DROSOPHILA BEHAVIOUR & GENE EXPRESSION IN ALTERED GRAVITY CONDITIONS: COMPARISON BETWEEN SPACE AND GROUND FACILITIES

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ABSTRACT

Previous experiments in space (unmanned satellites, space shuttle and the International Space Station, ISS), have shown that adult Drosophila flies change their motile behaviour in microgravity. A consistent increase in motility in space was found in these experiments, but mature flies (two weeks old) showed less increase than recently hatched flies. In the case of relatively long exposure to microgravity, the aging of male flies measured upon return to Earth was increased, with flies dying earlier than the corresponding in-flight 1g centrifuge or ground controls. The older flies, which experienced a smaller increase in motility, did not show this acceleration in the aging process. More recently we have performed comparative experiments using ground simulation facilities. Preliminary experiments using a random positioning machine (RPM) indicate that the effects of this simulation approach on the behavior of Drosophila are of smaller magnitude than the corresponding exposure to real microgravity. Further experiments are in progress to confirm this effect. However, when exposed to magnetic levitation, flies exposed to simulated weightlessness increased markedly their motile behavior compared with 1g controls both inside and outside the magnet. This altered gravity-related increase in motility was also less pronounced in more mature flies. This motility effect at the levitation position reproduces the results in real microgravity indicating the interest for space science of this simulation approach. Similar experiments are being performed in the Larger Diameter Centrifuge (LDC) located in ESTEC (the Netherlands) and indicate that 6g, 12g and 20g are key points in the hypergravity response in flies. Our experiments have shown that developmental processes from embryo to adult proceeded normally in the magnet, the RPM and the LDC. In terms of gene expression, preliminary results indicate that the affected set of genes under hypergravity responds in general in an opposite direction than that induced by the real or simulated microgravity exposure. The interest in conducting comparative parallel experiments in the complete spectrum of ground simulation methods is shown in the above studies and will be achieved in the near future.

1. INTRODUCTION

1.1 Previous results on Drosophila Aging on Space: Drosophila flies change their motile behaviour in microgravity in space. A consistent increase in motility was found in those experiments, but more mature flies (two weeks old) showed less increase in motility than recently hatched flies. The results of Drosophila Motility video-recording in the IML-2 experiment [1] indicates also that while the flies in microgravity walk very actively, they rarely jump (this is the initial movement to initiate flying).

1.2 Previous results on Drosophila gene expression on Space: Although very interesting, our results investigating changes in gene expression profile in space need to be validated in the same or at least similar conditions. Our preliminary results using simulated microgravity (RPM) or hypergravity conditions (10g midicar centrifuge) are also indicating novel changes in gene expression of exposed flies [2], but these results needs further validation using other molecular analysis. There exists an inverse correlation in the expression levels for all the genes present (MAS5) in samples exposed to RPM and to hypergravity conditions. This preliminary data, obtained in a single 10 g experiment, will be checked with the ongoing LDC experiments.

1.3 From Space to Ground Support Facilities: Our previous results in the "Gene" experiment performed in the ISS during the "Cervantes" Mission have made it essential to replicate similar experiments in European ground simulation facilities, some of them already ongoing.

Clinorotation: The RPM is an upgraded piece of equipment derived from the 3D clinostat. Gravity is neutralized almost completely or as required (0.16g as on the Moon, 0.36 g as on Mars) by submitting the samples to a randomly rotating movement that is selected by a computer program producing the selected level of residual gravity on the samples [3].

Magnetic levitation: This is an alternative approach for ground simulation of different gravity environments [4], complementary to RPM but affecting different molecular components in living organisms.

2. INITIAL *DROSOPHILA* RESULTS IN LDC

During April - June 2008, we performed studies in the LDC located in ESTEC [additional details in 5], the first goal being to establish key thresholds in the hypergravity response. Using the profile indicated in Fig. 1, and evaluating the flies' graviresponse in males and females separately, we have determined different behaviour patterns at 2g, 6g, 12g and 20g respectively. We have detected different behaviour precisely at these g levels and also slight gender differences (probably due to the larger size of females).

Future experiments, including behaviour and gene expression analysis using microarrays (whole *Drosophila* genome), will be performed with samples collected at various time points (from 12 h to 4 d) and developmental stages. The initial data about the overall survival and climbing ability of flies exposed up to 20g has been obtained, and is being analysed.

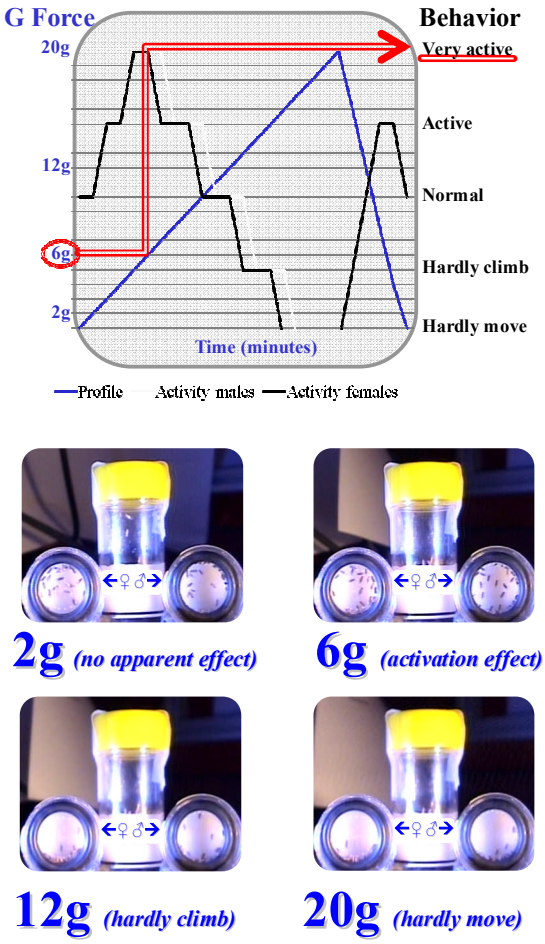

Fig. 1. Detection of threshold values for LDC experiments with Drosophila. Drosophila behaviour changes with the g force, first with an activation effect (6g) and then becoming slower because of their weight. Females show a poorer performance than males.

3. CONCLUSIONS

This is the first report of a systematic biological utilization of the new LDC facility, now fully operative and available for biological and physical science experiments in the ESTEC TEC-MMG section. Long term use of LDC, not only with *Drosophila* but also with the plant model system, *Arabidopsis*, will greatly support and justify further uses of this facility for the research community and promises to be a source of novel findings in the field.

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REFERENCES

1. Benguria A., et al. Microgravity Effects on *Drosophila melanogaster* behavior and aging. Implications of the IML-2 Experiment, *J. Biotechnol,* Vol. 47, 191–202, 1996.
2. Marco R., et al. Exposure to Microgravity in the ISS or in the ground produces an extensive change on gene expression during Drosophila pupae development. *Science* (Submitted).
3. van Loon J.J.W.A. Some history and use of the random positioning machine, RPM, in gravity related research. *Adv. Space Research.* Vol 39, 1161–1165, 2007.
4. Brooks J.S., et al. New opportunities in science, materials, and biological systems in the low-gravity (magnetic levitation) environment. *J. Applied Physics.* Vol 87, 6194-6199, 2000.
5. van Loon J.J.W.A., et al. A brief overview of animal hypergravity studies. *J. Grav. Physiol.* Vol 12, 5-10, 2005.