Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2018; 6(3): 1519-1522
© 2018 JEZS
Received: 26-03-2018
Accepted: 27-04-2018

Kush Shrivastava
Department of Animal Genetics and Breeding, College of Veterinary Science and Animal Husbandry, Jabalpur, Madhya Pradesh, India

Rebeka Sinha
Dairy Cattle Breeding Division, ICAR – NDRI, Karnal, Haryana, India

Mohan Singh Thakur
Department of Animal Genetics and Breeding, College of Veterinary Science and Animal Husbandry, Jabalpur, Madhya Pradesh, India

Correspondence
Rebeka Sinha
Dairy Cattle Breeding Division, ICAR – NDRI, Karnal, Haryana, India

Molecular DNA markers in conservation of wildlife: A brief review

Kush Shrivastava, Rebeka Sinha and Mohan Singh Thakur

Abstract
Molecular DNA markers are now gaining importance in wildlife conservation. Use of markers in identification of gene/ region in DNA, studying polymorphism, trait association and in marker assisted breeding schemes have now become ubiquitous in animal and plant breeding systems. Nuclear and mitochondrial genomic DNA are now being utilized in estimation of inbreeding, recent bottlenecks, genetic structure of population, migration and evolution pattern. The current review aims at providing a brief information about the use of mitochondrial and nuclear DNA in wildlife conservation and discuss in brief about non invasive DNA genotyping.

Keywords: Molecular DNA markers, conservation, wildlife

Introduction
Molecular DNA marker technology have developed drastically in the past few decades with the advent in modern biotechnological tools. Use of markers in identification of a gene/ region in DNA, studying polymorphism, trait association and in marker assisted breeding schemes have now become ubiquitous in animal and plant breeding systems. With this technological boom markers are now playing a significant role in studying variability and diversity, genetic trends, inbreeding effect, migration pattern in wild natural populations also. The drawbacks of classical morphological markers of being restricted to few traits, low level of polymorphism and environment sensitiveness have now become more apparent [1]. The second category of markers that were used after morphological markers were the biochemical markers particularly isoenzymes that can be easily tracked by electrophoresis [2]. However, isoenzymes carried a drawback that a limited number of polymorphic isoenzymes can be tracked by electrophoresis that again decrease the degree of polymorphism and distinction between two individuals could be merged, secondly the expression of various enzymes in the body systems is highly variable and depends on the environmental factors [2]. DNA markers however doesn't carry these limitations, they are highly polymorphic and are not affected by environmental circumstances. They tend to generate distinct band for each individual (creates fingerprint) that can be detected by silver staining or labelling. Development of molecular DNA markers started with the advent of RFLP in human genetics [3]. The method exploited the variations due to cut sites of restriction enzymes in nuclear DNA, now we know that these variations mainly arise due to changes in a single base pair within the recognition sequence of any particular restriction enzyme. The method generates fragments of DNA corresponding to the number of cuts that were detected by hybridization with a labelled probe and autoradiography. With the invention of PCR, only the desired regions were first amplified and then digested by specific restriction enzyme and the fragments were resolved and detected on agarose gel containing ethidium bromide in a UV transilluminator (PCR-RFLP). Later on, more marker systems were developed like RAPD (Randomly Amplified Polymorphic DNA) [4], VNTR (Variable number of tandem repeats or minisatellite), STR (Short tandem repeats, or SSR; simple sequence repeat or microsatellite), AFLP, retrotransposons, ISSR (Inter-simple sequence repeats), SNP chip etc. These markers, as discussed earlier, are now being used to study the genetic structure in wild animals. RAPD and AFLP markers are the commonest marker that are used in conservation genetics, besides them certain regions in mitochondrial DNA (mtDNA) are also being applied in species identification and divergence studies. In this brief review we will focus on various nuclear and mitochondrial DNA markers that can be used in conservation genetic studies with discussion on their possible benefits and drawbacks.

2. Nuclear DNA markers

Nuclear DNA markers are widely used in wildlife conservation. The most commonly employed markers for this purpose are RAPD, AFLP and SSR markers. RAPD and AFLP markers do not require any prior molecular information and are dominant in nature, whereas, SSR marker require a preliminary sequence information for primer designing. Because of their ease of application and genotyping RAPD markers have gained much popularity in wildlife conservation studies. A comparison between zoo and wild Far – Eastern leopard subspecies using RAPD markers revealed a low level of diversity in zoo animals as compared to their wild counterparts [5]. Freitas *et al.* [6] have shown the effect of recent bottleneck and inbreeding on genetic variation and its loss in Pacific white shrimps using 5 decamer RAPD primers. These markers have also been used in threatened species for estimation of genetic diversity, RAPD analysis of Iberian Imperial eagle population was done using 45 RAPD primers which amplified 614 loci in 25 individuals [7]. The diversity analysis and relationship among individuals would help in designing of proper mating plan for species that are being captively bred for conservation purpose. AFLP markers are quite similar but they can be automatized using PCR. Both the markers are multi locus but due to more stringency in reaction in AFLP they are more reliable than RAPD markers. Genetic diversity in sand tiger shark (*Carcharodon taurus*) and great white shark (*Carcharodon carcharias*) was assessed by AFLP marker, which showed high levels of allelic diversity between the two species [8]. AFLP markers have been used in reptiles for construction of relation tree and was suggested to be a valuable tool in determining phylogenetic relationships at fine levels [9]. Dasmahapatra *et al.* [10] has shown that AFLP markers can be effectively used to estimate inbreeding coefficient in natural populations and for determination of heterozygosity and fitness. However, RAPD and AFLP markers are dominant and thus not all the genetic variation can be captured by them as heterozygotes cannot be separately identified.

The drawbacks of AFLP and RAPD markers can be very well overcome by using microsatellite markers. Microsatellites are small tandemly repeating units of nucleotides in DNA (2 -4 bp long), the variation in number of repeating units create alleles at a locus. Microsatellites are single locus multi – allelic markers and hence tend to highly polymorphic in nature. Moreover, microsatellite markers can be easily optimized by PCR and alleles can be discriminated using denaturing PAGE or labelled primers. Microsatellite makers are mostly used in genetic diversity analysis and in molecular phylogenetic studies. Genetic diversity between captive bred and reintroduced population of Arabian orynx was assessed by seven microsatellite loci by Arif *et al.* [11]. Genetic diversity and population structure in giant pandas China using 10 microsatellite markers in Tangjiahe Nature Reserve (NR), there was low level of genetic differentiation between the subpopulations [12]. Microsatellite markers therefore are choice of marker for diversity analysis, they have been used in analysis of genetic structure and diversity in population of Vietnamese Sika deer [13], in development of species specific loci in woolly monkeys [14] to study hybridization, in assessment of within population diversity in Dutch Roe deer [15], in Chital deer [16, 17], in Norwegian red deer [18] etc. Besides mammals, microsatellites have been used in other species also, a panel of microsatellite markers was used to assess genetic variability within and among three ostrich breeds [19], population diversity in kakerori endangered forest bird [20], in characterization of Neotropical harpy eagle [21], genetic diversity in population of black tiger shrimp [22].

3. Mitochondrial and other markers

Besides the nuclear DNA markers, mitochondrial DNA markers have been extensively used in wildlife conservation. The mitochondrial protein coding regions are extensively used in molecular phylogenetic research as they evolve faster than the nuclear genes. The mitochondrial regions are also used in identification and wildlife forensics [23], the cytochrome b sequence was used in meat identification and slaughter weapon identification in endangered bird by Gupta *et al.* [24]. The cytochrome region has attracted the maximum part of researches, the cytochrome b region was used to assess the divergence between Korean, Chinese and Russian goral [25], the NADH dehydrogenase subunit with 16s rRNA gene were used for phylogenetic analysis of 35 species of Felidae family [26]. Moreover, the non-coding or the control region (CR) sequences are also being used for comparison and diversity among species. These CR sequences have been in, genetic status and evolution assessment in Tibetan gazelle population [27] and sun bear [28], gene flow in black muntjac [29], diversity and population structure analysis in Chinese water deer [30], phylogenetic analysis of oryx species [31] and in population genetic structure analysis in desert bird (*Chlamydotis undulata*) [32].

Another candidate that is a potential DNA marker in mitochondrial genome is the 12S and 16S ribosomal DNA. The 12S rDNA is highly conserved among taxa and is usually applied for higher level of phylogenetic studies like in phyla or subphyla, the 16S rDNA is less conserved and is applied in mid-level taxonomic studies like at families or genera level. This 12S fragment has been used, in phylogenetic analysis of Indian muntjac [33], in population structure analysis in spur-thighed tortoise [34], in species identification of Indian leopard [35]. 12S and 16S regions has been used in determining the phylogenetic status of Przewalski's gazelle [36]. The 16S region of mitochondria has also been used for establishing relationship pattern and taxonomic status of 4 genera [37]. Guha and Kashyap [38] has developed PCR assay based on 16S polymorphism to distinguish between Black buck, Goral, Nilgai, Hog deer, Chital, Sambhar and Thamin deer.

4. Source of DNA for wild animals genotyping

One of the major problems to genotype wild animal is the obtaining enough of the biological sample that can be utilized for isolation of genomic DNA. DNA in wild animals can be obtained from non-invasive techniques, the best of which is to use faeces as the source of DNA other sources include hair, skin, feather, saliva, postmortem material etc. [39]. Utilization of faecal DNA for genotyping animals have been proven to be quite useful in numerous studies [40-42, 17]. However, obtaining a superior quality DNA from faecal sample for genotyping is challenging. For faecal DNA isolation there are myriad of methods as well as commercial kits available. The quantity and quality of DNA and removal of inhibitors in important for each method. Also, once the DNA is obtained there are chances of genotyping error when using faecal DNA, amplification of false allele and allelic dropout [17]. Contaminating DNA, artifacts etc. can lead to allelic slippage or amplification of false alleles [43]. However, a standard multiple tube approach has been devised to nullify these drawbacks of faecal DNA, the approach is using of multiple PCR amplifications per reaction so that the false amplification can be identified in a sample [44]. Using the red deer

population it was suggested to employ 6 PCR amplifications per sample (per locus) to achieve 97 % accuracy and correct genotype [45]. Genotyping error rates may be important determinants of the outcome of noninvasive studies and hence should be carefully computed and reported.

5. Conclusion

Genotyping the wild animal population carries some difficulties with it, first being the sample size, which is often small, second is the sampling area and third is the obtaining of sample as DNA source. It is important that if the source of DNA has been chosen by noninvasive methods, genotyping error rates should be accounted and computed. The next part comes the choice of marker for the study. Dominant markers like RAPD, AFLP will be less stringent, less costly but will form an excellent choice for starting a project or for estimating the diversity between subpopulations. Codominant marker like microsatellite will be expensive and tedious to develop but will be excellent for establishing a phylogenetic relationship and taxonomic position due to their higher polymorphism and biallelic nature. Co-dominant marker allows for estimation of hybridization, inbreeding and recent bottlenecks during evolution. However, the quality of DNA required for genotyping by microsatellites should be good and poor quality DNA gives error in genotyping and amplification. The mitochondrial DNA markers are useful in phylogenetics, between phyla, subphyla or families as the rate of evolution is higher in these genes. The other major use of mitochondrial DNA markers has been in the identification of species, in case of illegal hunting or trade. Therefore, applicability of each marker depends on the objective or goal of our study and the choice of source material for DNA. Whatever the case may be, DNA markers are now being proving useful in conservation genetic and wildlife forensic studies.

6. References

1. Schulman AH. Molecular markers to assess genetic diversity. Euphytica. 2007; 158:313-321.
2. Tanksley SD. Molecular markers in plant breeding. Plant Mol Biol Rep. 1983; 1:3-8.
3. Botstein D, White RL, Skolnick M, Davis RW. Construction of a genetic linkage map using restriction fragment length polymorphisms. Am J Hum Genet. 1980; 32:314-331.
4. Williams JG, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucl Acids Res. 1990; 18:6531-6535.
5. Chelomina GN, Spiridonova LN, Kozyrenko, MM, Artiukova EV, Chelomin IuV, Zhuravlev IuN. Use of RAPD-PCR-analysis of cellular DNA for the evaluation of genetic polymorphism and subspecies diagnostics of the Far Eastern leopard *Panthera pardus orientalis*. Genetika. 1999; 35:681-687.
6. Freitas PD, Calgaro MR, Galetti Jr PM. Genetic diversity within and between broodstocks of the white shrimp *Litopenaeus vannamei* (Boone, 1931) (Decapoda, Penaeidae) and its implication for the gene pool conservation. Braz. J. Biol. 2007; 67:939-943.
7. Padilla JA, Martı´nez-Trancon M, Rabasco A, Parejo JC, Sansinforiano ME, Guijo MI. Genetic variability in the Iberian imperial eagle (*Aquila adalberti*) demonstrated by RAPD analysis. J. Hered. 2000; 91:495-499.
8. Zenger KR, Stow AJ, Peddemors V, Briscoe DA, Harcourt RG. Widespread utility of highly informative AFLP molecular markers across divergent shark species. J. Hered. 2006; 97:607-611.
9. Giannasi N, Thorpe RS, Malhotra A. The use of amplified fragment length polymorphism in determining species trees at fine taxonomic levels: analysis of a medically important snake, *Trimeresurus albolabris*. Mol. Ecol. 2001; 10:419-426.
10. Dasmahapatra KK, Lacy RC, Amos W. Estimating levels of inbreeding using AFLP markers. Heredity. 2008; 100:286-295.
11. Arif IA, Khan HA, Shobrak M, Al Homaidan AA, Al Farhan AH, Al Sadoon M. Measuring the genetic diversity of Arabian Oryx using microsatellite markers: implication for captive breeding. Gene. Genet. Syst. 2010; 85:141-145.
12. Yang J, Zhang Z, Shen F, Yang X, Zhang L, Chen L *et al*. Microsatellite variability reveals high genetic diversity and low genetic differentiation in a critical giant panda population. 2011; 57(6):717 -724.
13. Thevenon S, Thuy LT, Ly LV, Maudet F, Bonnet A, Jarne P *et al*. Microsatellite Analysis of Genetic Diversity of the Vietnamese Sika Deer (*Cervus nippon pseudaxis*). Journal of Heredity. 2004; 95(1):11-18.
14. Di Fiore A, Fleischer RC. Microsatellite markers for woolly monkeys (*Lagothrix lagotricha*) and their amplification in other New World primates (Primates: Platyrrhini). Molecular Ecology Notes. 2004; 4:246-249.
15. Postma E, Van Hooft WF, Van Wieren SE, Van Breukelen L. Microsatellite variation in Dutch roe deer (*Capreolus capreolus*) populations. Netherlands Journal of Zoology. 2001; 51(1):85-95.
16. Gaur A, Singh A, Arunabala V, Umapathy G, Shailaja K, Singh L. Development and characterization of 10 novel microsatellite markers from Chital deer (*Cervus axis*) and their cross–amplification in other related species. Molecular Ecology Notes. 2003; 3:607-609.
17. Shrivastava K, Thakur MS, Parmar SNS, Shrivastav AB. Microsatellite characterization of Chital deer (*Axis axis*) by using faecal DNA. Vet World. 2013; 6(2):83-86.
18. Haanes H, Rosef O, Veiberg V, Røed KH. Microsatellites with variation and heredity applicable to genetic studies of Norwegian red deer (*Cervus elaphus atlanticus*). Anim Genet. 2005; 36(5):454-455.
19. Kawka M, Horbanczuk JO, Sacharczuk M, Zieba G, Lukaszewicz M, Jaszczak K *et al*. Genetic characteristics of the ostrich population using molecular methods. Poult. Sci. 2007; 86:277-281.
20. Chan CH, Zhao Y, Cheung MY, Chambers GK. Isolation and characterization of microsatellites in the kakerori (*Pomarea dimidiata*) using feathers as source of DNA. Conserv. Genet. 2008; 9:1067-1070.
21. Banhos A, Hrbek T, Gravena W, Sanaiotti T, Farias IP. Genomic resources for the conservation and management of the harpy eagle (*Harpia harpyja*, Falconiformes, Accipitridae). Genet. Mol. Biol. 2008; 31:146-154.
22. Li Y, Wongprasert K, Shekhar M, Ryan J, Dierens L, Meadows J *et al*. Development of two microsatellite multiplex systems for black tiger shrimp Penaeus monodon and its application in genetic diversity study for two populations. Aquaculture. 2007; 266:279-288.
23. An J, Lee MY, Min MS, Lee MH, Lee H. A molecular genetic approach for species identification of mammals and sex determination of birds in a forensic case of poaching from South Korea. Forensic Sci. Int. 2007;

167:59-61.
24. Gupta SK, Verma SK, Singh L. Molecular insight into a wildlife crime: the case of a peafowl slaughter. Forensic Sci. Int. 2005; 154:214-217.
25. Min MS, Okumura H, Jo DJ, An JH, Kim KS, Kim CB *et al*. Molecular phylogenetic status of the Korean goral and Japanese serow based on partial sequences of the mitochondrial cytochrome b gene. Mol. Cells, 2004; 17:365-372.
26. Johnson WE, O'Brien SJ. Phylogenetic reconstruction of the Felidae using 16S rRNA and NADH-5 mitochondrial genes. J Mol. Evol. 1997; 44:S98-S116.
27. Zhang F, Jiang Z. Mitochondrial phylogeography and genetic diversity of Tibetan gazelle (*Procapra picticaudata*): implications for conservation. Mol. Phylogenet. Evol. 2006; 41:313-321.
28. Onuma M, Suzuki M, Ohtaishi N. Possible conservation units of the sun bear (*Helarctos malayanus*) in Sarawak based on variation of mtDNA control region. Jpn. J Vet. Res. 2006; 54:135-139.
29. Wu HL, Wan QH, Fang SG. Population structure and gene flow among wild populations of the black muntjac (*Muntiacus crinifrons*) based on mitochondrial DNA control region sequences. Zool. Sci. 2006; 23:333-340.
30. Hu J, Fan, SG, Wan QH. Genetic diversity of Chinese water deer (*Hydropotes inermis inermis*): implications for conservation. Biochem. Genet. 2006; 44:161-172.
31. Iyengar A, Diniz FM, Gilbert T, Woodfine T, Knowles J, Maclean N. Structure and evolution of the mitochondrial control region in oryx. Mol. Phylogenet. Evol. 2006; 40:305-314.
32. Idaghdour Y, Broderick D, Korrida A, Chbel F. Mitochondrial control region diversity of the houbara bustard *Chlamydotis undulata* complex and genetic structure along the Atlantic seaboard of North Africa. Mol. Ecol. 2004; 13:43-54.
33. Shukla MS, Pidiyar V, Bhave NA, Patole MS, Shouche YS. PCR amplification and sequencing of mitochondrial 12S rRNA gene fragment from *Muntiacus muntjak* (Indian muntjac). Curr. Sci. 2001; 80:617-618.
34. Van der Kuyl AC, Ballasina DLP, Zorgdrager F. Mitochondrial haplotype diversity in the tortoise species *Testudo graeca* from North Africa and the Middle East. BMC Evol. Biol. 2005; 5:29.
35. Pandey PK, Dhotre DP, Dharne MS. Evaluation of mitochondrial 12S rRNA gene in the identification of *Panthera pardus fusca* (Meyer, 1794) from field-collected scat samples in the Western Ghats, Maharashtra, India. Curr. Sci. 2007; 92:1129-1133.
36. Lei R, Jian Z, Hu Z, Yang W. Phylogenetic relationships of Chinese antelopes (sub family Antilopinae) based on mitochondrial Ribosomal RNA gene sequences. J Zool. 2003; 261:227-237.
37. Turan C. Molecular Systematic Analyses of Mediterranean Skates (Rajiformes). Turk. J Zool. 2008; 32:1-6.
38. Guha S, Kashyap VK. Development of novel heminested PCR assays based on mitochondrial 16s rRNA gene for identification of seven pecora species. BMC Genet. 2005; 6:42.
39. Shrivastava K, Parmar SNS, Shrivastav AB. Faeces as a source of DNA for wildlife conservation: A Brief Perspective. Life Science Bulletin. 2012; 9(1):52-54.
40. Wasser SK, Houston CS, Koehler GM, Cadd GG, Fain SR. Techniques for application of faecal DNA methods to field studies of Ursids. Molecular Ecology. 1997; 6:1091-1098.
41. Okello JBA, Wittemyer G, Rasmussen HB, Hamilton ID, Nyakaana S, Arctander P *et al*. Noninvasive genotyping and mendelian analysis of microsatellites in African savannah elephants. J. Hered. 2005; 96(6):679-687.
42. Chu J, Lin Y, Wu H. Applicability of non-invasive sampling in population genetic study of Taiwanese Macaques (*Macaca cyclopis*). Taiwania. 2006; 51(4):258-265.
43. Frantzen MAJ, Silk JB, Ferguson JWH, Wayne RK, Kohn MH. Empirical evaluation of preservation methods for faecal DNA. Molecular Ecology. 1998; 7:1423-1428.
44. Taberlet P, Griffin S, Goossens B. Reliable genotyping of samples with very low DNA quantities using PCR. Nucleic Acids Research. 1996; 24:3189-3194.
45. Valiere N, Bonenfant C, Toigo C, Luikart G, Gaillard JM, Klein F. Importance of a pilot study for non-invasive genetic sampling: genotyping errors and population size estimation in red deer. Conservation Genetics. 2006; 8:69-78.