Rapid Communication

Effects of reduced natural background radiation on *Drosophila melanogaster* growth and development as revealed by the FLYINGLOW program†

P. Morciano[1,2], R. Iorio[3], D. Iovino[3], F. Cipressa[1,2], G. Esposito[4], A. Porrazzo[1], L. Satta[2], E. Alesse[3], M.A. Tabocchini[2,4] and G. Cenci[1,2,5]

[1] SAPIENZA Università di Roma, Rome, Italy, [2]Museo Storico della Fisica e Centro Studi e Ricerche ''Enrico Fermi'', (Rome, Italy); [3] Dipartimento di Scienze Cliniche Applicate e Biotecnologie, Università dell'Aquila (Aquila, Italy). [4]Istituto Superiore di Sanita` (ISS) and Istituto Nazionale di Fisica Nucleare (INFN), Sezione Roma 1, Rome, Italy,

Running title:

FLYINGLOW: biological effects of protracted low radiation doses in *Drosophila melanogaster*

[5]Corresponding Author
Giovanni Cenci
giovanni.cenci@uniroma1.it
Tel +39 0649912477
Fax +39 0649912343

†This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: [10.1002/jcp.25889]

Received 06 February 2017; Revised 20 February 2017; Accepted 02 March 2017
Journal of Cellular Physiology

DOI 10.1002/jcp.25889

ABSTRACT

Natural background radiation of Earth and cosmic rays played a relevant role during the evolution of living organisms. However, how chronic low doses of radiation can affect biological processes is still unclear. Previous data have indicated that cells grown at the Gran Sasso Underground Laboratory (LNGS, L'Aquila) of National Institute of Nuclear Physics (INFN) of Italy, where the dose rate of cosmic rays and neutrons is significantly reduced with respect to the external environment, elicited an impaired response against endogenous damage as compared to cells grown outside LNGS. This suggests that environmental radiation contributes to the development of defense mechanisms at cellular level. To further understand how environmental radiation affects metabolism of living organisms, we have recently launched the FLYINGLOW program that aims at exploiting *Drosophila melanogaster* as a model for evaluating the effects of low doses/dose rates of radiation at the organismal level. Here, we will present a comparative data set on lifespan, motility and fertility from different Drosophila strains grown in parallel at LNGS and in a reference laboratory at the University of L'Aquila. Our data suggest the reduced radiation environment can influence Drosophila development and, depending on the genetic background, may affect viability for several generations even when flies are moved back to normal background radiation. As flies are considered a valuable model for human biology, our results might shed some light on understanding the effect of low dose radiation also in humans. This article is protected by copyright. All rights reserved

INTRODUCTION

All living organisms have to cope with the natural level of radioactivity on the Earth as well as with cosmic rays. Natural variations of background radiation likely represented a critical role during the evolution and contributed to the development of still poorly characterized defense mechanisms to minimize genotoxic damage. Several evidence indicated that that exposure of cells or organisms to low dose/dose rates of ionizing radiation above background might induce adaptive responses that reduced the deleterious consequences of DNA-damaging events (Yu et al., 2011; for reviews see Mitchel, 2009; Mitchel, 2015). These lines of evidence challenge the linear no-threshold model (LNT) which states that the radiation dose-risk relationship is linear across all doses and which is used for cancer risk extrapolation from high-medium doses to those of interest in radiation protection (below 100 mGy, where epidemiological data are of poor significance). A strictly related question is whether a reduction of natural background radiation could affect the metabolisms of living organisms. This question has been addressed by exploiting deep underground laboratories, which provide experimental science (including biology) with large areas almost free of interference from cosmic rays. Indeed, the permanence in a low-radiation environmental laboratory in the Pyrenees Mountains has been proven to inhibit growth of protozoan and cyanobacterium cells (Planel et al., 1987). In addition, a strong stress response has recently been observed in bacteria and rodent cells kept under reduced radiation environmental conditions at the Waste Isolation Pilot Plant (WIPP), USA (Smith et al., 2011). Certainly, the experiments carried out at the underground Gran Sasso National Laboratory (LNGS) of the Italian Institute of Nuclear Physics (INFN) on cultured mammalian cells of rodent and human origin represent the largest evidence on the effects of reduced environmental radiation on eukaryotic cellular systems (Carbone et al., 2009; Fratini et al., 2015; Satta et al., 2002; Satta et al., 1995). The LNGS laboratory, which has been carved inside the Gran Sasso dolomite mountain, is shielded by approximately 1,400 m of rock overburden, and as a consequence cosmic radiation is almost absent (The MACRO Collaboration 1990). Moreover, given that dolomite rocks are poor in both thorium and uranium content, the neutron flux within the laboratory area is one thousand time reduced with respect to external environment (Belli et al., 1989; Rindi et al., 1988). In addition, the sedimentary origin of the mountain and a low concrete lining of the laboratory walls keep both γ and μ radiation significantly reduced. Finally, efficient ventilation systems guarantee low concentration of radon, which is pumped outside the LNGS. These unique characteristics of LNGS where the presence of radiation is strongly reduced, made this

laboratory the ideal place to host physic experiments and study rare events such as solar neutrino detection and proton decay as well as to explore the effects of low radiation on biological systems. Pioneer observations by Satta and coworkers have indicated that *S. cerevisiae* exhibited reduced repair efficiency when grown at LNGS for 1 week (Satta et al., 1995). Successive experiments indicated that rodent and human cells maintained several months at the LNGS displayed altered activity of several antioxidant enzymes, increased radiation-induced mutation frequency and low efficiency at scavenging reactive oxygen species when compared to parallel cultured cells grown in reference background laboratories (Carbone et al., 2009; Fratini et al., 2015; Satta et al., 2002). Collectively these experiments suggest that environmental radiation contributes to the development of defense mechanisms at cellular level. However, whether this holds true at the organismal level remains still practically unaddressed.

Here we employed *Drosophila melanogaster* as a multicellular model organism to investigate whether the reduced background radiation at the LNGS affects development and growth of a complex multicellular organism. Drosophila is emerging as one of the most effective tools for analyzing the function of human disease genes, including those responsible for developmental and neurological disorders, cancer, cardiovascular disease, metabolic and storage diseases, and genes required for the function of the visual, auditory and immune systems (Bier, 2005; Bilen and Bonini, 2005; Kounatidis and Ligoxygakis, 2012; Miles et al., 2011; Moulton and Letsou, 2016; Ocorr et al., 2007). Flies have several experimental advantages, including their rapid life cycle and the large numbers of individuals that can be generated, which make them ideal for sophisticated genetic screens, and in future should aid the analysis of complex multigenic disorders. Drosophila has been also used as an *in vivo* model to study radiation induced oxidative stress and radioprotective agents. More recently, many of the important neurotoxic side effects resulting from radiation therapy during development in humans have been mimicked in Drosophila suggesting that fruit flies can serve as a useful experimental model for studying the neurotoxic consequence of radiation exposure, and ultimately for identifying specific genes and proteins involved in the molecular mechanism of radiation-induced damage (Nakamura et al., 2013). Although humans and fruit flies may not look very similar, it is widely accepted that most of the fundamental biological mechanisms and pathways that control development and survival are conserved across evolution between these species. In the framework of the FLYINGLOW project, funded by the Museo Storico della Fisica and Centro Studi e Ricerche Enrico Fermi, we compared life span extension and fertility of

wild type flies and flies bearing mutations in selected DNA damage repair genes maintained for different generations at the LNGS underground laboratory (Low Radiation Environment, LRE) with respect to parallel Drosophila cultures grown at the reference Laboratory at University of L'Aquila (Reference Radiation Environment, RRE). Our results indicate that LNGS environment influences Drosophila lifespan and fertility and provide further insights on how low dose radiation can affect growth and development of complex multicellular organisms.

MATERIALS AND METHODS

Drosophila strains and culture

To perform the *in vivo* experiments at LNGS underground laboratory (LRE) a dedicated lab has been designed to host small animals (e.g. insects, worms, fishes). It consists of module room, provided with temperature, humidity and light control systems as well as with an independent ventilation system. The facility is equipped with a dissecting microscope and a CO_2-based anesthesia system (AIR^2 Concert Bullettin, Issue 3, December 2015 http://www.concert-h2020.eu/en/Concert_info/Access_Infrastructures). Flies at LRE and RRE (University of L'Aquila) laboratories were maintained on standard cornmeal medium at 25°C, A standard wild-type line (Oregon-R) was used as a control. The mutant strains *Dp(1;Y)B^S; spn-A/TM3, tefu$^{ZIII\text{-}519}$/TM6B,* and *mei41^{29D}/FM7* were previously described (Laurencon et al., 2003; Morciano et al., 2013; Oikemus et al., 2004; Staeva-Vieira et al., 2003). To estimate the frequency of homozygotes, 10 heterozygous virgin females were crossed to 10 heterozygous males in a vial with standard medium at both LRE (LNGS) and RRE laboratories. After 7 days parental flies were discarded. Up to the 21st day after mating, adults from the resulting progeny were sorted, counted and removed. At least 4 independent crosses for each LRE generation (at least 36 crosses in total) and 23 crosses at RRE were set up and ~1800 adult flies examined and scored. For the transgenerational analysis, crosses at LRE were set up as above from selected generations and then transferred to RRE laboratory where they were maintained for 2 more generations. The frequency of homozygotes was calculated by dividing the total number of homozygotes over the total number of progeny. To generate the *newly balanced* (nb) *tefu$^{ZIII\text{-}519}$/TM3 Sb Ser* line, the original *tefu$^{ZIII\text{-}519}$/TM6b* was crossed to *CyO/Sco; MKRS/TM3 Ser* stock. The resulting *CyO/+; tefu$^{ZIII\text{-}519}$/TM3 Sb Ser* males were then mated to the *+/Sco; tefu$^{ZIII\text{-}519}$/TM3 Sb Ser* sisters to establish the final *CyO/Sco; tefu$^{ZIII\text{-}519}$/TM3 Sb Ser* line in which both original chromosomes 2 and the chromosome 3 balancer have been replaced.

Lifespan analysis

Flies were collected up to a 2-3 days after eclosion and sorted males transferred to fresh vials (10 males per vial) containing standard medium and aged at 25°C in both LRE and RRE laboratories. Flies were transferred to fresh food every 3 days, at which time the

number of surviving males was recorded. Each experiment was carried out on at least 30 flies for generation.

Fertility and female fecundity test.

Single females or males were mated to two control males or females *OR-R*, respectively, in a vial with standard medium in parallel at both LRE and RRE laboratories. Parental files were discarded after seven days and after 21 days all adult progeny was counted. Crosses presenting dead parental flies were not considered for the analysis. To measure the egg-to-adult fecundity, 4 virgin females were crossed to 4 males overnight. The following day, the adults were discarded and the number of eggs counted under the dissecting microscope. After 3 days, the hatched larvae were sorted, counted and transferred to a fresh vial. Adult flies resulting from each vial after 8 days were then scored.

Climbing test

The climbing assay was performed according to Iuso at al. 2014 (Iuso et al., 2014) with some modifications. Briefly, 10 one-day old male flies were placed in an empty plastic vial (95 mm high×24 mm in diameter) sealed at the top with parafilm to prevent fly escape. After a1-min recovery, flies were gently tapped to the bottom, and the number of adults that were able to climb vertically beyond a 7-cm mark within 10 s was recorded. The test was repeated five times for each 10 flies group of flies with 1-min rest periods between each test. To check possible age-related changes, the same assay was performed after aging the same flies up to one month.

Dosimetry

Radiation dosimetry at RRE and at LRE was obtained by measurement and evaluation of γ rays of any origin, cosmic rays (neutrons and directly ionizing particles), α-particles from ^{222}Rn and its daughters as described in Fratini et al. 2015 (Fratini et al., 2015).
Briefly, the neutron dose rate calculated at RRE was 2.5 nGy/h while at LRE, being neutron flux reduced by a factor of 10^3 compared to RRE, it was considered negligible.
The dose rates related to the γ rays component, measured by TLD dosimeters, were 34

nGy/h at RRE and about 1.6 times lower at LRE. Measurements of ^{222}Rn concentration in air were obtained using a Radon meter (alphaGUARD, Genitron Instruments GmbH). In the present experiments for both RRE and LRE, the values of Radon concentration have been higher than those obtained in the past. They varied in the range (10-70) Bq m^{-3} at RRE and (70-100) Bq m^{-3} at LRE. However, we are confident that the biological results described in this paper are not dependent on these differences. The reason is that for all endpoints considered, the results obtained at the RRE laboratory (University of L'Aquila) used here as controls, were almost indistinguishable from those found at Sapienza University of Rome, where the average radon concentration is about 100 Bq m^{-3}, i.e., the same level measured at LRE (data not shown). Lastly, the dose rate due to directly ionizing component of cosmic rays (mainly muons) is negligible for the underground laboratory LRE, and of about 39 nGy/h at RRE.

Statistical Analysis

Data are presented as mean ± standard error (SE), except where indicated, of at least three independent determinations. All statistical analyses were performed with Sigmaplot software version 11.0 (Systat Software, Inc., Chicago, IL, USA). Comparison of more than two group means were carried out by ANOVA test followed by Holm-Sidak test. Statistical comparison of life span using a log-rank test was performed with Kaplan–Meier survival analysis and the Holm-Sidak test was used for multiple comparisons. Level of $p < 0.05$ was regarded as statistically significant.

RESULTS

Reduced background radiation alters life span but not the locomotive behavior of adult flies.

At LRE we set up to maintain wild-type *Oregon/R (OR/R)*, and other Drosophila lines (see below) that were checked for life span analysis, locomotion activity and fertility for up to 9 months. Starting from April 2016, single populations (referred to as A, B, and C) were consecutively raised at LRE with A indicating the population brought to LRE the first month and B the population brought the next month and so on. Each new generation was then transferred to new vials while the parental flies were discarded. For sake of clarity, each generation was indicated as the exponent of each population so that, for instance, B^4 indicates the 4^{th} generation from population B. We had also maintained the same fly lines in the RRE, which is very close to LNGS (5 km). These flies were referred to as R.

Previous works had shown that Drosophila life span is sensitive to modulation of low dose radiation (Zhikrevetskaya et al., 2015). We thus sought to understand whether also the LNGS reduced background radiation would affect life span. To this aim, log-rank testing was used to evaluate differences in life span groups of five males from *OR/R* generation at LRE (populations A^6, A^8 and B^1) and from R population. We found that the median life span in A^6, A^8 and B^1 generations was significantly increased by 20%, 7% and 15%, respectively, relative to that of R wild-type flies indicating that reduction of natural background radiation alters the survival ratio (Figure 1).

Chronic low dose γ-irradiation has been also shown to enhance locomotive behavior in flies (Kim et al., 2015). To assess whether LNGS background radiation influenced locomotion, we examined climbing activity of adults from A^9, A^{12}, B^1 and B^7 and from R flies (see Materials and Methods). Climbing activity tests were then repeated on the same individuals that were aged for additional 30 days. These analyses revealed that the locomotive behavior of LRE flies was not different from that exhibited by R flies even after aging. As locomotion activity during aging is an important quality-aging indicator, these analyses indicate that, unlike chronic low dose γ-irradiation above the background, reduced background radiation does not influence quality of life of adult flies (data not shown).

Reduced background radiation is detrimental for both male and female fertility.

We then asked whether the fertility was affected by low radiation dose. To this purpose, we have isolated 3 day's old males from A^{8}, A^{12}, B^{2} and B^{6} generations as well as 3 day's old virgin females from A^{11}, B^{2}, B^{4}, B^{6} (the choice of these generations was totally random) and from the line R. Single males and females were individually mated to 2 females and 2 males respectively, from the R population and the crosses maintained at LRE (A and B) or at RRE (R). We found that whereas the R females and males produced an average number of offspring of 49.5 and 50.1 respectively after 21 days, the average number of offspring from A and B males and females single crosses was 35.4 and 34.7, respectively suggesting that the LNGS background radiation reduced by ~30% fertility of both male and female adults, respectively (Figure 2). Interestingly, the observation that A^{11} females or A^{12} males exhibited the same reduction in fertility as B^{1} and B^{3} flies indicates that the reduction effect occurs irrespective of the generation time at the LNGS and it is kept constant within a threshold of 30% reduction.

Reduced background radiation favors positive selection of homozygotes for a semi-viable mutation in the Drosophila ATM-encoding gene.

To verify if the reduced background radiation would affect survival of flies with impaired DNA damage response, in addition to wild-type *OR/R* flies, we decided to maintain at LRE different generations of *Dp(1;Y)B^{S}; spn-A/TM3, tefu$^{ZIII\text{-}519}$/TM6B,* and *mei41^{29D}/FM7* viable mutant lines. These mutant flies bear weak recessive mutations in key DNA damage response genes encoding the Drosophila orthologs of human RAD51 *(spn-A/rad51)* and of two essential kinases, Ataxia Telangectasia Mutated (*tefu/atm)* and Ataxia Telangiectasia and Rad3 related (*mei41/atr)* (Laurencon et al., 2003; Morciano et al., 2013; Oikemus et al., 2004; Staeva-Vieira et al., 2003). To verify if the survival of flies homozygous for either *spn-A, tefu* or *mei41* mutation was affected by LNGS environment, each mutant line was monitored for the frequency of homozygotes. This frequency was then compared to that observed in the mutant lines with same genotypes kept in parallel at RRE (*spn-A* (R*), tefu* (R) and *mei41* (R) lines). We found that for *spn-A* A^{10} and *spn-A* B^{2} generations and for *mei41* A^{3} and *mei41* B^{1} generations, the frequency of *spn-A/spn-A* (20% both males and females, N= 266) and *mei41^{29D}/ mei41^{29D}* homozygotes (1.7 %, only females, N= 232) did not significantly differ from the homozygote frequency measured in the corresponding R lines (20% N=175 for *spn-A* and 1,5%, N= 130 for *mei41^{29D}*). Conversely, *tefu$^{ZIII\text{-}519}$/tefu$^{ZIII\text{-}519}$* homozygotes were much more numerous (~20%; N= 150) in the *tefu* A^{3} and *tefu* A^{5}

than in *tefu* R lines (~2%; N= 250) suggesting that the LNGS environment positively favors viability of flies with little ATM (Figure 3). To further investigate this unexpected finding, we measured the number of *tefu* homozygous and heterozygous progeny that resulted from crossing ten 3-day old *tefu$^{ZIII-519}$/TM6b* females to ten 3-day old *tefu$^{ZIII-519}$/TM6B,* both at LRE and at RRE. At LRE, crosses were set up using flies from *tefu* A^{10}, A^{11}, B^{2}, B^{3} and C^{4} generations. By scoring ~1500 individuals at LRE and ~600 at RRE, we found that each cross produced in average the same number of progeny (53 at LRE and 49 at RRE) indicating that, differently from wild-type flies, *tefu* mutants did not exhibit reduction in the fertility. Yet, the frequency of *tefu$^{ZIII-519}$/tefu$^{ZIII-519}$* homozygotes in the *tefu* A^{10}, A^{11}, B^{2}, B^{3} and B^{4} generations ranged from 16 to 20% while at RRE remained as low as 2.1% (Figure 4). This analysis further confirmed that LNGS environment is beneficial to flies with residual ATM activity. To rule out the presence of a second non complementing site with a either dominant or recessive effect in *tefu* flies' genome, *tefu$^{ZIII-519}$/TM6b* females were crossed to *CyO/Sco; MKRS/TM3Ser* males and the resulting *CyO/+; tefu$^{ZIII-519}$/TM3Ser* flies kept at LNGS for 2 generations. Also in this case, we observed that the number of *tefu$^{ZIII-519}$* homozygotes (18.7%; n= 80) in generation 2 (nb *tefu$^{ZIII-519}$* A^{2}) did not differ from that observed for the other *tefu* generations kept at LRE indicating that this effect is strongly associated to the *tefu$^{ZIII-519}$* mutation. To further evaluate whether the increased number of *tefu$^{ZIII-519}$* homozygotes at LRE was due to differences in either fecundity or egg-to-adult viability, we compared the laid-egg/female and the adult/egg ratios of ten *tefu$^{ZIII-519}$* heterozygous females from the A^{10} generation with those from 10 *tefu$^{ZIII-519}$* heterozygous females from the R population. We observed that the adult/egg ratio for the *tefu$^{ZIII-519}$* A^{10} heterozygous females (20.0, N= 174) was similar to that of *tefu$^{ZIII-519}$* R heterozygous females (22.7; N=220). In addition, in both A^{10} and R lines 77% of eggs successfully hatched giving rise to 134 (26 homozygotes and 108 heterozygotes) and 169 (2 homozygotes and 167 heterozygotes) adults, respectively. Thus, giving the fact that *tefu$^{ZIII-519}$ /tefu$^{ZIII-519}$* homozygous females remain totally sterile even at LRE, we can envisage that this significant deviation of the homozygotes /heterozygotes ratio for the *tefu$^{ZIII-519}$ /TM6B* line was mainly due to a greater availability of *tefu$^{ZIII-519}$* haploid oocytes during oogenesis of *tefu$^{ZIII-519}$* heterozygous females. Interestingly, an increased frequency of *tefu$^{ZIII-519}$ /tefu$^{ZIII-519}$* homozygotes was also observed by analyzing offspring from two consecutive progenies resulting from 2 different *tefu$^{ZIII-519}$ /TM6B* generations, previously kept at LRE and then moved to RRE (Figure 4). This indicates that whatever the

mechanism that underlies this positive selection, this selective effect on *tefu*$^{ZIII\text{-}519}$ /*tefu*$^{ZIII\text{-}519}$ is trans generational.

DISCUSSION.

Although the biological effects of both natural and man-made sources of ionizing (including low-dose) radiations have been extensively addressed in different systems, little is known on whether reduced natural background radiation affects life, especially at the organismal level. In this context, the LNGS underground laboratory offers a unique opportunity to tackle this task. At the Europe's largest underground LNGS laboratory where the overall radiation rate is significantly reduced with respect to reference laboratories (i.e. University of L'Aquila), we have recently launched the FLYINGLOW program. The preliminary results from this project revealed that decreasing the rate of environmental radiation might affect the development of *Drosophila melanogaster*. These results, which served as an extension of Pulex/Cosmic Silence experiments on cultured cells (Carbone et al., 2009; Fratini et al., 2015; Satta et al., 2002) represent the first data set ever obtained from a complex model organism in a underground laboratory and therefore can be considered as a foot hold for deep underground biology of complex multicellular systems.

Several papers have reported that a chronic exposure to low radiation dose enhanced locomotive behavior and extended life span in flies suggesting that γ-irradiation at dose 5 and 40cGy can induce a beneficial effect in Drosophila (Kim et al., 2015; Zhikrevetskaya et al., 2015). Surprisingly, we observed that also a sharp reduction of environmental radiation, in particular for the directly ionizing cosmic rays and neutron components, increases life expectancy, but not locomotion, indicating that the influence of the environmental radiation on the biology of living systems is a very complex issue to address. Interestingly, both male and female fertility was also altered by LRE. Curiously, fertility was reduced right after few generation cycles (B^2 and B^4) and kept constantly reduced even after a long number of generation cycles (i.e A^{12} and B^6) indicating that it does not result as consequence of spontaneous LRE-induced harmful mutations, which accumulate with the generation time and gradually affect fertility. Alternatively, we can envisage that a further deviation from this threshold response could increase embryo lethality thus favoring only the survival of individuals with a fertility rate close to the threshold. The reason why LRE affects fertility in flies remains an open question. It has been suggested that environmental radiation contributes to the development and maintenance of DNA repair mechanisms in living organisms (Fratini et al., 2015). This

could be particularly relevant for the completion of specific biological processes such as spermatogenesis and oogenesis of Drosophila, which rely on a very large number of DNA repair factors (Blanton and Sekelsky, 2004; Joyce et al., 2011; Peretz et al., 2009).

Our FLYINGLOW program is also revealing that the LRE might confer positive selection on flies with specific genetic background. The increase of the number of *tefu*$^{ZIII\text{-}5190}$, but not of *me41*^{29D}, homozygotes indicates that reference background radiation exerts a strong selective pressure on flies in which ATM is limiting. Although we don't know the molecular mechanisms underlying this effect, we can certainly state that this positive selection is not related to the ATM kinase activity, which in these hypomorphic mutants is as normal as wild type (Morciano et al., 2013). Moreover, since *tefu*$^{ZIII\text{-}5190}$ homozygous females at LRE remain totally sterile, the increment of homozygote/total progeny ratio does not result from a potential progeny of *tefu*$^{ZIII\text{-}5190}$ homozygous females. This also suggests that LRE has no positive effect on telomere fusions normally found in embryos from *tefu*$^{ZIII\text{-}5190}$ homozygous females and that is responsible of the strong maternal effect associated with the mutation (Morciano et al., 2013). The enhanced survival of *tefu*$^{ZIII\text{-}5190}$ homozygotes that appear in a Mendelian 1:2 homozygous/heterozygous ratio suggests that the LNGS growth conditions suppress the semi lethality phenotype. Switching both chromosomes II and the third chromosome balancer gives rise to the same homozygotes' fraction making the possibility of a second site-dependent effect very unlikely. Interestingly, we found that the increase of homozygous/heterozygous ratio was also observed when flies from A^{12} and B^{4} LRE generations were moved to RRE and cultured for 2 more generations. This trans generational feature of the semi-lethality suppression induced by LNGS conditions is not totally unexpected. Chinese hamster V79 cells cultured at LRE were shown to keep memory of their state when moved to RRE (Fratini et al., 2015). Our data on Drosophila indicate that this maintenance of memory of the positive selection can also apply to complex organisms.

Acknowledgments

We thank M. Balata and L. Ioannucci for assistance and logistic support at LNGS and Prof. Y. Rong for providing the *tefu*$^{ZIII\text{-}519}$*/TM6B* mutant line. F.C is a Postdoctoral Researcher funded by Centro Fermi. This work was supported by grants of Italian Association for Cancer Research (AIRC IG 12479) to G.C and of Museo Storico della Fisica e Centro Studi e Ricerche ''Enrico Fermi'' to M.A.T. and G.C.

Figure Legends

Figure 1. Reduced background radiation extends the lifespan of males flies. Kaplan-Meier survival curves are shown for the line R, and LNGS flies from generation A^8, A^6, and B^1, with *p* values calculated using the log-rank test. Pairwise multiple comparison by the Holm-Sidak method demonstrated a significant extension in median life span in A^8 (median survival = 71 days; log-rank = 6,339; P = 0,0118), A^6 (median survival 63 days; log-rank = 6,590; P = 0,0103), and B^1 (median survival 68 days; log-rank = 6,283; P = 0,0122) flies compared with line R (median survival = 59). Survival was assessed from 30 males flies.

Figure 2. **Flies at LRE exhibit reduced fertility**. Fertility tests of Oregon R females (a) and males (b) that were isolated from two independent lines (A and B) after different generations (exponents) kept at LRE. Columns indicate the fraction of progeny, relative to the progeny observed in the RRE control which has been set a 100%, from 4 independent crosses of either one LRE female or male with 2 RRE males or females, respectively. Note that for all generations tested, the progeny/adult ratio from each fertility is significantly reduced ($p<0.01$) compared to that observed at RRE (R). (*) $p<0.01$ (ANOVA test followed by Holm-Sidak test). Bar indicates SEM.

Figure 3. Effects of reduced background radiations on DNA-repair gene mutants. Columns show the percentage of homozygotes/total progeny from independent *spn-A , tefu* and *mei41* lines maintained either at LRE (A, B) or at RRE (R). Homozygotes were scored at different generation times (exponents). Note that only for *tefu* lines, the number of homozygotes at LRE drastically increased with respect to RRE. (*)$p<0.01$ (ANOVA test followed by Holm-Sidak test). Bar indicates SEM.

Figure 4. LRE is beneficial to *tefu* mutants. Columns indicate the proportion (%) of *tefu* homozygotes obtained from mating ten 3-day old *tefu* heterozygous females to ten 3-day old *tefu* heterozygous males. Each column represents results from at least 4 independent crosses. Capital letters refer to *tefu* mutant lines brought to LRE at different times, while exponents indicate the number of generations from each progenitor line. *nb* (new

balanced) A^2 refers to a *tefu* mutant line in which the original chromosomes and balancers have been replaced by other multi inverted chromosomes (see Material and Methods), while *t* (transgenerational) A^{12} and $-B^4$ columns refer to the A^{12} and B^4 LRE lines moved to RRE and maintained for 2 more generations. See text for further details. R= RRE lines. Bar indicates SEM.

References

Belli P, Bernabei R, Dangelo S, Depascale MP, Paoluzi L, Santonico R, Taborgna N, Iucci N, Villoresi G. 1989. Deep Underground Neutron-Flux Measurement with Large Bf3 Counters. Nuovo Cimento A 101(6):959-966.

Bier E. 2005. Drosophila, the golden bug, emerges as a tool for human genetics. Nat Rev Genet 6(1):9-23.

Bilen J, Bonini NM. 2005. Drosophila as a model for human neurodegenerative disease. Annu Rev Genet 39:153-171.

Blanton H, Sekelsky J. 2004. Unique invasions and resolutions: DNA repair proteins in meiotic recombination in Drosophila melanogaster. Cytogenet Genome Res 107(3-4):172-179.

Carbone MC, Pinto M, Antonelli F, Amicarelli F, Balata M, Belli M, Conti Devirgiliis L, Ioannucci L, Nisi S, Sapora O, Satta L, Simone G, Sorrentino E, Tabocchini MA. 2009. The Cosmic Silence experiment: on the putative adaptive role of environmental ionizing radiation. Radiat Environ Biophys 48(2):189-196.

Fratini E, Carbone C, Capece D, Esposito G, Simone G, Tabocchini MA, Tomasi M, Belli M, Satta L. 2015. Low-radiation environment affects the development of protection mechanisms in V79 cells. Radiat Environ Biophys 54(2):183-194.

Iuso A, Sibon OC, Gorza M, Heim K, Organisti C, Meitinger T, Prokisch H. 2014. Impairment of Drosophila orthologs of the human orphan protein C19orf12 induces bang sensitivity and neurodegeneration. PLoS One 9(2):e89439.

Joyce EF, Pedersen M, Tiong S, White-Brown SK, Paul A, Campbell SD, McKim KS. 2011. Drosophila ATM and ATR have distinct activities in the regulation of meiotic DNA damage and repair. J Cell Biol 195(3):359-367.

Kim CS, Seong KM, Lee BS, Lee IK, Yang KH, Kim JY, Nam SY. 2015. Chronic low-dose gamma-irradiation of Drosophila melanogaster larvae induces gene expression changes and enhances locomotive behavior. J Radiat Res 56(3):475-484.

Kounatidis I, Ligoxygakis P. 2012. Drosophila as a model system to unravel the layers of innate immunity to infection. Open Biol 2(5):120075.

Laurencon A, Purdy A, Sekelsky J, Hawley RS, Su TT. 2003. Phenotypic analysis of separation-of-function alleles of MEI-41, Drosophila ATM/ATR. Genetics 164(2):589-601.

Miles WO, Dyson NJ, Walker JA. 2011. Modeling tumor invasion and metastasis in Drosophila. Dis Model Mech 4(6):753-761.

Mitchel RE. 2009. The dose window for radiation-induced protective adaptive responses. Dose Response 8(2):192-208.

Mitchel RE. 2015. Adaption By Low Dose Radiation Exposure: A Look at Scope and Limitations for Radioprotection. Dose Response 13(1).

Morciano P, Zhang Y, Cenci G, Rong YS. 2013. A hypomorphic mutation reveals a stringent requirement for the ATM checkpoint protein in telomere protection during early cell division in Drosophila. G3 (Bethesda) 3(6):1043-1048.

Moulton MJ, Letsou A. 2016. Modeling congenital disease and inborn errors of development in Drosophila melanogaster. Dis Model Mech 9(3):253-269.

Nakamura N, Suyama A, Noda A, Kodama Y. 2013. Radiation effects on human heredity. Annu Rev Genet 47:33-50.

Ocorr K, Akasaka T, Bodmer R. 2007. Age-related cardiac disease model of Drosophila. Mech Ageing Dev 128(1):112-116.

Oikemus SR, McGinnis N, Queiroz-Machado J, Tukachinsky H, Takada S, Sunkel CE, Brodsky MH. 2004. Drosophila atm/telomere fusion is required for telomeric localization of HP1 and telomere position effect. Genes Dev 18(15):1850-1861.

Peretz G, Arie LG, Bakhrat A, Abdu U. 2009. The Drosophila hus1 gene is required for homologous recombination repair during meiosis. Mech Dev 126(8-9):677-686.
Planel H, Soleilhavoup JP, Tixador R, Richoilley G, Conter A, Croute F, Caratero C, Gaubin Y. 1987. Influence on cell proliferation of background radiation or exposure to very low, chronic gamma radiation. Health Phys 52(5):571-578.
Rindi A, Celani F, Lindozzi M, Miozzi S. 1988. Underground Neutron-Flux Measurement. Nucl Instrum Meth A 272(3):871-874.
Satta L, Antonelli F, Belli M, Sapora O, Simone G, Sorrentino E, Tabocchini MA, Amicarelli F, Ara C, Ceru MP, Colafarina S, Conti Devirgiliis L, De Marco A, Balata M, Falgiani A, Nisi S. 2002. Influence of a low background radiation environment on biochemical and biological responses in V79 cells. Radiat Environ Biophys 41(3):217-224.
Satta L, Augustitocco G, Ceccarelli R, Esposito A, Fiore M, Paggi P, Poggesi I, Ricordy R, Scarsella G, Cundari E. 1995. Low Environmental Radiation Background Impairs Biological Defense of the Yeast Saccharomyces-Cerevisiae to Chemical Radiomimetic Agents. Mutat Res Lett 347(3-4):129-133.
Smith GB, Grof Y, Navarrette A, Guilmette RA. 2011. Exploring biological effects of low level radiation from the other side of background. Health Phys 100(3):263-265.
Staeva-Vieira E, Yoo S, Lehmann R. 2003. An essential role of DmRad51/SpnA in DNA repair and meiotic checkpoint control. EMBO J 22(21):5863-5874.
Yu X, Wang H, Wang P, Chen BP, Wang Y. 2011. The Ku-dependent non-homologous end-joining pathway contributes to low-dose radiation-stimulated cell survival. J Cell Physiol 226(2):369-374.
Zhikrevetskaya S, Peregudova D, Danilov A, Plyusnina E, Krasnov G, Dmitriev A, Kudryavtseva A, Shaposhnikov M, Moskalev A. 2015. Effect of Low Doses (5-40 cGy) of Gamma-irradiation on Lifespan and Stress-related Genes Expression Profile in Drosophila melanogaster. PLoS One 10(8):e0133840.

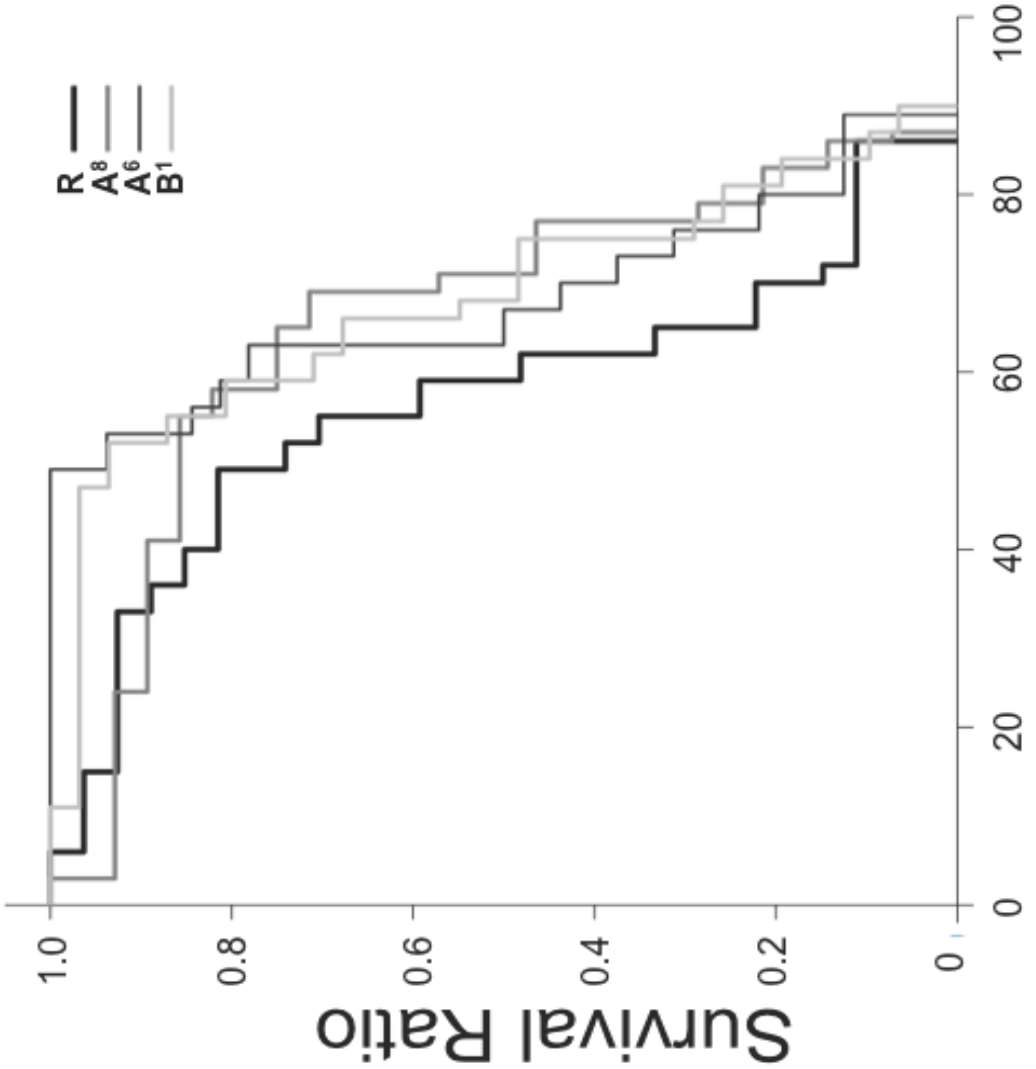

Figure 1

Figure 1

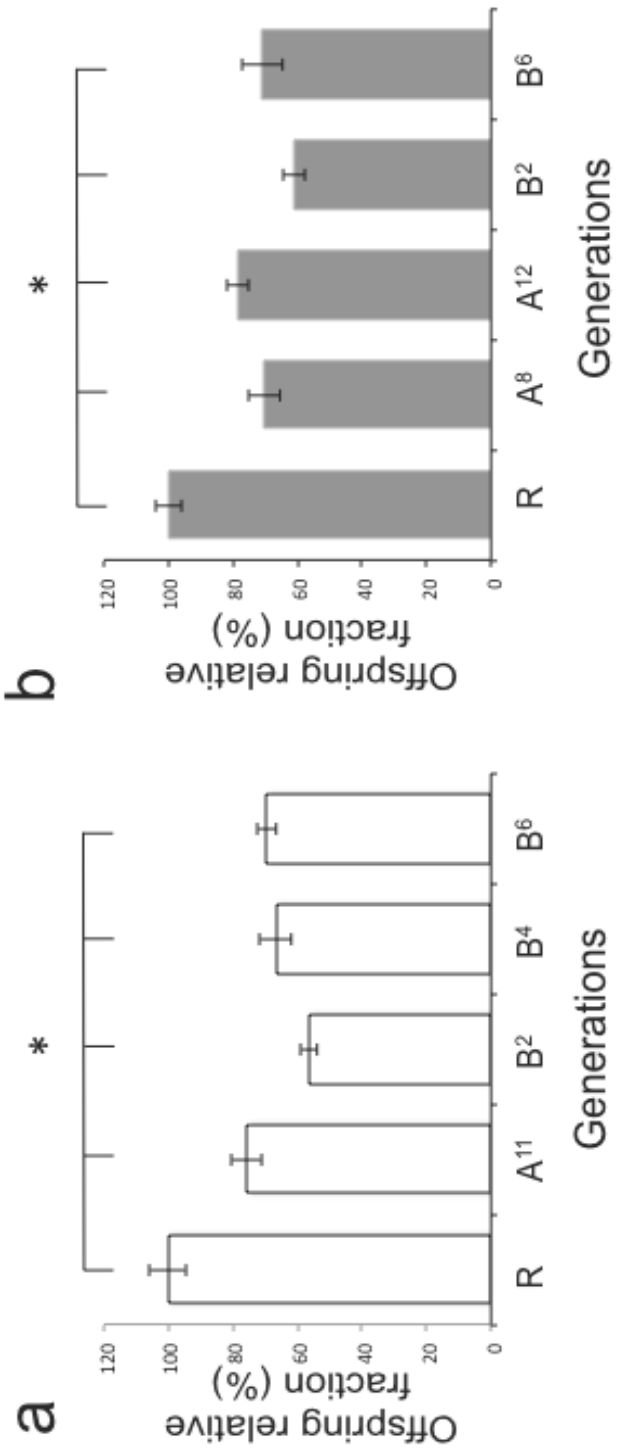

Figure 2

Figure 2

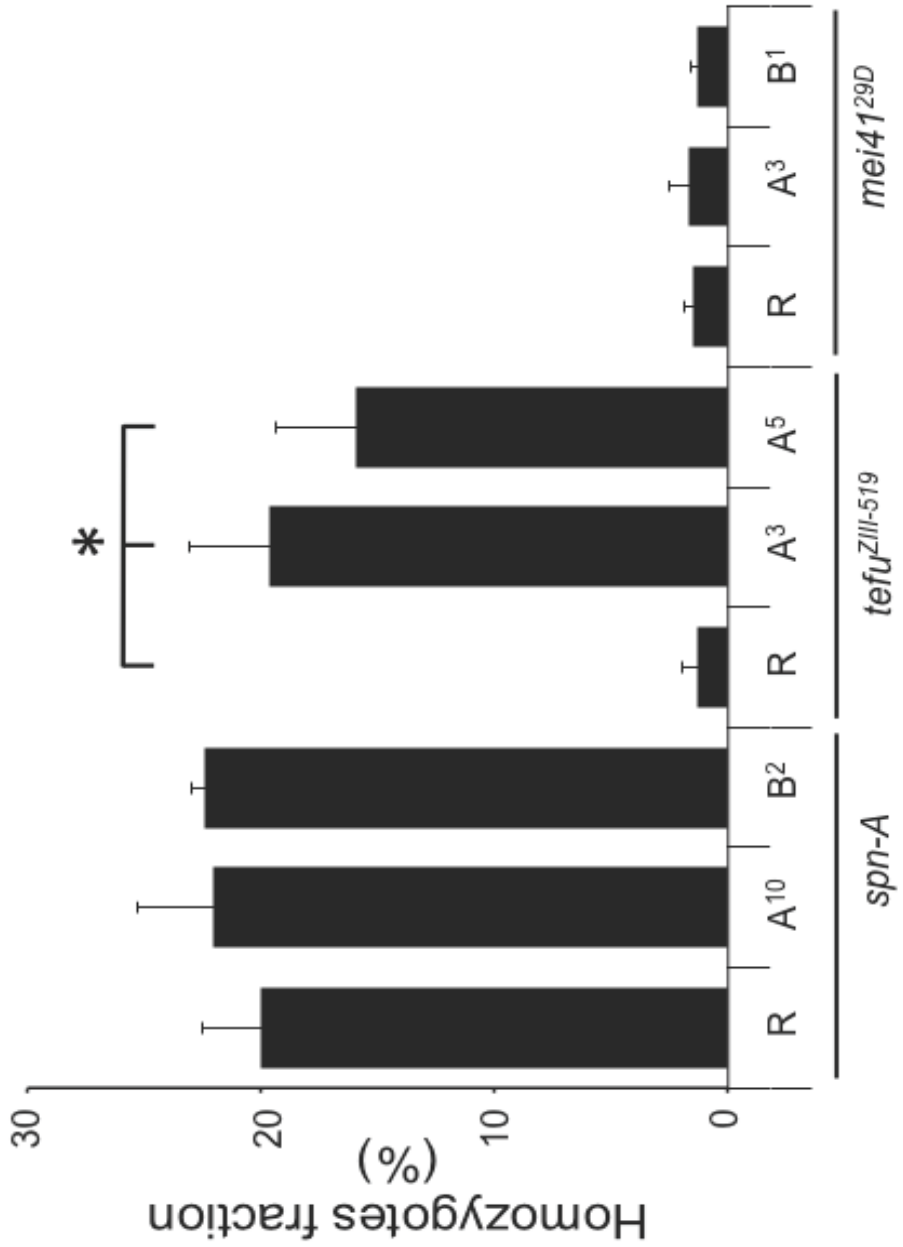

Figure 3

Figure 3

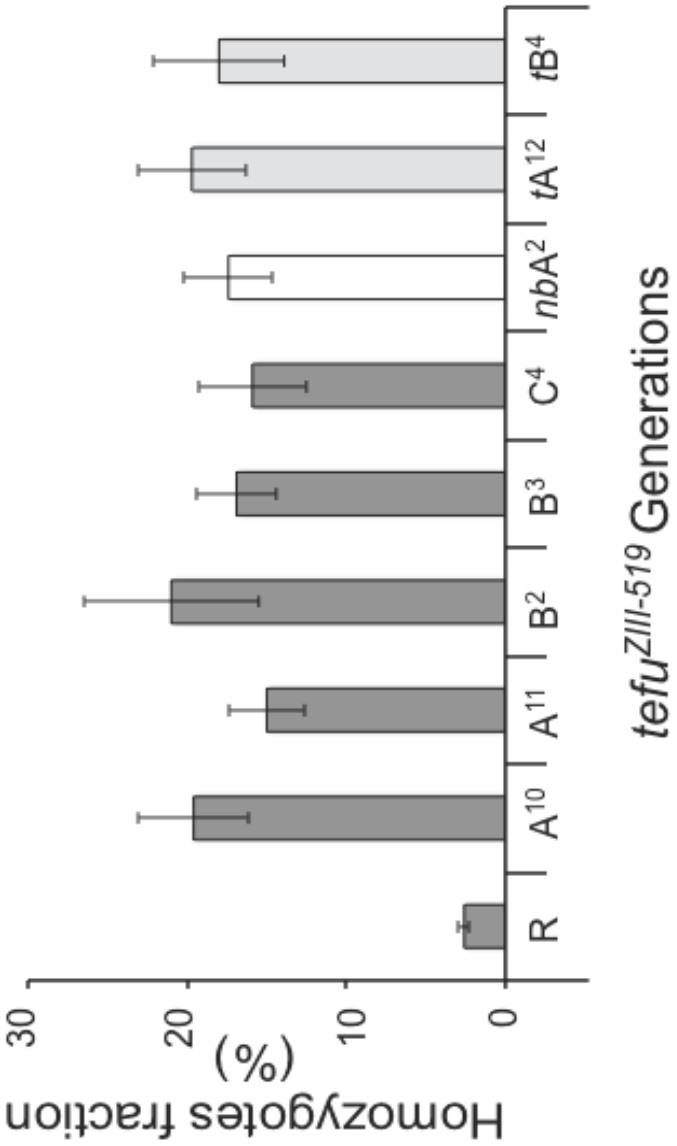

Figure 4

Figure 4