Design and development of hardware for long term cultivation of *Drosophila melanogaster* in the International Space Station.

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ABREVIATIONS: International Space Station (ISS), Experimental Support Equipment Item 1 (ESE–1), European Space Agency (ESA), European Modular Cultivation System (EMCS), Embryo Collecting Unit (ECU), Embryo Fixation Unit (EFU), Embryo/Adult Fixation Unit (E/AFU)

Abstract

The International Space Station is currently in an advanced state of construction. The possibility of conducting in the Space environment longer term experiments than previously possible is becoming a reality, but there are several obstacles in this undertaking, some purely based on the availability of suitable hardware, some more technical. To eliminate some of them and make possible investigations using one of the more important model systems, *Drosophila melanogaster*, we have developed an automatic device to grow and carry out biological experiments in Space with *Drosophila*. The Experimental Support Equipment Item 1 (ESE–1) is an automated culture system for multigenerational experiments with insects developed for the ESA International Space Station facilities. The concept could be generalized for preparing culture units for small invertebrates.

Introduction

A substantial quantity of data on the response of animal organisms, tissues and cells to the Space environment has been obtained in the last decades, taking advantage of the space Shuttle, the orbital MIR station, the biosatellites and platforms, the sounding rockets and parabolic flights (Pletser, 1995; Seibert, 2001). Our group has been involved in the study of these problems since more than a decade ago using as experimental system *Drosophila melanogaster*, an organism often used in the earlier history of Space research. We have shown that adults exposed to microgravity markedly increase their motility, a behavioral response that has been consistently observed (Benguria *et al.*, 1996; Miller and Keller, 2000). Possibly related to this change in behavior, their life-spans on the ground are shortened after their recovery from space (Marco *et al.*, 1996). On the other hand, we have shown that larvae and adult fruit flies are correctly developed in Space but only during short-term experiments which allowed no more than one generation to be developed in Space (Marco *et al.*,

1986; Marco *et al.*, 1992; Marco *et al.*, 1996). Nevertheless, experiments with other living models and isolated cell cultures have repeatedly shown that cells sense the absence of gravity and that important cellular functions are altered in weightlessness (Hughes-Fulford, 1991; Hemmersbach-Krause *et al.*, 1994; Kordyum, 1997; Klymchuk, 1998; Schatten *et al.*, 2001), functions believed to be critically involved in the developmental process itself. To get a better understanding on the adaptation or lack of adaptation of complex living organisms to Space, we are preparing experiments to monitor the phenotypic, genotypic, and behavioral modifications that *Drosophila* will undergo in long-term cultivation in the Space environment.

The previous hardware used by us in Shuttle or in Biosatellite flights required continuous servicing by the crew (Marco *et al.*, 1996) or was not appropriate for multigeneration experiments (for example, the hardware used in Biosatellites). The Multiple Generation Separation Unit (MGSU) designed and developed by NASA (Pence *et al.*, 1999). had similar problems. It required handling, *i.e.*, crew involvement, for all the separation steps and the accessibility to embryos was difficult or even impossible. The absence of a killing mechanism to eliminate the excess of embryos and larvae complicates its utilization since there is an increased risk of contamination as the larvae are capable of crawling from one tube to the next in the MGSU as reported (Pence *et al.*, 1999). The concept presented here can be complemented to the advanced version of this insect habitat as described and updated in Bhattacharya *et al*. (2004).

The availability of improved biological laboratories in the International Space Station (ISS) will soon allow getting further insights on the influence of gravity in cell processes and organism adaptation and evolution. Among advantages of ISS, one will reside in the possibility of conducting long-term experiments that will allow studying in detail the adaptation of living organisms to the Space environment. Since *Drosophila* has a generation time of about 12 to 15 days from adult to adult, it will be possible in a relatively short-experiment to obtain several generations of flies in Space. We are preparing experiments in which *Drosophila* will be cultured during several months, or even as long as one to two years in Space (24 to 48 generations), opening the way to evolutionary adaptation experiments.

The Biolab facility is the laboratory designed to support biological experiments on microorganisms, cells, tissue cultures, small plants and small invertebrates onboard the ISS (Figure 1A). The Biolab will be installed inside the European Columbus module originally intended for launch in 2004 but currently rescheduled to be launched when the Shuttle missions to the ISS are resumed. In addition to its general facilities such as glovebox, incubators, coolers and freezers, the Biolab includes standard Experiment Containers (Figure 1B) mounted on two centrifuge rotors that provide either microgravity or a defined preprogrammed acceleration level (for example, a 1g reference control). They can also be programmed at different g levels allowing studying the specific effects of weightlessness. The experiment hardware located inside the experiment containers, the Experimental Support Equipment, is provided by the investigators. Ten types of Experimental Support Equipment have been tested by ESA as bread-boards to be used inside the Experimental containers for experiments with cell and tissue cultures and with small animals (ESA Human Space Flight - Biolab Laboratory Web Page: http://www.esa.int/export/esaHS/ESA8EG0VMOC_iss_0.html).

We have designed, developed and tested the Experimental Support Equipment item 1 (ESE-1) for *Drosophila melanogaster*. It is fully compatible with the Biolab Experiment Containers and facilities. In the ESE-1, the special constraints linked to an ISS mission have been taken into account, such as relatively low energy, storage volume limitations, and crew-time availability. Among them, it is important to point out that sample exchange and equipment refurbishment will take place when a Shuttle will be expected to visit the Space Station (probably every three months under the best conditions). Consequently, any experiment in the ISS requires the development of specific

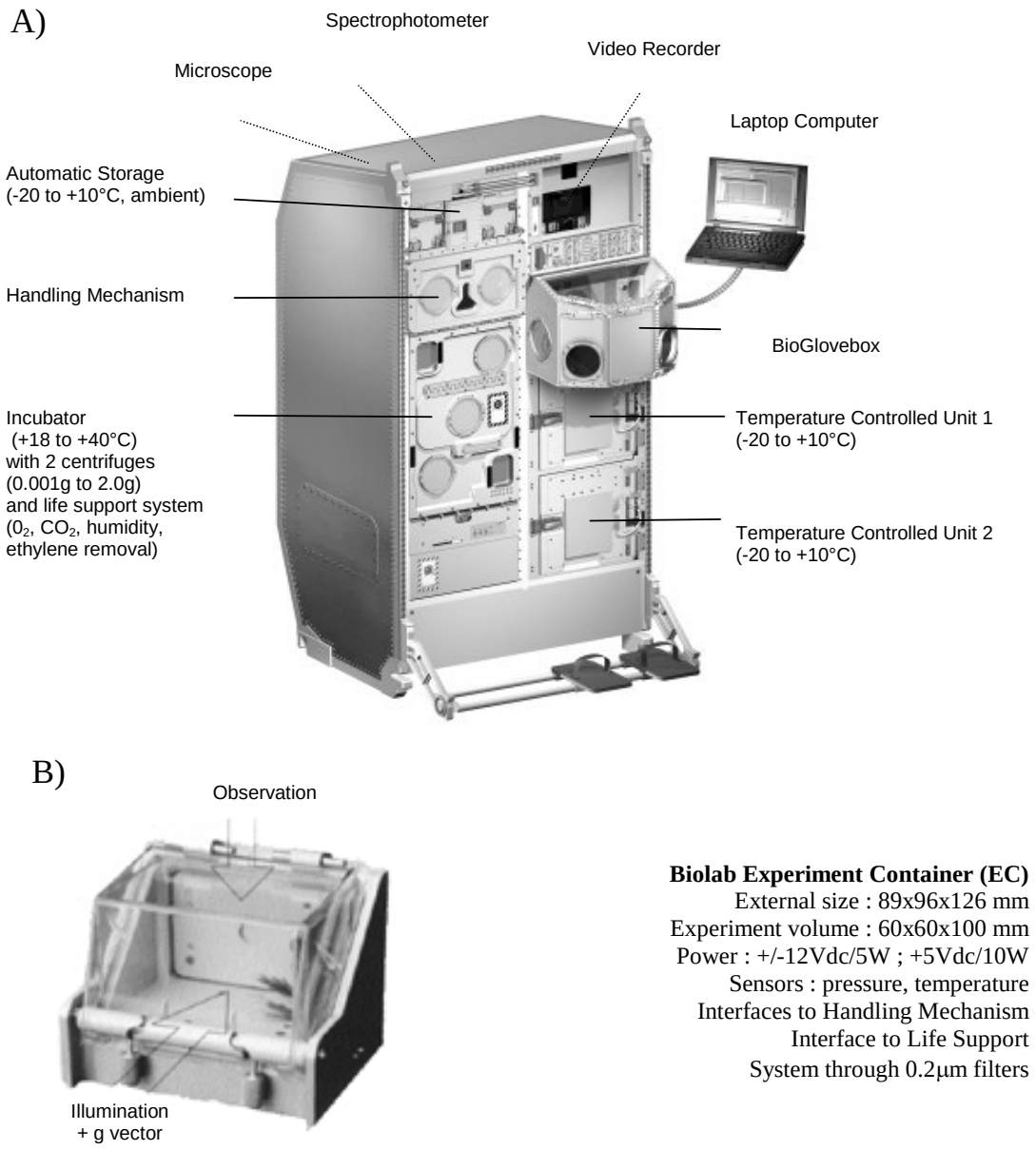

Figure 1. Required Hardware on ISS. (Pictures adapted from ESA / D. Ducros). A) BIOLAB. Part of ESA's microgravity facilities for Columbus, Biolab is a modular system divided into an automated section where experiments will be performed automatically, and a manual section dedicated to sample handling by the crew and storage. Although crew-time available will be extremely reduced, some experimental operation will be performed in the glovebox.. Different temperature units will give the possibility to store samples before and after the experiments. The facility is adapted for telescience operations. The experimental containers are located inside the incubator. B) Biolab Experiment Container (EC). Dimensions and features of this container are shown. The Advanced Experiment Container (AEC) has a video connection in addition to all the standard EC interfaces. In this case, the external size is : 125 × 175 × 147 mm. The EC interface plates supply the experiments with power and data lines and a controlled atmosphere (CO_2, O_2, humidity). The containers can be tested and handled safely by the crew in the glove box.

methodologies and equipment. On the one hand, we have modified basic handling techniques to make possible experiments with *Drosophila* in Space conditions (Herranz *et al.*, submitted). On the other hand, the present contribution describes the hardware we have developed to carry out *Drosophila* multigeneration experiments in the ISS, taking advantage of the Biolab facility. In addition to this main unit, we are designing devices for embryo preparation and observation in flight, as well as for fixation, preservation, or storage. We are convinced that the ESE-1 hardware presented here will provide the opportunity to study the adaptation of *Drosophila* to the Space environment in terms of development and function, at the organismal, cellular and molecular levels of analysis.

Drosophila multigeneration and functional studies with the ESE-1 unit

The Experimental Support Equipment Item 1 (ESE–1) of ESA is an automated culture system for multigenerational experiments with insects. Based on a concept that maximizes automation, ESE-1 will allow the culture in space of many generations of fruit flies to study adult behavior and embryo development with minimal participation of the crew. The main unit consists of two cultivation chambers and a central drum carrying a set of six food trays that can be automatically transferred and opened to the cultivation chamber(s) at specific times to feed the flies. Photos and a scheme of the ESE-1 unit are shown in Figure 2.

Inside the unit chamber, an infrared light and detector can be used to monitor fly activity automatically in combination with the video images obtained through the transparent wall of the chambers. To complete these observations, an analysis of the adaptation of the flies to normal gravity after a long-term space flight should be performed, in terms of development, viability, geotaxic response, mating, and fertility.

The embryos laid in the food trays can have different fates: a) to be discarded and killed by the specific killing actuator incorporated in the Unit (Figure 2E); b) to be transferred, opened to the second chamber and allowed to develop to obtain a new fly generation; c) to be removed for in-flight observation with the Biolab microscope; and d) to be removed for fixation and storage until the post flight analysis.

The current concept and tests have been performed with *Drosophila melanogaster*. It is obvious that it can be used with other small insects that have similar sizes and life cycles as *Drosophila*. Appropriate changes in the feeding medium and in the timing of the drum operation can easily be incorporated in the unit.

Complementary to this unit, we will have to develop specific hardware units for a) embryo preparation and *in vivo* observation of the development during the space flight with the microscope coupled to telescience Biolab facilities; b) embryo treatments for permeabilization, fixation and storage until morphological, structural, and molecular post flight analysis; and c) adult and embryo fixation and storage for the post-flight genetic expression analysis. With ESE-1 and these associated units, only limited activities during each cycle of around 14 days will require crew time. First, the ESE-1 unit would be transferred into the glovebox to remove the drum and the trays for embryo preparation and/or fixation, then the ESE-1 units would be transferred back of to the respective incubators. Secondly, time is required for sample preparation for microscopic observation or fixation and storage. The requirements of temperature control in a range of 18-25°C for adult and embryo survival, and 4°C for sample storage, are all in accordance with the Biolab facilities.

An Embryo Collecting Unit to prepare embryos for in-flight microscopic observation of *in vivo* development could work as follows. In ESE-1, the trays will be programmed to be exchanged for shorter periods of time (about 4 to 8 hours) to collect undeveloped embryos. The trays with the embryos and the feeding medium will be introduced in the Embryo Collecting Unit. This device, currently under development, is a closed chamber coupled with a system of fluid injection. There, a

hypochlorite dechorionating solution could be flushed and allowed to act for a few minutes. In addition to the dechorionating effect, this treatment liberates the embryos from the feeding medium enabling them to be transported by the fluid. A hypochlorite neutralizing solution would be then flushed through the unit, transferring the embryos into a second removable chamber, limited by transparent surfaces, which can be transferred and mounted in the Biolab Advanced Miniature Microscope or any alternative one in the ISS. The microscope offers fluorescent illumination allowing *in vivo* green fluorescent protein observation during embryo development. As the microscope has full telescience capability, the conditions will allow to the investigator to monitor the experiment and to take decisions or to assist the crew in the operations.

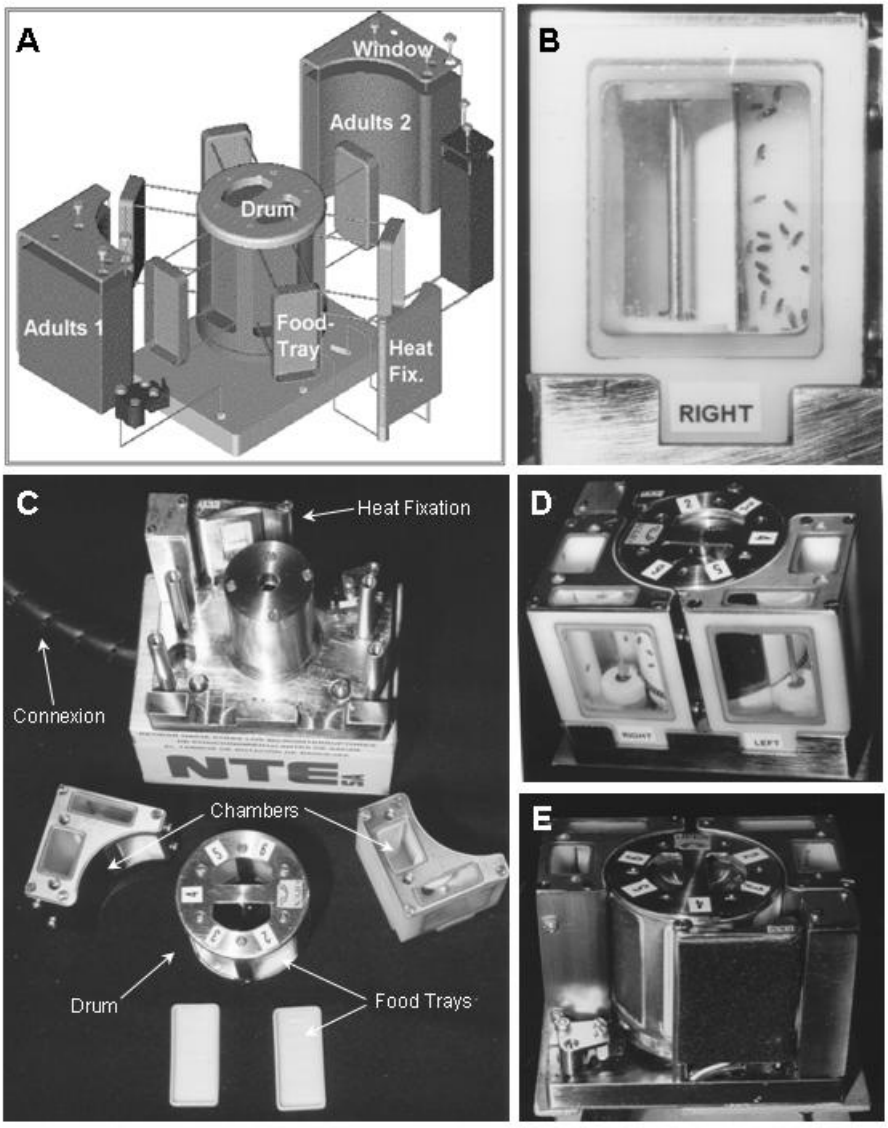

Figure 2. The Experimental Support Equipment-item 1 (ESE-1). A) Scheme of the different separated parts. B) Detailed view of a chamber with *Drosophila* imagoes inside. C) General view of the separate parts of the unit. D,E) Two views of the assembled unit (D, front; E, back). The main components of the system are two animal chambers and a drum with six food trays. Groups of flies, of the order of 50 per container (40 females and 10 males) can be handed-over in one of these units. Mobility of the adults is monitored by infra-red beams and video recordings. The adult chambers are

separated by doors from the food trays. Each food tray can be positioned in front of an adult chamber automatically and the doors can be opened on command. The motor assembly provides power to actuate the drum assembly, the doors chambers mechanisms. Single food trays can be subjected to a heat treatment to kill embryos or larvae by keeping them during 30 minutes at 50°C, or transfer them into specific embryo units for observation or fixation (not shown in the Figure). One of the last tray collections should be transferred into the second chamber of the ESE-1 unit to allow the development of a new generation of flies, that will allow the initiation of a new round of experiments. The drum is exchanged every two weeks with a new set of feeding trays, allowing the unit to be ready for a new generation. ESE-1 is fully compatible with the BIOLAB Experiment Container, and a different Biological Facility, the European Modular Cultivation System (EMCS) with limitations due to the lack of a handling mechanism. ESE-1 size: 100 mm × 60 mm × 60 mm including microcontroller (ESE Item-6 is the microprocessor used to perform these activities independently of the crew).

Linked to or even part of the previous unit, the Embryo Fixation Facility will allow fixing and storing embryos until post-flight morphological, structural and specific staining analysis. Taking advantage of the simplification of the current techniques of *Drosophila* permeabilization, fixation and storage (Herranz *et al.*, submitted), the embryos could be transported to a fixation chamber where a few permeabilization and fixation liquids could be delivered to the embryos. The fixation chamber could be detached and stored at 4ºC. The Unit is provided with the actuators to inject the different fluids.

This embryo/adult molecular preservation module is an extension of the units already discussed and should allow storing embryos and adults until post-flight for specific genetic expression analysis. As described elsewhere (Herranz *et al.*, submitted), the simpler type of successful treatment is based on the dehydration of the samples with acetone and storage at 4°C (Fujita *et al.*, 1987). The preservation of the proteins and RNAs is extremely good and quantitative with flies or embryos stored during more than 4 months at –20°C or 4°C. In the case of embryos, the tray from the main device ESE-1 would be inserted in the unit. There, acetone is injected allowing liberating embryos from the feeding medium. Fresh acetone is injected and changed twice before placing the unit at 4°C. If the direct acetone treatment fails to liberate the embryos, bleach dechorionization will achieve this objective and acetone treatment will be performed on the liberated embryos collected in a storage chamber. In the case of adults, a sucking device in the glove box will allow capturing the flies in the chambers of the ESE-1 unit, once they have been immobilized by exposing them to 4°C for 30 min. On the other hand, the Adult Fly Anesthetization and Collection Unit discussed by Pence *et al*. (1999) could provide an alternative approach to this problem. The flies will then be transferred to the storage chamber where they will be treated with a solution of acetone and finally stored at 4ºC. Crew time will only be needed for the transfer of the flies into the fixation unit and of the unit into the freezer and coolers.

ESE-1 was developed and tested with *Drosophila*, but it could be easily adapted with small modifications to other small invertebrates. Nevertheless, *Drosophila* is certainly one of the biological models of higher interest in Space Research. Its small size, resilience, relatively short life-cycle, and the knowledge of its genetic organization and regulation argue in favor of the utilization of *Drosophila* in the next phase of Space Biology Research, namely the establishment of autonomous small ecosystems outside the earth, to consider the difficulties encountered in a long distance exploration by space living organisms. Obviously, the project of long-term cultivation of *Drosophila melanogaster* in Space includes different experiments, progressively more complex, starting from shorter to longer *Drosophila* culture periods. The first step in this sequence would consist in testing

the complete hardware in specific ground facilities, as hypergravity (centrifuges), random positioning machine (3D clinostat), and eventually magnetic levitation. An updated version correcting of ESE-1 is currently available. Preliminary testing of the other concepts presented in this paper has been successful. As the Biolab facility is scheduled to be launched onboard ISS soon after the resumption of Shuttle flights to the ISS, we should soon be ready to start multi-generation experiments of *Drosophila* in the ISS facilities with ESE-1 and its sub-units.

In addition to clarifying the mechanisms involved in the effects of Space environment on behavior, development, and cell functions, the availability of ISS opens the way for the long-term cultivation of certain model systems in space and the study of their adaptation to this alien environment during these multi-generation experiments. For the first time it would be possible to study the effect of Space on complex organisms in evolutionary terms. Even if the time periods of exposure to such an environment are certainly small in terms of evolutionary mechanisms, it is important to carry them out if the whole enterprise of Space exploration involving human beings is going to be pursued in the 21st Century. First of all, such studies are linked to the development of effective life support systems necessary for the long maintenance of living organisms outside the earth and the challenge of long-distance manned space exploration, since we need to know how stable life systems are in these long-term conditions. Furthermore and with the same logic, if long-term exposure to alien environmental conditions can produce adaptations and changes in the properties of biological systems, they should be detected using faster growing biological systems than humans before colonization of external worlds is adopted as a sensible goal for the 21st Century.

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45th Annual *Drosophila* Research Conference

The 45th Annual *Drosophila* Research Conference was held on 24-28 March 2004 at the Marriott Wardman Park Hotel, Washington, DC. The 2004 Organizing Committee was Paul Lasko (McGill University, Montreal, Canada) and Howard Lipshitz (Hospital for Sick Children, Toronto, Canada). The conference was sponsored by The *Drosophila* Board in association with the Genetics Society of America, 9650 Rockville Pike, Bethesda, MD 20814-3998. The Program and Abstracts Volume lists 923 presentations, including 153 platform session talks and 757 posters.

Keynote Address

Peter A. Lawrence (Medical Research Council, Cambridge, UK). Pattern formation: From flake to avalanche.

Plenary Lectures

Mark A. Krasnow (Stanford University, CA). Making and shaping the tiny tubes of the *Drosophila* tracheal system.

Kristin White (Massachusetts General Hospital, Charlestown). The regulation and execution of apoptosis in development.

Matthew Freeman (Medical Research Council, Cambridge, UK). Investigating rhomboids in *Drosophila* and beyond.

Talila Volk (Weizmann Institute of Science, Rehovot, Israel). How: a post-transcriptional regulator of tissue differentiation.

Francis S. Collins (National Human Genome Research Institute, Bethesda, MD). Biology in the era of complete genomes.

Henry M. Krause (University of Toronto, Ontario, Canada). Looking for needles in a haystack: *Drosophila* nuclear receptors and the elusive ligands.

Trudi Schüpbach (Princeton University, NJ). Regulation of Gurken expression in oogenesis.

J. Lawrence Marsh (University of California, Irvine). Can flies help man treat neuro-degenerative diseases?

Kevin P. White (Yale University, New Haven, CT). Systematic mapping of developmental genomic regulatory networks.

Linda Partridge (University College, London, UK). Drosophila as a model organism for research into aging.

Ed Kravitz (Harvard Medical School, Boston, MA). Genetic manipulations at the Fruit Fly Fight Club.

Erika L. Matunis (Johns Hopkins University School of Medicine, Baltimore, MD). Balancing spermatogonial stem cell self-renewal and differentiation.

Workshops

Ecdysone

Organizers: Laurie H. von Kalm (University of Central Florida, Orlando) and Lucy Cherbas (Indiana University, Bloomington).

Genetics of Non-Drosophilid Insects: Emerging Models
Organizers: Jack Warren (University of Rochester, NY) and Claude Desplan (New York University, NY).

Functional Genomics of Mitochondria
Organizer: David Rand (Brown University, Providence, RI)

Olfactory Learning and Memory
Organizers: Scott Waddell (University of Massachusetts Medical School, Worcester) and Efthimios M.C. Skoulakis (Institute of Molecular Biology and Genetics, Vari, Greece)

RNA Biology
Organizer: Susan R. Haynes (Uniformed Services University of the Health Sciences, Bethesda, MD)

Chromatin Structure and the Cell Cycle
Organizer: John Manak (Stanford University School of Medicine, CA)

Stem Cells
Organizers: Sumana Datta (Texas A&M University, College Station) and Haifin Lin (Duke University Medical School, Durham, NC)

Drosophila Research and Pedagogy at Primarily Undergraduate Institutions
Organizers: Karen Hales (Davidson College, NC), Cris Cheney (Pomona College, CA), Bev Clendening (Hofstra University, Hempstead, NY), and Elaine Reynolds (Lafayette College, Easton, PA)

Mechanisms of Translation
Organizers: Rolando V. Rivera Pomar (Max Planck Institute for Biophysical Chemistry, Göttingen, Germany)

Cell Cycle Checkpoints
Organizers: Tin Tin Su (University of Colorado, Boulder) and Claudio Sunkel (Universidade do Porto, Portugal)

Immunity, Pathogenesis, and Hematopoiesis
Organizer: Catherine Brennan (Sloan-Kettering Institute, New York, NY)

Extracellular Matrix Interactions and Signaling
Organizers: Rolf Bodmer (The Burnham Institute, La Jolla, CA) and Hannele Ruohola-Baker (University of Washington, Seattle)

Gravity and the Fly
Organizers: Kathleen M. Beckingham (Rice University, Houston, TX) and Sharmila Bhattacharya (NASA Ames Research Center, Moffett Field, CA)

The *Drosophila* Board

The Board's duties include: overseeing community resource centers and addressing other research and resource issues that affect the entire *Drosophila* research community. The Board also administers the finances for the annual North America *Drosophila* Research Conference and its associated awards, and it chooses the organizers and the site of the annual meeting. The Board consists of nine regional representatives, eight from the U.S. and one from Canada, who serve 3-year terms. It also has three elected officers including a President, a President-Elect and a Treasurer. President-Elect is a new position that began in Fall 2004. In addition, the Board has *ex officio* members who represent *Drosophila* community resource centers or international *Drosophila* communities. For more information about the Board and the summaries of the annual Board meetings, see: http://flybase.net/.data/docs/DrosBoard.

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