



Molecular markers: A review

Komal Duhan^{1*}, Rachna Gulati², Manoj³, Arvind malik⁴

¹⁻⁴ Department of Zoology, CCS HAU Hisar, Haryana, India

Abstract

This review represents an attempt to highlight the different types of molecular markers by introducing a brief summary on the development of genetic markers including both the classical genetic markers and more advanced DNA-based molecular markers. This review could be helpful to better understand the characteristics of different genetic markers and its role in specifying the genetic diversity of animal genetic resources.

Keywords: DNA, animal genetic, molecular markers

Introduction

What are they?

A genetic marker is a gene or DNA sequence with a known location on a chromosome and associated with a particular gene or trait. It can be described as a variation, which may arise due to mutation or alteration in the genomic loci that can be observed. A genetic marker may be a short DNA sequence, such as a sequence surrounding a single base-pair change (single nucleotide polymorphism, SNP), or a long one, like mini & micro satellites ^[1]. The main aim of the breeder is to select animal with superior genetic potential as parents for the next generation. The first attempt to improve animals used the phenotype of an animal for a specific trait as a tool for selection. This application uses external animal characteristics as a marker that called morphological markers (i.e. udder shape, coat color, body shape, skin structure, and anatomical characteristics) ^[2]. These markers depend on visual observation and measurement to identify, classify, and characterize the genetic evolution of different species or populations. The conclusions reached through applying morphological markers are often not completely accurate when they used for the evaluation of farm animal genetics, because these markers based on subjective judgments and descriptions. Another type of markers represents by using of cytological markers that were included several criteria such as chromosome karyotypes, bandings, repeats, translocations, deletions, and inversions to investigate the genetic resources of animals ^[3, 4, 2, 5, 6]. The third type of markers is biochemical markers, such as the blood type and isozymes. These markers represent biochemical traits that could be analyzed by protein electrophoresis. The differences in the amino acid composition of isozymes and soluble proteins were used to investigate the genetic variation within species and phylogenetic relationships between species ^[7].

Restriction fragment length polymorphism (RFLP)

The RFLP is a technique that is not widely used now, but it was one of the first techniques used for DNA analysis in forensic science and several other fields. The RFLP is defined

by the existence of alternative alleles associated with restriction fragments that differ in size from each other ^[8]. The molecular basis of the RFLP is that nucleotide base substitutions, insertions, deletions, duplications, and inversions within the whole genome can remove or create new restriction sites ^[9]. Despite the fact that it is less widely used now, there have been numerous benefits to RFLP analysis. It plays an important role in allowing scientists to map the human genome as well as provide information on genetic disease ^[10, 11, 12].

The RFLP was also one of the first methods used for genetic typing - also known as genetic fingerprinting, profiling or testing. Despite that the RFLP have many benefits but it is still a slow and more tedious process compared to some of the newer DNA analysis techniques. It is also requires substantially larger sample sizes than other forms of analysis.

Random amplification of polymorphic DNA (RAPD)

In the last decade, the RAPD technique based on the polymerase chain reaction (PCR) has been one of the most commonly used molecular techniques to develop DNA markers ^[13, 11]. The RAPD technology provides a quick and efficient screen for DNA sequence based polymorphism at a very large number of loci. The major advantage of RAPD includes that, it does not require pre-sequencing of DNA ^[14, 2 15]. The RAPD analysis has been extensively used for various purposes which include identification and classification of accessions ^[26], identification of breeds ^[16] and genetic diversity analysis ^[17]. The principle of RAPD is that, a single, short oligonucleotide primer, which binds to many different loci, is used to amplify random sequences from a complex DNA template.

Amplified fragment length polymorphism (AFLP)

AFLP markers have found the widest application in analyses of genetic variation below the species level, particularly in investigations of population structure and differentiation ^[18]. AFLP methods rapidly generate hundreds of highly replicable markers from DNA; thus, they allow high-resolution

genotyping of fingerprinting quality. However, AFLPs are dominant bi-allelic markers,^[19] and are unable to distinguish dominant homozygous from dominant heterozygous individuals^[20]. The AFLP method is an ideal molecular approach for population genetics and genome typing, it is consequently widely applied to detect genetic polymorphisms, evaluate, and characterize animal genetic resources^[21, 22, 23].

Microsatellites

Microsatellites or simple sequence repeated (SSR) loci, which have been referred to in the literature as variable number of tandem repeats (VNTRs) and simple sequence length polymorphisms (SSLPs), are found throughout the nuclear genomes of most eukaryotes and to a lesser extent in prokaryotes^[11, 22]. The number of repeats is variable in populations of DNA and within the alleles of an individual. The sequence below has a 20 dinucleotide repeat (40bp) stretch of CA that is shown in bold.

```
CGTTCAATAAGCAAAAATCCATAGTTTTAGGAA
TGTGGGCT
GCTTGGTGTGATGTAGAAGGCGCCAATGCATCT
CGACGTAT
GCGTATACGGGTTACCCCCTTTGCAATCAGTGC
ACACACAC
ACACACACACACACACACACACACACACACAG
TGCCAAGCA
AAAATAACGCCAAGCAGAACGAAGACGTTCTC
GAGAACACC
AGAAGTTCGTGCTGTCGGGGCATGCGGCGAGT
AAAGGGGAT
```

Single-nucleotide polymorphism (SNP)

In 1996, Lander proposed a new molecular marker technology named SNP. In other words, it refers to a sequence polymorphism caused by a single nucleotide mutation at a specific locus in the DNA sequence^[2]. This sort of polymorphism includes single base transitions, transversions, insertions and deletions^[11], and the least frequent allele should have a frequency of 1% or greater. The more recent SNP concept has basically arisen from the recent need for very high densities of genetic markers for the studies of multifactorial diseases^[20]. In 1996, Lander proposed a new molecular marker technology named SNP. When a single nucleotide (A, T, C, or G) in the genome sequence is altered this will represent the SNP. In other words, it refers to a sequence polymorphism caused by a single nucleotide mutation at a specific locus in the DNA sequence. This sort of polymorphism includes single base transitions, transversions, insertions and deletions), and the least frequent allele should have a frequency of 1% or greater. Transitions are the most common (approx.2/3) among all the SNP mutation types. SNP markers are one of the popular approach, despite they can be considered as a step backwards (simple bi-allelic co-dominant markers) when compared to the highly informative multi-allelic microsatellites^[22, 21]. The more recent SNP concept has basically arisen from the recent need for very high densities of genetic markers for the studies of multifactorial diseases. The fundamental principle of SNPs is to hybridize detected DNA fragments with high-density DNA probe arrays (also called

SNP chips); the SNP allele is then named according to the hybridization results. SNPs are third generation molecular marker technology coming after RFLPs and SSRs it was successfully performed to investigate genetic variation among different species and breeds.

Advantages of molecular markers

- Molecular markers are abundant while biochemical markers are very few and morphological markers still fewer in number and insufficient for using for breeding purposes.
- Morphological and biochemical markers are subject to variation due to environmental factors while molecular markers are not.
- Biochemical markers may show tissue specificity while molecular markers show no such bias.
- Morphological markers are affected by epistasis while molecular markers are not.
- Molecular markers can exhibit high polymorphism compared to the other types of markers

Conclusion

The accurate genetic evaluation of animals is the primary target for their conservation and utilization. Different methods have been developed and tested at the DNA sequence level. These methods provide a large number of markers and opening up new opportunities for evaluating diversity in farm animal genetic resources. Among all these methods, microsatellites remained the marker of choice for the past 15 years^[77-80], due to their highly polymorphic and hence informative nature^[81]. The continuous development of molecular markers along with innovation of new statistical methods and the available of software could be end the debate about this subject by identification which of them is the best. This certainly will lead to more progress in application of molecular markers in animal breeding.

Future perspectives

Faced with major challenges in increased production because of global challenges new biotypes of diseases and arming, often reduce insects and several abiotic stresses which often reduce crop yield. The progress made in biotechnology crop yield. The progress made in biotechnology and genomics is preparing the path to meet the and genomics is preparing the path to meet the challenges; besides that new genes for resistance to challenges; besides that new genes for resistance to major biotic and abiotic stresses are constantly being identified using DNA markers. Integration of desired genes from different backgrounds led to the development of crops gene pool.

References

1. Adams WT. Application of isozymes in tree breeding. In: D. Tanksley and T. J. Orton (eds.), *Isozymes in plant genetics and breeding*, part A Elsevier Science, Amsterdam, the Netherlands, 1983, 381-400.
2. Ajmone-Marsan P, Negrini R, Milanese E, Bozzi R, Nijman IJ, Buntjer JB, *et al.* Genetic distances within and across cattle breeds as indicated by biallelic AFLP markers. *Anim Genetics*. 2002; 33:280-286.

3. Avise JC. Molecular markers, natural history and evolution. Chapman and Hall, New York, 1994.
4. Becak ML, Becak W, Roberts FL. Fish, amphibians, reptiles and birds. Berlin, Heidelberg, New York: Springer-Verlag, 1973.
5. Bitgood JJ, Shoffner RN. Cytology and cytogenetics. Poult breeding Genet. 1990; 22:401-427.
6. Brumlop S, Finckh MR. Applications and potentials of marker assisted selection (MAS) in plant breeding. Final report of the F+E project Applications and Potentials of Smart Breeding (FKZ 350 889 0020) On behalf of the Federal Agency for Nature Conservation, 2010.
7. Buvanendran V, Finney DJ. Linkage relationships of egg albumen loci in the domestic fowl. Br Poult Sci. 1967; 8:9-13.
8. Dekkers JC. Commercial application of marker- and gene-assisted selection in livestock: strategies and lessons. J Anim Sci. 82 E-Suppl: E313-328, 2004.
9. DeYoung RW, Honeycutt RL. The molecular toolbox: genetic techniques in wildlife ecology and management. J Wildl Manage. 2005; 69:1362-1384.
10. Drinkwater RD, Hetzel DJS. Application of molecular biology to understanding genotype-environment interactions in livestock production. In Proc. of an International Symposium on Nuclear Techniques in Animal Production and Health. Vienna: IAEA, FAO, 1991, 437-452, 15-19.
11. Emadi A, Crim MT, Brotman DJ, *et al.* Analytic validity of genetic tests to identify factor V Leiden and prothrombin G20210A. Am J Hematol. 2010; 85(4):64-270, ISSN 0361-8609.
12. Ewing B, Green P. Analysis of expressed sequence tags indicates 35,000 human genes. Nat Genet. 2000; 25:232-4.
13. Fischer RA. The correlation between relatives: the supposition of mendelain inheritance. Transactions of the royal society of Edinburgh. 1918; 52:399.
14. Goodfellow PN. Variation is now the theme. Nature. 1992; 359:777-778.
15. Hajibabaei M, Janzen DH, Burns JM. DNA barcodes distinguish species of tropical Lepidoptera. Proc Nat Acad Sci. USA. 2006; 103:968-971.
16. Hebert PDN, Cywinska A, Ball SL, de Waard JR. Biological identifications through DNA barcodes. Proc R Soc. Biol. Sci. 2003; 270:313-321.
17. Hedrick P. Shooting the RAPDs. Nature. 1992; 355:679-680.
18. Henderson CR. Applications of linear models in animal breeding. Can. Catal. Publ. Data, Univ Guelph, Canada, 1984.
19. Jonker J, Meurs G, Balner H. Typing for RhLA-D in rhesus monkeys: II. genetics of the D antigens and their association with DR antigens in a population of unrelated animals. Tissue Antigens. 1982; 19:69-78.
20. Kumar NS, Gurusubramanian G. Random amplified polymorphic DNA (RAPD) markers and its applications. Sci Vis. 2011; 11(3):116-124.
21. Lander ES. The new genomics: global views of biology. Science. 1996; 274:536-539.
22. Lynch M, Milligan BG. Analysis of population genetic structure with RAPD markers. Molecular Biology. 1994; 3:91-99.
23. Morin PA, Luikart G, Wayne RK. The SNP American Association of Blood Banks, Arlington, workshop group. SNPs in ecology, evolution VA, USA, pp. 277D280. And conservation. Trends Ecol. Evol. 2004; 19:208-216.
24. Nandani KN, Thakur SK. randomly amplified polymorphic DNA- a brief review. American Journal of Animal and Veterinary Sciences. 2014; 9(1):6-13.
25. Payseur BA, Cutter AD. Integrating patterns of polymorphism at SNPs and STRs. Trends Genet. 2006; 22:424-429.
26. Peter G. An assessment of the utility of single nucleotide polymorphisms (SNPs) for forensic purposes. Int J Legal Med. 2006; 114:204-210.
27. Rothschild MF, Larson RG, Jacobson C, Pearson P. PvuII polymorphisms at the porcine oestrogen receptor locus (ESR). Anim Genet. 1991; 22(5):448.