NORTHERN ILLINOIS UNIVERSITY

Hot Dogs and Somatic Mutagenicity in Drosophila Melanogaster

A Thesis Submitted to the
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Department of Biological Sciences
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INTRODUCTION

Drosophila Melanogaster is convenient and easy to handle in testing for mutagenic effects of chemicals. The reason is that the fly is easily cultured and its generation time is only two weeks at 21 degrees Celsius. Induced somatic mutations are seen in the eyes as well as wings in genetics. In this experiment, wings were analyzed for somatic mutations because they can be mounted permanently and hence are convenient to work with. Also, in the wing system there are literally thousands of target cells potentially at risk in every exposed imaginal disc (Würgler, 1984, 154). The goal of this project was to analyze the mutagenic effects of nitrites in hot dogs by looking for induced somatic mutations and recombinations of the multi-wing hair (mwh) and flare (flr) genes on the wing blades. Induced mutations lead to single mosaic spots (mwh) and induced recombinations, which are rarer, lead to mwh and flr twin spots and also, to a certain extent, mwh single spots. Multi-wing hairs are described as three or more hairs arising from a single cell; flare hairs are short, thick, stubbly hairs; and a twin spot has both multi-wing hairs and flare hairs.

MATERIALS AND METHODS

Treatment: Two hot dogs, one nitrite-free and the other with nitrite, were used as the experimental groups. I was not told at the beginning which one was

which, to remove the possibility of bias. The nitrite hot dog (#1) was Vienna brand, purchased from the Dill Pickle. The nitrite-free hot dog (#4) was a chicken hot dog. Both hot dogs had been broiled. There was a control group of untreated flies (+ control), fed regular Drosophila food. The hot dogs were liquefied and homogenized by Polytron, which vibrated them and made them into "soupy" mixtures. They were then mixed with 1 gram each of finely ground Drosophila food and 4 ml of water per vial. Yeast was added to the vials also. Flies that were 3 days old ± 3 hrs and heterozygous for flr^3/mwh were added to the food. The parents were the following genotypes: (♀) $\frac{+\ +\ flr^3}{+\ ser\ +}$ X $\frac{mwh\ +\ +}{mwh\ +\ +}$ (♂)

There were two groups of larvae:

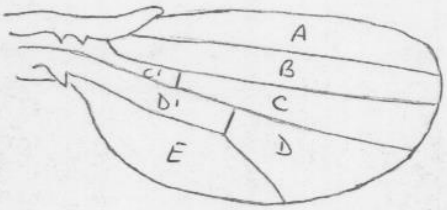
1. $\frac{+\ ser\ +}{mwh\ +\ +}$ and 2. $\frac{+\ +\ flr^3}{mwh\ +\ +}$

The first group was discarded because the project's stated purpose was to look at mutations of the flr and mwh genes.

Preparation of Wings: The Hot Dog #1 flies, Hot Dog #4 flies, and + control flies were stored in vials filled with 70% ethanol. For preparing the wings for microscopic analysis, the wings were separated from the body with forceps, transferred into Faure's solution (which is like glue) on a slide, and then lined up. Twenty wings were mounted on each slide. The slides were allowed to dry overnight in a dry heat box. Then, a large drop of Faure's solution was put on the wings and a coverslip was mounted.

The slides with coverslips were again put in the dry heat box in order to dry and harden the preparations permanently.

Microscopic Analysis of Wings: The wings were analyzed under a compound microscope at 400x (high dry) magnification. Only certain regions of the wing (A-E) were scored for mutant spots:



On the wing, one looks at the hairs, each of which are single cell processes. If the genetic markers, mwh and flr, are exressed, then the final differentiation of the hairs is altered. If not, then the hairs are wild-type, i.e. normal. Spot-initiating events change genetic information in one of the continuously dividing primordial wing cells in the larva or early pupa (Würgler, 1984, 157). A clone can form if the cell divides a number of times and the appropriate markers are phenotypically expressed. A large clone usually appears as a contiguous, noninterrupted spot. Size of the spot corresponds to the extent of mutation. Small single (1-2) mwh spots have high spontaneous frequencies. Large single (≥ 2) mwh spots have lower spontaneous frequencies. Twin spots have very low spontaneous frequencies.

DATA*

Hot Dog #1

Wings with spots

N	Type	n	%
275	s	240	88.2
	s 1-2	27	9.8
	s ⩾ 2	6	2.2
	t	2	.73

Hot Dog #4

Wings with spots

N	Type	n	%
272	s	251	92.3
	s 1-2	16	5.9
	s ⩾ 2	5	1.8
	t	0	0

+ Control

Wings with spots

N	Type	n	%
359	s	333	92.8
	s 1-2	25	7.0
	s ⩾ 2	1	.28
	t	0	0

*Classification Table symbols: s=0 mwh; s 1-2=small single spot, 1-2 mwh; s ⩾ 2=single large spot, ⩾ 2 mwh; t= twin spot
N= total # wings; n= # wings in Type spot

RESULTS

Since small single spots (1-2 mwh) have high spontaneous frequency even in the + control, I decided to just look at large single spots ($\succ$ 2 mwh) in order to conclude whether or not H.D. 1 and H.D. 4 are actually mutagenic relative to the + control. Following is a statistical analysis of the H.D. 1 and H.D. 4 populations using 2x2 contingency tables. Twin spots are analyzed separately.

	#Wings with $\succ$ 2 mwh spots	# Wings with no 2 mwh spots	Proportion with $\succ$ 2 mwh
H.D. 1	6 = a	267 = b	.022
+ Control	1 = c	358 = d	.0028
	7 = a+c	625 = b+d	

$$X^2 = \frac{N(ad-bc)^2}{(a+b)(c+d)(a+c)(b+d)}$$

i.e. $X^2 = 5.22$ at .05 significance level, and 1 degree of freedom

$X^2 \succ 3.84 \Rightarrow$ the 2 populations are different

Since $5.22 \succ 3.84$, the 2 populations are different. The probability that the data are consistent with the Null Hypothesis: that the 2 populations are not different is less than .025.

	#Wings with 2 mwh spots	# Wings with no 2 mwh spots	Proportion with 2 mwh
H.D. 4	5 = a	267 = b	.018
+ Conrol	1 = c	358 = d	.0028
	6 = a+c	625 = b+d	

$X^2 = 3.997$ at .05 significance level and 1 degree of freedom

Since $X^2 \geq 3.84$ the 2 populations are different. The probability that the data are consistent with the Null Hypothesis: that the 2 populations are not different is less that .05.

	# Wings with Twin spots	# Wings with no Twin spots	Proportion with Twin spots
H.D. 1	2 = a	273 = b	.0073
+ Control	0 = c	359 = d	.0000
	2 = a+c	632 = b+d	

X^2 = 2.62 at .05 significance level and 1 degree of freedom

Since $2.62 < 3.84$, the 2 populations are probably not different with respect to twin spots, which is consistent with the Null Hypothesis.

	# Wings with Twin spots	# Wings with no Twin spots	Proportion with Twin spots
H.D. 4	0 = a	272 = b	.0000
+ Control	0 = c	359 = d	
	0 = a+c	631 = b+d	

X^2 = 0 at .05 significance level and 1 degree of freedom

Since $0 < 3.84$, we Fail to reject the Null Hypothesis.

DISCUSSION

As can be seen from the results, Hot Dog #1, the one with nitrites, is more mutagenic than Hot Dog #4 because it induced more large mwh spots and twin spots (somatic mutations and recombinations respectively). X^2 analysis reveals that H.D. 4, which is nitrite-free, is also mutagenic rela-

tive to the + control (which was completely untreated), in inducing large mwh spots, but less so than Hot Dog 1. According to the data, the + control had a higher frequency of small single spots than Hot Dog 4, but this could be due to some inconsistency in reading and classifying small spots (since my data includes other people's data). Neither Hot Dog 1 or Hot Dog 4 are significant in inducing twin spots relative to the control, especially Hot Dog 4. It could be possible that Hot Dog 4 is slightly mutagenic, according to the X^2 analysis for large single spots, because of the broiling treatment. Broiling could also be responsible for some of the somatic mutagenicity of Hot Dog 1. Interestingly enough, Hot Dog 1 did induce twin spots (which are normally rare in controls), though the difference relative to the control is not significant according to the X^2 analysis.

BIBLIOGRAPHY

Würgler, F.E., U. Graf, et al. "Somatic Mutation & Recombination Test in Drosophila Melanogaster." Environmental Mutagenesis, vol. 6, 1984, pp. 153-188.

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