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

H.H. Salem1, B.A. Ali1, T.H. Huang1 and D.N. Qin2
1Cell Biology and Medical Genetics Department, Shantou University, Medical College, 22 Xinlin Road, Shantou, Guangdong 515031, People’s Republic of China
2Physiology 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 oligonucleotides. The use of DNA marker technology in poultry as a strain 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 (Marie-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 multilocus 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 (Ermara 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).

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

804
Salem et al.: Use of Randomly Amplified Polymorphic DNA (RAPD) Markers in Poultry Research

(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 (Plotisky 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., 1998). 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 14.2% 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 Rhode 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 Montaza and Mandaragh) ranged from 72.4 to 85.4%. While the genetic similarity between the two chicken strains selected for meat production (Bahej and El-Salam) is 88.9% (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 oligonucleotides 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 KLO (Sharma 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 58 scorable 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 (Maciuszonnek 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 Blagovarski 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 Biagovskii 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 pallasii) was studied by Kukikova 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 Enrich, 2000). RAPDs (Welsh et al., 1991; Bernardi and
Talley, 2000; Fischer et al., 2000; Garcia-Mas et al., 2000; Lehmann et al., 2000; Mohd-Azri 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 Sitibinski, 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 (Tankslke 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 (Zhitovotovsky, 1999). At the molecular level, heteroduplex formation of homologous sequences (Ayliffe et al., 1994; Novy and Verssa, 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; Halden 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 (Halden 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.

808
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


