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MOLECULAR METHODS APPLIED TO DETERMINING GENETIC DIVERSITY IN GEESE

Research Article

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Abstract: Genetic diversity, refers to the total number of genetic traits in the gene pool of any species and races belonging that species adapted to a particular geographic region. Molecular markers allow us to obtain information about the unique functional genes of the races and the characteristics of these genes. In the detection of genetic diversity are used such as methods polymerase chain reaction (PCR), Amplified Fragment Length Polymorphism (AFLP), Restriction Fragment Length Polymorphism (RFLP), Randomly Amplified Polymorphic DNA (RAPD), Simple Sequence Repeat (SSR), Short Tandem Repeats (STR) and Single Nucleotide Polymorphism (SNP). In poultry such as goose, chicken, quail, duck and turkey; different methods are used depending on the type of animal, its characteristics and the purpose of the study. In the studies conducted to in geese; Microsatellites are more preferred because they require a small amount of DNA, show high polymorphism, are reproducible and provide more information. In this study, it is aimed to compile the researches on the use of molecular markers in other poultry, especially geese.

Key Words: Goose, genetic diversity, molecular markers, mikrosatellits, PCR

1. Introduction

Genetic diversity, refers to the total number of genetic traits in the gene pool of any species and races belonging that species adapted to a particular geographic region. It is also a concept that includes the ability of populations of that species to adapt to changing environmental conditions. Genetic diversity and variation that occurs under environmental and other conditions; it allows some individuals in the population to have allelic variations suitable for environmental conditions. This allows the population to be maintained under that environmental conditions by obtaining progeny with the desired characteristics through selection processes. Detection of genetic diversity not only allows the protection of native breeds, but also provides an opportunity to improve breeding studies. Meeting the food needs of the ever-increasing world population depends on interracial and intraracial genetic diversity and constitutes the most basic resource for breeders (Mercan, 2010). The selection process has an important place in the emergence of genetic differences between races. Thanks to selection processes and breeding studies, it is possible to improve the yield characteristics of any breed. Firstly environmental stress factors, adaptation factors such as reproduction, survivability of parents and offspring can cause genetic differences between races (Mercan, 2010).

DNA polymorphism analyzes have an important place in determining the genetic diversity of farm animals (Baumung et al, 2004). In this context; In addition to basic research such as DNA markers, phylogenetic analyzes and the search for beneficial genes, it also constitutes the most important materials for practical studies such as marker-assisted selection, parental tests, and determination of

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homozygosity in populations. Detection of interracial and intraracial genetic diversity in farm animals, identification of breeds and revealing kinship relations between populations are carried out with the help of microsatellite markers (Kırıkçı, 2017). Today, in revealing the genetic diversity of geese in general; microsatellite markers are isolated and used in scientific studies from wild forms of geese such as Greylag goose (*Anser anser*), Canada goose (*Branta canadensis L.*), Swan goose (*Anser cygnoides L.*), White-fronted goose (*Anser albifrons*). It has been stated that these markers are also used to can also be found in such as Chinese, Hungarian, Embden and Zatorska etc. detect genetic diversity in geese species (Weib et al, 2008; Cathey et al, 1998; Li et al, 2007; Tu et al, 2006; Mindek et al, 2014b).

It is stated that molecular markers give us information about the unique functional genes of the races and the characteristics of these genes (Anonymous, 2007; Toro et al, 2009). Molecular applications provide researchers with alternative sources for the determination of population size (Ne). Mercan (2010), effective population size; stated that it is an indicator used in the estimation of the number of productive (effective) animals that ensure the reproduction of a population and the transfer of possessed genes to the next generation (Mercan, 2010). Population size (Ne) is considered in inbreeding practices and genetic drift. Therefore, the (Ne) value gives us an idea about whether the current population is in danger of extinction or not. Reliable estimation of the Ne value in traditional livestock farming is usually made using either pedigree records or animal censuses. However, in populations, these data are not sufficient in terms of variables such as reproductive efficiency and time between generations, making it very difficult to reach reliable data (Anonymous, 2007). In this context, molecular applications are very effective in terms of the existence-continuity of the population and the reliability of the data obtained. However, due to the high costs of molecular applications, these studies cannot be realized at the desired levels, especially in developing countries.

In this study; it was aimed to compile the molecular methods currently used in animal husbandry and the studies carried out on the determination of genetic diversity in goose breeding with these methods.

2. Molecular Methods

In the scientific world, there are many different techniques for detecting genetic diversity. Along with technological developments, new ones are added to these techniques every day. In addition to detecting genetic diversity, these methods are also used for molecular typing of microorganisms. If the typing is briefly explained; It is a more advanced identification process that is performed at the subspecies level in microorganisms and has no effect on naming (Akın et al, 2020). Typing methods can generally be divided into two subgroups, which are phenotypic and genotypic typing (Açık and Öztürk, 2010). Today as phenotypic typing methods are used biochemical and antigenic profile, antibiotic resistance tests of bacteria, phage typing, serotyping, multioccise enzyme electrophoresis profile detection (Akın et al, 2020). In genotypic typing methods are included Polymerase Chain Reaction (PCR), Amplified Ribosomal DNA Restriction Analysis (ARDRA), plasmid fingerprinting, Restriction Fragment Length Polymorphism (RFLP), Amplified Fragment Length Polymorphism (AFLP) and Pulsed-Field Gel Electrophoresis (PFGE) (Akın et al, 2020; Aydın and Sudagidan, 2016; Tenover et al, 1995).

We can explain the characteristics and application procedures of genetic diversity and molecular typing methods as follows.

2.1 Polymerase Chain Reaction (PCR)

It was first used in 1983 this technique by Dr. Kary B. Mullis, it is based on the principle of reproducing the target nucleic acid chains of organisms in the laboratory using special primers and

heat-stable enzymes. PCR is a unique and reliable technique that consists of a simple in-vitro chemical reaction. It allows an unlimited number of targeted nucleic acids and sequences to be synthesized. For a problem-free PCR process, it is possible with the activation of DNA polymerase, which can copy the DNA chain under suitable conditions. It is a powerful tool for in-vitro amplification and microbiological diagnosis of the DNA in PCR of a microorganism isolated from any organism (Cilo Dalyan, 2011; Çetinkaya and Akman, 2012). PCR technique; It allows any genetic material that has been researched to be replicated and turned into a homogeneous DNA material, despite being among many DNA molecules. Thus, researchers can easily identify the investigated genetic material (Çetinkaya and Akman, 2012; Schochetman and Jones, 1988).

In the PCR technique, opposite sequences of DNA are synthesized by the reaction polymerase enzyme, and it is possible to suppress unwanted sequences while the selected DNA sequence is amplified. Thus, identification of the DNA sequence is not difficult at all. (Çetinkaya and Akman, 2012; Saiki et al, 1988). Most molecular-based methods (AFLP, RAPD, SSR, SNP) are PCR-based markers. Today, there are many optimized and modified PCR methods for different usage purposes. With the discovery of the PCR technique, it has been possible to develop many technologies for polymorphism detection at the DNA level (Provan et al, 1999).

2.2 Amplified Fragment Length Polymorphism (AFLP)

Genotyping method based on the principle of amplified fragment length polymorphism of genetic material was first introduced in 1995 by Vos et al it has been proposed as a new and high-resolution fingerprinting method for bacterial species and has attracted great interest since its publication (Vos, et al, 1995; Lindstedt et al, 2000). The AFLP method consists of amplifying the DNA fragments by PCR by cutting the fragment containing the target DNA material being studied with restriction enzymes. In this method, two-stage PCR is applied. Thus, labeling of DNA and limiting the level of polymorphism are ensured (Zabeau and Vos, 1993). It is seen that this method is especially used in determining the degree of closeness of species and is frequently used in species identification studies (Gözel et al, 2016).

2.3 Restriction Fragment Length Polymorphism (RFLP)

The RFLP method is the first and oldest molecular marker method used in genetic studies. In this method, it is aimed to detect the differences in the DNA sequence. Restriction enzymes recognize special 4-8 base pair (bp) nucleotide sequences in the DNA chain and cut them from the restriction sites. The distribution of DNA fragments of different lengths cut by enzymes is detected in agarose gel electrophoresis. In addition to the advantages of repeatability of this method, such as being easy, fast and cheap; we can say that as the biggest disadvantage of the method is that the restriction enzyme used cuts a large number of DNA, making it difficult to interpret the bands. In order to overcome and minimize the mentioned problems, probes are used in the hybridization process. After this stage, the band profile entering the hybridization process with the probe is evaluated and interpreted (Liu and Cordes, 2004; Busch and Nitschko, 1999).

2.4 Amplified Ribozomal DNA Restriction Analysis (ARDRA)

The ARDRA method was developed as a result of combining PCR-based DNA typing method with restriction enzyme analysis. The principle of application of the method is based on the cutting of rDNA fragments obtained in PCR with the combination of selected restriction enzymes in the next step. After this process, the obtained fragments are separated in an agarose or polyacrylamide gel, and then the band profiles are evaluated and the grouping process is started (Açık and Öztürk, 2010; Torsvik et al, 1998).

2.5 Randomly amplified polymorphic DNA (RAPD)

This technique was developed in 1990 by Williams et al. (Williams et al, 1990). The method is performed by randomly amplifying regions on the genome of the studied organism in PCR using single primers of 6-10 bases. As in other techniques, there is no target DNA region to be replicated in the RAPD method. In PCR, DNA fragments are amplified and then run in the electrophoresis, for can detect polymorphism according to the bands that appear (Gözel et al, 2016).

2.6 Single nucleotide polymorphism (SNP)

Single nucleotide polymorphism (SNP), can be expressed as a change in a single nucleotide pair in the DNA sequence seen in a very small part of a huge population. SNP's, which are mostly found in non-coding regions of DNA, occur frequently in the genome. If the high number of single nucleotide polymorphisms, makes it easy for us to identify variations among individuals. The emergence of a single nucleotide polymorphism in DNA is due to the addition or deletion of one or more bases. It has been stated that the SNP technique, it is preferred in mapping and durability studies with the quantitative trait locus (QTL) (Gözel et al, 2016).

2.7 Microsatellites

Microsatellites were discovered by Tautz (1989) and defined as the smallest repeating units of DNA sequences with repeat motifs 1-6 bp long (Tautz, 1989). They are also known as Simple Sequence Repeats (SSR) or Short Tandem Repeats (STR). SSR marker system, in biodiversity studies; High polymorphism rate, reproducibility and codominant character have made them the most widely used PCR-based method (Ün et al, 2000; Budak et al, 2004). Microsatellites show high level of polymorphism because most microsatellite loci have the frequency of mutations in a generation varies from 10-2 to 10-6 (mean 5x10-4) in per locus, for each gamete. It has been stated that microsatellites are one of the most important markers of polymorphic DNA structure (Tautz, 1989; Goldstein et al, 1995).

Microsatellites allow the detection of genetic diversity within and between populations, identification and monitoring of allelic polymorphism. It allows the comparison of pedigree records by evaluating them at the genomic level, and it has features such as keeping and keeping these records, allowing to obtain versatile and detailed information. This has made microsatellites more preferable than other methods. (Takezaki and Nei, 1996; Pariset et al, 2003). When compared to other techniques; It is a very useful method for researchers because it requires a small amount of DNA, shows high polymorphism, is reproducible, and contains more information (Powell et al, 1996). In addition to all these advantages, the most important disadvantage of the method is that it requires genome information and sequence analysis. (Morgante and Olivieri, 1993). In addition, the development of new SSR markers is not easy and requires high costs. In recent years; microsatellite libraries have been created and contributed to the partial elimination of this deficiency.

The most common use of microsatellites in genetic studies is population studies. SSR's are found more common in non-coding regions. They occur as a result of mutations such as replication shift and cross-over and are characterized by highly levels repetitive length polymorphisms (Mercan, 2010; Schlötterer and Tautz, 1992). Differences in individuals in a population result from differences in the non-coding DNA sequence between genes. As a result of genome studies; Since

the sequences of microsatellite primer pairs are conserved between species, it has been found to be suitable for evaluations between related species and races (Navani et al, 2002).

Inter Simple Sequence Repeat (ISSR) method was created by modifying the SSR method. ISSR is known as the region between microsatellite loci in a genome region, and the ability of the DNA fragments between these two microsatellites to be amplified by PCR using primers allowed the emergence of the method (Zietkiewicz et al, 1994). The most important advantage of the method is that there is no need for sequence information, and it allows primer design. However, it is known as the most important shortcoming of the method that it is dominant, has low reproducibility, and particles of similar size are not homologous.

3. Using of Molecular Markers in Geese and Other Poultry

Molecular markers are used such as in chickens, quail, duck, goose, etc. to reveal the genetic characteristics of many different poultry. Different methods are applied according to the characteristics of the animal being studied and the purpose of the study. In a study investigated the genetic diversity and population structure of Taihu geese in China, 26 microsatellite markers were used, a total of 83 alleles were detected, and the average number of alleles per locus was reported as 3,107. In addition, it has been explained the mean observed heterozygosity was 0.829, and the expected mean heterozygosity was 0.545. Thanks to the study, it has been revealed Taihu geese have significant genetic diversity and low level of inbreeding, despite the decrease in their breeding regions (Qing-Ping et al, 2009).

By Mindek et al (2014a) to investigate the genetic diversity and genetic characterization of the Tesedik goose, were used 6 microsatellite loci (Aalµ1, Bcaµ1, CKW21, TTUCG5, Ans02, Ans25) and make genotyping of 50 individuals, 28 different alleles have been reported (Mindek et al, 2014a). In another study of the researchers; it has been reported low heterozygosity values and genetic diversity of the endangered Suchovska and Slovak goose breeds from Slovakia. They explained that low population size and ineffectiveness of heterozygosity reduce genetic diversity and this situation causes the two races to be endangered (Mindek et al, 2014b). In a different study, 29 microsatellite markers were used to determine the evolutionary relationship between 5 domestic and 1 foreign origin Chinese goose breeds. It was stated that 334 alleles were observed among a total of 6 races, and 45 of these alleles (13.5%) were specific to only one race, and the genetic diversity of the foreign race was higher than the native one. (Li et al, 2012). The researchers also explained that there is a decrease in genetic diversity among some of the indigenous breeds in China due to too much cross-breeding.

Andres and Kapkowska (2011) conducted a study to develop molecular markers to be used in detecting genetic diversity, starting from the idea that although there are many domestic and commercial breeds of geese spread throughout the world, genetic diversity cannot be adequately determined at the molecular. Researchers stated that 24 microsatellite markers, which are currently used in many birds, can be used effectively in population genetics and genetic diversity studies on geese. Pellegrino et al (2015), using mitochondrial DNA and microsatellites, obtained samples from mating areas in Norway and the Netherlands and wintering areas in northern and southern France to determine the genetic diversity of Greylag goose populations on the European Atlantic coastline. As a result of their mtDNA analysis, they reported that there was low genetic diversity among the samples taken from the Netherlands, Norway and Northern France, and that the Southern France population exhibited a partially different genetic change from the other 3 populations. As a result of microsatellite analysis, they stated that it is not possible to group geographically, and there is a high amount of genetic mixing in all populations except genetically very similar populations in mating areas in Norway (Pellegrino et al, 2015).

In a study using mitochondrial DNA on 26 native goose breeds and 6 Landaise geese in China; It was stated that the average haplotype diversity and nucleotide diversity were 0.1384 and 0.00029, respectively, and it was determined that one of the domestic goose breeds originated from the Greylag Goose breed and the remaining 25 breeds originated from the Chinese Goose. In addition, it was also stated that Linxian White Goose and Wanxi White Goose showed a lot of genetic similarity with Rhine Goose and Landaise Goose, which originated from Gray Goose (Li et al, 2011). Mitochondrial DNA method was used to determine gene flow among snow goose populations distributed in West Africa. At the end of the study, it was determined that gene flow between populations is possible when the populations overwinter in a general place, and it has been stated that wintering regions play an important role in the preservation of genetic diversity among populations (Shorey et al, 2011).

In a study conducted by Tu et al (2006), using microsatellite markers to determine the genetic diversity of 14 native Gray Goose breeds in China; It was stated that 25 out of 31 microsatellites showed moderate polymorphism and the mean heterozygosity was between 0.4985 and 0.6916. At the end of the research, it was explained that the characteristics of indigenous breeds in East China should be investigated and these breeds should be protected (Cathey et al, 1998). In a study in which Canadian geese (*Branta canadensis*) were scanned with clones containing various microsatellite motifs, 460 Canadian geese were used. For the study, 5 primer pairs showing polymorphism in more than one population were developed and it was stated that two of these 5 primers were monomorphic. While the number of alleles per locus varied between 7 and 24, the mean heterozygosity levels were reported to vary between 0.34 and 0.78. It has been emphasized that these markers will contribute to revealing the existing genetic problems in Canada geese thanks to the high levels of polymorphism (Cathey et al, 1998). Poyarkov et al, (2010) examined the genetic diversity of Swan Geese in Russia by mitochondrial DNA method and defined a total of 11 haplotypes. He explained that the genetic diversity of Swan Geese is lower than that of some other geese that are in danger of extinction in the world.

Sun et al, (2014) examined the mitochondrial DNA control regions of 245 domestic goose and stated that goose species in the European originated from the Greylag goose (*Anser anser*), while the Chinese domestic goose breed originated from the Swan goose (*Anser cygnoides*). It was isolated 10 new polymorphic microsatellite loci from Greylag geese by Weib et al, (2008) and 5 new primer pairs were designed based on previous microsatellite loci from those closely genetically related. They explained that the number of alleles per locus was between 2-12, and the observed heterozygosity was between 0.07-0.85. They stated that these marker sets would be more effective in determining the origins and proportions of new individuals belonging to the Greylag goose population. Basha et al (2016), in their study to reveal the genetic diversity and phylogenetic relationship between the randomly amplified polymorphic DNA method and the native Egyptian geese and ducks (Muscovy, Sudani and White Pekin); 19 random primers were designed. Of these primers, 16 primers were noted to produce 189 reproducibly amplified fragments. Of these, 169 DNA bands were stated to be 89.41% polymorphic, and it was explained that primers OPA04 and OPA03 produced high polymorphic bands.

Demirtaş (2018) obtained blood samples from 110 geese from Yozgat province in Turkey in order to determine the genetic diversity of domestic goose populations belonging to White, Pied, Pure Domestic goose genotypes and Embden x Chinese hybrid genotype. Genetic diversity status of populations were analyzed using microsatellite loci Ans02, Ans25, Ans17, Aalµ1b, Aph19b and TTUCG5. The polymorphism information content (PIC) values of these loci were reported

respectively; 0.919, 0.925, 0.917, 0.907, 0.879 and 0.904, It was stated the most allele was in Ans25 (28), and the least allele was Aph19b (14) detected in the microsatellite locus. The researcher stated that the average allele counts in the White, Pied, Pure Domestic and Embden x Chinese cross populations were 37.0, 40.3, 41.8 and 38.5, respectively, and that the populations had a rich allelic diversity. Devrim et al, (2007) conducted a study to determine the genetic structure, evolutionary relationships and genetic diversity among local geese, which are defined by four feather colors as white, black, tawny and yellow in the transition region between the Caucasus Mountains and Anatolia. For this purpose, DNA samples were taken from 100 animals and randomly screened with 50 primers. They stated that genetic relationships showed 40 polymorphic bands (83.33%) among all populations, and randomly amplified polymorphic DNA polymorphisms obtained from a total of 48 loci were detected. A dendrogram they created for this study revealed a close relationship between geese with white and black feathers. They also stated that the tawny geese closely resembled the white and black geese, and the yellow colored geese differed significantly from the other three populations.

Recently, microsatellite markers are one of the most widely used methods in the analysis of chickens' genotypes and populations at the molecular level, in determining the genetic diversity, genetic similarities and differences of chickens, and in the genome studies of chickens. The fact that microsatellite markers have significant advantages over other methods in determining genetic similarity and difference has an important role in the preference. Besides chickens, such as duck, quail, turkey etc. some studies on the use of microsatellite markers in determining genetic diversity, genetic similarity and difference in poultry and the results obtained from these studies are briefly explained below.

In a study, hybrid genetic diversity and diversity of 8 chicken lines (3 lines of different origin White Legorn and Finland native lines, one Rhode Island Red and Broiler genotype) were investigated by using 9 microsatellite markers. The number of alleles seen; While it varied between 4-13 per locus and 1-10 per line, the observed heterozygosity value was reported to be between 0.00-0.91. It has been reported that the allele distribution pattern is quite irregular at different loci, the lowest value of average heterozygosity per line was observed at 0.29 in the Makela White Legorn, and the highest value was observed in the Broiler line with 0.67 (Vanhala et al, 1998). Zhou and Lamont (1999) investigated the genetic characterization of 23 chicken lines from Legorn, grouse, Fayoumi and Hispanic breeds obtained from intensive inbreeding program. In this study, it was revealed that the kinship coefficient of 13 lines was 99 %, 4 lines were 60%, and 4 lines were Major Congenic* lines. Mercan (2010) stated that in this study, the researchers aimed to determine the genetic profile of 23 lines using 42 microsatellite loci and to compare the genetic diversity within and between lines. He also stated that they aimed to determine the phylogenetic relationship between the lines by identifying line and breed specific primers (Mercan, 2010).

In the study by Wimmers et al, (2000) was investigated the genetic diversity among different local populations from Bolivia, India, Nigeria and Tanzania. It was aimed to genetically evaluate chicken populations obtained from different tropical and subtropical countries and to determine genetically different ones in this study. The research team used 12 microsatellite loci in the study, and reported that all populations showed high heterozygosity at the end of the study. They stated that the lowest heterozygosity rate was observed in the population named Aseel originating from India with 45%, and the highest rate was observed in the Arusha population originating from Tanzania with 67%. In a study by Li et al (2006), conducted to determine Chinese domestic ducks which the genetic

^{*} Although have a genetic background from the same paternal line, lines which differ from this line in terms of particular gene

diversity levels and genetic relationships between different populations; 28 microsatellite markers were used, the total number of alleles was found 236, the mean number of alleles was 8.429, and the mean PIC value was 0.725. They reported that the phylogenetic relationships of the samples showed a compatible classification in terms of geographical location and historical relevance. In a study, it was aimed to detect for the genetic relationships of ancestral turkey varieties with commercial turkeys, and 5 ancestral turkey varieties and 1 commercial turkey line were analyzed using 10 microsatellite loci. With the findings of the study, it was determined that the Blue Slate, Bourbon Red, Narragansett ancestral turkey varieties are closely related to the commercial turkey line. It was concluded that Royal Palm and Spanish Black varieties are the two most distant varieties from the commercial line, and that the ancestral varieties can be used successfully in the genetic breeding of commercial lines (Kamara et al, 2007). It was conducted by Kim et al, (2007) in the study, compared the genetic diversity of the randomly mated quail line and the line produced from this line and included in the inbreeding program. They used 24 loci to compare the kinship values calculated from the pedigree records with microsatellite marker analysis. It was stated that the observed heterozygosity value, was 0.43 in the randomly mated line and 0.21 in the inbreeding line (Kim et al, 2007).

4. Result and Conclusion

In order to determine the genetic diversity of poultry such as goose, chicken, quail, duck, turkey; different methods were preferred according to the type and characteristics of the animal studied and the purpose of the research to be conducted. Microsatellites have been preferred more than other methods because of its advantages such as showing high polymorphism with less DNA, high reproducibility and containing more information. However, the application of the method requires experience, since genome information and sequence analysis are essential. Molecular methods cannot be applied at desired levels especially in developing countries due to their high costs. In this context, developed countries will contribute to the applicability of these methods all over the world with the support of experienced scientists as well as financial support. It can be said that a sustainable livestock activities can be possible by determining and recording the genetic information of world heritage animals. In Turkey, it has been observed that there is not enough research to determine the genetic diversity of geese. In this regard, it was concluded that the genetic diversity of our domestic geese should be determined, recorded and protected.

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Authors' Contributions

YA: data collection, research area, project design, drafting the article; MFÇ: manager of the project, statistical analysis, translation control.

Conflict of Interest Declaration

The authors have no conflict of interest.

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