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## RESEARCH ARTICLE

# AGE DEPENDENT ALCOHOL ADDICTION IN SEXUALLY DEPRIVED FRUIT FLIES

**Sanketh Dhumal .S**

Jr.Embryologist, Santhathi Centre for Reproductive Medicine, Mangalore-575002

### ARTICLE INFO

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### ABSTRACT

Alcohol abuse is a pervasive problem known to be influenced by various factors, yet our understanding of the mechanisms underlying alcohol addiction is far from complete. *Drosophila melanogaster* has been established as a model for studying the molecular mechanisms that mediate the acute and chronic effects of alcohol. However, the *Drosophila* model has not yet been extended to include more complex alcohol-related behaviors such as self-administration and addiction. We recently established a paradigm to characterize ethanol consumption and preference in flies. We demonstrated that flies prefer to consume ethanol-containing food over regular food. The mated males showed less preference to alcohol and sexually deprived flies showed much preference to alcohol. And we had even found that the age will effect on the alcohol preference or not, and we found that younger sexually deprived flies showed fluctuations in preference of alcohol food over normal food but whereas older flies showed stronger and stabilized preference to alcohol containing food over normal food. And the sexually deprived and then mated flies showed decreased preference to ethanol food over regular food according to their age. Thus, ethanol preference in flies provides a new model for studying important aspects of addiction and their underlying mechanisms.

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### INTRODUCTION

The fruit fly *Drosophila melanogaster* is one of the most widely used and successful genetic model systems for studying development and behaviour. The usefulness of this model system is based on the fact that the genes and biochemical pathways underlying development and behavior have largely been conserved during evolution. As a result, many genes first identified in *Drosophila* have provided major insights into human and other vertebrate development and disease. *Drosophila* has a relatively sophisticated nervous system and is capable of many complex behaviors. For example, the flies can learn to associate certain events and to remember that association (Davis 1996; Dubnau and Tully 1998). Furthermore, they have sophisticated courtship behaviors (O'Dell and Kaiser 1997). Another advantage of *Drosophila* is that they are easy to rear and have a generation time of only approximately 2 weeks, allowing researchers to explore the heritability of certain traits or behaviors over many generations in a short period of time.

The natural habitat of *Drosophila* includes fermenting plants, which often contain high alcohol levels (i.e., 3 or more percent). Accordingly, fruit flies are resistant to alcohol's toxic effects and can metabolize alcohol efficiently for use as an energy source or as a starting material (i.e., substrate) for the production of lipids (Geer *et al.* 1993). *Drosophila* display many behaviors resembling acute intoxication in mammals,

such as impaired motor control when exposed to alcohol vapors.

*Drosophila melanogaster* is one of the most genetically accessible model organisms. Behavioral responses to ethanol are conserved between flies and mammals: both exhibit locomotor stimulation at low doses and motor incoordination and sedation at high doses (1). Flies also exhibit tolerance with repeated ethanol exposures (2,3). Importantly, several molecular pathways shown to mediate acute responses to ethanol in flies, such as the cAMP (4), neuropeptide F (neuropeptide Y in mammals)(5), and EGFR13 pathways, also regulate mammalian ethanol responses (6,7). *Drosophila melanogaster* frequently encounters significant levels of ethanol produced by fermenting plant materials (1). Flies have evolved mechanisms to process the ethanol they ingest, by efficiently degrading it for use as an energy source or a precursor for lipid biosynthesis (1).

We used two distinct cohorts, one cohort mated male and the other is sexually deprived cohort. The mated male cohort is generated by allowing the virgin male flies to copulate with virgin female flies according to their age we had chosen 2 days old, 12 days old and 22days old flies. Those succeeded in copulating were isolated and kept in separate bottles according to their age. And those failed to copulate are isolated and kept in separate culture vials.

\*Corresponding author: **Sanketh Dhumal .S**

Jr.Embryologist, Santhathi Centre for Reproductive Medicine, Mangalore-575002

## MATERIALS AND METHODS

### Fly lines and culture

Flies were raised at 25°C in a 12-h-light/12-h dark cycle and maintained on cornmeal, yeast, dextrose, and agar medium. Oregon k were used as the wild type strains and were provided by Dr. Krishna (U. Mysore) and by *Drosophila* Stock center, Dept. of Zoology, University of Mysore, Mysore.

### Behavioral assays

All behavioral assays were performed at 25°C unless indicated otherwise.

### Generation of rejected, virgin and mated males

**Conditioning Protocol:** Virgin male flies and female flies were collected and here we have followed pupal method of isolation that is males were isolated based on the presence of sex comb and kept in separate culture bottles and those pupae showed absence of sex comb were considered as females and transferred to separate culture bottles. Virgin male flies taken in separate vials and placed with mated females (previous day). The males those succeeded in copulating (2 hour conditioning trials) were separated and kept in new culture bottles and those rejected where separated and were kept in separate culture bottles and individual male fly was placed with mated female in glass vials and subjected for rejection finally causing sexual deprivation (repeated for thrice a day with minimum of 2 hrs time interval) then both the mated male and sexually deprived male flies were subjected to two choice ethanol preference assay method.

**Virgin males:** Virgin males were collected through the pupal isolation method and kept separately in culture bottles. And then single male flies are kept in a food vial (VWR culture glass tubes 10 x 75 mm) according to their age (2, 12 and 22 days) until they were assayed for ethanol preference.

**Mating protocol:** To generate the “mated group” cohort, virgin male flies were collected based on the pupal method of isolation and kept in a groups in regular culture bottles. They were supplied with virgin females for 5 hours every day and female flies were removed after each mating period. And those males succeeded in copulating were separated and kept in separate culture bottle until they were assayed for ethanol preference.

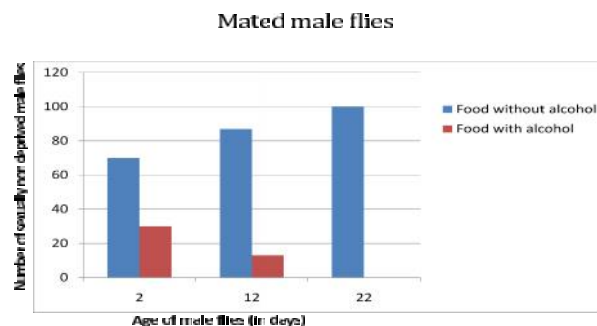
**Rejected males:** The virgin male flies were placed in separate glass vial individually with mated female through mouth pipetting for two hours and after the rejection for copulation the female is removed and new mated female is placed (for 2 hrs) same protocol is repeated thrice a day.

## RESULTS

### Alcohol preference by the mated male flies

It was noticed from the graph 1 that in the age group of 2 days old sexually non deprived(mated) male flies preferred food

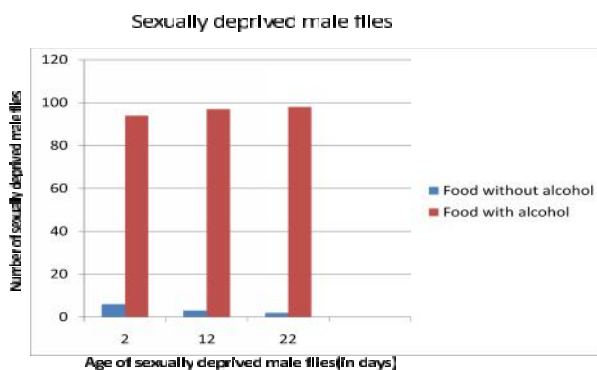
without alcohol in 70% remaining 30% preferred food with alcohol( $\chi^2=16$  df=1  $p<0.001$ ). Similarly at 12 days 87% sexually non deprived male flies preferred food without alcohol while in 13% preferred food with alcohol ( $\chi^2=54.76$ , df=1  $p<0.001$ ). In contrast to this 22 days old 100% of sexually non deprived male flies preferred food without alcohol. Thus this study suggests that sexually non deprived males preferred food without alcohol and this preference increased with increasing male age.



GRAPH 1 Alcohol preference by the mated male flies

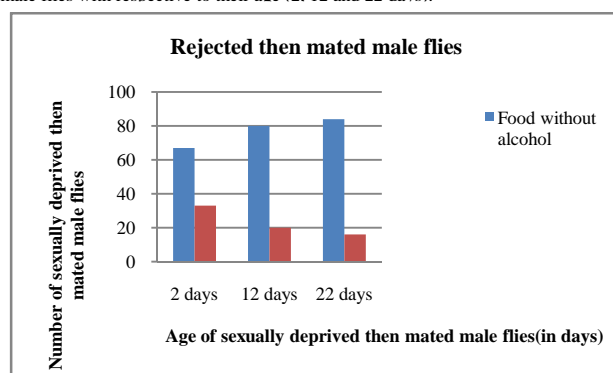
Graphical representation of alcohol preference in sexually non-deprived (mated) male flies with respective to their age (2, 12 and 22 days).

### Alcohol preference by sexually deprived male flies



GRAPH 2 Alcohol preferences by sexually deprived male flies

Graphical representation of alcohol preference in sexually non-deprived (mated) male flies with respective to their age (2, 12 and 22 days).



GRAPH 3 Alcohol preference by rejected then mated male flies

Graphical representation of alcohol preference in rejected, then mated male flies with respective to their age (2, 12 and 22 days).

It was noticed from the graph 2 that in the age group of 2 days old sexually deprived male flies(unmated) preferred food with alcohol in 94% and remaining 6% preferred food without alcohol( $\chi^2=77.44$  df=1  $p<0.001$ ). Similarly at 12 days in 97% sexually deprived male flies preferred food with alcohol while

in 3% preferred food without alcohol ( $\chi^2=88.36$  df=1  $p<0.001$ ). In contrast to this at 22 days 98% sexually deprived male flies preferred food without alcohol and 2% of them preferred food without alcohol ( $\chi^2=98.16$  df=1  $p<0.001$ ). Thus this study suggests that sexually deprived males preferred food with alcohol and this preference increased with increasing male age.

#### **Alcohol preference by rejected then mated male flies**

It was noticed from the graph 3 that at 2 days upon mating 67% of sexually deprived males preferred food without alcohol and in 33% preferred food with alcohol ( $\chi^2=60.84$  df=1  $p<0.001$ ). Similarly at 12 days upon mating 80% of sexually deprived male preferred food without alcohol and only 20% sexually deprived male preferred food with alcohol ( $\chi^2=64$  df=1  $p<0.001$ ). In contrast at 22 days upon mating 84% of sexually deprived males preferred food without alcohol while in 16% preferred food with alcohol ( $\chi^2=57.76$  df=1  $p<0.001$ ). Thus this study suggests that upon mating sexually deprived males preferred food without alcohol and this preference was increased with increasing male age.

## **DISCUSSION**

We used three distinct cohorts, one cohort is mated males and the other is sexually deprived males cohort and the third type includes initially sexually rejected then mated males. The mated male cohort is generated by allowing the virgin male flies to copulate with virgin female flies according to their age we had chosen 2 days old, 12 days old and 22days old flies. Those succeeded in copulating were isolated and kept in separate bottles according to their age. And those failed to copulate are isolated and kept in separate culture vials. And the second cohort which involves in generating the sexually deprived males involved allowing the unmated males with freshly mated female flies, and in nature this kind of mating attempt is generally rejected by the freshly mated female flies within less than two hours and male flies those failed in making successful copulation were isolated and subjected to the fresh batch of mated females for every 2 hours a day and those which failed to mate were isolated and maintained in separated group. And third cohort involves initially rejected then mated males. And all the three cohorts were given choice between food with alcohol and food without alcohol and the preference was recorded in all the three cohorts.

And those flies which were initially succeeded in copulation showed preference to food without alcohol (Graph-1) and those flies which are subjected for rejection and sexually deprived showed preference to food with alcohol (Graph-2).

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The third cohort which is initially sexually deprived and succeeded in mating showed preference to food without alcohol rather than food with alcohol (Graph-3). In consideration of all the three cohorts the preference towards alcohol was gradually increasing in the deprived males as the age increases. And likewise the preference towards food without alcohol was more in male flies those succeeded in mating and it was gradually increased as the age of the flies increased. And surprisingly those males initially rejected and sexually deprived upon mating reverted back to food without alcohol when the choice was given among the food with and without alcohol, and even this reversal increased gradually as the age of the male flies increased.

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