FIRST APPROACH FOR STUDYING THE IMPACTS OF THE NUCLEAR TESTS ON INSECTS IN REGGANE, ALGERIA

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ABSTRACT

Herein, we report the initial findings of a study of two insect species (the Sahara bee *Apis mellifera sahariensis* and the silverfish *Lepisma saccharina*) collected from the site of the 1960 Reggane nuclear test site in Algeria. These samples were analysed via single-cell electrophoresis for DNA damage resulting from radiation exposure due to nuclear tests. Our results show statistically significant variation ($p<0.001$) in DNA damage in the silverfish compared to control organisms, but no such effect was found in the Sahara bee. This study represents what we believe to be the first of its kind regarding the ecological effects of the 1960 Reggane nuclear test and will be expanded upon in future research. Overall we call for further in-depth studies of the desert populations of various taxa including birds and mammals.

KEYWORDS

Algeria, Reggane, comet assay, DNA damage, ionizing radiation.

INTRODUCTION

Since the first test of a nuclear device (the 1945 “Trinity” test), numerous similar tests of fission and fusion weapons have been performed in twenty one countries (Barrillot, 2007) . In the early 1960’s, the French government conducted

a series of above and below ground nuclear tests in the south of Algeria at the Reggane and Ekker sites. These nuclear tests resulted in the release of radioactive material and a dispersal of these materials to regions far from the testing sites (Danesi *et al.* 2008). Despite extensive research into the consequences of nuclear weapon testing on human health, the impact of such testing on terrestrial ecosystems has received less attention (Koliado *et al.* 2001). Among the research that has been conducted, a consensus exists that radiation has varying effects within and across taxa. These studies include those of the Semipalatinsk underground nuclear tests in Kazakhstan, as well as of the meltdowns of the Chernobyl and Fukushima nuclear power plants, in 1986 and 2011, respectively, (Møller and Mousseau 2015; Galván *et al.* 2011; Geraskin *et al.* 2003; Garnier-Laplace *et al.* 2011)

It is well-known that exposure to ionizing radiation can damage organic molecules (e.g. lipids, nucleic acids, proteins etc.), thus affecting the living cell that are reliant on these molecules. In general, high doses of ionizing radiation can cause breakage of the bonds of such macromolecules. This breakage is followed by an increased generation of low-mass reactive chemical species of oxygen and nitrogen (referred to as ROS and RNS). In turn, these reactive radicals may cause chemical alterations to other biologically important macromolecules. Normally, these molecules are repaired, although these repair mechanisms may be overwhelmed by further irradiation (Murphy *et al.* 2011) . In particular, oxidation of phospholipids may cause a destabilization of the cell membrane, triggering apoptosis. (Spitz *et al.* 2004). Of potentially greater concern is the damage caused to DNA, as such damage can result in heritable mutations that may be passed to offspring, thus increasing genetic load in the population. (Møller & Mousseau 2013)

In this study, we investigated the consequences of the 1960 French nuclear tests on the desert ecosystem of the Algerian Sahara (specifically the Reggane region). To the best of our knowledge, this work is the first investigation of the eco-genetic effects of increased radiation caused by nuclear tests in the Algerian Sahara. Here, we present an analysis of two insect species (the Sahara bee, *Apis*

mellifera sahariensis, and the silverfish, *Lepisma saccharina*) collected from the radioactively contaminated sites of the Sahara desert and from a location distant from the test site. We performed this analysis using single cell gel electrophoresis (SCGE, also known as the Comet Assay) to identify DNA damage in interphase cells. The results of this study show that while the silverfish show a statistically significant increase of DNA damage compared to the control population, the Sahara bee showed no such statistically significant increase, but we suggest that this may be due to our limited sample size, differences in the life histories of these two species, or more likely, the much smaller differences in background radiation between the experimental and control locations.

MATERIALS AND METHODS

Study area and sampling

Fully mature silverfish were sampled from a region 20 km away from the Hammoudia nuclear testing site near the town of Reggane, where the background dose rate was previously estimated at about 2.7 µSv/h (Danesi *et al.* 2008). Control individuals were collected near Algiers, Algeria which is located 1,800 km away from the blast site and has much lower levels of background radiation (0.3 uSv/h). Mature Saharan bees, *Apis mellifera,* were captured from the Adrar territory 170 km from the explosion area. Laboratory bred individuals of the same subspecies of *A. mellifera* were used as controls.

Ten silverfish were captured at the Hammoudia and six individuals of *Apis mellifera sahariensis* were sampled near the territory of Adrar. The same numbers of samples for each species were used for the control groups. The geographical locations of the sampling sites in relation to the Hammoudia site, the town of Reggane, Adrar, and Algiers are shown in Figure 1.

Upon capture, each specimen was placed into an individual tube filled with RNAprotect (Qiagen, USA) for immediate stabilization of cellular nucleic acids. Samples were stored at 4 ^{o}C in this solution until further processing.

Cell preparation and Comet assay

The preserved insects were gently homogenized using microtube pestles in order to obtain a cell suspension. Debris (such as chitin) was sedimented during centrifugation at 1000 g for 1 minute and the supernatant was transferred into a new vial.

Neutral comet assays were performed according to the standard procedure (Singh et al, 1988) with minor modifications, as described herein. Cells were embedded in a 1% low-melting point agarose (IBI Scientific, USA), set on a microscope slide, and lysed overnight at 4 ^{o}C. Following lysis, samples were placed into a Tris-acetate buffer (0.3M NaAc*3H_2O, 0.1M Tris, pH 10) left for 10 minutes, and then electrophoresed at 0.75 V/cm for 20 minutes. After electrophoresis, the slides were rinsed in Tris-HCl buffer (pH 7.5) three times and then were fixed in 75% ethyl alcohol for fifteen minutes. After drying, the samples were stained using SYBR® Gold (Life Technologies, USA) at a dilution of 1:30,000 for 15 minutes.

DNA damage was determined using an Imager Z2 Zeiss fluorescent microscope and the Metafer Slide Scanning System (MetaCyte). Slides were scanned using the 20x objective. Data from at least 100 cells were obtained per individual. DNA damage was assessed using the estimates of the DNA percentage in the comet tail, which were obtained for each selected cell. For every individual, the mean and 90th percentile of the percentage of DNA present in tail was estimated.

Statistical analysis

According to Lovell *et al.* (1999), the unit of comparison in statistical analyses is the individual animal for *in vivo* studies. Genotoxic effects detected by the comet assay are usually inferred from the mean values and dispersion of the data. Data, obtained from the comet assay, were analysed using SAS 9.2. Data from the two species were tested independently for normality using the command PROC UNIVARIATE. Following this, the difference between control and tested group was inferred using the ANOVA procedure.

RESULTS

We obtained estimates of DNA damage using an average of 298 cells from each silverfish individual, and 218 cells from Sahara bees. The mean and 90th percentile values of DNA present in the comet tails for each species are reported in Table I. Examples of typical comet tail sizes for the silverfish and Sahara bees are shown in Figures 2 and 3, respectively. The mean percent of DNA damage varied significantly between the control and experimental groups of silverfish ($F=15.19$, $p<0.0011$), but did not vary between the control and experimental groups of Sahara bees ($F=1.65$, $p<0.2280$). The ANOVA analysis of the 90th percentile of DNA damage yielded $F=22.00$, $p<0.0002$ and $F=0.17$, $p<0.6882$ for the silverfish and the Sahara bee, respectively. Graphical representations of ANOVA analyses for each species are shown in Figure 4.

DISCUSSION

The Hammoudia test site is located in the Sahara desert about 1500 km south of Algiers and 50 km south of the town of Reggane, an oasis village of a few thousand inhabitants. Recently measured dose rates varied near the site of detonation, reaching a maximum of 2.7µSv/h (Danesi *et al.* 2008). According to a report by the International Atomic Energy Agency (IAEA, 2005), the measured radiation comes from the activity of residual plutonium incorporated into the sand. Also, radioactivity may have accumulated in plant material via the absorption of nuclides from contaminated soil (Koranda & Robison 1978). The erosion of sand and soils, caused by frequent sandstorms and tornadoes, contributes to the distribution of radioactive material further from the testing site.

Our qualitative census of the wildlife around the Reggane region, which we have been conducting for the past few years, shows a significant diversity of the ecosystem. There are more than 60 species of invertebrates, as well as some species of mammals (e.g. gerbils) and birds (e.g. the strand raven, the shrike, the barn owl, kestrel and turtle doves etc). To our knowledge, there is little research

evaluating the effects of low-dose ionizing radiation on this desert wildlife (Chebli A., pers. comm.).

Research suggests that, among invertebrate species, the different taxa of insects demonstrate significant variation in radiation sensitivity. Studies have been reported that used insect imagoes to study the effects of acute doses of ionizing radiation on DNA integrity. These studies included the common fruit fly *D. melanogaster* (Jordan *et al.* 2012), the weevil *Curculio sikkimensis* (Todoriki *et al.* 2006), Colorado potato beetle *Leptinotarsa decemlineata* (Filipas *et al.* 2008), and several others. These studies suggest that intense irradiation is needed in order to disrupt the DNA integrity of exposed cells (Hinton *et al.* 2007). However, most of these studies used laboratory-bred organisms and controlled irradiation protocols. These factors make it difficult to interpret the ecological effects of high background radiation using the trends put forth by these studies. In turn, the research that has been conducted in the Chernobyl Exclusion Zone (CEZ) and around the Fukushima disaster site reported that insect taxa responded to background radiation by a change in species abundance and by alteration of the development of adult insect. (Hiyama *et al.* 2013; Mousseau & Møller 2014; Bezrukov *et al.* 2015; Akimoto 2014) . At the same time, the offspring of the grasshoppers (*C. albomarginatus*) from Chernobyl did not demonstrate a significant effect due to irradiation of their parents. (Beasley *et al.* 2012). Thus, further research is needed to derive a strong data pool with which to study the impacts of radiation impact on the invertebrates.

In this study, we report what we believe to be the first evidence of possible radiation impact on inhabitants of the desert ecosystem of Algeria. Samples of silverfish, collected at contaminated site of Hammoudia demonstrated significantly increased disruption of the DNA integrity compared to control samples. However, we did not find such a significant difference in DNA integrity in the samples of Sahara bees, collected near Adrar. We suggest that our of lack of significant differences among the bee populations may be related to our relatively low sample sizes compared to other studies of this nature; although DNA damage

was on average higher in individuals collected from the experimental site (Figure 3), variability among samples rendered this difference statistically insignificant. Also, given that bees may forage over much larger geographic areas than silverfish, exposure to radioactive contaminants may have been on average much lower for this group. In any case, much greater replication at both the individual and population levels, combined with much better characterization of received dose, would be needed before generating rigorous claims concerning any direct causal links between radioactive contaminants and organismal effects in this system. Thus, the findings reported here, although suggestive, are very preliminary and we call for further research of this ecosystem to elaborate upon these findings. However, the methods reported here, when combined with more extensive replication and dosimetry, could be useful tools for the exploration of ecological consequences of radioactive fallout at nuclear bomb test sites around the world. Such research could prove generally useful given past and likely future nuclear accidents.

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Available:

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Figure 1. Map of the sampling sites. The cross marks the Hammoudia site, the triangle marks the site of silverfish capture, and the diamond marks the site of Sahara bee capture.

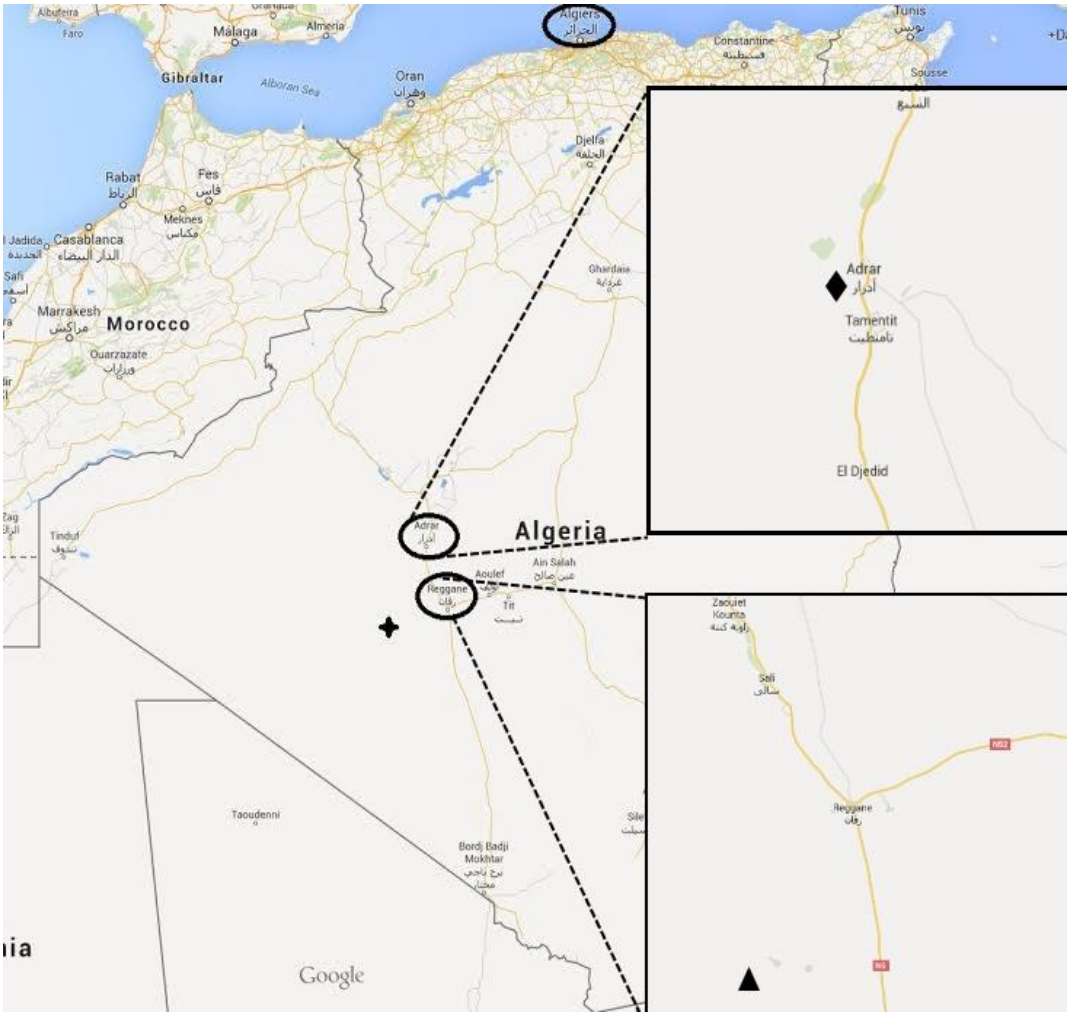
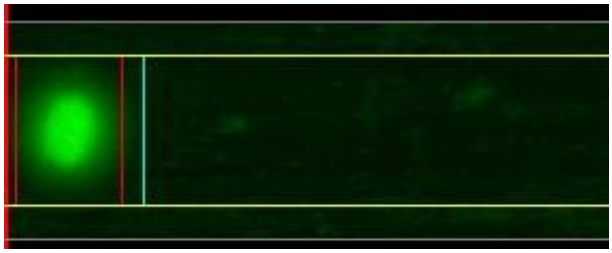


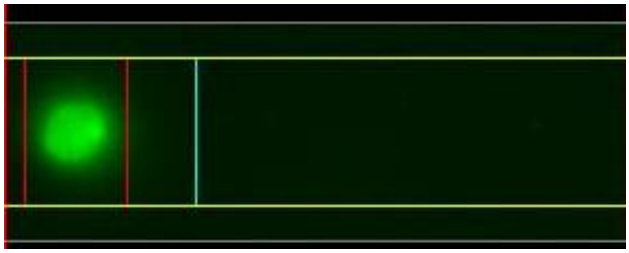
Table I. Log-transformed values of the mean percent DNA in tail and 90th percentile of the mean DNA (m±SE). The exposed silverfish group showed a statistically significant variation from control groups in both mean and 90th percentile values, ($p<0.001$)

Species	Testing groups	Mean Percent DNA in tail	90th Percentile of the Percent DNA in tail
L. saccharina	Control (n=10)	1.680±0.287	2.529±0.273
	Hammoudia (n=10)	2.249±0.362	3.147±0.314
A. mellifera	Control (n=6)	1.565±0.133	2.204±0.058
	Adrar (n=6)	1.481±0.091	2.190±0.060

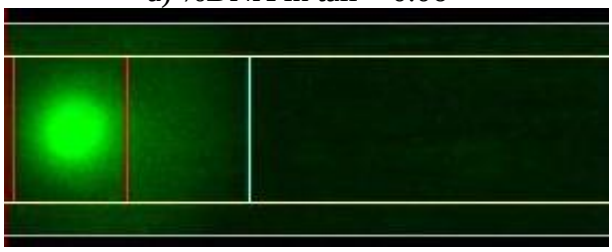
Figure 2. Typical comet images for *L. saccharina*. DNA damage levels increase from image a to image d, as shown by increasing tail size (marked by the white bar) relative to the head size (marked by the red bars).



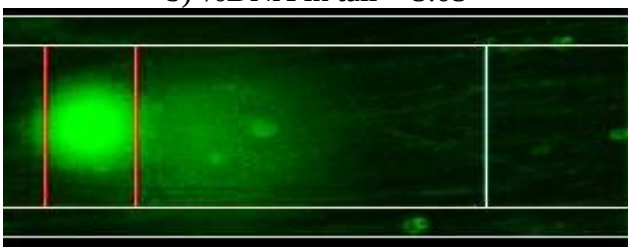
a) %DNA in tail = 0.06



b) %DNA in tail = 5.05



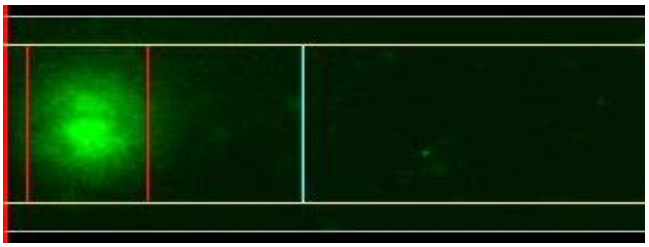
c) %DNA in tail = 22.16



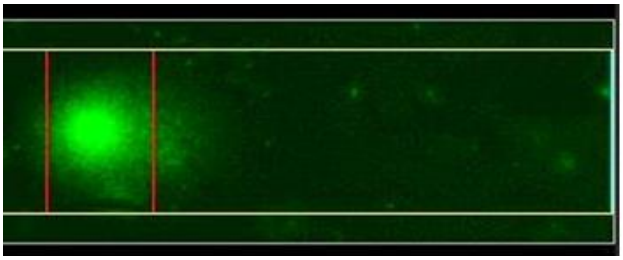
d) %DNA in tail = 48.83

Figure 3. Typical comet images for *A. mellifera*. .

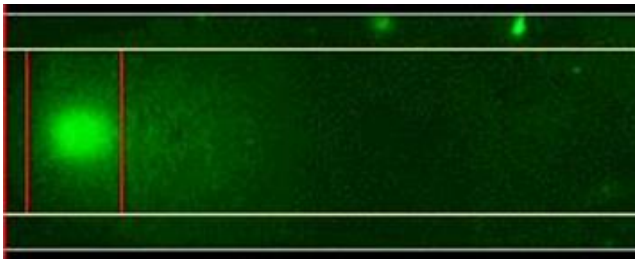
e) %DNA in tail = 0.00



f) %DNA in tail = 8.98



g) %DNA in tail = 17.05



h) %DNA in tail = 48.14

Figure 3. Graphical representation of ANOVA analyses of *L. saccharina* and *A. mellifera*