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Genetic stability under stresses expected in a space station environment: Effect of hypergravity and vibration in *Drosophila melanogaster*.

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Genetic and developmental systems will be challenged by new stresses when organisms begin to adapt to long-term habitation of a space environment, such as that on the International Space Station (ISS). Previous assays of mutation and chromosome damage in response to hypergravity or stress (Pence, 1999) have yielded conflicting results or have used extremes that are unlikely to be experienced in actual space exposures. Our initial ground-based studies in *Drosophila melanogaster* are designed to estimate mutation rates, aneuploidy, somatic mutation, and developmental stability under some of the stress exposures that organisms can experience in a space environment like that on the ISS. In addition to providing valuable information about genetic and developmental stability and about the capacity of an organism to adapt to a space environment, these experiments yield initial ground control data for possible multi-generation mutation rate experiments on the ISS or other space environment.

Experimental cultures were exposed to hypergravity and vibration stresses at NASA/Ames Research Center, and genetic breeding programs were then completed at the University of Oklahoma and at Bowling Green State University. The hypergravity conditions we have chosen to test first are near the high end of the range that organisms might experience on vehicle launch and travel. *Drosophila* are exposed to hypergravity using the 1-Foot Diameter Centrifuge (2 - 5 g), which is designed to maintain carefully regulated low-level hypergravity conditions for extended periods. Our treatments were typically 2-hour or 4-hour exposures, although pilot studies with other treatments not reported here have also been done. Vibration conditions are modeled on the mid-deck vibration of a shuttle launch (Figure 1). Vibration exposures (typically 5 minutes in duration) were done on a computer-controlled Vibration Table (20 - 2000 Hz). "Full Range" refers to a five-minute exposure to random vibration that has the cumulative frequency profile shown in Figure 1. Five-minute exposures to just low range (20-150 Hz), mid-range (150-1000 Hz), and high range (1000-2000 Hz) are also reported here.

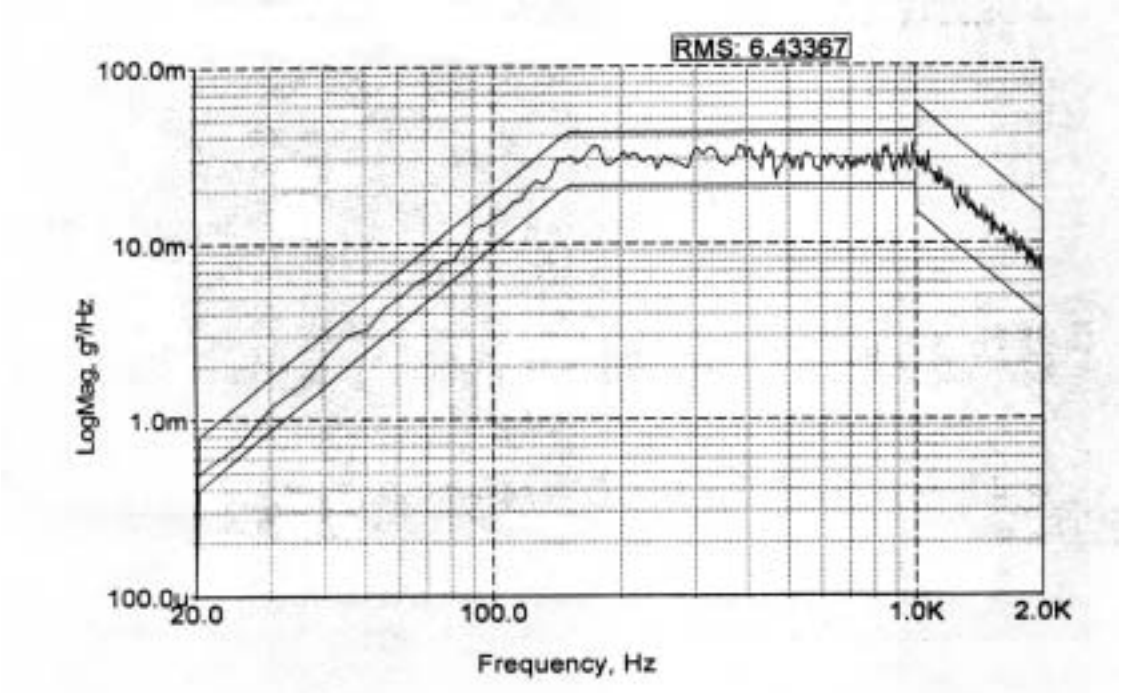

Figure 1. Random vibration profile (Full Range) as established in Interface Definitions Document NSTS-21000-IDD-MDK for a shuttle mid-deck. This representative profile printout is from an experimental treatment conducted in January 2001 (Log #2529).

Table 1. Nondisjunction in the *zeste* test: Vibration and hypergravity.

Treatment	Normal Adults	Exceptional		Total	% Exceptional
		Female	Male		
Control	99,788	7	12	99,807	0.0190
Hypergravity:					
2 g – 2 hr	17,089	0	3	17,092	0.0180
4 g – 2 hr	5,053	0	2	5,055	0.0400
5 g – 2 hr	24,762	5	9	24,776	0.0565[a]
5 g – 4 hr	114,627	12	31	114,670	0.0375[b]
Vibration:					
Full Range	79,143	5	22	79,170	0.0341[c]
20-150 Hz	17,265	0	4	17,269	0.0232
150-1000 Hz	17,102	2	7	17,111	0.0526[d]
1000-2000 Hz	7,828	1	1	7,830	0.0260
Total:		32	91	382,780	

[a] Normal Test, $P < 0.05$; Fisher's exact $P = 0.003$; $\chi^2 = 10.52$, $P < 0.005$
[b] Normal Test, $P < 0.05$; Fisher's exact $P = 0.008$; $\chi^2 = 6.29$, $P < 0.025$
[c] Normal Test, $P < 0.05$; Fisher's exact $P = 0.034$; $\chi^2 = 3.90$, $P < 0.05$
[d] Normal Test, $P < 0.05$; Fisher's exact $P = 0.015$; $\chi^2 = 6.87$, $P < 0.01$

The *zeste* test (Zim-mering, *et al*., 1990) is a genetic breed-ing program designed to detect chromo-some loss and gain in male and female progeny from parental fe-males exposed to stress or control condi-tions. Data from the *zeste* test (Table 1) show an in-creased rate of aneuploidy from nondisjunction in flies exposed to extended periods of 5 g (2h: 0.056%, $P < 0.01$; 4h: 0.038%, $P < 0.01$) and to vibration, particularly in the 150-1000 Hz range (full range: 0.034%, $P < 0.05$; 150-1k range: 0.053%, $P < 0.05$), compared to the control frequency.

Sex-linked lethal mutation rate estimates using the *Basc* balancer stock indicate about a two-fold increase at 5 g (2h: 0.28%, P < 0.05; but marginally non-significant at 4h: 0.24%) compared to 1 g controls (0.14%). The 2g, 4g, and vibration treatments may be slightly elevated, but are not significantly different form the controls at the present sample sizes. Additional replicates will be completed soon.

Table 2. X-Linked lethals: Vibration and hypergravity.

Treatment	Normal	New Lethals	Total	%
Control	10,859	15	10,874	0.1379
Hypergravity:				
2 g – 2 hr	2,848	5	2,853	0.1752
4 g – 2 hr	1,347	2	1,349	0.1482
5 g – 2 hr	6,105	17	6,122	0.2777[a]
5 g – 4 hr	10,307	25	10,332	0.2420[b]
Vibration:				
Full Range	2,606	5	2,611	0.1915

[a] Normal Test, $P < 0.05$; Fisher's exact $P = 0.036$; $\chi^2 = 4.07$, $P < 0.05$.
[b] Normal Test, $P < 0.05$; Fisher's exact $P = 0.056$; $\chi^2 = 3.04$, $P > 0.05$.

There is no experimental evidence for chromosome breakage due to either hypergravity or vibration stresses (Table 3), although there is significant chromosome breakage caused by gamma radiation, as expected.

In conclusion, it appears that exposures to some of the stress conditions that can be experienced in a space environment might cause an increase in genetic damage, but the degree of that damage is not necessarily very large. This might help account for some of the disagreement in results from earlier studies.

Table 3. Summary of spontaneous, gamma ray, hypergravity, and vibration induced chromosomal breakage in *Drosophila melanogaster* males using the hyperploidy test [C(1)DX, *y w f* females × treated Canton-S males] [scoring female progeny].

	# Breaks	# Chromosomes Scored	% Breakage
Spontaneous			
Total from 8 replicates	0	10,184	0
Gamma Rays			
1,000 R	2	275	0.73***
2,000 R	1	348	0.29*
4,000 R			
Total from 2 replicates	11	1,962	0.56***
8,000 R	8	866	0.92***
Hypergravity			
2g for 2 hours			
Total from 2 replicates	0	7,230	0
5g for 2 hours			
Total from 4 replicates	0	6,995	0
5g for 4 hours			
Total from 1 replicate	0	458	0
8g for 1 hour			
Total from 2 replicates	0	4,505	0
Vibration Full			
Total from 2 replicates	0	7,257	0

*Fisher's exact $P < 0.05$; ***$P < 0.001$.

Additional ex-periments are now being done to explore other treat-ment levels and stresses, such as continuous expo-sure to low level radiation, and possible interac-tion effects among these stress conditions.

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