ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

International Journal of Poultry Science 4 (10): 804-811, 2005
ISSN 1682-8356

Use of Randomly Amplified Polymorphic DNA (RAPD) Markers in Poultry Research

H.H. Salem[1], B.A. Ali[1], T.H. Huang[1] and D.N. Qin[2]
[1]Cell Biology and Medical Genetics Department, Shantou University, Medical College, 22 Xinlin Road, Shantou, Guangdong 515031, People's Republic of China
[2]Physiology Department, Shantou University, Medical College, 22 Xinlin Road, Shantou, Guangdong 515031, People's Republic of China

Abstract: Random amplified polymorphic DNA (RAPD) markers can be generated using short arbitrary primers to amplify genomic DNA, giving a genotype-specific pattern of bands. RAPD analysis should lead to the saturation of the genome without the requirement of previous genetic information and using few expensive oligonucleiotides. The use of DNA marker technology in poultry as a strains identification has progressed rapidly during the last decade. This review summarizes the use of RAPDs as molecular markers in poultry research, especially in some genetic resources of economically important species such as chickens, quails, ducks, goose, turkey and other birds. Also we discuss its limitations and benefits including its simplicity and ease of use in the laboratory.

Key words: Applications, RAPD, molecular markers, poultry, species

Introduction

The development of molecular techniques has been created new possibilities for the selection and genetic improvement of livestock. The discovery of the PCR had a major impact on the research of eukaryotic genomes and contributed to the development and application of various DNA markers (Marle-Koster and Nel, 2003). Molecular markers derived from polymerase chain reaction (PCR) amplification of genomic DNA are an important part of the toolkit of evolutionary geneticists (Holsinger *et al*., 2002). By detecting genetic variation, genetic markers may provide useful information at different levels: population structure, levels of gene flow, phylogenetic relationships, patterns of historical biogeography and the analysis of parentage and relatedness (Feral, 2002). PCR-based multi-locus DNA fingerprints represent one of the most informative and cost-effective measures of genetic diversity and are useful population-level biomarkers of toxicologic and other anthropogenic impacts (Bagley *et al*., 2001). Random amplification of polymorphic DNA (RAPD) markers are polymorphic DNA separated by gel electrophoresis after PCR using short random oligonucleotide primers (Welsh and McClelland, 1990; Williams *et al*., 1990). It has been particularly used for genetic and molecular studies as it is a simple and rapid method for determining genetic diversity and similarity in various organisms. It also has the advantage that no prior knowledge of the genome under research is necessary (Fischer *et al*., 2000; Klinbunga *et al*., 2000). It has been concluded that although AFLP analysis is superior in terms of efficiency, RAPDs may still be used as reliable markers in small low-tech laboratories (Kjolner *et al*., 2004).

This technology is currently being adopted for the discrimination of genetic resources of economically important such as poultry, and other farm animals. In poultry, RAPDs has been used to detect specific markers to estimate genetic relatedness among various poultry species, as well as for genome mapping. The aim of this paper is to summarize the applications of RAPD molecular markers in most important poultry species and to discuss limitations and benefits of these molecular markers.

Applications of RAPD molecular markers in different poultry species

Chickens: The current chicken genetic map contains at least 1,965 loci within 50 linkage groups, and it covers about 4,000 cM. About 235 of these loci have homology with known human or mammalian genes. The remaining loci are anonymous molecular DNA markers, including microsatellites, amplified fragment length polymorphism (AFLP), randomly amplified polymorphic DNA (RAPD), CR1 elements, and others (Emara and Kim, 2003). The effectiveness of RAPD in detecting polymorphism between chicken populations and their applicability in population studies and establishing genetic relationships among chicken populations has been reported by Sharma *et al*. (2001). Ali and Ahmed

Corresponding author: B.A. Ali, Nucleic Acid Research Department, Genetic Engineering and Biotechnology Research Institute, Mubarak City for Scientific Research & Technology Applications, New Borg El-Arab City 21934, Alexandria, Egypt

(2001) and Ali *et al.* (2003) have also presented some preliminary data showing molecular differences between Egyptian chicken strains, and indicating the potential use of RAPD markers for a wide range of applications in poultry breeding. Genetic map of the chicken Z chromosome RAPD markers has been studied. Thirteen Z-linked RAPD markers were identified, mapped, and linked to two RFLPs and one phenotypic marker. The linkage distances obtained suggest that the RAPD markers are widely distributed throughout the Z chromosome and are likely to be linked to most or all traits of interest on this chromosome. The map provides a preliminary estimate of genetic to physical distance of about 0.5 Mb per centimorgan for the Z chromosome in chickens (male-specific recombination) (Levin *et al.*, 1993). Cheng *et al.* (1995) developed a genetic map of the chicken with markers of high utility using RAPD markers. They identified 11 new random amplified polymorphic DNA (RAPD) markers and 24 new markers based on the chicken CR1 element were mapped. The addition of these markers increases the total number of markers on the East Lansing genetic map to 273, of which 243 markers are resolved into 32 linkage groups. The map coverage within linkage groups is 1,402 cM with an average spacing of 6.7 cM between loci. Thirteen highly inbred chicken lines were genetically characterized by DNA fingerprinting and polymerase chain reaction using arbitrary primers. The RAPD-PCR band-sharing values ranged from 0.66 to 0.99 for all between-line comparisons. The DNA fingerprinting (DFP) band-sharing (BS) values among lines from different breeds (Leghorn, Fayoumi, Spanish) ranged from 0.10 to 0.20. The DFP BS values among Leghorn lines from different genetic backgrounds ranged from 0.42 to 0.79. The DFP BS values among lines selected for different major histocompatibility complex serotypes from a common genetic background ranged from 0.70 to 0.95. Thus, the ability to detect biodiversity at the DNA level was greater in this study for DFP than for RAPD-PCR (Plotsky *et al.*, 1995). Methods of multilocus genome fingerprinting (DNA fingerprinting) and the polymerase chain reaction with random primers were used to detect genome variability of eleven chicken breeds. The diagnostic value of the markers used for differentiating breeds and detection of origin of several breeds of Russian selection was demonstrated (Semenova *et al.*, 1996). The variation in polymorphic DNA (RAPD and minisatellite) and protein markers was compared for nine Russian chicken breeds differing in morphological and productivity types and in origin, three European egg breeds, and three broiler breeds of the Asian origin. In all breed groups, minisatellites and RAPD markers revealed higher genetic diversity as compared with protein markers (Semenova *et al.*, 2002). A total of 60 random primers were used in the RAPD analyses to evaluate genetic polymorphism and relatedness within and among four chicken breeds and two turkey populations (Smith *et al.*, 1996). Seventy percent of primers tested amplified patterns with at least one polymorphic fragment in one or more of the populations. About 14.29% of primers amplified polymorphic fragments in each of the six strains with a within- and between-population average band-sharing frequency of less than one but above zero ($P < 0.05$). Reciprocal crosses and backcrosses were conducted between inbred Rhode Island Red and White Leghorn chickens differentiated for egg production and egg quality traits. RAPD markers distinguishing inbred lines were detected. Twenty-two polymorphic bands were found from screening 120 single 10-mer random primers of which two were consistent with sex-linked markers (Wei *et al.*, 1997). Investigation RAPD markers used for evaluating genetic relationships among three varieties of guinea fowl (Lavender, Pearl and White) (Sharma *et al.*, 1998). The estimates, measured as BS were high for within and between population genetic similarities. The results indicated a very low level of intra and inter varietal genetic variation in these guinea fowl varieties. Sharma *et al.* (2001) used RAPD markers to detect polymorphism among five breeds of chicken [White Leghorn and Rhodes Island Red (selected for part period egg production and egg mass respectively), Red Cornish and White Plymouth Rock (selected for early body weights) and Kadaknath (native breed)]. Twelve of the fifty random primers screened yielded distinct polymorphic RAPD profiles. Of the total 96 fragments amplified, about 25% showed polymorphism. Genetic variability through RAPD markers has been detected within and between-strain in White Leghorn population (Singh and Sharma, 2002). Using 50 random decamer primers, only 12 primers detected polymorphism between the strains. Between-strain genetic similarity estimates, based on band sharing (BS) as well as on band frequency (BF) ranged from 0.756 to 0.958 and from 0.830 to 0.996, respectively. Comparative analysis of allozyme, random amplified polymorphic DNA, and microsatellite polymorphism on Chinese chickens (five native populations, two fast-growing broiler lines, and one layer line) has been reported. The genetic distances among all populations measured by three methods were different. While, genetic distances from RAPD showed a close relationship between Chinese native chickens and broiler and layer chickens (Zhang *et al.*, 2002a). Genetic diversity of Chinese native chicken breeds was investigated using protein polymorphism, randomly amplified polymorphic DNA (RAPD), and microsatellite polymorphism. Imported broiler and layer breeds were included in the analysis. The results from protein polymorphism did not show distinct differences. The data obtained from RAPD indicated that gene diversity within a population was large in Chinese native chickens, intermediate in broilers, and low in layers and that there were small differences between Chinese

native chickens and both broilers and layers (Zhang *et al.*, 2002b). The genetic lineage of four ubiquitous port-city (Haimen) chicken populations in China has been detected using RAPDs and microsatellite markers (Olowofeso *et al.*, 2005). The results suggested that these chickens are likely to have originated from the same source. RAPD technique was applied to detect genetic similarity between five local chicken strains that have been selected for eggs and meat production in Egypt. Based on six oligonucleotide primers, the genetic similarity between the egg-producing strains (Anshas, Silver Montazah and Mandarah) ranged from 72.4 to 85.4%. While the genetic similarity between the two chicken strains selected for meat production (Baheij and El-Salam) is 86.9% (Ali *et al.*, 2003). DNA alterations include DNA adducts formation, breaks, point mutations, large rearrangements, and other changes such as structural distortion induced by chemical or physical agents following direct and/or indirect interaction with the genomic DNA. Such alteration can be detected by RAPD. RAPD technique was used to detect DNA band variations between both normal and abnormal male of broiler chicken based on RAPD marker. DNA polymorphisms between normal and mutant birds were detected using fifteen oligonucleiotides primers. Using these primers, DNA band loss ranged from 25 to 75%. Data demonstrated that RAPD marker could detect DNA alterations (Ali, 2003a).

Quails: Quail are an economically important avian species and provide an alternative to the more commonly used chicken. They require less space and low initial investment and have good export potential. Quail are in the genus Coturnix, family Phasianidae and order Galliformes (Sharma *et al.*, 2000). Japanese quail (*coturnix coturnix japonica*) is the smallest avian species farmed for meat and egg production and it has also assumed worldwide importance as laboratory animals (Panda and Singh, 1990; Baumgartner, 1990). There are various well-developed lines, which are used commercially. The knowledge about the genetic characterization of these lines and amount of genetic diversity among them is minimal; hence studies are needed to characterize these lines genetically and to estimate the genetic variability between them for selection and breeding (Howard and Moore, 1984).

To detect polymorphism in various quail lines, RAPD markers were tested and were found to be effective. Twenty decamer primers were selected at random and tested, and 6 of these generated distinct polymorphic patterns between the quail lines. Out of a total of 60 bands amplified using 6 selected primers, 19 (31•7%) were found to be polymorphic. Genetic similarity estimates between the populations ranged from 0•709 to 0•808. Genetic similarity within the lines ranged from 0.726 in HBW to 0.836 in KLQ (Sharama *et al.*, 2000). DNA fingerprints of Japanese quail male and female pure line breeders were obtained with three probes and they yielded a total of 59 scoreable bands. The results indicated that the two selection methods had different effects on the genetic constitution of the lines, in agreement with previous observations made from the analysis of biochemical polymorphisms with the same set of birds. Therefore, the relationship of heterosis with BS may also depend on the past history of selection in the lines (Minvielle *et al.*, 2000). Data for 17th generation of three lines: two selected quail lines (egg and meat) and control line were analyzed at the DNA level using RAPD-PCR. This study was carried out using seven random primers. Six of them introduced polymorphic bands between the two selected quail lines and control one. The data suggests that RAPD-PCR Markers are useful tool for studying the genetic variability among selected quail's lines (Ali *et al.*, 2002).

Goose, Ducks and Turkeys: Bednarczyk *et al.* (2002) studied the DNA polymorphism among pooled DNA of eight goose lines by RAPD-PCR. The number of bands amplified by each primer ranged from 1 to 8, within a mean of 2.86. Some bands appeared specific for the line or genetic background. The results indicated that 10 generations selected for egg production and body weight under various pressure, resulted in genetic variation among goose lines as detected by RAPD. Selection for meat traits caused greater genetic diversity than selection for egg production. Random primers were used for RAPD fingerprinting in Chinese, White Roman and Landaise geese to detect female specific DNA sequences (Huang *et al.*, 2003). They found that one of the primers used in this study produced a 938-bp sex-specific fragment in all females and in no males of Chinese geese only. Also data showed that a simple and effective PCR-based sexing technique could be used in the three goose breeds studied. Four indigenous Polish goose breeds, Kartuska (Ka), Lubelska (Lu), Kielecka (Ki) and Podkarpacka (Pd) were genetically analysed by the RAPD-PCR method in order to determine the band-sharing frequency as well as bands characteristic of the evaluated breeds. For each genetic group specific bands with given primers were obtained, suggesting their potential for use as population-specific markers (Maciuszonek *et al.*, 2005). RAPD-analysis of genetic polymorphism of ducks to estimate differences in breeds and to determine interlineal differences in a Peking duck species have reported. The genetic differentiation of breeds of Peking and Musk ducks maintained at the Blagovarskii State Farm for Pedigree Poultry was studied. A comparison of genetic distances estimated by two different methods, as well as similarity dendrograms based on these estimates, was performed. The similarity dendrograms obtained using different primers and methods of construction were found to be similar. The pattern of breed clustering on these dendrograms adequately

reflects their actual state known from the history and genealogy of breeds. The possibility of using RAPD markers for the detection of differences between lines of Peking duck constituting a local population maintained at the Blagovarskii State Farm for Pedigree Poultry was demonstrated. Genetic distances based on the RAPD markers precisely and accurately reflect even small changes that occurred in the genetic structure of Peking duck lines during breeding of parental forms. The pattern of inheritance of RAPD markers obtained using primer HM13 was studied in the F1 progeny of two families. The results can be used for improvement of available high-productivity lines of ducks breeding of new lines, and promotion of the combining abilities of lines (Dolmatova *et al*., 2000a, b).

A study involving the use of random amplified polymorphic DNA (RAPD) was conducted to evaluate genetic polymorphism and relatedness within and among two turkey populations, a long-term random bred and a commercial strain. A total of 60 random primers were used in the RAPD analyses. Forty-two of the 60 primers tested amplified patterns with at least one polymorphic fragment in one or more of the populations. Differences among the six primers for genetic distance among populations were significant ($P < 0.05$). The results provide evidence of the applicability of RAPD to determining genetic relatedness within and among different poultry populations and in developing reproducible markers useful in evaluating individual variation in turkeys (Smith *et al*., 1996).

On the other hand, Zhu *et al*. (1996) conducted an experiment to estimate genetic parameters in six experimental and five commercial primary breeding turkey lines using DNA fingerprinting, but RAPD-PCR has not been used in this study. Eighteen individual DNA samples per line were digested with an HaeIII restriction enzyme and hybridized with Jeffrey's' 33.6 probe. The band sharing (BS) Within lines ranged from 0.39 to 0.62 and reflected the history of the experimental lines. Among lines, BS ranged from 0.21 to 0.33 with an average of 0.26. The computer program used in this study made DNA fingerprinting easier to use in population analysis.

Other bird species: The use of RAPDs has been reported in other bird species research. RAPD phenotypes generated by 13 primers were scored for 101 individuals in 14 populations of the endangered red-cockaded woodpecker (*Picoides borealis)*. Although no population-specific markers were found, the frequencies of several markers differed significantly among populations. The results of this study indicated that RAPDs could be helpful in differentiating populations at the phenotypic level even when small sample sizes, estimation bias, and inability to test for Hardy-Weinberg equilibrium complicate the genotypic interpretation. Also this study suggested that lack of large differences among populations of red-cockaded woodpeckers may allow flexibility in interpopulation translocations, provided factors such as habitat preference, latitudinal direction of translocation, and status of donor populations are considered (Haig *et al*., 1994). Bello and Sanchez (1999) described the identification of a sex-specific DNA marker in the ostrich using RAPD assay. They reported in this study the identification of a W-linked marker in the ostrich (*Struthio camelus*), which allows gender diagnosis in chicks or juvenile birds. DNA from 10 females and 11 males was used to prepare two pools for each sex. Two hundred different 10-mer primers of arbitrary sequence were used to screen those pools. One primer generated a female-specific band. Sex specificity was confirmed by testing the 21 animals individually. The candidate DNA fragment was cloned and sequenced. Longer primers were designed to optimize a sex-specific PCR that will be useful in diagnosis. Genetic variability in the Iberian imperial eagle (*Aquila adalberti*) population has been demonstrated by RAPD analysis (Padilla *et al*., 2000). The Iberian imperial eagle is one of the most threatened bird species in the world. Forty-five (75%) of 60 arbitrarily designed primers amplified 614 loci in 25 individual eagles, 59.7% of which were polymorphic. The RAPD method has revealed a high level of heterozygosity in this species. The genetic distances estimated between 25 eagles can serve to establish more adequate mating in order to preserve genetic variability. Using RAPD-PCR, the genetic diversity in the local population of the Manchurian pheasant *(Phasianus colchicus pallasi*) was studied by Kulikova *et al*. (2002). Based on the DNA patterns obtained in PCR with five arbitrary decanucleotide primers, genetic polymorphism of this population has been assessed, estimated genetic distances between individuals, and constructed a phylogenetic tree, and an UPGMA Dendrogram of genetic similarity. The population was shown to exhibit high average genetic polymorphism ($P = 79.4\%$) and genetic distances ($D = 0.267$.

Discussion

There are various well-developed strains of poultry that are used commercially. However, information about the genetic characterization of these strains and the amount of genetic diversity among them is minimal. Hence more studies are needed to characterize these strains genetically and to estimate the genetic variability between them in order to enhance selection and breeding (Howard and Moore, 1984). The RAPD method appeared a decade ago as an alternative in genetic relationship studies. The technique generates polymorphic band patterns, produced by PCR using arbitrary DNA sequence primers. If total DNA is used, RAPD yields abundant information about the analyzed genome in a rapid and inexpensive way (Xena de Enrech, 2000). RAPDs (Welsh *et al*., 1991; Bernardi and

Talley, 2000; Fischer *et al.*, 2000; Garcia-Mas *et al.*, 2000; Lehmann *et al.*, 2000; Mohd-Azmi *et al.*, 2000) and fluorescence *in situ* hybridization (FISH) (Menke *et al.*, 1998; Brutovska *et al.*, 2000) have been so far used to analyze genetic similarity and diversity in genetics and breeding research of animal, plant and microbes. RAPD analysis also has been used to evaluate genetic diversity for fish: species and subspecies identification in guppy (Dinesh *et al.*, 1993), tilapia (Bardakci and Skibinski, 1994; Dinesh *et al.*, 1996), brown trout and Atlantic salmon (Elo *et al.*, 1997), largemouth bass (Williams *et al.*, 1998) and Ictalurid catfishes (Liu *et al.*, 1998), Indian major carps (Barman *et al.* (2002). RAPD markers that were shown to genetically link to a trait of interest could be used for individual and pedigree identification, pathogenic diagnostics, and trait improvement in genetics and breeding programmes. Morphological criteria (Bernardi and Talley, 2000), biochemical data (Jensen, 2000), isozyme electrophoresis (Smith *et al.*, 1997; Begg *et al.*, 1998; Cagigas *et al.*, 1999), restriction fragment length polymorphism (RFLP) (Hallerman and Beckmann, 1988; Garcia-Mas *et al.*, 2000), minisatellites and microsatellites (Taggart and Ferguson, 1990; Li *et al.*, 2000). The linked markers can be used for marker-assisted selection in breeding programmes (Hansen *et al.*, 1997) or for the screening of genomic libraries for map-based cloning (Tanksley *et al.*, 1995). After a definition of genetic diversity among other aspects of biodiversity, special features of the marine environment and processes governing genetic diversity are given together with the molecular tools required to study it (Feral, 2002). Some uses of genetic diversity for assessment, conservation and protection purposes are also needed.

With respect to RAPD-PCR limitations, a number of drawbacks of RAPD markers have been reported. Most RAPD markers are dominant, and thus, are less suitable for linkage analysis than codominant markers, e.g. RFLP markers (Williams *et al.*, 1993; Sall and Nilsson, 1994). The analysis of population structure with RAPD data is hampered by the lack of complete genotypic information resulting from dominance, since this enhances the sampling variance associated with single loci as well as induces bias in parameter estimation. Lynch and Milligan (1994) have suggested a modified procedure that reduces bias introduced by the square-root transform. However, the procedure recommends ignoring those samples in which fewer than four null-homozygotes are observed. This may lead to significant bias in estimates of genetic diversity (Zhivotovsky, 1999). At the molecular level, heteroduplex formation of homologous sequences (Ayliffe *et al.*, 1994; Novy and Vorsa, 1996) is a potential source of error. The most serious error that occurs in the RAPD assay, however, is competition between different DNA fragments for amplification (Williams *et al.*, 1993; Halldén *et al.*, 1996). This phenomenon, in which a RAPD band successfully amplified in one genotype, is undetected in another, results in incorrect genotype interpretations. For polymorphic bands, the frequency of errors due to competition has been found to be approximately 15% in genomes of both high and of low complexity (Halldén *et al.*, 1996). Three amplification protocols were analyzed for error rate and generation of polymorphisms during RAPD analysis. Using a set of 240 primers, the protocols detected similar frequencies of polymorphisms in two inbred sugar beet lines. The error rate was investigated by including a 1:1 mixture of DNA from the two lines in all analyses. Similar error rates, approximately 18%, were detected by the three protocols. Thus, altered amplification conditions did not substantially affect the error rate during RAPD analysis. For each of the three possible pairs of protocols, a positive correlation was obtained for primer and number of polymorphisms. (Hansen *et al.*, 1998).

Concerns about reproducibility of RAPDs have limited their wider use in environmental biology. Several studies have reported poor reproducibility for RAPD markers (Weeden *et al.*, 1992; Penner *et al.*, 1993; Skroch and Nienhuis, 1995). Bagley *et al.* (2001) assessed polymorphism and reproducibility of the two common fingerprinting techniques, RAPD and AFLP in pedigreed populations of rainbow trout (*Oncorhynchus mykiss*) to derive general rules for selective removal of problematic fingerprint bands. They found that by excluding bands that comprised less than 1% of total intensity, and by excluding the largest and smallest 10% of the bands, they could achieve nearly 100% reproducibility of AFLP fingerprints. Similar application of band exclusion criteria to RAPD fingerprints did not significantly enhance their reproducibility, and at least 15% of RAPD bands were not fully repeatable, heritable, or transmittable.

In the case of investigation of polymorphism in closely related strains, the highest possible complexity of the patterns obtained by RAPD-PCR is required to assure revealing of limited polymorphism. Most parameters (reaction components concentration, additives, different polymerases, and thermal profiles) affecting RAPD-PCR should be examined, in an effort to increase pattern complexity (Diakou and Dovas, 2001). Fraga *et al.* (2002) analyzed the effect of changing concentrations of the primer, template DNA and *Taq* DNA polymerase with the goal of determining their optimum concentration for the standardization of the RAPD technique for genetic studies of Trichomonas vaginalis. To ensure that the amplified DNA bands originated from genomic DNA, and not primer artifacts, negative control should be carried out for each primer/breed combination (Ali, 2003b). No amplification was detected in control reactions. All amplification products were found to be reproducible when reactions were repeated using the same reaction conditions.

Conclusion and future prospects: The effectiveness of RAPD in detecting polymorphism between different poultry species, their applicability in population studies, and the establishment of genetic relationships demonstrated with this review. It is important to mention the fact that data results from RAPD assays can be extended to further dissect traits in a more refined way to exactly knowledge on specific genes and genetic pathways using other molecular methodologies. There is also the opportunity and need to study sequences of specific polymorphic bands, to determine the genes detected by RAPD experiments. Further studies with other molecular methodologies are essential to clarify and confirm genetic relationships among fish species depicted using RAPDs.

References

Ali, B.A. and M.M.M. Ahmed, 2001. Random amplified polymorphic DNA in some chicken strains. In: Proceeding of the Congress of Role ofBiochemistryinEnvironment and Agriculture. Part I, p. 23-31. 6th–8th February, Cairo Univ., Cairo, Egypt.

Ali, B.A., M.M.M. Ahmed, M. Bahie El-Deen and H.M. Shalan, 2002.Genetic variability in the 17th generation of Japanese quail selected for high eggs and meat production. Egypt. Poult. Sci. J., 22: 59-71.

Ali, B.A., 2003a. Detection of DNA alteration in abnormal phenotype of broiler chicken male by random amplified polymorphic DNA (RAPD). Afr. J. Biotechnol., 2: 153-156.

Ali, B.A., 2003b. Genetics similarity among four breeds of sheep in Egypt detected by random amplified polymorphic DNA markers. Afr. J. Biotechnol., 2: 194-197.

Ali, B.A., M.M.M. Ahmed and O.M. Aly, 2003. Relationship between geneticsimilarity and some productive traits in local chicken strains. Afr. J. Biotechnol., 2: 46-47.

Ayliffe, A.A., G.J. Lawrence, J.G. Ellis and A.J. Pryor, 1994. Heteroduplex molecules formed between allelic sequences cause nonparental RAPD bands. Nucleic Acids Res., 22: 1632–1636.

Bagley, M.J., S.L. Anderson and B. May, 2001. Choice of methodology for assessing genetic impacts of environmental stressors: polymorphism and reproducibility of RAPD and AFLP fingerprints. Ecotoxicology 10: 239-244.

Barman, H.K., A. Barat, B.M. Yadav, S. Banerjee, P.K. Meher, P.V.G.K. Reddy and R.K. Jana, 2002. Genetic variation between four species of Indian major carps as revealed by random amplified polymorphic DNA assay. Aquaculture, 62093: 1-9.

Bardakci F. and D.O.F. Skibinski, 1994. Application of the RAPD technique in tilapia fish: species and subspecies identification. Heredity, 73: 117-123.

Baumgartner, J., 1990. Japanese quail as Laboratory Animals (in Slovak). PublishingHouse of Slovak Academy of Science, Bratislava, pp: 100.

Bednarczyk, M., M. Siwek, A. Mazanowski and P. Czekalski, 2002. DNA polymorphism in various goose lines by RAPD-PCR. Folia Biol., (Krakow) 50: 45-48.

Begg, G.A., C.P. Keenan and M.J. Sellin, 1998. Genetic variation and stock structure of school mackerel and spotted mackerel in northern Australian waters. J. Fish. Biol., 53: 543-559.

Bello, N. and A. Sanchez, 1999. The identification of a sex-specific DNA marker in the ostrich using a random amplified polymorphic DNA (RAPD) assay. Mol. Ecol., 8: 667-669.

Bernardi, G. and D. Talley, 2000. Genetic evidence for limited dispersal in the coastal California killifish, Fundulus parvipinnis. J. Exp. Mar. Biol. Ecol., 255: 187-199.

Brutovska, R., E. Cellarova and I. Schubert, 2000. Cytogenetic characterization of three *Hypericum* species by *in situ* hybridization. Theor. Appl. Genet., 101: 46-50.

Cagigas, M.E., E. Vazquez, G. Blanco and J.A. Sanchez, 1999. Combined assessment of genetic variability in populations of brown trout (*Salmo trutta* L.) based on allozymes, microsatellites and RAPD markers. Mar. Biotechnol., 1: 286-296.

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

Diakou, A. and C.I. Dovas, 2001. Optimization of random-amplified polymorphic DNA producing amplicons up to 8500 bp and revealing intraspecies polymorphism in Leishmania infantum isolates. Anal. Biochem., 288: 195-200.

Dinesh, K.R., T.M. Lim, K.L. Chua, W.K. Chan and V.P.E. Phang, 1993. RAPD analysis: an efficient method of DNA fingerprinting in fishes. Zool. Sci., 10: 849-854.

Dinesh, K.R., T.M. Lim, K.L. Chua, W.K. Chan and V.P.E. Phang, 1996. Genetic variation inferred from RAPD fingerprinting in three species of tilapia. Aquac. Int., 4: 19-30.

Dolmatova, Iu.I., T.F. Saitbatalov and F.T. Gareev, 2000a. RAPD-analysis of genetic polymorphism of ducks: differences in breeds. Genetika, 36: 682-687.

Dolmatova, Iu.I., T.F. Saitbatalov and F.T. Gareev, 2000b RAPD-analysis of duck genetic polymorphisms. Interlineal differences in a Peking duck species. Genetika, 36: 805-812.

Elo, K., S. Ivanoff, A. Jukka, J.A. Vuorinen and J. Piironen, 1997. Inheritance of RAPD markers and detection of interspecific hybridization with brown trout and Atlantic salmon. Aquaculture, 152: 55-56.

Emara, M.G. and H. Kim, 2003. Genetic markers and their application in poultry breeding. Poult. Sci., 82: 952-957.

Feral, J.P., 2002. How useful is the genetic markers in attempts to understand and manage marine biodiversity. J. Exp. Mar. Biol. Ecol., 268: 121-145.

Fischer, M., R. Husi, D. Prati, M. Peintinger, M.V. Kleunen and B. Schmid, 2000. RAPD variation among and within small and large populations of the rare clonal plant *Ranunculus reptans* (Ranunculaceae). Am. J. Bot., 87: 1128-1137.

Fraga, J., L. Rojas, I. Sariego and C.A. Sarria, 2002. Optimization of random amplified polymorphic DNA techniques for its use in genetic studies of Trichomonas vaginalis isolates. Infect. Genet. Evol., 2: 73-75.

Garcia-Mas, J., M. Oliver, H. Gomez-Paniagua and M.C. Vicente, 2000. Comparing AFLP, RAPD and RFLP markers for measuring genetic diversity in melon. Theor. Appl. Genet., 101: 860-864.

Haig, S.M., J.M. Rhymer and D.G. Heckel, 1994, Population differentiation in randomly amplified polymorphic DNA of red-cockaded woodpeckers Picoides borealis. Mol. Ecol., 3: 581-595.

Halldén, C., M. Hansen, N.O. Nilsson, A. Hjerdin and T. Sall, 1996. Competition as a source of errors in RAPD analysis. Theor. Appl. Genet., 93: 1185-1192.

Hallerman, E.M. and J.S. Beckmann, 1988. DNA-level polymorphism as a tool in fisheries science. Can. J. Fish. Aquat. Sci., 45: 1075-1087.

Hansen, M., C. Halldén, N.O. Nilsson and T. Sall, 1997. Marker assisted selection of restored male-fertile *Brassica napus* plants using a set of dominant RAPD markers. Mol. Breed., 3: 449-456.

Hansen, M., C. Halldén and T. Sall, 1998. Error Rates and Polymorphism Frequencies for Three RAPD Protocols. Plant Mol. Biol. Rep., 16: 139-146.

Holsinger, K.E., P.O. Lewis and D.K. Dey, 2002. A Bayesian approach to inferring population structure from dominant markers. Mol. Ecol., 11: 1157-1164.

Howard, R. and A. Moore, 1984. A complete checklist of birds of the WorldRevised ed. London, MacMillan.

Huang, M.C., W.C. Lin, Y.M. Horng, R. Rouvier and C.W. Huang, 2003. Female-specific DNA sequences in geese. Br. Poult. Sci., 44: 359-364.

Jensen, S.R., 2000. Chemical relationships of Polypremum pro-umbens,Tetrachondra hamiltonii and Peltanthera flori-bunda. Biochem. Syst. Ecol., 28: 45-51.

Kjolner, S., S.M. Sastad, P. Taberlet and C. Brochmann, 2004. Amplified fragment length polymorphism versus random amplified polymorphic DNA markers: clonal diversity in Saxifraga cernua. Mol. Ecol., 13: 81-86.

Klinbunga, S., P. Ampayup, A. Tassanakajon, P. Jarayabhand and W. Yoosukh, 2000. Development of species-specific markers of the tropical oyster (*Crassostrea belcheri*) in Thailand. Mar. Biotechnol., 2: 476-484.

Kulikova, I.V., G.N. Chelomina, Iu.N. Zhuravlev, 2002. RAPD-PCR analysis of genetic diversity in the Manchurian pheasant. Genetika, 38: 836-841.

Lehmann, D., H. Hettwer and H. Taraschewski, 2000. RAPD-PCR investigations of systematic relationships among four species of eels (Teleostei: Anguillidae), particularly *Anguilla anguilla* and *A. rostrata*. Mar. Biol., 137: 195-204.

Levin, I., L.B. Crittenden and J.B. Dodgson, 1993. Genetic map of the chicken Z chromosome using random amplified polymorphic DNA (RAPD) markers. Genomics, 16: 224-230.

Liu, Z., P. Li, B.J. Argue and R.A. Dunham, 1998. Inheritance of RAPD markers in channel catfish (*Ictalurus punctatus*), blue catfish (*I. furcatus*) and their F1, F2 and backcross hybrids. Anim. Genet., 29: 58-62.

Li, X., K. Li, B. Fan, Y. Gong, S. Zhao, Z. Peng and B. Liu, 2000. The genetic diversity of seven pig breeds in China, estimated by means of microsatellites. J. Anim. Sci.,13: 1193-1195.

Lynch, M. and B.G. Milligan, 1994. Analysis of population genetic structure with RAPD markers. Mol. Ecol., 3: 91-99.

Maciuszonek, A., B. Grajewski and M. Bednarczyk, 2005. RAPD-PCR analysis of various goose populations. Folia Biol., (Krakow) 53: 83-85.

Marle-Koster, E.V. and L.H. Nel, 2003. Genetic markers and their application in livestock breeding in south Africa: A review. S. Afr. J. Anim. Sci., 33: 1-10.

Menke, M., J. Fuchs and I. Schubert, 1998. A comparison of sequence resolution on plant chromosomes: PRINS versus FISH. Theor. Appl. Genet., 97: 1314-1320.

Minvielle, F., J.L. Coville, A. Krupa, J.L. Monvoisin, Y. Maeda and S. Okamoto, 2000. Genetic similarity and relationships of DNA fingerprints with performance and with heterosis in Japanese quail lines from two origins and under reciprocal recurrent or within-line selection for early egg production. Genet. Sel. Evol., 32: 289-302.

Mohd-Azmi, M., A.S. Ali and W.K. Kheng, 2000. DNA fingerprinting of red jungle fowl, village chicken and broilers. J. Anim. Sci., 13: 1040-1043.

Novy, R.G. and N. Vorsa, 1996. Evidence for RAPD heteroduplex formation in cranberry: implications for pedigree and genetic-relatedness studies and a source of co-dominant RAPD markers. Theor. Appl. Genet., 92: 840–849.

Olowofeso, O., J.Y. Wang, K.Z. Xie and G.Q. Liu, 2005. Phylogenetic scenario of port-city chickens in China based on two-marker types. Int. J. Poult. Sci., 4: 206-212.

Padilla, J.A., M. Martinez-Trancon, A. Rabasco, J.C. Parejo, M.E. Sansinforiano and M.I. Guijo, 2000. Genetic variability in the Iberian imperial eagle (Aquila adalberti) demonstrated by RAPD analysis. J. Hered., 91: 495-499.

Panda, B. and R.P. Singh, 1990. A decade of research and development on quail. ResearchBulletin, Central Avian Research Institute, Izat-Nagar, India.

Penner, G.A., A. Bush, R. Wise, W. Kim, L. Domier, K. Kasha, A. Laroche, G. Scoles, S.J. Molnar and G. Fedak, 1993. Reproducibility of random amplified polymorphic DNA (RAPD) analysis among laboratories. PCR Methods Appl., 2: 341-345.

Plotsky, Y., M.G. Kaiser and S.J. Lamont, 1995. Genetic characterization of highly inbred chicken lines by two DNA methods: DNA fingerprinting and polymerase chain reaction using arbitrary primers. Anim. Genet., 26: 163-170.

Sall, T. and N.O. Nilsson, 1994. The robustness of recombination frequency estimates in intercrosses with dominant markers. Genetics, 137: 589-596.

Semenova, S.K., A.L. Filenko, V.A. Vasil'ev, M.I. Prosniak, A.A. Sevast'ianova and A.P. Ryskov, 1996. Use of polymorphic DNA markers for differentiation of chicken breeds of varying origin. Genetika, 32: 795-803.

Semenova, S.K., I.G. Moiseeva, V.A. Vasil'ev, A.L. Filenko, A.A. Nikiforov, A.A. Sevast'ianova and A.P. Ryskov, 2002. Genetic polymorphism of Russian, European, and Asian chicken breeds as revealed with DNA and protein markers. Genetika, 38: 1304-1308.

Sharma, D., K.B. Appa Rao, H.P. Singh and S.M. Totey, 1998. Randomly amplified polymorphic DNA (RAPD) for evaluating genetic relationships among varieties of guinea fowl. Genet. Anal., 14: 125-128.

Sharma D, K.B. Appa Rao and S.M. Totey, 2000. Measurement of within and between population genetic variability in quails. Br. Poult. Sci., 41: 29-32.

Sharma, D., K.B. Appa Rao, R.V. Singh and S.M. Totey, 2001. Genetic diversity among chicken breeds estimated through randomly amplified polymorphic DNA. Anim. Biotechnol., 12: 111-120.

Singh, R.V. and D. Sharma, 2002. Within and between-strain genetic variability in White Leghorn population detected through RAPD markers. Br. Poult. Sci., 43: 33-37.

Skroch, P. and J. Nienhuis, 1995. Impact of scoring error and reproducibility of RAPD data on RAPD based estimates of genetic distance. Theor. Appl. Genet., 91: 1086-1091.

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

Smith, P.J., P.G. Benson and S.M. McVeagh, 1997. A comparison of three genetic methods used for stock discrimination of orange roughy, *Hoplostethus atlanticus*: allozymes, mitochondrial DNA, and random amplified polymorphic DNA. Fish. Bull., (Wash. D. C.) 95: 800-811.

Taggart, J.B. and A. Ferguson, 1990. Minisatellite DNA fingerprints of salmonid fishes. Anim. Genet., 21: 377-389.

Tanksley, S.D., M.W. Ganal and G.B. Martin, 1995. Chromosome landing: a paradigm for mapbased gene cloning in plants with large genomes. Trends Genet., 11: 63-68.

Weeden, N.F., G.M. Timmerman, M. Hemmat, B.E. Kneen and M.A. Lodhi, 1992. Inheritance and reliability of RAPD markers. In: Hoisington D, McNab A (eds), Proc Symp Appl RAPD Tech Plant Breeding, pp 12–17. November 1st , Minneapolis MN, USA. Crop Sci. Soc. Am., Madison, WI, USA.

Wei, R., M.R. Dentine and J.J. Bitgood, 1997. Random amplified polymorphic DNA markers in crosses between inbred lines of Rhode Island red and White Leghorn chickens. Anim. Genet., 28: 291-294.

Welsh, J. and M. McClelland, 1990. Fingerprinting genome-using PCR with arbitrary primers. Nucleic Acids Res., 18: 7213-7218.

Welsh, J., C. Petersen, M. McClelland, 1991. Polymorphisms generated by arbitrarily primed PCR in the mouse: application to strain identification and genetic mapping. Nucleic Acids Res., 19: 303–306.

Williams, J.G.K., A.R. Kublik, K.J. Livak, J.A. Rafalski and S.V. Tingey, 1990. DNApolymorphism amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res., 18: 6531–6535.

Williams, J.G.K., M.K. Hanafey, J.A. Rafalski, S.V. Tingey, 1993. Genetic analysis using random amplified polymorphic DNA markers. Meth. Enzymol., 218: 705-740.

Williams, D.J., S. Kazianis and R.B. Walter, 1998. Use of random amplified polymorphic DNA (RAPD) for identification of largemouth bass subspecies and their intergrades. Trans. Am. Fish. Soc., 127: 825–832.

Xena de Enrech, N., 2000. A decade of the RAPD method: possibilities and limitations for plant genetics relationship studies. Acta Cient. Venez., 51: 197-206.

Zhang, X., F.C. Leung, D.K. Chan, Y. Chen and C. Wu, 2002a. Comparative analysis of allozyme, random amplified polymorphic DNA, and microsatellite polymorphism on Chinese native chickens. Poult. Sci., 81: 1093-1098.

Zhang, X., F.C. Leung, D.K. Chan, G. Yang and C. Wu, 2002b. Genetic diversity of Chinese native chicken breeds based on protein polymorphism, randomly amplified polymorphic DNA, and microsatellite polymorphism. Poult. Sci., 439-446.81: 1463-1472.

Zhivotovsky, L.A., 1999. Estimating population structure in diploids with multilocus dominant DNA markers. Mol. Ecol., 8: 907-913.

Zhu, J., K.E. Nestor, R.A. Patterson, D.J. Jackwood and D.A. Emmerson, 1996. Measurement of genetic parameters within and between turkey lines using DNA fingerprinting. Poult. Sci., 75: 439-446.