



MICROSATELLITES: A VERSATILE MARKER FOR GENETIC/EVOLUTIONARY/ECOLOGICAL STUDIES

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ABSTRACT

Microsatellites arise in regions consisting of short runs of repetitive nucleotides and show extreme polymorphism. Microsatellites are excellent molecular markers to address ecological questions. Versatile characteristics of microsatellites such as their presence in genomes of all living organisms, high level of allelic variation, PCR typability and co-dominant way of inheritance have resulted in rapid expansion of the power of molecular markers to address ecological questions. Microsatellites are of great interest especially to ecologists because microsatellite repeats are important in genomic organization, function and their association with disease conditions. In biological analyses, single tandem repeats involved in molecular functions such as recombination or regulation of transcription factors are extensively used for purposes like genetic mapping, analyzing relationships between species and population studies. Microsatellites have high mutation rate and analysis of these mutation events aid in evolutionary studies, human disease studies such as neurological disease and cancer. The newest techniques based on microsatellite sequences are easy approaches for studies of population genetic structure, evolutionary studies and ecological studies.

KEYWORDS: Microsatellites; Genetic Markers; Genetic/Evolutionary/Ecological Studies.

INTRODUCTION

Microsatellite abundance is currently been examined in a range of species and their importance has been investigated in evolution, personal identification, population genetic analysis, tumor biology and the construction of human evolutionary trees. Microsatellite mutations are sufficient enough to make large-scale studies of evolutionary and mutational process. Several researchers therefore embarked upon sequencing homologous microsatellite loci in different species to be able to study mutations accumulated over evolutionary time periods. Microsatellites are of particular interest to ecologists because they are highly versatile genetic markers. The advent of polymerase chain reaction (PCR) technology, which aids in the analysis of samples with limited DNA has opened new research areas especially in population genetics. A large proportion of the growing family of repetitive DNA sequences is accommodated by microsatellites and it is extensively dispersed in the genome. Versatile characteristics of microsatellites like

their existence in genomes of all living organisms, high level of allelic variation, PCR typability, co-dominant way of inheritance *etc.*, have given rise to the rapid growth of molecular markers to tackle a range of purposes such as ecological questions, disease diagnosis, personal identification, population genetic analysis and the construction of human evolutionary trees.

Microsatellites as Markers

Microsatellites occur in high numbers in every eukaryotic genome and consist of tandem repetitive units of DNA typically less than five base pairs in length, with a high variability due to different repeat numbers [*e.g.* (CA)_n]. Microsatellite loci are amplified with specific PCR primers and the different alleles observed are separated along an electrophoretic gradient in routine laboratory procedures (Goldstein *et al.*, 1999) or genotyped on a Sequencer (Schulke, 2000). Microsatellite allele variation in CA repeats with an example of three different alleles is given in figure1.

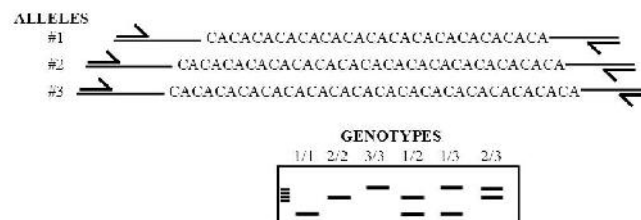


FIGURE 1. Microsatellite Alleles and Their Detection # The half-arrows in the figure represent the locus-specific primers used for amplification. Illustrations of the various gel patterns that would be observed with different allele combinations are also shown.

As compared to allozymes, DNA-based techniques like microsatellites utilize PCR to amplify the marker of interest from a small tissue sample. An ideal marker allows the use of tiny amounts of tissue samples, which are easily preserved for future use. It allows the use of simple tissue preservatives such as 95% of ethanol for storage. Moreover, since microsatellites are DNA sequence stretches shorter in length than sequenced loci (100–300 vs. 500–1500 bp), they could still be amplified with primers flanking the variable microsatellite region in spite of some DNA degradation (Taberlet *et al.*, 1999). When DNA degrades, it splits into smaller pieces and the probability of effectively amplifying a long segment is proportional to its length (Frantzen *et al.*, 1998). This trait signifies the use of microsatellites as markers permitting to apply fast effective and cheap DNA extraction protocol with degraded DNA or DNA from hair and fecal samples used in non-invasive sampling (Taberlet *et al.*, 1999). Furthermore, since microsatellites are locus-specific, co-dominant, highly polymorphic and species-specific, cross-contamination by non-target organisms is much lesser compared with techniques that employ universal primers (*i.e.*, primers that would amplify DNA from any species). The primers used in PCR based marker analysis are usually highly conserved within species and at times, even across broad taxonomic groups. This essentially reduces the work required for primer optimization for a new species. On the contrary, since same microsatellite primer sets rarely works across taxonomic groups, the primers has to be developed anew for each species (Glenn and Schable, 2005). Nevertheless, microsatellite marker isolation has now become faster and less expensive, significantly reducing failure rate and/or cost of new marker isolation in many cases (Glenn and Schable, 2005). Cross-species amplification of microsatellite loci from primers developed from other species in the same genus or even family, especially in vertebrates, reptiles and mammals occur mostly due to the presence of conservative sequence of flanking sequence across taxa. Success rate of primers may decrease proportionally due to the genetic distance between the focal species and the species of origin. In addition, the allelic diversity often decreases when primers are used in non-source species, a type of ascertainment bias that can be accounted for on the basis of requirement (Rico *et al.*, 1996). However new marker isolation from certain taxa such as some marine invertebrates (Cruz *et al.*, 2005), lepidopterans (Meglecz *et al.*, 2004) and birds still has considerable failure rate (Primmer *et al.*, 1997).

Microsatellites and Genetic/Evolutionary/Ecological Studies

Most of the published literature focused on the frequency of microsatellite regions in the genomes of animals, plants, fungi and prokaryotes and concludes that the success rate of isolating microsatellite markers is proportional to their frequency in the genome. Repeated analysis of microsatellite frequency in the genomes of various organisms has shown the occurrence of microsatellites which are relatively rare in lepidopterans, birds, bats, prokaryotes whereas fishes and most mammals tend to have a high frequency of repeat motifs. In addition, low population sizes, species with high rates of inbreeding and severe or frequent bottlenecks typically have low

heterozygosity and low average polymorphism and also on average short repeated sequence (Toth *et al.*, 2000).

Another study by Toth *et al.* (2000) on simple sequence repeats (SSRs) in several eukaryotic taxa, from fungi to humans, revealed highly taxon-specific patterns in the distribution of various repeat types for different motifs in coding and non-coding sequences and also introns and intergenic regions. This specificity can partly be explained by interaction of mutation mechanisms and differential selection. The accumulated empirical evidence seems to indicate that SSRs are more abundant and longer in vertebrates than in invertebrates, and among vertebrates longer SSR tracts are observed in cold-blooded species (Chambers and MacAvoy, 2000). It is interesting to note that among the taxa compared by Toth *et al.* (2000), maximum abundance of microsatellites was displayed by rodents, while *C. elegans* displayed minimum microsatellites.

Li *et al.* (2003) proposed the structure, function and evolution of microsatellites. In recent times, an abundant number of microsatellites, also known as simple sequence repeats (SSRs) have been recognized and represented within protein-coding genes and their untranslated regions (UTRs). These data offer a unique opportunity to the direct study of possible SSR functions. Here, SSR distributions are accommodated by expressed sequence tags (ESTs) and genes including protein-coding, 3'-UTRs and 5'-UTRs, and introns. These distributions discuss the consequences of changes in SSRs of those regions of both eukaryotes and prokaryotes. An evidence-based experiment by Garza *et al.* (1995) also shows that SSRs are non-randomly distributed across UTRs, introns and protein-coding regions. Natural selection is very important constraint acting on SSR allele sizes. In theory, natural selection could act against long alleles, introducing a form of length ceiling. The micro-geographical study by Li *et al.* (2002) in wild wheat implied that natural selection might act as high and low limits. Morgante *et al.* (2002) provided evidence of positive selection for specific repeats in the transcribed regions by examining whether complementary motifs were equally represented in the transcribed strand in *Arabidopsis thaliana* genome, and suggested that very different selection pressures seem to act on the 5'UTR, open-reading frames and 3'UTR regions of the genes.

Some of the important applications of microsatellites in different areas of genetic/evolutionary/ecological studies are summarized under the following headings below:

- a. microsatellites in population genetic analysis;
- b. microsatellites in genome mapping;
- c. microsatellites in detection of paternity and gene flow;
- d. microsatellites in conservation genetics;
- e. microsatellites in cancer detection; and
- f. microsatellites in detecting demographic bottlenecks.

a) Microsatellites in Population Genetic Analysis

Diverse studies generally show that high allelic variation enables the use of microsatellites as a versatile molecular marker in population biology. So far, the isolation strategy of species-specific microsatellites has been dependent on time and labor-consuming laboratory protocols providing typically a few hundred sequences.

By reason of the widespread abundance of microsatellites in genomes and their relative uniform distribution, mutation dynamics and high degree of polymorphism, microsatellites have emerged as valuable tool in genetic mapping, forensic identity testing and population studies (Arcott *et al.*, 1995). Brown *et al.* (2003) has demonstrated nuclear microsatellite and mitochondrial marker estimates of population structure in striped bass (*Morone saxatilis*) and found that this highly migratory anadromous and long-lived fish has recently recovered from a severe decline in population size.

Microsatellites enable direct access to genetic information from wild populations. Evolutionary Significant Units (ESUs), an operational level of organization for assessing biodiversity independent from taxonomic hierarchy have facilitated the description of several previously unrecognized species in the amphibian fauna (Beerli *et al.*, 1994). Recently, a large number of microsatellite repeats have been found to be associated with *Alu* interspersed repeated DNA elements. *Alu* elements play a direct role in the origin of microsatellite repeats and various studies put forward the association of an *Alu* element with a microsatellite repeat. This could result from the integration of an *Alu* element within a pre-existing microsatellite repeat. *Alu* repeats are considered as a microsatellite source for genomes of primates.

Microsatellite markers have been found to be an effective tool in the analysis of genetic differentiation among a wide range of populations. Molecular marker analysis provides an effective measure of genetic relationship based on genetic characteristics. Researches on genetic relationship among populations were conventionally based on the allele frequencies at different loci. The use of microsatellites has revealed high levels of genetic diversity amongst the huge pool of genetic resources of pigs (Blott

et al., 2003), sheeps (Arranz *et al.*, 2001), nematodes (Plantard *et al.*, 2008), lions (Gaur *et al.*, 2006), and beetles (Demuth *et al.*, 2007).

Several other studies have also demonstrated a link between geographical distance of populations and genetic dissimilarity. These studies were generally carried out on large sample of population from different geographical locations. Genetic differentiation can be measured on a microscale and if genetic differentiation could be correlated with the connectivity of the landscape, it would be an effective tool to enumerate dispersal (McCauley, 1991). Various other studies have also documented the genetic differentiation among population of the moor frog (*Rana arvalis*) on a spatial scale where some dispersal between populations is likely to occur in a landscape (Rafinski *et al.*, 2000).

In recent years, microsatellites have become one of the most powerful genetic markers in biology due to its sufficient mutation rate. A study conducted by Schlotterer *et al.* (1992) demonstrated that high mutation rate of long microsatellite allele in *Drosophila melanogaster* provides evidence for allele specific mutation rate. Another study by Rothschild *et al.* (1994) comparing allelic variability between a hatchery strain (DEBYTM) and a wild Chesapeake Bay population (James River) revealed low genetic variation in the DEBYTM strain and high genetic differentiation with the wild population and DEBYTM strain. All these studies highlight the apparent differences in the frequency of microsatellite regions in various genomes thus proving the use of microsatellite loci as an effective tool for upcoming population genetic studies. Few examples of studies conducted in different species whose genetic diversity has been measured by using microsatellites is given in Table 1.

TABLE 1. Microsatellites in Genetic Diversity Analysis in Different Species

No.	Species	Source
A	SHEEPS	
	Alpine sheep, <i>Biellese</i>	Bozzi <i>et al.</i> (2009)
	Spanish sheep, <i>Latxa</i>	Juan-Jose <i>et al.</i> (2001)
	Greek sheep, <i>Skopelos</i>	Ligda <i>et al.</i> (2009)
B	FISHES	
	Atlantic cod, <i>Gadus morhua</i>	Brooker <i>et al.</i> (1994)
	Atlantic salmon, <i>Salmo salar</i>	Slettan <i>et al.</i> (1995)
	Brown trout, <i>Salmo trutta</i>	Estoup <i>et al.</i> (1993)
C	BEEPLES	
	Red flour beetle, <i>Tribolium castaneum</i>	Demuth <i>et al.</i> (2007)
	Milkweed beetle, <i>Tetraopes tetraophthalmus</i>	McCauley <i>et al.</i> (1987)
	Ground beetle, <i>Carabus problematicus</i>	UGent <i>et al.</i> (2003)
D	PIGS	
	Iberian pig, <i>Guad yerbas</i>	Alves <i>et al.</i> (2006)
	Chinese breed, <i>Huai</i>	Blott <i>et al.</i> (2003)
	Brazilian pig, <i>Moura</i>	Sollero <i>et al.</i> (2003)
E	WHALES	
	Bowhead whale, <i>Balaena mysticetu</i>	Morin <i>et al.</i> (2010)
	Killer whale, <i>Orcinus orca</i>	Morin <i>et al.</i> (2006)
	Pilot whales, <i>Globicephala melas</i>	Amos <i>et al.</i> (1991)

b) Microsatellites in Genome Mapping

Microsatellites have been widely used for genome mapping in a variety of species. Quantitative genetic traits that are typically determined by multiple loci with various

and additive effects forms the basis for great majority of ecological and demographic characteristics relevant for population variability. Quantitative Trait Loci (QTL) is an approach currently used for mapping of species on the

basis of microsatellites. Microsatellites play a very significant role in the upcoming field of 'population genomics', where numerous QTLs across the whole genomes are sampled with locus-specific effects of population genetic parameters being distinguishable from the general sample distribution across all loci (Falconer *et al.*, 1996). For the purpose of mapping QTLs, a comprehensive linkage map is needed, with variable markers distributed across the genome. Linkage between a marker locus and a segregating QTL allele can only be steadily detected at or below a map distance of 20 centimorgans (Soller *et al.*, 1976) though the generation of higher density maps does not do much to intensify the power of QTL detection (Halley and Knot, 1994). Microsatellites have a unique credit of offering the first practical tool that can be used to generate robust high density linkage maps in many species including fishes (Rico *et al.*, 1996), grain species (Chen *et al.*, 1997; Akagi *et al.*, 1996) and humans (Dib *et al.*, 1996).

c) Microsatellites in Detection of Paternity and Gene Flow

Paternity analysis and gene flow studies have mostly used microsatellite markers for the reason that allozyme loci do not have enough variability to confirm parentage by exclusion. Gerber *et al.*, (2000) showed that polymorphic microsatellite markers are excellent tool for detecting paternity by comparing 159 AFLP loci and six polymorphic microsatellite markers. In order to assess paternity exclusion in *Pithecellobium (Mimosoideae)*, Chase *et al.*, (1996) has adapted four microsatellite loci and six allozyme loci. The study suggests that in addition to being powerful tools for the analysis of population structures, microsatellites also provides a means for accurately examining both gene flow and paternity- two important parameters in conservation biology. Dakin *et al.* (2004) discussed the importance of microsatellite null alleles in parentage and population genetic analyses.

The comparison of microsatellite alleles provides two kinds of information, i.e., allele identity/non-identity; and allele size difference. Allele size based measures of

differentiation is widely used in assigning species, sex and individual identity in a population and difference in size between two different alleles might be informative. The larger the difference, the higher the number of mutation events is expected to occur since common ancestry. Allele identity information gives the estimates of gene flow. Identity among genotypes in populations of Canadian polar bears (Paetkau *et al.*, 1995) and *Apis mellifera* (Estoup *et al.*, 1995) are estimated by using microsatellite loci.

d) Microsatellites in Conservation Genetics

Conservation genetics employs a wide range of possible technical approaches and genetic marker types. Population genetic differentiation in protected/managed populations thus becomes an essential prerequisite for the identification of evolutionarily significant units (ESUs) and management units (MUs). In this context, Parker *et al.* (1998) point out that though too much of focus has been put on different conceptual definitions of ESUs and MUs, the population differentiation estimates or lack thereof that are prerequisites to using these concepts might not be so clear. Comparative analysis of nuclear and organelle genetic markers may help delineate management units or evolutionarily significant units though population differentiation estimates from multiple genomes can also conflict.

Hedrick (2001) explained the genetic considerations are probably most useful when incorporated early in a species' conservation plan, when the existence of some robust population across a species' geographical range offers the possibility of a variety of creative solutions to conservation problems. Many European amphibian species suffer serious declines in population but are not yet exposed to the imminent risk of extinction, rendering timely molecular studies. However, an increased knowledge of the population genetic structure alone is not sufficient enough to guarantee conservation, and new scientific findings based on high variation genetic markers would help amphibian conservation when integrated into current and future action plans.

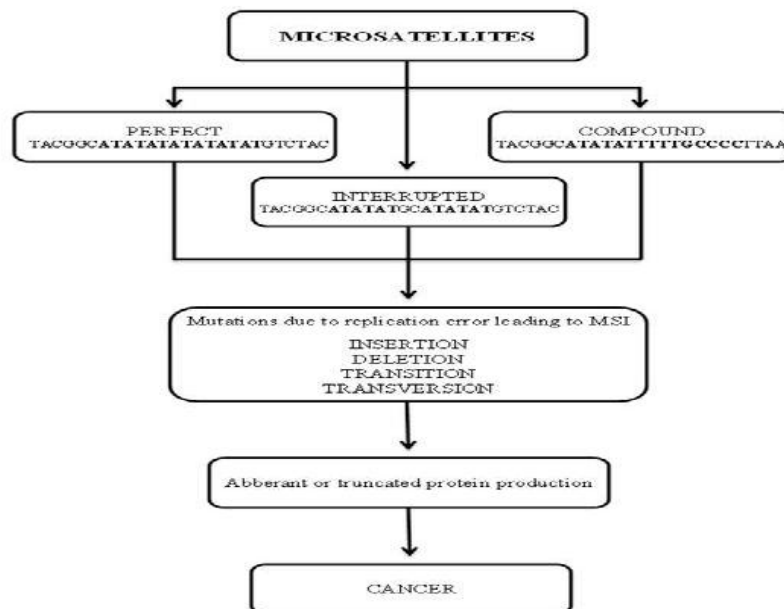


FIGURE 2.Types of Microsatellites and Different Aberrations Involved in the Incidence of Cancer

e) Microsatellites in Cancer Detection

Epidemiological studies of past few decades have shown that the risk for each individual to develop cancer is closely linked to his/her own genetic potentialities. Some populations that are defective in DNA repair processes are more prone to develop cancer as compared to their counterparts due to the accumulation of mutations within the genome. Using molecular-based strategies, especially microsatellite instability (MSI), one can detect the cancer. MSI is a form of genomic instability associated with defective DNA mismatch repair in tumors. MSI has

become one of the important and widely accepted pathways in tumorigenesis. Tumors may be characterized on the basis of high-frequency MSI (MSI-H – i.e., having insertion/deletion mutations), and low-frequency MSI (MSI-L–i.e., not having as much insertion/deletion mutations) (Bapat *et al.*, 1999; Thibodeau *et al.*, 1993). Different types of microsatellites and different aberrations involved in the incidence of cancer, and the list of cancers detected by using microsatellite instability status are given in figure 2 and table 2 respectively.

TABLE 2. Cancers Detected By Using Microsatellite Instability Status

Type of Cancer Detected	Source of sample	Author
Proximal Colon Cancer	Colon Tissues	Thibodeau <i>et al.</i> (1993)
Colorectal Cancer	Peripheral Blood	Bapat <i>et al.</i> (1999)
Breast Cancer	Breast Tissues	Elisa <i>et al.</i> (1997)
Gastric Carcinoma	Gastric Carcinoma Tissues	Ja-Mun Chong <i>et al.</i> (1994)

f) Microsatellites in Detecting Demographic Bottlenecks

The ability of microsatellites to trace back population bottlenecks opens the door for addressing a variety of questions. The detection of past bottlenecks could, for example, indicate the decimation of a population due to disease, or the colonization of a newly formed habitat. Bottlenecks are also expected to occur during extinctions/ recolonization processes in metapopulations, which are of vital importance for the maintenance of overall genetic variation. Such events could now be reconstructed from genetic data, without historical knowledge of population demography. Furthermore, for studies that aim to trace population reductions over longer time scales, additional

maximum likelihood and coalescent-based methods are available (Beaumont *et al.*, 1999).

Microsatellites being a molecular marker that follow extreme polymorphism and Mendelian inheritance greatly facilitate detecting demographic patterns. These traits are considered as ideal for any marker. Putative distribution biases are usually checked for in exons, introns and intergenic regions. The probable relationships with other genomic elements including interspersed repeats are also verified (Arcot *et al.*, 1995; Li *et al.*, 2003; Lim *et al.*, 2004; Malpertuy *et al.*, 2003; Toth *et al.*, 2000). Some applications of microsatellites in genetic/evolution/ ecological studies discussed above are schematically given in figure 3.

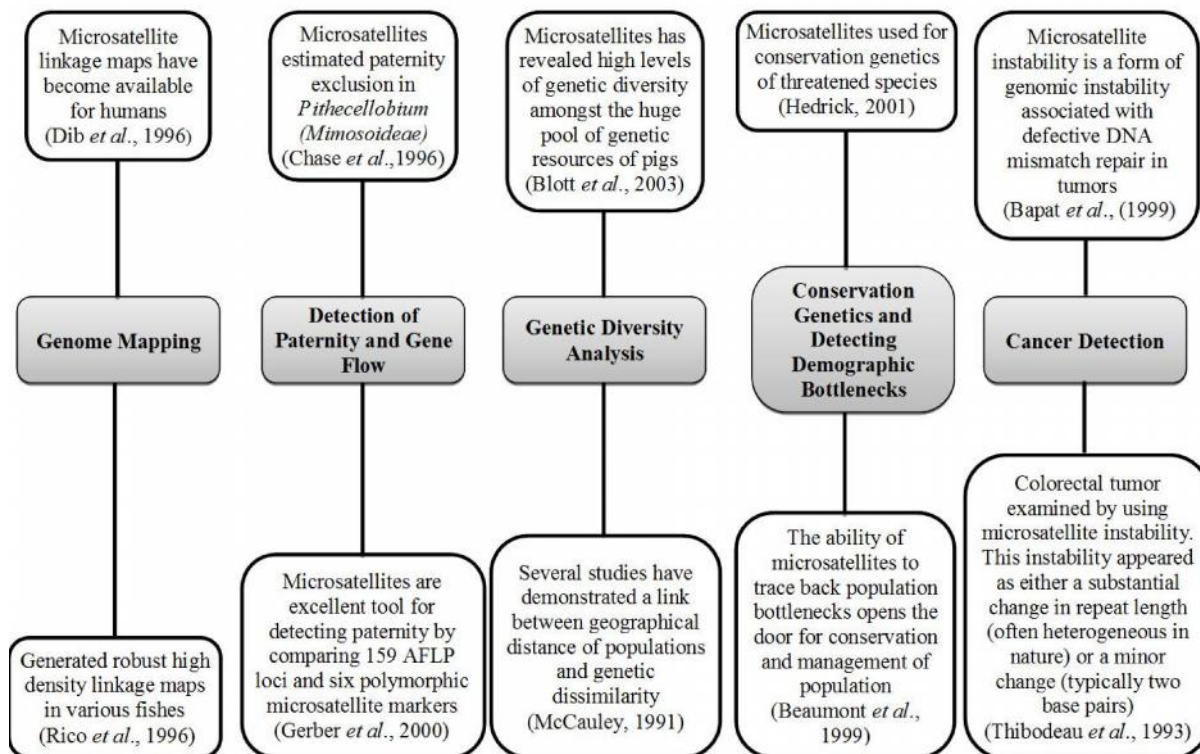


FIGURE 3. Different Applications of Microsatellites in Genetic/Evolution/Ecological Studies with Examples

Strategies for Microsatellite Isolation – Different Methods

The traditional approach was to clone small genomic fragments and using radiolabeled oligonucleotide probes of microsatellite repeats to identify clones with microsatellites. This technique was also found to be working well in organisms with abundant microsatellite loci (Tautz, 1989; Weber and May, 1989). But as this approach was observed to be not working well when microsatellite repeats are less abundant, alternatively, two classes of enrichment strategies came into being viz., i) uracil-DNA selection (Ostrander *et al.*, 1992) and ii) hybridization capture (Armour *et al.*, 1994; Kandpal *et al.*, 1994; Kijas *et al.*, 1994). Hybridization capture is the strategy which was predominantly used in this isolation process as it had an advantage of allowing selection prior to cloning, and hence is faster and easier to process multiple samples than uracil-DNA selection. Ostrander *et al.* (1992) and Paetkau (1999) proposed a unique strategy for the production of libraries enriched in microsatellite loci based on primer extension which primarily involves two reactions. The first reaction uses a biotinylated primer allowing vectors with microsatellite-containing inserts to

be selected with streptavidin-coated magnetic beads. This reaction may be dependent on the strand displacement activity of the Klenow fragment of DNA polymerase I. The second reaction is a strand extension reaction which is included to improve the relative transformation efficiency of clones containing microsatellites. In order to get rid of construction and screening, a few authors have suggested Random Amplified Polymorphic DNA (RAPD) approach for the amplification of unknown microsatellites by either using repeat-anchored random primers (Wu *et al.*, 1994) or using RAPD primers (Ender *et al.*, 1996). Another method of separating microsatellite-containing DNA fragments from genomic DNA is Fast Isolation by AFLP (amplified fragment length polymorphism) of Sequences Containing Repeats [FIASCO] that largely relies on effective digestion-ligation reaction. This method is said to be comparatively simple and rapid than the aforesaid methods. New advances in pyrosequencing have shed light on DNA sequencing and large amounts of sequences are produced from DNA sample at low cost. This has opened new avenues for the setup of markers for species without any previous genomic information.

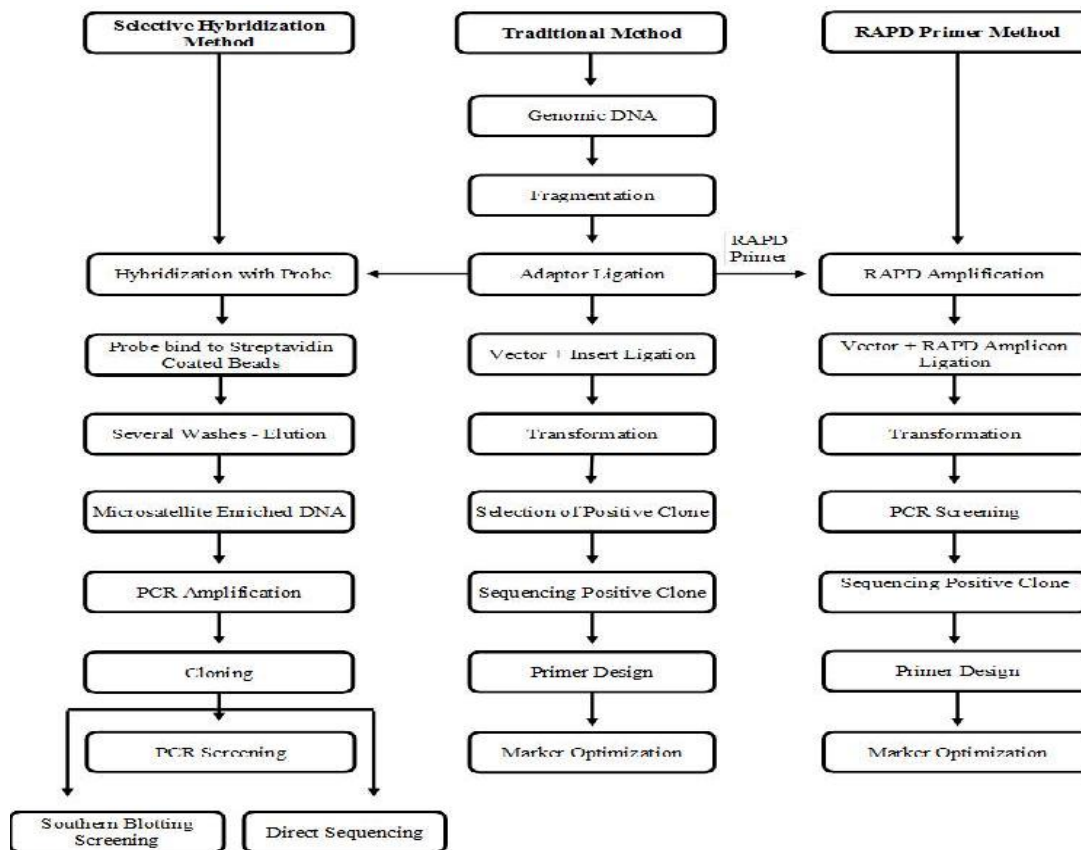


FIGURE 4. Schematic Representation of Microsatellite Isolation Protocols

There are many different strategies for obtaining microsatellite DNA loci. However, there is a considerable variation in the time taken for isolation and yield of microsatellites based on the different strategies of isolation protocols. Some of the important strategies of microsatellite isolation protocols are illustrated as figure

and table. A schematic representation of microsatellite isolation protocols is given in figure 4 and the variation in the time taken for isolation and yield of microsatellites based on the different strategies of isolation protocols is given in table 3.

TABLE 3. The Variation in the time taken for isolation and yield of microsatellites based on the different strategies of isolation protocols

Protocol	Time	Yield
Traditional	1 Month	Low
RAPD-Based	1 Week	Variable
Primer Extension	2 Weeks	Medium/High
Selective Hybridization	1-2 Weeks	Medium/High

Applications of Next Generation Sequencing (NGS) in Microsatellite Development

The introduction of Next Generation Sequencing (NGS) has brought a new revolution to genomic and transcriptomic approaches of biology. These new sequencing tools are also playing a very important role in the discovery, validation and assessment of genetic markers in populations. NGS technologies help in the easy identification of a large number of microsatellites at a fraction of the cost and effort of traditional approaches. The key advantage of NGS methods is that their ability to produce large amounts of sequence data from which numerous genome-wide and gene-based microsatellite loci can be isolated and developed. Availability of a number of different sequences may assist in optimizing the setup of microsatellite markers by permitting the selection of microsatellites which are not compound or interrupted and that may follow a simple mutation model. The identification of microsatellites using NGS is comparatively a new approach and only a handful of studies have been reported in this area so far. To cite a few studies where species-specific microsatellites are developed by using NGS are, Endangered Dwarf Bulrush (*Typha minima*) (Csencsics *et al.*, 2010) and *Ceanothus Roderickii* (Rhamnaceae) (Burge *et al.*, 2012).

Rapid advancement of sequencing technologies has broadened the applications of genomic information and its high cost prevents the duplication or triplication of workload by using multiple independent gene sequences in parallel (Zhang and Hewitt, 2003). SNP markers are a new marker type for such polymorphic studies that hold great promise for future and have gained high popularity though their use in non-model organisms are still nascent (Morin *et al.*, 2004). Studies conducted by Mitton *et al.* (1984) and Cho *et al.* (2000) prove that microsatellites derived from genomic libraries shows higher level of polymorphism than those derived from ESTs contained in the GenBank database. Occasionally, microsatellites are linked with mobile element and this link has an effect on amplification of microsatellite by polymerase chain reaction (PCR). Most of the available evidence has proved that the large number of sequences allows the detection of putative mobile elements by spotting sequence similarity groups and eliminating them. As a result, the primers allow amplification of specific sequence at unique loci with a high clear banding that would avoid time-consuming primer tests. The existence of null alleles is also an issue in microsatellite amplification. Unless numerous copies of the identical loci are detected, and the sequencing was based on pooled DNA from many individuals, some of the null alleles can be identified and avoided in case consensus sequence construction is stringent. Every marker locus can be regarded as a sample of the genome. Relying only on a single locus to calculate

ecological traits from genetic data might often lead to a high chance of sampling error. Therefore, in order to avoid such a sampling error and to avail a more precise and statistically strong method of comparing populations and individuals, multiple samples of the genome should be taken into consideration by combining the results from multi-loci. Moreover, statistical approaches to the queries of significant concern to ecologists often need multiple, comparable loci.

Versatile characteristics of microsatellites such as their presence in genomes of all living organisms, high level of allelic variation, PCR typability and co-dominant way of inheritance makes it a very efficient tool in Genetic, Ecological and Evolutionary studies. Despite the fact that molecular techniques such as AFLP, Allozymes and RAPD are also multi-locus, none of these have the resolution and power of a multi-locus microsatellite study. Short length of microsatellite markers permits it to be an effective, fast and cheap tool in studies with degraded DNA. Furthermore, since microsatellites are locus-specific, co-dominant, highly polymorphic and species-specific, cross-contamination by non-target organisms is much lesser as compared to other techniques.

Along with aforesaid advantages microsatellites have disadvantages too. Microsatellite markers face many challenges and drawbacks that at best set hurdles to the data analysis, and at worst limit their applicability and utility and thereby confuse their analysis as well. Each and every marker types have some downsides, and the versatility of microsatellites to tackle with different types of ecological questions often offsets their drawbacks for several applications. Fortunately, many of the drawbacks that are unique to microsatellite markers can best be addressed by careful selection and screening of loci.

CONCLUSION

This review has brought forth available evidences to suggest that the detection of microsatellite variation plays an important role in the study of evolution. Today, the role of microsatellites has been increasingly recognized in the field of ecological research and studies of population genomics as well. Microsatellites have thus become an important tool for researchers working in the field of ecology and population biology. Microsatellites as molecular markers in molecular-ecology, population-genetics and genetic-mapping are actively utilized for the conservation of threatened species in a population by comparing the level of variability in microsatellite loci. Traverse through the aforesaid studies have enabled us to explore an array of ecological questions and various levels of population structure in terms of microsatellite markers. Among them, a considerable number of studies have also proved that microsatellite markers are extremely useful not only in examining the evolution of different kinds of

population but also the rates of genetic divergence among these populations. All these studies have generally revealed that the success rate of isolating microsatellite markers largely depend on their frequency in the genome. To sum up, through the present review we have attempted to justify that microsatellites are efficient and versatile markers for genetic, evolutionary and ecological studies and thus contribute towards making concrete datasets and relevant information in molecular ecology, evolutionary biology and ecological genomics as well.

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