COSMIC SILENCE

DULIAbio 2019

Low dose radiobiology at Laboratori Nazionali del Gran Sasso: results, challenges and future plans

INFN LNGS
Istituto Nazionale di Fisica Nucleare
Laboratori Nazionali del Gran Sasso

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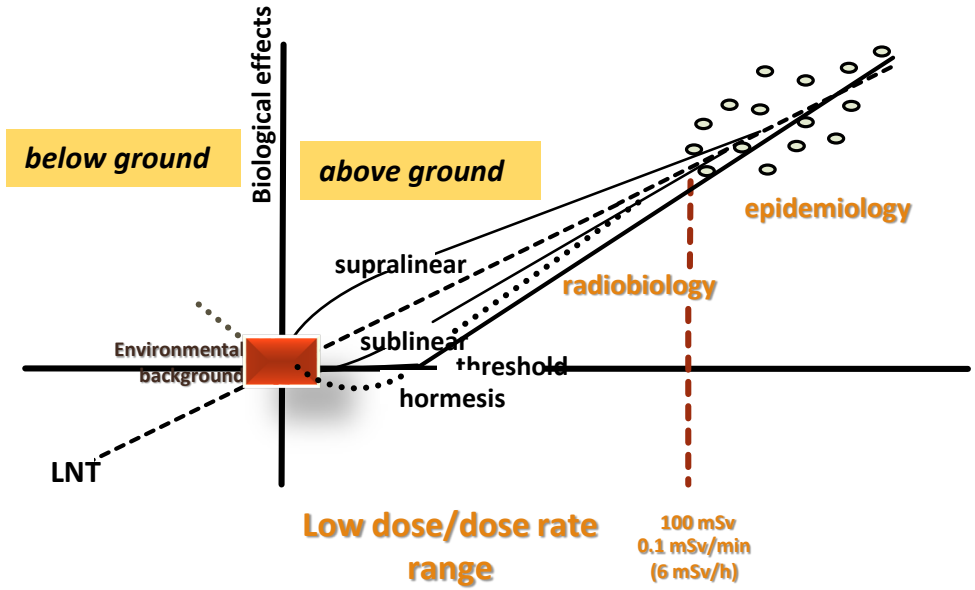
LNGS

BASIC INSIGHTS FROM LOW DOSE RADIOBIOLOGY CAN BE APPLIED TO OUR ORDINARY LIFE:

- Cancer risk estimation from medical, occupational or space travel radiation exposure
- Development of new more medical applications (radiodiagnostic, radiotherapy)
- Radioprotection issues: dose-dose rate relationship, radiation quality



Understanding how environmental radiation influences living matter is an important dowel to elucidate the biological effects of low dose/dose rate radiation exposures.

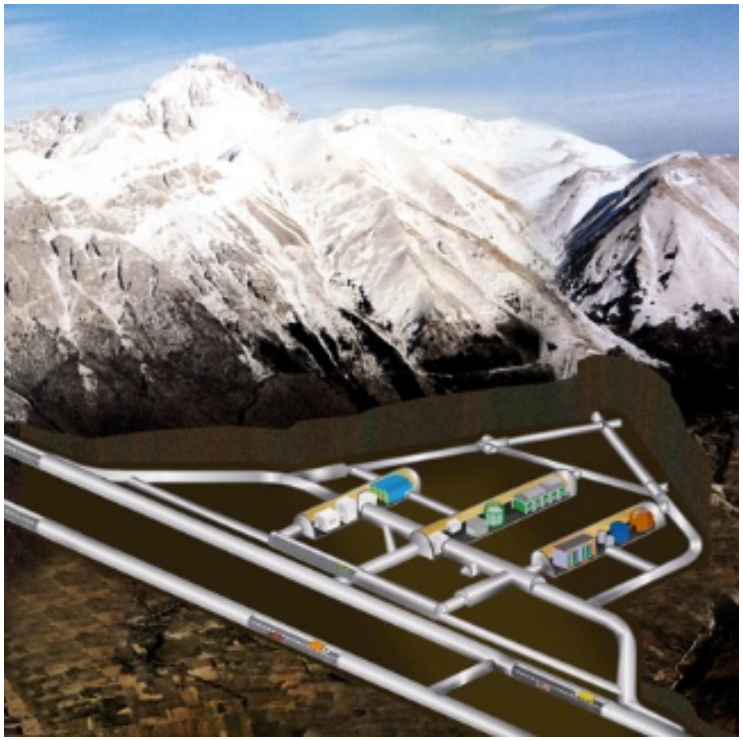
'Underground biology'

Life on Earth has evolved in the presence of ionizing radiation referred as **natural background radiation** (cosmic rays, crust's radionuclides, radon)

What happens to living organisms when
natural background radiation
is reduced?

Deep Underground Laboratories (DULs)
are unique places where it is possible to investigate
the effects of reduced natural background radiation

LABORATORI NAZIONALI DEL GRAN SASSO



The 1400 metre-rock natural coverage provides a **cosmic ray flux reduction by one million times**;
moreover, the **flux of neutrons** in the underground halls **is about thousand times less than on the surface** due to the very small amount of uranium and thorium in this rock.

Radon concentration is kept at a very low level by an efficient ventilation system that pumps fresh air from the outside into the laboratory.

- **ONE OF THE LARGEST UNDERGROUND RESEARCH CENTERS IN THE WORLD**
- **THREE LARGE UNDERGROUND HALLS THAT HAVE BEEN EXCAVATED IN THE GRAN SASSO MASSIF (CENTRAL ITALY) UNDER 1400 M OF ROCK: 20M WIDE X 18M HIGH X 100M LONG**
- **TOTAL VOLUME AND AREA OF THE LAB ARE 1.8×10^5 M^3 AND 1.35×10^4 M^2, RESPECTIVELY**

https://www.lngs.infn.it/en/lngs-overview

LABORATORI NAZIONALI DEL GRAN SASSO

Designed and built to host particle, astroparticle and nuclear physics experiments

In 1989, the first experiment **MACRO** started with the aim of perform measurements in areas of astrophysics, nuclear, particle and cosmic ray physics (in 2000 MACRO stop its measurements).

Few years later, the first biology experiment called **PULEX**, in contrapposition, started (thank to the brilliant intuition of Prof. Satta)

PULEX

Low environmental radiation background impairs biological defence of the yeast *Saccharomyces cerevisiae* to chemical radiomimetic agents

L. Satta [a,b,*], G. Augusti-Tocco [c], R. Ceccarelli [c], A. Esposito [b], M. Fiore [d], P. Paggi [c], I. Poggesi [d], R. Ricordy [d], G. Scarsella [c], E. Cundari [d]

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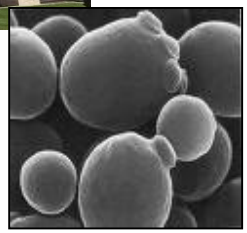
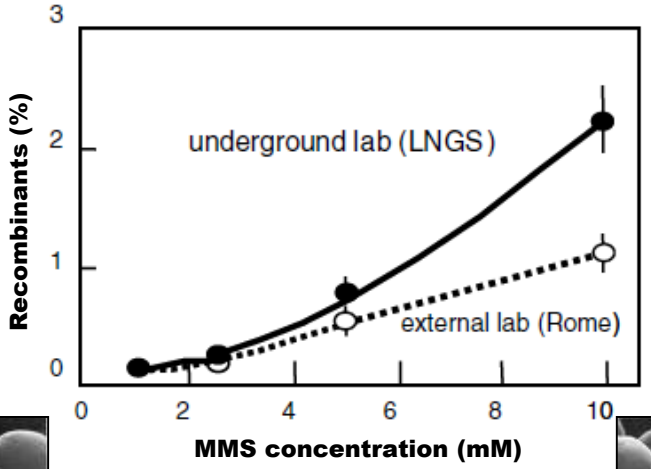
Received 9 April 1995; revised 24 May 1995; accepted 26 May 1995

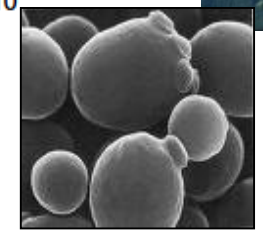
Reference Radiation Environment



yeast



Low Radiation Environnment



yeast

Environmental radiation represents a constant daily stimulus incorporated in the biology of living organisms during evolution and contributes to the development of defence mechanisms

EXPERIMENTAL APPROACH:

Set Up of Parallel Experiments Under Different Radiation Environments

Underground laboratory (low background lab)

Directly ionizing cosmic rays: 10^{-6} nSv/h
Neutrons: 10^{-3} nSv/h
Radon: units – 10-70 Bq/m^3
Gamma rays: 10 - 100 nSv/h

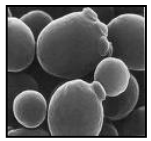
Aboveground Laboratory (reference lab)

Directly ionizing cosmic rays: tens of nSv/h
Neutrons:~ 10 nSv/h (at 1 km above sea level)
Radon: tens - hundreds Bq/m^3
Gamma rays: tens - hundreds nSv/h

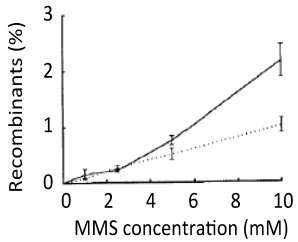
Temperature
Relative humidity
Atmospheric pression
Medium for culture

Long term underground experiments on *in vitro* models

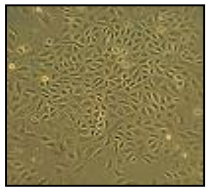
Satta et al., 1995



S. Cerevisiae



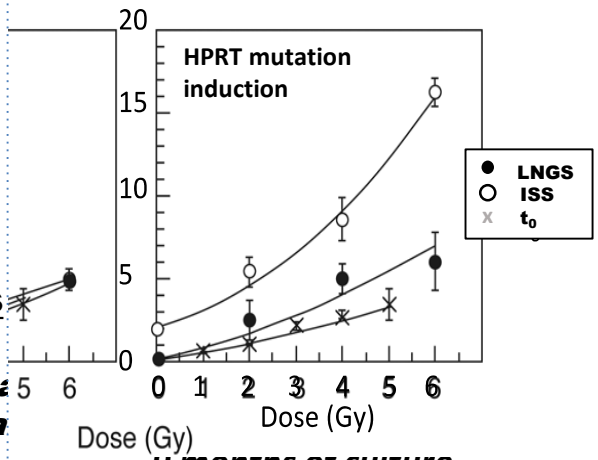
Satta et al., 2002
Antonelli et al., 2008
Fratini et al., 2015



Chinese hamster cells

Similar results at LNGS externa
reference la

Pulex

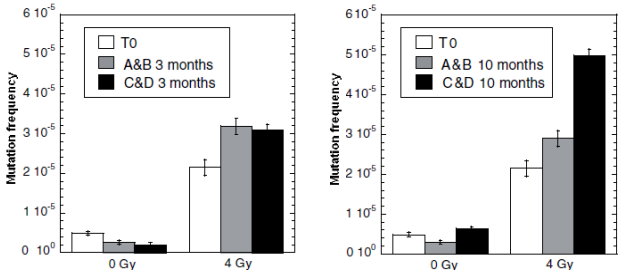


9 months of culture

Cells cultured in reduced environmental radiation conditions for several months are:

- *Less tolerant to radiation-induced DNA damage*
- *Less efficient in scavenging reactive oxygen species*

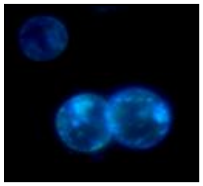
Pulex-2



A&B: external cultures at LNGS
C&D: underground cultures

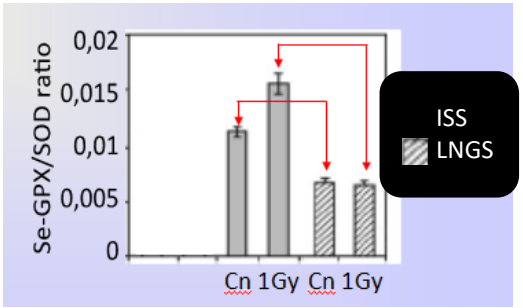
10 months of culture

Carbone et al., 2009
Carbone & Pinto et al., 2010



Human cells

Cosmic Silence



6 months of continuous culture

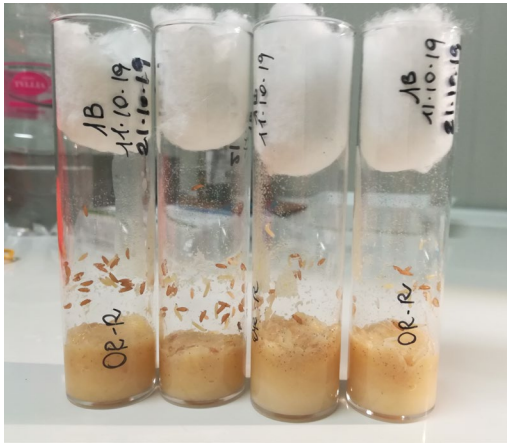
From *in vitro* … to *in vivo* model system

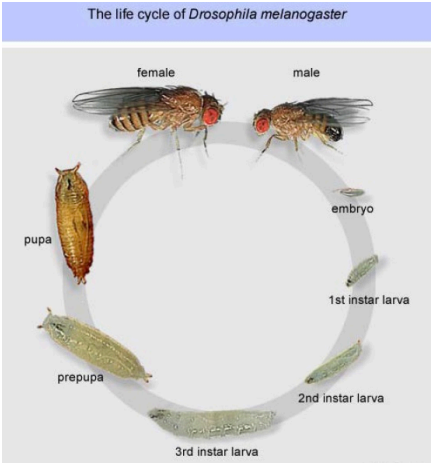
The Flyinglow project

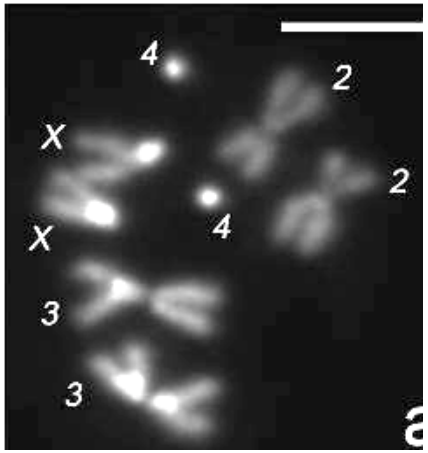
Recently, we started employing ***Drosophila melanogaster***, the common fruit fly, as a multicellular model system to investigate whether the **reduced background radiation** at the LNGS **affects development and growth of a complex multicellular organism**

Drosophila as model organism

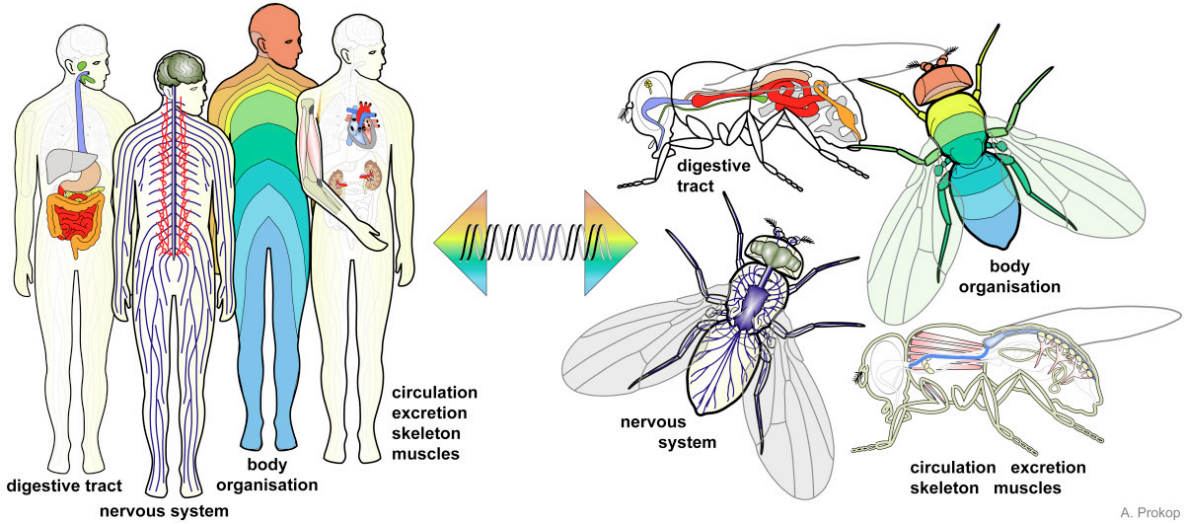
- Easy to grow in the lab with low overall costs
- Short life cycle: about 10 days from fertilization to adult (rapid multigenerational studies)







- Small dimensions: adults 3 mm and eggs 0.5 mm in length
- High fecundity: a female lays up to 100 eggs/day
- Drosophila has only 4 pairs of chromosomes and genome has been sequenced in 2001



Most of the fundamental biological mechanisms and pathways that control development and survival **are conserved across evolution in humans and flies**

- About **75% of human disease genes** have a recognizable **match** in fruit flies
- For almost every **organ** in humans there is a match in flies, and common genes regulate their development, organization and function
- Many fly proteins are encoded by a **single gene** whereas mammalian genomes are more redundant making genetic manipulations more complicate

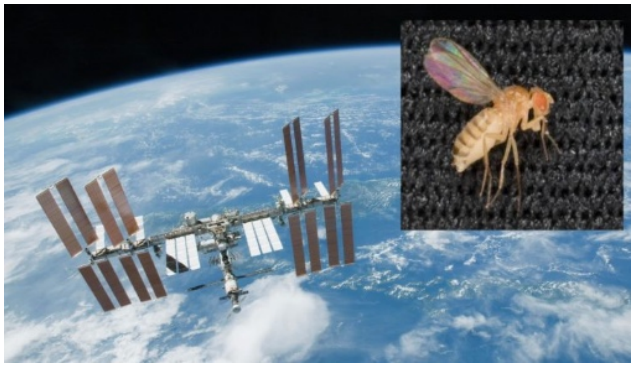
Drosophila in radiobiology

The Fly Room at UT-Austin; Muller is at the right, with loupe (Lilly Library, Indiana University)

In 1927, Hermann J. Muller demonstrates that X-rays can induce mutations using fruit flies as experimental model. He received Nobel prize in 1946

- Drosophila was the first animals sent in the space (109 km) in 1947 aboard a U.S.-launched V-2 rocket. The purpose of the experiment was to explore the effects of radiation exposure at high altitudes.

- **https://www.nasa.gov/ames/fruit-fly-lab**:
  Fruit Fly Lab-01 (FFL-01) in Juanary 2015
  Fruit Fly Lab-02 (FFL-02) in June 2017
  Fruit Fly Lab-03 (FFL-03) in April 2018



The Flyinglow project

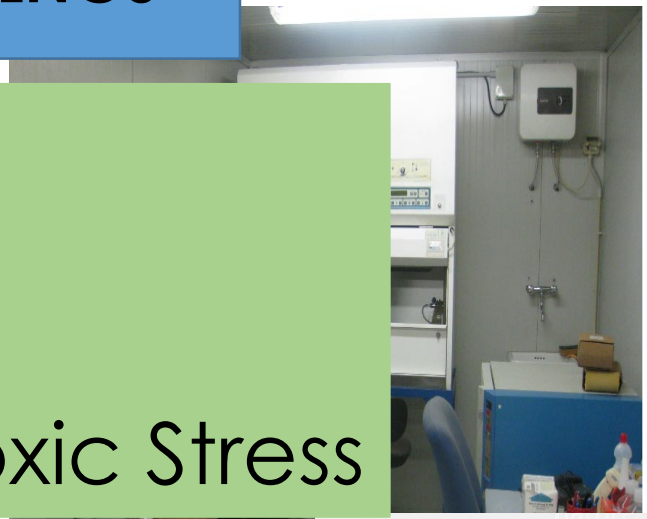
Set Up Of Parallel Experiments Under Different Radiation Environments

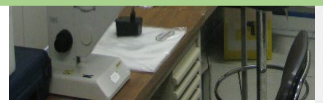
Reference Radiation Environment

University of L'Aquila

Low Radiation Environnment

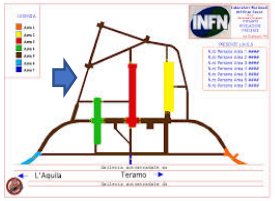
LNGS



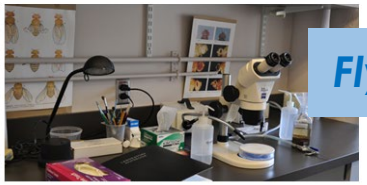


Endpoints:
Lifespan
Fertility
Response To Genotoxic Stress

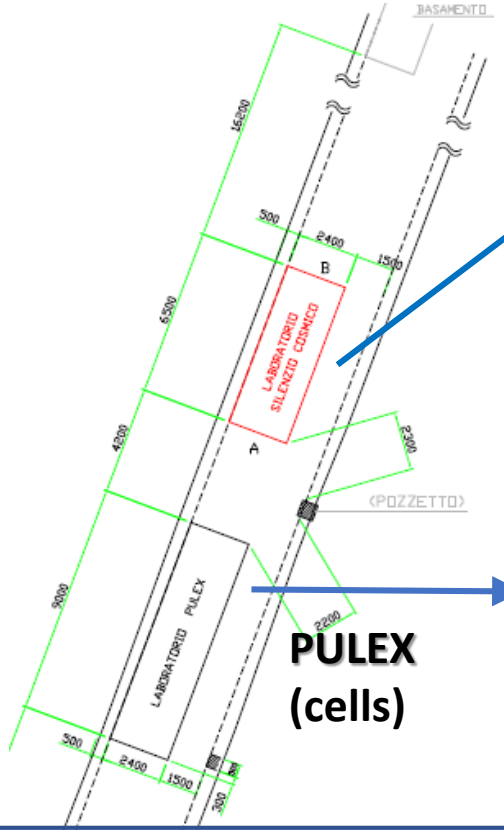
Cosmic Silence at LNGS



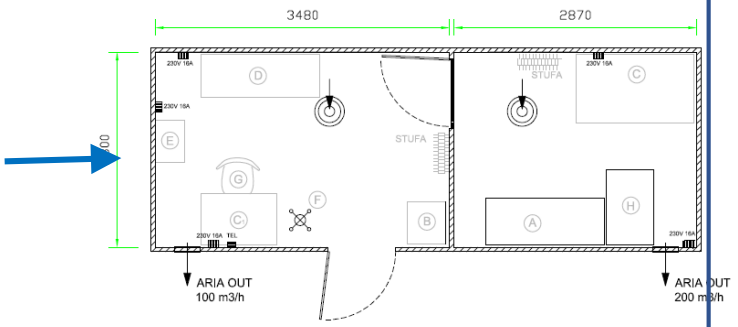
DROSOPHILA workstation



Flyinglow project

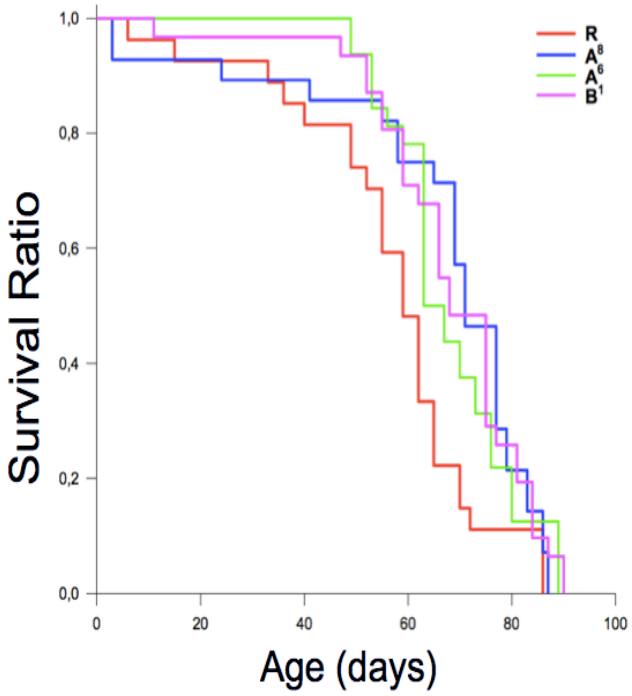


COSMIC SILENCE
(organisms)



PULEX
(cells)

Reduced background radiation extends lifespan in adult flies

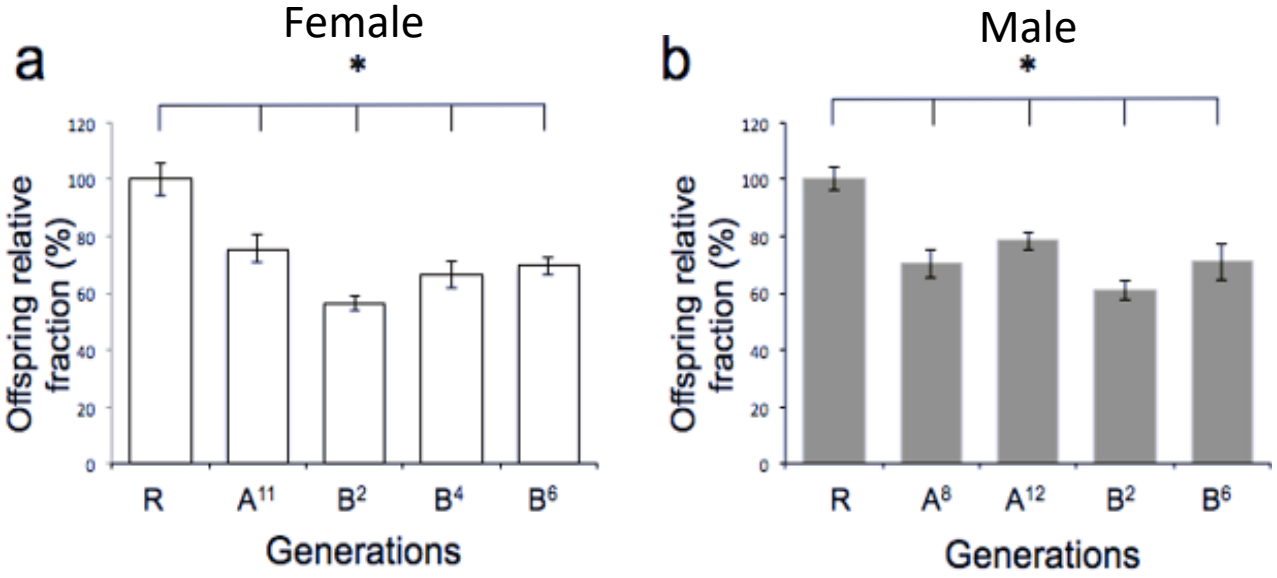


- **Analysis over different generations (1,6,8) and populations (A and B) of wild type OR-R strain males**
- **Kaplan-Meier survival curve** was used
- *p-value* was calculated by **log-rank test**
- Pairwise Multiple comparison was done by **Holm-Sidak method**

The statistical analysis shows a significant extension in median life span (about 15%) in flies maintained at underground lab compared to control flies R maintained at University of L'Aquila

(Morciano et al., Journal of Cell Physiology 2017)
(Morciano et al., Radiation Research 2018)

Reduced background radiation reduces fertility of male and female flies

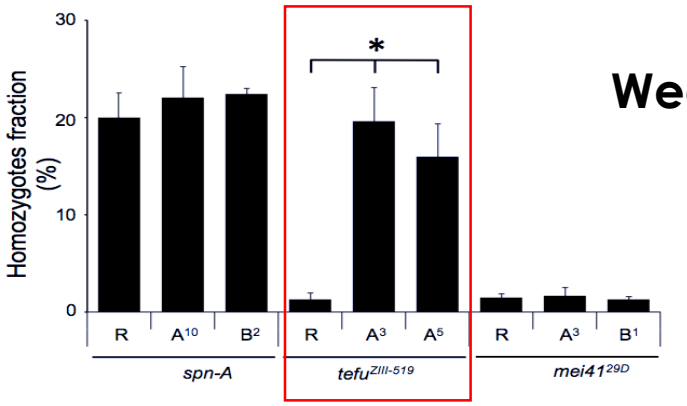


~30% reduction the male and female fertility at LNGS reduced background radiation. Fertility reduction is an early effect (B2 generation) and remained unchanged along next generations.

(Morciano et al., Journal of Cell Physiology 2017)
(Morciano et al., Radiation Research 2018)

Reduced background radiation affects the survival of DNA damage response mutants

To verify the effect of reduced background radiation on survival of DDR mutant homozygous, mutant lines were monitored for the frequency of homozygotes



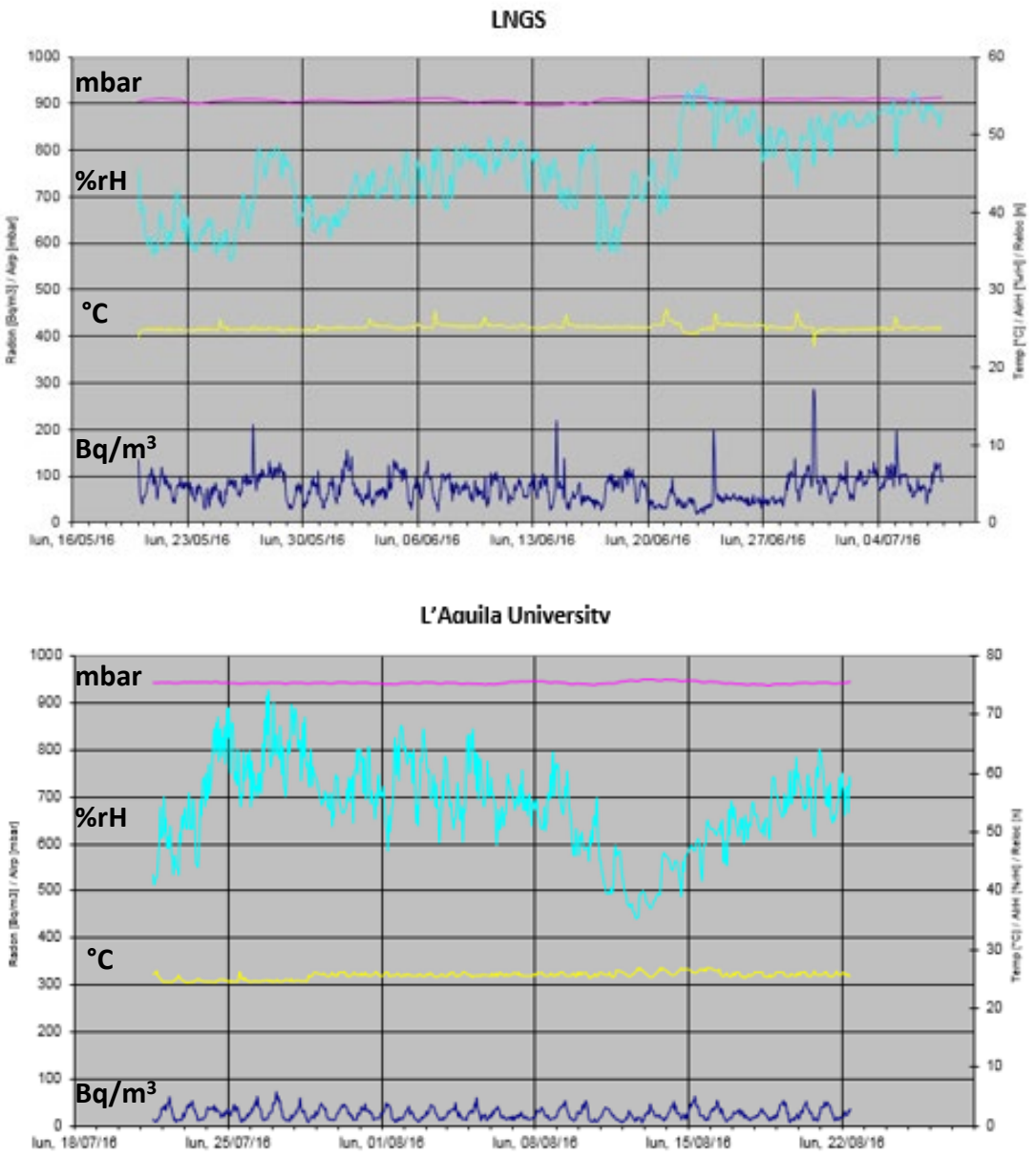
Weak mutants of DNA Damage Response (DDR)

spn-A mutant → RAD51 gene
tefu mutant → ATM gene
mei-41 mutant → ATR gene

Frequency of *tefu*$^{ZII-5190}$ homozygous adults was much higher at LNGS underground lab (about 20%) compared to that of the reference lab R (1.3%)

(Morciano et al., Journal of Cell Physiology 2017)
(Morciano et al., Radiation Research 2018)

Other environmental parameters are constantly monitored



Alphaguard device:

- Pressure
- Relative humidity
- Temperature
- Radon activity concentration

Mesurements during Flyinglow experiments

L'Aquila Univ. vs LNGS

941.9 ± 2.7 mbar vs 906.7 ± 4.0 mbar

54.1 ± 7.6 % vs 45.5 ± 5.6 %

25.6 ± 0.6 °C vs 25.2 ± 0.4 °C)

Radon Concentration range

25 Bq m^{-3} vs 70 Bq m^{-3}

At the present

In 2018, an **Operative Collaboration** R&D in the field of radiobiology **between ISS and INFN** has been established

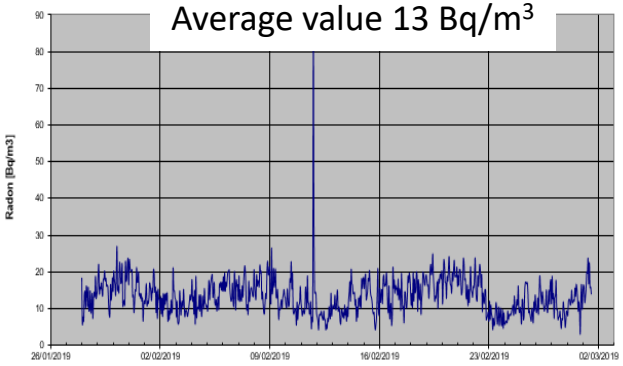
In this framework, the ***RENOIR*** project was submitted and funded (starting from 2020) by INFN-Commission V

The **external lab** is now located at the LNGS Servizio di Chimica e Impianti Chimici

Ventilation system in the underground lab was improved and works efficently keeping the **radon concentration** at comparable value with respect to the external reference environment

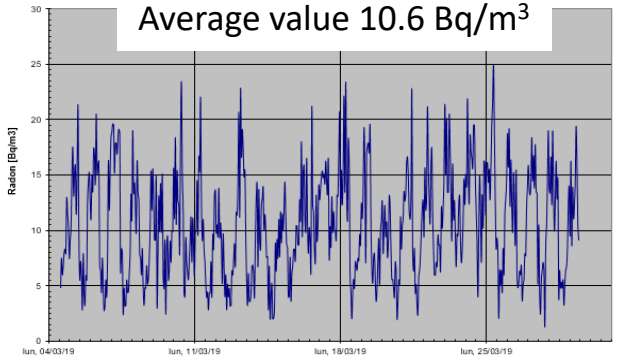
LNGS Underground

Average value 13 Bq/m^3



LNGS External

Average value 10.6 Bq/m^3



March, 2019

CHALLENGES and possible solutions

SAME CONDITIONS IN TWO DIFFERENT LABS

- Two identical *Drosophila* incubators inside the underground and the external lab
- Underground and external lab at same location (LNGS): same operator to performing the experiments in parallel
- "Ready-to use" standard medium from the same batch; same water for reagent preparation

ACCURATE DOSIMETRY

- Spectroscopic/dosimetric measurements and simulations of the environmental radiation
- New approaches for neutron measurements
- Implementation of a *Drosophila* geometric model in the simulations

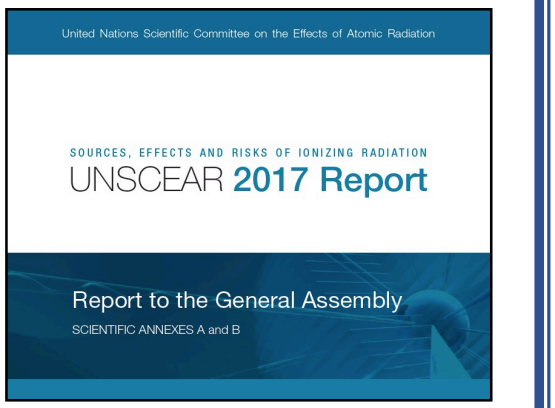
Perspectives

1. Investigation of the **molecular mechanisms** underlying the influence of the enviromental radiation in the biological response of *Drosophila*: Comparative RNA-seq analyses between external and underground flies' total RNAs and qPCR-based validation

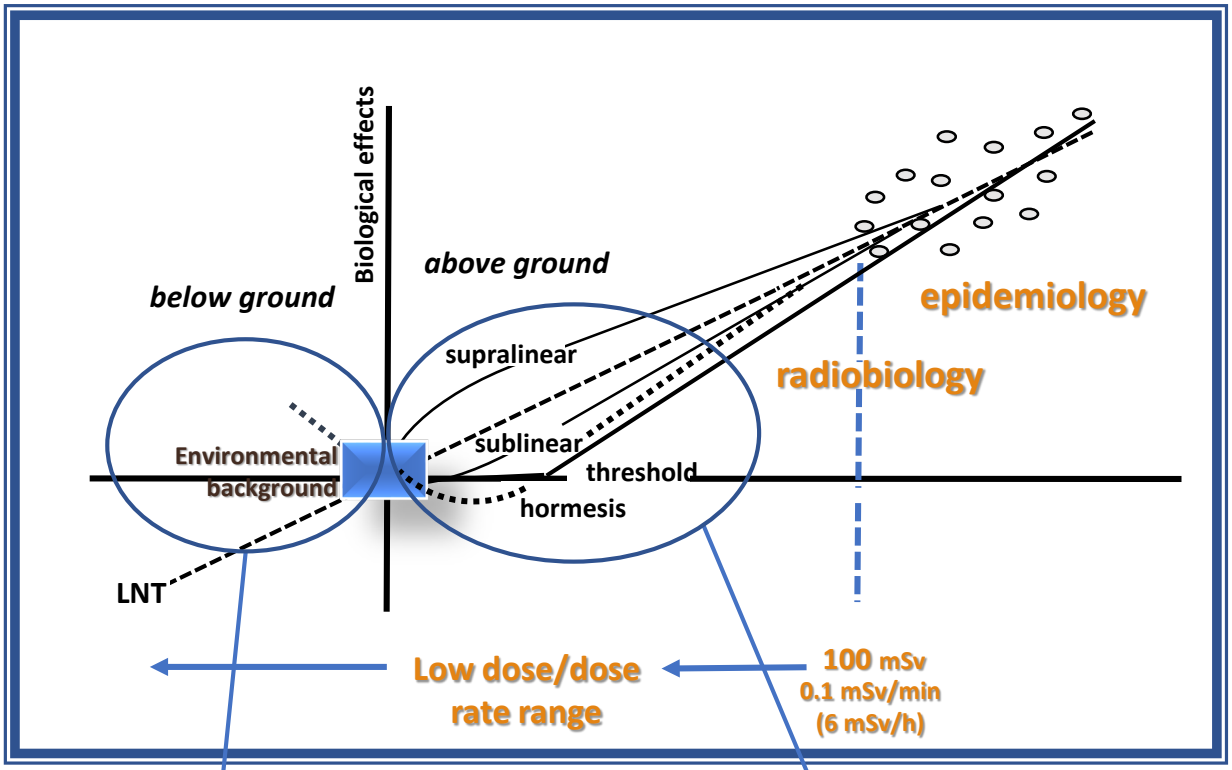
2. Improvement the **knowledge of the radiation field** inside the external and underground labs at LNGS (dosimetric, spectrometric and simulation approach)

3. Obtain information about the involvement of the **different components of the radiation** field on *Drosophila* biological response. In particular the role of the gamma component (*RENOIR* project)

Investigation on the effects of protracted exposure to very low dose rate is a hot research topic in radiobiology



" A systematic examination of the risks associated with radiation exposure at low doses and low dose rates is, therefore, particularly timely. "



Laboratori Nazionali del Gran Sasso, LNGS

LIBIS ISS Rome

The LIBIS facility at ISS

(Low dose/dose rate gamma Irradiation facility for in vitro BIological Systems)

THREE CS-137 SOURCES

37 MBq	→	2 µGy/h	(125 cm distance)
		40 µGy/h	(28 cm distance)
740 MBq	→	40 µGy/h	(125 cm distance)
		793 µGy/h	(28 cm distance)
18.5 GBq	→	1 mGy/h	(125 cm distance)
		20 mGy/h	(28 cm distance)

Environmental background at ISS: 0.3 µGy/h

- The availability of very low dose rate **facilities (*LIBIS* and *LNGS***) allows us to perform a **systematic study** in different exposure senarios …

- … and provides a potential opportunity to address important radiation protection questions: the **shape** of the **low dose/dose rate relationship for biological effects;** the **possible role** of **radiation quality** in triggering the biological response.

COLLABORATORS

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Claudia Tomei

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Cosmic Silence and
RENOIR experiments

Cosmic Silence and Flyinglow
experiments

COSMIC SILENCE

THANK YOU
for your ATTENTION!

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