Phylogenetic Relationships Among Japanese Quail Phenotypes Based On RAPD and ISSR Molecular Markers

Ahmed Mansour[1]*and Iman E. El-Araby[2]

[1]Genetics Department, Faculty of Agriculture, Zagazig University, Zagazig, Egypt.

[2]Animal Wealth Development Department, Fac. of Vet. Med., Zagazig Univ., Zagazig, Egypt.

*For correspondance: alzohairy@yahoo.com ; amansour@zu.edu.eg

ABSTRACT

Japanese quail is an important poultry bird in Egypt and allover the world. Since its immigration to Egypt, new breeds with new phenotypes were appeard. For improving quail production and its attributes as poultry bird, different phenotype and genotype are often produced and evaluated under different conditions. In this regards, utilization of molecular marker analysis has provided breeders with new insights for molecular assisted selection (MAS). Depending on the marker system used, the genetic similarity analyses varied dramatically. Knowledge about genetic diversity and phylogenetic relationships among breeding quail could be an invaluable aid in improvement strategies of such poultry birds

In this report, genomic variation within four isolated phenotypes of Japanese quail in Egypt, were investigated using two different molecular marker systems which targets different genomic regions; RAPD (random amplified polymorphic DNA) and ISSR (inter-simple sequence repeat). Different dendrograms were constructed based on RAPD and ISSR results individually and collectively revealed that similarity and clustering is much dependant on the markers system used.

INTRODUCTION

The Japanese quail belongs to the order Galliformes, genus coturnix, and species japonica. The scientific designation for Japanese quail is *Coturnix coturnix japonica* *(1)* and now renamed *Coturnix japonica (2)*. The Japanese quail is different from the common quail (*Coturnix coturnix*) and found in Japan, Korea, Eastern China, Mongolia and Sakhalin as migrating birds.

Japanese quail (*Coturnix japonica*), as the chicken (*Gallus gallus*), belongs to the order Galliformes and the family Phasianidae. Both species have a similar genome size and karyotype. Japanese quail karyotype composed of 2n= 78 chromosomes, some of which are morphologically distinguishable macro chromosomes , 1-8 and the ZW sex chromosomes, and the rest are indistinguishable micro chromosomes *(3)*. Moreover, Japanese quail, as poultry bird, is appreciated for meat and eggs as well as a valuable laboratory species because of its small body size, short generation interval and high prolificacy.

Genetic analyses were performed with plumage color and plumage color mutations (roux, brown and white) in Japanese quail. Roux and brown quail have similar plumage color, but roux quail is paler. Three plumage color loci have been reported on the Z chromosome of the Japanese quail. The first locus (AL) has two kinds of mutations: imperfect albinism *(4-6)* or sex-linked white *(7, 8)*, and red-eyed brown (8) or cinnamon *(6, 9, 10)*. The second locus has only one mutation, brown *(6, 8, 11)*, which is caused by a recessive allele. Another sex-linked plumage color mutation roux has been independently discovered in France and is reported to be controlled by a recessive gene *(6)*.

Knowledge about studying genetic diversity and phylogenetic relationships among quail phenotypes could be an invaluable aid in its improvement strategies. A number of methods are currently available for analyses of genetic diversity in breeding strains and populations. These methods have relied on pedigree data, morphological data, performance data, biochemical data and more

recently molecular (DNA-based) data. With DNA-based markers, it is theoretically possible to exploit the entire diversity in DNA sequence that exists in any cross *(12)*. Molecular typing methods provide an opportunity for a powerful and reproducible approach of estimating relatedness within and among genotypes based on DNA sequence variation. Different types of locus specific markers are available, i.e., minisatellite, microsatellite *(13, 14)* and random amplified polymorphic DNA (RAPD) *(15)*.

Microsatellites, also known as simple sequence repeats (SSRs), are tandem repeats of 1-6 bases in length that occur abundantly and at random in most eukaryotic genomes and co dominance mode of inheritance. Microsatellites can be typed using the polymerase chain reaction (PCR), have made them the markers of choice in genome mapping and linkage analyses **(16)** as well as analyses of genetic diversity and evolution of animal genomes *(17-19)*.

Random amplified polymorphic DNA (RAPD) technique provides a powerful tool for identification of populations and detection of genetic variability within and among populations. The RAPD procedure is relatively simple, fast, inexpensive and without requirement for target DNA sequence information *(20)*. However, the potential of the original RAPD assay to generate polymorphic DNA markers with a given set of primers was further increased by combining two primers in a single PCR and three sets of primers *(21)*.

In this investigation, we study genomic variation within four isolated phenotypes of Japanese quail in Egypt using two different molecular marker systems; RAPD (random amplified polymorphic DNA) and ISSR (inter-simple sequence repeat) to reveal similarity and evolution of Japanese quail in its new inhabitant in Egypt.

MATERIAL AND METHODS

Quail genotypes

A total of four quail genotypes were used in the present study. This includes, Plumage color quail wild type, Roux color quail (mutant type), Brown color quail (mutant type) and White color quail (mutant type).

All bird samples were phenotypically normal and healthy. The studied quails birds were obtained from Faculty of Agriculture, El-Azhar University.

Blood samples

Blood samples from different genotypes were collected from wing vein of individual birds, blood samples for DNA extraction were collected in plastic tubes containing EDTA and stored at -20°C.

DNA extraction

DNA samples were extracted from blood samples using (Promega Genomic Wizard kit) following the manufacturer instructions. method followed by an RNase-A treatment (Sigma, St. Louis, MO; R-4875) for 30 min at 37°C in each case *(21)*. The quality and quantity of extracted DNA was measured (2 µl) by a NanoDrop ND-1000 UV-Vis spectrophotometer (NanoDrop Technologies, Delaware, and USA). DNA samples were adjusted to a concentration of 50 ng/µl with ddH2O before subjected to PCR amplification.

RAPD PCR reactions

Amplification reactions were performed *(15)* in volumes of 25 µl. Briefly the reaction mixture containing 10 mM Tris-Cl, pH 8.3, 50 mM KCl, 2 mM $MgCl_2$, 0.001% gelatin, 100 µM of each dATP, dCTP, dGTP and TTP (Pharmacia), 0.2 µl primer, 25 ng of genomic DNA, and 0.5 unit of *Taq* DNA polymerase (Promega). The amplification was performed in a Perkin Elmer 2400 Thermal Cycler programmed for 5 minutes at 94°C followed by 40 cycles of 1 min at 94°C, 1 min at 34°C, 2 min at 72°C, using the fastest available transitions between each temperature (ramp time), followed by one cycle of 72°C for 20 min; and 4°C thereafter. The annealing temperature varied according to the melting temperature (™) for the primer (Table 1). The core program increased from 40 to 45 cycles, if amplification was weak, to get a slight increase in the amount of PCR products *(21)*.

ISSR PCR reactions

A set of 15 anchored microsatellite primers was procured from Metabion, Germany, (Table 1). PCR amplification was performed according to *(23)*. Briefly, 20 ng of DNA was added with 10 mM Tris-HCl pH 7.5, 50 mM KCl, 1.5 mM $MgCl_2$, 0.5 mM spermidine, 2% formamide, 0.1 mM dNTPs, 0.3 uM primer and 0.8 U of *Taq* DNA polymerase (Promega Taq DNA polymerase, USA) in a 25 µl reaction using Perkin Elmer 2400 thermocycler. All the chemicals required for the reaction mixture were obtained from (Sigma-Aldrich, USA). After initial denaturation at 94°C for 5 min, each cycle consisted of 30 sec denaturation at 94°C, 45 sec of annealing at 50°C, 2 min extension at 72°C along with 5 min extension at 72°C at the end of 40 cycles. It is worth mentioning that the annealing temperature varied according to the melting temperature (TM) of each primer. Moreover, the core program increased from 40 to 45 cycles, if amplification was weak, to get a slight increase in the amount of PCR products.

Table 1. Sequence of the RAPD and ISSR primers applied

RAPD	Sequences (5′- 3′)	ISSR	
P1	GTA GAC CCG	**814**	$(CT)_8$TG (#814)
P2	GGA CCC TTAC	**844A**	$(CT)_8$AC (#844A)
P3	GTC GCC GTC A	**844B**	$(CT)_8$G (#844B)
P4	GGT CCC TGA C	**17898A**	$(CA)_6$AC(#17898A)
P5	TGG ACC GGT G	**17898B**	$(CA)_6$GT (#17898B)
P6	AGG GGT CTT G	**17899A**	$(CA)_6$AG (#17899A)
P7	TTC CCC CGC T	**17899B**	$(CA)_6$GG (#17899B)
P8	TTC CCC CCA G	**HB8**	$(GA)_6$GG (#HB8)
P9	ACT TCG CCA C	**HB9**	$(GT)_6$GG (#HB9)
P10	CAA TCG CCG T	**HB10**	$(GA)_6$CC (#HB8)
P11	AGG GAA CGA G	**HB11**	$(GT)_6$CC (#HB11)
P12	TGC GCC CTT C	**HB12**	$(CAC)_3$GC(#HB12)
P13	TTC GCA CGG G	**HB13**	$(GAG)_3$GC (#HB13)
P14	GTG AGG CGT C	**HB14**	$(CTC)_3$GC (#HB14)
P15	CAA ACG TCG G	**HB15**	$(GTG)_3$GC (#HB14)
P16	CTG CTG GGA C		
P17	GTG ACG TAG G		
P18	CCA CAG CAG T		
P19	TGA GCG GAC A		
P20	GTG AGG CGT C		

Gel electrophoresis

Amplified fragments, 10 µl, were separated by agarose (1.6%) gel electrophoresis, stained with ethidium bromide (0.5 ng/µl) at 80 V in 1X TBE buffer and photographed on a UV transilluminator (Pharmacia) by Canon S5 digital camera with UV filter adaptor. A negative control which contained all the necessary PCR components except template DNA was included in the PCR runs.

Data analysis

Fragment analysis was conducted *(21)*. Briefly, amplification products were scored independently as 1 and 0 for presence and absence of bands, respectively, and the obtained binary data were used for the analyses. Only sharp PCR fragments were scored. Fragments at low intensities were only scored as present when they were reproducible in repeated experiments using GelAnalyzer 3 (Egygene) software. Unequivocally reproducible bands were scored and entered into a binary character matrix (1 for presence and 0 for absence). The genetic similarity among accessions was determined by Nei's genetic distance *(24)* modified to accommodate dominant (e.g., RAPD-like) markers. A dendrogram was constructed based

on the matrix of distance using Unweighted Pair Group Method with Arithmetic averages (UPGMA). All the calculations were performed using the NTSYS-pc 2.02 software package (Numerical Taxonomy System, Exeter Software) *(25)*. The statistical stability of the clusters was estimated by a bootstrap analysis with 1000 replications using Winboot software *(26)*.

RESULTS AND DISCUSSION

Genetic diversity based on RAPD markers

Single, random oligodeoxyribonucleotide primers were used to generate PCR-amplified fragment markers, from bulked genomic DNA of each genotype. The twenty primers from the initial screening process were listed in Table 1 that are highly variable in DNA sequence and therefore chosen for this study. A fragment was considered polymorphic when absent in at least one genotype. These primers detected scoreable polymorphisms in banding patterns among the four quail genotypes Fig. 1. All Polymorphic RAPD products were confirmed by repeating the PCR reaction. Each of twenty primers used for analysis of individual quail genotype amplified different number of bands. The average numbers of amplified fragments from all primers for all genotype are summarized in Table 2. The RAPD technique was successfully used to detect individual variation and estimate relatedness within and between populations *(27, 28)*. The high frequency of species-specific fragments may be due to the random primers annealing to different forms of the same or similar gene *(29)*.

Phylogenetic Relationships among Quail genotypes based on amplified RAPD fragments (bands)

The potential application of RAPD analysis to relationships among quail genotype was assessed through cluster analysis of these four different genotypes, with large number of scorable polymorphic markers from twenty different random primers. The dendrogram of genetic distances among quail genotypes based on band polymorphisms generated by RAPD-PCR after using all primer is shown in Fig. 2. This dendrogram clusters the quail genotypes into three clusters which are given in Table 3. Cluster III was the largest having two genotypes, while clusters I and II had single genotype. RAPD markers were used to detect polymorphism in various quail lines which were found to be effective and 31.7% of primers were found to be polymorphic *(30)*.

Genetic diversity based on ISSR markers

The genetic diversity (allele frequency, mean polymorphic information content (PIC) of preserved population of 4 genotypes of quail was analyzed by 15 microsatellite markers, and exhibited high polymorphism Fig. 3. The clustering dendrogram was obtained eventually based on data of 15 microsatellite primers. The results showed that the average number of alleles of each locus was 10 ± 2 and the range of allele frequencies was 0.333 and 4 genotypes of quail shared 13 ± 2 alleles as average number of each locus. The average Polymorphic (without unique) of 15 microsatellite loci was 3 ± 2 (Table 2). The dendrogram of genetic distances among quail genotypes based on band polymorphisms generated by RAPD-PCR after using all primer is shown in Fig. 4. This dendrogram clusters the quail genotypes into two clusters which are given in Table 4. Cluster II was the largest having three genotypes, while clusters I had single genotype. Several studies *(31-34)* showed that 28% of primers were polymorphic and results indicated that the usefullness of microsatellite markers to characterize the quail genome. The development of a genetic map for this economically valuable species would facilitate eventual construction of a comparative genetic map in Phasianidae, which comprises a number of important species of poultry.

Genetic diversity of quail genotypes as determined by combined analysis of ISSR and RAPD band differences

The dendrogram produced from genetic distances between quail genotypes is shown in Fig. 5. The linkage dendrogram provide visual idea about clustering and variability present in the population. The grouping pattern and

distribution of quail genotypes into different clusters is given in Table 5. Cluster II was the largest having three genotypes, while clusters I had single genotype.

CONCLUSION

Depending on the consumer's needs and desires, quail breeder usually prefers to breed one color than others. Thus, it is important to understand color development and evolution of quail inside its new habitat. However, the plumage color phenotype was the original color from which the other colors were segregated, results from RAPD and ISSR markers indicated that Roux, and White mutants quail phenotypes are genetically closer to Plumage than the Brown mutant phonotype. This result reveals that some of the quail segregates with different colors is more genetically closer to the original plumage color which they have originally evolved from. The genetic basis and inheritance of those different colors is yet to be studied extensively. However, more research still needed to explain the genetic basis behind the development of one color to another. In this case, the brown color seemed to be very distant phonotype from the original.

Table 2. Comparison of DNA marker systems in quail phenotypes

Marker system	№ of Primers	Gel polymorphism			Average № of Bands / primer	Polymorphism (%)	Mean of band frequency
		Polymorphic (without unique)	Unique bands	Polymorphic (with unique)			
Single-primer RAPD	20	8 ± 2	17 ± 2	25 ± 2	20 ± 2	85.31	0.361
ISSR	15	3 ± 2	13 ± 2	15 ± 2	10 ± 2	94.10	0.333
Total ≈	35	11	30	40	30		0.694

Table 3. Grouping pattern of the studied four quail genotypes based on analysis of their RAPD band differences using all primers.

Cluster	Number of genotypes	Quail genotypes falling in cluster
I	1	Brown
II	1	Roux
III	2	Plumage and White

Table 4. Grouping pattern of the studied four quail genotypes based on analysis of their ISSR band differences using all primers.

Cluster	Number of genotypes	Quail genotypes falling in cluster
I	1	Brown
II	3	Roux, Plumage and White

Table 5. Grouping pattern of the studied four quail genotypes based on analysis of their ISSR and RAPD band differences using all primers.

Cluster	Number of genotypes	Quail genotypes falling in cluster
I	1	Brown
II	3	Roux, Plumage and White

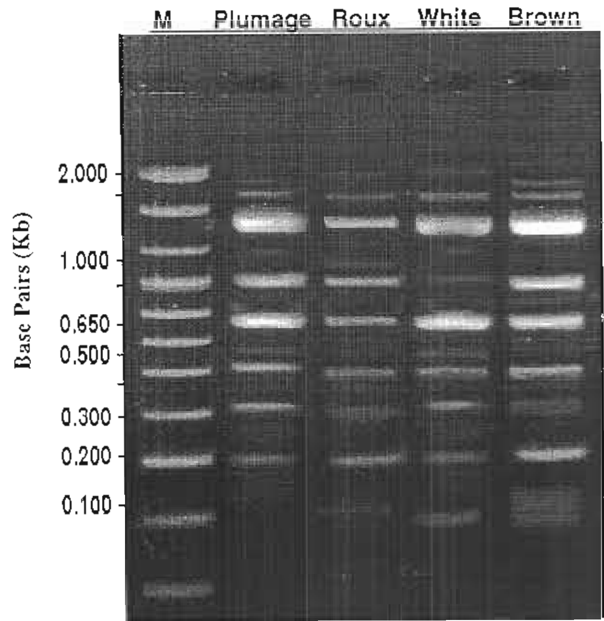

Fig. 1. Ethidium bromide stained RAPD fragments using bulked genomic DNA extracted from the quail breeds.

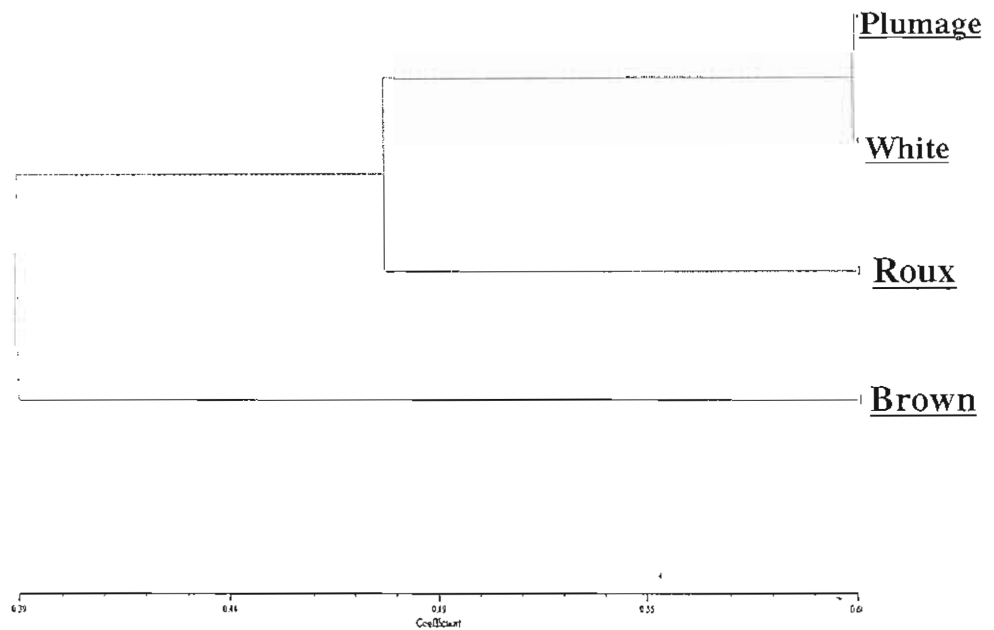

Fig. 2. Linkage dendrogeram of studied 4 *quail* breeds based on RAPD PCR products after using all primers.

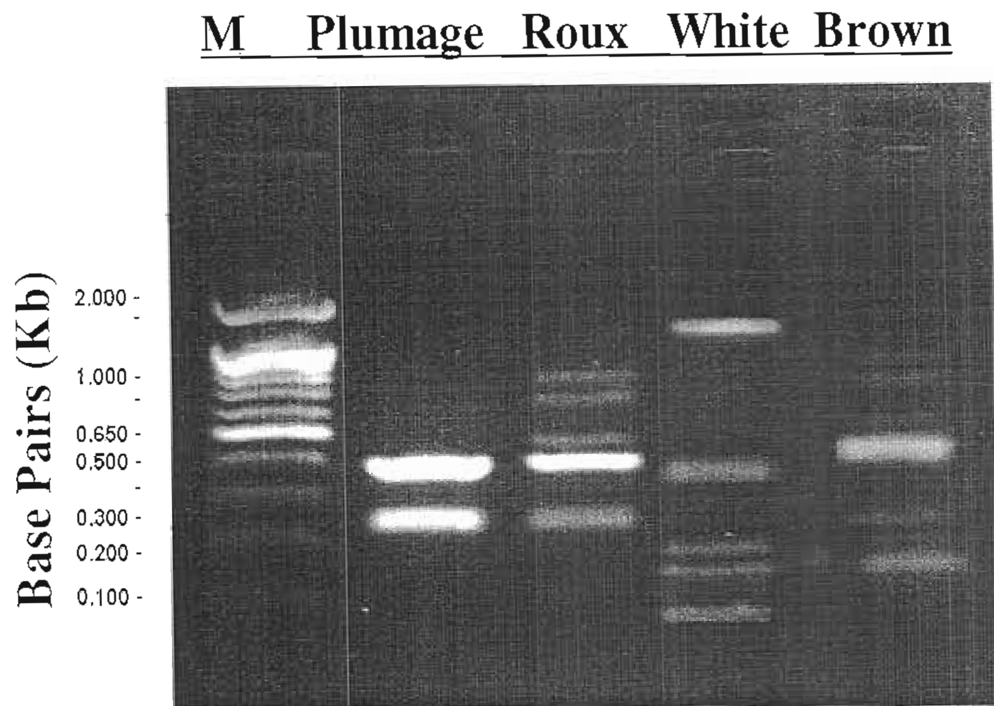

Fig. 3. Ethidium bromide stained ISSR fragments using bulked genomic DNA extracted from the quail breeds.

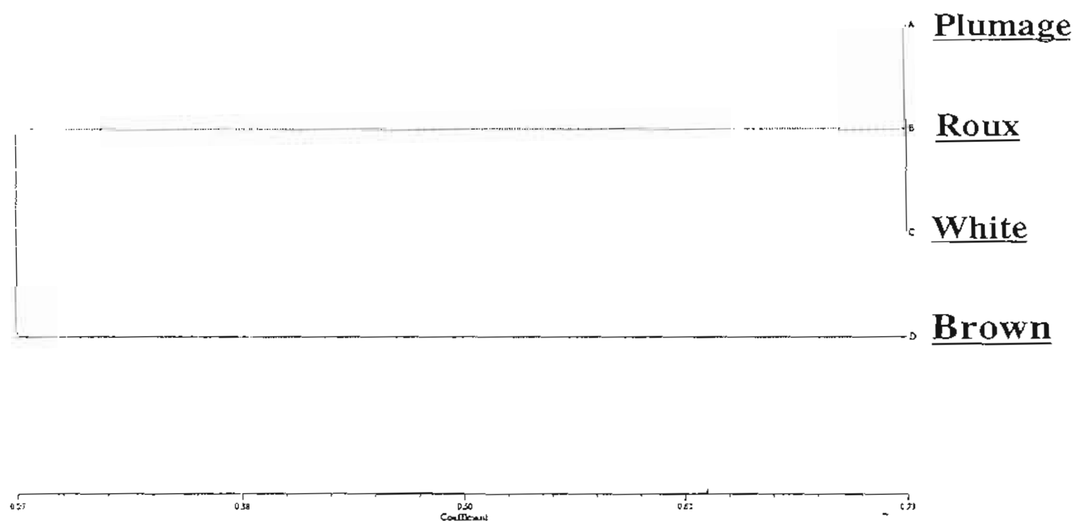

Fig. 4. Linkage dendrogeram of studied 4 *quail* breeds based on ISSR PCR products after using all primers

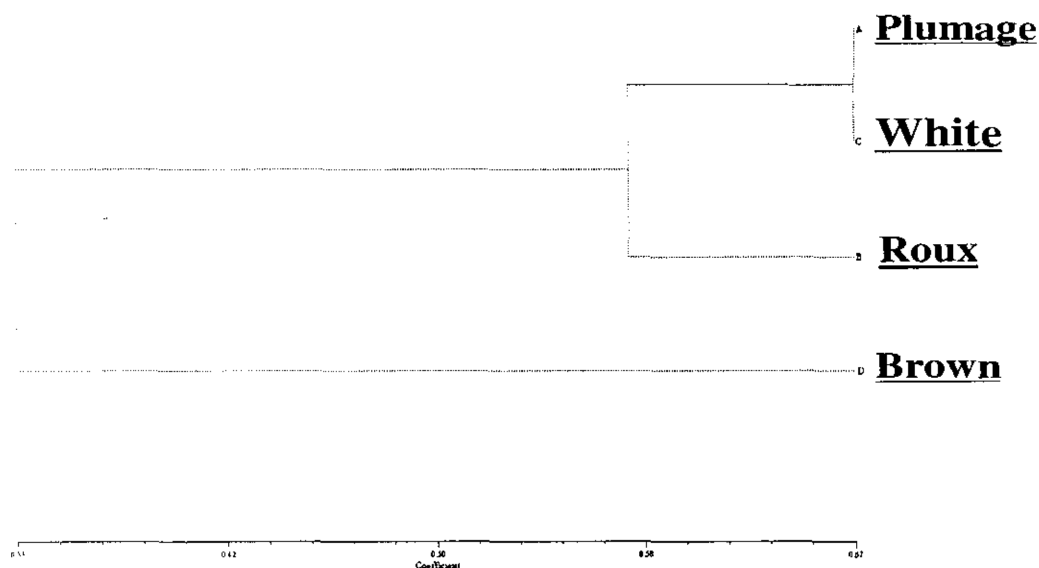

Fig. 5. Linkage dendrogeram of studied 4 *quail* breeds based on combined analysis of all RAPD and ISSR-PCR data.

REFERENCES

1. ***Wakasugi, N (1984):*** Japanese quail. In: Evolution of domesticated animals (Mason Il, ed). New York: Longman; 319-321.

2. ***Crawford, R D (1990):*** Origin and history of poultry species, In: Poultry breeding and genetics (Crawford RD, Ed). Amsterdam: Elsevier; 1-41.

3. ***Ryttman, H and Tegelstrom, H (1981):*** G-banded karyotypes of three Galliformes species, Domestic Fowl (Gallus domesticus), Quail (Conturnix coturnix Japonica), and Turkey (Meleagris gallopavo). Hereditas, 49:165-170.

4. ***Lauber, J K (1964):*** Sex-linked albinism in the Japanese quail. Science 146: 948-950.

5. ***Sittmann, K, Wilson, W O and Mc Farland, L Z (1966):*** Buff and albino Japanese quail. J Hered 57: 119-124.

6. ***Somes, R G (1988):*** International registry of poultry genetic stocks. Storrs, Agricultural Experiment station.

7. ***Homma, K, Jinno, M, Sato, K and Ando, A (1968):*** Studies on perfect and imperfect albinism in the Japanese quail (Coturnix coturnix japonica). Jpn J Zootech Sci 39: 348-352.

8. ***Wakasugi, N and Kondo, K (1973):*** Breeding methods for maintenance of mutant genes and establishment of strains in the Japanese quail. Exp Anim 22 (suppl): 151-159.

9. ***Homma, K and Jinno, M (1969):*** Sex-linked plumage characters in the Japanese quail. In: Proceedings of the 57 th meeting of the Japanese Society of Zootechnical Science, Kagoshima. Tokyo: Japanese Society of Zootechnical Science; 82.

10. ***Truax, R E and Johnson, W A (1979):*** Genetics of plumage color mutants in Japanese quail. Poult Sci 58: 1-9.

11. ***Homma, K (1968):*** Sex-linked dilute character in the Japanese quail. In: Proceedings of the 55 th meeting of the Japanese Society of Zootechnical Science, Obihiro. Tokyo: Japanese Society of Zootechnical Science; 47.

12.Jerry, B, Cheng, H and Okimoto, R (1997): DNA marker technology: A Revolution in animal genetics. Poultry science 76: 1108-1114.

13.Meyer, W, Mitchell, T G, Freedman, E Z and Vilgays, R (1993): Hybridization probes for conventional DNA fingerprinting used as single primers in the polymerase chain reaction to distinguish strains of *Cryptococcus neoformans. Journal of Clinical Microbiology* 31, 2274–2280

14.Gupta, M, Chyi, Y S, Romero-Severson, J and Owen, J L (1994): Amplification of DNA markers from evolutionarily diverse genomes using single primers of simple-sequence repeats. *Theoretical Applied* Genetics 89, 998–1006

15. Williams, J G K, Kubelik, A R, Livak, K L , Rafalski, J A and Tingey, S V (1990): DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research* 18, 6531-6535.

16.Cheng, H H, Levin, I, Vallejo, R L, Khatib, H, Dodgson, J B , Crittenden, L B and Hillel, J (1995): Development of a genetic map of the chicken with markers of high utility. Poult Sci 74; 1855-1874.

17. Mac-Hugh, D E, Shriver, M D, Loftus, R T, Cunningham, P and Bradley, D G (1997): Microsatellite DNA variation and the evolution, domestication and phylogeography of taurine and zebu cattle (Bos taurus and Bos indicus). Genetics 146: 1071-1086.

18.O'Brien, S L, Womack, J, Lyons, L A , Moore, K L, Jenkins, N A and Copeland, N G (1993): Anchored reference loci for comparative genome mapping in mammals. Nat Genet 3: 103-112.

19.Takahashi, H, Nirasawa, K, Nagamine, Y, Tsudzki, M and Yamamoto, Y (1998): Genetic relationships among Japanese native breeds of chicken based on microsatellite DNA polymorphisms. J Hered 89:543-546.

20.Zhang, X, Leung, F C , Chan, D K, Yangm G and Wu C (2002): Genetic diversity of Chinese native chicken breeds based on protein polymorphism, randomly amplified polymorphic DNA, and microsatellite polymorphism. Poultry sci. Oct; 81 (10): 1463-72.

21.Mansour, A, Omayma M Ismail and Mohei EL-Din M (2008): Diversity Assessment among Mango (*Mangifera indica* L.) Cultivars in Egypt using ISSR and Three-Primer Based RAPD Fingerprint. *African journal of plant science and biotechnology* 2(2), 87-92.

22.Snedecor G W and Cochran W G (1967): Statistical methods. 6th ed. The Iowa State, University Press. Iowa, U. S. A.

23.Dangi, R S , Lagu, M D , Choudhary, L B, Ranjekar P K and S Gupta V (2004): Assessment of genetic diversity in *Trigonella foenum-graecum* and *Trigonella caerulea* using ISSR and RAPD markers. *BMC Plant Biology* 4 (13), 1-11.

24.Nei, M (1987): Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **83**,583-590

25.Rohlf, F J (2000): NTSYSpc: numerical taxonomy and multivariate analysis system, version 2.02. Setauket (New York), Exeter Publishing.

26. Yap I V and Nelson R J (1996): Winboot: a program for performing bootstrap analysis of binary data to determine the confidence limits of UPGMA-based dendrograms, Manila, IRRI.

27. Plotsky, Y, Kaiser, M G and Lamont, S J (1995): Genetic characterization of highly inbred chicken lines by two DNA methods: DNA fingerprinting and polymerase chain reaction using arbitrary primers. Anim Genet. Jun; 26 (3): 163-70.

28. Zhang, X , Mcdaniel G R and Giambrone J J (1995): Random amplified polymorphic DNA comparisons among

broiler lines selected for incidence of tibial dyschondroplasia. Poultry science; 74: 1253- 1258.

29. *Smith, E J, Jones, C P, Bartlett J and Nestor K E (1996):* Use of randomly amplified polymorphic DNA markers for the genetic analysis of relatedness and diversity in chickens and turkeys. Poultry science 75: 579-584.

30. *Sharma, D, Appa Rao , K B and Totey, S M (2000):* Measurement of within and between population genetic variability in quails. Br Poul Sci. Mar; 41 (1): 29-32.

31. *Gruszczynska, J, Michalska E and Sobierajska K (2002):* Microsatellite polymorphism selecting loci in a Japanese quail population. Cell. Mol. Biol. Lett. Vol. 7. No. 1.

32. *Kayang, B B, Inoue-Murayama, M , Hoshi, T, Matsuo, K, Takahashi, H, Minezawa, M, Mizutani M and Ito, S (2002):* Microsatellite loci in Japanese quail and cross-species amplification in chicken and guinea fowl. Genet Sel Evol. Mar-Apr; 34 (2): 233-253.

33. *Kayang, B B, Inoue-Murayama, M, Nomura, A , Kimura, K, Takahashi, H, Mizutani, M and Ito S (2000):* Fifty microsatellite markers for Japanese quail. J Hered. Nov-Dec; 91 (6): 502-505.

34. *Pang, S W, Ritland, C , Carlson, J E and Cheng, K M (1999):* Japanese quail microsatellite loci amplified with chicken-specific primers. Anim Genet. Jun; 30 (3): 195- 199.

الملخص العربي

الواسمات الجزيئية المرتبطه بأشكال مظهرية جديدة للسمان في مصر

احمد منصور[١] ، ايمان السيد العربي[٢]

١- قسم الوراثة- كلية الزراعة- جامعة الزقازيق

٢- قسم تنمية الثروة الحيوانية- كلية الطب البيطري- جامعة الزقازيق

يعتبر السمان من أهم الطيور الموجودة فى مصر و العالم فمنذ هجرته الى مصر ظهرت سلالات جديدة. و من أجل تحسين انتاجية السمان نقيم الاشكال الجديدة المنتجة تحت الظروف المختلفة.

من أجل ذلك تم أستخدام تحليل العلامات الوراثية التى تساعد المربين على انتخاب سلالات جديدة بناءا على المعلومات الوراثية الناتجة من استخدام المجسات الوراثية لمعرفة مدى التشابه الموجود بين سلالات السمان و بالتالى فأن معرفة الاختلافات الوراثية و علاقات القرابة بين سلالات السمان سوف يساعد فى التحسين الوراثي لهذا الطائر.

فى هذا البحث نجد أن الاختلافات الوراثية بين الاشكال المختلفة للسمان فى مصر قد تم دراستها بأستخدام طريقتين مختلفتين على المستوى الجزئي باستخدام تقنية RAPD-PCR و أستخدام تقنية ISSR-PCR. و اعتمادا على ذلك تم رسم شجرة القرابة بين الأنواع الأربعة المدروسة اعتمادا على التشابه بينهم و على التقنية المستخدمة.