Hardware Prototype and Remote Operations Testing in Preparation for Multiple Generation *Drosophila* Experiments on the International Space Station.

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FOREWORD

This document has been prepared and submitted under NASA Contract No. NAS2-14263, Task Order SSBRP 3.0-2, Subtask 3 and is a part of the Space Station Biological Research Project (SSBRP) documentation at Ames Research Center, Moffett Field, California.

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Note: All Geneve and Taipei web pages and images referenced in this paper have been relocated and can now be found at: http:// spaceprojects.arc.nasa.gov/space_projects/flylab/flyhome.html

Acronym Table

CDS – Communications and Data Systems
CSA – Canadian Space Agency
FCU – Fly Collection Unit
HTML – Hypertext markup language
MG – Multiple generation test group

MGSU – Multiple generation separation unit
NASA – National Aeronatics and Space Administration
PI – Principal Investigator
RH – Relative humidity
SB – Stock bottle test group
SHARP –Summer High School Apprenticeship Research Program
SSBRP – Space Station Biological Research Project
STELLAR – Science Training for Enhancing Leadership and Learning through Accomplishments in Research
UOF – User Operations Facility
VD – Food volume and depth test group
WWW- World Wide Web

INTRODUCTION

Life sciences research during the Space Station era will allow for much longer experiment duration than was ever possible on the Space Shuttle. The capability of having a full time laboratory in space will allow biologists to study the effects of long duration exposure to the space environment on biological systems. In order to conduct long duration experiments with insects in space, fruit flies in particular, a suitable method must be devised to separate successive generations of flies for planned 90 day Space Station increments.

NASA plans to provide a habitat that will allow for separation of successive generations on orbit. This capability is required by the international insect science community so that multiple generation experiments can be conducted on long duration Space Station increments (Givens and Wade, 1996). Several methods of separating generations have been examined by the Space Station Biological Research Project and other groups (Pence *et al.*, 1994; Fish *et al.*, 1995; Pence, 1996a). Based on test results, a natural selection method was eliminated because it relies on the insects' attraction to light or some other locomotive behavior. This method is dependent upon the behavior, which may not be reliable and may not work with all strains of a species. A specific example of this problem could occur with a gene expressed in the larval stage of development known as the foraging (*for*) gene in *D. melanogaster*. There are two types of alleles for this gene: rover (a dominant allele) and sitter (a recessive allele). This locus is also acted upon by other modifier genes and the environment. The *for* gene is responsible for the larvae either migrating through medium or staying localized in one area of the medium (Sokolowski and deBelle, 1990). Therefore, a technique that relies on larval motility would not be desirable. If a larvae separation technique was utilized to allow larvae to migrate through food from one container to another container, the technique may select for or against groups of genes that are linked to the same chromosome as the *for* gene. Similar situations could occur with other techniques that rely on light attraction, smell attraction or some other behavioral response.

Two previous tests were completed using a mechanical method multiple generation separation unit (MGSU) prototype. Separation of generations in the first test failed due to a behavioral preference of the females to deposit eggs into the old food cylinder rather than into the new food cylinder that was being provided. The second test used food with a furrowed surface and was successful in stimulating the females to lay eggs onto the new food in the outgoing cylinder. The second test succeeded in separating generations, but demonstrated that overcrowding during development was due to overpopulation and a finite amount of food. The study found that access to the new food must be controlled to maintain a stable population (Pence, 1996a).

The objectives of the third test were to better define the method of separating multiple generations of fruit flies by developing a food slide cover mechanism or other suitable method that limits access to food during larval development and egg deposition, and helps to control overpopulation and mixing of generations. Other factors also necessary in conducting multiple generation experiments in space include evaluating remote video monitoring to observe development and determine separation time of generations, and evaluating techniques, collection prototypes devices, and operations which would simulate the process astronauts would use to collect adult flies and return them to Earth.

MATERIALS AND METHODS

MGSU Prototype

The MGSU prototype is based on a sequential specimen container configuration joined by a food filled cylinder system (Figure 1). The MGSUs are composed of two parts: a specimen container top and a medium cylinder base. The specimen container top is composed of five 48 mm × 33 mm × 50 mm (79 cc) Lexan sheet cut containers arranged in a "staggered" configuration. The medium cylinder base is constructed from solid Lexan and holds five medium cylinders in parallel. Each cylinder contains a nutrient medium volume of 10 cc (20 mm depth) and an exposed surface area of 3.65 cm^2. Six MGSUs and one spare were used in the test. Each specimen container, except the last, provides space for two food cylinders. One cylinder is the incoming cylinder that brings in egg laden food and provides the same food for the developing larvae. The second cylinder is the outgoing cylinder. It is also filled with food and is used as a receptacle to collect eggs deposited by the newly developed and matured females. When adults become mature, the old food is covered and the new food is exposed. Once the females deposit their eggs, the second cylinder is moved to a new container.

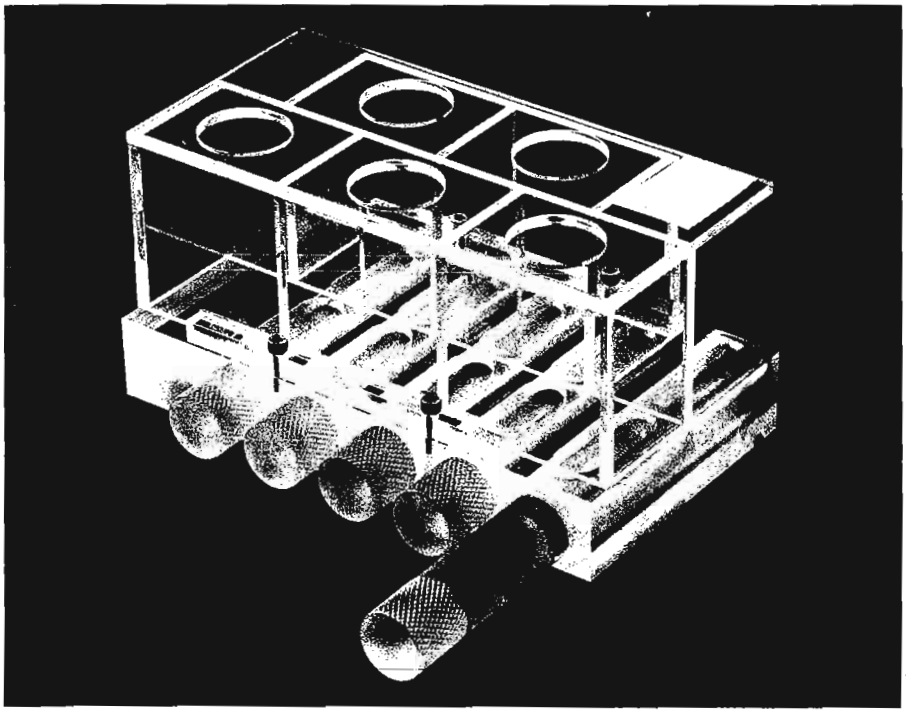

Figure 1. Multiple Generation Separation Unit (MGSU) prototype.

World Wide Web Data Collection System Development and Testing

To simulate the Insect Habitat space data collection operations and to simulate data collection by the principal investigator (PI), a web-based environmental and video data collection system was developed (Sun *et al.*, 1996). For the multiple generation separation unit (MGSU) prototype testing, the plan was to simulate data collection on orbit and use the images to monitor fly development and to make operational decisions regarding movement of food cylinders. The system is comprised of computer hardware, as well as software components, and several laboratory instruments.

The hypertext markup language (HTML) user interface for the development of the web site pages was designed using the WebMagic application (Silicon Graphics, Inc., Mountain View, CA) and BBEdit for Macintosh (Bare Bones Software, Bedford, MA). A Java applet to view real-time environmental data was compiled using Java Development Kit for SGI Irix (Sun Microsystems Inc., Santa Clara, CA). The web site was maintained on a SGI Indy workstation (Taipei: 100 MHz MIPS R4000 processor, 64 MB RAM running Irix version 5.3) in the insect laboratory with video data routed to the main computer collection system loaded on the SGI workstation Geneve in the User Operations Facility (UOF) at Ames Research Center. The data collection system on Geneve is a searchable database (Oracle Inc., Redwood City, CA). Video data was

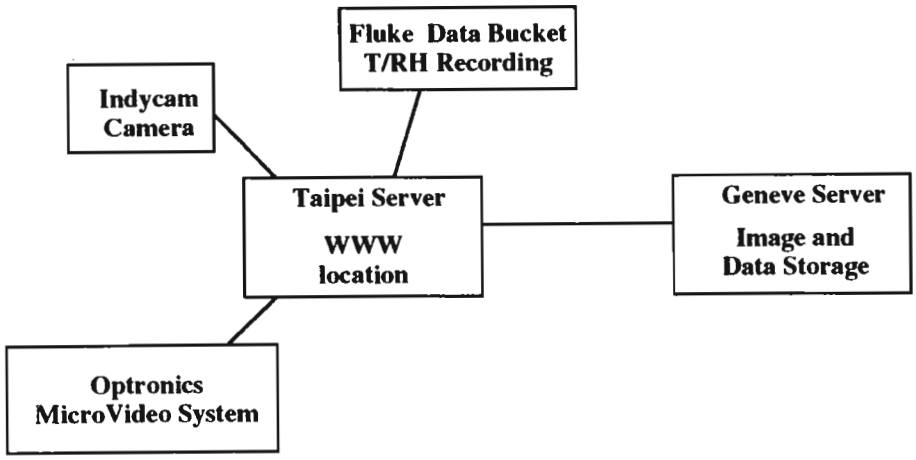

Figure 2. WWW system schematic.

collected by a SGI Indycam (Model # CMNB006C, Silicon Graphics, Inc., Mountain View, CA) and the environmental data (temperature/ humidity) was collected by Optronics dual probes (model # HT46R, Rotronic Instrument Corp., Huntington, NY) and stored via a Fluke Hydra Data Bucket (Model 2635A and 2620A-100 Input Module, John Fluke Mfg. Co., Inc., Everett, WA) to a PCMCIA card (IC Memory Card, Prod # 927512 256 KB Seiko Epson Corporation, Torrance, CA) and converted on Microsoft Excel. Later testing data was routed to Geneve and embedded with the collected images by a Common Gateway Interface shell script (Appendix I). The video and environmental data system was tested and images were collected during the MGSU food isolation mechanism test (Figure 2).

During functional testing, the video camera was positioned over the multi-generation slide cover test unit and images were collected at 20 minute intervals. The collection interval was remotely adjustable through a password-protected section of the web site. Prior to experiment use, the camera system was examined for proper lighting, options for camera positioning, necessary frame rate and resolution of images. The web site was tested for ease of use and accessibility of data and images.

Magnified Video Imaging

In addition to the Indycam video imaging system, a video microscope camera system (VI-470 CCD Video Camera System and VI-470 Camera Head, Optronics Engineering, Goleta, CA) (PVM-1351Q Video Display, Sony Electronics, Park Ridge, NJ) (Haines, 1994) was connected to the World Wide Web data collection system to test the feasibility of using magnified video on *Drosophila melanogaster* to visualize the Drosophila. A Unix shell script (Appendix II) was added to the site to simultaneously capture video from the Indycam and video micro-camera to support the multi-generation test and magnified video images collected. Images were collected over a five day period of a metamorphosing pupa in a 15 mm × 30 mm Petri dish positioned in front of the 10x objective and stored on the Taipei server. Movies were created using SGI IRIS Movie Maker 2.1 then converted to mpeg using mv2mpg shareware created by Andreas Paul (1996) http://wwwzenger.informatik.tu-muenchen.de/persons/paula.html.

Adult Fruit Fly Anesthetization and Collection Unit Prototype (FCU) Testing and Collection Operations Development

To better understand the equipment required and the amount of time necessary to collect flies, a simple prototype system was constructed to anesthetize and remove adults from the MGSU (Figures 3 and 4). The system consisted of two components: an anesthetic section and a vacuum section. The anesthetic section consisted of a small hand-held bicycle tire inflator device (Superflate, Innovations in Cycling Inc., AZ) that dispensed compressed CO_2 from a gas cylinder (CO_2 Powerlet -Model 2311, Copperhead Co., East Bloomfield, NY) via a gas flow regulator (Nupro Co., Willoughby, OH). The vacuum section was comprised

of a commercial mini-vacuum (Model # 440, MicroComputer Accessories Inc., Ingelwood, CA) and an insect aspirator (Bioquip Products, Gardena, CA). Adult flies were collected in snap cap 1.5 ml microcentrifuge tubes (Applied Scientific, South San Francisco, CA) that were attached to the modified aspirator. A small "finger" brush was also constructed from a small paint brush to aid in collecting flies stuck in crevices and corners of the test units.

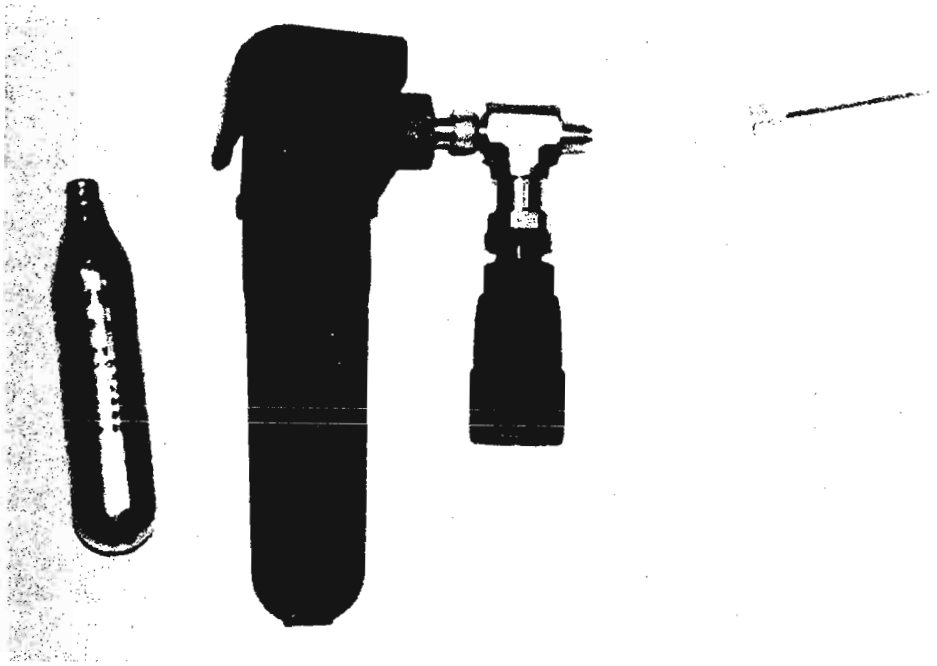

Figure 3. Anesthetization section of the FCU with CO_2 cylinder at left.

Figure 4. FCU prototype hardware. Test vacuums (upper left, center, and upper right) were used during prototype testing. The unit on the right was selected for the multiple generation test. The anesthetization unit (lower left) and 1.5 ml collection vials (lower right) are shown.

Several vacuums were tested prior to the final system selection for tne FCU system manufacture and functional testing (Model # MS-4, Metropolitan Co., Suffern, NY). Operation of the system was practiced prior to the multi-generation test and during the food tests.

During the integrated multi-generation test, two "crew members" conducted the "on-orbit" adult collection while a third observer recorded the times for each operation. Operations were split into functional categories. The shared tasks for all groups were sterilization of the hood, readying the units, gassing the flies, removing the flies, replacing the fly collection vial, and sterilizing the lab work bench after each collection. Tasks included moving cylinders, sterilizing the new chamber covers, and replacing the CO_2 cylinders. This data was collected to give an estimate of how much crew time will be necessary to perform an adult collection during a multigeneration experiment and gauge the level of difficulty it may take to complete a specimen collection task in orbit. No attempts were made to accelerate tasks during collection procedures and procedures were not practiced between collections. Operations were performed by the same personnel at each collection interval.

Food Isolation Mechanism Test

Due to their small size, fruit fly larvae are capable of squeezing through very tight spaces to gain access to food. This behavior was observed during medium volume tests when larvae squeezed between a container wall and a food retaining wall of less than 0.5 mm. The food isolation test was conducted to verify a possible method of eliminating access of the developing larvae and adults to new food.

To conduct the test, one of the prototypes was fitted with five square Lexan sheets (dimensions: 2.3 mm × 3.3 mm × 0.3 mm and cover rail dimensions: 4.7 mm × 0.3 mm × 0.5 mm) in each of the five sequential generation specimen chambers. The assessment criterion was set that if even one larva breached the slide cover, the current slide cover would be considered unsatisfactory for the complete five generation test. The prototype was equipped with the food isolation covers to limit access to the food and a second prototype was equipped only with food cylinders that were rotated 180° to limit access to the food. Four groups of one hundred adults (Oregon R wild type, P: 60 females/40 males) were each housed in the two separate specimen containers in the two multiple generation prototype units. Flies were raised and housed in a temperature and lighting controlled incubator (L:D 12:12, 24.9 ± 0.4 SD °C, 52.7 ± 11.3 SD % RH). RH was not controlled. The adults were allowed to mate and lay eggs for twenty-four hours with the intention that the female adults would lay enough eggs to create an overcrowded environment. The adults were removed with the collection prototype and the embryos were allowed to develop through the adult stage (fourteen days). The developing larvae were observed and video images were recorded with the World Wide Web video collection system to observe possible larval breach of the food isolation cover.

Integrated Five Generation Test

The multiple generation separation unit five generation test included biological testing of the MGSU and FCU prototypes, use of the web video data collection system, and performance of crew operations.

The MGSUs were biologically tested for information about population dynamics and development over five successive generations by collecting data on adult number, adult female group mass, and total development time for each generation. Adults were counted and sexed at the end of each generation and the females were dried and weighed *en masse*. Flies that were stuck in the food during transfers or collection, or pupae that were uneclosed at time of collection, were counted in the group population totals, but were not included in the female mass measurements. Development was scored by the appearance of a development stage in each test container at lights on each morning and in the late afternoon. Each measured parameter was used to assess possible stress conditions of overcrowding (Ohba, 1961). Statistical analysis was completed using unpaired student t test between and within the test groups. The prototype was also assessed for its ability to maintain a stable population by controlling food exposure through all five generations. The minimum criterion was one mating pair in each prototype to perpetuate generations.

Two other groups were maintained during the five generation test: a food volume and depth (VD) group and a standard *Drosophila* stock bottle group (SB). The VD control group consisted of six acrylic containers (TAP Plastics, Mountain View, CA) with a 40 mm × 40 mm × 72 mm outer dimension and a 100 cc volume 20 mm depth medium (10 cc), and 10 initial adults (6 female: 4 male). The SB group consisted of six standard laboratory polypropylene *Drosophila* containers (Applied Scientific, South San Francisco, CA), 103 mm height, 55 mm width at the bottom, tapering to 35 mm at the lid, with a total volume of 170 cc, 20 mm depth medium (45 cc) and 10 initial adults (6 female: 4 male). The *Drosophila* stock bottle container (SB) was assumed as the control container for housing flies. The multi-generation prototype is based on a system of moving food with newly laid fly eggs on the food surface. The control containers are based on an adult moving scheme allowing adults to emerge, then moving the new adults to new food containers. Due to the differences between the multigeneration prototype and the "controls," the plan was that the two groups would be coupled to one another chronologically to account for differences from generation to generation when adults emerge, when they are exposed to the new food, and when the food cover and cylinder is moved.

Similar container covers were used for both test units and controls. The container openings were covered with a small sheet of acrylic fitted with a small foam filled slit to allow air flow and retain container food moisture. Sterile technique was used throughout the experiment. For the parental (P) generation of the MGSU and the control groups, flies were removed with normal collection techniques and tools, and not the FCU.

All food was made prior to the start of the 5 generation test. For the multi-generation cylinders, food was cooked, placed into cylinders while melted, wrapped in aluminum foil, taped together by generation of use, placed into an autoclave bag, then autoclaved. Stock bottles were filled with melted cooked food, plugged, autoclaved, then sealed with parafilm after cooling. Volume and depth control containers were ethanol sterilized, filled with sterile food, then parafilmed after cooling. All food was stored at 5°C until use.

Mature, axenic (microbe-free) adult fruit flies (5-10 days old) were utilized (Oregon R wild type strain, Mid-America Stock Center, Bowling Green, OH) and the experiment was conducted in an incubator at 24.9 ± 0.5°C, 38.5 ± 10.7% uncontrolled relative humidity, and an environmental lighting regime of light:dark 12:12 (Pence and Hult, 1997). Flies were reared and tested on a nutrient medium of cornmeal (Sigma C-6304) 135 g/L, Grandma's unsulfured molasses 100 ml/L, and *Candida utililis* yeast (Sigma YCU) 40 g/L, agar (Sigma A-9915) 20 g/L, p-hydroxy benzoate methyl ester (Sigma H3647, 10% in 95% EtOH, 1.5 ml/L) 15 ml/L and propionic acid (8 ml/L) in distilled H_2O.

Mating groups (6 females: 4 males 10-12 days adult age) were placed into the six test units and removed 24 hours later. Two groups of controls were also simultaneously started; the food depth/volume (VD) control and a *Drosophila* stock bottle (SB) baseline control. The video camera was set on unit #5 and image collection was set at a 20 minute interval.

RESULTS AND DISCUSSION

World Wide Web Data Collection System Development and Testing

The insect web data collection system was developed jointly between the User Operations Facility Computer Data System group and the insect science lead in an iterative process. The core functions of the web site consisted of a database query form (Figure 5) and a selectable functions page (Figure 7). The information that simulated "on orbit" data retrieval was the habitat monitor page (Figure 6) which listed the "habitat" camera image, data sample time, temperature, relative humidity, the MGSU #, chamber #, and notes of the image.

The selectable functions page allows the PI to select several options of data visualization, image frequency and data collection, "snapshot" imaging, instantaneous image and data collection, and graphic data visualization. The automatic image and data collection function allows the PI to select how often images are automatically collected and conceivably could control other habitat functions, such as temperature and lighting cycle. The collection frequency could be programmed from minutes to years. For the MGSU test, the image collection frequency was set to collect images automatically at 20 minute intervals. Increasing or decreasing

frequency is possible with the current system; however, there is a limit to data storage space. The snapshot image and data collection allows the PI to collect image and data instantaneously. Once the habitat monitor page is retrieved, the PI can add notes about the image, then store the image in the database. With the instantaneous data option, the habitat monitor page is "streamed" approximately every 10 seconds, without storing the data to the database. The graphic database option allows the PI to view the environmental data over a period of time.

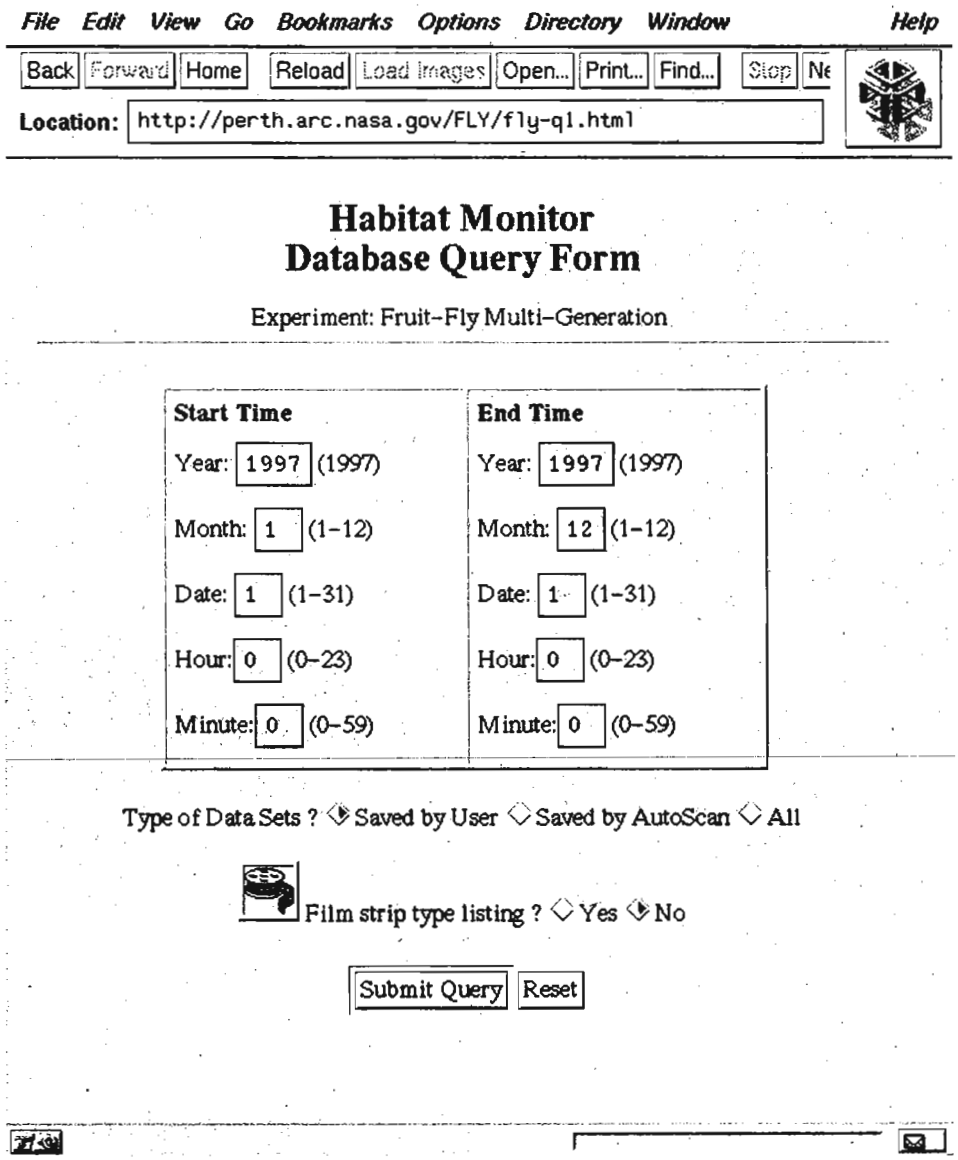

Figure 5. Database Query Form.

The database query form is contracted to allow the PI to retrieve all stored habitat monitor pages. The page is set up to allow the PI to select the start and end time of data to retrieve by year, month, day, hour and minute and gives options to collect autosaved or snapshot image data and the option to view the images as a film strip. (All habitat monitor pages for the food isolation mechanism test and the MGSU test are available through the database query form page on the Space Projects server. Images from the food isolation mechanism test are retrievable from (test dates: 9/17 - 10/1, 1996) and images from the MGSU test are

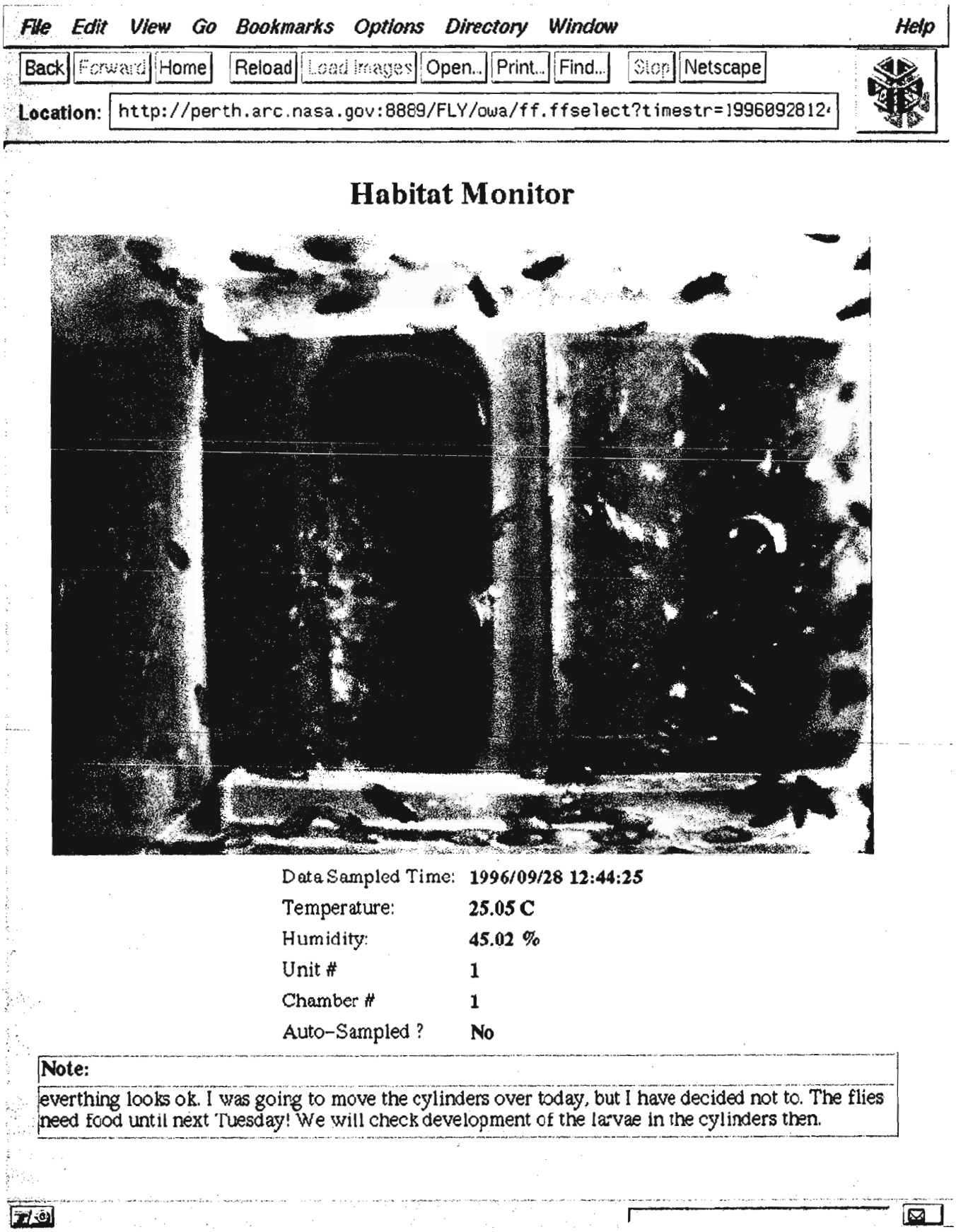

Figure 6. Habitat Monitor Page.

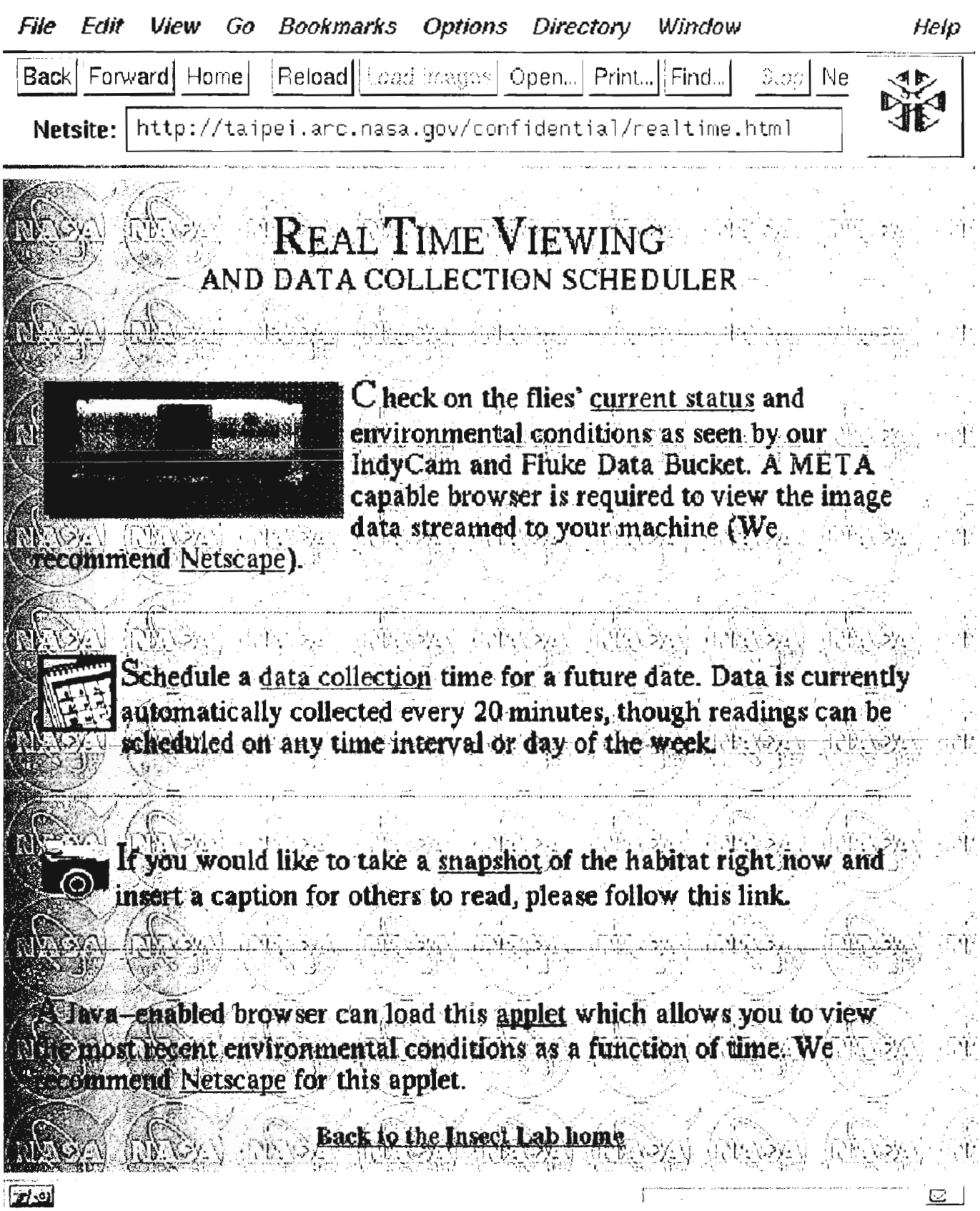

Figure 7. Selectable Functions Page.

retrievable from (test dates: 12/11, 1996 - 2/7, 1997).) A time-lapse video was made from the food isolation mechanism test collected images and is available on the Space Projects server (larvae.mpg).

World Wide Web System Performance

The data retrieval worked very well, but typing in the dates on the database query form was less than optimal and the numbers were reset to the lowest number in each group each time the page was reloaded. It may be better to set up the page as a mouse clickable set of numbers. During set up of the system, a conflict was found between the web data collection system and the laboratory temperature/humidity collection system. The Fluke Data Bucket was not designed to send data to more than one output and therefore could not send data to the PCMCIA card and send data to the web system simultaneously or even sequentially. The temperature and humidity data was critical for documentation of the incubator environment during the MGSU test. Due to the experimental nature of the web system, and as a safety precaution, the temperature/humidity data was not sent to the web data collection system. The realtime data transmittal to the web site was achieved successfully after completion of the multi-generation test. There were some reliability problems during the test including the loss of the main server one day, although collected images could be retrieved at a later date, and a data file problem at the start of the new year, in which all images were lost for approximately a 24 hour period, during a critical development period of the test. There was also a time stamp problem one day during the test, which caused no problem to the image collection, but did cause some confusion in temporal review of the images. Reliability is extremely important and the new system needs to be fully tested prior to system use in flight operations.

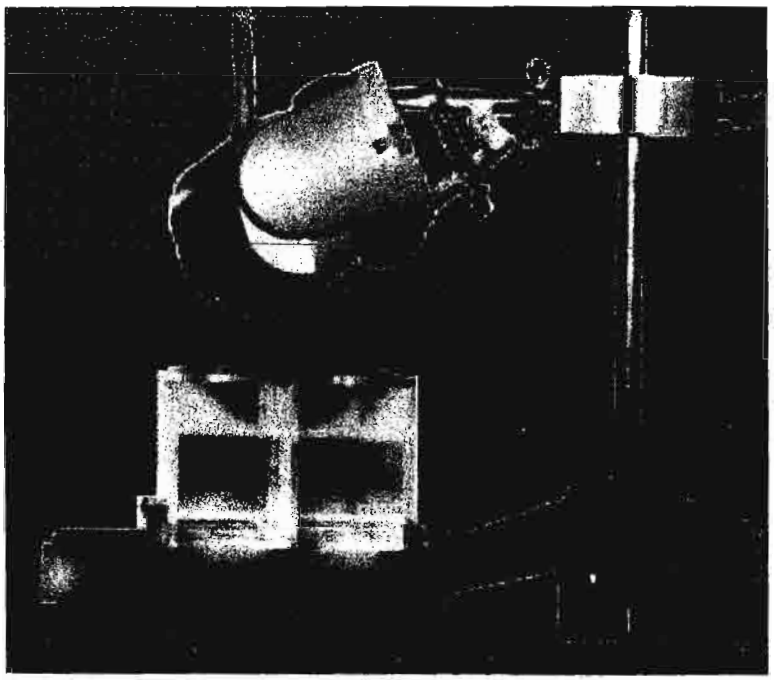

Figure 8. Indycam camera and MGSU configuration.

The Indycam camera images were of satisfactory quality, and lighting and positioning were adequate to observe fruit fly development. There were some difficulties with focusing the camera, maintaining color balance, and line dropouts of the images. The camera was focused by screwing the lens in and out of the camera, but it was difficult to get the exact focus needed to view developing larvae in the food. Autofocusing would have been desirable. For a space based system, serious consideration will have to be given to image quality. For the tests, the camera was positioned over the test units and a small foam filled acrylic plastic sheet (6 cm × 3 cm) was used to allow viewing of the test chamber and to allow passive air flow through the chamber (Figure 8).

An acrylic sheet was used on all the test units to allow image collection of the other units as needed. To maintain the proper image color, the camera was white balanced using camera software to return the camera to proper color after spectral drifting as needed. Drifting was usually caused by camera exposure to laboratory fluorescent lighting when the incubator door was opened, but the camera also exhibited an inherent drift. Another problem that occurred with the Indycam system was line

dropout of some of the images which resulted in reduced image quality. The reason for the dropout was unknown, but was probably caused by the supporting camera software.

Aside from the functional and feasibility testing of the web system, educational information was added to the web site by the addition of other html pages. Information added to the site includes past insect flight history and literature references, STELLAR (Science Training for Enhancing Leadership and Learning through Accomplishments in Research) and SHARP (Summer High School Apprenticeship Research Program) laboratory interns information, and project documentation. Links were made to the Canadian Space Agency (CSA); an international Insect Habitat development partner, NASA sites, and other fruit fly web pages.

The web site has also been extremely helpful in providing information to the CSA and international insect science advisors. This page has also been linked to the SSBRP home page and to Summer 1996 STELLAR teacher Judy Jones' classroom web site, to aid in Mrs. Jones' presentation to students of lessons prepared at NASA ARC.

Magnified Video Imaging

The magnified video imaging system was not used on the MGSU test units due to the small focal plane of the camera and close focusing range of the lens, the size of the MGSU containers, and the space needed for the Indycam. Images were collected of a stationary metamorphosing pupa in a 15 mm × 30 mm Petri dish. Time-lapse movies of the developing pupa were created and are available on the Space Projects server (pupdev.mpg, pupdev2.mpg). Magnified imaging of stationary specimens is probably feasible on orbit, and may be required for some developmental studies. 10× magnified imaging was adequate for visualization of a single fruit fly pupa. For mobile targets, however, a target tracking and focusing system would be necessary.

Adult Fruit Fly Anesthetization and Collection Prototype (FCU) and Collection Operations Development

Prototype Trials

The CO_2 gas system anesthetized groups of adult flies in stock bottles within 3-5 seconds and then flies were given a 30 second dose to maintain the anesthetic effect for collection. The flies stayed anesthetized for approximately 2-3 minutes after gassing. The total collection time for a single chamber was approximately 2 minutes, which was faster than expected.

Fly Collection During the Food Isolation Mechanism Test

On the second day of the food isolation mechanism test, fly collecting procedures were practiced to test the FCU. The FCU worked well, except for repeated activation of a motor shutoff switch. The switch was activated automatically when the motor overheated, due to continual high suction pressure created when collecting the flies in multiple containers. During the test, the operator had to wait for the motor to cool before the motor could start again. After the test, a carburetor was added to the vacuum line so that vacuum pressure could be controlled manually. This increased vacuuming time and the motor would still occasionally shut off. A more durable motor is needed for this task.

The average collection time of flies during the functional test was approximately six and one half minutes per multiple generation unit for the parental generation (Table 1) and two and one half minutes for the F1 generation (Table 2). Vacuum motor overheating created the delay between the P and F1 times. In the P generation only 1 gas cylinder was used, but during the F1 generation 1 cylinder changeout occurred. It required 2 minutes for cylinder changeout.

Table 1. Parental (P) generation FCU collection data.

Test Unit/chamber	gas time	tube change	vacuum/overheat?	total time
Unit 1/chamber 1	1.5 min.	1 min.	4 min./Y	6.5 min.
Unit 1/chamber 2	2 min.	1 min.	4 min./Y	7 min.
gas cyl. change	0 min.	N/A	N/A	0 min.
Unit 2/chamber 1	2 min.	1 min.	3 min./Y	6 min.
Unit 2/chamber 2	2 min.	0.75 min.	3.5 min./Y	6.25 min.
Average time	1.9 min.	0.9 min.	3.6 min.	6.4 min.
Total time				25.8 min.

Table 2. 1st Familial (F1) generation FCU collection data.

Test Unit/chamber	gas time	tube change	vacuum/overheat?	total time
Unit 1/chamber 1	1 min.	1 min.	1 min./N	3 min.
Unit 1/chamber 2	0.5 min.	0.75 min.	0.75 min./N	2 min.
gas cyl. change	2 min.	N/A	N/A	2 min.
Unit 2/chamber 1	0.5 min.	1 min.	0.75 min./N	2.25 min.
Unit 2/chamber 2	0.8 min.	1 min.	0.8 min./N	2.6 min.
Average time	0.7 min.	0.9 min.	0.8 min.	2.5 min.
Total time				11.9 min.

When using the fly collection prototype, fly collection was easier in the MGSU lacking the food isolation mechanism, although there were no large differences in the collection times. The food isolation cover rails created crevices for the adults to hide in, which made it difficult to remove the flies. After the test, a small "finger" brush was constructed to aid in collecting flies that were stuck in crevices and corners of the test units. An advantage of using the compressed CO_2 gas in the non-slide modified units was that you could use the gas stream to blow all of the adult flies into a corner, which made it extremely easy to vacuum them and you would not need the small brush.

Also during the test, there were some problems with flies becoming stuck in the vacuum tubing when the flies were vacuumed. To overcome this problem, clear tubing was added to the vacuum line to make viewing of the flies easier as they were vacuumed. The clear tubing was later used during the five generation integrated test.

Biological Testing of the Food Isolation Mechanism

The food slide cover mechanism failed to stop the developing larvae from entering the new food cylinder. As the overcrowded larvae population developed and exhausted the food supply in the first cylinder, the larvae began searching for another source of food. Many larvae were able to crawl underneath the cover and access the food (Figure 9). The time-lapse video also shows that the larvae were able to physically move the slide to the side when locomoting between the slide and the chamber wall. Individual images of the larvae breaching the slide are available at the Space Projects web site (test date 09/23 -9/24/96).

With the failure of the slide cover, it was decided to rotate the food cylinders by 180° to limit access to the food and continue the five generation test without a redesign of a slide cover system. Another problem that occurred during the later portion of the test was food drying in the exposed cylinders in both test units. This was due to the small food volume, large chamber volume and air exchange within the incubator. During the test, test chambers were capped with a foam plug. For the five generation test, the foam plug was replaced by

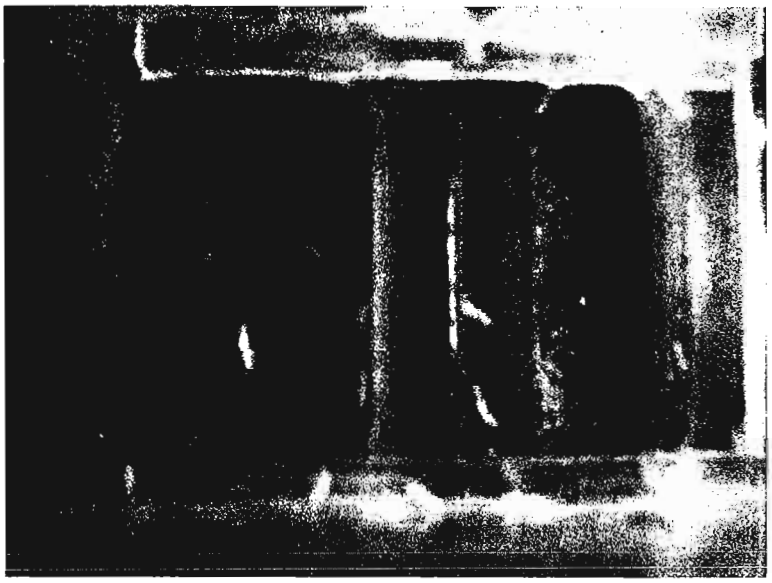

Figure 9. Overhead view of a food isolation cover slide within an MGSU chamber. 3rd instar larvae can be seen crawling beneath the slide on the left.

a clear acrylic sheet perforated with small foam-filled holes to maintain air exchange and to allow video imaging.

During the testing of the food isolation mechanism, problems persisted with bacterial growth on the fly food (Pence, 1996c). At this time a microbially sterile population protocol was developed based on a technique described by Michael Ashburner (Ashburner, 1989) and a Test/Operation Procedure was created (Pence and Hult, 1997) and used for the five generation integrated test.

A rectangular food container could provide sufficient food for a larger population whereas the cylinder does not provide an adequate quantity of food to sustain a large population of developing adults. Further engineering design and manufacture will be required to implement a larvae-proof seal.

Five Generation Integrated Test

Detailed generation to generation test results are described in Appendix III.

Assessment of Development: Population, Female Mass, and Development Time

Population

The population was adequately maintained in four of the six MGSUs during the test and it appears that the exposure time to the food (Figure 11) was adequate to help maintain the population. It is unclear if the decrease in exposure time at the F2/F3 transition made any difference to the next generation, since the vast majority of eggs were probably laid immediately after lights off. Flies were killed and their offspring not transferred to the next generation, due to entrapment in the food during anesthetization to transfer flies to new SB and VD containers, or when collecting the adults. A few flies were also killed in moving cylinders in the MGSU and several flies also became stuck in the MGSU food as well. In the F3 generation there was death of flies in the VD units due to the inadequate warming of food and the resultant accumulation of fluid in the bottom of the chambers. (Table 3). The most significant loss of flies occurred in the SB F2 and F4 and the VD F1, F2, and F3. In the SB F2, most flies were uneclosed pupae. The high number of uneclosed may be due to overcrowding, but the mass and population data do not support overcrowding (Figure 12). The only statistically significant difference between group populations occurred between the SB and VD groups ($P < 0.005$) in the F3 generation (Table 4) and the difference in mass of these groups was also statistically significant (Table 6). No conclusion can be drawn, however, since no other groups exhibited change in population with a correlation to a difference in mass. When comparing groups from generation to generation (Table 5), there was a significant increase in the SB from F1 to F2 ($P< 0.05$). The VD had a significant increase from F1 to F2 ($P < 0.0001$) a significant decrease from F3 to F4 ($P < 0.001$), and another significant increase from F4 to F5 ($P < 0.01$). The VD group population seemed to be the most unstable population, but

there were no significant differences in groups from generation to generation except in the F3 between the VD and SB. The MGSU appeared to be the most stable population from generation to generation, with no statistically significant differences. This may be due to the fact that the MGSUs were used to determine length of food exposure and timing of generation interval.

There was no particular evidence of stressed development, although there were uneclosed pupae in the F2 and F4 SB groups at the time of exposing the generations to new chambers.

Table 3. Uncollected flies % of total population. Dead and uneclosed.

Unit	F1	F2	F3	F4	F5
MG 1	0	2	0	0	0
MG 2	0	16/15u	0	0	0
MG 3	4	1/2u	0	0	0
MG 4	0	0	0	0	10u
MG 5	0	0	0	0	0
MG 6	0	0	-	-	-
avg. %	3%	8%	0%	0%	2%
SB 1	3	2/5u	0	21/11u	0
SB 2	1	3/14u	0	6/6u	0
SB 3	4	3/49u	0	1/5u	0
SB 4	4	1/38u	0	13/4u	0
SB 5	5	1/10u	0	4/7u	0
SB 6	1	1/7u	0	2/5u	0
avg. %	9%	20%	0%	22%	0%
VD1	2	1/13u	12	1	0
VD2	2	2/24 u	8	2/1u	0
VD3	0	4/16u	17	2u	0
VD4	0	18	3	18	0
VD5	0	3/18u	5	8/1u	0
VD6	1	41	0	2	0
avg. %	2%	21%	15%	13%	0%

u = uneclosed pupae

Table 4. Population unpaired student t test analysis between test groups (P values).

	F1	F2	F3	F4	F5
MG vs. VD	ns	ns	ns	ns	ns
MG vs. SB	ns	ns	ns	ns	ns
VD vs. SB	ns	ns	< .005	ns	ns

Table 5. Population unpaired student t test analysis within test groups (P values).

	F1 to F2	F2 to F3	F3 to F4	F4 to F5
MG	ns	ns	ns	ns
SB	< 0.05	ns	ns	ns
VD	< 0.0001	ns	< 0.001	< 0.01

Female Mass

It was hard to define the standard control mass in the test groups, although the SB group had consistently higher mean mass than the VD or MGSU groups in all of the generations, which could indicate that the SB group had the healthiest flies. The mean mass of the SB however varied from generation to generation, peaking at the F2 and decreased continually afterwards. In the F1, the MGSU and VD groups were both significantly less than the SB group ($P < 0.05$ and < 0.01, respectively), although the population (Figure 10) between the three groups was not different, which could indicate stressed development. In the F2 generation, the mass of the VD ($P < 0.0001$) was significantly less than the SB. The large standard error in the MGSU was due to weighing the two extremely small populations in the MGSUs, #3 and #6 which skewed the data. The removal of these two populations probably would have made the MGSU significantly less than the SB control. In the F3 generation, there was no significant difference between the MGSU and SB groups. There was a significant difference between the MGSU and VD ($P < 0.0001$) and the SB and VD ($P < 0.0001$). The VD group mean was at an all time population high with a mean of 120 adults, which could indicate stressed development with a decreased mean mass. The VD did display some delayed development, but not different than the MGSU group (Figure 13). In the F4 generation, there were no significant differences in female mass. This was also the generation when the mean population was at its lowest for the VD and MGSU groups, although, as stated before, the mean populations were not significantly different than the other generations (Figure 10). In the F5 generation, all three group's female masses were significantly different with the VD and SB groups being the most significantly different ($P < 0.0001$), followed by the VD and MG groups ($P < 0.002$) and MG and SB groups ($P < 0.01$).

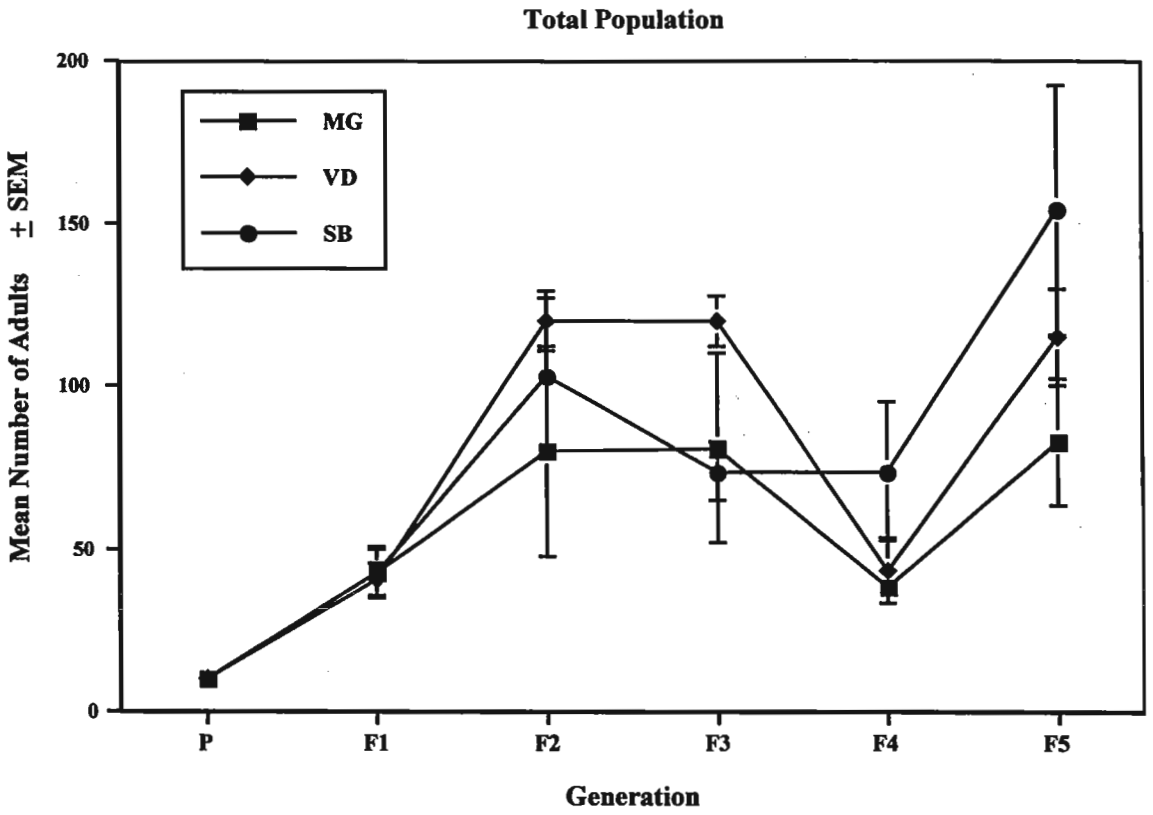

Figure 10. Total Population.

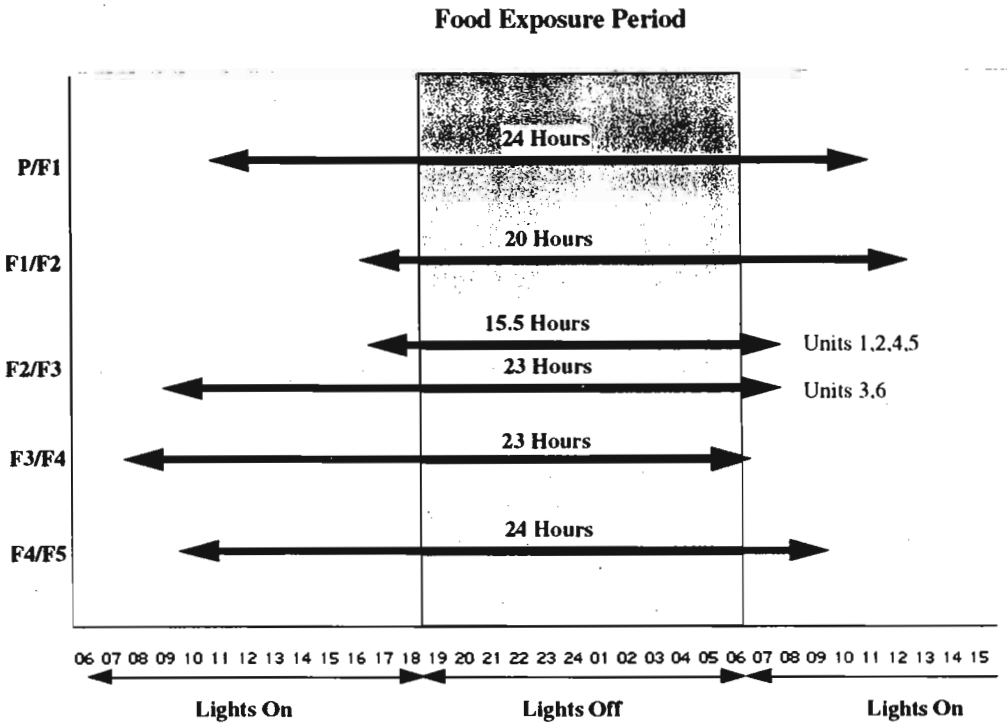

Figure 11. Food exposure periods.

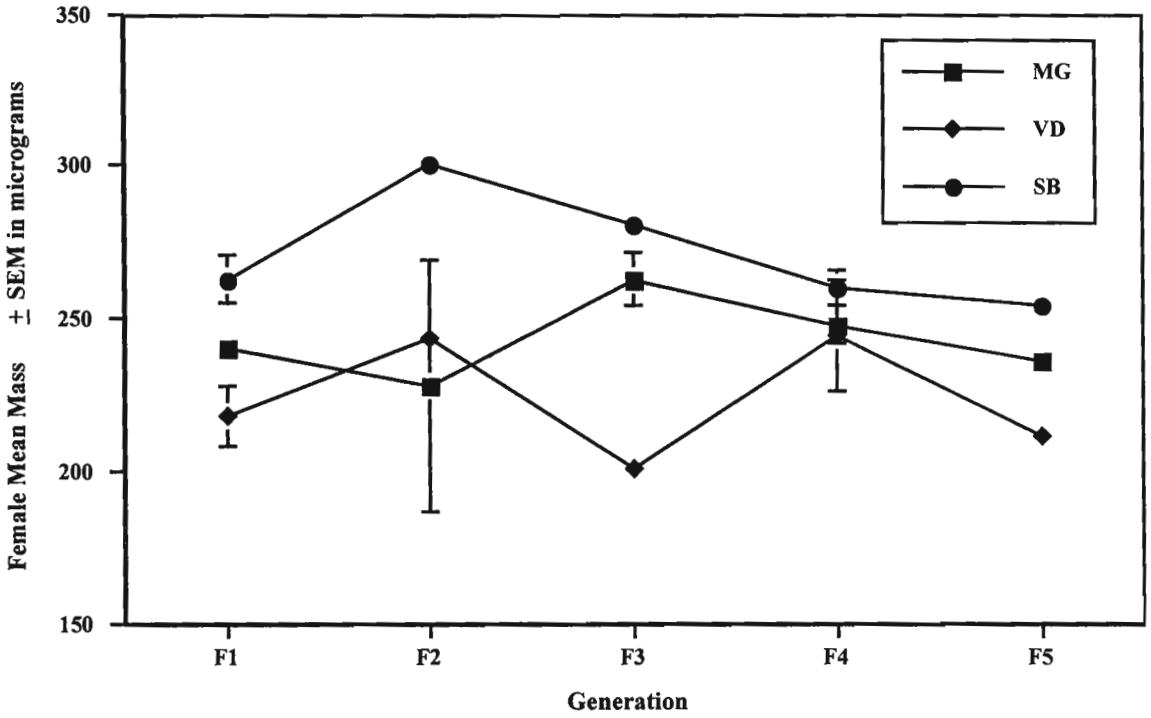

Figure 12. Female Mass.

Table 6. Female mass unpaired student t test analysis between test groups (P values).

	F1	F2	F3	F4	F5
MG vs. VD	ns	ns	< 0.0001	ns	< 0.002
MG vs. SB	< 0.05	ns	ns	ns	< 0.01
VD vs. SB	< 0.01	< 0.0001	< 0.0001	ns	< 0.0001

Development Time

In the F1 generation, it appeared that the majority of the SB and VD controls were 12-18 hours in advance of the MGSUs at the time of adult emergence. In the F2 generation, there appeared to be a 12-24 hour delay in development of the MGSUs as compared to both control groups, although some uneclosed pupae were still present in the SB controls when adults were collected. In the F3 generation, it appeared that, for the first time in the start of the experiment, all of the test groups were developing at approximately the same rate. In the F4 generation, the SB controls were slower in development, but it did not appear to be caused by an exceptionally large population. In the F5 generation, the VD group appeared to develop at the fastest rate.

There was a definite observed difference in development timing between groups, which was somewhat unexpected, unless there was profound delayed development due to overcrowding, but this does not seem to be the case. The VD and MGSUs were expected to develop at the same rate due to the same food volume and depth dimensions. It is also interesting how the fastest developing group varied from generation to generation. Overall, the F2 and F3 groups developed slower than the other three generations and the adults were collected one day later in the F2 and F3 generations. There is no apparent reason why these generations were slower or faster, and the decision to collect adults was based upon overall readiness of the generations.

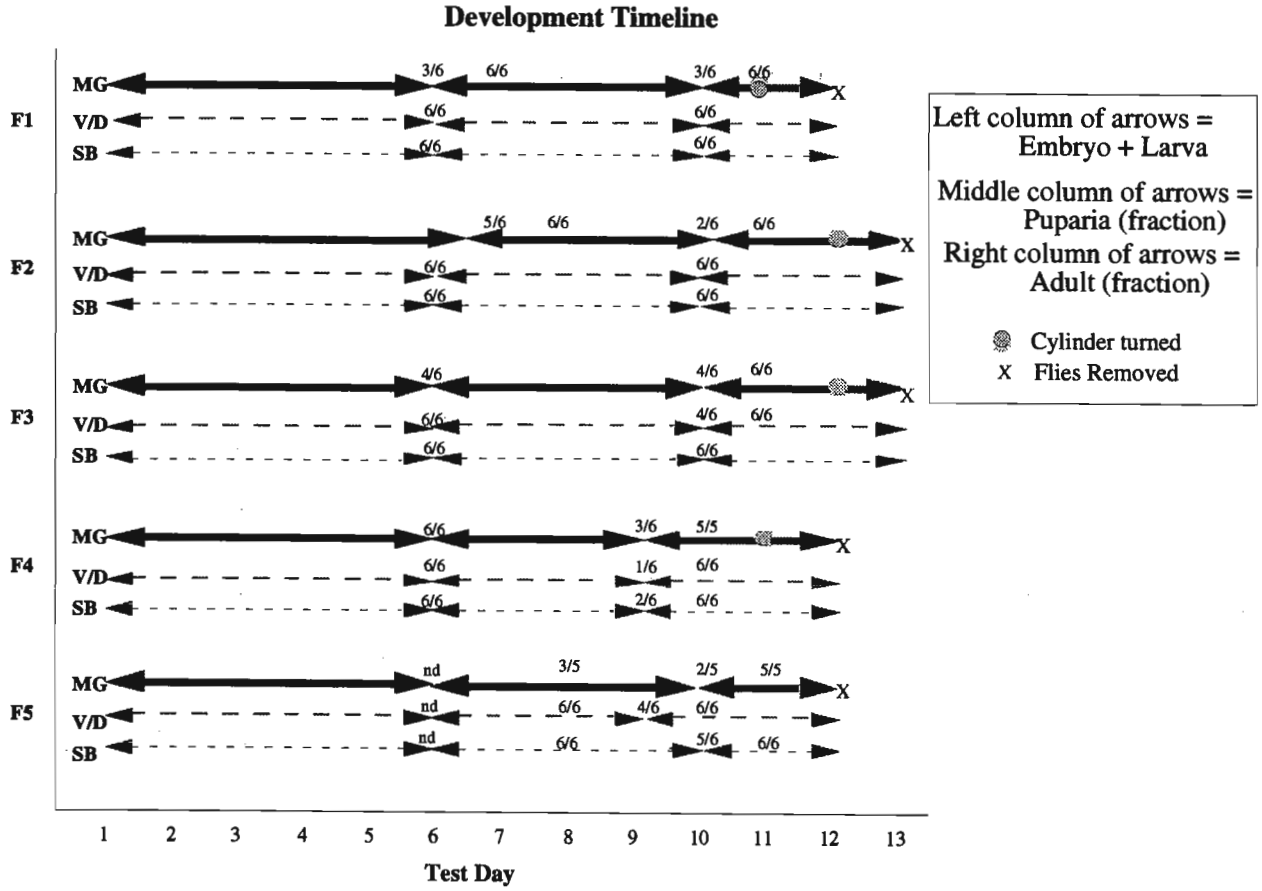

Figure 13. Development Timeline.

Crew Operations

Figure 14 shows the total time it took to perform the operations necessary to collect the flies from all test chambers for each generation. MGSU flies were collected with the FCU prototype and the operations also included the time necessary to move new food cylinders to the next chamber. The operation time for the MGSUs in the F1-F3 took significantly longer, due to the problems in moving the cylinders in two of the units, and due to the FCU motor overheating. Time to perform the operations significantly decreased when one malfunctioning unit was replaced and the other was discontinued (Figure 14). At the worst case, collection from six MGSUs, took approximately 1.5 hours to complete. The total operation time may have decreased due to familiarity of performing the operations after multiple collections. This can be seen in the VD and SB groups over the five generations of collecting the flies with the fly collection tools. Figure 15 shows the amount of time it took to perform each task of the operation. The extra operations of moving cylinders, replacing cylinders, removing flies, and sterilizing covers, took longer than control groups due to the vacuum unit problems, and replacing collection vials took longer due to reattachment of the aspirator to the vacuum.

A significant amount of time was necessary during operations in sterilization of equipment and facilities for all groups. On average, it appears that it took twice as long to perform operations with the MGSUs than with the two other groups.

It may be difficult to perform multiple generation experiments with replicate groups, but options exist to automate some parts of the operations or to collect only the last generation of adults. Video observation was essential in monitoring developmental timing in order to perform generation separation operations. Flexible

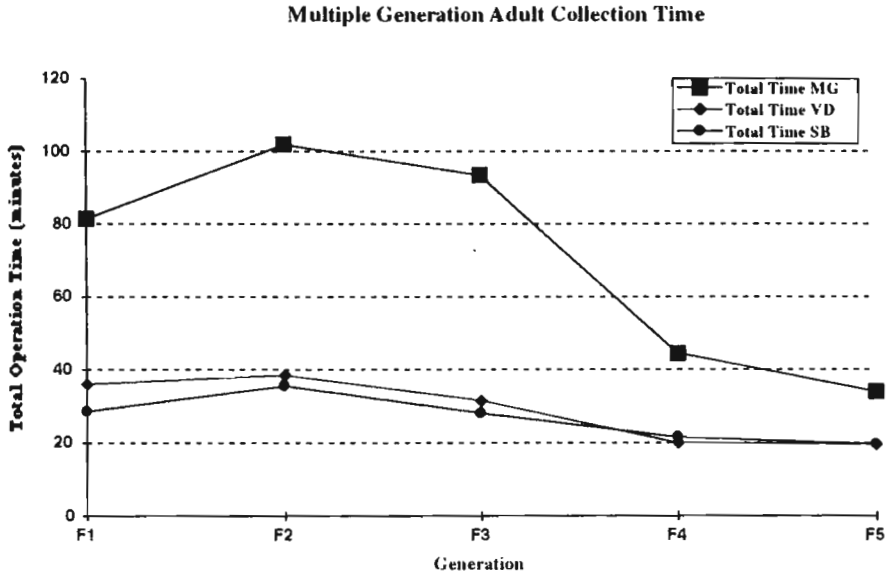

Figure 14. Operation Duration. Notice that the MGSU generations F4 and F5 are reduced. This is due to removal and or replacement of malfunctioning units.

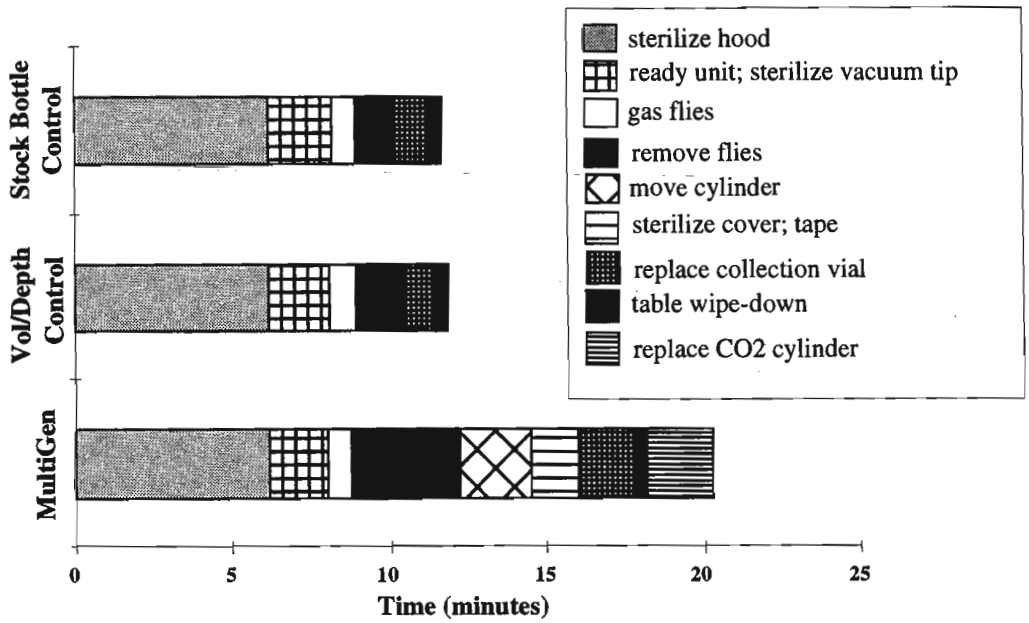

Figure 15. Mean adult removal procedure times.

scheduling with the crew may be difficult and moderately intensive training will be necessary to perform the operations.

Flexibility in crew scheduling will be necessary, but operations can assuredly be performed during a normal crew shift. It is recommended that two crew members will be necessary to perform the tasks. Crew member 1 performs the activities of collecting flies and crew member 2 monitors procedures, operates the

collection vacuum, and tracks collected specimens. Since the adult collections are a fairly infrequent event, approximately every 2 weeks, it is feasible for the crew to perform the operations, although significant training would be necessary, as well as continued refresher training while on orbit.

One of the most critical aspects of conducting multiple generation experiments is maintaining a stable population. To control the population the containers need continual monitoring. The key parameters to monitor were early development duration, eclosion timing of adults, adequate mating duration, adequate egg laying duration, and total population from generation to generation. The health of the population was the key issue in maintaining the generations. Overall during the five generation integrated test, all six units were monitored and alterations were made in the planned schedule to maintain a steady population in all six units. Populations varied between the units, and in order to maintain a steady population, operations in some units had to be altered on a unit by unit basis. For instance, due to mechanical difficulties in two of the units, the population decreased in these two units and the exposure time of females to the food for egg laying had to be extended. Equipment must also be mechanically sound to prevent failure. Delays in development resulted in the necessity of delaying cylinder advance until all units were ready to advance. All groups were held in the old containers until most adults were mature before the adults were exposed to the new food for each generation. This was done so the newly laid eggs were of synchronized age. It will be a challenge to maintain a steady population over multiple generations and to maintain multiple populations.

Table 7. CO_2 cylinder used.

Generation	cylinders used
1	2
2	2
3	3
4	2
5	2

FCU Prototype

Overall the prototype developed for the test worked extremely well, and has good potential for future applications, when development of an adult collection device is necessary on orbit. One of the problems that persisted in the MGSU test was vacuum motor shutoff. More work will have to be done to correct this problem, as engine shutoff persisted even with removal of the circuit breaker during the multiple generation test. This problem can easily be fixed with testing of other commercial motors that are not part of a vacuum designed to clean computer keyboards.

Some other additions that may be beneficial to a FCU device and crew operation would be to supply pre-sterilized vacuum tips that can be changed after each use, if sterile operations are required. A transparent vacuum hose would be a requirement to be able to view flies in the tube to make sure they are all collected. Another possible change to the current prototype design would be to use the vacuum tube as the collection tube instead of using a microcentrifuge tube. The tubing would have to be changed after each use, but it may make design of the FCU easier and may give other options in preserving the specimens for return to Earth. A flexible tube may give more options than a flip-cap microcentrifuge tube. Something that may be required to make collecting flies easier is to add a brush to the end of the vacuum tip instead of a separate brush that has to be utilized by the crew.

The bigger challenge is to make a crew and fruit fly "friendly" device that is compatible with chamber design. Another challenge is to make the system operational in space. It is unknown how fly behavior and response to the anesthetic in space will impact the ability to collect specimens.

When collecting flies it is still unknown how the fly behavior in space and reaction to the anesthetic will be altered as compared to the ground. For example, when collecting flies with the FCU, it was very easy to use the CO_2 gas flow to push the anesthetized flies into a corner and then to quickly vacuum them into a vial, but in reduced gravity, it is unknown if the flies will adhere to the walls or float within the chamber. Other designs may need to be considered to accommodate this issue. Air flow through the chamber may be a way to gather flies for collection.

Stowage of supplies will also be a consideration when performing multiple generation experiments. It is recommended that "kits" will have to be made to store the necessary supplies for collecting adult fruit flies and for processing of tissues as needed. Kits that may be necessary include: a FCU box, a gas cylinder kit and

vacuum tubing kit, a vial/specimen storage kit for each generation, a facilities and equipment sterilization kit with sterile towels and sterile gloves, and possibly a tool kit for special tools needed to collect flies, operate the FCU, or to gain access to specimen chambers. For instance, in collecting flies during the five generations, a total of 11 CO_2 cylinders were used (Table 7), which would require stowage space.

SUMMARY/CONCLUSION

- A World Wide Web site was created in conjunction with the SSBRP Communications and Data System group for the purpose of simulating on-orbit observation of fruit fly multiple generation experiments. The system was designed to collect and archive video images and environmental data and was used to monitor development in the five generation experiment to aid in decision making for performing experimental operations. During the five generation test, the system worked very well to provide simulated remote observation capability of the experiment. There were a few reliability problems during the test with data storage, but overall the system exceeded the original expectations. More user friendly options for retrieval of data and the need for camera coverage of more test units is necessary. The 10× magnified video system attached to the web site also performed well. At the higher magnification, a World Wide Web capable system should be developed for cameras to track and focus on individual specimens for successful remote observations.

  After the development of the site for the five generation test, the function of the site expanded to provide information about the SSBRP insect laboratory, the studies conducted in the lab, and as a database for past insect space research information. The site was also expanded to provide information about SHARP and STELLAR interns. The Summer '96 STELLAR teacher used the site to educate her grade school students about the work she performed at NASA Ames Research Center. The site was also recently enhanced to provide server access to Canadian Space Agency personnel for document review and distribution.

- A prototype adult fruit fly collection unit (FCU) was developed with modified commercial off-the-shelf products to collect flies during the five generation test. The prototype was designed to anesthetize fruit flies with gas from a portable CO_2 cylinder regulated by a small flow valve, then to collect anesthetized flies into 1.5 ml tubes using a small vacuum driven aspirator. The prototype system performed extremely well and "crew" operation times were collected during the five generation test. There were a few problems with the commercial vacuum motor performance, although the system was functional during testing. The concept is feasible for specimen collection on orbit by crew members, although the weightless environment may have an impact on fly behavior to anesthetic and crew operation techniques will have to be refined. Design of specimen chambers and interfaces with a FCU will also need to be considered. More design work and testing will be necessary to create a unit for flight.

- The food cover mechanism added to a Multiple Generation Separation Unit (MGSU) prototype that was designed to eliminate developing larvae from gaining access to food failed to work, but the backup method worked very well. Previous testing had shown that a mechanism was needed to keep larvae from accessing food designated for a future generation of fly eggs in a specimen chamber with two food cylinders. Due to their small size, fruit fly larvae were capable of squeezing through a very small gap between the cover and the unit, to gain access to the food. The food isolation test was conducted to verify a possible method of limiting access of the developing larvae and adults to new food on the previously developed MGSUs. During the five generation test, a backup method of rotating food cylinders was used to eliminate larval access to the food with complete success. If a food presentation design solution other than a cylinder is used, the seal/slide system will have to be created.

- A five generation test utilizing the MGSU and two control groups was successfully completed. The five generation units worked very well to separate generations and to maintain populations. There were some mechanical difficulties with two of the units due to sterilization of the units. This resulted in the loss of one of the six populations of fruit flies by the 3rd generation.

Fly populations were adequately maintained in the MGSUs with no clear evidence of delayed development due to overcrowding. Development in the MGSUs was 12-24 hours slower than controls in the F1 and F2 generations, but this was not due to a large population. It appears that limiting access to food appeared to be successful in limiting the population growth. There were no significant differences between populations of the MGSU or controls, from generation to generation, except between the volume/depth control and stock bottle control in the F3 generation. The MGSUs had the most stable mean population from generation to generation, while the VD control appeared to have the most unstable population. Flies were killed during the experiment when they became stuck in the food during anesthetization for transfer or collection, or during movement of food cylinders in the MGSUs, which could have had an impact on future generations. The fly mass data showed that the SB controls consistently maintained a higher mean female mass from generation to generation which may indicate a healthier population when compared to the other two groups.

The fly collection unit worked very well in the five generation test and crew operations were performed with two "crew members." One operator handled the FCU and MGSUs and the second operator helped to operate the vacuum, load collection vials, and unload and label the collected flies. The maximum amount time to perform adult collections and move cylinders in six MGSU on Earth was approximately 1.5 hours in an 11-12 day period, which also includes the time it took to move cylinders in mechanically defective units. Collection of adult flies is probably possible on orbit, but further development of the unit and supporting supplies would be necessary.

The World Wide Web system worked well to aid in decision making regarding the development of flies in the MGSUs and direct visual observation helped to corroborate the web images. Images of all of the test group's development times would have significantly aided the decision to expose food, move cylinders and collect flies. In a space-based multiple generation system, video data will be indispensable in monitoring the development of individual test chambers to assess the need to perform experiment operations. Time critical operations will need to be quickly viewed and assessed by the PI to plan crew or automated system operations and video will probably be the most important data to collect.

During the five generation test, making decisions to maintain the six populations of flies was a somewhat complex task to perform due to the variation between populations and differences in development between the test groups. Similar problems may be encountered on orbit and when maintaining control populations on the ground.

Acknowledgments: I would like to thank Charles Sun, May Windrem, and Lou Picinich for design and development of the World Wide Web site data collection system. I would also like to thank Kjell Hult for significant development of the web page, web video script development, and for help in development of the multiple generation test operations. I would also like to thank Mayur Trivedi and Sam Black for assisting in the muliple generation test. I would also like to thank Mayur Trivedi, Gabrielle Meeker and Melissa Kirven-Brooks for review of the final report.

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and L. Picinich 1996, Application of World Wide Web (W3) Technologies in Payload Operations. Proceedings of the Fourth International Symposium on Space Mission Operations and Ground Data Systems (SpaceOps 96). Article # SO96.2.033. Munich, Germany, http://www.esoc.esa.de/external/mso/SpaceOps/2_33/2_33.htm.

APPENDIX I

Script to collect timed video data from the micro video system and download to the SGI Indy workstation.

```
#!/bin/sh
# A simple shell script to take video snapshot from SGI Indy video system.
# Uses input from S-VHS CCD camera
# Reduces image size 50% and saves as .rgb for movie compilation
# original source: unknown
# modified by Charles Sun 4/22/96
# modified by Kjell Hult 12/20/96
# set TIME as variable to alphabetize pictures in order taken.
TIME=`date '+%Y%m%d%H%M'`
cd /usr/people/guest/public_html/pupdev
# Save .rgb image to pupdev/ directory and use TIME variable as name
# Redirect vidtomem output to /dev/null/usr/sbin/vidtomem -v 1 -f $TIME 2>/dev/null
# Scale new image file down 50% and remove -00000 suffix
/usr/local/bin/convert -geometry 50%x50% $TIME*.rgb $TIME.rgb
# Copy large image to other directory for web access and convert to .jpg
cp /usr/people/guest/public_html/pupdev/$TIME-00000.rgb \
/usr/people/guest/public_html/pupdev/current/current.rgb
/usr/sbin/imgcopy
/usr/people/guest/public_html/pupdev/current/current.rgb \
/usr/people/guest/public_html/pupdev/current/current.jpg 2>/dev/null
# cat "current.jpg"
# Delete old (large) image files
rm -f $TIME-0*rm -f /usr/people/guest/public_html/pupdev/current/current.rgb
```

APPENDIX II

Script to collect temperature and humidity data from the Fluke Data Bucket and download it to an SGI workstation.

```
#!/bin/sh
# A simple shell script to replace fly-a1.html for easier access to
# other executables via shell
# Charles Sun 4/22/96
# HTTP handshake w/ client
echo Content-type: text/html
echo
# define vars
name=$REMOTE_HOST
date=`date`
# read temp and humidity sensors from ch5 & ch3
temphigh="29"
templow="15"
```

```
#temp="25.05"
temp=`/usr/people/csun/fly/flydaq 5 `
#hum="55.02"
hum=`/usr/people/csun/fly/flydaq 3 `
echo "<HTML><META HTTP-EQUIV=\"Refresh\" CONTENT=\""
echo $QUERY_STRING
echo "\">"
echo "<HEAD><TITLE>Habitat Snapshot</TITLE></HEAD>"
if test $temp -gt $temphigh
then echo "<BODY BGCOLOR=\"red\">"fi;
if test $temp -lt $templow
then  echo "<BODY BGCOLOR=\"red\">"fi;
echo "<CENTER>"
echo "<H2>Habitat Monitor</H2><HR>"
echo "<IMG SRC=\"http://taipei.arc.nasa.gov/cgi-bin/capture.cgi\">"
echo "<TABLE><TR><TD>Data Sampled Time: <TD><STRONG>"
echo $date
echo "</STRONG><TR><TD>Temperature: <TD><STRONG>"
echo $temp
echo " C</STRONG><TR><TD>Humidity: <TD><STRONG>"
echo $hum
echo " %</STRONG></TABLE>"
echo "</CENTER></BODY></HTML>"
echo
```

APPENDIX III

Five Generation Integrated Test Notes

The following sections address the performance of each of the 5 generations and the parental generation in the MGSU and controls. Performance is divided into the four categories of Biology, MGSU prototypes, FCU Prototypes and Operations, and the web system.

Parent (P) Mating and Egg Deposition

Biology- Flies were added to the multiple generation separation unit, SB and VD controls, and exposed to the new food on the morning of Day 0. The flies were exposed to the new food for 24 hours. After removal of the flies on the morning of Day 1, an adequate number of eggs (20-50) were seen in all 6 units as well as the controls.

MGSU Prototypes- Most MGSUs functioned well, although there was some trouble in loading the starting food cylinders into two of multiple generation units and a small hammer had to be used to load the cylinders. It appears that the cylinder ports were slightly distorted when the bottom portion of the units were autoclaved.

FCU Prototypes and Operations- Adult flies were added on test day 0 and removed on test day 1 with the normal fly collection tools and CO_2 system. There were no anomalies in the test start operations, other than cylinder loading described in the prototype section.

WWW- The system worked well and baseline images of the units and controls were taken during the beginning of the experiment. The Indycam camera was set to collect images at 20 minute intervals on MGSU #5. There was a problem with the image date stamp command on day 0 that was fixed by CDS personnel.

First Generation (F1) Development

Biology- On the morning of day 6, pupariation had occurred in 50% of the MGSUs, and 100% of the SB controls. Pupariation had occurred in 100% of the VD controls, but all of the pupae were seen in the food. In all groups, most pupae formed close to the food and at most, crawled to only the bottom 3rd of the container walls to pupate. Some of the SB controls also had pupae in the food. On the morning of day 7, most larvae had pupated in MGSUs, although some 3rd instar larvae were still seen in the MGSUs and the SB controls. On the afternoon of test day 9, wing pads and eyes were clearly present in all of the SB controls and most of the VD controls. Wing pads were visible in most of the MGSU pupae. On the morning of day 10, the MGSUs had no adults. All the VD and SB controls had adults, with each still having some pupae uneclosed. Late on day 10, MGSUs only had adults in 50% of the containers. VD and SB controls all had adults with some pupae still uneclosed. On the morning of day 11, adults had appeared in all the MGSUs, although there were still some uneclosed pupae. The VD and SB controls had all adults eclosed, although one container of the SB controls had 2 uneclosed pupae. Overall it appeared that the majority of the SB and VD controls were 12-18 hours in developmental advance of the MGSUs.
MGSU Prototypes- By the completion of the generation, there was moderate drying of the food in the units and controls due to low RH in the incubator.
FCU Prototypes and Operations- Nothing to report.
WWW- Images were collected of all MGSUs and control groups during the F1, in addition to the continual 20 minute interval image collection of MGSU # 5.

F1 Finish and F2 Start

Biology- Adults were exposed to the new food on the afternoon of day 11 and the egg-laden cylinders were moved to the F2 chambers on the afternoon of day 12. Adults from all groups were only exposed to the food after the slowest groups, which were in the MGSUs, had time to reach maturity. More than 100 eggs were visible in each test unit and controls. During the transfer process, some flies became stuck in the food and were not transferred (Table 3). Some eggs were also lost when moving the cylinder.
MGSU Prototypes- Most units operated well when new cylinders were inserted at day nine, exposure of new food and closing of old food on day 11, and transfer of food to the F2 chambers on day 12. It was still very difficult to move the cylinders without the aid of a small hammer and vise. Four adults were mashed in the process of turning the cylinder, and some of the egg-laden food was splattered onto the wall of the F1 chamber when the cylinder was moved on day 12. Some flies were also stuck in the food of the control groups and not moved to the next generation containers (Table 3).
FCU Prototype and Operations- The timing of operations was dependent upon the development time of the adults in the MGSUs and the control groups. The needed information was gathered by video observation and by direct visual observation of the groups. These scheduling issues persisted as the experiment progressed and the fly population fluctuated from generation to generation. The sequence of decisions that required continual monitoring of development to determine when to perform the operations were:

1. When to add new food cylinders: The cylinders were not added until after the entire population of 3rd instar larvae had pupated (if possible). For the F1 generation, new food cylinders were added on day 9.

2. When the cylinders are rotated and for how long: The best time to rotate cylinders (expose new food and close old food) is after all of the adults are eclosed and a reasonable amount of time has passed for the adults to sexually mature. Cylinders were rotated on day 11 in the F1 generation. The number of adults that emerge, and their ability to lay eggs, are the most important factors in deciding how long to expose the new food. Another factor that was not considered was that it will probably not be feasible to expose or move food in the dark cycle of the photoperiod, unless the system is automated. The females lay the most eggs immediately after onset of the dark cycle, which makes it difficult to monitor the exposure period.

3. When to vacuum flies and move food cylinders to the next chamber: For this experiment , the decision was made to remove the adults prior to moving the cylinders to eliminate the risk of adults moving into the next generation chamber and mating with their offspring. Turning of the cylinder prior to vacuuming also made collection easier. Until an effective method of preventing adults from moving to the next chamber is

devised, this was the safest method. Adult collection with the gas and vacuum units and cylinder movement was generally successful. Flies became stuck several times when the vacuum tip was dipped in ethanol and not given adequate time to evaporate.
WWW- Ideally, remote observation of all test units and controls would be helpful to gauge development rate in all groups. By using the images collected during the F1, it was possible to make reasonable estimates of when to perform the specific operations.

Second Generation (F2) Development

Biology- On the morning of F2 day 1 (test day 12) eggs were visible in all groups. It was difficult to see any eggs in one of the MGSU because of the disturbed medium. On the afternoon of test day 17 (F2 day 6), pupae began to appear in the VD and SB controls. Pupae were not yet visible in the MGSUs. On the afternoon of day 7, pupae were present in most of the MGSUs. On day 9, wing pads were present in the VD and SB controls. Wing pads were not present in the MGSUs until the afternoon. Late on day 10, adults were present in both the VD and SB controls. Adults were present in only 2 of the 6 MGSUs. On the morning of day 11, flies were present in all units, except one MGSU, although it had a large number of pupae ready to eclose (215 total final adults), which may have caused a delay in development. Overall, there appeared to be a 12-24 hour delay in development of the MGSUs as compared to both control groups, although some uneclosed pupae were present in the SB controls.
WWW- The system continued to work well, however 20 minute interval collected images were lost on January 1st (test day 21, F2 day 10) due to the lack of a directory to accept images with a 1997 date. Images could still be accessed from the web page to see fly activity, but they could not be stored. The error was not corrected until January 2nd.

F2 Finish and F3 Start

Biology- Adults were exposed to the new food on the afternoon of day 22, F2 day 11 and on the morning of day 12. Flies were collected and the egg-laden food cylinders were moved to the F3 chambers on test day 24/F2 day 13. There were some differences noticed in the population sizes of two of the six test units. Food exposure times were split between the two low populated units to allow the other four units with smaller populations to have more time to lay eggs. The well developed units were exposed for approximately 14 hours to limit any overpopulation, while the two lower population units were exposed for approximately 23 hours. There were still only five adults visible in one unit at the time of egg laying. On day 13 at the time of cylinder rotation there were still some uneclosed pupae seen in all the units and some flies became stuck in the food during collection (Table 3). On the morning of day 13, there appeared to be plenty of eggs in most of the units except.
MGSU Prototypes- There were major difficulties in loading food cylinders and rotating food cylinders in two MGSUs. At the start of the F3 generation, the decision was made to replace one MGSU with the backup unit. There were no problems with the backup unit when the egg laden food cylinder was transferred to it.
FCU and Operations- New food cylinders were inserted on the morning of F2 test day 11. During adult collection with the FCU, there were some difficulties with the vacuum motor due to overheating as described previously. This delayed the collection times.
WWW- The remote video collection system continued to provide the needed information on the developing flies.

Third Generation (F3) Development

Biology- On the morning of F3 day 1, test day 24, there appeared to be plenty of eggs in four of the six units. On the afternoon of test day 4, one unit still had no signs of larval development, and the food was still unworked. On the morning of day 6, pupae were present in four of the six MGSUs and controls. It is believed that the mechanical striking of the cylinders when moving them to the F3 chambers, severely affected the developing eggs and caused extremely low or non-existent populations. On the morning of day 7, there were

some pupae in MGSU #3. On the late afternoon of day 9, wing pads and eyes were visible on MG units and controls, although no adults had appeared. There were still very active 3rd instar larvae in MGSU #2. Adults began to appear on the morning of day 10. Adults were present in all SB controls, 66% of the VD controls and 66% of the MGSUs. On the morning of day 11, almost all adults had eclosed in all groups, although many of the adults looked very recently eclosed. It appeared that for the first time since the start of the experiment, that all of the test groups were developing at the same rate.
MGSU Prototypes- Nothing to report.
FCU and Operations- Nothing to report.
WWW- The remote video collection system continued to provide the needed information on the developing flies.

F3 Finish and F4 Start

Biology- The flies in one MGSU appeared to have made a good comeback after a low population in the F2 generation. One unit had no flies. The populations in the four other units were variable. During presentation of the new food to the VD controls, some of the adults became stuck in condensed food fluids that were created during warming (Table 3). The reduced exposure time may have helped lower the SB population.
MGSU Prototypes- There were no hardware problems with the prototypes at this time.
FCU and Operations- Due to the large number of flies in most test and control containers, it was planned to expose the flies to the food only during the light cycle and collect flies late in the day. The flies however, laid very few eggs during the day and adult collection was delayed until the next morning. Therefore, the flies were exposed to the new food for 23 hours. It was not planned to conduct fly collection during lights out, due to the difficulty in performing operations under red filtered light. It is also unlikely that crew members could perform these operations. Adults were collected on the morning of day 13 with no new anomalies. As with the last collection, the motor shut off, after intermittently overheating.
WWW- The remote video collection system continued to perform well.

Fourth Generation (F4) Development

Biology- Eggs were visible on the food surfaces of the MGSUs and controls. On the evening of day 5, no pupae were present in any of the groups. On the morning of day 6, pupae were present in all of the containers of all of the groups, except one MGSU. One container of the VD control had all of the pupae in the food. On the morning of day 8, wing pads and eyes were visible in some of the containers of all of the groups.

By the afternoon of day 9, adults were visible in 50% of the MGSU containers, 17% of the VD containers, and 33% of the SB containers. On the morning of day 10, adults were present in all containers, although a lot of the adults in the containers appear to be recently eclosed and callow. The SB control group still had a large number of unclosed pupae and some 3rd instar larvae were still visible. In the F4 generation, the MGSUs and VDs appeared to be developing a slightly faster than the SB controls. The SB controls were slower in development, but it did not appear to be caused by an exceptionally large population.
MGSU Prototypes- Nothing to report.
FCU and Operations- Nothing to report.
WWW- The remote video collection system continued to provide the needed information on the developing flies to determine how the flies were developing in the MGSUs.

F4 Finish and F5 Start

Biology- Nothing to report.
MGSU Prototypes- One MGSU was discontinued from testing due to the absence of any new flies in the unit. All other units performed well.
FCU and Operations- There were no new FCU problems.
WWW- Nothing to report.

Fifth Generation (F5) Development and F5 Collection

Biology- On the morning of F5 day 1, test day 47, eggs were visible in all the containers of all groups. On the morning of day 5, 3rd instar larvae in the VD control were crawling on the walls and preparing to start pupariation, although no pupae were seen yet. Other groups only had larvae crawling in the food. No information was collected on days 6 or 7. On the morning of day 8, pupae were present in 60% of the MGSUs, and 100% of the VD and SB groups. 3rd instar larvae were still present in the MGSU and the SB groups. On the afternoon of day 9, 67% of the VD group had adults, while no adults were present in either the MGSU or SB groups, although wing pads and eyes were visible in the pupae. On the morning of day 10, adults were present in 87% of the SB group and 40% of the MGSU group. On the morning of day 11, adults were in 100% of the SB and MGSU groups, although uneclosed pupae were still present in some of the individual containers. On adult collection at day 12, there were still 10 uneclosed pupae in one MGSU.

MGSU Prototypes- Nothing to report.

FCU and Operations- Adult flies were collected on the morning of day 12 with no problems. The FCU worked flawlessly at this time.

WWW- Images were not stored for the entire day of test day 53, for an unknown reason.