important, because there are several important elements that had to be present in the flies' diet to have optimal survival and reproductive behavior.

Sugar and yeast are the main factors influencing reproduction in *Drosophila melanogaster*. These insects direct their egg production according to the availability of yeast because it is a rich source of protein and the acetic acid that is the fermentation product of yeast is a very attractive odorant stimulus for flies.

Diet not only influences egg production, but also has an effect on mating duration in males. On the other hand, copulation was observed to stimulate female food intake, as mated females consumed more food than virgin females.

Another important aspect that is influenced by diet is lifespan. It was observed that when the flies mated, they also consumed a larger amount of food, and this led to a shorter life span.

In conclusion, diet is key to many activities of *Drosophila melanogaster* flies, and feeding activity is favored when insects must choose between feeding, sleeping, or mating.

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