SANATAN DHARM COLLEGE MUZAFFARNAGAR
(Affiliated to Maa Shakumbhari University, Saharanpur)

# Topic

# QUANTITATIVE METHODS FOR WILDLIFE MANGEMENT (WLM)

**For**

## Subject Zoology

## &

## B.Sc. VI Sem

**DR. ARUN RATN, Ph.D.**
[CSIR-JRF/SRF, CSIR-NET/LS
GATE, CEEB-BIT (DBT-JNU)]
**(ASSISTANT PROFESSOR)**
(arunratnbt14@gmail.com)
**DEPARTMENT OF ZOOLOGY,**
**SANATAN DHARM (P.G.) COLLEGE,**
**MUZAFFARNAGAR-251001,**
**UTTAR PRADESH (INDIA).**

# ADVANCED QUANTITATIVE METHODS FOR WILDLIFE MANAGEMENT (WLM)

- **Molecular-genetic technology** and **statistical methods** based on principles of population genetics provide valuable information to wildlife management.
- New approaches, using contemporary **molecular techniques (mainly Random Amplified Polymorphic DNA or RAPD)** in conjunction with **demographic statistical data**, can be extremely useful for improving the understanding of the dynamics, population structure and social biology of many invasive species during wildlife management (Taylor et al. 2000).
- **Molecular Genetic Techniques for WLM-given as follows-**

  1. **Nuclear *vs.* Mitochondrial Genomes**

  All genetic techniques and molecular markers described to examine portions of DNA at some scale. **Two different genomes** are used in genetic studies of animals.

  - The nuclear genome is **bi-parentally** inherited and is found in the cell nucleus. It is large and not well mapped in most species.
  - The mitochondrial genome is housed in the mitochondrion, an organelle involved in cellular metabolism. It is small compared to the nuclear genome and is a circular, **maternally inherited molecule** that has been well mapped in many species. Nuclear DNA on average evolves slowly although some portions (e.g., microsatellites) evolve quickly.
  - Mitochondrial DNA (mtDNA) on average evolves more quickly than the nuclear genome and some areas (e.g. control region) evolve very rapidly. These features make mtDNA and some regions of nuclear DNA suitable targets for certain genetic studies (Avise 1994).

  **2. Investigating Genetic Variation**

  - Some molecular techniques consider gene products (e.g., proteins), and some examine DNA variation at the nucleotide level (e.g., DNA sequencing, fragment analysis).
  - In the past, analysis of certain proteins has been easy and economical; however, quantifying variation at the nucleotide level has become a more powerful molecular tool for population genetics and systematics.
  - Some techniques look for differences in actual nucleotide sequence, while others infer relatedness based on analysis of fragments and restriction sites.

- The advent of the Polymerase Chain Reaction (PCR) has revolutionized molecular biology. Essentially, PCR is a reaction in which a region of DNA is targeted and amplified exponentially.
- PCR reaction requires development of unique primers, which flank both sides of the targeted region of DNA. Once amplified to large quantities, the targeted region (usually between 100–2,000 base pairs) is available for study with a wide variety of molecular techniques. We briefly review several molecular techniques that have been and are currently used in wildlife management programmes (Table 1).

**Table 1.** Applicability of common types of molecular markers for wildlife biologists. The number of X's indicates the relative applicability of each technique to a specific question (modified from Mace et al. 1996).

| Type of marker | Taxonomic delineations | Regional/ subspecific population structure | Genetic diversity and sub-population structure | Individual ID and paternity/ maternity analysis |
|---|---|---|---|---|
| Allozymes | XXX | XXX | XXX | X |
| MtDNA sequences and RFLP | XXXX | XXXX | XX | X |
| Microsatellites | X | XX | XXXX | XXXX |
| Minisatellites | X | X | XX | XXXX |
| RAPD | X | XX | X | X |
| AFLP | X | X | XX | XXX |

- 

## 3. Analysis of Gene Products

- Protein electrophoresis is a technique that can be used to examine population subdivision or structure. Proteins are a series of amino acids joined by peptide bonds. Each amino acid has a distinctive side chain, some of which are either positively or negatively charged. Thus, when an electric current is present these proteins migrate differentially through a matrix based on their charge and on their size and shape. Proteins can then be visualized through histochemical staining or other methods.
- Mutations cause changes in the DNA sequences of amino acids forming proteins, which in turn cause changes in the shape, net charge, and migration rates of proteins.
- Such changes can be revealed through electrophoresis and provide information showing **variability between individuals, populations, or species**. While inexpensive, this

technique can examine only a small proportion of the variation present in the DNA that codes for the proteins; differences in proteins are not necessary detected.

- The subset of proteins typically studied with this approach is called allozymes. These proteins, however, may be under selective pressure and may not represent the diversity and divergence present in other genes.
- Further, the tissue required for this type of analysis typically requires highly invasive/destructive sampling and is logistically difficult to manage in field situations.

**4. Fragment Analysis**

- Fragment analysis comprises a group of different genetic techniques that explore nucleotide variation indirectly **by comparing the size of DNA fragments** electrophoretically.
- Although fragment analysis offers less resolution than **direct DNA sequencing**, it is cost effective when examining many individuals and many different loci. Among fragment analysis techniques, some cut (cleave) DNA in certain areas, **(i.e., Restriction Fragment Length Polymorphisms [RFLPs] and minisatellite fingerprinting),** while others **amplify many different loci (Random Amplified Polymorphic DNAs [RAPD], Amplified Fragment Length Polymorphisms [AFLP], and microsatellites**).
- With the exception of microsatellites, these techniques produce multiple fragments (bands) per individual. In these cases, **individuals are compared by the extent of band sharing among individuals.**
- These markers, with the exception of microsatellites, are considered **dominant** which refers to the fact that they document presence or absence of an allele.
- **Co-dominant markers** are those that reveal both alleles at a given locus (i.e., heterozygotes can be distinguished from homozygotes). Thus, they provide much more information and allow for the **documentation of heterozygosity and tests of Hardy-Weinberg Equilibrium and Mendelian inheritance**.
- For **RFLP** analysis, the **template DNA** is typically a small portion of the nuclear or mitochondrial genome that has been amplified using PCR. RFLPs characterize genetic variation using **restriction endonucleases**, which are enzymes that cleave at specific locations within DNA sequences. Restriction enzymes cleave at a specific recognition sequence, usually 4–6 base pairs long. The enzyme EcoRI, for example, cuts between G and A when it comes across the sequence GAATTC. Thus, every string of GAATTC

in the PCR product will be cut in the same location and will produce many fragments of different sizes.

- **Mutations** that cause changes in the cleavage site (e.g., GATTC changed to GATAC) prevent the enzyme from cutting at that location **thereby producing a different series of fragments** (different numbers or sizes of fragments). The series of fragments is then compared to examine the similarity of individuals or populations. While RFLPs look for variation within a single targeted segment of DNA, other fragment-based methods examine variation throughout the genome.
- **Minisatellites** refer to portions of DNA that have **variable numbers of tandem repeats** (sometimes called VNTRs); the length of each repeat unit is approximately **20 base pairs long**. Typically, genomic DNA is digested into many fragments with restriction enzymes. These fragments are then separated by size using electrophoresis. The number of fragments produced by this process precludes visualization of individual bands, so radioactive or fluorescent probes specific for the minisatellite repeat are used to visualize and compare these sequences. Because such "repeats" are commonly repeated in the genome, it is not unusual for this technique to produce dozens of bands.
- Although **DNA fingerprinting** with minisatellites has typically involved analysis with restriction enzymes and labeled probes, PCR-based approaches are becoming more common. **Rapid amplified polymorphic DNAs (RAPD)** provide an alternate approach to documenting variation among individuals by generating a set of PCR products (bands) using **short, random PCR primers**. Variation is presumably due to sequence changes in priming sites or length changes in the PCR products.
- Analysis involves examining the presence or absence of bands of a particular size. RAPD analysis has the advantage of being **a multi-locus technique**, whereby hundreds of **polymorphic loci** can be scored with minimal a priori knowledge of the target genome. Two disadvantages with this technique limit its utility: it is **a dominant marker** system (**heterozygotes cannot be distinguished from homozygotes**), and its repeatability has been questioned (Ellsworth et al. 1993, Muralidharan and Wakeland 1993).
- Amplified fragment length polymorphism (AFLP) analysis is **another multilocus technique** that involves **randomly primed loci** and requires no a priori knowledge of the target genome. Analysis of AFLPs involves cutting the genomic DNA with restriction enzymes, and ligating (attaching) short "**adapters**" of known sequence to

the fragment ends. PCR is then used to selectively amplify subsets of these fragments. As with RAPDs, AFLPs produce a series of hundreds of bands on a gel. Scoring is based on the presence or absence of a particular PCR product. AFLP analysis is also a **dominant marker system**, but has the advantage of being highly repeatable and is being used with increasing frequency.

- Microsatellite analysis, another PCR-based technique, differs from most other fragment analyses because the attempt is **to identify diploid (co-dominant**) genotypes for specific loci. Like minisatellites, **microsatellites are VNTRs**; however, the repeated sequence is short (2–5 base pairs). Mutation rates of these regions are high and the number of alleles (versions of a particular sequence) per locus in a population is also typically high. Allelic-variation is usually in the form of length polymorphism, which can easily be detected on a high-resolution gel.
- Amplification results in either 1 (homozygote) or 2 (heterozygote) bands per individual. Microsatellite primers are specific to a single locus and are usually specific to a particular species or group of closely related species. Because of this primer specificity, the development of primers for an individual species can be expensive. The advantages of microsatellite analysis include **co-dominance** and high levels of polymorphism. Typically, data from several microsatellite loci are used in a particular study.

**5. DNA Sequence Analysis**

- Direct DNA sequencing (nuclear or mitochondrial) is one of the most widely used techniques today because it is highly informative and, recently, has become much easier and less expensive to perform.
- It is also appealing because evolutionary processes can be modeled and integrated into analyses. Further, because the genome is so vast, the amount of information gleaned from sequencing may be quite large.
- DNA sequencing involves amplifying a target region and then creating a series of labeled (either radioactively or fluorescently) DNA fragments that correspond to each nucleotide in that region.
- The DNA fragments are then separated using electrophoresis and visualized. Recent technological advances have automated the sequencing process using fluorescently labeled DNA fragments that are read by a laser and interpreted by computer software.

- Usually, steps used in the **study of polymorphic microsatellite loci** as follows-

1. These microsatellites have been extensively characterized and amplified using 10 bp long random primers in polymerase chain reactions (PCR) for whole DNA or per individual.
2. Then, the DNA fragments were separated on a 5% polyacrylamide gel or 1.5% agarose gel containing the ethidium bromide (EtBr).
3. At last, fragments were seen under the UV light in Gel Documentation system and amplified DNA images were taken out for further uses.

- All these parameters are important to quantify because the effective management of any species requires at least some basic understanding of their dynamics.
- Genetic data analyzed in **a hierarchical, spatial context** among individuals and among populations at micro- and macro-geographic scales.
- Genetic data has been widely used to provide information on the **degree of population structure and to estimate rates of dispersal.**
- Our goals were-

  (1) To provide **an overview of spatial statistics** commonly used in empirical population genetics.

  (2) To introduce **analytical designs** that can be employed to extend hypothesis-testing capabilities by incorporating **space-time interactions** and by using information on habitat quality, distribution, and degree of connectivity.
- We show that genetics data can be used **to quantify the degree of habitat permeability to dispersal and to qualify the negative consequences of habitat loss**.
- We highlight empirical examples that use information on **spatial genetic structure** in areas of harvest derivation for **admixed migratory species, wildlife disease, and habitat equivalency analysis**.

# CONSERVATION GENETICS (WLM)

- A focus of **conservation genetics** has been **preservation of genetic diversity within** and **between populations**, especially **in rare or endangered taxa**. Genetic diversity can be estimated using **molecular markers or morphological measurements**.
- While studies have examined the underlying genetic variation and heritability of specific morphological traits in both captive and free-ranging wildlife (Kruuk et al. 2000, Réale and Festa-Bianchet 2000), intensive investigations are difficult to implement for many species.
- Frankham et al. (2002) reviewed using **quantitative genetic approaches** in a conservation context to study the effects of multiple genes and environmental variation on complex traits like morphology and behavior.
- Our primary focus is on what **molecular markers tell us about the demography and genetics of a population** and **how that information can be applied to issues in wildlife conservation.**
- Genetic diversity and genetic variation are often used interchangeably to refer to a dizzying array of population characteristics. We use genetic diversity **to refer to variation in frequencies of alleles at individual genes.** It is difficult to quantify total genetic diversity in populations; most studies look at surrogates of this measure based on variation at molecular markers.
- Four processes are generally thought to influence patterns of genetic diversity: **mutation, gene flow, drift, and selection**.
- Most conservation geneticists promote maintaining **large effective sizes of populations to prevent loss of genetic variation** and possible associated reductions in population viability.

## Conclusion

1. Molecular genetic techniques represent a relatively new and powerful set of tools that can address both research and management issues in wildlife science. These approaches have shown their utility in wildlife management **by helping identify species and appropriate units for conservation**.
2. Knowledge gained about the factors affecting distribution and loss of genetic variants has led to refinements in population management such as maintaining effective population sizes and connectivity between reserves.
3. More recently, the introduction of PCR has allowed noninvasive collection of genetic material from a variety of sources such as **hair, feathers, and feces**. Together with the ability **to examine highly polymorphic loci and gender-specific markers, noninvasive sampling** has allowed **genetic assays** to contribute to ecological studies of sex ratios, food habits, population size, and mating systems.
4. We provide general theory of population genetics and have identified those techniques and applications currently used in wildlife studies. This body of literature is expanding rapidly and readers are referred to more detailed accounts of population genetic theory, techniques, and applications.
5. With rapid development of DNA-based technologies, it is likely that currently unforeseen applications of genetic approaches will soon be available to assist wildlife scientists addressing a wide variety of problems, eventually, helping in the conservation of wildlife species by their proper management.