

Study of Chromosomal and DNA Damage Baseline Level in Two Subspecies of *Chamaeleo chamaeleon* in Egypt Using Micronucleus and Comet Assays

Hanan R.H Mohamad, Mohamed A. M. Kadry

Zoology Department, Faculty of Science, Cairo University, Egypt

Abstract: As any alteration in the genetic material of wild organisms pose significant threats to the persistence of wild animal populations affecting individuals, populations, genetic diversity and ultimately ecosystem biodiversity. Thus variations in chromosomal and DNA damage baseline levels in two subspecies of *Chamaeleo chamaeleon*; *C. chamaeleon chamaeleon* inhabiting El-Dabaa (Marsa Matrouh) and *C. chamaeleon musae* inhabiting El-Arish (North Sinai) of Egypt was studied in our study using micronucleus and comet assay. Despite slight variations in tail moment and %tail DNA in blood, both of micronuclei and DNA damage levels was homogeneous in the two studied subspecies in blood and liver cells. We concluded that both of chromosomal and DNA damage baseline level were identical in both *Chamaeleo chamaeleon* subspecies; *C. c. chamaeleon* and *C. c. musae* confirming the safety of their habitat. Molecular studies both on DNA and protein levels will be done to study the differences in adaptations of these two subspecies to their habitats.

Keywords: chromosomal damage, DNA damage, comet assay, micronucleus assay and chamaeleonidae

1. Introduction

Chamaeleo is a genus of chameleons belongs to the family Chamaeleonidae that found primarily in the mainland of sub-saharan Africa. Chameleons are a distinctive and highly specialized clad of lizard that are easily distinguishable by their zygodactyl feet, their separately mobile and stereoscopic eyes, their very long tongues, prehensile tail, crests on their distinctively shaped heads, and the ability to change skin coloration, laterally compressed body and special leg adaptations for grasping vegetation [1-3].

The Common Chameleon has the broadest distribution of all chameleon species, found from Morocco and the southern Iberian Peninsula over the whole of North Africa, to the Near East, Turkey, Cyprus and Southern Arabia [4]. The genus *Chamaeleo* contains 4 recognized subspecies: *C. c. chamaeleon*, *C. c. musae*, *C. c. orientalis* and *C. c. retriocrista*. The subspecies; *C. c. chamaeleon* and *C. c. musae* allocate from North Africa, Middle East, Morocco, Algeria, Tunisia, Libya, Egypt, Israel, Palestine, Jordan, Western Sahara, Saudi Arabia, Yemen, Lebanon, Syria, Iraq, and Iran [5].

The study of the damages inflicted by contaminants to the genetic material of wild populations of animals is a useful target to assess the threat that such contaminants pose to individual fitness, as well as to the persistence of natural populations. Comet and micronucleus assays are currently the most widely used methods that allow the characterization of DNA damage induced by physical and chemical agents in wild species [6,7]. The comet assay has rapidly become the most common technique for the detection of DNA strand breakage because it is simple, inexpensive and reliable, and can be applied to virtually any nucleated cell line. Moreover, DNA damage is detected on the level of single cell as in cytogenetic assays [8-10].

Micronuclei (MN) are small bodies that originate outside the nucleus by chromosome breakage and centromere or spindle dysfunction during cell division that are generated by both

aneugens and clastogens which induce spindle damage and cause chromosome to break, respectively [11]. Thus micronucleus assay has long been used as a biomarker of exposure, and is generally considered to be reliable and sensitive test.

Therefore, the present work was designed to study the variations in chromosomal and DNA damage baseline levels in two subspecies of *C. chamaeleon* (*C. chamaeleon chamaeleon* and *C. c. musae*) inhabiting the coastal and Sinai desert of Egypt, respectively.

2. Materials and Methods

Taxon sampling and study area

A total of 10 individuals from 2 Egyptian subspecies of chamaeleonid lizards; *C. c. chamaeleon* and *C. c. musae* were collected from El-Dabaa (Marsa Matrouh) and El-Arish (North Sinai) respectively [31° 01' 37.49"N 28° 26' 8.48"E and 31° 07' 55.53"N 33° 48' 11.79"E respectively] (Fig. 1).



Figure 1: Photos of *C. c. chamaeleon* (a) and *C. c. musae* (b) inhabiting El-Dabaa (Marsa Matrouh) and El-Arish (North Sinai) respectively

Micronucleus Assay

Micronucleus assay described by Schmid [12] was used to detect chromosomal damage baseline. In briefly, blood cells were mixed with filtered fetal bovine serum and spread on clean slide. Air-dried, fixed and finally stained for 5 minutes in May-Grunwald – Giemsa stain mixture then mounted with Distrene 80, Dibutyl phthalate, Xylene (DPX). 2000 intact erythrocytes were counted and micronuclei were considered as small inclusions of nuclear material inside erythrocytic cytoplasm with well-defined outline and coloration similar to that of the main nucleus and a size from 1/3 to 1/20 in relation to that of the main nucleus [13]. Results were expressed as mean \pm SD.

Comet Assay

DNA damage baseline level was estimated in both blood and liver tissues of the two studied *Chamaeleo chamaeleon* subspecies using alkaline (pH >13) comet assay. According to Tice *et al.* [8] a small piece of liver was minced using cold mincing solution (Hanks balanced Salt Solution (HBSS) Ca⁺⁺ and Mg⁺⁺ free with 20 mM EDTA, 10% DMSO) and then 10 μ l aliquot of either blood or liver cell suspension containing approximately 10000 cells was mixed with 75 μ l 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 4°C 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 according to the following endpoints measurements: **Tail length**: it is used to evaluate the extent of DNA damage away from the nucleus and expressed in μ m, **% DNA in tail**: intensity of all tail pixels divided by the total intensity of all pixels in the Comet and **tail moment**: calculated as: tail moment= tail length \times %DNA in tail/100.

Statistical Analysis

Results were expressed as mean \pm SD Statistical analysis was performed using the T-test to test the significance level between the two subspecies in the tested parameters. All statistics were carried out using Statistical Analysis Systems (SAS) program [14] ®.

3. Results and Discussion

It is very useful to study the diversity of genetic damage baseline in two subspecies of *Chamaeleo chamaeleon* inhabiting different habitats as DNA damage resulting in alteration of the genetic material of wild organisms that pose

significant threats to the persistence of wild animal populations causing impairment of the ecosystem health and its provision of services to human society [7, 15]. Micronucleus test and Comet assays are currently the most widely used methods that allow the characterization of DNA damage induced by physical and chemical agent's genetic damage in wild species [16, 17].

Using micronucleus assay blood cells of *C. chamaeleon musae* inhabiting El-Arish (North Sinai) have slightly higher micronuclei level than that found in *C. c. chamaeleon* inhabiting El-Dabaa (Marsa Matrouh) but without any statistical difference between them as shown in Fig. 2. Therefore, both two Chameleons subspecies inhabiting different habitats have the same chromosomal damage baseline that reflecting the environmental safety.

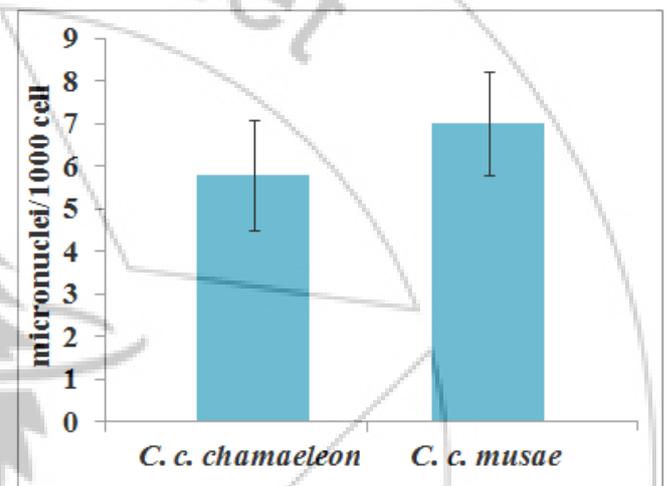


Figure 2: Micronuclei number in blood cells of *C. c. chamaeleon* and *C. c. musae*. Results are expressed as mean \pm SD

Results of comet assay evidenced that the DNA damage baseline level was nearly the same in the studied two subspecies of *Chamaeleo chamaeleon* (*C. c. chamaeleon* and *C. c. musae*) as shown by the observed non significantly differences in DNA damaged measured parameters (tail length, %DNA in tail and tail moment) in liver and tail length only in blood (Fig. 3). Thus our results confirmed the environmental safety and safety of diet applied to apply to both subspecies of chamaeleons in agreement with the recorded null variations in the activity of hepatic α -esterases isoenzymes in our previous study [18]. However, the observed slight significant increases in %tail DNA and tail moment in blood of *C. c. musae* over that of *C. c. chamaeleon* could be attributed to other environmental factors and/or by inter-individual differences in age, sex, nutritional state or physiological condition [15].

Conclusion: the genetic damage baseline level was identical in both *C. c. chamaeleon* inhabiting El-Dabaa (Marsa Matrouh) and *C. c. musae* inhabiting El-Arish (North Sinai) of Egypt that confirmed the safety of their habitats.

References

Keren-Rotem, T.; Bouskila, A. and Geffen, E. (2006): Ontogenetic habitat shift and risk of cannibalism in the common chameleon (*Chamaeleo chamaeleon*). *Behav. Ecol. Sociobiol.*, 59: 723-731.

[1]

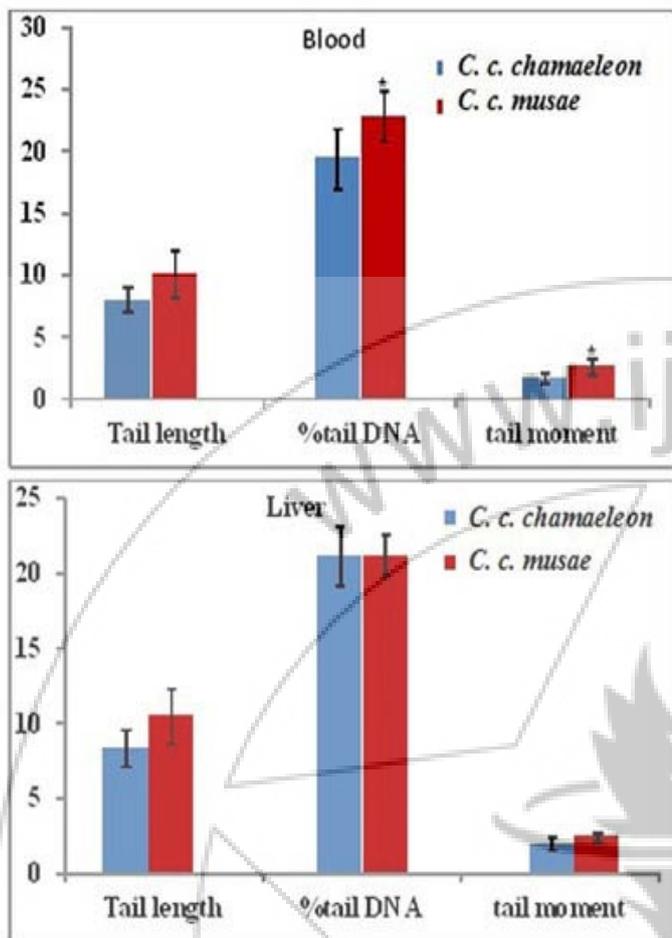


Figure 3: comet assay in *C. c. chamaeleon* and *C. c. musae* subspecies. Results are expressed as mean \pm SD indicating by error bars in blood and liver tissues. * indicating statistical significant difference at $p < 0.05$

- [2] Stuart-Fox, D. and Moussalli, A. (2008). Selection for social signaling drives the evolution of chameleon color change. *Public Library of Science Biology*, 6, e25.
- [3] Anderson, C.V., Sheridan, T. and Deban, S.M. (2012). Scaling of the ballistic tongue apparatus in chameleons. *Journal of Morphology*, 273: 1214–1226.
- [4] Dimaki, M.; Valakos, E. and Legakis, A. (2000): Variation in body temperatures of the African chameleon *Chamaeleo africanus* Laurenti, 1768 and the common chameleon *Chamaeleo chamaeleon* (Linnaeus, 1758). *Belgium J. Zool.*, 130: 87-91.
- [5] Baha El Din, M. (2006): A Guide to Reptiles and Amphibians of Egypt. Cairo: American University in Cairo Press. 359 pp.
- [6] Kleinjans J.C.S and van Schooten F.J. (2002). Ecogenotoxicology: The evolving field. *Environ. Toxicol. Phar.*, 11: 173–179.
- [7] 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.
- [8] 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.

- [9] Dhawan, A., Bajpayee, M. and Parmar, D. (2009). Comet assay: A reliable tool for the assessment of DNA damage in different models. *Cell Biol. Toxicol.*, 25: 5–32.
- [10] Frenzilli, G., Nigro, M. and Lyons, B.P. (2009). The Comet assay for the evaluation of genotoxic impact in aquatic environments. *Mutat. Res.-Rev. Mutat.*, 681: 80–92.
- [11] Fenech, M. (2000). The *in vitro* micronucleus technique. *Mutat. Res.*, 455: 81–95.
- [12] Schmid, W. (1975). The micronucleus. *Mutation Res.*, 31: 9–15
- [13] Al-Sabti, K. and Metcalfe, C.D. (1995) Fish micronuclei for assessing genotoxicity in water. *Genet. Toxicol.*, 343: 121-135.
- [14] Statistical Analyses Systems (SAS) (2005). SAS Program ver. 9.12, SAS institute incorporation, Cary, NC 27513 USA.
- [15] Bonisolti-Alquati, A. (2014). Avian genetic ecotoxicology: DNA of the canary in a coalmine. *Current Zoology* 60 (2): 285–298, 2014
- [16] Galindo, T.P. and Moreira, L.M. (2009). Evaluation of genotoxicity using the micronucleus assay and nuclear abnormalities in the tropical sea fish *Bathygobius soporator* (Valenciennes, 1837) (Teleostei, Gobiidae). *Genetics and Molecular Biology*, 32(2): 394-398.
- [17] Torres-Bugarin O, Zavala-Cerna MG, Nava A, Flores-García A, and Ramos-Ibarra ML (2014). *Review Article: Potential Uses, Limitations, and Basic Procedures of Micronuclei and Nuclear Abnormalities in Buccal Cells. Disease Markers*, Volume 2014, Article ID 956835, 13 pages
- [18] Kadry M.A.M. and Mohamad. H.R.H. (2014). Genetic and metabolic variability between two subspecies of *Chamaeleo chamaeleon* (Reptilia: Chamaeleonidae) in Egypt. In press