Understanding the changes that occur in living organisms to bring about adaptation to the space environment is essential to supporting future plans for long-term missions to the Moon and Mars. The European Modular Cultivation System (EMCS), a life science research facility developed by the European Space Agency (ESA), can serve as a habitat for culturing multiple generations of Drosophila melanogaster. Based on the results obtained from previously tested prototype hardware, a new Prototype Container III (PIII) was developed (Figure 1-A) and tested. Data from Prototype containers I and II were published previously (M.E. Sanchez, et al., 2004).

The primary differences between the new design and the previous models were: A. Increase in growth chamber volume. B. Modification of air holes in order to fit the holding frame for the Experiment Container or EC (Figure 1-B), C. Addition of stainless steel membranes to cover air holes, and D. Addition of a rubber band covering the junction between the growth chamber and food cylinder in order to prevent larval escape.

The goal of these experiments was to optimize growth of Drosophila cultures and to ensure biocompatibility of the cultures within the containers. During the development phase, modifications to the containers were made as required to resolve any suboptimal performance issues. Fly behavior, humidity levels, and hardware mechanics were assessed. Flies were grown in the containers using standard techniques for fly handling and videotaped using the video capabilities of the EMCS ERM (Experiment Reference Module) camera system.

Assessing optimal humidity levels within the growth chamber is critical for culture health. Adults could adhere to container surfaces and die if the humidity levels dramatically increase, while larvae might dessicate if humidity levels decrease. In order to find an optimal material that allows sufficient airflow and maintains normal levels of humidity, we grew flies in the containers using three different types of breathable membranes: stainless steel mesh, Nytex™ (nylon mesh), and a paper membrane. Growth in these conditions was compared to standard culture vials covered with the three different types of membranes (Figure 2).

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While all three membranes allowed air exchange (Figure 2), only the Nytex membrane covered prototype containers showed a reduction in progeny size (Figure 3). Since the integrity of the paper and Nytex membranes are affected by the humidity levels, the stainless steel membrane was selected for further experiments.

Selecting a food media that can withstand long-term storage without losing its nutritional value is also critical for space flight experiments. Making fresh media on orbit is impractical and would require significant crew time, therefore pre-prepared fly media will need to be stored for several weeks prior to conducting multi-generation experiments in space. In this test, four media recipes were made and stored in Mylar™ bags (Impak Corporation PAKVF 3.5M Silver, available from www.sorbentsystems.com). We tested the following four media recipes: A. Semidefined (1% Agar, 8% Brewer’s Yeast, 2% yeast extract, 2% peptone, 3% sucrose, 6% glucose, 0.05% magnesium sulfate, 0.05% calcium chloride, 0.6% propionic acid, 1% of 10% p-Hydroxy-benzoic acid methyl ester in 95% ethanol) B. Semi-
defined with cornmeal (same as semidefined with the addition of 60g of cornmeal) C. EMCS Fab Feast 1:1 (6.47% dextrose 6.47% molasses, 0.93% agar, 6.12% cornmeal, 3.24% yeast, and 2.0% Tegosept) D. EMCS Fab Feast 3:1 (Same as EMCS Fab Feast 1:1, with the exception of 9.7% dextrose and 3.23% molasses).

The media was prepared and packed under sterile conditions. In addition, we used proton beams at a range of 25-33 kgrays to sterilize a number of our samples. This was done through the company Nutek at Hayward California. The additional irradiation was conducted in order to assess if additional sterilization was necessary for samples that will be stored for long periods of time prior to use.

The data shown in Figure 3 shows that the food is usable for 2 months. We have also tested these media for longer periods of time, and find that the food can be stored in this way at room temperature for periods of over a year (data not shown) and still sustain fly growth.

Having completed a considerable amount of testing to optimize the Prototype Fly Containers, we proceeded to test the Prototype Containers within the Experiment Reference Model (ERM) hardware in order to optimize performance within the full flight hardware configuration. The ERM hardware provides the interfaces and environmental controls similar to the flight EMCS research facility that will eventually be used for conducting Drosophila research in space. Temperature levels were set at 23°C during the light cycle and 25°C during the dark cycle. This delta of 2°C was to neutralize the heat generated from the LED board immediately above the EC. Growth under these conditions was compared to standard culture vials that were maintained in our laboratory incubators.

The ERM was able to support fly growth (Figure 4). Both locations of the EC, top and bottom, produced nearly the same numbers of flies. These positions of the hardware were described earlier in Fig 1B. Temperature and humidity levels were monitored throughout the experiment (data not shown). About three days before the 1st generation emerged, we removed the parental population. Figure 4 shows the counts from the 1st generations that emerged from each container.

**Summary**

A prototype container has been developed for conducting experiments with Drosophila melanogaster in the EMCS hardware on the International Space Station. This container is capable of supporting larger populations of flies than standard laboratory vials, as depicted in figures 2 and 4. Our studies have helped optimize humidity levels, food formulations, and container materials in order to effectively support fly populations in this hardware. Tests that should continue in the future include multigenerational growth testing and the development of sampling devices.