International Journal of Astrobiology (2024), **23**, e26, 1–22
doi:10.1017/S1473550424000223

CAMBRIDGE UNIVERSITY PRESS

REVIEW ARTICLE

Drosophila melanogaster: a promising system for space biology research

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Received: 3 October 2023; **Revised:** 15 August 2024; **Accepted:** 13 October 2024

Keywords: Astrobiology, *Drosophila melanogaster*, microgravity, space radiation

Abstract
Drosophila melanogaster has given enormous contributions to Space Biology Research. This organism is an important tool to be manipulated in genetic engineering and molecular experiments in order to understand different biological processes homologous to other multicellular systems, including humans. Their milestone contribution in microgravity conditions and radiation, the two most important variables in space, have allowed new knowledge and perspectives on the positive and negative effects on cellular, molecular and genetic levels. In this review, we expose the historical contribution of *Drosophila melanogaster* in Astrobiology.

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Introduction

Drosophila melanogaster L. (Diptera: *Drosophilidae*) has been an important organism in research for the last century. It has contributed to understand several genetic mechanisms and it still continues being used as a biological model for uncovering the behaviour of genes in different scientific areas such as space biology. Indeed, this organism is one of the most used systems in bioinformatics, signal transduction research, and omics sciences in general. The presence of orthologs genes in other multicellular systems allows generating phylogenetic trees that examine divergent patterns between species including humans. On the other hand, Space biology is a relatively new scientific discipline whose objectives include the understanding of the effects of the extreme conditions on biological systems including microgravity and cosmic radiations (Des Marais *et al*., 2008). These ones play a limiting role in the development and survival of any living being that leaves our planet. In this context, *D. melanogaster* has been an excellent tool for the study of these effects, analysing not only the development, genetics changes, aging and survival, but generating future strategies in the future space exploration. Since the International Space Station (ISS) was built, *D. melanogaster* has been a tool for several research projects carried out by scientists, astronauts and others on the ground improving exponentially the comprehension in biology. In this context, in 2015 NASA launched The Fruit Fly Lab-01 mission, the first flight containing a new research platform for long-term experiments aboard the ISS. This mission provided new insights into genomics, proteomics, metabolomics, cell biology, immunology, cancer biology, population genetics, developmental biology, histology, pathology, and radiobiology in space, examining mainly how the lack of gravity and the radiation could affect these insects, and therefore providing relevant information to human health in the Space (Keller, 2015). In this review, we present the information about the significant contributions of *D. melanogaster* in the field of Space Biology, highlighting key studies on microgravity and radiation conducted aboard the ISS, satellites and/or under simulated conditions on Earth. Furthermore, this work emphasizes important examples and concepts in evolutionary biology of ecological development (eco-evo-devo), which integrates ecology, developmental biology, and evolutionary biology closely linked to multiple biological mechanisms described here. The eco-evo-devo effects produced by developmental plasticity (rooted phenotypes in response to the environment), symbiotic relationships (interactions between organisms), or those inherited epigenetically, and their overlap are presented throughout the different sections of the review showing the complexity of these processes and the now growing discipline of ecological evolutionary developmental biology.

Genetic of *Drosophila melanogaster*

In 1909, Thomas Hunt Morgan, interested in understanding the Mendelian genetics much more deeply, built the first fruit fly laboratory at Columbia University. This 'fly room', as he called it, would be a milestone for the start of genetic studies in *Drosophila*, indeed, since each fly is a unique individual, its advantage over larger animal models was evident (Stephenson and Metcalfe, 2013). One example of these findings was the discovering of mutant flies with white eyes in large communities of red eyes flies (Moore, 1983). It led to formulate the chromosome theory of inheritance discovering the mechanism by which gender-related genes are transmitted and the importance of the transmission on the behavior. Importantly, it gave the principles to understand how the body develops from a single cell to become the complex variety of tissues that built the adult organism and regulatory mechanisms involved at genetic level (Matthews *et al.*, 2005). Although the complete genome of *Drosophila melanogaster* was only sequenced in 2000 and despite having a size of 180 Mb, which is 95% smaller than that of any average mammal (Adams *et al.*, 2000), the alignment obtained a genetic homology rate of 60% with humans establishing that *D. melanogaster* could be a good experimental model for studying gene expression (Chien, 2002; Yamamoto *et al.*, 2014; Mirzoyan *et al.*, 2019). Importantly, 75% of the genes responsible for human diseases had homologues in *D. melanogaster* and thanks to the wide range of flies with genetic mutations available in laboratories, it became an animal model to investigate the genetic bases of diseases. For example in neurodegeneration, tumour development, immunity, muscle degeneration and cachexia, and intestinal dysfunction (Verheyen, 2022). Since 1982, novel approaches have been employed to investigate genetic mechanisms, including site-directed mutagenesis in specific genes, oligonucleotide mutagenesis, P-element insertion mutagenesis, and, more recently, genomic editing with CRISPR-Cas9. The fruit fly, *Drosophila*, has been particularly useful for such studies due to its short life cycle and ability to produce a substantial number of offspring (Stephenson and Metcalfe, 2013). These experiments have yielded detailed insights into the regulation of numerous signalling pathways during early developmental stages in flies, and as a result, *D. melanogaster* has become one of the most popular experimental systems among eukaryotic species (Fig. 1) (Abbott *et al.*, 1992). Thanks to this model and the possibility of being able to generate a large number of repetitions, the contribution to topics related to Astrobiology that see adaptation processes of

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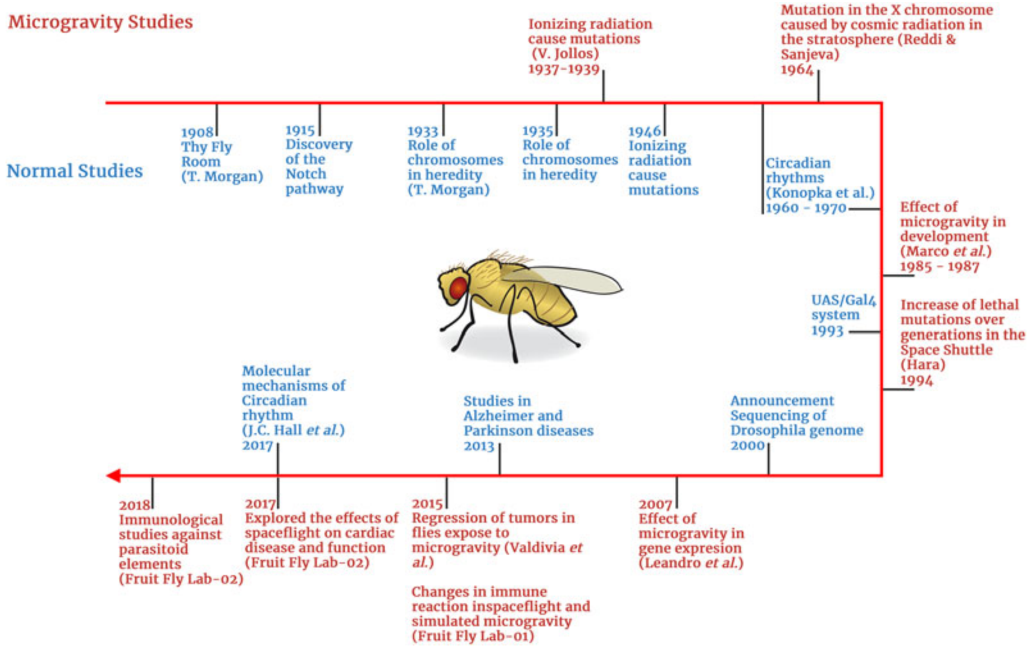

Figure 1. *Timeline contrasting the major milestones in biologic research and space research on* Drosophila melanogaster *in normal and microgravity conditions.*

living organisms to space and the effects of radiation and microgravity have been enormous (Vernós *et al*., 1989*a*).

Drosophila as an experimental model in Space radiobiology

The biological consequences of ionizing radiation on humans participating in Space missions are studied by the multidisciplinary field of Space radiobiology (Radiology (ACR), 2022). The environment of space radiation is a complex mixture of radiation species dominated by highly penetrating charged particles from various sources such as galactic cosmic rays (GCRs) that originate outside the solar system, particles trapped in the Earth's magnetic field (i.e. Van Allen Belts), and particles emitted by our Sun (SPE) as a result of solar activity (Ohnishi and Ohnishi, 2004; Onorato *et al*., 2020). This is why dose-effect models of radioactive species are essential to understand and minimize damage to biological systems, including humans. In this sense, *D. melanogaster* debuted as a model to examine the effects of radiation at the molecular level, since Morgan and his students managed to identify ionizing radiation as a causal agent of genetic mutations (Stephenson and Metcalfe, 2013). These findings were instrumental in designing experiments that employed genes marked with mutations induced by ionizing radiation to investigate phenomena such as translocations and population dynamics (Muller, 1927; Muller and Altenburg, 1930). Indeed, during the first experiments on *Drosophila* existed the hypotheses that mutations were directly linked to chromosomal rearrangements. However, after Muller and Altenburg (1930) measured the frequency of translocations among *Drosophila's* four pairs of chromosomes, he found a number of translocations occurring between Chromosomes C1, C2 and C3 only after exposure to X-rays. Several years later, Demerec and Fano (1944) proposed a similar study irradiating *D. melanogaster* sperm with different intensities of X-rays trying to test this hypothesis. Their results confirmed that the link between mutations and chromosome rearrangements did not exist (Muller and Altenburg, 1930; Demerec and Fano, 1944). So, the use of the fruit fly in different life stages was so successful that, based on the availability of the technology, three types of studies were conducted as a model to examine the effects of ionizing radiation on living organisms: (i) research exposing flies to X, gamma, or beta radiation using radioactive isotopes; (ii) research

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employing balloons or exposing flies to suborbital radiation levels in peaks; and (iii) research exposing flies to cosmic radiation in the low-Earth orbit radiation environment. Experiments in this sense allowed to expose larvae of *D. melanogaster* to high-frequency X-ray radiation revealing that exposure to ionizing radiation extended the duration of their prepupal stage. Furthermore, the researchers found that this effect was more pronounced when the larvae were exposed to radiation during later stages of development (Hussey *et al*., 1932). As the researchers continued their investigations, they ratified that ground radiation was negligible in the generation of spontaneous mutations, prompting them to investigate the impact of cosmic radiation on genes (Muller, 1927; Jollos, 1937).

Later studies led by King *et al*. (1956; King, 1957) focused on studying the effects of gamma radiation on the reproductive capabilities of *D. melanogaster*. The application of radiation on eggs of different strains showed no significant effects on hatchability, but the irradiation on females flies produced the apparition of lethal mutations in their offspring, and several morphological effects on their tissues, including the degeneration of nurse cells and the cytolysis of the ovaric chambers (King *et al*., 1956; King, 1957). Other studies related to reproductive abilities showed stronger correlations between the appearance of lethal mutations in sex chromosomes (Ives, 1959) and the opposite effects of high and low doses of radiation that included advantages in heterozygous populations (Blaylock and Shugart, 1972). These authors irradiated homozygous populations of *D. melanogaster* and *D. simulans* with lethal doses of 5000 mGy, and observed greater viability in the heterozygous populations of both flies due to chromosomal mixing, in addition to obtaining a greater number of individuals for 20 generations of flies exposed to low doses of radiation (Ives, 1959; Mukai *et al*., 1966; Blaylock and Shugart, 1972). Interestingly, genetic models with the fruit fly throughout history have also shown that the changes observed due to radiation are not necessarily linear to the radiation dose. As mentioned previously, this is due to the various variables that affect the biological systems (eco-evo-devo). Importantly, a recent study which analysed some stress-sensitive genes subjected to gamma radiation in *D. melanogaster* showed nonlinear effects of low dose radiation versus sex-specific radio-resistance of the post-mitotic cell state of the imago. This meant that the observed positive changes in life expectancy (hormesis) were not related to the changes of organism physiological functions after the exposure to low doses of ionizing radiation (Zhikrevetskaya *et al*., 2015).

On the other hand, altitude studies referred to exposition to suborbital cosmic radiation were started with Jollos (1937). The researcher analysed the radiation exposure of fruit flies at more than 4000 m above sea level (more than 14 000 feet) for 44 days, finding that there was an increase in sex-linked mutations in comparison to control. Two years later, the same author discovered that the presence of metals, such as lead, increased the amount of radiation, due to secondary radiation, which was perceived by the flies leading to a greater number of mutations (Jollos, 1937, 1939). These studies were also important to enhance the nascent high altitude medicine and the effect of radiation on populations that live in high mountains such as the Andes or the Alps. Then, in the search to a better understanding of recessive mutations and translocations due to radiation, several studies were initiated with balloons that can reach the stratosphere containing *D. melanogaster* flies. Since cosmic radiation is 5 times greater in the stratosphere than on the sea level, Reddi and Sanjeeva (1964) analysed the effects of the exposure of male flies divided to test for mutations by breeding in six successive batches for 3 days in stratospheric balloons at 32 km altitude. To test for dominant lethality, each male was tested individually in mating and a reduction in hatchability relative to controls was determined. They found no evidence of recessive lethal mutations or translocations, but found a constant reduction in hatchability which they explained as an effect of the production of dominant lethal mutations mainly by chromosome breakage (Reddi and Sanjeeva, 1964).

Although the study of the effects of radiation on organisms was very popular in the eras prior to the installation of the ISS, after this, the radiation studies were mixed with the search for the effects of the almost absence of gravity known as microgravity (as we will describe later) (See Table 1). A relevant study analysed two types of mutations, one sex-linked recessive lethal mutations induced in male reproductive cells and other somatic mutations which give rise to morphological changes in hairs growing on the surface of wing epidermal cells. For this research the authors used a wild-type strain of *D.*

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Table 1. *Space Radiation studies with* Drosophila melanogaster

Space Mission	Objectives	Results	Source
V-2 rocket (1947)	Explored the effects of radiation in space	Undetectable changes	Joosse (2023)
Biosatellite II (1968)	Studied mutations in mature reproductive cells of *Drosophila* adults and pupae irradiated during flight	Mature sperm cells present recessive lethal mutations in the paternal X chromosome	Thimann (1968)
Salyut-6 (1978)	Evaluated The effect of weightlessness on chromosomal aberration. The frequency of nondisjunction and loss of X chromosomes in pre-flight irradiated *Drosophila melanogaster*	Acceleration significantly amplified radiation damage.	Vaulina *et al.* (1981), Filatova *et al.* (1983)
STS-72 (1996)	Evaluated possible effects of space radiation in the fruit fly, analysing two types of mutations, sex-linked recessive lethal mutations induced in male reproductive cells and somatic mutations that give rise to morphological changes in the hairs that grow on the surface of the fruit flies, wing, epidermal cells.	The flight groups of Canton-S and mei-41 strains exhibited higher sex-linked recessive fatal mutation frequencies compared to ground control groups, suggesting potential effects of the space environment on specific cell types.	Ikenaga *et al.* (1997)

melanogaster and another radiation-sensitive called *mei-41* loaded on the US Space Shuttle Endeavour. It's interesting to note that the research revealed that while the frequencies of somatic wing-hair mutations were no significantly different in both the flight and control groups, the frequencies of sex-linked recessive fatal mutations in flight were greater than those in ground control groups. These findings raised the prospect that specific cell types, including male reproductive cells, may have undergone alterations as a result of the space environment (Hara, 1994) (Fig. 2).

By the time the ISS was launched into low Earth orbit (LEO), the relevance of studying *Drosophila melanogaster* as a model organism was already recognized, and a specific module, named 'the insect hab' was installed for this purpose (Santos, 2001) allowing to better resolve several questions that came to light from the research carried out previously. For example, studies by Ikenaga *et al.* (1997), a few years before the ISS, suggested that microgravity could induce an increase in mutation rates caused by radiation. Ohnishi *et al*. (2002) began a more in-depth study to corroborate these hypotheses by analysing the role of DNA repair systems, antioxidants and protective proteins in fruit flies on the ISS. Although other organism models such as bacteria, fungi, and cell cultures gave contradictory results and effects. *Drosophila* was able to demonstrate sustained increased sensitivity to changes during space travel and suggested that space radiation may depress the recovery of DNA damage induced by space radiation. This was one of the pioneering results in seeing biological effects of the interaction between microgravity and radiation. Another study using *D. melanogaster* also suggested that radiation is important for certain normal functions at the genetic level. The authors showed that flies placed in an underground laboratory in Russia had significant deficiencies in various molecular and cellular

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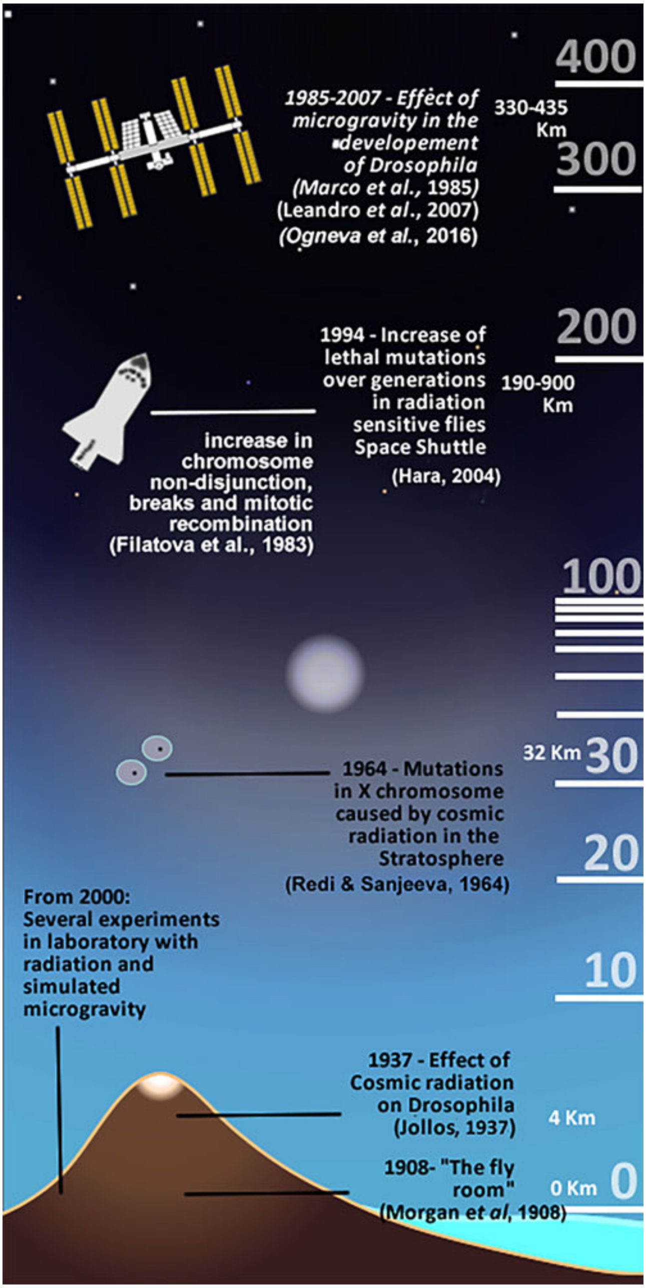

Figure 2. *Experiments to test the effects of cosmic radiation and microgravity measured at different altitudes. Over time it has been possible to study these conditions separately with the development of more sophisticated methods.*

functions (Zarubin *et al*., 2021). These results could be related to previous findings where experiments conducted at Fruit Fly Lab-01 (FFL-01) revealed that chronic low-dose radiation (16.7 mGy h^{-1} exposure dose rate) caused behavioural changes in *D. melanogaster.* Indeed, despite impairing climbing activity, exploratory movement, effects on development (pupation and hatching rates), some changes

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in the expression of genes involved in the regulation of metabolism resulted in a surprising extension of lifespan of life (Kim *et al*., 2015).

For other types of radiation, Alexandrov *et al*. (2022) studied the nature of hereditary recessive genetic mutations induced by ionizing radiation (γ rays and neutrons) examining five sex-linked genes on chromosome 2 and discovered that neutrons were 2.5 times more effective than γ-radiation in causing mutations. Conversely, they discovered that, in contrast to neutrons, γ-radiation frequently caused clusters of changes in DNA, sometimes even in a single double-stranded helix. Curiously, up to fourteen genetic alterations at the chromosomal and intragenic level were present in mutants with the same visible phenotype. This phenomenon is known as cryptic genetic variation in which they do not produce phenotypic differences under normal conditions but during periods of stress or environmental changes, they could express (Gilbert *et al*., 2015).

Drosophila melanogaster, due to its versatility in handling for experimentation, has proven to be very useful for the study of radiation and its effects on living beings. The different forms of radiation and the different variables in the environment in the various experiments with fruit flies were tried to control to understand parts of the phenomenon in biological systems. The different experiences presented and summarized here aim in some way to expose the importance of *Drosophila* in achieving milestones in the field of mutations linked to sex chromosomes, which are without a doubt, together with the analysis of radiation-sensitive genes, the findings most important aspects of using this model.

***Drosophila* and microgravity**

Microgravity refers to conditions in which the gravitational acceleration acting on an object is much lower compared to Earth's gravity, which is approximately $9.8\,\mathrm{m\,s^{-2}}$ at the Earth's surface (1 G) (National Research Council, 2000). Physics originally predicted that the acceleration due to gravity would not be enough to produce any change in living things; however, studies in many living organisms and tissues have shown the opposite, showing effects on early life cycle, tissue-cell interactions, and gene expression (Morey-Holton, 2003; Kiss *et al*., 2012).

The experiments conducted by Roberto Marco and his team on the ISS have shown that *Drosophila* alters its behaviour when exposed to microgravity, resulting in developmental disruptions during oogenesis and embryogenesis (Abbott *et al*., 1992; de Juan *et al*., 2007; Herranz *et al*., 2008). Specifically, hatched flies exhibited increased motility in comparison to adult flies, which in turn experienced reduced motility by the time they reach two weeks of age. In addition, the experiment included a hypergravity control via centrifugation (2 G). Notably, *Drosophila's* locomotor activity increased in microgravity, but was repressed under 2 G hypergravity, with both conditions having detrimental effects on the overall survival of the flies (Serrano *et al*., 2010).

Other studies about the development of *D. melanogaster* and *Carasius morosus* exposed to microgravity at low orbit at 2000 m.a.s.l at the ISS, showed changes on the embryonic hatching rates and a delay in the development of larvae and adult insects after a 7 days flight (Vernós *et al*., 1989*a*, 1989*b*). The authors observed stimulation of the oogenesis and a decreased number of hatching of larvae for *D. melanogaster* exposed to microgravity. Following their return to Earth, several *Drosophila* from these tests showed delayed larval and adult development as well as a decline in the number of flies that reached maturity (~25%) (Fig. 3).

As previously described, since 1937, *Drosophila* has been one of the most studied multicellular organisms (on Earth and Space). Scientists have been able to investigate the impact of weightlessness on living things, particularly in terms of genetic regulation, thanks to technological advancements. For gene expression profile of fruit flies subjected to microgravity circumstances was thoroughly analysed by the researchers using next-generation sequencing techniques. These methods allow the investigation of thousands of genes at once and offer complete details on how their activity varies in response to microgravity. On the other hand, the precise monitoring of morphological and structural changes in Drosophila's cells under microgravity has also been possible thanks to advances in microscopy. It is possible to examine how the absence of gravity affects the expression of important genes and proteins

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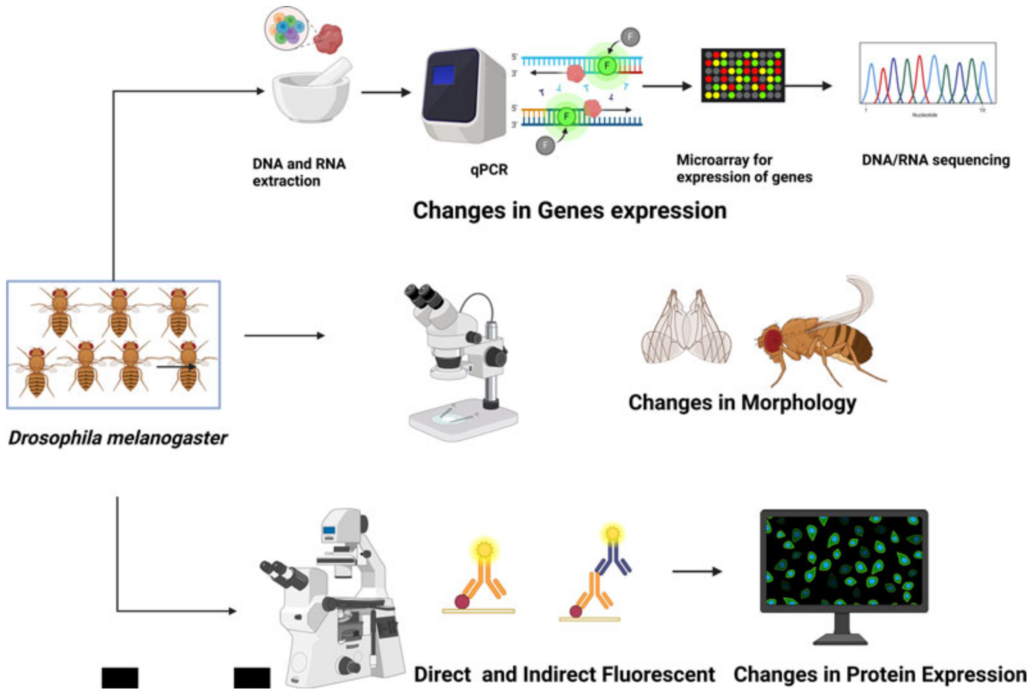

Figure 3. *Workflow of gene expression after microgravity experiments.*

in various tissues and organs, thanks to confocal and fluorescence microscopes, which allow viewing and following biological activities in real time.

Several studies related to understand the phenotype under microgravity conditions were developed on the ISS. For example, the Fruit Fly Lab-02 had the objective to study physiologic and genetic changes in the development of the heart in *Drosophila* under microgravity. Although the fruit fly cardiovascular system is less complex than that of mammals, there are shared structural, functional, and genetic similarities with flies that help researchers to understand the possible effects and risks of new and stressful environments associated with long-duration Space missions (Keller, 2017). The hearts of flies grown under these conditions showed exhibited myofibrillar remodelling, constricted heart and in the isolated hearts, RNA sequencing (by RNA-seq) indicated decreased sarcomeric/ECM gene expression and dramatically elevated proteasomal gene expression, which was consistent with the damaged and smaller hearts, suggesting aberrant proteostasis (Walls *et al.*, 2020). Another interesting example was the Fruit Fly Lab-03. This mission aboard the ISS investigated the effects on the innate immune response against parasites (Keller, 2018). Since the immune system dysfunction and infections are major risks for astronauts on long-duration space exploration missions, the model parasite-host in *Drosophila* was important to determine changes in this interaction and determine whether spaceflight alters the ability of the parasite to infect the host. The results of these experiments and others are summarized in Table 2.

It is commendable for these investigations with *Drosophila* that they allowed the phenotypic results to be extended to other insects and animals on the ISS. Later studies evaluated the development and changes in morphology based on these antecedents found, and therefore made an enormous leap in understanding several processes that generated the bases of Space Biology. However, given that the research carried out was almost 80% more related to the phenotype than that carried out for the genotype, perhaps due to technological difficulties in their initial stages, the studies only focused on evaluating specific genes or mutations due to radiation. These points came full circle when new studies began to show that microgravity alone caused changes at the genetic level. Therefore, this led to studies

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Table 2. *Microgravity Studies with Drosophila melanogaster in the space*

Special Mission	Objectives	Results	Source
Biosatellite I (1966)	Studied newly hatched *Drosophila* larvae for effects on rapidly growing cells as they develop to adulthood. Chromosomal abnormalities were determined at recovery.	It was not possible to recover the capsule. No survivors	Oster (2022)
Bion 3/Cosmos 782, (1975)	Studied the effects of aging on fruit fly livers and plant tissues with grafted cancerous growths were also studied	Microgravity exposure had minimal effects on fly development but potentially accelerated aging processes and reduced glycogen granules in immature flies during spaceflight.	Williams (2020)
Bion 4/Cosmos 936 (1977)	Studied the effect of weightlessness on the emergence and development of cell organelles and genetic structures	Mutation frequencies, including recessive fatal mutations, were comparable between offspring of flies flown in Cosmos and control groups, with no deletions or recessive lethals observed in either.	Parfyonov *et al.* (1979)
Spacelab mission D1 (1985)	Investigated at the unicellular and multicellular level respectively.	Microgravity experiments involved a larger number of embryos compared to control experiments, but resulted in reduced hatching of larvae and shorter lifespan (up to 25%) in male flies.	Volkmann (1988)
Cosmos 2044 (1989)	Investigated the possible effects of microgravity on the development and aging of *Drosophila melanogaster*. Also understand how normal development could occur in microgravity even though many cellular processes are altered in microgravity, such as cell proliferation and response to mitogens and other signalling molecules.	Despite unforeseen circumstances, including launch delays and temperature differences, the study demonstrated that wild type *Drosophila melanogaster* embryos undergo normal development in a microgravity environment.	Marco *et al.* (1992)
Misión IML-1 (1992)	Confirmed that exposure to microgravity of young *Drosophila melanogaster* imagos accelerates their aging, to relate this effect to the mitochondrial theory of aging, which suggests that an increase in locomotor activity should produce an increase in respiratory activity and a corresponding acceleration of mitochondrial processes leading to impaired function.	In space, there is a stimulation of oogenesis, resulting in slightly delayed development compared to synchronous terrestrial controls, while young male flies exposed to microgravity exhibited accelerated aging.	Marco *et al.* (1996)
Mission IML-2 (1994)	Evaluated the effects of microgravity on *Drosophila melanogaster* behaviour and aging	Microgravity-exposed male flies displayed accelerated aging, reduced vitality, and shorter lifespan, potentially associated with mitochondrial metabolism, while females exhibited increased locomotor activity without significant lifespan changes.	Benguría *et al.* (1996)

(*Continued*)

Table 2. (*Continued.*)

Special Mission	Objectives	Results	Source
STS-93 (1999)	Assessed the effect of spaceflight on neuronal development associated with muscle fibres in *Drosophila melanogaster* embryos and larvae in Petri dishes placed in an incubator; experiment flown on STS-106	No results obtained due to hardware malfunction in the Commercial Generic Bioprocessing Apparatus (CGBA) incubator.	KSC (2006)
STS-106 (2000)	1. Investigated the impact of space flight on nervous system development and neuromuscular synapse formation using GFP visualization to study motor neurons, muscle targets, and overall nervous system development. 2. Analysed the specific developmental periods of *Drosophila* embryos and larvae to understand how microgravity during spaceflight affects critical stages of their development.	Preliminary data indicated satisfactory operation of the CGBA hardware, despite two containers experiencing unexpected temperature increases, while the final data, has not been publicly released.	Keshishian (2000)
Spanish Soyuz – Cervantes (2003)	Investigated the effect of microgravity during *Drosophila* metamorphosis.	Determined how microgravity affects the organism rebuilding processes that occurs during *Drosophila* metamorphosis.	Herranz *et al.* (2005, 2007) 2008, 2010
STS-129 (November 2009)	Investigated the effects of spaceflight on the innate immune responses of *Drosophila melanogaster.*	Spaceflight altered the innate immune responses of *Drosophila melanogaster* reduced levels of imd and target genes attacins and drosocin, as well as changes in the expression of serine proteases SPE.	Marcu *et al.* (2011)
China's Shenzhou-9 spaceship (2012)	Investigated the effect of spaceflight on the circadian rhythm, lifespan, and gene expression o*f Drosophila melanogaster.*	Space flight did not significantly affect the expression levels and beat of major clock genes, but differential regulation of circadian output genes in space-flewn flies indicated an impact on the circadian output pathway. There was also a slight overlap in gene expression among control groups, possibly due to stress-related effects on gene expression.	Ma *et al.* (2015)
Heartflies SpaceXCRS-3 (2014)	Evaluated the effects of microgravity in Hearts and cardiovascular system	Cardiac remodelling and proteostatic stress are a fundamental response of heart muscle to microgravity.	Ocorr (2020)
	Studied the effects of microgravity in the development of *Drosophila*	The results of the spatial analysis of cytoskeletal proteins and transcriptome detection demonstrate that exposure to	Ogneva *et al.* (2016)

Foton-M and M-25M424 spacecraft (2014)		weightlessness increases the transcription of metabolic genes and cuticle components while decreasing the transcription of genes involved in morphogenesis, cell differentiation, cytoskeleton organization, and genes related to the plasma membrane. The transcription of the same genes was significantly increased or decreased 'post' exposure to microgravity.	
International Space Station and NASA's Ames Research Center in California, USA. Fruit Fly Lab – 01 on SpaceX-CRS 5 mission 2015	Investigated the effects of spaceflight and simulated microgravity conditions on the virulence of *Serratia marcescens* in the *Drosophila melanogaster* infection mode	Found that the *Serratia marcescens* was significantly more lethal to the fruit flies after growth on the International Space Station than ground-based controls, indicating that spaceflight and simulated microgravity conditions can increase the virulence of pathogens.	Gilbert *et al*. (2020)
FFL-02 (2017)	Studied the impact of microgravity on heart structure, function, and gene expression in *Drosophila*	Fruit flies with mutations in the seizure gene and wild-type fruit flies experienced structural and functional cardiac remodelling in response to microgravity.	Walls *et al*. (2020)
FFL-03 SpaceXCRS-1 (2018)	*Drosophila* is used as the host, and two species of *Leptopilina* wasps – natural fruit fly parasites – as the pathogen – to explore host-parasite interactions. in the space	Findings from the FFL-03 mission will provide insight into the effects of spaceflight on innate immune systems	Keller (2018)
ISS-65 (October, 2021) ISS-66 (Marzo, 2022)	Determined the effects of space flight on reproductive function and to provide insights into maintaining and/or restoring the motility of male sperm cells for preserving species during long-term space exploration.	They found that the movement speed of the spermatozoa decreased in the early period of readaptation after space flight, but cellular respiration and the content of respiratory chain proteins remained at control levels.	Ogneva *et al*. (2022)

evaluating genomics in a more comprehensive manner, which is evident in the low production of studies only focused on genetic effects due to radiation. Studies analysing the joint effects of microgravity and radiation began to be carried out on missions to the ISS, and new base-ground tools on Earth such as magnetic systems or microgravity simulators made their appearance with greater participation in this research field. In this regards, Leandro *et al*. (2007) performed microarray analysis for the transcriptome on flies (*D. melanogaster*) and nematodes (*Caenorhabditis elegans*) grown in the ISS, concluded that there were significant differences in gene expression levels in response to spaceflight (Leandro *et al*., 2007). They found that the set of genes with changes in their expression levels were different between these species. Studies like this brought others related to analysing which genes changed, and seeing if these were related to important biological functions that included aging (de Juan *et al*., 2007; Herranz *et al*., 2008). For instance, Herranz *et al.* (2010) discovered notable changes in gene expression in comparison to 1 g controls when examining the impact of microgravity (experiments conducted on the ISS) on the pupal development of *Drosophila*. It was shown that decreasing gravity levels significantly impacted gene expression, that non-ideal environmental conditions on the ISS and spaceflight preparation processes entail additional pressures that also impacted gene expression. In turn, experiments performed on the ground under ideal simulated gravity conditions using the random position machine (RPM) showed more subtle effects on gene expression, But by repeating the ground experiments under conditions that replicated the additional environmental stresses of spaceflight procedures, 79% of the differentially expressed genes (DEGs) detected on the ISS were reproduced in the RPM experiment. The genes that underwent variation were related to breathing, developmental processes and stress-related changes. In turn, hypergravity simulation induced a similar transcriptome response but in the opposite direction, where genes promoted in microgravity were generally suppressed in hypergravity. From which they deduced that the transcriptome of pupal-stage *Drosophila* is finely tuned to normal gravity and that microgravity, together with the environmental constraints associated with space experiments, can have profound effects on gene expression (Herranz *et al*., 2010).

Other investigations reported about genes encoding proteins involved in morphogenesis, cytoskeletal organization, cell motility, and transcriptional regulation, cuticle genes, and genes involved in proteolytic processes, which changed from microgravity conditions to Earth standard gravity. The genes Cp11B, Cpr92F, Cpr56F, TwdlF, Cpr67Fb that codify cuticular proteins are overexpressed leading to a higher percentage of chitin in the larva compared to regular flies (Herranz *et al*., 2013). Importantly, these studies argue the complexity of epigenetic factors in the final results that must be better studied.

Base-ground studies in microgravity

The difficulty of transferring systems to the ISS, either due to the costs related to transportation or due to the limited availability of access and space in its laboratories, the microgravity simulator systems on Earth sought to somewhat equate the research in this field. Different strategies were designed for the study of short or prolonged exposition to microgravity conditions such as *parabolic flights*, which allow to observe the effects of the exposition to short periods of time to microgravity followed by hypergravity periods; powerful *electromagnets* or superconducting *solenoids* provided to cause a repulsive diamagnetic force on water that could balance the weight of several samples and cause it to float them (diamagnetic levitation) (Herranz *et al*., 2012); *rotating wall vessels* (RWV) bioreactors where cells are suspended in medium that is constantly rotating; or/and *clinostats* and *random positioning machines* (RPM) which consists of two or three axis that rotate generating a simulated microgravity environment at their centre (Blaber *et al*., 2010; Laván *et al*., 2015) (See Table 3). Importantly, low orbit facilities and parabolic flights are been associated with numerous stressful factors that can affect the organism in study. Because of that these based-ground experiments minimized key factors related to the environment such as temperature, pH, pressure, oxygen, CO_2 concentration, etc., which require to be controlled all the time in the other processes. However, some researchers disagree with this argument, suggesting that there are too many variables to control in any case. Another debate caused by studies on Earth

https://doi.org/10.1017/S1473550424000223 Published online by Cambridge University Press

Table 3. *Types of study of Micrograving on ground*

Type of simulation	Description	Objectives	Results	Source
Random positioning machine (RPM)	The RPM is made up of two independently rotating platforms that are capable of rotating at various rates and in any direction. The sample is positioned between the two platforms and its orientation with respect to gravity changes continuously and arbitrarily. This produces a nearly weightless atmosphere that is comparable to the microgravity seen in space.	Determine the effects of microgravity simulation and oral administration of essential phospholipids on the cellular respiration and contents of the main cytoskeletal proteins in the ovaries of *Drosophila* flies that had undergone a full oogenesis.	The study found that after a full cycle of oogenesis under simulated microgravity, the rate of cellular respiration in the fruit fly ovaries increases, apparently due to complex II of the respiratory chain	Ogneva and Usik (2021)
		Investigate alterations in the activity and sleep of *Drosophila melanogaster* under simulated microgravity,	In normal photoperiod (LD), increased activity and sleep resulted under simulated microgravity, while for the constant dark (DD) condition, activity and sleep rhythms appeared disordered and activity thus decreased the probability of waking up during the day. The results indicated that normal day length could alleviate the effects of simulated microgravity on fruit fly activity and sleep.	
		Study on the changes in mouse and fly sperm motility under modelling microgravity.	After 6 h of simulated microgravity, the motor activity of *Drosophila melanogaster* spermatozoa increased, while for mouse spermatozoa, there was a decrease in motor activity. Changes in the content of actin-binding proteins in fly and mouse spermatozoa were also observed, which may indicate different mechanisms of gravireception and/or different rates of formation of the adaptive pattern of proteins.	
		To determine the effect of simulated microgravity and hypomagnetic conditions for 1, 3 and 6 h on sperm	Increased velocity of movement of sperm tails after 6 h in simulated microgravity. The levels of proteins that make up the	Ogneva *et al.* (2020)

(*Continued*)

https://doi.org/10.1017/S1473550424000223 Published online by Cambridge University Press

Table 3. (*Continued.*)

Type of simulation	Description	Objectives	Results	Source
		motility of the fruit fly *Drosophila melanogaster.*	axoneme of the sperm tail did not change, but cellular respiration was altered. Exposure to hypomagnetic conditions led to decreased motility after 6 h against the background of decreased cellular respiration rate due to complex I of the respiratory chai	
Magnetic levitation	Commonly referred to as magnetic levitation or maglev, it employs magnetic fields to lift and move items without making direct contact with them. Magnet levitation can be utilized in microgravity to manipulate items without the interference of gravity.	To evaluate the effects of diamagnetic levitation on the transcriptional profile of *Drosophila*.	The results are compared with those of similar experiments carried out with a Random Positioning Machine (RPM). Observing a developmental delay in fruit flies from embryo to adult. Microarray analysis showed changes in overall gene expression from images that developed from larvae under diamagnetic levitation and also under simulated hypergravity conditions. Significant changes were observed in the expression of immune, stress and temperature response genes	Herranz *et al.* (2012)
Multi-axis space flight simulator (Gravite®, GC-USRCE010001)	A multi-axis space flight simulator that can provide a continuous state of free fall while rotating the payload in a controlled and programmed manner to mimic the microgravity environment.	Study the effects of microgravity on cellular respiration and cytoskeletal proteins in fruit fly ovaries.	• The increase in histone acetylation correlated with an increase in the expression of actin and alpha-actinin. • An increase in the rate of cellular respiration due to complex I of the respiratory chain was observed in the ovaries under microgravity conditions. • The intensity of cellular respiration was significantly higher in the ovaries of flies that underwent a full cycle of gametogenesis under simulated microgravity compared to controls.	Usik *et al.* (2021)

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Diamagnetic levitation	When a diamagnetic material is exposed to a powerful magnetic field, a type of magnetic levitation known as diamagnetic levitation happens. Materials that are diamagnetic, such as copper, gold, silver, and graphite, have a negative magnetic susceptibility, meaning that a magnetic field only weakly repels them. A diamagnetic substance will experience a force that resists the magnetic field when it is exposed to a strong magnetic field, which will cause it to levitate. The magnetic field and the electrons in the diamagnetic substance interact to produce the levitation.	Demonstrate the usefulness of diamagnetic levitation as a viable alternative to more established ground-based techniques for simulating the effects of microgravity on a complex organism	The results of the study show that the gait speed of fruit flies and their 'activity' are significantly altered by counteracting the gravitational force. Diamagnetic levitation allowed to recognize the disturbance of effective gravity as the cause of the anomalous behaviour. The study demonstrates how diamagnetic levitation can be used to quantitatively assess the behavioural response of a macroscopic organism to zero gravity.	Hill *et al.* (2012)

with microgravity simulators versus those carried out in real environments like the ISS is the representativeness and significant similarity of genetic processes in both environments. This fact, although it has given controversial results, has shown that in the case of *D. melanogaster*, the genes and mechanisms that were detected in studies on the ISS could be partially or almost completely repeated in the simulators on Earth. For example, using a clinostat to simulate microgravity on Schneider S-1 cells originating from *Drosophila*, it was observed that there were changes in the mitochondrial structure and cristae morphology as a consequence of microtubule disruption. All these results matched those attained by exposure to cultured human lymphocyte (Jurkat) cells during spaceflights (Schatten *et al*., 2001).

Although gravity modification appears to have minimal influence on gene expression under ideal environmental conditions, when those criteria are far from optimal, gene expression must be effectively controlled and the effects become more predominant. This could be due either to the lack of experience that animals have with new ecosystems, such as those that do not have gravitational attraction or perhaps the opposite, that the organisms spent an indeterminate amount of time under microgravity and therefore the return to those previous conditions a biological response was generated. Either way, research in this field using *D. melanogaster* has led us to better understand these effects (Herranz *et al*., 2013).

Although radiation is a factor considered mutagenic for inducing genetic alterations that lead to a greater risk of the appearance of malignant tumours (carcinogenesis), and despite the number of studies that show that radiation seems to worsen its effects if microgravity coexists, Other studies such as those presented by Grimm's group in human cancer cells suggest that the mechanisms are more complex than it seems (Grimm, 2021). This point allows us to emphasize here that in this review we focus on the contributions of the fruit fly to space research and that the extrapolation of these processes and mechanisms in humans should not be so direct but rather be used as guides for a better understanding of the variables studied. Indeed, the effects of microgravity on human cancer cells had controversial results showing pro- or anti-tumoural effects in different studies. Importantly, at the beginning the objective to analyse tumours in Space was generate 3D models capable to explain the mechanisms of invasion and metastasis in cancer. The initial studies on this were also developed in *Drosophila melanogaster* models (Becker and Souza, 2013). Finally, to understand the mechanisms at the molecular level, studies by our group suggested the presence of genetic sensors that, to our understanding, could be the most sensitive in determining gravitational waves (Laván *et al*., 2015). Six genes found in our study showed significant different patterns of expression against microgravity, compared to the thousands of genes analysed molecularly. In addition, these genes suggested to be microgravity 'sensors' in *Drosophila* have orthologous genes in humans very related to those found in the main types of cancers (Valdivia-Silva *et al*., 2015).

Possible radiation-microgravity synergistic effects on **Drosophila melanogaster**

The simultaneous effects of radiation and microgravity on biological systems encompass the two essential components of the Space environment thus improving the understanding of the contribution of both variables in the effects observed separately. As previously indicated, the work carried out on *D. melanogaster* for radiation was mostly replaced by mixed studies together with microgravity (see Tables 1 and 2). According to Beckingham (2010) the synergy between microgravity and any other stress factor positively affects the main effects of microgravity. The effects of microgravity are stronger when other stress factors are involved, in this case radiation is considered a stress factor. For example the RhogEF2 gene is subexpressive during microgravity conditions which leads to a possible reduction in the wound regeneration (Beckingham, 2010; Gilbert *et al*., 2020). Since then, several studies included the interaction of these variables in their designs and conclusions, although they left open the possibility of new options related to the importance of each of them in the observed phenomenon. It is so that, for example Ikenaga *et al.* (1997), analysed male *D. melanogaster* flies from the US space shuttle Endeavour after the flight in order to examinate some somatic mutations and sex-linked recessive fatal mutations caused in male reproductive cells. The results showed that the prevalence of deleterious sex-linked recessive mutations was two to three times higher in the flight groups than in the ground control ones irradiated with X-ray to different doses. Doses on the ground had to be 100 times higher to achieve similar effects to those seen in flight. As already seen previously, the genetic consequences of Space radiation in male reproductive cells seemed to be substantially magnified under microgravity, which may explain these enhanced mutation frequencies in the flight samples. Another example is shown by (Yatagai and Ishioka, 2014). They exposed *Drosophila* to X-rays emitted from ^{85}Sr during a spaceflight and compared with base-ground controls. In the control groups, the survival percentage of *Drosophila* larvae irradiated at 8 Gy was reduced by 26.5%, while at the same dose in flies in orbit the fraction of survivors was reduced by 38.5%, suggesting a synergistic effect of radiation with microgravity which enhance the works previously reported about genetic mutations found in *Drosophila* (Shank, 1974).

These studies have suggested that although the effects of microgravity on *D. melanogaster* could be minor, they are sufficient to make a difference between stay and no stay in Space. Importantly, the implications of these results for human Space exploration could allow quantifying the damage of ionizing radiation in human tissues and predict some harmful mutations to which certain human genes could be subjected (Blaylock and Shugart, 1972).

Concern that this could lead to male infertility prompts further studies of the combined effects in astronauts.

Implications of *Drosophila* research in Space human biology

Fruit flies and humans have 60% similarity in the genome, and around 75% of human disease-causing genes have *Drosophila* homologous (orthologous) (Ugur *et al*., 2016; Verheyen, 2022). Due to, several of the fundamental biological mechanisms and pathways that regulate development and survival have been conserved throughout evolution in these two species (Jennings, 2011), These characteristics, along with the fruit fly's quick generation time, minimal care requirements, and accessibility to potent genetic tools, make it possible to explore intricate processes important to biological studies, including aerospace biology (Mirzoyan *et al*., 2019). Indeed, the studies of the effects of ionizing radiation in *D. melanogaster* initiated in the first half of the 20th century and the possibility of extrapolating the results to humans achieved its maximum potential when the fly genome sequence was published in 2000 (Adams *et al*., 2000).

The effect of radiation reported by Blaylock and Shugart (1972) in a homozygous population of *D. melanogaster* could be of importance for the future establishment of humans on celestial bodies such as Mars, where a small population may give rise to a group of Martians with poor genetic pool because of inbreeding effects. In accordance with these studies, there would be a radiation threshold where the generation of mutations could be beneficial to the population, because it would generate genetic diversity.

Studies focused on germ-line cells found that spontaneous mutation rates per generation are similar among *D. melanogaster* and humans and these rates are not related to time (Drost and Lee, 1995) In the case of *D. melanogaster*, the average limit number of cell divisions throughout its life is 19, with no major difference between sexes. It has been seen that the average mutation rate in humans is approximately 2.5^{-8} mutations per nucleotide site or 175 mutations per diploid genome per generation and it has been estimated that the genomic deleterious mutation rate (U) is at least 3, a higher value than that estimated for *Drosophila* ($U = 0.02 - 1$) (Nachman and Crowell, 2000). These data about comparative mutation rates are important to validate the use of *Drosophila melanogaster* as a model to study the effects of the Space environment at the genetic and molecular level. Potentially, the results obtained in *Drosophila* about possible genetic damage caused by cosmic radiation or microgravity could mean that in humans, those effects could be similar or worse. In addition, the base substitution mutation rates are substantially higher in males than in females and rise with paternal age, this is explained as male germline cells divide often (Crow, 1997). In this sense, *Drosophila* could also be used for evaluating spatial effects on the germline genome, due to the similarities in mutation rates and the conservation of several metabolic pathways (Drost and Lee, 1995). Of course, the research in the line of reproduction is very important to future projects of other planets or satellites colonization, and *Drosophila* can help us predict possible harmful effects on humans in Space.

Laván *et al.* (2015) showed interesting implications for understanding the microgravity effects at the transcriptomic level. They found a group of six genes of *D. melanogaster* which are inhibited in real and simulated microgravity and probably could be the keys to understanding the roots of multiple effects on cells. These genes and their human orthologues are currently the focus of research in our group as gravity sensors which would imply an important advance to correlate physiological or patho-physiological processes in living organisms with changes in gravitational waves (Laván *et al.*, 2015).

Conclusions

Drosophila melanogaster is undoubtedly an organism that has proven to be a great biological model to the study of the hazards in extraterrestrial extreme environments. These organisms at different levels, are a tool that facilitates the understanding of the possible effects of these conditions and variables on all living beings, especially humans. Indeed, thanks (i) to the easy handling of cultures of flies on Earth or in Space conditions, (ii) to the extensive knowledge of their anatomy, physiology, biochemistry, immunology, genomics, proteomics, and other related omics sciences, (iii) to the increasing number of molecular manipulation technologies, and (iv) to the possibility of extrapolating their biological mechanisms between various organisms; the fruit flies will continue to be a tool of choice for research in the field of Space Biology and Astrobiology in general. It is true that at the system level, *Drosophila melanogaster* has some limitations as a model against different human diseases, for example; such as the absence of acquired immunity. However, the molecular mechanisms of this model help visualize specific processes and increase the clarity of others (Dionne and Schneider, 2002; Verghese and Su, 2018).

There is still a lot of work to be done to determine the full effects that ionizing radiation and micro-gravity on Space, but it is certain that the human being, a born explorer, will at some point colonize other moons or planets. *Drosophila melanogaster* will always be the spearhead to understand what could await us and will be there to support us to decide what measures we could take to adapt to that new home.

Finally, the authors emphasize that the focus and objective of the article does not seek to analyse all *Drosophila* studies in science, but specifically to the field of Space Biology, where access to research is more restricted not only by the required infrastructure but for the possibility of having access to space flights.

Acknowledgements. We thank the members of the Sociedad Científica de Astrobiología del Peru (SCAP) for their suggestions in this review. The research project was supported by the Programa Nacional de Innovación para la Competitividad y Productividad (Innóvate Perú), under contract 450-PNICP-BRI-2014.

Author contributions. The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Conflict of interest. The authors report no conflict of interest.

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https://doi.org/10.1017/S1473550424000223 Published online by Cambridge University Press

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