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# Genetic and Histological Diversity between the Coastal and Desert *Chamaeleo chamaeleon* Subspecies in Egypt

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**Abstract:** ***The biodiversity between the two Chamaeleo chamaeleon (C.c.) subspecies (chamaeleon and musae) inhibiting the coastal and desert habitats of Egypt, respectively, was estimated using random amplification of genomic DNA (PCR-RAPD), histological and histochemical examinations. PCR-RAPD analysis using six arbitrary primers evidenced the genetic diversity. The observed slight histological variations including lightly stained neural nuclei and higher renal glomerulae and blood sinusoids in addition to higher pigment cells in C.c. chamaeleon than that observed in C.c. musae showed that C. c. chamaeleon subspecies is more active and adaptable to its coastal habitat. This was further evidenced by the observed higher histochemical localization of either lipid in C. c. chamaeleon or glycogen in C. c. musae.***

**Keywords:** Biodiversity, PCR-RAPD, *Chamaeleo chameleon chameleon*, *Chamaeleo chameleon musae* and histochemical localization.

## 1. Introduction

Chameleons are the old world lizard best known for their ability to change body colors easily in response to differences in temperature, light, and the chameleon's mood. Their body is flexible range in length from 1 inch (2.5 centimeters) to 26.8 inches (68 centimeters) that enable them to bend easily. Chameleons have large eyes that protrude, or stick out, long slim legs, with four feet, five toes on each foot and the tail is formed in a way to help the chameleon hold on to twigs and branches. Moreover, their sticky tongue can extend the length of its entire body, or even longer fast enough to catch a fly in midair **[1]**.

The Common Chameleon or *Chamaeleo chamaeleon* (family Chamaeleonidae) is insectivorous, capturing insect and even eat young chameleons and fruits. It is easily distinguishable from other lizards by their unique zygodactyl feet and laterally compressed body. Their adjacent digits are fused on each foot, forming opposing grasping pads **[2]**. Common chameleons are widely distributed like all chameleon species from Morocco and the southern Iberian Peninsula over the whole of North Africa, to the Near East, Turkey, Cyprus and Southern Arabia **[3]**.

The genus *Chamaeleo* contains 4 recognized subspecies: *C. c. chamaeleon*, *C. c. musae*, *C. c. orientalis*, and *C. c. rectricrista*. The two subspecies; *C. c. chamaeleon* and *C. C. musae* are allocated from North Africa, Middle East, Morocco, Algeria, Tunisia, Libya, Egypt, Israel, Palestine, Jordan, Western Sahara, Saudi Arabia, Yemen, Lebanon, Syria, Iraq, and Iran **[4]**.

In our previous studies metabolic and genomic variabilities between the two *Chamaeleo chamaeleon* subspecies; *C. c. chamaeleon* and *C. c. Musae* were indicated by the observed higher lactate dehydrogenase activity and protein and lipid accumulations in *C. c. chamaeleon* than *C. c. musae* **[5,6]** and now we are going for further investigations of the differences and variations between these two subspecies on higher levels.

Genetic variability is very important for the animals' survival maintainability by increasing its accommodation with the changeable environmental conditions and stress **[7-10]**. Despite, several techniques are used to analyze and study genetic variability, polymerase chain reaction - random amplified polymorphic DNA (PCR-RAPD) is the most widely, simple, cheap, speed and highly efficient used molecular tool in screening the genetic diversity in various populations **[11, 12]**. Moreover, genetic diversity of individuals affects the histological structure of various organs and their functions.

Thus this study was designed to investigate the molecular diversity and histological variability between the *C. c. chamaeleon* inhabiting the coastal desert and *C. c. musae* inhibiting the Sinai desert of Egypt using PCR-RAPD technique and histological and histochemical examinations of some tissues.

## 2. Materials and Methods

### Taxon Sampling and Study Area

A total of 10 samples 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] (**Figure 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.

**PCR-RAPD analysis**

Small portions of liver were removed and washed with cold PBS to remove excess blood and stored at −20˚C until used to study molecular variability between these two subspecies using RAPD-PCR analysis.

**Extraction of the genomic DNA**

Extraction of genomic DNA was performed using the GeneJET Genomic DNA Purification kit (Thermo Scientific #K0721, #K0722) according to the manufacturer's instructions. DNA quantity and purity were measured spectrophotometrically by reading the absorbance at 260 nm for DNA quantity and its purity was estimated by ratio of absorbance reading between 260 and 280 nm.

**PCR-RAPD Reactions**

Polymerase chain reactions for random amplification of the genomic DNA (PCR-RAPD) were performed using the previously designed six arbitrary primers by **Roehrdanz and Flanders [13]**. PCR mixture (25 µL) containing DNA sample (1 µL), primer (2 µL), master mix (9 µL) and water (13 µL) were initially denaturated at 92°C for 5 min, fourty cycles of denaturation (92°C for 30 sec), annealing (35°C for 1min) and extension (72°C for 2 min) and Finally extended at 72°C for 10 min to complete amplification in the Thermal Cycler (**Veriti® 96-Well Thermal Cycler**).

| List of primers used in PCR-RAPD | | |
|---|---|---|
| | Primer name | Primer sequence |
| 1 | C1 | TTCGAGCCAG |
| 2 | P13 | GGAGTGCCTC |
| 3 | N8 | ACCTCAGCTC |
| 4 | B12 | CCTTGACGCA |
| 5 | H5 | AGTCGTCCCC |
| 6 | P8 | GGAGCCCAG |

Final reactions products were electrophoresed on a 1.5% ethidium bromide-treated agarose gel (Sigma, UK) according to the standard protocol described by **Sambrook *et al*. [14]** and visualized using Gel documentation **(G:BOX) system (SYNGENE model 680XHR).**

**Analysis of RADP data**

The RAPD pattern of the amplified six primers were analyzed to study genetic diversity between the two studied *Chamaeleo chamaeleon* subspecies (*C. c. chamaeleon* and *C. c. musae*) by direct comparison of the amplified profiles. Data were scored by 1 for the presence and 0 for the absence of bands with various molecular weights in the form of binary matrix. Manually total RAPD bands, monomorphic bands, and polymorphic bands between two subspecies were calculated by directly scoring the RAPD amplified banding profiles from gel photographs. Genetic variability was estimated by calculating the genetic diversity and percentage of polymorphism. Using the SIMQUAL program, a common estimator of genetic similarity (Jaccard's coefficient) was calculated as follow S= *N*AB/(*N*AB + *N*A + *N*B), where *N*AB is the number of bands shared by samples, *N*A represents amplified fragments in sample A, and *N*B represents fragments in sample B. The genetic variabilities within and between chameleon populations were obtained as *V = 1 – S*.

**Histological and histochemical examination**

Small pieces of liver, kidney and brain were removed immediately, washed and fixed in neutral buffered formalin (10%) for further processing by the ordinary routine work: dehydration, clearing, embedding and finally sectioning into thin slices (4–5 µm thickness). After hematoxylin–eosin staining, the stained sections were evaluated by a histopathologist using light microscopy (U-III Multi-point Sensor System; Nikon, Tokyo, Japan). Histochemical localization of total lipid and glycogen was also done using Sudan Black and periodic acid Schiff's (PAS) reactions respectively.

## 3. Results

**PCR-RAPD**

Results of PCR-RAPD were summarized in **tables 1&2** and **figure 2**. Random amplification using six arbitrary primers resulted in total 97 (average 16.17) bands in *C. c. chamaeleon* and 76 (average 12.67) bands in *C. c. musae* **(Table 1 and Figure 2)**. The bands number ranged from 11 (H5) to 23 (B12) bands with molecular weight ranged from 250 to 2000 (C1), 300 to 1900 (P13), 250 to 2500 (N8), 160 to 2100 (B12), 320 to 1500 (H5) and 400 to 3000 (Pg) bp in *C. c. chamaeleon* and from 9 (N8 & H5) to 18 (B12) bands with molecular weight ranged from 140 to 2500 (C1), 300 to 1900 (P13), 550 to 2000 (N8), 160 to 2000 (B12), 300 to 1300 (H5) and 600 to 3000 (Pg) bp in *C. c. musae* **(Figure 2)**.

**Table (1): The banding pattern of random amplification (PCR-RAPD) using six arbitrary primers in *C. c. chamaeleon and C. c. musae*.**

| Primer name | *C. c. chamaeleon* | | | *C. c. musae* | | | C. c. (*chamaeleon* and *musae*) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Total number of bands | Total number of monomorphic bands | Total number of polymorphic bands | Total number of bands | Total number of monomorphic bands | Total number of polymorphic bands | Total number of bands | Total number of monomorphic bands | Total number of polymorphic bands |
| C1 | 12 | 8 | 4 | 11 | 7 | 4 | 14 | 8 | 6 |
| P13 | 19 | 11 | 8 | 15 | 9 | 6 | 21 | 12 | 9 |
| N8 | 15 | 8 | 7 | 9 | 4 | 5 | 17 | 8 | 9 |
| B12 | 23 | 11 | 12 | 18 | 11 | 7 | 26 | 12 | 13 |
| H5 | 11 | 7 | 4 | 9 | 6 | 3 | 13 | 7 | 6 |
| P8 | 17 | 10 | 7 | 14 | 9 | 5 | 19 | 10 | 9 |
| Total | 97 | 55 | 42 | 76 | 46 | 30 | 110 | 57 | 52 |
| Average | 16.17 | 9.17 | 7.00 | 12.67 | 7.67 | 5.00 | 18.33 | 9.50 | 8.67 |

Table 2: Percentage of polymorphic loci and genetic diversity within and between *C. c. chamaeleon* and *C.c. musae*.

| Primer name | % polymorphism | | | Genetic diversity | | |
|---|---|---|---|---|---|---|
| | *C. c. chamaeleon* | *C. c. musae* | C. c. (chamaeleon and musae) | C. c. chamaeleon | C. c. musae | C. c. (chamaeleon and musae) |
| C1 | 33.33 | 36.36 | 42.86 | 0.38 | 0.27 | 0.37 |
| P13 | 42.10 | 40.00 | 42.86 | 0.27 | 0.15 | 0.54 |
| N8 | 46.67 | 55.55 | 52.94 | 0.31 | 0.33 | 0.76 |
| B12 | 52.17 | 38.89 | 50.00 | 0.37 | 0.33 | 0.55 |
| H5 | 36.36 | 33.33 | 46.15 | 0.22 | 0.27 | 0.42 |
| P8 | 41.18 | 35.71 | 47.37 | 0.15 | 0.25 | 0.44 |
| Average | 41.97 | 39.97 | 47.03 | 0.28 | 0.27 | 0.51 |

The total number of monomorphic was 55 (average 9.17), ranged from 7 (H5) to 11 (P13 & B12) and that of the polymorphic bands was 42 (average 7.29), ranged from 4 (C1) to 12 (B12) in *C. c. chamaeleon*. While, the total number of monomorphic was 46 (average 7.67), ranged from 4 (H5) to 11 (B12) and that of the polymorphic bands was 30 (average 5.00), ranged from 3 (H5) to 7 (B12) in *C. c. musae* **(Table 1)**.

The total number of scored shared RAPD fragments between the two studied *C. chamaeleon* subspecies was 110 (average 18.33), ranged from 13 (H5) to 26 (B12); the scored number of monomorphic bands was 57 (average 9.50), ranged from 7 (H5) to 12 (P13 & B12) and that of the polymorphic bands was 52 (average 8.67), ranged from 6 (C1 & H5) to 13 (B12) **(Table 1)**.

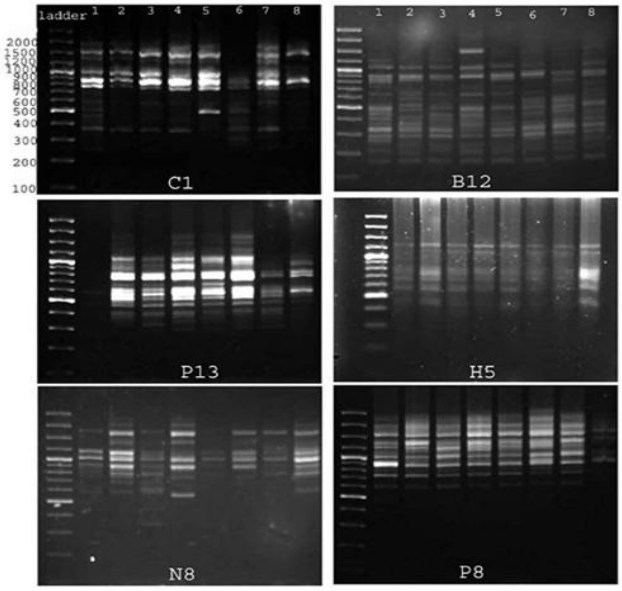


Fig. 2: PCR-RAPD fragments of the genomic DNA of *C.c. chamaeleon* (lanes 1-4) and *C.c. musae* (lanes 5-8) electrophoresed on 1.5% agarose gel using six arbitrary primers.

The average percentage of polymorphic loci was 41.97 (33.33-52.17) in *C. c. chamaeleon*, 39.97 (33.33-55.55) in *C. c. musae* and 47.03 (42.86-52.94) between these two subspecies **(Table 2)**. Indeed, the estimated genetic diversity depending on genetic similarity was 0.28 (0.15-0.38) in *C. c. chamaeleon*, 0.27 (0.15-0.33) in *C. c. musae* and 0.51 (0.37-0.76) between the two *C. chamaeleon* subspecies **(Table 2)**.

**Histological and histochemical examination**

As shown in **Figure 3**, brain, kidney and liver tissues of both *C. c. chamaeleon* and *C. c. musae* have the basic characteristic histological structure with slight variations. Nuclei of neural cells of *C. c. musae* are most intensively stained compared with that of *C. c. chamaeleon*. Higher number of *glom*erulae and Bowman's capsules was observed in *C. c. chamaeleon* kidney and finally the blood sinusoids and pigment cells observed in *C. c. chamaeleon* was higher than that observed in *C. c. Musae*. Histochemical localization of lipid and glycogen showed that lipid is highly widely distributed in *C. c. chamaeleon* in reverse to its localized accumulation in *C. c. musae*. On contrary glycogen accumulation was mild in *C. c. chamaeleon* and sever in *C. c. musae* **(Figure 4)**.

## 4. Discussion

Genetic variability increases the stability of ecosystem functions through time by allowing the generation of new traits and providing successful progress in populations' adaptations with their natural environment **[15].** Therefore, the present study was designed to study the genetic diversity between the two *C. chamaeleon* subspecies; *C. c. chamaeleon* and *C. c. musae*. Histological and histochemical variations were also investigated between them.

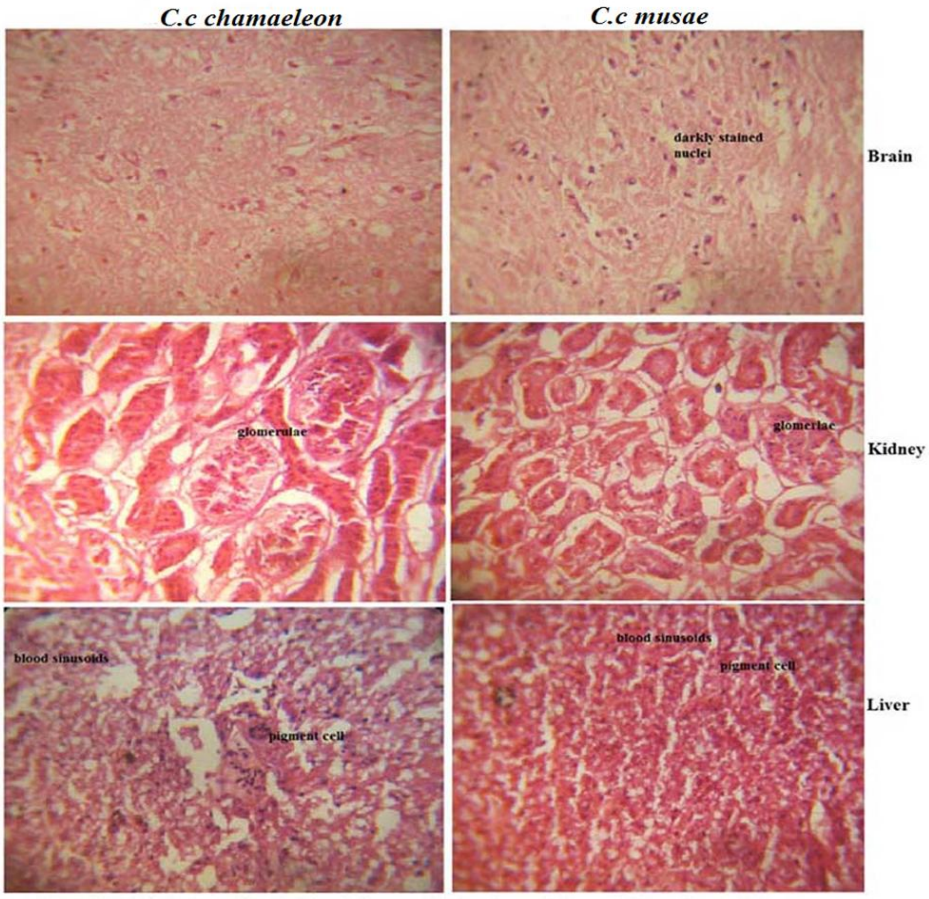


**Fig. 3: Histological examination of liver, kidney and brain tissues of both *C.c chamaeleon* and *C. c musae***

The pattern of PCR-RAPD using six arbitrary primers evidenced the genetic diversity between *C. c. chamaeleon* inhibiting El-Dabaa (Marsa Matrouh) and *C. c. musae* inhabiting El-Arish (North Sinai) of Egypt by the observed high percentage of polymorphic loci and genetic diversity index **(Table 2)** in a harmony with our previous study **[5]** which evidenced the genetic variability between *C. c. chamaeleon* and *C. c. musae* using polyacrlamide gel electrophoresis. Indeed, low genetic variations were observed within any of the two subspecies by the observed low genetic diversity index **(Table 2)**.

Our examination of the liver, brain and kidney histological structures revealed histological variations between the two studied *Chamaeleo chamaeleon* subspcies (*chamaeleon* and *musae*). These histological variations evidenced that *C. c. chamaeleon* is more active and adaptable to its coastal habitat than *C. c. musae*. Indeed, higher pigmentation of *C. c. chamaeleon* enable it for more coloration that increasing its adaptability. This evidence was further supported by our finding of higher histochemical accumulation of either lipid in *C. c. chamaeleon* or glycogen in *C. c. musae* **(Figure 4)**. These results were supported by our previous finding of higher hepatic total lipid and proteins and lactate dehydrogenase activity in *C. c. chamaeleon* than that in *C. c. musae* **[5]**.

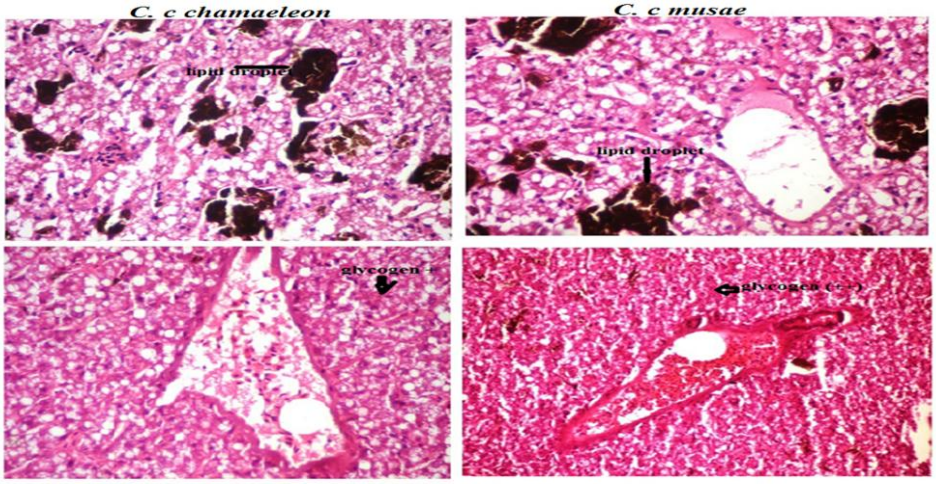


**Fig. 4: Histochemical localization of lipid and glycogen in *C. c chamaeleon* and *C.c musae***

## 5. Conclusion

Our results of PCR-RAPD evidenced the genetic diversity between the two studied *C. chamaeleon* subspecies (*chamaeleon and musae*). However, histological and histochemical variations between these two subspecies showed that *C. c. chamaeleon* is more active and adaptable to its coastal habitat than *C. c. musae* that inhabiting desert habitat.

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