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Genotoxicity testing for radon exposure: *Dolichopoda* (Orthoptera, Rhaphidophoridae) as potential bio-indicator of confined environments

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Abstract Radon represents the major source of natural radioactivity in confined environments. Despite the clear evidence of a direct association between residential exposure and human lung cancer provided by case-control studies, results relating indoor exposure and genotoxic/mutagenic effect induction are still contradictory. The present study attempts to estimate the genotoxic effects induced by exposure to radioactive radon in wild cricket populations sampled from caves where varying concentrations of radon are present. Cave crickets are also tested as possible bio-indicator organisms of genotoxic potential of contaminated residential and confined environments. Six caves in Central Italy are considered covering a broad spectrum of radon radioactivity concentration (221–26,000 Bq/m^3). *Dolichopoda* specimens were sampled from each cave; both haemocytes and brain cells taken from individuals were tested for responsiveness to DNA damage induced by radon through the Comet assay. Specimens from the least radioactive cave, housed in controlled conditions for 60 days before analysis, were used as control group. Statistically significant increase of DNA damage was found in all groups of individuals from each cave, for both cell types. Very low values of all Comet parameters were found in control group individuals, which gave indications of a good responsiveness of the organism to the variable environmental levels of radioactive contamination. Results indicate that cave crickets represent a reliable tool for the detection of genotoxic potential induced by radioactive contamination of confined environments and can be proposed as a possible bio-indicator system for air (-radioactive) pollution related to indoor exposure [*Current Zoology* 60 (2): 299–307, 2014].

Keywords High-LET natural-radioactivity, Indoor exposure, Cave crickets, DNA primary damage, Comet assay

Radon emanates naturally from the ground and appears principally with the decay chain of the radium and uranium series; it is often the single largest contributor to an individual's background radiation dose, being also the most variable one from location to location (Al-Zoughool and Krewski, 2009). For example natural caves are characterized by very low concentrations of ^{238}Uranium and therefore by very low radon emission, while caves of volcanic origin may show extremely high levels of radon (Baubron et al., 1991; Aytekin et al., 2006), as a consequence of the decay chain products of uranium series. Besides these, artificial sites such as vaults and archaeological sites are often characterized by the presence of tuff, a lithic substrate of volcanic origin characterized by a high uranium content. In such environments radioactive radon concentrations may reach very high levels (Bigu et al., 2000; Tuccimei et al., 2006; Iwaoka et al., 2013). Radon gas from natural sources can accumulate in buildings, especially in confined areas such as attics and basements; it can also be found in some spring waters. In the open air its concentration ranges from 1 to 100 Bq/m^3 and even less (0.1 Bq/m^3) above the ocean; in caves or aerated mines, or ill-aerated houses, it may rise up to 20–2,000 Bq/m^3, reaching much higher values in mining contexts. Typical domestic exposures are about 100 Bq/m^3 indoors, and 10-20 Bq/m^3 outdoors (Sperrin et al., 2001). Guideline protection strategies have been established which define concentration limits of radon for domestic areas, above which actions have to be taken: such limits vary depending on the organization, the European Union recommending actions at 400 Bq/m^3 (11 pCi/L) for old houses and 200 Bq/m^3 (5 pCi/L) for new ones, while the US-EPA (2007) recommends actions at concentrations as low as 74 Bq/m^3(2 pCi/L).

Although radon is chemically inert and electrically uncharged, its radioactive decay generates electrically charged atoms, also called radon progeny. Once genera-

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ted, radon progeny can attach themselves to any surface as well as to tiny dust particles in indoor air. These particles can easily be inhaled and adhere to the lining of the lung; the deposited atoms decay by emitting alpha radiation, which interact with bronchial epithelial cells and can damage their DNA (Schmid et al., 2010).

A clear link between breathing high concentrations of this radioisotope and incidence of lung cancer is ascertained by a great number of epidemiological evidences (Al-Zoughool and Krewski, 2009). Human exposure to radon represents the second most frequent cause of lung cancer after cigarette smoking in the United States (Turner et al., 2011). Due to its alpha particle emission of radioactive radon progeny, a radon-contaminated environment is therefore expected to exert a highly mutagenic potential. In this context, in spite of the large body of evidence about the causative effect of radon exposure on cancer induction, results relating radon exposure to the induction of genotoxic effects in humans are still contradictory, especially if indoor exposure is considered (Al-Zoughool and Krewski, 2009). Therefore the possibility of a direct estimation of the actual genotoxic effect potentially induced by environmental exposure to radon is still of great concern for human health risk estimation and decision making against related hazards. In this view, the present investigation is aimed to the evaluation of genotoxic potential of environments with different concentrations of radon in wild populations of cave crickets (genus *Dolichopoda*). As a consequence, these organisms are proposed as possible bio-indicators for genotoxicity in radon-polluted environments.

In recent years, increasing attention is being paid to insects as potential bio-indicators for environmental contamination (Beasley et al., 2013), and a few studies exist focused on genotoxic effects of contaminants such as pesticides (Mukhopadhyay et al., 2004; Augustyniak et al., 2006) and heavy metals in industrial wastes (Siddique et al., 2005a) revealed by Comet assay, which is largely employed for the evaluation of the induced primary DNA damage. Validation studies for mutagenesis testing have also been carried out both in *Drosophila* (Bilbao et al., 2002; Siddique et al., 2005b; Carmona et al., 2011) and in the weevil *Curculio sikkimensis* (Todoriki et al., 2006; Hasan et al., 2008).

Among animal species that colonize and stably live in subterranean environments, crickets belonging to the genus *Dolichopoda* (Orthoptera, Rhaphidophoridae) are very typical and common in Central Italy. In the present study we considered two species of *Dolichopoda*, *D. laetitiae* and *D. geniculata*, coming from seven different caves in Central Italy that were selected on the basis of information concerning their radon concentration. Populations of these two species are strictly allopatric and, as shown by previous studies (Sbordoni et al., 1985; Allegrucci et al., 2005, 2009, 2011), are genetically very similar, especially in the inland core of peninsular Italy from Tuscany to Basilicata, where they form a homogeneous genetic group (Sbordoni et al., 1985).

The main objective of our work consisted in assessing the induction of pre-mutagenic DNA-damage through the Comet assay in cells from individuals of *Dolichopoda* environmentally exposed to radioactive radon. Since the absence of any radioactive contamination and/or that of other possible genotoxicant in natural caves could not be ensured, a truly unexposed group of crickets to be used as reference control sample was not achievable. For this reason an adequate negative control was set up using individuals captured in one of the caves (MTR2) and reared for a recovery time, following an accurate protocol. In order to verify the existence of a relationship between the yield of induced DNA damage and radioactivity levels in the corresponding caves, ^{222}Rn concentrations inside the cavities under study were measured. Finally, possible differences in sensitivity to the genotoxic effects of Radon exposure of cells taken from different body areas were also investigated in order to determine the most suitable tissue for the application of the Comet assay in *Dolichopoda* crickets. Therefore two different cell types, haemocytes and brain cells, were selected on the basis of 1) the close contact of haemocytes with the radioactive gas introduced in the body trough passive diffusion, also taking into account that these cells have been used in a previous study on larval specimens of *Drosophila* (Carmona et al., 2011); 2) the documented sensitivity of brain cells to genotoxicant exposure and their large use in environmental mutagenesis investigations carried out in other insects (Augustyniak et al., 2006; Bilbao et al., 2002).

1 Materials and Methods

1.1 Sampling sites and measurements of natural radioactivity

A minimum number of 7 individuals per cave were collected by view hunting. Overall, the study material consisted of five *Dolichopoda geniculata* and two *Dolichopoda laetitiae* populations. The range in sample sizes in all populations examined is indicated in Table II. As stated in the introduction, these two species are ge-

netically very similar and we can reasonably assume that their response to ionizing radiation is the same. In particular, *D. geniculata* samples were collected from four caves in Central Italy, namely Fiume Coperto (FCP), Pastena (two rooms: PAS-1 and PAS-2), Collepardo (CLP) and Tuscolo (TUS), while *D. laetitiae* samples were collected from Monterano (two rooms: MTR-1 and MTR-2), as shown in Fig. 1. Immediately after sampling the specimens were temporarily collected in polythene bags and transferred to special cages for transport, keeping them in the dark and under conditions of constant humidity. In total, 50 individuals were analyzed. Measurements of radioactive-radon concentrations were carried out through a passive Track-Film detector (Alpha track detector LR115) over a period of at least 30 days (GEOEX s.a.s., Rome, Italy); the working values were referred to the average houry concentrations/m^3 in air (certificate numbers from 13,843 to 13,849, in accordance with U.S. EPA National Radon Proficiency Program EPA - CFA Recommended Test Report Format). Radioactivity measurements and animal samplings were carried out between late winter and spring. Table I shows the distribution of radioactivity levels, in which a central range of radiation doses can be identified, namely from 1,305 and 2,677 Bq/m^3 (ACP and MTR-2 caves, respectively) where the highest value doubles the lowest one. Two extreme values are also present, which are one order of magnitude below (CLP = 221 Bq/m^3) and one order of magnitude above (MTR-1 = 25,997 Bq/m^3) the central range, respectively, expanding the scale of radioactivity over two orders of magnitude between its minimum and maximum values.

1.2 Control sample: housing and rearing conditions

Four specimens taken from MTR-2 were used for the negative control group. These crickets were maintained for 60 days in cages at a temperature ranging between 15°C and 18°C inside a chamber under constant darkness and humidity, mimicking the average conditions of cave environments. During this period they were fed with Tetramin (Spectrum Brands, USA) and watered with well water. The other 6 specimens were processed immediately.

1.3 Tissue collection and cell preparation

For the Comet assay cell samples were taken from two separate areas of the body, namely legs at the metathorax region, between coxa and trochanter (haemolymph cells) and behind the neck, at a membranous cylinder between skull and chest (brain cells) (Fig. 2).

Fig. 1 Map of Lazio (central Italy) showing the localization of the five sites under study (symbols)
◘ = Monterano (rooms1 and 2); ◊ =Tuscolo; ★ = Fiume Coperto; ◇ = Collepardo; △ = Pastena (rooms 1 and 2).

Table I Natural Radon radioactivity values detected in each cave expressed in Becquerel (Bq/m^3)

Cave (code)	Radioactive exposure kBq/m^3	Average hourly concentration Bq/m^3 ± *SE*	Δt (days)
Collepardo (CLP)	170	221±35	32
Fiume coperto (ACP)	1002	1305±91	32
Tuscolo (TUS)	2745	1906±76	60
Pastena-2 (PAS-2)	1785	2324±116	32
Pastena-1 (PAS-1)	1832	2385± 119	32
Monterano-2 (MTR-2)	2120	2677±134	33
Monterano-1 (MTR1-1)	20590	25997±520	33

Total radioactive concentration and average hourly values ± standard error (*SE*) are shown, registered over a period (Δt) of 32 and 60 days (minimum and maximum, respectively).

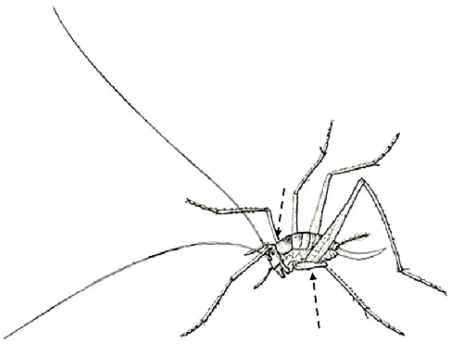

Fig. 2 Localization (dashed arrows) of points for cell extraction in *Dolichopoda*

The haemolymph was collected by exploitation of a common feature in *Orthoptera*, known as auto-haemorrea: after anesthesia with chloroform, the individual was punctured with an insulin syringe through the membrane which allowed leakage of haemolymph in an unpredictably variable quantity, between 0 and 20 μl. Immediately after, a new syringe was used to pierce the neck membrane, sliding the needle upwards towards the cephalic zone, gently aspiring the tissue. The sampled tissues/fluids (average volumes between 15–20 μl) were suspended in 20 μl of 10X phosphate buffered saline (PBS) in separate tubes and kept on ice until completion of samplings (i.e., less than ten minutes), then centrifuged for ten seconds to allow cells to settle to the bottom, facilitating optimal density of cell suspensions before slide preparation.

For Comet analysis it was not always possible to get a sufficient amount of both tissues from the same individual; therefore, in a few cases there is no correspondence between the number of individuals and that of sampled tissues.

1.4 SCGE analysis (Comet assay)

The standard alkaline (pH>13) Single Cell Gel Electrophoresis (SCGE), or Comet assay, was performed as described earlier (Tice et al., 2000). Cells extracted from *Dolichopoda* tissues were suspended in 5μl of 1X phosphate buffered saline (PBS) and kept on ice during subsequent samplings. Two slides for each tissue were prepared. After cell lysis, electrophoresis was conducted at 25 V and 300 mA for 20 min at 4°C. Nucleoids were stained with ethidium bromide (20 μg/ml) (Sigma, Italy) and examined at 400x magnification with an automatic image analyser (Comet Assay III, Perceptive Instruments, UK) connected to a fluorescence microscope (Axioskop 2, Zeiss, Germany). To evaluate the yield of DNA damage, computer generated tail moment (TM) values and percentages of DNA damage, such as tail length (TL) and tail intensity (TI), were used. For each experimental point one hundred cells/specimen/tissue were scored from two slides (fifty each).

1.5 Statistical analysis

Comparison of DNA damage in wild vs. control populations

The working hypothesis was that, transferring the environmentally exposed *Dolichopoda* crickets in a low- radioactive environment for a sufficient time for DNA repair mechanisms to occur would significantly reduce the amount of DNA damage, so as to reach a control level.

The effect of removal of crickets from their radioactive environment in reducing the DNA damage was tested by separate non-parametric Wilcoxon Rank Sum tests (also known as Mann-Whitney U test) (one-tailed). The analysis was performed for each tissue from wild crickets just caught from the MTR2 cave and those captured from the same cave and maintained in the laboratory under controlled conditions before analysis. The mean value of the natural logarithm (*ln*) of the tail length (TL) values of the Comet assay, for each tissue and each individual was taken as a measure of DNA damage.

Environmental radioactivity effect on DNA damage induction

The effect of environmental radioactivity on the

induction of DNA damage in exposed wild crickets was tested by fitting two separate Linear Mixed Models (LMMs) to data from the two tissues, with the mean value of the natural logarithm (*ln*) of the TL, calculated for each tissue and each individual, as the response variable. The *ln* of radioactivity measures (in Bq/m^3) was the only fixed effect. In order to account for non independency of data from the same cave, the identity of each cave (population) as a random effect was included. The significance of the fixed effect was tested by a likelihood ratio test (LRT) between the full model and a null model comprising only the intercept and the random effects. Visual inspection and Shapiro-Wilk tests revealed no significant deviation of the model residuals from a normal distribution and no apparent heteroskedasticity.

Implementation

All statistical analyses were performed in *R* version 3.0.1 (R Core Team, 2013**).** The function wilcox.test (package 'stats', R Core Team, 2013) was used to perform Wilcoxon Rank Sum tests. LMMs were fitted using the *lmer* function from (package 'lme4' version 1.0-5, Bates et al., 2013). LRTs were computed by the *anova* function and Shapiro-Wilk by the *shapiro.test* function (both in package 'stats', R Core Team, 2013).

2 Results

Results of Comet parameters in terms of Tail Length (TL), Tail Intensity (TI) and Tail Moment (TM) are summarized in Table II. Mean values of the total number of cells analysed in all individuals per tissue are shown for each cave for both cell types; besides, mean radioactivity concentrations of the corresponding caves are also indicated.

Very low values of Comet TM, TL and TI are found in control cricket population, while a general increase in the genotoxic damage is detectable in each exposed cave-cricket population in both haemocytes and brain cells, with respect to the control one.

Table II Mean of average values (±*SD*) on 100 cells/specimen/tissue of the three Comet parameters: Tail Length, in µm (TL), % Tail Intensity (TI) and Tail Moment (TM) in haemocytes (HC) and brain cells (BC) of *Dolichopoda* cave crickets from control and exposed groups. Number of specimens analysed per cave and tissue is also shown.

^{222}Radon AHC [a] (Bq/m^3) --- Site	N. of individuals analysed/cell type (HC; BC)	HAEMOCYTES (HC)			BRAIN CELLS (BC)		
		TL (µm)	TI (%)	TM[b]	TL (µm)	TI (%)	TM[b]
0 --- Control	4; 4	17.96 (3.03)	4.63 (1.08)	0.66 (0.18)	22.03 (2.32)	4.87 (1.78)	0.86 (0.33)
221 --- CLP	4; 4	79.60 (19.51)	36.61(12.89)	17.92 (13.02)	59.86(10.33)	32.32 (6.05)	10.50 (3.54)
1.305 --- ACP	3; 5	41.39(9.76)	7.54 (6.97)	2.16 (2.22)	57.20(14.66)	16.65(8.10)	6.45(4.62)
1.906 --- TUS	4; 4	47.50 (11.48)	14.71 (2.45)	3.73(1.36)	69.13(22.98)	31.47(16.09)	14.14(12.54)
2.324 --- PAS-2	3; 4	58.51 (19.94)	26.44 (8.10)	7.59 (3.05)	62.22 (13.96)	27.59 (5.25)	9.27 (3.34)
2.385 --- PAS-1	4; 4	46.43 (10.76)	19.78 (8.21)	5.40 (3.15)	56.34 (17.53)	28.36 (13.21)	8.19 (5.42)
2.677 --- MTR-2	6; 6	66.73 (30.72)	22.61 (20.19)	12.58(13.79)	64.73 (21.20)	19.88 (15.77)	8.40 (8.41)
25.997 --- MTR-1	6; 7	88.13 (17.89)	24.58 (14.83)	11.84 (11.41)	82.86 (26.35)	22.45 (14.25)	11.27 (13.29)

CLP=Collepardo; ACP=Fime Coperto; TUS=Tuscolo; PAS-1=Pastena-1; PAS-2=Pastena-2; MTR-1=Monterano-1; MTR-2=Monterano-2.

[a] = Average Hourly Concentration (AHC) values of radon radioactivity, expressed as Bq/m^3; [b] = TM: arbitrary value.

Fig. 3 shows results from statistical analyses of DNA damage data in terms of TL values, obtained from individuals of the different cricket populations. In particular, in Fig. 3A, the comparison of results from wild *vs*. control cricket populations is shown. Statistical analysis indicates that the amount of DNA damage was significantly lower in crickets maintained in the laboratory under controlled conditions than that detected in wild individuals caught from the same cave, both for brain cells (Wilcoxon Rank Sum test, $N_{wild} = 6$, $N_{control} = 4$, $W = 0$, $P = 0.005$) and for haemocytes ($N_{wild} = 6$, $N_{control} = 4$, $W = 0$, $P = 0.005$). Fig.3B shows a scatter-plot representation of data from exposed cricket populations. Results obtained by LMM analysis shows a significant regression line ($P = 0.022$, Table III) for brain cells of wild cricket populations, indicating a dose-effect increase of DNA damage related to the radioactivity levels measured in the caves. The same significant effect is not found for haemocytes ($P = 0.521$, Table III).

3 Discussion

Aims of the present study were the assessment of DNA damage induced by radon radioactivity in cells from environmentally exposed *Dolichopoda* cave crickets as well as the ascertainment of a possible dose-effect relationship between the yield of the induced

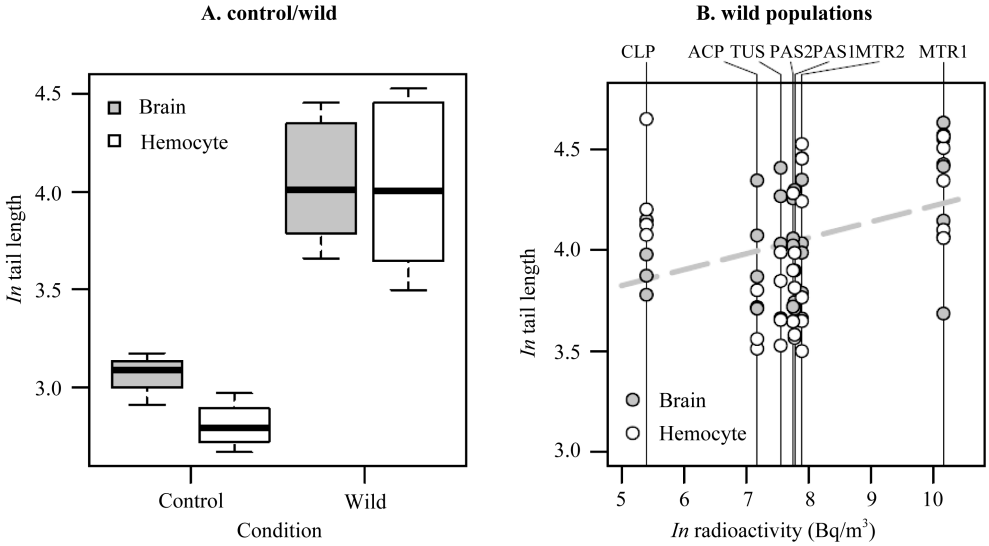

Fig. 3 Boxplots (A) and scatterplots (B) of mean *ln* of tail length values of Comet assay (in ordinate) obtained from the Comet assay performed on both haemocytes (white areas) and brain cells (gray areas) of *Dolichopoda* crickets sampled from the caves, with the different levels of radioactive Radon exposure (in abscissa)

In **A)** Boxplots of mean *ln* tail length obtained for each tissue and each individual captured from MTR-2 cave and divided in two populations made of specimens maintained in laboratory under controlled conditions (boxes on the left, 'control') and of wild specimens (boxes on the right, 'wild'). In **B)** Scatterplots of mean *ln* tail length obtained for each tissue and each individual of wild populations from the 7 caves as a function of *ln* radioactivity (Bq/m^3). Individual values from the same cave population are connected by thin vertical lines and the code of each cave is indicated above the graph.The gray dashed line indicates the significant regression line estimated from Linear Mixed Model (LMM) on brain cell data. Regression for haemocytes (not significant) is not shown.

Table III Estimated values, standard errors (*SE*), *t*-values and significance (*P*-values) obtained by Likelihood Ratio Test (LRT) of full vs. reduced models, for the coefficients of fixed effects in Linear Mixed Models (LMMs) for brain cells and haemocytes in wild populations (see text for details)

Brain cells.						
Response variable: *ln* mean tail length (per individual).	Observations of 34 individuals in 7 populations					
Fixed effect	Estimate	*SE*	*t*-value	LRT *df*	LRT χ^2	*P*-value
Intercept	4.053	0.046	87.320			
ln radioactivity[1]	0.110	0.047	2.340	1	5.261	0.022
Hemocytes.						
Response variable: *ln* mean tail length (per individual).	Observations of 30 individuals in 7 populations					
Fixed effect	Estimate	*SE*	*t*-value	LRT *df*	LRT χ^2	*P*-value
Intercept	3.973	0.113	35.230			
ln radioactivity[1]	0.063	0.120	0.530	1	0.412	0.521

[1] centered and scaled (z-transformed).

damage and the different radioactivity concentrations. In this context, the priority need was both to construct an appropriate control sample and to identify a suitable cell system in cricket populations that could provide a good sensitivity to the genotoxic effects of Radon exposure. The present results show that both haemocytes and brain cells of *Dolichopoda* cave crickets responded well to DNA damaging effects of radioactive Radon exposure. This is indicated by the statistically significant difference, for both cell types, of TL values detected in control vs. wild cricket groups of individuals captured in MTR2 (Fig. 3A). In particular, the result obtained in brain cells, that indicate their responsiveness to the radon induced genotoxic insult, is in agreement with Augustyniak and coworkers (2006) who applied the Comet assay in larval neuroblasts of grasshoppers exposed to heavy metals. In addition, a good responsiveness to radon effects is detected also in haemocytes (Fig.3A) representing a new achievement, since these cells have been tested only once before, in larval stage of *Drosophila* (Carmona et al., 2011). These findings offer the possibility to use any of the two cell systems for environmental monitoring in wild crickets.

Comet results showed an increase of TL, TI and TM in both tissues of exposed crickets with respect to control ones (Table II) and variation of the three parameters are consistent with a general trend. In detail, TL shows a clear increase with the increasing doses, while TI and TM values do not follow the same trend (TM being an arbitrary parameter that includes both TL and TI). Consequently, it can be argued which one among the three Comet parameters may better represent the actual extent of the induced DNA damage in the present experimental approach. This question is also present in the literature about the best descriptor for the induced DNA damage (Collins et al., 2008), to which a 'conventional' agreement often falls on the Tail Moment adoption, as a unifying parameter such as in human lymphoblastoid cell studies (Berni et al., 2008). Indeed, TM can acceptably represent a compromise between TL and TI, yet actual dimensions of damaged DNA (Collins et al., 2008), while in recent environmental studies the increase in DNA damage given as % Tail DNA is largely preferred (Lewis and Galloway, 2008; Lacaze et al., 2010; Carmona et al., 2011). A possible interpretation of the apparent discrepancy between TL and TI of the present results stands on the induction of clustered DNA damage generated by high-LET (Linear Energy Transfer) radiations such as those of radioactive radon, as formerly proposed by Hada and Georgakilas (2008). The authors argue about the induction of oxidative clustered DNA lesions (OCDL) by high LET radiations in a proportionally reduced rate at the increasing energy, in which many lesions are in close proximity to each other. Thus, an increasing number of lesions in each cluster gives rise to a decrease in the overall OCDL yield. This concept has been recalled more recently by other authors (Asaithamby and Chen, 2011; Sage and Harrison, 2011) and demonstrated by experimental evidence (for a review see Okayasu, 2012). In the present context, at increasing doses of radioactive Radon an increasing number of smaller sized fragments would be generated, giving rise to increased TL values, but not necessarily to a parallel increase of the Comet tail intensity (TI). According to these observations, the TL parameter was adopted for statistical analysis of data.

On the basis of the significant reduction of TL values observed in the housed (control) cricket population (Fig. 3A), this group was considered a suitable, reference control group for this investigation. Such result indicates that the adopted conditions for housing, i.e., its duration after their removal from the radioactive environment and diet, have been effective in reducing their level of genotoxic damage.

Results of regression analysis of Comet data showed a significant dose-effect increase for radon exposure only for brain cells (Fig. 3B, Table III). However, outlier values of haemocytes detected in CLP (Fig. 3B) appear to be too high to be justified only by the radon dose. Indeed, it is likely that some other genotoxic agent(s) might be present in this cave, affecting mainly the TL values of haemocytes. This finding could be explained by the characteristics of this cell type, being haemocytes the first cells entering in close contact with any genotoxicant introduced in the body through passive diffusion before distribution to other tissues. Therefore, a deeper analysis on environmental conditions of this cave is necessary to elucidate the cause of such results. Conversely, the results for all the other caves strongly suggest that the radon-radioactive environment represents the actual genotoxic agent. From the present results TL represents the most reliable parameter, particularly consistent with the exposure to alpha radiation.

Experimental data of the current investigation show that for of alpha radiation exposure, namely ^{222}Radon, a quite high level of genotoxic effect is detectable. Even if the Comet results cannot be considered as equivalent to an actual mutagenic effect, they are consistent with human epidemiological data from indoor exposure to

radon, from which striking evidence of an increase level of genetic and cytogenetic effects is not available (Al-Zoughool and Krewski, 2009). Lastly, from data obtained in the described experimental system it can be concluded that cave crickets might represent a good bio-indicator of radioactive-radon genotoxicity, especially for doses that are typical of confined environments.

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