



## Clonal Analysis and Genetic Diversity of Cat Breeds from both Local and Foreign Breeds

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### Abstract

**S**TUDYING the genetic diversity of cat populations provides insights into their evolutionary history, migration patterns, and population dynamics. In order to clarify the genetic relationships between cat breeds, a total of 17 cat species (8 local and 9 imported from foreign countries) were subjected to blood samples, and whole genomic DNA samples were extracted from their RBCs. “Random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR)” was performed to fingerprint their genomes. The resulting gel DNA bands were analysed using GelJ software. The clustering of cat species was performed according to their type and country of origin. Two (groups were identified (groups 1 and 2) with 13 genotypes, in which cluster 1 contained all local species while the other cluster was grouped with all imported species (the similarity between the two groups were 53%). Most of the local cat species were genetically similar at 100%. While most of the cats that were imported were genetically diverse (most of them were grouped under different genotypes), The study suggests the genetic mixing of local species resulted from mating with each other, while reduced opportunities for mating between imported and local cats resulted in limited genetic relatedness. Understanding the factors that influence mating capabilities and genetic mixing among local and imported cat species is essential for managing and conserving these populations effectively. It highlights the complexities of interspecies interactions and underscores the importance of considering ecological, behavioral, and genetic factors in conservation planning and management strategies.

**Keywords:** Clonal analysis; cat breeds; genetic factors; RAPD-PCR.

### Introduction

The wide variety of genetic variations among them, cats, which comprise a large number of species and breeds, display a remarkable range of morphological, physiological, and behavioral traits [1]. Species-specific features that are suited for survival in a variety of settings have been shaped by evolutionary forces, including environmental adaptations and predator-prey dynamics. On the other hand, several varieties of domestic cats with distinctive phenotypic traits and temperaments have resulted from centuries of artificial selection applied to their wild ancestors [1]. Cat populations have an intricate genetic diversity, which is a result of the interaction of natural selection, genetic drift, and gene flow. Furthermore, thorough investigations of feline genomes have been made possible by recent developments in genomic technologies, providing insight into the genetic basis of a variety of

characteristics, including coat color and pattern, disease susceptibility, and behavioral proclivities (Xu et al., 2016). Comprehending the subtleties of feline genetic variation is crucial for conservation initiatives, veterinary care, and animal welfare, highlighting the significance of multidisciplinary research projects attempting to decipher felid genomic complexity [2].

Normal cellular functions and interactions with the environment can induce mutations in all organisms, resulting in genetic variety or polymorphism. A species requires variation in genetics so as to survive and be able to respond to its changing surroundings. Variation in genes occurs between individuals and contributes to populations, different species, and more advanced biological group divergence as well as to other mechanisms of evolution like mutation and selection [3]. Molecular markers and the creation of new statistical methods have completely changed

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the analytical capacity required to investigate genetic variation in both native populations and captive groups. Many novel molecular methods have recently been investigated and documented for cat [4]. Initially, the Random amplified polymorphic DNA (RAPD) method was presented by Williams and colleagues [5]. One of the most widely used molecular techniques for taxonomic and systematic assessments of different organisms is the RAPD technique, which has had significant benefits diversity [5]. Even with endangered species, RAPD has been utilized to quantify the genetic diversity and variability required to research cat management and conservation techniques [6]. Its foundation is the PCR amplification of specific genomic areas using arbitrary-sequence short oligonucleotide primers. For genetic investigations, the features evaluated by RAPD are helpful since they offer a variety of data, such as taxonomic population and inheritance pattern of different organisms, including cats [7]. For taxonomic studies, knowledge of cat morphometric measures and the analysis of their statistical relationships are crucial. Morphometric features are also often employed to identify commercially important cat species and to determine the origin of stocks or the separation of stocks [8]. Understanding the interplay between environment, selection, and heredity on the body sizes and forms within a species is facilitated by these kinds of research. Numerous research have been carried out to compare the morphometrics of various cat populations [9].

This technique was used in the current study to examine the genetic relationships among populations of different local and imported foreign cats, as there has been relatively little research on the genetic diversity of feline by utilizing Random Amplified Polymorphic DNA, this study aims to investigate genetic variety.

## **Material and Methods**

### *Cat breeds and blood sample collection*

A blood samples of 17 cats; 8 local, and 9 foreign cats (4 Van, 2 Shirazi, 2 Himalayan and 1 Scottish) from different Veterinary clinics in Duhok city were taken with care. One ml blood from each cat were collected from saphenous vein and putted into heparinized tubes and transported to the "Duhok research Centre, college of Veterinary Medicine, University of Duhok" in ice box in stored at -20 °C for subsequent DNA extraction [10].

### *DNA extraction and RAPD PCR*

The DNA was extracted by using "AddPrep Genomic DNA Extraction Kit" (AddBio /Korea) according to the manufacture instruction. Using primer sequences "(RAPD 1: 5'-

GGTGCGGGAA-3' and RAPD 2: 5'-AACGCGCAAC -3')", this was used to find similar cat breeds and discover variations described by Velasco-Ramírez et al. [11]. Two microliters of each primer (10 pmol/μl), 12.5 microliters of "hot start master mix" (AddBio, Korea), two microliters of DNA at an average concentration of 50–100 ng/μl, and 6.5 microliters of "nuclease-free water (Qiagen, Germany)" were used in the PCR reaction, which had a total volume of 25 μl. The "PCR system 9700 GeneAmp (Applied Biosystem, USA)" was used to carry out the PCR. The "PCR setting" was utilized with minor modification according to Erfanmanesh et al. [12], in which the annealing temperature was increased to 48 °C. At last, the PCR products were labelled using a "red safe DNA staining solution (GeNetBio, Korea)" and placed into a 1.5% agarose with "1X Tris-acetate-EDTA (TAE) buffer". A 100-bp DNA ladder "(Genedirex, Taiwan)" was employed to find the band sizes [13].

### *Clonal Relatedness and Diversity Analysis of cat species*

After first observing an electrophoresis image using 17 different species of cats to confirm the presence of the DNA band in the "RAPD gel", the dendrogram was created using the "GelJ software version 2.0 (available at <https://sourceforge.net/projects/gelj/>)". Using a tolerance of 1% and the "Dice similarity coefficient, the Unweighted Pair Group Method with Arithmetic Mean (UPGMA)" methodology was used to establish the species typing [14]. For clustering of DNA fingerprint in RAPD-PCR, those with similarity limits of 90% or more were assigned to identical genotypes or group. The clustering of cat species was performed according to their type and country of origin.

## **Results**

The results demonstrated that the matching ranged from 53 to 100% depending on both the number and size of "RAPD sequence" varieties as well as the RAPD-PCR profiling analysis observed across all species. Two clusters were identified (cluster 1 and 2) with 13 genotypes, in which the cluster 1 have contained all the 8 local species, while the other cluster were grouped with all 9 imported species (similarity between the two cluster was 53%) (Fig.1).

Most of the local species in cluster 1 were genetically similar at 100% (species No. 17 with 16; 15 with 14; 13 with 12). For the imported species, only two of them were classified under the same genotype (No. 1 with 7 from Van, Turkey, and 1 from Scotland), while all the others

were genetically diverse (most of them were grouped under different genotypes) (Fig.1).

### **Discussion**

There are several reasons of determining the cat's genetic similarity or diversity; one of these is to help breeders to make informed decisions about the breeding programs in which breeding cats with similar genetic backgrounds can help maintain desirable traits within a breed while minimizing the risk of genetic disorders caused by inbreeding [15]. Other reason it can aid in identifying genetic predispositions to certain diseases or conditions within specific cat populations [16]. Conservation efforts are another important reason for making the cat's diversity, this will reduce the risk of inbreeding depression, ultimately ensuring the long-term survival of the species [17]. The last one is related for forensic investigations, such as identifying lost or stolen pets [18].

For the reasons listed above, the purpose of this study was to ascertain the degree of genetic diversity among various cat species breeds, both native as well as frequently imported from foreign countries, and whether or not there was a breeding relationship between them.

In this study, there was a lot of genetic relatedness in the local cat's species; this is an indication that the possibility of mating is high. Generally, cats in our area live in outdoor enclosures that provide a stress-free environment, and this may provide a comfortable space for mating, which reduces the stress level and increases the likelihood of successful mating [19, 20]. During mating, male cats transfer sperm containing genetic material (DNA) to female cats. This genetic material combines with the female's genetic material, resulting in the creation of offspring with a unique combination of genetic traits inherited from both parents [15].

There was a considerable genetic diversity between the local and foreign cat breeds. This could be attributed to the fact that imported foreign cat species may be physically isolated from local cat populations, either through geographic barriers or human intervention. This isolation can prevent opportunities for mating and gene flow between the two groups [21].

In contrast to the local species, most of the imported foreign ones showed considerable genetic diversity. This could be attributed to several factors. One is related to the introduction of unrelated cats into

breeding programs helps increase genetic diversity by bringing new alleles into the population [22]. Another reason is related to habitat diversity, in which cats living in diverse habitats are exposed to different selective pressures, leading to the development of diverse genetic adaptations. Preserving various habitats and ecosystems ensures that cat populations have access to a range of environmental conditions, promoting genetic diversity [23]. Another factor is concerned with migration and gene flow. The movement of individuals between populations, either naturally or through human intervention, facilitates gene flow and increases genetic diversity. Migration helps prevent isolated populations from becoming genetically homogeneous [24]. In general, our sampled cats were imported from diverse countries, and this may give them a greater chance of having such genetic heterogeneity.

### **Conclusion**

By assessing the genetic similarity of cat species, researchers, breeders, conservationists, and healthcare professionals can make informed decisions to promote the health, diversity, and welfare of cat populations, both domestic and wild. Additionally, understanding the genetic makeup of cats enhances our appreciation of their evolutionary heritage and contributes to their conservation and management in the face of ongoing environmental challenges.

### ***Ethical approval***

This study was approved by the ethical committee of the College of Veterinary Medicine, University of Duhok, Duhok, Iraq, with reference number VM2023/1503UD; date: March 15, 2023.

### ***Acknowledgment***

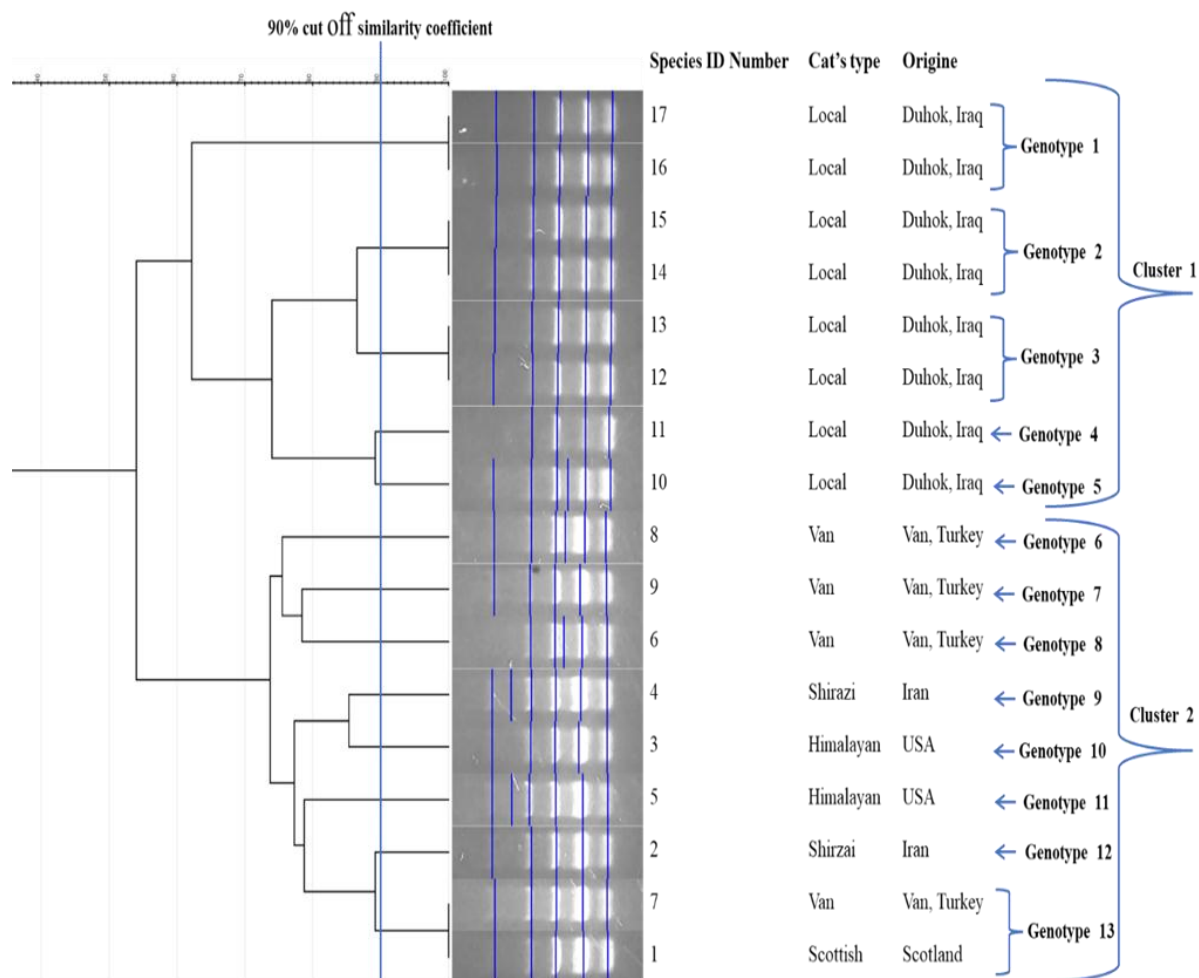
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### ***Conflict of interest***

No conflict of interest.

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**Fig. 1.** A dendrogram obtained from RAPD-PCR shows the banding pattern of 17 cat species from both native and imported countries.

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## التحليل النسيلى والتنوع الوراثي لسلاسل القطط من السلالات المحلية والأجنبي

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### الملخص

توفر دراسة التنوع الجيني لمجموعات القطط نظرة ثاقبة حول تاريخهم التطوري، وأنماط الهجرة، وديناميكيات السكان. من أجل توضيح العلاقات الوراثية بين سلالات القطط، تم إخضاع 17 نوعاً من القطط (8 محلية و9 مستوردة من دول أجنبية) لعينات الدم، وتم استخلاص عينات الحمض النووي الجينومي الكامل من كرات الدم الحمراء الخاصة بها. تم إجراء "تفاعل سلسلة بوليميراز الحمض النووي متعدد الأشكال العشوائي" RAPD-PCR " (لأخذ بصمات الجينوم الخاص بهم). وقد تم تحليل نطاقات الحمض النووي الهلامية الناتجة باستخدام برنامج GelJ. تم تصنيف أنواع القطط حسب نوعها وبلدها الأصلي. تم تحديد مجموعتين (المجموعتين 1 و 2) تحتويان على 13 تركيبياً وراثياً، حيث ضمت المجموعة الأولى جميع الأنواع المحلية بينما تم تجميع المجموعة الأخرى مع جميع الأنواع المستوردة (بلغ التشابه بين المجموعتين 53%). معظم أنواع القطط المحلية كانت متشابهة وراثياً بنسبة 100%. في حين أن معظم القطط التي تم استيرادها كانت متنوعة وراثياً (معظمها تم تجميعها تحت أنماط وراثية مختلفة)، تشير الدراسة إلى أن الاختلاط الوراثي للأنواع المحلية نتج عن قدرتها على التزاوج، في حين انخفضت فرص التزاوج بين القطط المستوردة والمحلية أدى إلى محدودية الارتباط الوراثي. إن فهم العوامل التي تؤثر على قدرات التزاوج والاختلاط الجيني بين أنواع القطط المحلية والمستوردة أمر ضروري لإدارة هذه المجموعات والحفاظ عليها بشكل فعال. وهو يسلط الضوء على تعقيدات التفاعلات بين الأنواع ويؤكد على أهمية مراعاة العوامل البيئية والسلوكية والوراثية في تخطيط الحفظ واستراتيجيات الإدارة.