Torgerson, Brian T., and James N. Thompson, jr. 2002. Measurement of fluctuating asymmetry in *Drosophila melanogaster* due to hypergravity stress. *Dros. Inf. Serv*. 85: 92-95.

Measurement of fluctuating asymmetry in *Drosophila melanogaster* due to hypergravity stress.

<u>Torgerson, Brian T., and James N. Thompson, jr</u>. Department of Zoology, University of Oklahoma, Norman, OK

It is easy to take for granted the environmental variables that seldom vary, such as gravitational force. But these become quite important when evaluating the ability of an organism to adapt to a radically different environment, such as that on the International Space Station or other future multi-generation space environment. A central goal of NASA's Life Science Division is to understand the effect of deviations from normal gravity on living systems from the cellular level to the whole organism. Opportunities to study the effects of microgravity are generally limited by space flight scheduling and appropriate habitat support. But hypergravity stresses, such as those associated with a shuttle launch, can be simulated in a variety of centrifuges and can provide insights to predict effects across the gravitational range. This pilot study was designed to explore the effects of hypergravity stress on developmental homeostasis, as reflected by changes in fluctuating asymmetry (FA), following a modest exposure to hypergravity.

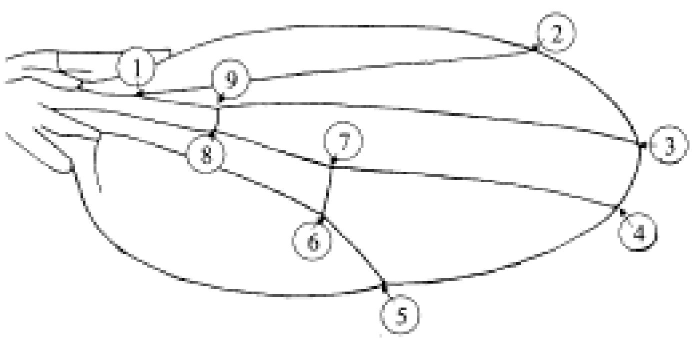

Figure 1. Nine landmarks digitized on the *Drosophila* wing to assess shape parameters. These yielded nine length measurements and three angles reported in Tables 1-4.

Stress can be defined as a factor that increases the "noise" in an animal's development, which could potentially create long term damaging effects. Environmental stresses like adverse temperature and nutritional limitations have been shown to affect its developmental homeostasis adversely, as measured by deviations from symmetrical development (Palmer and Strobeck, 1986; Hoffmann and Parsons, 1991; Parsons, 1990, 1992; Markow, 1994; Palmer, 1994, 1996; Møller and Swaddle, 1997). There is concern, however, that published results on fluctuating asymmetry principally report positive effects and may not be completely representative of the phenomenon. Recent attention has been paid to the sensitivity and statistical power of fluctuating asymmetry (Merila and Bjorklund, 1995; Whitlock, 1998; van Dongen, 1999; van Dongen, *et al*., 1999; Bjorksten, *et al*., 2000; Houle, 2000; Hoffmann and Woods, 2001), and we are incorporating these ideas into the design of a study of the effects of a space station environment on developmental stability. The work reported here is part of that pilot study.

We measured fluctuating asymmetry in the wings of progeny from stressed and control females to quantify their developmental stability. Virgin Canton-S wild type females were exposed to 5 g

Table 1. "Repeated Measurement" Effect of a 5g for 2 hours stress on fluctuating asymmetry for wing morphology measures in *Drosophila melanogaster*. ANOVA results for analyses of both the signed difference between left and right wings and the absolute value of that difference are represented here by their F values (d.f. for "Measures" = 1; d.f. for the "Residual" or error term = 432). Measurement abbreviations are defined in terms of the landmarks shown in Figure 1. (n = 10 pairs of wings per sex per culture tube).

Measurement	(L – R)/[mean(L + R)]		|(L – R)|/[mean(L+R)]	
	F	P	F	*P*
Length 1-3	0.07	ns	0.01	ns
Width 2-5	0.08	ns	0.22	ns
Length 2-3	< 0.01	ns	0.32	ns
Length 3-4	0.01	ns	0.03	ns
Length 4-5	0.01	ns	0.02	ns
Length 1-9	0.05	ns	0.18	ns
Length 7-8	0.03	ns	< 0.01	ns
Length 6-7	0.04	ns	0.01	ns
Length 6-5	0.10	ns	1.09	ns
Angle 2-1-3	0.04	ns	< 0.01	ns
Angle 2-1-4	0.07	ns	0.04	ns
Angle 2-1-5	< 0.01	ns	0.46	ns

Table 2. "Replicate" Effect of a 5g for 2 hours stress on fluctuating asymmetry for wing morphology measures in *Drosophila melanogaster*. ANOVA results for analyses of both the signed difference between left and right wings and the absolute value of that difference are represented here by their F values (d.f. for "Replicate" = 5; d.f. for the "Residual" or error term = 432). Measurement abbreviations are defined in terms of the landmarks shown in Figure 1. (n = 10 pairs of wings per sex per culture tube).

Measurement	(L – R)/[mean(L + R)]		|(L – R)|/[mean(L+R)]	
	F	*P*	F	*P*
Length 1-3	0.56	ns	0.70	ns
Width 2-5	1.36	ns	2.08	ns
Length 2-3	0.21	ns	0.90	ns
Length 3-4	4.18	<0.01	1.30	ns
Length 4-5	1.73	ns	3.27	<0.01
Length 1-9	2.47	<0.05	1.27	ns
Length 7-8	0.62	ns	2.13	ns
Length 6-7	0.55	ns	0.96	ns
Length 6-5	2.03	ns	0.98	ns
Angle 2-1-3	2.21	ns	0.63	ns
Angle 2-1-4	0.64	ns	0.40	ns
Angle 2-1-5	0.72	ns	1.70	ns

hypergravity for a period of two hours (a treatment abbreviated as 5g2h), using the 1-Foot Diameter Centrifuge at NASA/Ames Research Center, Moffett Field, California.

This centrifuge is a modified Beckmann with a sensitive controller that allows long-term maintenance of fractional low-level gravitational forces applied to specimens that can fit on a plate holder the size of a 96-well tissue culture dish. A similar group of virgin females was placed in the same type of containment but then left on a bench near the centrifuge. The treated and control flies were individually mated to Canton-S males to generate isofemale lines. A total of three 3-day broods of offspring were collected, but the present analysis focuses only on offspring from the first brood. The remaining broods are being stored in a 4:1 ethanol:glycerol solution for later study.

From the progeny of ten females exposed to 5g2h hypergravity, the left and right wings of 10 male and 10 female F1 progeny were removed and permanently mounted on microscope slides using D.P.X. mountant (Gurr Chemical Company). Similar slides were made from the progeny of ten control females; six broods of each condition were used for this analysis. Each wing was photographed with an Olympus C-4040Zoom digital camera mounted on a dissecting microscope. Images were then transferred to a computer, where nine wing

Table 3. "Treatment" Effect of a 5g for 2 hours stress on fluctuating asymmetry for wing morphology measures in *Drosophila melanogaster*. ANOVA results for analyses of both the signed difference between left and right wings and the absolute value of that difference are represented here by their F values (d.f. for "Treatment" = 1; d.f. for the "Residual" or error term = 432). Measurement abbreviations are defined in terms of the landmarks shown in Figure 1. (n = 10 pairs of wings per sex per culture tube).

Measurement	(L – R)/[mean(L + R)]		|(L – R)|/[mean(L+R)]	
	F	*P*	F	*P*
Length 1-3	12.29	<0.001	5.14	<0.05
Width 2-5	0.32	ns	8.50	<0.01
Length 2-3	0.20	ns	0.60	ns
Length 3-4	0.52	ns	6.58	<0.05
Length 4-5	1.69	ns	0.44	ns
Length 1-9	0.67	ns	<0.01	ns
Length 7-8	15.16	<0.001	0.20	ns
Length 6-7	2.85	ns	3.80	ns
Length 6-5	4.90	<0.05	0.32	ns
Angle 2-1-3	0.26	ns	0.16	ns
Angle 2-1-4	0.18	ns	5.11	<0.05
Angle 2-1-5	23.42	<0.001	6.10	<0.05

Table 4. "Sex" Effect of a 5g for 2 hours stress on fluctuating asymmetry for wing morphology measures in *Drosophila melanogaster*. ANOVA results for analyses of both the signed difference between left and right wings and the absolute value of that difference are represented here by their F values (d.f. for "Sex" = 1; d.f. for the "Residual" or error term = 432). Measurement abbreviations are defined in terms of the landmarks shown in Figure 1. (n = 10 pairs of wings per sex per culture tube).

Measurement	(L – R)/[mean(L + R)]		|(L – R)|/[mean(L+R)]	
	F	*P*	F	*P*
Length 1-3	0.25	ns	2.00	ns
Width 2-5	0.03	ns	11.52	<0.001
Length 2-3	13.27	<0.001	1.57	ns
Length 3-4	5.89	<0.05	0.01	ns
Length 4-5	0.88	ns	11.93	<0.001
Length 1-9	0.09	ns	36.56	<0.001
Length 7-8	10.72	<0.01	7.10	<0.01
Length 6-7	0.03	ns	1.56	ns
Length 6-5	1.60	ns	2.28	ns
Angle 2-1-3	10.06	<0.01	0.07	ns
Angle 2-1-4	0.91	ns	3.71	ns
Angle 2-1-5	1.40	ns	0.61	ns

landmarks were digitized (Figure 1) independently two times. Morphometrics software was obtained from the website of F. J. Rohlf at SUNY Stony Brook (http://life.bio.sunysb.edu/morph/; tpsDIG was used for data acquisition and tpsUtil provided the utility programs). Analysis of the data was performed using the Morpheus *et al.* program (Slice, 2000). The total sample was therefore 960 wing data sets (10 pairs of left and right wings per tube × 2 treatments × 2 sexes × six lines each × two independent digitizations).

The data for each of 12 linear and angular measurements derived from the nine landmarks were analyzed for four different factors: treatment, sex, replicate (*i.e.*, isofemale tube), and repeated measures (*i.e.*, independent digitizations) by ANOVAs that were programmed into an Excel spreadsheet (Sokal and Rohlf, 1969).

In Table 1, "Repeated Measurements", we can see the high degree of reproducibility in marking the landmarks. There were no significant differences between any of the repeated measurements. Table 2, "Replicate", shows only three where there was significant variation among isofemale lines within a treatment. Two of the differences were Length 3-4 and Length 4-5, which might be explained by variation in just the longitudinal vein 4 (L4). Thus, repeatability and replicate variation are low, indicating that significant differences among treatments are biologically relevant.

In Table 3, “Treatment”, nine different measurements were found to be significantly different between the 5g2h treatment and the control group. In three of these the differences were very highly significant ($P < 0.001$). Each reported difference comes from the F value of an independent ANOVA, but the pattern measurements themselves are not necessarily independent. Still, the data suggest that hypergravity stresses development in a way that affects wing shape, with the overall wing length and width changing significantly. There is also variation between sexes (Table 4), as expected due to differences in sizes of females versus males.

“Gravity shapes life!” This statement on report materials from the Life Sciences Division of NASA Ames Research Center, helps us remember that adaptability has some dimensions that many biologists have probably taken for granted when focusing on adaptation in an earth environment. But as space exploration opens new opportunities for life to expand beyond this planet, the effects of novel environmental stresses, such as deviations from 1 g, are important to understand.

Acknowledgments: We thank Jenna Hellack for helping program the morphometrics analysis. Ron Woodruff, Wade Dressler, Keith Anderson, Morsal Tahouni, and Matt Potthoff provided helpful discussions. We also thank Sharmila Bhattacharya, Max Sanchez, Ruth Globus, and others at NASA Ames Research Center for access to equipment and for their encouragement. This work is supported by NASA grant NAG 2-1427.

References: Bjorksten, T.A., K. Fowler, and A. Pomiankowski 2000, Trends in Ecol. and Evol. 15: 163-166; Hoffmann, A.A., and P.A. Parsons 1991, *Evolutionary Genetics and Environmental Stress*. Oxford University Press, Oxford, England; Hoffmann, A.A., and R. Woods 2001, Ecology Letters 4: 97-101; Houle, D., 2000, J. Evol. Biol. 13: 720-730; Markow, T.A., 1994, *Developmental Instability: Its Origins and Evolutionary Implications*. Kluwer Acad. Publ., Dordrecht, The Netherlands; Merila, J., and M. Bjorklund 1995, Syst. Biol. 44: 97-101; Møller, A.P., and J.P. Swaddle 1997, *Asymmetry, Developmental, Stability, and Evolution*. Oxford University Press, Oxford, England; Palmer, A.R., 1994, In: *Developmental Instability: Its Origins and Evolutionary Implications*. (Markow, T.A., ed.). Kluwer Acad. Publ., Dordrecht, The Netherlands; Palmer, A.R., 1996, BioScience 46: 518-532; Palmer, A.R., and C. Strobeck 1986, Ann. Rev. Ecol. Syst. 17: 391-421; Parsons, P.A., 1990, Biol. Rev. 65: 131-145; Parsons, P.A., 1992, Heredity 68: 361-364; Sokal, R.R., and F.J. Rohlf 1969, *Biometry*. W. H. Freeman and Company. San Francisco, California; van Dongen, S., 1999, J. Evol. Biol. 12: 547-550; van Dongen, S., G. Molenberghs, and E. Matthysen 1999, J. Evol. Biol. 12: 94-102; Whitlock, M.C., 1998, Proc. Roy. Soc. London, Ser. B., 265: 1429-1431.