Study of genomic DNA damage in four-spotted gecko, *Tarentola annularis* one species inhabiting two different habitats

Hanan R.H. Mohamed[1]*, Mohamed A. Kadry[2]

[1,2]: Zoology Department, Faculty of Science, Cairo University, Giza, Egypt

***Corresponding author Tel: 0235676689, Fax*: 0235727556**

email*: hananesresm@yahoo.com

Abstract

The genus *Tarentola* is widely distributed; lives mainly in arid and semi-arid habitats and comprises 21 species with low inter-specific morphological variations. However, polluted habitats damage DNA and changes the genetic materials of living organisms that threaten the persistence of animal populations and affect on individuals, populations, genetic diversity and ultimately ecosystem biodiversity. Therefore, our study was aimed to assess the DNA damage level in two natural populations of *Tarentola annularis* inhabiting two different habitats in Egypt; El-Faiyum and El-Beheira. Comet assay was used to assess single and double strand breaks and Laddered DNA fragmentation assay was used to study the DNA fragmentation on agarose gel stained using ethidium bromide. A higher DNA damage level in the natural population of *T. annularis* inhabiting El-Faiyum compared with that inhabiting El Beheira was revealed by higher strand breaks and fragmentation pattern. Thus, we concluded that the highly polluted natural habitat of El-Faiyum threatens the persistence of the wild natural population of *T. annularis* by increasing the damage of its DNA that decreases its genomic stability. As the pollution threatens animal populations' persistence and ultimately affect on ecosystem biodiversity, we recommended to protect provide suitable protections to the natural population of *T. annularis* inhabiting El-Faiyum by placing them in nature reserves to protect it from the destructive human activities.

Keywords

Genetic diversity, DNA damage, comet assay, laddered DNA fragmentation, *Tarentola annularis*, El-Faiyum and El-Beheira.

Introduction

The genus *Tarentola* belongs to the family Gekkonide of the order squamata that is distributed throughout the world and representing one of the largest vertebrate groups among squamates (Vidal and Hedges, 2005). Gekkonidae is divided into four subfamilies: Diplodactylinae, Gekkoninae, Eublepharinae and Sphaerodactylinae with 1130 species and 108 genera (Han et al., 2004).

The genus *Tarentola* lives mainly in arid and semi-arid habitats, widely distributed in Libya, Sinai, Ethiopia and Somali land, Countries and Islands bordering the Mediterranean (Baldo et al., 2008). Moreover, it comprises 21 species with low inter-specific morphological variations (Sprackland and Swinney, 1998; Carranza et al., 2002; Diaz and Hedges, 2008). However, genetic variations between them were evidenced by molecular analysis of their protein and their nuclear and mitochondrial genes (Carranza et al., 2002; Jesus et al., 2002; Perera and Harris, 2010; Ali, 2012**).**

The *Tarentola annularis* is distributed in a wide area across Africa, central Sudan, to the north along the Nile to the Nile Delta and Sinai Peninsula (Egypt) (Baha El Din, 2006). It is a common rock dwelling species inhabiting rocky wadis, ruins and old buildings.

It is essential to study the damages inflicted by contaminants to the genetic material of wild populations of animals to assess the threat that such contaminants pose to individual fitness, as well as to the persistence of natural populations. Comet and Laddered DNA fragmentation assays are widely used methods to estimate DNA damage inductions in several populations (Kleinjans and van Schooten, 2002; Alarifi et al., 2013; Mohamed, 2014).

Therefore, the current study was aimed to estimate the DNA damage level in the two natural populations of the four-spotted gecko, *Tarentola annularis*; the first population inhabiting El-Faiyum and the second inhabiting El-Beheira in Egypt using comet and Laddered DNA fragmentation assays.

Materials and methods

Animals' collection and study area

A total of 10 individuals of two natural populations of the four-spotted gecko, *Tarentola annularis* were collected from two different habitats in Egypt. The first population was collected from El-Faiyum (low land = 40 m below sea level) [29° 18′ 30.25 ″ N 30° 50′ 34.26 ″ E]. It is one of the governorates of Egypt in the middle of the country. Its capital is the city of Faiyum, located about 81 mi (130 km) south west of Cairo. It is characterized by the large fertile Faiyum Oasis, which comprises farmland. South of the Faiyum Oasis, there is a smaller depression called El Gharaq el Sulţāni and irrigated from the Nile. A dry barren depression named Wadi Elrayan covers 280 mi² (725 km²), west of the El Gharaq el Sulţāni depression. Desert and dry mountains mostly surround the depressions.

While the other population was collected from El-Beheira (near sea level = latitude "coastal" area) [30° 50′ 53.16 ″ N 30° 20′ 36.78 ″ E]. El-Beheira is located within the Alexandria Region, which encompasses Alexandria, Matrouh, and El Beheira govemorates. Geographically, El-Beheira govemorate is characterized by a vast desert in the south and the west, cultivated areas stretching to the eastem borders of the Rosetta Branch of the Nile, and Edco Lake and the Mediterranean Sea in the north.

Both natural populations of *T. annularis* have circular pupil of the eye, dilated digits, mid-dorsal tubercles are lower than lateral tubercles with low inter-specific morphological variations between them except only in body coloration where the natural population of *T. annularis* inhabiting El-Faiyum showed a lighter coloration than that inhabiting El-Beheira (Fig. 1).

Our Institutional Animal Care and Use Committee (IACUC) at Zoology Department, Faculty of Science, Cairo University has approved this study protocol from the ethical point of view and according to Animal welfare Act of

the Ministry of Agriculture in Egypt that enforces the humane treatment of animals and the IACUC permit number is **CUFS F Ecol. 4 15**.

Comet assay

Single and double strand breaks were detected by using alkaline comet assay (Tice et al., 2000). In brief, small piece of liver tissue was minced with cold mincing solution (Hanks balanced Salt Solution (HBSS) Ca++ and Mg++ free with 20 mM EDTA, 10% DMSO) and 10 µl aliquot of cell suspension containing approximately 10000 cells was mixed with 70 µl of 0.5% low melting point agarose (Sigma) and spread on a fully frosted slide pre-dipped in normal melting agarose (1%). After solidification, the slides were placed in cold lysis buffer (2.5 M NaCl, 100 mM EDTA and 10 mM Tris, pH 10) with freshly added 10% DMSO and 1% Triton X-100) for 24 hours at 4ºC in dark. Subsequently, the slides were incubated in fresh alkaline buffer (300 mM NaOH and 1 mM EDTA, pH>13) for 20 min. The unwinding DNA was electrophoresed for 20 min. at 300 mA and 25 V (0.90 V⁄cm) and neutralized in 0.4 M Trizma base (pH 7.5) and finally, fixed in 100% cold ethanol, air dried and stored at room temperature until they were scored. The extent of DNA migration for each sample was determined by simultaneous image capture and scoring of 100 cells at 400 x magnification using Komet 5 image analysis software developed by Kinetic Imaging, Ltd. (Liverpool, UK). The extent of DNA damage was evaluated using the most common DNA damage indicators: tail length, %DNA in tail and tail moment.

Laddered DNA fragmentation assay

Apoptotic DNA fragmentation was assessed according to Sriram *et al.* (2010) as follow: liver cells were lysed in TE lysis buffer containing 0.5% sodium dodecyl sulfate, then added 0.5 mg/mL RNase A and incubated at 37°C for one hour. Incubated after that with 0.2 mg/mL proteinase K at 50°C overnight. Phenol extraction of DNA and precipitated by 7.5 M ammonium acetate and isopropanol.

Electrophoresed fragmentized DNA in a 1% agarose gel at 70 V, visualized by UV transiluminator and photography.

Statistical analysis

In this study the Statistical Program for Social Science (SPSS) version 20 package software was used for statistical analysis. Student *t-test* was to test the significance level between the two natural populations of *T. annularis* in two different habitats in Egypt.

Results

Level of DNA damage

The DNA damage level in *Tarentola Annularis* was estimated in this study using tail length, %DNA in tail and tail moment as DNA damage endpoints. Various grades of DNA damage were observed in two populations of *T. annularis* (Fig. 2) however, the values of tail length, %DNA in tail and tail moment were statistically significantly higher in *T. annularis* population inhabited El-Faiyum area than those observed in population inhabited El-Beheira area of Egypt (Fig. 3).

Laddered DNA fragmentation

Results of laddered DNA fragmentation were shown in Fig .4. Running of the genomic DNA of two populations of *T. annularis* on agarose gel showed that DNA fragmentation in population inhabited El-Faiyum area was higher than that inhabited El-Beheira area as indicated by the highly smeared pattern of genomic DNA of El-Faiyum inhibited population compared with the other population

Discussion

Assessment of DNA damage level in two populations of the same species inhabiting two different areas is very important to ensure the safety of the environment as any disturbance in the environmental conditions resulting in alteration of the genetic material of wild organisms that threatens the persistence of wild animal populations causing impairment of the ecosystem health and its

provision of services to human society (Bonisolti-Alquati, 2014; Mohamed and Kadry, 2015; Schaumburg et al., 2015). Thus, the present study investigated the DNA damage level that reflecting the genomic stability in two populations of *T. annularis* inhabiting two different areas; EL-Faiyum and El-Beheira in Egypt.

Results of the comet assay revealed the higher DNA damage induction level in a population inhabiting EL-Faiyum compared with that in El-Beheira by the statistical significant elevations in the tail length, %DNA in the tail and tail moment (Fig. 3). Indeed, the smeared appearance of the running genomic DNA on agarose gels of *T. annularis* inhabiting EL-Faiyum compared with that of inhabiting El-Beheira (Fig. 4) further confirmed the higher DNA damage level in Tarentola annularis inhibiting El-Faiyum compared with that inhibiting El-Beheria habitat.

These results could be attributed to the difference in the nature of the two habitats as the environment in EL-Faiyum is highly polluted with many pollutants such as heavy metals compared with that in El-Beheira (Shendi, 2000; Abd El_Motaleb, 2002). These heavy metals accumulate with time in the living organisms and react with the macromolecules leading to reactive oxygen species (ROS) generations.

Consequently, ROS generations can oxidize double bonds on fatty acid tails of membrane phospholipids resulting in lipid peroxidation and also cause protein carbonylation, mitochondrial alterations, a decrease in endogenous and exogenous antioxidants, and an increase in DNA oxidation (Ivanoviene et al., 2004; Celik et al., 2009; Cambier et al., 2010). Indeed, this increases membrane permeability and fluidity, making cells more susceptible to osmotic stress or hindering nutrient uptake (Cabiscol *et al*., 2000). Peroxidized fatty acids can trigger reactions that generate other free radicals, leading to more cell membrane and DNA damage including DNA strand breaks, cross-linking, and adducts of the bases or sugars therapy leading to mutations that threatens the existence of *Tarentola annularis*.

Conclusion

The highly polluted environment in EL-Faiyum compared with El-Beheira resulting in significant elevations in DNA damage level that threatens the persistence of *Tarentola annularis* inhabiting this habitat. Thus it is recommended to solve the pollution problem to protect ecosystem biodiversity.

References

Abd El_Motaleb M.H. (2002) Contribution of Geographic Information Systems for studying some environmental problems in EL-FaiyumGovernorate, Egypt. Ph.D. Thesis, Fac. of Agric. At Fayoum, Cairo University, Egypt.

Alarifi S, Ali D, Al-Doaiss AA, Ali BA, Ahmed M, Al-Khedhairy AA (2013) Histologic and apoptotic changes induced by titanium dioxide nanoparticles in the livers of rats. *Int J of Nanomedicine* 8: 3937–3943

Ali R.A.M., (2012) Genetic variation among nine Egyptian gecko species (Reptilia: Gekkonidae) based on RAPD-PCR. Life Sci. J. 9: 154–162.

Baha El Din M (2006) A Guide to Reptiles and Amphibians of Egypt. Cairo: American University in Cairo Press pp 359

Baldo D, Borteiro C., Brusquetti F., García E. and Prigioni C. (2008) Notes on geographic distribution, Reptilia, Gekkonidae, *Hemidactylus mabouia*, *Tarentola mauritanica*: Distribution extension and anthropogenic dispersal. Check List 4: 434–438.

Bonisolti-Alquati, A. (2014) Avian genetic ecotoxicology: DNA of the canary in a coalmine. *Current Zoology* 60 (2): 285–298, 2014

Cabiscol E, Tamarit J and Ros J. (2000). Oxidative stress in bacteria and protein damage by reactive oxygen species. *Int Microbiol* 3:3–8.

Cambier S, Gonzalez P, Durrieu G, and Bourdineaud J-P (2010) Cadmium-induced genotoxicity in zebrafish at environmentally relevant doses. *Ecotoxicology and Environmental Safety*, 73(3): 312–319.

Carranza S., Arnold N., Mateo A. and Geniez P. (2002) Relationships and evolution of the North African geckos, *Geckonia* and *Tarentola* (Reptilia: Gekkonidae), based on mitochondrial and nuclear DNA sequences. Mol. Phylogenet. Evol 23: 244- 256.

Celik A, B¨uy¨ukakilli B, Cimen B, Tasdelen B, Ozturk M.I, and Eke, D (2009) Assessment of cadmium genotoxicity in peripheral blood and bone marrow tissues of maleWistar rats, *Toxicology Mechanism and Methods* 19(2): 135–140

Diaz M. and Hedges B. (2008) A new gecko of the genus *Tarentola* (Squamata: Gekkonidae) from eastern Cuba. Zootaxa 1743: 43-52.

Han, D., Zhou, K., Bauer, A.M (2004) Phylogenetic relationships among gekkotan lizards inferred from C-mos nuclear DNA sequences and a new classification of the Gekkota. Biol. J. Linn. Soc. 83: 353–368.

Ivanoviene L, Sadauskiene I, Lesauskaite V, Stapuloinis R and Ivanov L (2004) Induction of apoptosis by cadmium chloride in mouse liver. Biologija Nr 2: 42-45

Jesus J., Brehm A. and Harris J. (2002) Relationships of *Tarentola* (Reptilia: Gekkonidae) from the Cape Verde islands estimated from DNA sequence data. Amphibia-Reptilia 22: 235-242.

Kleinjans J.C.S and van Schooten F.J. (2002) Ecogenotoxicology: The evolving field. Environ. Toxicol. Phar 11: 173–179.

Mohamed H. R. H. (2014) Evaluation of DNA Damage and Oxidative Stress Inductions by Excessive Medical Intake of Saline in Mice Bone Marrow Cells. International Journal of Sciences: Basic and Applied Research (IJSBAR) 15(1): 37-56

Mohamed H.R.H and Kadry MA (2015). Genetic and Histological Diversity between the Coastal and Desert *Chamaeleo chamaeleon* Subspecies in Egypt. International Journal of Science and Research **4 (7);** 613-617

Pereira A. and Harris J. (2010) Genetic variability within the Oudri's fan-footed gecko *Ptyodactylus oudrii* in North Africa assessed using mitochondrial and nuclear DNA sequences. Mol. Phyl. Evol. 54: 634–639.

Schaumburg, L.G, Poletta G.L., Siroski, P.A. and Mudry, M.D. (2012) Baseline values of micronuclei and comet assay in the lizard Tupinambis merianae (Teiidae, Squamata). Ecotoxicol. Environ. Saf. 84: 99-103.

Shendi, M. M. (2000) Geographic soil data-base for Sinnuris District, Fayoum Governorate, Egypt. 2nd International Conference on Earth Observation and Environmental Information. 11- 14 November 2000, Cairo, Egypt.

Sprackland G. and Swinney N. (1998). A new species of giant gecko of the genus *Tarentola* (Reptilia: Squamata:Gekkonidae) from Jamaica. J. Zool., 245: 73–78.

Sriram MI, Kanth SBM, Kalishwaralal K and Gurunathan S (2012) Antitumor activity of silver nanoparticles in Dalton's lymphoma ascites tumor model. *Int. J of Nanomed* 5: 753–762

Tice R.R., Agurell E., Anderson V, Burlinson B., Hartmann A., Kobayashi H., Miyamae Y., Rojas E., Ryu J.C. and Sasaki Y.F. (2000). Single cell gel/comet assay: guidelines for in vitro and in vivo genetic toxicology testing, Environ. Mol. Mutagen. 35: 206–221.

Vidal, N. and Hedges S.B (2005). The phylogeny of squamate reptiles (lizards, snakes, and amphisbaenians) inferred from nine nuclear protein coding genes. CR Biol. 328: 1000–1008.

Figures capatations

Fig. 1: Photos of *Tarentola annularis* inhibiting El Faiyum (a) and El Beheira in Egypt

Fig. 2: Comet assay in *Tarentola annularis* population inhibiting El Faiyum area and in other popluation inhibiting El Beheira area of Egypt

Fig. 3: Representative photo for comet assay showing undamged (a) and damaged (b) nuclei

Fig. 4: Genomic DNA pattern of *Tarentola annularis* inhibiting El Faiyum (F) and El Beheira (B) of Egypt

Fig. 1: Photos of *Tarentola annularis* inhibiting El Faiyum (a) and El Beheira in Egypt

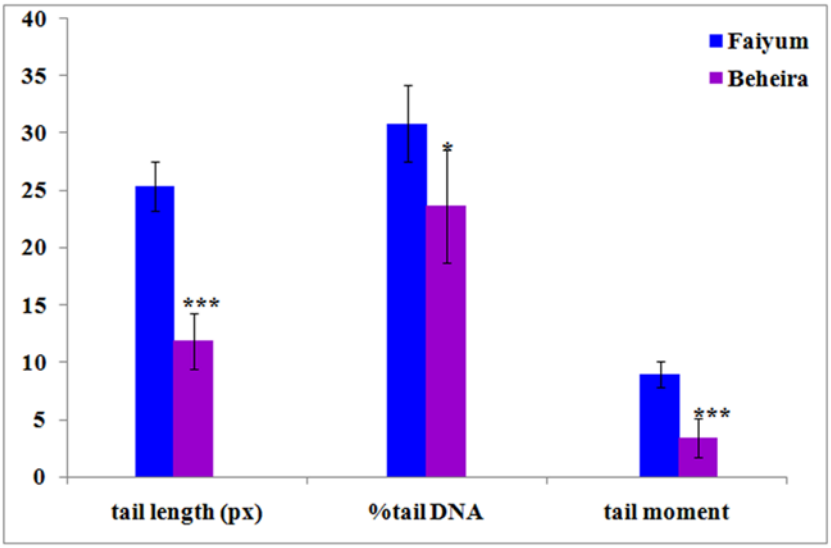

Fig. 2: Comet assay in *Tarentola annularis* population inhibiting El Faiyoum area and in other population inhibiting El Beheira area of Egypt

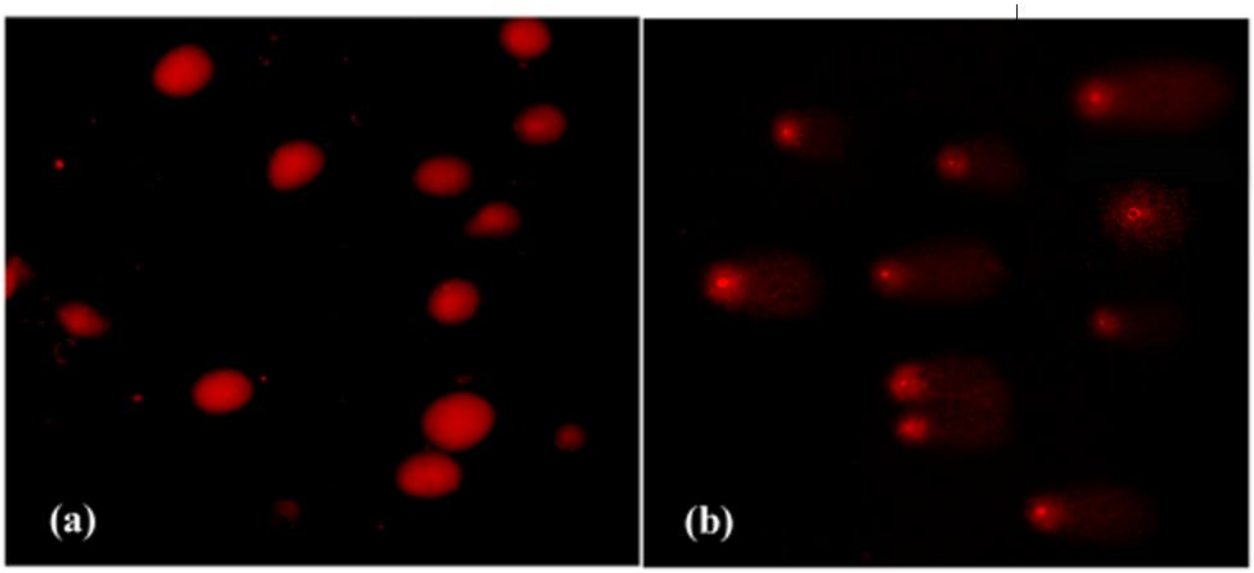

Fig. 3: Representative photo for comet assay showing undamaged (a) and damaged (b) nuclei

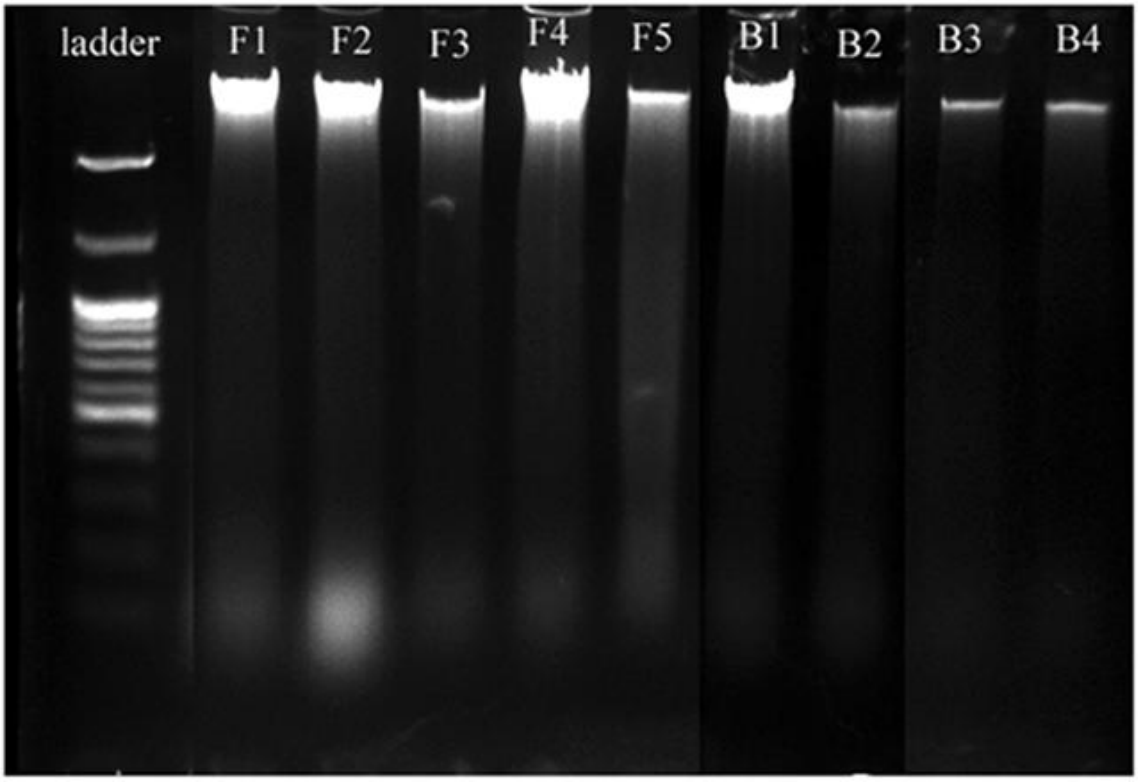

Fig. 4: Genomic DNA pattern of *Tarentola annularis* inhibiting El Faiyum (F) and El Beheira (B) of Egypt