Molecular markers for biodiversity analysis of wildlife animals: a brief review

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Abstract

Molecular markers for biodiversity analysis of wildlife animals: a brief review.— Molecular markers are indispensable tools for determining the genetic variation and biodiversity with high levels of accuracy and reproducibility. These markers are mainly classified into two types; mitochondrial and nuclear markers. The widely used mitochondrial DNA markers with decreasing order of conserved sequences are 12S rDNA > 16S rDNA > cytochrome b > control region (CR); thus the 12S rDNA is highly conserved and the CR is highly variable. The most commonly used nuclear markers for DNA fingerprinting include random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and microsatellites. This short review narrates the application of these molecular markers for biodiversity analysis of wildlife animals.

Key words: Molecular markers, Biodiversity, Wildlife animals, Conservation.

Resumen

Uso de los marcadores moleculares para el análisis de biodiversidad de los animales salvajes: una breve revisión.— Los marcadores moleculares constituyen unas herramientas indispensables para determinar la variación genética y la biodiversidad con un alto grado de precisión y reproductibilidad. Dichos marcadores se clasifican principalmente en dos tipos: marcadores mitocondriales y nucleares. Los marcadores de DNA mitocondrial, que se utilizan mucho, son en orden decreciente de secuencias conservadas ADNr 12S > ADNr 16S > citocromo b > región de control (RC); así pues, el ADNr 12S es muy estable y el RC es muy variable. Los marcadores nucleares más utilizados para las huellas genéticas incluyen al ADN polimórfico ampliado al azar (RAPD), el polimorfismo ampliado de la longitud del fragmento (AFLP) y los microsatélites. Esta corta revisión describe las aplicaciones de estos marcadores moleculares en el análisis de la biodiversidad de animales salvajes.

Palabras clave: Marcadores moleculares, Biodiversidad, Animales salvajes, Conservación.

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Introduction

Conservation genetics or the application of genetics to the preservation of species has received increasing attention in recent years (Allendorf & Luikart, 2007; Frankham, 2003). In conservation genetics, knowledge of the relatedness between individuals is particularly important in captive breeding programs that seek to reduce incestuous matings in order to minimize inbreeding and the loss of genetic variation (Frankham et al., 2002). It is well established that a decline in genetic variation reduces the ability of a population to adapt to environmental changes and therefore decreases its long term survival. The loss of genetic diversity also results in lower individual fitness and poor adaptability (Lande, 1988). The fate of small populations is linked to genetic changes. The captive breeding of endangered wildlife animals is often necessary for their conservation; however, this strategy potentially increases the chances of inbreeding that, in turn, causes poor fitness of these populations (Ralls & Ballou, 1983; Crnokrak & Roff, 1999). Inbreeding is known to decrease genetic diversity and to reduce reproductive and survival rates leading to increased extinction risk. Genetically impoverished endangered populations often fail to exhibit signs of recovery until crossed with individuals from other populations (Land & Lacy 2000; Westemeier et al., 1998). Moreover, wildlife populations with lower genetic diversity are at greater risk of extinction (Saccheri et al., 1998). Knowledge and studies on genetics can reduce the extinction risk by helping to develop appropriate population management programs that can minimize the risks implied through inbreeding. Breeding programs are often started assuming that the wild founders initiating the captive population are unrelated. However, threatened animals brought into captivity often have small population sizes and therefore the founders may be related to each other (Geyer et al., 1993; Haig et al., 1994). Assessment and preservation of biodiversity of wild populations is crucially important to minimize the loss of initial genetic variation as a consequence of inbreeding (Russello & Amato, 2004). Molecular methods play an important role in estimating the genetic diversity among individuals by comparing the genotypes at a number of polymorphic loci (Avise, 2004). Several types of molecular markers, including mitochondrial DNA (mtDNA) and nuclear DNA markers, are available but none of them can be regarded as optimal for all applications (Sunnucks, 2000). The characteristic features of various mtDNA and nuclear DNA markers are summarized in tables 1 and 2, respectively. A large number of studies have utilized approaches with mtDNA sequencing; however, mtDNA only represents the geneology of particular genes that are only inherited maternally (some exceptions do exist as explained in the caption of table 1). Additional markers targeting nuclear DNA therefore need to be used for more accurate interpretation of population genetics and biodiversity. This short review summarizes recent studies on the application of various molecular markers for the analysis of molecular diversity in wildlife species.

Mitochondrial ribosomal RNA markers

Animal mitochondria contain two ribosomal RNA (rRNA) genes, 12S rDNA and 16S rDNA. Mitochondrial 12s rDNA is highly conserved and has been applied to understand the genetic diversity of higher categorical levels such as in phyla. On the other

Table 1. Characteristics of various mtDNA markers: * Not all mitochondria in animals are inherited maternally. Rare instances of paternal leakage have been found in some species including mice, birds and humans. However, the extent of paternal leakage is thought to be low in most animals, with the exception of certain mussel species which follow double biparental inheritance (Freeland, 2005).

*Tabla 1. Características de varios marcadores de ADNm: * En los animales no todas las mitocondrias se heredan por vía materna. Se han hallado algunos casos de un cierto grado de filtración de ascendencia paterna en algunas especies de ratones, aves y en el hombre. No obstante, se cree que dicha filtración es muy pequeña en la mayoría de los animales, a excepción de ciertas especies de mejillones, que siguen un patrón de herencia biparental doble (Freeland, 2005).*

Molecular marker	Characteristics
mtDNA	Inherited from the mother (maternal lineage); rare exceptions do exist*
mtDNA	Degrades slower than nuclear DNA. It can be used in degraded or old samples
mtDNA	Evolves about 10–fold faster than nuclear DNA
12s rDNA	Highly conserved; used for high–category levels: phyla and subphyla
16S rDNA	Usually used in mid–category differentiation such as families
Protein–coding genes	Conserved; used in low–categories such as families, genera and species
Control region	Hypervariate; used for identification of species and sub–species

Table 2. Characteristics of various nuclear DNA markers: * Dominant markers can identify only one allele (presence or absence of a band) and are therefore unable to determine heterozygosity; co–dominant markers are able to identify both the alleles. ** Multi–locus markers can visualize many genes simultaneously in contrast to only one region amplification by single–locus markers; however, the latter can easily be multiplexed for more reliable fingerprinting.

*Tabla 2. Características de diversos marcadores de ADN nuclear: * Los marcadores dominantes sólo pueden identificar un alelo (ausencia o presencia de una banda) y por lo tanto son incapaces de determinar la heterozigosidad; los marcadores codominantes pueden identificar ambos alelos. ** Los marcadores multi–locus pueden visualizar simultáneamente muchos genes, al contrario de los marcadores de un solo locus, que amplifican sólo una región; no obstante, estos últimos pueden multiplexarse para obtener unas huellas genéticas más fiables.*

	RAPD	AFLP	Microsatellites
Allelic information*	Dominant	Dominant	Co–dominant
Locus presentation**	Multi–locus	Multi–locus	Single locus
DNA required (µg)	0.02	0.50	0.05
PCR–based	Yes	Yes	Yes
Restriction digestion	No	Yes	No (Yes for development)
Reproducibility	Low	High	High
Development cost	Low	Moderate	High
Cost per assay	Low	Moderate	Low

hand, the 16s rDNA is often used for studies at middle categorical levels such as in families or genera (Gerber et al., 2001). For molecular analysis, these markers are first amplified by PCR using conserved primers and the amplicons are sequenced. Sequencing data are then aligned and compared using appropriate bioinformatics tools. Alvarez et al. (2000) have suggested specific haplotypes of 12S rRNA gene to study the effects of geographical isolation on genetic divergence of endangered spur–thighed tortoise *(Testudo graeca*). The sequence analysis of a 394–nucleotide fragment of 12S rRNA gene has been used to examine the genetic variation in *Testudo graeca* using 158 tortoise specimens belonging to the four different subspecies (Van der Kuyl et al., 2005). The mitochondrial DNA haplotyping has suggested that the tortoise subspecies of *Testudo graeca graeca* and *Testudo graeca ibera* are genetically distinct with a calculated divergence time in the early or middle Pleistocene; however, other proposed subspecies could not be recognized based upon their mitochondrial haplotypes (Van der Kuyl et al., 2005). The 12S rRNA fragment of *Testudo graeca* was found to be somewhat less variable than the D–loop fragment, due to the inherently slower evolutionary rate of rRNA genes than the variable parts of the D–loop (Pesole et al., 1999). Lei et al. (2003) examined the mitochondrial rRNA genes of Chinese antelopes and observed that average sequence divergence values for 16S and 12S rRNA genes were 9.9% and 6.3% respectively. A single base in the 16S rDNA sequences from the endangered species *Pinna nobilis* was found to be different in all analyzed individuals from a single population sample differentiating it from others (Katsares et al., 2008). Mitochondrial 16S rRNA was used to elucidate the pattern of relationships and systematic status of 4 genera, including nine species of skates living in the Mediterranean and Black Seas (Turan, 2008). Molecular studies on endangered Pecoran have shown lower sequence diversity in 16S rRNA gene as compared to cytochrome b gene, both between and within species; however, the 16S rRNA gene harbored a larger number of species–specific mutation sites than cytochrome b gene (Guha et al., 2006). NaNakorn et al. (2006) have assessed the level of genetic diversity in critically endangered Mekong giant catfish species using 570 bp sequences of 16S rRNA from 672 individuals of nine species. In all species studied, haplotype diversity and nucleotide diversity ranged from 0.118–0.667 and 0.0002–0.0016, respectively. Four haplotypes were detected among 16 samples from natural populations of Mekong giant catfish. The findings from this study have important implications for conservation of the Mekong giant catfish, especially in designing and implementing artificial breeding program for restocking purposes (NaNakorn et al., 2006).

Mitochondrial protein–coding genes markers

Due to their faster evolutionary rates compared to ribosomal RNA genes, the mitochondrial protein–coding genes are regarded as powerful markers for genetic diversity analysis at lower categorical levels, including families, genera and species. Animal mitochondria

contain 13 protein–coding genes; however, one of the most extensively used protein coding genes of the mitochondrial genome for molecular analysis is cytochrome b (cyt b). Mitochondrial cyt b sequences have been used to understand the genetic diversity for better conservation management of Tibetan gazelle (*Procapra picticaudata*), a threatened species on the Qinghai–Tibet Plateau of China (Zhang & Jiang, 2006). The sequence analysis of 46 samples collected from 12 geographic locations identified 16 cyt b haplotypes, to be used as molecular markers for conservation planning (Zhang & Jiang, 2006). Partial cyt b based molecular analysis of genetic distances has revealed that there is considerable genetic divergence between the Korean goral and the Chinese goral, but virtually none between Korean and Russian gorals (Min et al., 2004). The Korean gorals possessed two haplotypes with only one nucleotide difference between them, while the Japanese serows showed slightly higher sequence diversity with five haplotypes. These data highlight the importance of conservation of the goral populations of these regions, and the need to reconsider the taxonomic status of Korean and Russian gorals (Min et al., 2004). Another important mitochondrial protein coding gene, NADH dehydrogenase subunit 5 (318 bp), has been used for phylogenetic analysis of multiple individuals of different species from Felidae family and successfully differentiated eight clades reflecting separate monophyletic evolutionary radiations (Johnson & O'Brien, 1997). Mitochondrial cytochrome oxidase I (COI) gene has recently gained more attention in developing DNA barcodes for species identification and biodiversity analysis; the relevant studies on this topic are discussed below under a separate heading.

DNA barcoding

DNA barcoding has become a promising tool for rapid and accurate identification of various taxa and it has been used to reveal unrecognized species in several animal groups. Animal DNA barcodes (600–800 base–pair segments) of the mitochondrial cytochrome oxidase I (COI) gene have been proposed as a means to quantify global biodiversity. DNA barcoding has the potential to improve the way the world relates to wild biodiversity (Janzen et al., 2005). Moreover, the introduction of DNA barcoding has highlighted the expanding use of the COI as a genetic marker for species identification (Dawnay et al., 2007). Fisher & Smith (2008) evaluated the role of DNA barcoding as a tool to accelerate species identification and description of arthropods. They performed morphological and CO1 DNA barcode analysis of 500 individuals to recognize five species of *Anochetus* and three species of *Odontomachus* (Fisher & Smith, 2008). The potential of character–based DNA barcodes has been demonstrated by analyzing 833 odonate specimens from 103 localities belonging to 64 species (Rach et al., 2008). The unique combinations of character states within only one mitochondrial gene region (NADH dehydrogenase 1) reliably discriminated 54 species and 22 genera. It was concluded that the DNA barcodes are able to identify entities below the species level that may constitute separate conservation units or even species units (Rach et al., 2008). Fleischer et al. (2006) have conducted DNA analysis of seven museum specimens of the endangered North American ivory–billed woodpecker (*Campephilus principalis*) and three specimens of the species from Cuba to document their molecular diversity. The sequences of these woodpeckers have been shown to provide an important DNA barcoding resource for identification of these critically endangered and charismatic woodpeckers. Witt et al. (2006) have employed DNA barcoding to examine *Hyalella*, a taxonomically difficult genus of amphipod crustaceans, from two distant sites. The extent of species diversity was assessed using a species screening threshold (SST) set at 10 times the average intrapopulation COI haplotype divergence. These findings have been suggested to have important implications for the conservation of life in desert springs that are threatened due to groundwater over–exploitation (Witt et al., 2006). The COI–DNA barcode has been testified as a tool for species identification, biodiversity analysis and discovery for morphospecies of *Belvosia* parasitoid flies (Smith et al., 2006). Barcoding not only discriminated among all 17 highly host–specific morphospecies of *Belvosia*, but it also raised the species count to 32 by revealing that each of the three generalist species were actually the arrays of highly host–specific cryptic species. Lorenz et al. (2005) have suggested that depositing barcode sequences in a public database, along with primer sequences, trace files and associated quality scores, would make this technique widely accessible for species identification and biodiversity analysis.

Mitochondrial control region markers

Mitochondrial DNA contains a non–coding region termed the control region (CR or D–loop) due to its role in replication and transcription of mtDNA. The D–loop segment exhibits a comparatively higher level of variation than protein–coding sequences due to reduced functional constraints and relaxed selection pressure. The length of the D–loop is approximately 1 kb and it can easily be amplified by PCR prior to sequencing to determine the molecular diversity. Sequence analysis of the CR of the sun bear has been used to measure molecular diversity and to identify conservation units for better management of this species (Onuma et al., 2006). Wu et al. (2006) have sequenced a portion of mitochondrial CR (424 bp) to assess the population structure and gene flow among the populations of black muntjac (*Muntiacus crinifrons*) using 47 samples collected from three large populations. A total of 18 unique haplotypes (15 of them as population–specific) were defined based on 22 polymorphic sites. It has been suggested that the coexistence of distinct haplotypes in a specific population was induced by historical population expansion after fragmentation and that

the current genetic differentiation should be attributed to the reduction of female–mediated gene flow due to recent habitat fragmentation and subsequent loss (Wu et al., 2006). Hu et al. (2006) have studied the genetic diversity and population structure of the Chinese water deer (*Hydropotes inermis inermis*) by analyzing the 403 bp fragment of mitochondrial D–loop. They have detected 18 different haplotypes in 40 samples demonstrating the haplotype diversity of 0.923 and nucleotide diversity of 1.318, whereas no obvious phylogenetic structure among haplotypes was found for samples of different origin (Hu et al., 2006). Iyengar et al. (2006) have performed a comparative study of CR sequences from several captive oryx species and concluded a close grouping of *Oryx leucoryx* with *Oryx gazelle* instead of *Oryx dammah*. Idaghdour et al. (2004) have sequenced the 854 bp of the CR from the houbara bustard (*Chlamydotis undulate*) to describe the molecular diversity of this threatened cryptic desert bird, whose range extends from North Africa to Central Asia. Zhang & Jiang (2006) have used CR sequences to investigate the genetic diversity and evolutionary history of the Tibetan gazelle. A total of 25 CR haplotypes with high frequencies of both CR haplotype and nucleotide diversities were identified. These findings have suggested that the present population structure is the result of habitat fragmentation during the recent glacial period on the Qinghai–Tibet Plateau and it is likely that the present populations of Tibetan gazelle exhibit a pattern reminiscent of several bottlenecks and expansions in the recent past (Zhang & Jiang, 2006).

RAPD markers

Random amplified polymorphic DNA (RAPD) markers are analyzed by using PCR to amplify the segments of nuclear DNA. The use of a single primer (usually 8–10 bp long) that attaches to both strands of DNA and low annealing temperatures increase the likelihood of amplifying multiple regions representing a particular locus (multi–locus). Although RAPD is a simple and inexpensive technique its major limitation is the inability to differentiate between homozygote and heterozygote; this marker is therefore regarded as a dominant type. Padilla et al. (2000) have analyzed the genetic diversity of the highly endangered Iberian imperial eagle (*Aquila adalberti*) using 45 arbitrary primers producing about 60% polymorphic bands among the total 614 amplified loci. In contrast to the traditional allozyme analysis, the RAPD method has revealed a high level of heterozygosity in this species (H = 0.267). The genetic distances estimated between 25 eagles can serve to establish more adequate mating in order to preserve genetic variability and to aid conservation efforts to protect this species (Padilla et al., 2000). The genetic diversity and population structure of endangered Blanca Cacereña bovine have been evaluated by RAPD markers comprising 71 primers with relevancy to specific amplification of 1,048 loci (Parejo et al., 2002). RAPD produced a number of polymorphic loci (30.44%) and it has been proved to be a useful method for evaluating polymorphisms in this breed. The findings from this study have been suggested to assist in planning the most adequate mating strategy to maintain the genetic diversity and to improve the efficiency of conservation for the Blanca Cacereña bovine breed (Parejo et al., 2002). Gouin et al. (2001) have studied the genetic diversity among 21 populations of a threatened freshwater crayfish (*Austropotamobius pallipes*) native to Europe, using four primers capable of generating six well–resolved polymorphic bands. The genetic diversity within populations of *A. pallipes,* estimated by Shannon's diversity index, ranged from 0 to 0.446 with a mean of 0.159. Freitas et al. (2007) have amplified 52 polymorphic loci using five primers to investigate the genetic variation in Pacific white shrimp. The loss of genetic variation observed in this study has been related to probable bottleneck effects and inbreeding. Moreover, the genetic divergence values between the different samples may also reflect the initial founder composition of such stocks, suggesting a putative importance of interbreeding for the establishment of genetic improvement programs for these broodstocks (Freitas et al., 2007). Thus, genetic variation monitoring using RAPD could play an important role in the gene pool conservation of aquaculture species. Maciuszonek et al. (2005) have applied the population–specific RADP markers to resolve the genetic group specific bands for four indigenous Polish goose breeds. A total of 102 scorable bands, specific to particular genetic groups, were obtained suggesting their potential application as population–specific markers, especially in ex–situ conservation methods. The same investigators have also suggested that keeping endangered geese as separate flocks is relevant for their preservation (Maciuszonek et al., 2005). RAPD markers have also been used to study the population divergence by analyzing the genetic variation within and between two populations of endangered Pampas deer (*Ozotoceros bezoarticus*) using 15 primers specific to 105 polymorphic bands (Rodrigues et al., 2007). There was no differentiation and about 96% ($P < 0.00001$) of the total variance was attributable to variation within populations. The findings of this study have been suggested to be potentially useful for the future monitoring of the genetic variation within these populations and for the development of management guidelines for their conservation (Rodrigues et al., 2007).

AFLP markers

Amplified fragment length polymorphism (AFLP) is a multi–locus technique that involves restriction digestion and PCR amplification. Similar to RAPD, these markers are also dominant. However, the main strengths of AFLP are high specificity and reproducibility due to the use of restriction digestion of DNA, specific adaptors and high annealing temperatures for selective amplification. Recently, an AFLP method has been validated to determine genetic diversity and inbreeding coefficient in old–field mice (*Peromyscus polionotus subgriseus*), suggesting the usefulness of

AFLP markers to estimate the inbreeding coefficient in natural populations (Dasmahapatra et al., 2008). Based on the comparative evaluation of pedigree–based empirical data from 179 wild and captive–bred mice with genetic data from 94 AFLP markers and 12 microsatellites, the inbreeding estimates resulting from both AFLP and microsatellite markers were found to correlate strongly with pedigree–based inbreeding coefficients (Dasmahapatra et al., 2008). Giannasi et al. (2001) have reported the application of AFLP for species determination using 24 specimens of a medically important snake, *Trimeresurus albolabris*. They have suggested that AFLPs may prove a valuable aid in determining species trees as opposed to gene trees at fine taxonomic levels and this should facilitate the incorporation of molecular data into such activities as antivenom production and conservation management (Giannasi et al., 2001). AFLP markers have also been used to evaluate the genetic diversity in the endangered sand tiger shark (*Carcharodon taurus*) and the great white shark (*Carcharodon carcharias*) (Zenger et al., 2006). A total of 59 and 78 polymorphic loci were resolved in *C. taurus* and *C. carcharias*, respectively. A major constraint to obtaining much needed genetic data from sharks is the time–consuming process of developing molecular markers; the general use of the AFLP technique, however, provides large numbers of informative loci in these animals (Zenger et al., 2006). Takami et al. (2004) have used AFLP markers to compare the genetic diversity of butterfly species from urban and rural environments and observed significant genetic variation among species. Lucchini (2003) has compared the results of AFLP markers with microsatellites and mitochondrial DNA sequences and suggested that the AFLP technique could be very useful for genetic diversity evaluation, especially for conservation management. Thus, owing to their ease of amplification in any species, AFLP markers may prove to be a valuable tool to estimate genetic diversity.

Microsatellite markers

Microsatellites are multiple copies of short tandem repeats, generally 1–5 bp long, located in both coding and non–coding regions and fairly evenly distributed throughout the eukaryotic genomes. Microsatellites are co–dominant markers with bi–allelic or multi–allelic presentation in an individual or a population, respectively. These markers are highly polymorphic and abundant; they can easily be amplified by PCR, rendering them highly versatile markers for molecular fingerprinting. An optimized characterization of microsatellite markers has been carried out for molecular profiling and conservation management of wild and captive harpy eagle (*Harpia harpyja*) (Banhos et al., 2008). Of the 45 microsatellites tested, 24 were polymorphic, six monomorphic, 10 uncharacterizable due to multiple bands and five did not amplify. The gene diversity observed in the analyzed sample of *H. harpyja* was low. While a large proportion of the microsatellite markers were highly variable, individuals of *H. harpyja* could be differentiated by a joint analysis of just three or four markers (Banhos et al., 2008). Microsatellite markers have been used to assess genetic variation within and among three ostrich breeds (Kawaka et al., 2007). The genetic diversity within the breeds was found to be low. Population analysis showed the highest variability potential for black–necked ostriches (mean diversity = 29.04%, mean heterozygosity = 0.30) and the lowest for the red–neck ostriches. The largest genetic similarity was recorded between red– and blue–necked ostriches, but the greatest genetic distance was between the red– and black–necked ostriches (Kawaka et al., 2007). Chan et al. (2008) have developed 10 microsatellite markers using feather DNA, suggesting the usefulness of isolated loci in studying population genetics of the endangered forest bird, kakerori (*Pomarea dimidiata*). Seven of the loci were found to be polymorphic in 42 individuals examined while the number of alleles per locus in the polymorphic loci varied from three to five. The observed and expected heterozygosities ranged between 0.57–0.74 and 0.50–0.74, respectively. The investigators believed that these loci will be useful in studying kakerori conservation genetics (Chan et al., 2008). Li et al. (2007) have demonstrated the usefulness of microsatellite markers for genetic diversity studies on two populations of black tiger shrimp (*Penaeus monodon*). The two multiplexed systems containing six and seven microsatellites respectively have been developed based on allelic size range and compatibility of the fluorescent labeling dyes. These markers specific to 13 polymorphic loci have detected high levels of genetic variability in both populations. This methodology has been suggested as a significant step in the development of high throughput systems for genetic diversity study, parentage identification and genetic mapping in *Penaeus monodon* (Li et al., 2007). Ryberg et al. (2002) used microsatellite loci to investigate patterns of genetic variation within and between populations of alligators distributed at coastal and inland localities in Texas. The mean heterozygosities across seven loci for both the populations ranged from 0.50–0.61 revealing similar patterns of variation; however, significant population differentiation among all populations was observed, while each population contained unique alleles for at least one locus. These genetic data have clear implications for management by suggesting considerable subdivision among alligator populations, possibly influenced by demographic and life history differences as well as barriers to dispersal (Ryberg et al., 2002). Yue et al. (2004) have applied microsatellite and AFLP markers for monitoring of genetic diversity of highly endangered fish species, Asian arowana (*Scleropages formosus*). Microsatellite analysis of 32 randomly collected individuals from each of the three stocks showed high allelic and gene diversity in all three varieties; the green stock showed higher allele number (100) and higher gene diversity (0.75) than the red (98 and 0.74) and golden ones (85 and 0.71), respectively (Yue et al., 2004). Lougheed et al. (2000) have compared the genetic differentiation among populations of the threatened massasauga rattlesnake using microsatellites and

Table 3. Conclusion: which marker is suitable for a particular study? (* This depends on objective, molecular information, facilities, etc.).

Tabla 3. Conclusión: ¿qué marcador es adecuado para cada estudio en particular? (Depende del objetivo, de la información molecular, de las facilidades, etc.).*

Molecular marker	Suitable for*
mtDNA markers	Useful to study evolutionary relationship and biodiversity
RAPD and AFLP	Do not require prior molecular information
AFLP	Has greater differentiation power than RAPD
RAPD	Is a simple method and is the least–expensive
Microsatellite and AFLP	Are highly powerful in determining genetic diversity
Microsatellites	Are best for parentage and strain analysis

RAPD. Both types of markers have been found to be suitable for defining broad–scale genetic structures in snake populations and can provide important inputs into conservation initiatives of focal taxa; however, microsatellites are superior for detecting structure at limited spatial scales (Lougheed et al., 2000).

Conclusion

There are several molecular markers for biodiversity analysis of wildlife animals; however, their selection depends on the objective, molecular information sought and the facilities available. The important aspects of various molecular markers, particularly in answer to the question "which marker is suitable for a particular study?" are summarized in table 3. It is virtually sensible to consider the expected level of variability when choosing a marker as some genetic regions are expected to evolve more rapidly than others and the desired variability will depend on the question that is being asked. Mitochondrial DNA markers are particularly useful for studying evolutionary relationship among various taxa. DNA barcoding based on mitochondrial genes (most often COI) has emerged as a powerful strategy for species identification. Among the nuclear markers, AFLP has greater differentiation power than RAPD, though RAPD is a comparatively more simple and least–expensive method. Both microsatellite and AFLP are highly powerful markers in determining the genetic diversity. Microsatellites are rarely used for high–level systematics however, but are the best for parentage and strain analysis.

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