International Journal of Poultry Science 6 (2): 145-153, 2007
ISSN 1682-8356

Use of Microsatellite Markers in Poultry Research

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Abstract: Microsatellites or simple sequence repeats (SSRs), or short tandem repeats (STRs), discovered in 1981, are tandemly repeated motifs of 1-6 nucleotides found in all prokaryotic and eukaryotic genomes. They are present in both coding and non-coding regions. In addition to being highly variable and polymorphic, microsatellites are also easy to genotype and densely distributed throughout eukaryotic genomes, making them the preferred genetic marker for high resolution genetic mapping. The use of DNA marker technology in poultry as a strains identification has progressed rapidly during the last decade. This review summarize the use of microsatellite 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 easy use in the laboratory.

Key words: Molecular markers, microsatellite, poultry species

Introduction

The development of DNA based markers has had a revolutionary impact on gene mapping and more generally on all of animal and plant genetics. The discovery of the polymerase chain reaction (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 appear particularly useful for 1) measuring local gene flow and migration, 2) assigning individuals to their most likely population of origin, 3) measuring effective population size through the between generation comparison of allele frequencies, and 4) detecting past demographic bottlenecks through allele frequency distortions (Jehle and Arntzen, 2002). Microsatellite markers are now been widely used in the genetic appraisal of several species populations. Because of the relative ease of scoring and ability to exhibits high level of polymorphisms as well as higher heterozygosities, its application as genetic appraisal tool is quite significant (Olowofeso *et al.,* 2005a). Recent information in literature have revealed that microsatellite markers are useful in determining not only heterozygosity and estimating genetic distances among closely related species (Chen *et al.,* 2004), it is also suitable for measurement of genetic parameters such as number of effective alleles (Ne) as well as the polymorphism information content (PIC) in population and can detect rare alleles (Bartfai *et al.,* 2003). These markers can in addition be used to generate data suitable for the estimation of cumulative power of discrimination of any population including the avian species (Olowofeso *et al.,* 2005b). The aim of this paper is to summarize the applications of microsatellite markers in most important poultry species and to discuss limitations and benefits of these molecular markers.

Applications of Microsatellite molecular markers in different poultry species

Chickens: The first linkage map for the chicken reported by Bumstead and Palyga (1992) consisted of about 100 RFLP markers. Schmid *et al.* (2000) reported the first consensus linkage map for the chicken genome. Chromosomal locations for 1..... 965 markers forming 50 linkage groups were reported. In the Ark Database there are 2,483 loci for the chicken, of which 435 are unassigned genetic markers. (Jacobsson *et al.,* 2004). Chromosome-specific libraries for chicken Macrochromosomes 1, 2, 3, 4 were prepared in a phage vector. Fifty two additional unique microsatellite markers of the $(AC)_n$ type were developed from these chromosome specific libraries. Results of the study suggest that development of markers from chromosome specific libraries is very useful for constructing high density linkage maps for chicken macrochromosomes (Ambady *et al.,* 2002). Jacobsson *et al.* (2004) established a genetic map of 2,426.6 cM comprising 25 linkage groups based on 145 microsatellite markers. The average distance between adjacent markers assigned to linkage groups was 17.0 cM; however, there were 7 gaps greater than 40 cM. McElroy *et al.* (2005) identified QTL conferring resistance to marek's disease (MD) in commercial layer chickens. Microsatellite markers used in this study were chosen based on their associated with MD resistance in 2 preliminary selective DNA pooling. Their results showed that identification of and subsequent selection upon QTL affecting MD resistance will be useful to the poultry industry to reduce losses caused by MD virus infection. The effectiveness

of microsatellite in detecting polymorphism between chicken populations and their applicability in population studies and establishing genetic relationships among chicken populations has been reported by Zhang *et al.* (2002a,b). The genetic variability and divergence of eight chicken lines were evaluated using nine microsatellite markers. The chicken lines included three white Leghorn hybrids, three Finnish Landrace lines, a Rhode Island Red line, and a broiler hybrid line. All the microsatellite loci were found to be polymorphic, the number of alleles varying from 4 to 13 per locus and 1 to 10 per line, respectively. Observed heterozygosities ranged from 0.00 to 0.91. The highest (0.67) and lowest (0.29) mean heterozygosity per line was observed in the broiler and White Leghorn respectively. (Vanhala *et al.,* 1998). Method of polymerase chain reaction with microsatellite markers were used to detect polymorphism between and within broiler populations. The 59 primer sets selected for this study provided wide genomic coverage. The average allele number per line per microsatellite was 2.8 and 2.9 for populations L and C, respectively. Considering the 57 primer pairs generating product in both lines, 72.3 % of the total alleles were unique to one or the other population. (Kaiser *et al.,* 2000). The comparative analysis of allozyme, random amplified polymorphic DNA (RAPD), and microsatellite polymorphism on Chinese chickens (five native populations, two fast growing broiler lines, and one layer line) has been reported. The lowest average heterozygosity was obtained with allozyme analysis (0.2209), intermediate heterozygosity was obtained with RAPD (0.2632), and the highest heterozygosity was observed with microsatellite analysis (0.7591). The genetic distances among all populations measured by three methods were also different. Allozyme data showed close relationships between Chinese native chickens and two broiler lines, but they were both remotely related to the layer line. Microsatellite polymorphism analysis was similar to the allozyme analysis but genetic distance 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, RAPD and microsatellite polymorphism. Imported broiler and layer breeds were included in the analysis. The results from protein polymorphism did not show distinct differences. Microsatellite polymorphism data showed that genetic diversity was high in the Chinese native chickens and low in layers and that there was a close relationship between Chinese native chickens and broiler but a remote relationship between Chinese native chickens and layers (Zhang *et al.,* 2002b). Genetic variation was observed at the microsatellite loci for the three commercial broiler pure lines. The number of alleles at a single locus ranged from one to eight, and the average number of alleles per locus was 3.5, 2.8 and 3.1 for each of the lines, respectively. The observed heterozygosities ranged between 0 and 89 % in the lines (Emara *et al.,* 2002). A total of 15 primers were used in the microsatellite analyses to evaluate genetic variability within and between four Haimen chicken populations (Jiangchun, Rugao, Cshiqishi and Wan-Nan) (Olowofeso *et al.,* 2005a,b). 116 alleles were generated, the number of alleles per locus ranged from 2 to 16 and 1 to 15 per chicken population. The smallest genetic distance was obtained between Rugao vs Jiangchun (0.1691) and the largest distance was found between Rugao vs Cshiqishi (0.3372). Yonash *et al.* (2001) used microsatellite markers (linked to quantitative trait loci affecting antibody response and survival rate) in meat type chickens. Four out of twenty five markers, which represented 14 linkage groups covering about 800 cM (24%) of the genetic map of the chicken, were found to be associated with the measured traits. The finding of this study suggest that the reported markers could be used for marker assisted selection to improve antibody response of young broiler chicks to two important pathogens (*E. coli* and NDV) and their survival rate when challenged by *E. coli* and even to improve general early and high humoral immune response to any antigen. Investigation microsatellite markers were used for evaluating genetic diversity in Rhode Island Red (RIR) and Sussex (SX) chickens, divergently selected over six generations for high or low incidence of skeletal defects in embryos. At the 15 microsatellite loci examined, the total of allele numbers amounted to 44 and 40 for RIR and SX chickens, respectively. The number of alleles at a single locus ranged from 1 to 6, the average number being 2.9 and 3.6 for RIR and SX chickens, respectively. The selection for a high or low level of skeletal defects in embryos respectively, had different effects on the frequencies of alleles of the analyzed microsatellite loci (Wardecka *et al.,* 2004). Hillel *et al.* (2003) used microsatellite markers to assess the genetic variation within and between 52 populations from a wide range of chicken types. The polymorphism measures for the average, the least polymorphic population and the most polymorphic population were, respectively, as follow: number of alleles per locus, per population: 3.5, 1.3 and 5.2; average gene diversity across markers: 0.74, 0.05 and 0.064 and proportion of polymorphic markers: 0.91, 0.25 and 1.0. Kaiser and Lamont (2002) used microsatellite markers to evaluate specific genomic regions for resistance to salmonella enterica (SE) burden in young broiler–cross chicks. All four microsatellites had a significant main effect and interaction with sex or dam line on levels of spleen SE burden in one or more sire families. The genetic structure of local chicken ecotypes of Tanzania has been detected using 20 polymorphic microsatellite DNA markers. It was concluded that there were high genetic

relatedness within indigenous chicken ecotype than between ecotypes (Msoffe *et al.,* 2005). Microsatellite technique was applied to detect genetic relationship among ten Japanese native breeds of chicken and one imported breed. Most Japanese native chickens were divided into three groups that correspond to the origin of breeds, Jidori, Shokoku and Shamo (Takahashi *et al.,* 1998). Five microsatellite markers with high polymorphisms were selected to detect the genetic diversity of seven Shandong indigenous chicken breeds. Altogether, forty alleles were found in this experiment, the distribution of alleles was not balanced and each locus showed one or more dominant alleles. The average heterozygosity in the Shouguang chicken was the lowest (0.3327), and that in other breeds was also less than 0.4 (Cheng *et al.,* 2003). Thirty microsatellite markers with medium or high polymorphisms were selected to detect the genetic diversity of 8 indigenous chicken breeds in Sichuan. The results showed that 24 out of 30 microsatellite sites were highly polymorphic, so the 24 microsatellite markers were effective markers for analysis of genetic relationship among chicken breeds. The mean heterozygosity of 8 chicken breeds was all over 0.5. The highest was the Luning chicken (0.681), and the lowest was the Jiuyuan Dark chicken (Tu *et al.,* 2005). The genetic variability of various local chicken populations derived from Bolivia, India, Nigeria and Tanzania was evaluated with 22 microsatellites. Between two and 11 alleles per locus were detected. All populations showed high levels of heterozygosity with the lowest value of 45% for the population named Aseel from India and the highest value of 67% for Arusha from Tanzania (Wimmers *et al.,* 2000). Forty-two microsatellite loci were analyzed in 23 highly inbred chicken lines derived from Leghorn, Jungle Fowl, Fayoumi and Spanish breeds. The band-sharing (BS) values were calculated and the proportions of shared alleles distances were estimated. The BS values between each pair of noncongenic Leghorn lines were 0.32–0.97, and between Leghorn and exotic (Jungle Fowl, Fayoumi and Spanish) breeds were 0.03-0.55. (Zhou and Lamont, 1999). 728 blood samples were collected from 22 breeds (28 populations) of native Japanese chickens to examine the genetic variability and relationships by using microsatellite DNA polymorphisms. The mean number of alleles per locus, the proportion of the polymorphic loci, and the expected average heterozygosity ranged from 1.75 to 4.70, from 0.55 to 1.00, and from 0.21 to 0.67, respectively (Osman *et al*., 2006).

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). Gaining in popularity as an experimental animal in both research and education, the Japanese quail (coturnix japonica) is a small, early maturing, highly efficient egg and meat producer (Pisenti *et al.,* 1999). Inoue-Murayama *et al.* (2001) tested chicken microsatellite markers to see if they would be suitable as genetic linkage markers in Japanese quail. Twenty-six percent (31/120) of chicken primers amplified individual loci in Japanese quail and 65% (20/31) of the amplified loci were found to be polymorphic. The results showed that most chicken markers are not useful for studies in quail. They concluded that more effort should be committed to developing quail-specific markers rather than attempting to adapt chicken markers for work in quail. A total of 100 Japanese quail microsatellite markers were evaluated in a population of 20 unrelated quails. Ninety-eight markers were polymorphic with an average of 3.7 alleles per locus and a mean heterozygosity of 0.423 (Kayang *et al.,* 2002). Three microsatellite loci were used with four quail populations in east China for the detection of genetic diversity of the no genetic relationship resources. The results demonstrated that gene diversity among loci ranged from 0.4627±0.03 to 0.6345±0.05 and the average gene diversity observed in the populations were in the increasing order of 0.4627, 0.5146, 0.5549 and 0.6345, respectively (Olajide *et al.,* 2006). Genetic coadaptability of wild Japanese quail, wild common quail and domestic quail populations in China was studied using 7 microsatellite DNA markers and Monte Carlo method to test genetic disequilibrium. The results showed that genetic coadaptability dominated the genetic disequilibrium of the three quail populations, and totally 16.67%, 9.66% and 10.05% of non-allelic combinations were in the genetic disequilibrium in wild Japanese quail, wild common quail and domestic quail populations, respectively (Guobin *et al.,* 2006). A linkage map of the Japanese quail genome was constructed based on microsatellite loci in 433 F2 progeny of 10 half-sib families. Fifty-eight of the markers were resolved into 12 autosomal linkage groups and a Z chromosome-specific linkage group, while the remaining 14 markers were unlinked. (Kayang *et al.,* 2004). To detect polymorphism in Japanese quail, fifty microsatellite markers were tested and were found to be effective. Forty-six percent (23 of 50) of the markers revealed polymorphism in two unrelated quail individuals (one male and one female) randomly sampled from a population of wild quail origin. (Kayang *et al.,* 2000). Forty-eight primer pairs for chicken microsatellite loci were tested in PCR amplification of Japanese quail genomic DNA. Amplification products were obtained from 28 primer-pairs (58.3%). Eleven (22.9%) of these generated specific products and 17 yielded non-specific amplification products. Eight markers were polymorphic and three were

monomorphic in four Japanese quail populations (Pang *et al.,* 1999).

Turkey, goose and duck: Reed *et al.* (2002) described the characterization of twelve microsatellite loci for the turkey. Seven of the twelve loci were polymorphic in the individuals examined. The number of alleles ranged from one to six, with an average of 2.7 alleles per locus. Eighty-eight chicken microsatellite markers were tested for their ability to amplify polymorphic fragments using turkey genomic DNA. From the 61 markers that gave a product, only eight showed a length polymorphism while 37 were monomorphic on the three divergent commercial turkey lines used. The results showed that chicken microsatellite markers are not very useful for mapping purposes in turkey (Liu *et al.,* 1996). The efficacy of employing the chicken genome sequence in developing genetic markers and in mapping the turkey genome was studied. A total of 78 primer sets were tested for amplification and polymorphism in the turkey, and informative markers were genetically mapped. Sixty-five (83%) amplified turkey genomic DNA, and 33 (42%) were polymorphic in the Turkey (Chaves *et al.,* 2006). Reed *et al.* (2005) utilized the INRA ChickRH6 whole-genome radiation hybrid panel and chicken whole-genome shotgun sequence to map microsatellite markers from the turkey. Thirty-three of the 41 markers typed on the RH panel had significant linkage to at least one other marker and 83 of 100 sequences returned significant BLAST similarities. In order to increase the number of markers on a turkey genetic linkage map. Burt *et al.* (2003) have been mapped the new microsatellite sequences obtained from a GT-enriched turkey genomic library. 43% of all turkey primers tested were found to be polymorphic, in both commercial and wild type turkeys. Twenty linkage groups (including the Z chromosome) containing 74 markers have been established, along with 37 other unassigned markers. Kamara *et al.* (2007) used microsatellites to analyze the genetic relatedness among commercial and heritage domestic turkeys including Narragansett, Bourbon Red, Blue Slate, Spanish Black, and Royal Palm. The phylogenetic analysis showed that the Blue Slate, Bourbon Red, and Narragansett were genetically closely related to the commercial strain with Nei distance (D) of 0.30, and the Royal Palm and Spanish Black were the least related to the commercial strain with D=0.41 and D=0.40, respectively. The genetic diversity of six goose breeds (White Goose, Zi Goose, Huoyan Goose, Wanxi Goose, Rhin, Landoise) was analyzed using microsatellite markers. Results showed that 7 microsatellite sites were highly polymorphic, and could be used as effective markers for analysis of genetic relationship among different goose breeds. The mean heterozygosities were between 0.6617 (Rhin) and 0.8814 (Zi goose), Among six goose breeds, the lowest was Rhin goose (0.6617) and the highest (0.8814) was Zi goose (Shuang *et al.,* 2006). Yunjie *et al.* (2006) examined the genetic structure of 13 indigenous grey goose breeds using 31 polymorphic microsatellite markers. Of the 13 goose breeds, the highest mean heterozygosity was observed in the Shitou (0.6727), whereas the lowest heterozygosity was found in the Yan breed (0.4985). Pierson *et al.* (2000) compared data from seven microsatellite DNA loci and 143 base pairs of the control region of mitochondrial DNA from the two populations of Aleutian Canada Geese and another small-bodied subspecies, the Cackling Canada Goose (B.c. minima) which nests in western Alaska. The populations of Aleutian geese were genetically differentiated from one another in terms of mitochondrial DNA haplotype and microsatellite allele frequencies, suggesting limited contemporary gene flow and/or major shifts in gene frequency through genetic drift. Tu *et al.* (2006) studied the genetic structure research of 14 indigenous grey goose breeds using 19 developed and 12 searched microsatellite markers with middle polymorphism. The results indicated that 25 out of 31 microsatellite sites showed polymorphic at medium level. The mean heterozygosity was between 0.4985 and 0.6916. A total of 35 microsatellites primers were used to detect polymorphisms in 31 unrelated Peking ducks. Twenty-eight loci were polymorphic and seven loci were monomorphic. A total of 117 alleles were observed from these polymorphic microsatellite markers, which ranged from 2 to 14 with an average of 4.18 per locus. The frequencies of the 117 alleles ranged from 0.02 to 0.98 (Huang *et al.,* 2005). A genetic linkage map for the duck was developed within a cross between two extreme Peking duck lines by linkage analysis of 155 polymorphic microsatellite markers. A total of 115 microsatellite markers were placed into 19 linkage groups. The sex-averaged map spans 1353.3 cM, with an average interval distance of 15.04 cM. The male map covers 1415 cM, whereas the female map covers only 1387.6 cM (Huang *et al.,* 2006). Williams *et al.* (2004) assessed genetic variation among 225 mottled ducks and mallards using five microsatellite loci. In contrast only 3.4% of mallards were inferred to have been hybrids, suggesting asymmetric hybridization. Populations from different geographic areas within Florida exhibited hybridization rates ranging from 0% to 24%. Williams *et al.* (2002) studied nuclear DNA-based markers for Mottled Ducks and determined levels of subdivision among populations in Florida. They screened 13 microsatellite primer pairs and identified six microsatellite loci that were variable in Mottled ducks. These markers revealed a low level of genetic differentiation and a high level of genetic exchange among four Mottled duck subpopulations within Florida. The analysis of the Florida Mottled duck population indicated high levels of heterozygosity and no evidence of genetic subdivision among breeding units.

Other birds: The use of microsatellites has been reported in other bird species research.

Van Den Bussche *et al.* (2003) examined genetic variation within and among populations of lesser prairie chickens associated with 20 leks in Oklahoma and New Mexico through mitochondrial genome and by Microsatellite loci. Approximately 89 % of the variation was partitioned within leks, whereas 3.0 % (mtDNA) and 6.7 % (microsatellites) of the variation was partitioned among leks within Oklahoma and New Mexico. Piertney and Hoglund (2001) described the characterization of microsatellite polymorphism from black grouse. Mean observed heterozygosity was 0.74 and the number of alleles ranged from 5 to 16. No evidence for linkage disequilibrium or the presence of null alleles was found. Bo-Göran *et al.* (2003) observed Siberian Jay group composition using microsatellite analysis. They found that out of 311 groups that included at least one more individual than the territory holders, 74% were nuclear families, including breeding birds and 1-3 retained offspring. However, 26% of the groups were not families, but consisted of pairs accompanied only by individuals that were not their offspring. Six perfect GT microsatellites were characterized and optimized in 45 individuals of red-legged partridge. All loci revealed high levels of polymorphism with a number of alleles that ranged from three to 13. Observed heterozygosity ranged from 0.2 to 0.6. Cross-species amplification showed that all loci were also polymorphic in rock partridge. (Gonzalez *et al.,* 2005). Chen *et al.* (2006) used allelic variation at eight microsatellite markers to describe the genetic structure of Rock Partridge populations. The average value of expected heterozygosity (H_E) (0.455) was smaller than observed heterozygosity (H_O) (0.477). There was a heterozygote deficit at the MCW135 locus in the Lanzhou population and the Beidao population. Analysis of population structure revealed clear differentiation among the eight populations of Rock Partridge, suggesting strong isolation of these populations and correspondingly low levels of migration or gene flow. Polymorphic microsatellite loci were identified in order to study golden eagle population fragmentation. Fifteen loci were polymorphic with between two and six alleles detected per locus. Observed heterozygosity ranged from 0.15 to 0.77 among 177 unrelated individuals from Scotland. (Bourke and Dawson, 2006).

Discussion

Although the mapping of the chicken genome is almost complete, it is important that poultry and avian genetic stock be available for further genetic research studies. Compared to other DNA polymorphism analyses, the detection of microsatellite polymorphism results in the greatest expected heterozygosity (Powell *et al.,* 1996). Microsatellite polymorphisms enable a clearer differentiation, even between closely related breeds, and increase the accuracy of the predicted divergence (Zhang *et al.,* 2002a,b). Microsatellites are useful for a number of analyses. They were originally utilized for genetic mapping (Tuiskula *et al.,* 2002; Ambady *et al.,* 2002) and have been extensively used for linkage analyses in the association with disease susceptibility genes (McElroy *et al.,* 2005; Wardecka *et al.,* 2004). In addition they have proven to be useful in the analysis of paternity and kinship (Queller *et al.,* 1993) and in the probability of sample identity at both the individual (Edwards *et al.*, 1992) and population levels (Ya-Bo *et al.,* 2006). In the study of entire populations microsatellites are also very useful (Kashyap *et al.,* 2006; Takahashi *et al.,* 1998). Microsatellite variation has been used to study the amount of hybridization between closely related species (Gottelli *et al.,* 1994). In comparison of the levels of variation between species and populations they have also proven to be useful in the assessment of overall genetic variation (Gottelli *et al.,*1994; Paetkau and Strobeck, 1994; Taylor *et al.,* 1994). They can be used to estimate effective population size (Allen *et al.,* 1995) and to gain insight into the degree of population substructure including both the amount of migration between subpopulations (Allen *et al.*, 1995; Gottelli *et al.,* 1994) and genetic relationships among the various subpopulations (Bowcock *et al.,* 1994; Forbes *et al.,* 1995; Estoup *et al.,* 1996; Lade *et al.,* 1996). With respect to the microsatellite limitations, a number of drawbacks have been reported. There is a striking absence, however, of successful application to phylogenetic reconstruction. This almost certainly results from a combination of two complicating factors: (1) the existence of range constrains limiting the size of microsatellite alleles, and (2) the degradation of microsatellites loci over time. Preliminary studies indicate that the latter can sometimes make it difficult to find microsatellites which are polymorphic in multiple species. (Shriver *et al.,* 1995; Garza *et al.,* 1995) although in other instances polymorphic microsatellites can last over considerable phylogenetic divergences. In order to facilitate the use of microsatellites in phylogenetic reconstruction, the dependence of the microsatellite type and genomic location should be studied systematically (Pollock *et al.,* 1998). A number of authors have emphasized that range constrains critically influence the utility of microsatellte loci (Garza *et al.,* 1995; Feldman *et al.,* 1997). One of the major drawbacks of single locus microsatellite markers is the time and cost required to isolate and characterize each locus, a process typically involving library construction and screening, DNA sequencing, PCR primer design and PCR optimization. To capture some of the polymorphism associated with microsatellite loci without the expense of single locus isolation, two multi-locus DNA profiling approaches have been developed which

target microsatellite regions in the genome. One approach is to probe genomic DNA with oligonucleotides complementary to microsatellites. A second approach is PCR using primers containing microsatellite repeats (Fisher *et al.,* 1996). Microsatellites developed for particular species can often be applied to closely related species, but the percentage of loci that successfully amplify may decrease with increasing genetic distance (Jarne and Lagoda, 1996). Point mutation in the primer annealing sites in such species may lead to the occurrence of 'null alleles', where microsatellites fail to amplify in PCR assays (Jarne and Lagoda, 1996; Dakin and Avise, 2004). Null alleles can be attributed to several phenomena. Sequence divergence in flanking regions can lead to poor primer annealing, especially at the 3' section, where extension commences; preferential amplification of particular size alleles due to the competitive nature of PCR can lead to heterozygous individuals being scored for homozygosity (partial null). PCR failure may result when particular loci fail to amplify, whereas others amplify more efficiently and may appear falsely homozygous. However, stochastic effects of small populations and the possibility of sex linkage must also be considered in order not to give false evidence of a null allele due to increased homozygosity within population analysis. Allele size differences may not reflect true divergence i.e. mutation may result from addition or deletion of bases and overall microsatellites may be under certain constraints in length. Mutation rates are not standard, and the neutrality of some microsatellite regions are coming under question, perhaps due to quantitative trait variation or occurrence within exon regions of genes under selection (Jarne and Lagoda, 1996). When using microsatellites to compare species, homologous loci may be easily amplified in related species, but the number of loci that amplify successfully during PCR may decrease with increased genetic distance between the species in question. Mutation in microsatellite alleles is biased in the sense that larger alleles contain more bases, and are therefore likely to be mistranslated in DNA replication. Smaller alleles also tend to increase in size, whereas larger alleles tend to decrease in size, as they may be subject to an upper size limit; this constraint has been determined but possible values have not yet been specified. If there is a large size difference between individual alleles, the there may be increased instability during recombination at meiosis (Jarne and Lagoda, 1996). In tumour cells, where controls on replication may be damaged, microsatellites may be gained or lost at an especially high frequency during each round of mitosis. Hence a tumour cell line might show a different genetic fingerprint from that of the host tissue.

Conclusion: The microsatellite marker has been the most widely used, due to its easy use by simple PCR and to the high degree of information provided by its large number of alleles per locus. The effectiveness of microsatellite in detecting polymorphism between different poultry species, their applicability in population studies, and the establishment of genetic relationships demonstrated with this review. Benefiting from molecular cloning and PCR techniques, DNA markers have now became a popular means for identification and authentication of plant and animal species. The use of molecular techniques offers new opportunities and challenges for building and using more predictive and efficient statistical models for livestock improvement.

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