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Original Article

Microgravity effects on the oogenesis and development of embryos of *Drosophila melanogaster* laid in the Spaceshuttle during the Biorack experiment (ESA)

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ABSTRACT. The results obtained during the last successful flight of the Challenger Shuttle, in early November 1985, indicate that oogenesis and embryonic development of *Drosophila melanogaster* are altered in the absence of gravity. Two hundred forty females and ninety males, wild type Oregon R *Drosophila melanogaster* flies were flown in the Spaceshuttle during the 7-day D-1 mission and the embryos laid during the spaceflight were recovered and studied. Although some eggs developed into normal 1st instar larvae and many into late embryos in the 23±2 h collection periods throughout the flight, several interesting differences from the parallel ground and in-flight centrifuge controls were observed: 1) There was an increase in oocyte production and size. 2) There was a significant decrease in the number of larvae hatched from the embryonic cuticles in microgravity. 3) The majority of embryos were normally fertilized and at late stages of development, except in the space-flown containers in microgravity where a percentage of earlier stage embryos were recovered showing alterations in the deposition of yolk. 4) In correspondence with these results, at least 25% of the living embryos recovered from space failed to develop into adults. 5) Studies of the larval cuticles and those of the late embryos indicate the existence of alterations in the anterior, head and thoracic regions of the animals. 6) There was a delay in the development into adults of the embryos and larvae that had been subjected to microgravity and recovered from the space shuttle at the end of the flight. No significant accumulation of lethal mutations in any of the experimental conditions was detected as measured through the male to female ratio in the descendant generation. It seems that *Drosophila melanogaster* flies are able to sense and respond to the absence of gravity, changing several developmental processes even in very short space flights. The results suggest an interference with the distribution and/or deposition of the maternal components involved in the specification of the anterioposterior axis of the embryo.

KEY WORDS: Drosophila melanogaster, *development, oogenesis, microgravity, space flights*

Introduction

In spite of recent progress in the genetic, molecular and descriptive approaches of the embryology of certain systems, such as *Drosophila melanogaster* (Sambrock, 1985; Akam, 1988), the emergence of a complex organism from an apparently homogeneous fertilized oocyte remains a wonderful event which still challenges our current scientific understanding. The notion that physical factors may be playing an epigenetic role in the early events in animal development remains an interesting, although unproven possibility (Malacinski and Bryant, 1984; Nucitelli, 1986). Since Pflueger in 1884 and Schultze in 1900 (Schultze, 1900) proposed that in frog embryos gravity could be playing a role in these initital events of segregation of the zygotic cytoplasm, many experimental embryologists have been trying to test this hypothesis using, for example, higher g forces exerted on the embryonic cytoplasm by centrifugation (Kalthoff, 1976; Brown and Schubiger, 1977; reviews in Davidson, 1976; and Grant, 1978) or other experimental tricks like immobilizing the embryos in a fixed position vs the gravity vector (Kochav and Eyal-Giladi, 1971; Cooke, 1986). The issue remained practically unsolvable, however, until the availability of the biological laboratories in Space made it possible to test the hypothesis in what should, in principle, be an unequivocal way. It proved particularly difficult to test whether gravity could be playing a role in the organization of the oocyte cytoplasm during oogenesis, since, in many of the experimental systems used, this phase of development lasts long periods of time inside the female body (Taylor *et al.*, 1986). Thus, when in 1980, the Microgravity Program of the European Space Agency issued a Call for Proposals for Biological Experiments in Space, we decided to select the insect system *Drosophila* to make a preliminary test of any developmental effects caused by the unusual space environment (Marco *et al.*, 1983). *Drosophila* seemed particularly suited for this purpose, due to its short life cycle, its

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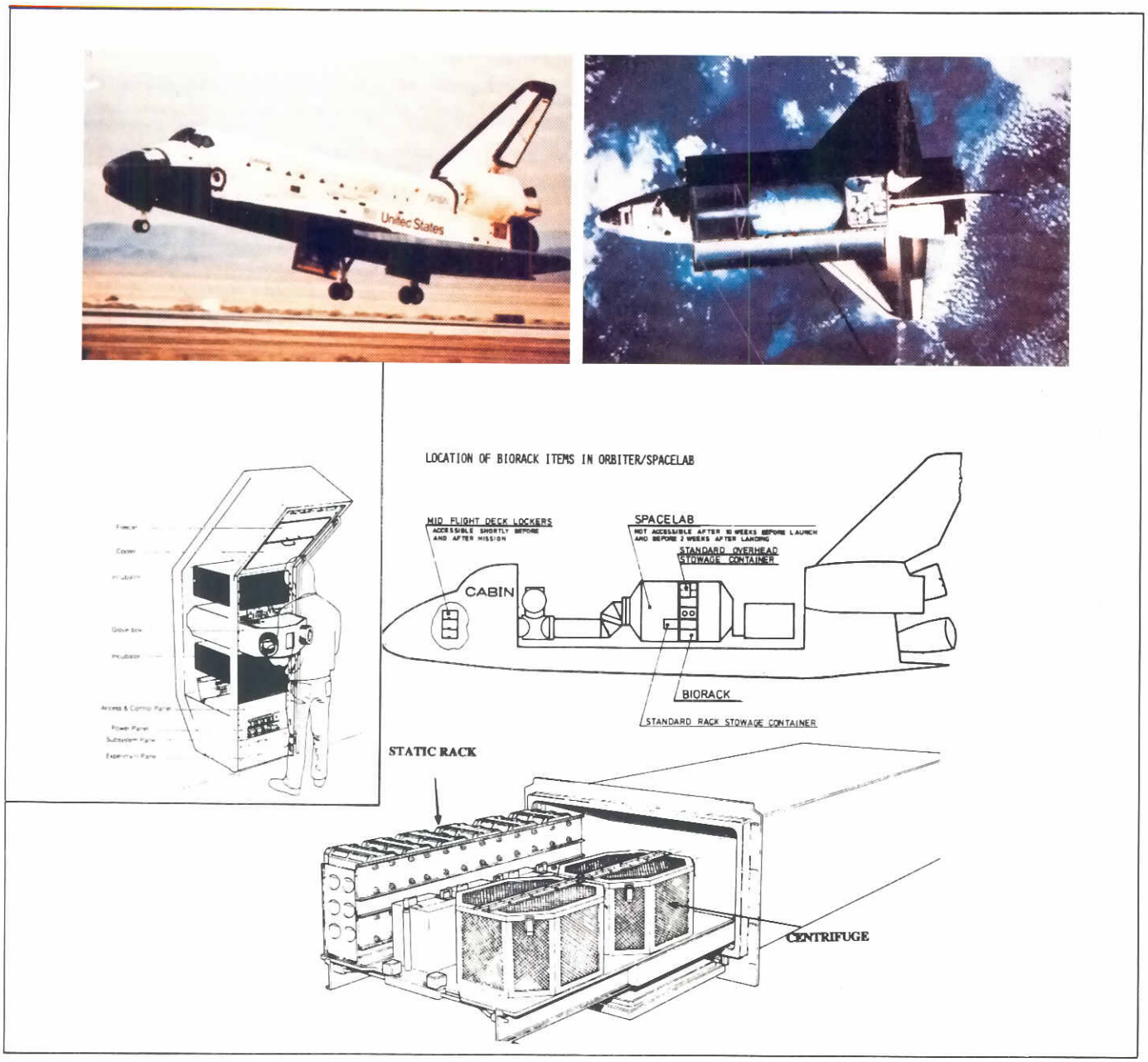


Fig. 1. The Biorack Facility in the Shuttle. *On the D-1 mission, several different experiments were flown together in these containers stored in the appropriate incubators: (A) Procaryotes:* B. subtilis *(growth and sporulation) and* E. Coli *(genetic recombination, transformation and transaduction and antibiotic sensitivity). (B) Unicellular and lower eucaryotes:* Chlamydomonas reinhardii *(cyrcadian rhythms of phototropic response),* Paramecium aurelia *(growth),* Physarum polycephalum *(cytoplasmic streaming), human lymphocytes (mitogen (Concavalin A) response) and transformed hybridoma plasma cells. (C)Complex eurcaryotes:* Drosophila, Carausius morosus *and* Xenopus *embryogenesis, plants (lentil roots graviperception). These photographs were kindly supplied to the authob by the European Space Agency.*

ability to survive during space flights and the possibility it offers of exploiting the wealth of genetic and developmental knowledge accumulated on this organism. Although the results of earlier space experiments (Parfyonov *et al.*, 1979; Miquel, 1984) seemed to indicate that *Drosophila* development could proceed normally in space, the type of experiments feasible in earlier space facilities made quantitative results difficult to obtain. Thus, in principle, this early evidence could not absolutely exclude an involvement of the physical environmental parameters altered in space in any of the many complex processes which participate in development.

TABLE 1

EFFECT OF GRAVITATIONAL FORCES ON *DROSOPHILA* EMBRYONIC SIZE

	Space Embryos				Ground Controls			
	0g (static racks)		1g (centrifuge)		1g (static racks)		1,4g (centrifuge)	
Length	0.4826±0.0018	*	0.4730±0.0039	*	0.4582±0.0062	**	0.4334±0.0048	
Width	0.1707±0.0010	ns	0.1676±0.0022	*	0.1567±0.0027	*	0.1466±0.0022	
number of embryos counted	391		103		74		137	

Data correspond to the pooled data of all the embryos recovered from the 4th and the 5th tray exchanges which were available for this type of measurement after the dechorionization, devitellinization and fixation procedures described in Materials and Methods. (Measurements are in mm)

* $p < 0.55$ and ** $p < 0.01$indicate the statistical significance of the adjacent numbers on each side.

In this article, we describe in detail the results on the effects of the space environment on the embryogenesis of *Drosophila* obtained in the better control conditions afforded by Biorack (Fig.1), a multiuser biological facility developed by the European Space Agency (ESA), during a 7-day spaceflight of the Shuttle. This spaceflight — the West German Spacelab D-1 mission — was the last successful flight of the Challenger Shuttle and took place during the first week of November 1985. The results indicate that although *Drosophila* development is feasible in the absence of gravity, it is altered in a way suggesting a modification in the deposition of the maternal information in the egg, particularly that utilized in the organization of the anterior end of the embryo. Furthermore, there is an increase in the size and number of eggs deposited in space, particularly under continuous microgravity conditions. A preliminary and partial communication of these results has already been published (Marco *et al.*, 1986).

Results

Drosophila *oogenesis in the Spaceshuttle*

Several different observations discussed below indicate that in the conditions of the Biorack experiment, egg laying was mostly induced as a response to the insertion of fresh medium at the beginning of the collection times (Figs. 2 and 3). These criteria include: a) the number of embryos recovered, which was relatively independent of the length of the collecting times; b) the relatively late stage of development of the embryos recovered and the number of hatched larvae in the controls, which was higher than expected for a uniform collection; and c) the fact that a few mistakes in the experimental procedure (three trays were inserted upside down) did not seem to affect the number of embryos recovered in the subsequent collection.

The laying periods varied from 12 h for the 2nd tray exchange, 21.5 h for the 3rd, 4th and 5th tray exchanges and 25.5 h for the 6th tray exchange (Fig. 3). The trays from the first exchange were not analyzed due to lack of time and to the fact that they contained mostly embryos laid during the 16 h preceding launch after late access fly selection and preparation of the experimental containers.

The comparison between the results obtained in space and those of the exact replica experiment carried out in the ground-based Biorack Training Module allow us to reach two main conclusions on the quantitative behavior of *Drosophila* oogenesis and egg laying in space:

A) There was a stimulation of oogenesis (Fig. 4). This stimulation is particularly clear in the case of the containers kept in microgravity conditions, which in all collections but the second exceeded the number of embryos recovered in the containers from the 1g centrifuges and ground controls (Fig. 4). The analysis (see legend of Fig. 2) also indicates that in the case of both types of space containers, there was an increasing number of embryos laid as the flight proceeded, suggesting a time-dependent reaction of the animals to the experimental (space) conditions. Although the effect is more clearly visible in animals maintained continuously in microgravity, the containers in the centrifuge in the flying Biorack seemed to give intermediate results. The results obtained in the ground controls, especially those of the containers in the static racks, were lower than the usual controls obtained by us in the regular laboratory conditions and corresponding to the range of the controls obtained one week after the experiment (Fig. 4) for reasons that may be related to the particular environment in the Biorack facility. In fact, the results also suggest that the maintenance of the flies in the on-ground centrifuge may also have a stimulatory effect on

TABLE 2

EFFECT OF MICROGRAVITY ON *DROSOPHILA MELANOGASTER* EMBRYONIC HATCHING
(% of larvae recovered in each collection)

A. INDIVIDUAL COLLECTIONS

Mission elapsed time (Start)	Collection (duration)	Flight Module (Space)			Training Module (Earth Control)		
		0g		1g Centrifuge	1.4g		1g Centrifuge
0 day 22h	Tray 2 (12+10h)	3.3±0.8	ns	3.7±1	8.4	ns	3.5
1 day 11h	Tray 3 (21.5h)	0.8±0.6	*	7.7±5.5	8.6±1.4	ns	15.3±1.4
2 day 8h	Tray 4 (21.5h)	0.7	ns	-	8.2±7.2	ns	11.7±3.5
3 day 5h	Tray 5 (21.5h)	1.1±0.4	*	10.7±0.7	7.1±0.7	ns	22.5±5.0
4 day 3h	Tray 6 (25.5h)	3.8±0.2	*	21.3±3.5	6.7±1.0	ns	22.5±5.0
Control 1 week after recovery (21.5h)		3.8±0.2	ns	8.4±3.6	5.3±1.5	ns	5.3±5.7

B. OVERALL POOLED RESULTS

	FREE CONTAINERS		CENTRIFUGE		CONTROL (1 week after landing)
FM (space)	0.9±0.2	**	9.6±2.4	**ns**	5.3±1.4
Percent larvae	**		**ns**		**ns**
TM (ground control)	20.5±4.3	*	8.0±2.0	**ns**	6.1±1.7

** Data differences significant $2p < 0.001$ among adjacent values
* Data differences significant $2p < 0.05$ among adjacent values
ns Data differences among adjacent values non significant

Drosophila oogenesis. Whether this stimulatory effect is a response to changed environmental conditions like humidity is made unlikely by the ground controls, performed under the same experimental conditions.

B) An additional change between the 0g, 1g centrifuge and ground control embryos was detected when the size of the embryos was measured. Although there is some variability in the size of embryos in normal wild type embryos, this parameter is quite constant and dependent on the strain used (Warren, 1924). Interestingly, as shown in Table 1, the embryos recovered from space, which had been subjected to continuous microgravity, showed greater size than those coming from the space 1g centrifuge and the two ground controls. Furthermore, the embryos recovered from the trays inserted in the containers housed in the ground control centrifuges showed even greater reduction in size than the normal 1g controls.

Drosophila *embryogenesis in the Spaceshuttle*

Although apparently normal embryos and larvae were found even after 6 days of space flight, several different experimental observations indicate that embryogenesis is probably modified in space:

1) Hatching of larvae from the embryonic envelope is greatly decreased under microgravity conditions (Table 2). In the containers in the Spaceshuttle, the percentage of hatched larvae was only 10% of the corresponding percentage found in the 1g centrifuges. Interestingly, this difference in the percentage of hatching was only observed in the 3rd and posterior collections in space, i.e., when the oogenetic process (normally requiring 2 days) had occurred completely in space. In Table 2, the statistical comparison of the pooled data is also shown.

2) The microscopic analysis of a significant number of embryos recovered from the experiment rules out some simple explanations for these results, such as an increase in the number of unfertilized eggs as the reason for the decrease in hatching under microgravity. As shown in Table 3, a high number of embryos were at the final stages of development — at or close to stage 17 of Campos-Ortega

TABLE 3

CLASSIFICATION OF SECTIONED *DROSOPHILA* EMBRYOS FROM THE MICROGRAVITY AND CONTROL CONDITIONS

	FLIGHT MODULE		TRAINING (Ground) Module
	Microgravity %	1g Centrifuge %	Static containers %
Final stages of development (Stages 16 & 17)[1]	46	32	73
Intermediate developed (Stages 14 & 15)[1]	21	21	<5
Germ band extension (Stages 8-10)[1]	23	<5	<5
Blastoderm	3	11	7
Unfertilized	8	36	15
Total number of embryos examined	39	17	15

[1] Stages described by Campos and Hartenstein (1985)

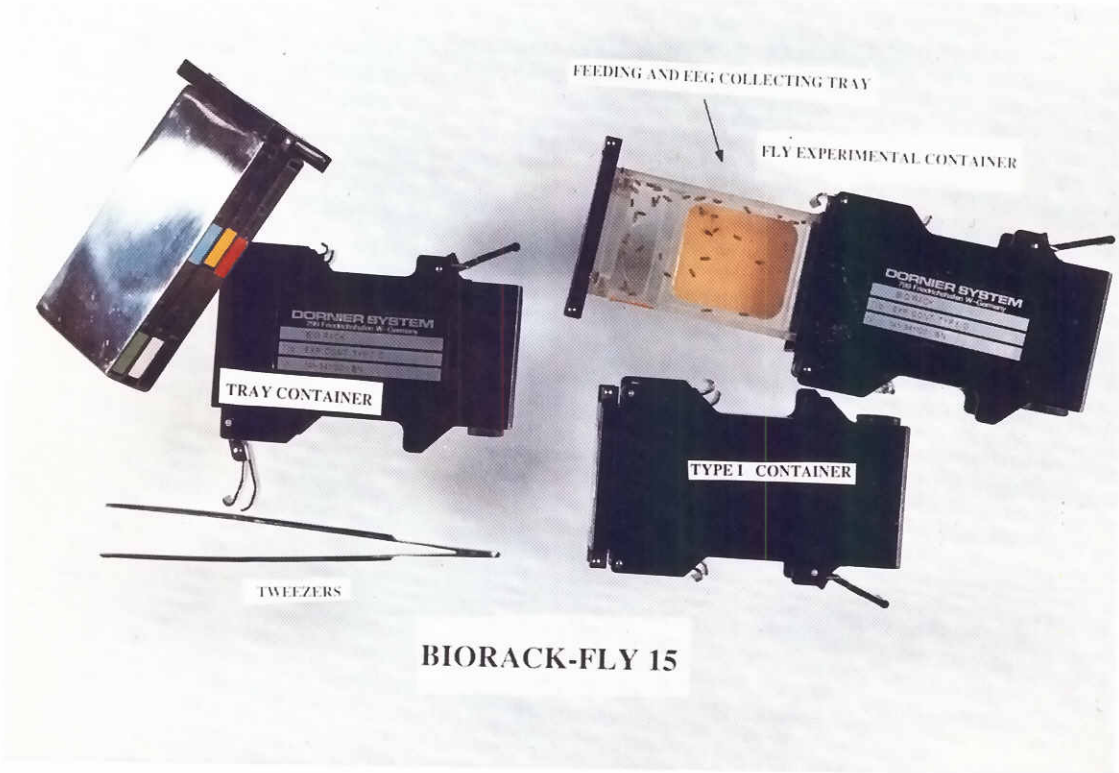
and Hartenstein (1985). The information obtained from these microscopic observations indicates that these developed embryos and/or hatched larvae showed normal interior organ structures (Fig. 5A). However, in the case of the space-flown embryos in microgravity, an additional set of embryos was recovered at earlier stages of development, but could not be easily classified since they did not correspond clearly to any of the described stages of normal development (Campos and Hartenstein, 1985). We think that these earlier embryos recovered from 0g containers from space (Fig. 5B) may correspond to germband extension embryos, in which the usual yolk granule-rich areas, normally very conspicuous in these early embryos, was less evident.

3) The study of the larval cuticle of the hatched larvae was complicated by the fact that we had to use freezing our method for storing the samples. Freezing induces some retraction in the anterior part of the larval cuticles (Table 4). Nevertheless, the larvae recovered from space showed a clear increase (Table 4) in the defects of the head and thoracic segments as shown in Fig. 5E, F, G. The defects consist in the presence of foldings and retractions of the head and thoracic segments, which in the stronger epiphenotypes modify the position of the mouth pieces. In Fig. 5E, F and G, a series of defects of increasing strength corresponding to the data in Table 4 are presented. This retraction syndrome as well as the decreasing percentage of hatching described above could be related to abnormalities in the head and thorax muscle development, since hatching is somewhat driven by head and larval movements in the egg case.

4) This effect was also visible in the cuticles from the developed embryos recovered in the experiment. In spite of the stimulation in the number of embryos recovered under microgravity, not many of the embryos reached the stage of development at which the cuticle could be observed. Nevertheless, the embryos recovered with observable cuticles from the microgravity containers showed conspicuous alterations at their anterior end and possibly at the posterior end as well. These alterations were not seen in the embryos from the controls. Examples of the alterations of the embryos are shown in Fig. 5H, I.

5) As mentioned in Materials and Methods, the last tray collection from space recovered about 17 h after landing at Kennedy Space Center was allowed to develop to adulthood to monitor whether any effects appeared at later stages of the *Drosophila* life cycle. Three types of observations could be made using these developed animals, two of which relate to the further development of the embryos and one to the mutational load during the experiment:

1) There was a decrease in the number of flies which reached adulthood in the case of the trays recovered from space, since nearly 25% of the embryos were unable to develop into adults. Due to the constraints experienced during the recovery of the experiment, we did not count the embryos and larvae recovered in the last egg collection in Cape Kennedy. Therefore, the number of adults hatched from the last tray collection was compared to the number of eggs recovered in the preceding collection which, because it was shorter, should have yielded a smaller number



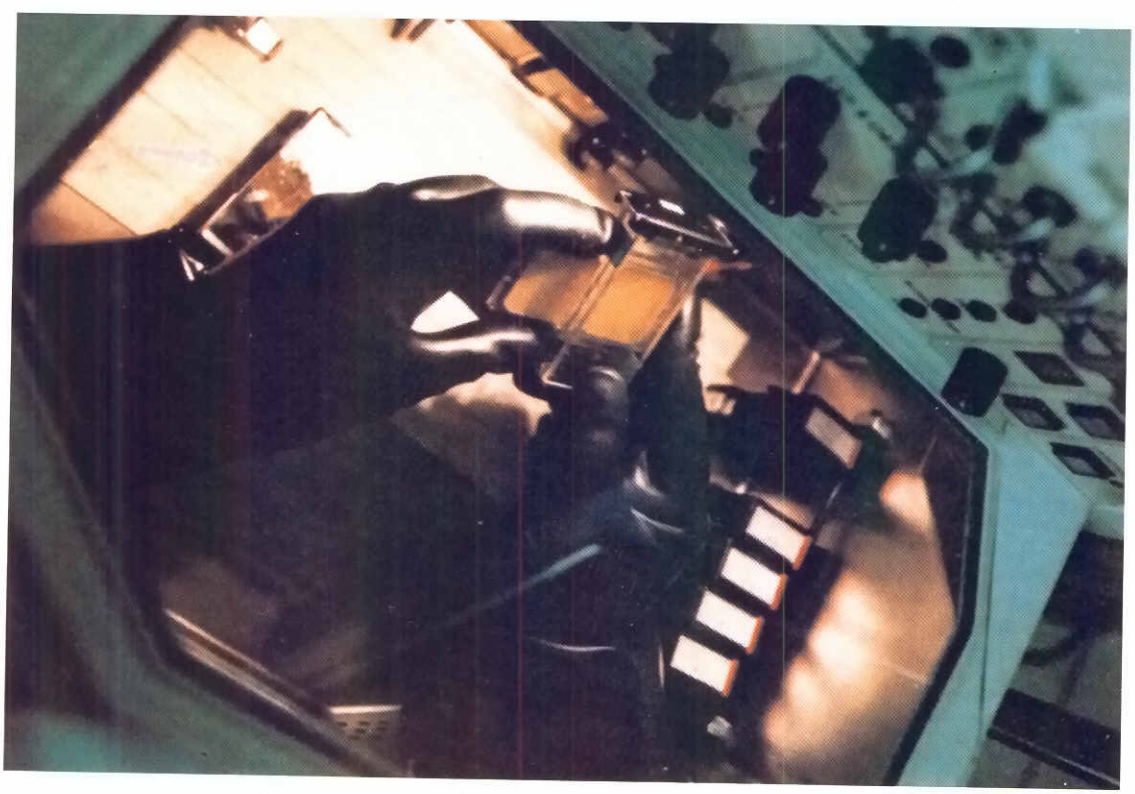


Fig. 2. Hardware of the biorack Fly 15 Experiment: *Type I experimental and tray holding containers. The trays were labeled with different colors to be matched with the colors in the containers to minimize possible mistakes in the operating protocol. Below, the Biorack with the operation of the tray exchange. These photographs were kindly supplied to the authors by the European Space Agency.*

of embryos. This comparison gave a ratio of 0.74 ± 0.05 for the space containers while in the ground control containers the number was 1.44 ± 0.16, reflecting the longer laying period of the last collection. This decrease in the number of adult flies recovered from the trays which had been kept continuously under microgravity was not much different from the ones in the 1g centrifuge. In fact, the decrease must have occurred at the embryonic and larval stages since there were hardly any black, unhatched pupae in any of the containers. The few black pupae recovered in the experiment were mostly visible in the developing populations of the space trays (8 per container from space vs 1 per container from the ground control). The mutational origin of this lethality is unlikely (see below). A possibility is that they may correspond to the embryos in earlier stages of development with unusual type of yolk deposition (compare with Table 3).

2) There was an additional difference between the trays in the 1g centrifuge and the ones in microgravity. As can be seen in Fig. 6, the emergence of the adults was delayed by about 1 day in the case of the trays from the 0g containers, a result which also may be related to the inhibition in hatching described above. This difference was also observed (but not precisely quantified) during the period of larval growth, in that there was a delay in the appearance of third instar larvae and in their pupation movements out of the feeding medium in the case of the containers coming from microgravity, i.e., from the static space rack containers.

Mutational load in short spaceflights

A comparison among the male vs female ratios in the first generation of flies emerged from the last collection trays rules out a relatively high accumulation of lethal mutations due, for instance, to space radiation levels as a cause of the described effects. As is well known, any detectable increase in lethal mutations immediately shows up in the decrease in the number of males in the first generation, due to the uncovering of the lethal mutations which may have occurred in their single X chromosome. In fact, if anything, the ratios of males to females were closer to unity in the progeny of the space-flown flies (female/male ratio = 1.01 ± 0.07 in the emerged flies from the last collection of the static rack containers and 1.05 ± 0.03 for the centrifuge) than in the corresponding ground control sibling flies (1.14 ± 0.07 for the static racks and 1.10 ± 0.15 for the centrifuges). Moreover, at least superficially, the emerged adults did not show any conspicuous alterations in morphology that could be observed under the dissecting microscope, making any later phenotypic or phenocopying effect of the space environment on the first generation of flies hatched on Earth insignificant at the present level of detection. This was in contrast to the effects of other physical phenocopying treatments applied

TABLE 4

EFFECT OF SPACE ENVIRONMENT AND/OR FREEZING ON LARVAL MORPHOLOGY

0g FM (Space Static Racks)	**Tray exchanges**		
	2nd	**4th**	**5th**
Normal	14	0	0
Head*	2	0	3
T1*	1	3	4
T2*	0	10	4
1g FM (Space centrifuges)			
Normal	13	1	0
Head*	0	5	4
T1*	1	1	13
T2*	0	0	5
1g TM (Ground control)			
Normal	7	19	23
Head*	5	8	19
T1*	0	5	12
T2*	0	0	0

*Number of larvae with Head, Head and T1, and Head, T1 and T2 abnormalities as described in Figure 3.

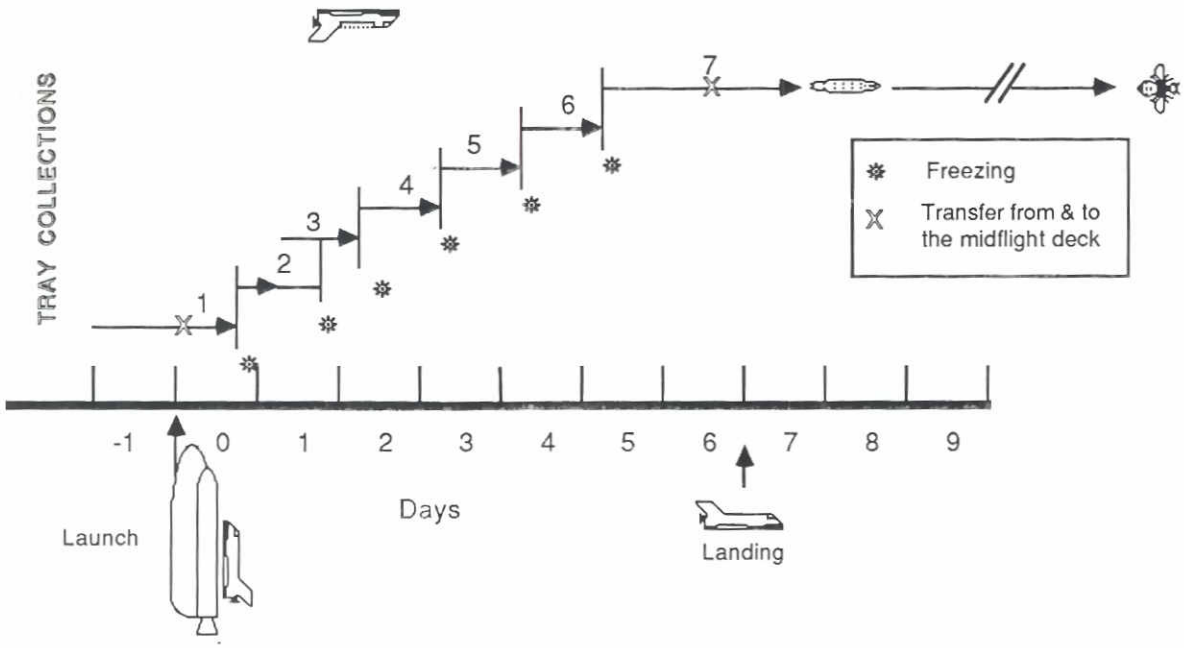


Fig. 3. Collecting protocol.

during early embryogenesis, for instance heat shock or ether treatment (Goldschmidt, 1935; Gloor, 1947; Capdevila and García-Bellido, 1978).

Discussion

The opportunity of carrying out better controlled experiments in the Biorack facility has made possible a quantitative analysis of several developmental parameters of *Drosophila* in space. Our results can be interpreted as a confirmation on firmer evidence of earlier reports (Dubinin *et al.*, 1973; Miquel and Philpott, 1978) concerning the development in space of *Drosophila* embryos. Moreover, in our experiment we have bonafide evidence of this capability for development even in the case of embryos laid after 6 days of spaceflight, i.e., when their oogenesis had been carried out entirely in the space environment. At least 75% of the embryos studied were at final stages of development, indicating that gravity is not playing an *absolute* epigenetic role in embryonic differentiation or morphogenesis, including the process of laying the maternal components in the egg during oogenesis.

Nevertheless, the overall data indicate that embryonic development and hatching is affected to some extent by the exposure to microgravity. In fact, it is interesting that the different morphologies of embryos and larvae found can be ordered in a series of decreasing epiphenotypic alterations, which are particularly clear in the case of the flies kept continuously under microgravity: alterations, which are particularly clear in the case of the flies kept continuously under microgravity:

a) A significant number of embryos kept under microgravity conditions in space appeared in an earlier abnormal phase of development (Fig. 5B); they did not show clearly the usual yolk-rich areas typical of the *Drosophila* embryos. Such embryos were not found in the sample studied from the 1g centrifuge in space and from the ground control sample. The number of these abnormal embryos correlates with the decrease in the number of adults emerging from the trays recovered from the space containers, suggesting that they may not be able to develop to completion. In the 1g controls in space, there is a small increase in the number of unfertilized oocytes.

b) A large number of embryos was delayed or blocked at late phases of development. The ones that developed until segregation of the cuticular landmarks showed an accumulation in space of abnormalities in the head and thorax of very late embryos and larvae, and some hints of small alterations in the posterior end of the late embryos (Fig. 5H). The interpretation of this result is complicated by the retraction effects of the freezing step used in the storage of samples. In subsequent experiments, for instance, in the International Microgravity Laboratory, programmed to fly shortly after the resumption of the Shuttle flights, we will try to minimize this freezing effect.

c) There is a decrease in the number of adults emerging from embryos laid in space, and a delay in their development, as well as a slight accumulation of dead black pupae.

All this is reminiscent of the series of expression phenotypes (Mohler and Wieschaus, 1986) described for some

dominant maternal-effect mutations of *Drosophila melanogaster* causing the production of double-abdomen embryos, Bicaudal C and Bicaudal D. In these mutations, incomplete penetrant hypomorphic phenotypes have been described which extend from unhatched normal embryos to a series of embryos with relatively small defects in the head development (Mohler and Wieschaus, 1986), bearing some similarity to the defects described in our experiments. Moreover, recent reports (Frohnhöfer and Nüsslein-Volhard, 1986; Lehmann and Nüsslein-Volhard, 1986; Driever and Nüsslein-Volhard, 1988) indicate that both in the case of anterior head and thorax structures and for the posterior end of *Drosophila* embryos, localized maternal components are stored in the anterior and posterior ends of the embryo and play an important role in setting the anterior-posterior axis of the embryo. Therefore, the important and intriguing suggestion can be made that microgravity may be interfering in some way with the normal organization or deposition of these components. These effects are more evident in the case of the flies kept continuously under microgravity, and in this case, in the samples recovered 36 h after the beginning of the spaceflight indicating that oogenesis has to be carried out under microgravity for these effects to become apparent. Whether additional space environmental parameters are also playing a role is uncertain. In general, the flies in the 1g centrifuge showed results intermediate between those of the microgravity and ground control flies, but it has to be pointed out that the flies kept in the 1g control centrifuges were also exposed to microgravity at the beginning and end of the mission during the periods of Biorack activation and deactivation (around 9 h each), and transiently during 30 min to 1 hour every day at the time of tray exchanging as well as during the times in which the centrifuges were stopped during the operations of the additional Biorack experiments. Furthermore, centrifugation is not an identical or perfect physical substitution of gravitational forces (Schatz, 1983).

Space radiation does not seem important since the level of radiation in the Biorack measured in a companion experiment (Bücker *et al.*, 1986a) gives for HZE particles a level of 40-50 hits per sq. cm per day, which indicates that only a small proportion of *Drosophila* embryos could be directly hit in our trays by these heavy ions. As explained elsewhere (Marco *et al.*, 1988), the Shuttle is also intermittently hit by a higher flux of protons, but the radiation levels by Earth standards is minimal (less than 200 μSv/day). Nevertheless, developmental effects on another insect, *Carausius morosus*, also flown in a companion experiment, Biostack (Bücker *et al.*, 1986b) and in a posterior biosatellite flight (Alpatov *et al.*, 1989; Bücker *et al.*, 1989) indicate a synergistic effect of radiation and microgravity on the development of the *Carausius* embryo. To show this inhibition in hatching, *Carausius* embryos have to be exposed to microgravity during the blastoderm and germ layer formation stages. An interesting point to notice is that, independently of the differences in the protocols of the *Carausius* and *Drosophila* experiments owing to their different developmental strategies, the same effects of exposure to microgravity (and space radiation?) have been found, i.e., a strong inhibition of embryonic hatching of both types of insect with an apparently complete embryonic development. The differences with the *Drosophila* results reported here are small, especially if one remembers that the stick insect, *Carausius morosus*, is a very slow developing insect (90 days from oviposition to hatching). Therefore, the oogenesis of the staged embryos used in the Biostack experiments (Bücker *et al.*, 1986b), as well as most of the organogenesis, occurred under Earth conditions in the presence of gravity. In the case of the fast-developing *Drosophila*, only when oogenesis has been

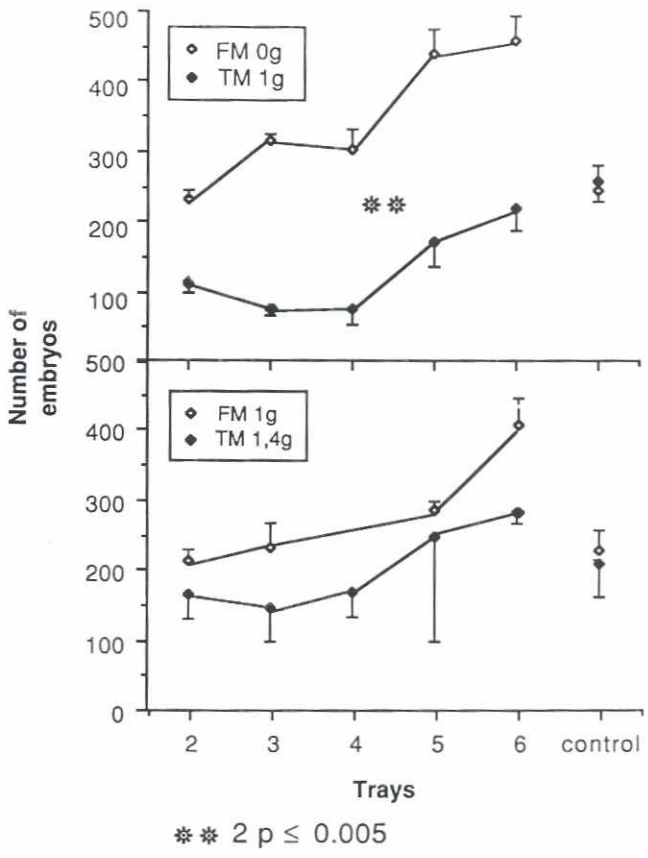


Fig. 4. Effect of microgravity on the number of eggs produced. *** indicate statistical significance 2p < 0.005. The number of embryos and larvae recovered in each tray collection is plotted against time. Upper panel compares the number obtained in the static racks collections of the Flight (space) and Training (ground) modules. These are averages of the 4 trays obtained in each collection. In the lower panel the number obtained from the containers in the centrifuges, 1g (space) and 1.4g (ground) are also shown. Since they correspond to an average of only two trays per collection, the statistics are weaker. Several statistical analyses performed on the data indicate that the data obtained from space (FM in the upper and lower panels) can be adjusted by linear regression lines with statistically significant slopes, while this is not the case for the data obtained in the ground control. The control at the end of the experiment corresponds to the number of embryos laid in Madrid one week after landing by the corresponding population of flies.*

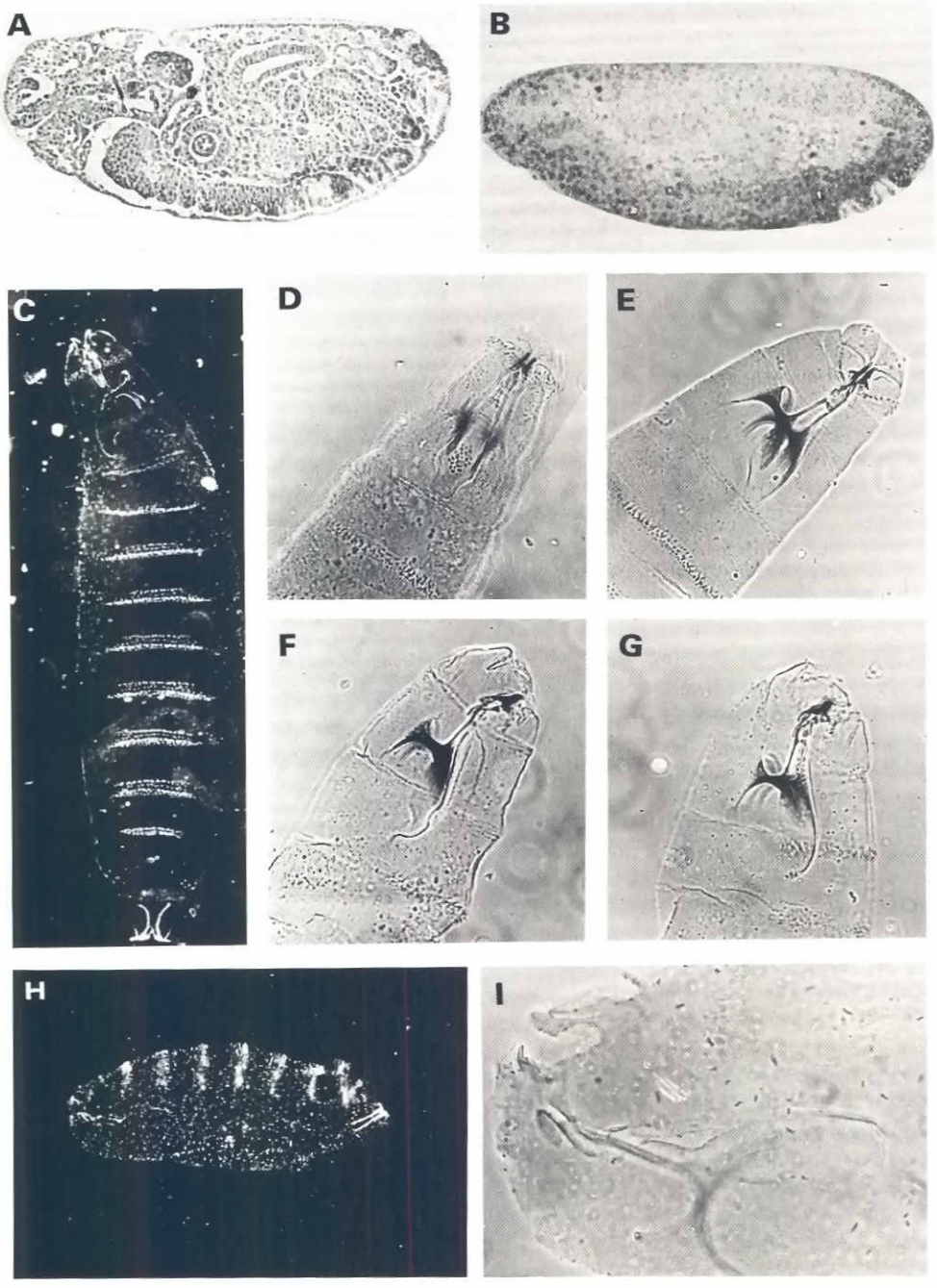


Fig. 5. Morphology of embryos and larvae recovered from space. *Section of a 17 stage embryo (**A**) of* Drosophila melanogaster *recovered from a space (Flight module) tray during the 6th collection at the end of the flight under microgravity conditions. (**B**) Sections across an unusual embryo recovered from a similar microgravity tray from space (see Table 3 for percentage). In the case of embryos, fixation was also far from optimal but sufficient to stage and classify them (see Table 3). (**C**) Cuticle from the practically normal larva recovered from a microgravity container in the last collection completed in space (FM6). (**D**) Detail of the head of another example of a larva with very slight alterations recovered from the fifth collection. (E, F and G) Examples of larvae recovered from microgravity containers in space in which head and thorax cuticular defects are visible to an incresing degree: (**E**) Cuticle in which head defects are visible. (**F**) Cuticle in which defects extend to the 1st thoracic segment. (**G**) Cuticle in which the defects extend to the 2nd thoracic segment (see Table 4). These alterations are examples of a head retraction syndrome in the otherwise normal developed cuticles, which sometimes made the observation of the thoracic segments and cuticular head structures difficult, induced by the freezing step but found in increased numbers in the case of the space larvae (Table 4). (**H** and **I**) Abnormalities encountered in embryos which had developed cuticles in space in absence of gravity. The larvae and embryos were treated and mounted as described in Materials and Methods.*

completed in space under microgravity do the inhibition of hatching and alterations of the anterior posterior axis begin to accumulate.

Additional effects on developmental systems have been also noted in the Biorack. A growing sample of *B. subtilis* did not sporulate (Menningmann and Lange, 1986). Cogoli's group (Bechler *et al.,* 1986) repeated earlier experiments showing a strong inhibition of the mitogenic response of human lymphocytes kept in microgravity. All these experiments indicate a wide effect on developmental processes of the decrease in the gravitational vector, whether coupled or not to other space environmental parameters.

In addition to the developmental effects of microgravity, there is also an increase in the oogenesis rate of females kept in microgravity conditions as indicated in Fig. 4. Interestingly, similar results of an enhancement of cell proliferation have been found in many parallel Biorack experiments in both procaryotic and eucaryotic systems. They include *B. subtilis* (Menningmann and Lange, 1986), *Chlamydomonas reinhardii* (Mergenhagen, 1986), *Paramecium aurelia* (Richoilley *et al.,* 1986). The experiments seem to contradict certain theoretical predictions (Moroz, 1984) involving the concept that since cell size may be sensed through gravity, cell size should increase in microgravity conditions, delaying the moment when cell division occurs, decreasing the cell proliferation rate. In fact, the difference in overall embryonic size found in this experiment follows the trend found in earlier space experiments in which increases in cell size attributable to microgravity have been reported (cited in Moroz, 1984). Our data indicate that this size effect is really attributable to the absence of gravity, since there is a clear difference between sizes of the embryos recovered from the 0g and 1g centrifuge space containers. Interestingly, *Drosophila* embryos exposed to slightly larger g forces (1.4 g in the ground centrifuges) were even smaller. Whether this size variation is at all linked to the reported developmental changes remains to be investigated. It must be pointed out, however, that the theoretical models coupling diffusion to chemical reactions that are candidates for theories explaining the establishment of inhomogeneities in the early embryo are in principle sensitive to changes in the overall size of the embryonic fields accessible to the putative diffusible morphogens (Meinhardt, 1982; Urquía 1984). Nevertheless, this claim has been called into question (see Harrison, 1987).

In conclusion, although the final interpretation of these results requires complementary future experiments, it is clear that there is some response of the female body to the microgravity conditions that changes the oogenetic process in such a way that the oogenetic rate and egg size, as well as the developmental process, are altered, probably through some change in the organization of information which has to be used to distinguish anterior vs posterior ends of the *Drosophila* embryo. The *Drosophila* and *Carausius* experiments will be repeated and extended in a Biokosmos 9 flight (planned for the end of this year) and in a second Biorack flight, in the International Microgravity Laboratory, unfortunately delayed by the accident of the Challenger. The question of mechanisms by which gravity and other environmental factors in space are sensed by the cells and organisms resulting in the effects reported in this article remains to be investigated in these follow up experiments.

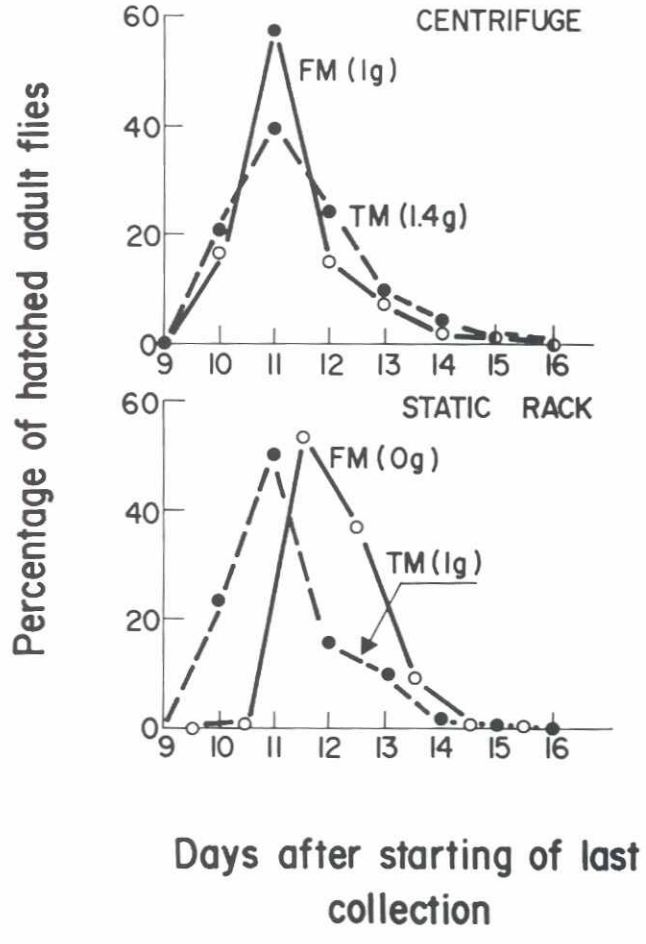


Fig. 6. Delay in the postflight emergence of *Drosophila melanogaster* reared in microgravity. *The trays recovered in Kennedy Space Center 17 hr after landing were transferred into* Drosophila *rearing tubes and monitored for the pupation and emergence of the imagos, counting males and females every day. In agreement with the results presented in the Figure, a delay in the pupation time of the tubes containing the embryos and larvae from space was also observed, particularly in the case of the samples from microgravity conditions. The total number of emerged flies was used to calculate the data summarized in the text about the number of flies hatched and male to female ratios. The broken lines correspond to the values obtained in the ground static control, from the containers in the centrifuge (upper panel) or in the racks (lower panel).*

Materials and Methods

The Biorack is a multiuser facility (Fig. 1) located inside the Spacelab. It is composed of several parts: two incubators (settled for this spaceflight at 22°C and 37°C), one cooler (kept at 4°C), one freezer (at -15°C) and one glove box (Figs. 1 and 2). Thus, the astronauts can manipulate the samples in a closed environment since the glovebox is provided with a circulating air system which continuously clears off the air over a litterbed. An important feature of the Biorack facility is the incorporation of 1g control centrifuges, two in each of the incubators which are adjusted to give an acceleration of 1g in microgravity, perpendicular to the rotation axis. Two types of containers, one large, type II (70x70x99 mm external dimensions) and one small, type I (90x58x24 mm external dimensions) were provided for the different experiments. The centrifuges only accepted the smaller type I containers, which were the ones used in this experiment. Accordingly, we distinguish two classes of type I containers — centrifuge containers and static rack containers. For our experiment, the type I containers were fitted in their interior with small transparent cages which housed the flies (Fig. 2). In the bottom of the cages small square trays were inserted containing the feeding and laying medium. These trays were exchanged by pushing the used tray with the embryos and larvae out one side with a fresh tray (Fig. 2). Ethanol washed, overnight UV sterilized trays were filled with 3 g of feeding and collecting medium made up from a mixture of 1.4% agar, 7.5% brown cane sugar, 5% wheat meal, 5% baker's yeast and 0.5% propionic acid. After hardening the melted medium, the trays were further UV sterilized during 1 h and stacked in an additional set of trayholder containers (Fig. 2). The whole preparation of the experimental containers was carried out under sterile conditions in a laminar flow cabin. Each tray was labeled with a color code which matched the corresponding experimental container so that we could always identify the set of trays coming from the same container.

Recently emerged, at most 12 h old, wild type Oregon R flies, two hundred forty females and ninety males, were sampled 15 h before launch in six experimental type I containers and transferred to the Challenger middeck stowage (Fig. 3) to carry out the experiment. After the successful launch of the Shuttle, at 9.5 h of Mission Elapsed Time (MET), these containers were transferred to the 22°C incubator in the Biorack; 4 were mounted in the static racks and 2 in one of the 1g centrifuges. At the times indicated in Fig. 1C, which were basically adjusted to match the experiment with the rest of operations in the D-1 mission, all six containers including those from the 1g centrifuge were removed from the incubator, transferred into the glovebox of Biorack, and the feeding and egg collecting trays were exchanged by new fresh trays that had been stored in the middeck stowage during 12 weeks before launch. The containers with the fresh trays were transferred back to their respective positions in the 22°C incubator, while the old trays with the collected embryos and/or larvae were frozen in the -15°C Biorack freezer. The second egg collection lasted only 12 h and the old trays were maintained in the 22°C incubator for an additional period of time of 10 h to normalize the development of the majority of trays. The rest of the laying periods lasted 21.5 h for the 3rd, 4th and 5th tray exchanges and 25.5 h for the 6th. The trays from the first exchange were not analyzed since they contained mostly embryos layered during the 16h preceding launch after late access preparation of the experimental containers.

Freezing is a far from optimal procedure for storage of living material, particularly for later microscopic observation, but it was a necessary compromise due to the limitation in crew time and the relatively difficult and time consuming operations involved in the fixation of *Drosophila* embryos and larvae. At the end of the D-1 mission, the frozen containers were transferred to the -12° C passive thermal units, provided by the ESA as a complement to the Biorack facility, and the containers with the living flies were transferred again to the middeck stowage with the passive thermal canisters to be unloaded from the Shuttle immediately after landing.

At the end of the spaceflight, the containers were recovered in the Kennedy Space Center 17 h after landing. Fresh trays were inserted immediately in the containers and the trays with the collections from the last 48 h collection in space were introduced in *Drosophila* rearing tubes to allow them to develop into adults to study the possible effects on later stages of development as well as the number and type of lethal mutations which might have been induced in space.

An exact replica experiment with sib flies was performed as a ground control. For this, we used the second Biorack located in the Biological Laboratories of Hangar L in the Kennedy Space Center, Florida, USA. This second unit, called the Training Module so as to distinguish it from the Flight Module, which was in the Spacelab, provided an identical ambient and experimental configuration, with the only difference being the space vs ground environment. The Biorack Training Module was provided also with a set of centrifuges, giving the parallel ground samples a resultant 1.4g acceleration, due to the compound effect with the Earth gravity vector parallel to the rotating axis. The ground control experiment was performed with a two-hour delay with respect to the space experiement to ensure the incorporation in the ground control of any changes due to unforeseen circumstances in the performance of the experiment in space.

Once in Madrid, the frozen trays were thawed and the embryos and larvae counted under the dissecting microscope. The larvae were treated and mounted to study their cuticles as described by van der Meer (1977). The embryos were fixed *en masse* by a modification of the procedure described by Mitchison and Sedat (1983). The embryos were dechorionated by incubation in 5% hypochlorite during 5 min and devitelinized by treatment with 4% paraformaldehyde in PBS saturated in heptane, followed by 50mM EGTA in 90% methanol saturated in helptane. The fixation of the devitelinized embryos was completed in 70% Methanol-EGTA, 30% of the PBS solution of paraformaldehyde during 5 min followed by an additional treatment of 3 min with fresh fixative. The embryos were postfixaed in 2% OsO4 and included in Araldite after dehydration. Semi-thin sections were obtained using a Sorval MT-2B ultramicrotome. The sections were stained with 1% Toluidine blue in 1% borax during 3 min at 50°C, dehydrated and mounted in Araldite.

To estimate the accumulation of lethal hits in the different fly population, the ratio of male to female flies in their descendants was determined after narcotizing the recently hatched adults.

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