GENETIC DIVERSITY IN SUSTAINABLE FISHERIES MANAGEMENT

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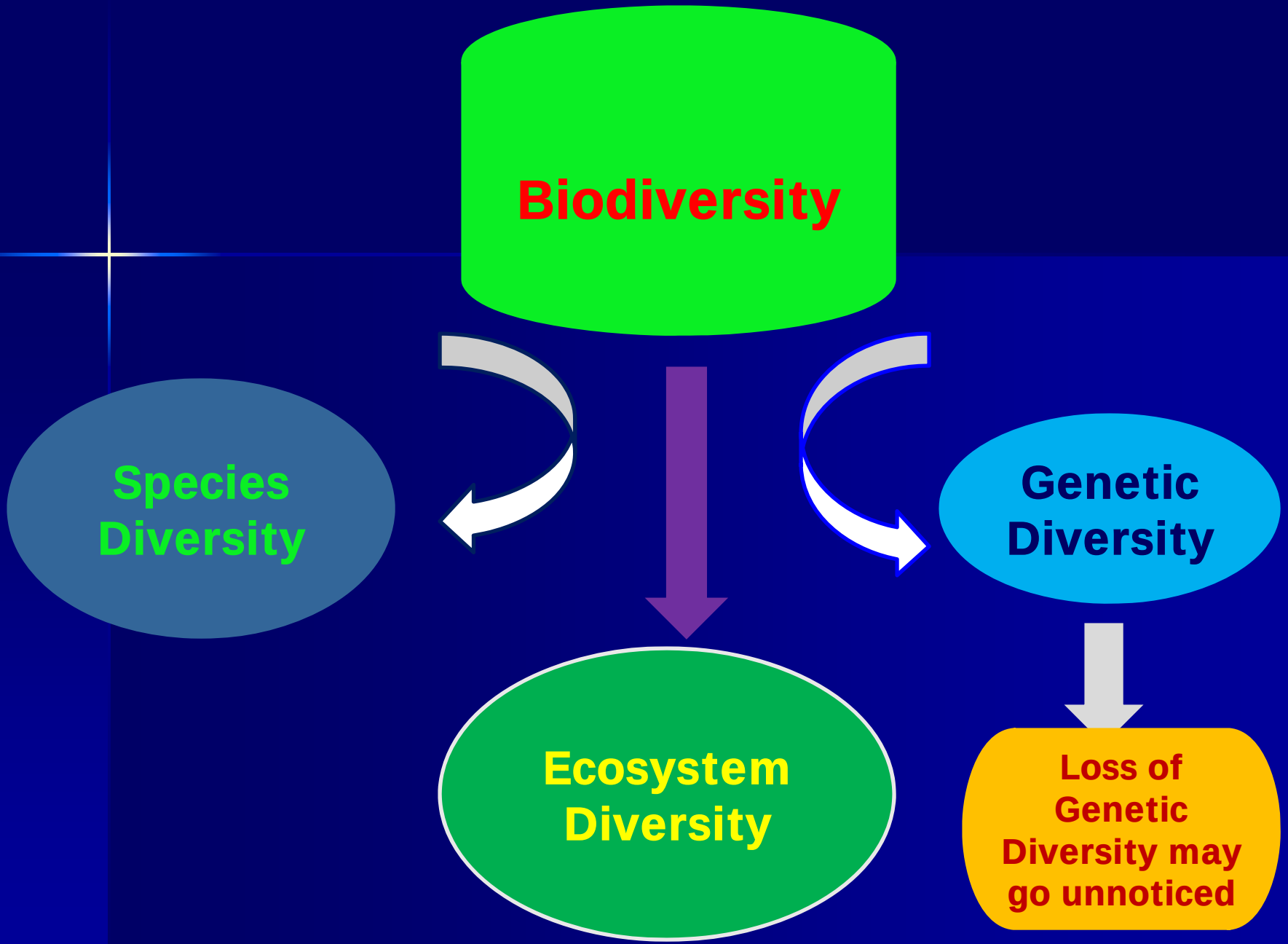

Biodiversity
Species Diversity
Genetic Diversity
Ecosystem Diversity
Loss of Genetic Diversity may go unnoticed

Genetic Diversity?

- Heritable variations observed in the individuals of one or more population of a species.

Genetic Diversity?

Genetic Diversity Manifested as:

- Change in DNA Sequence
- Protein Variations
- Morphological Changes

Why do we do research into Genetics Diversity?

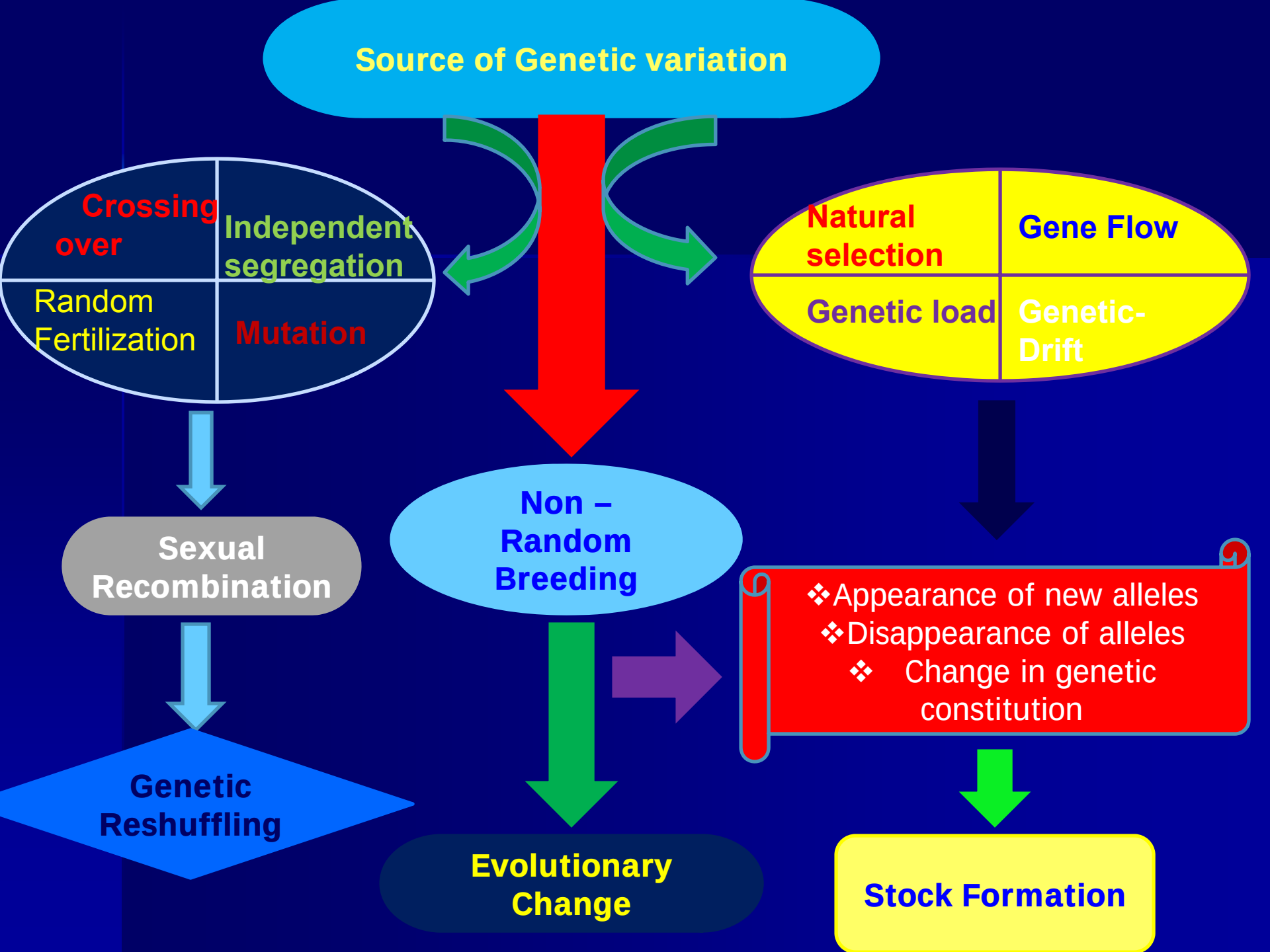
Aqua's research into Genetics Diversity aims to gain knowledge on :

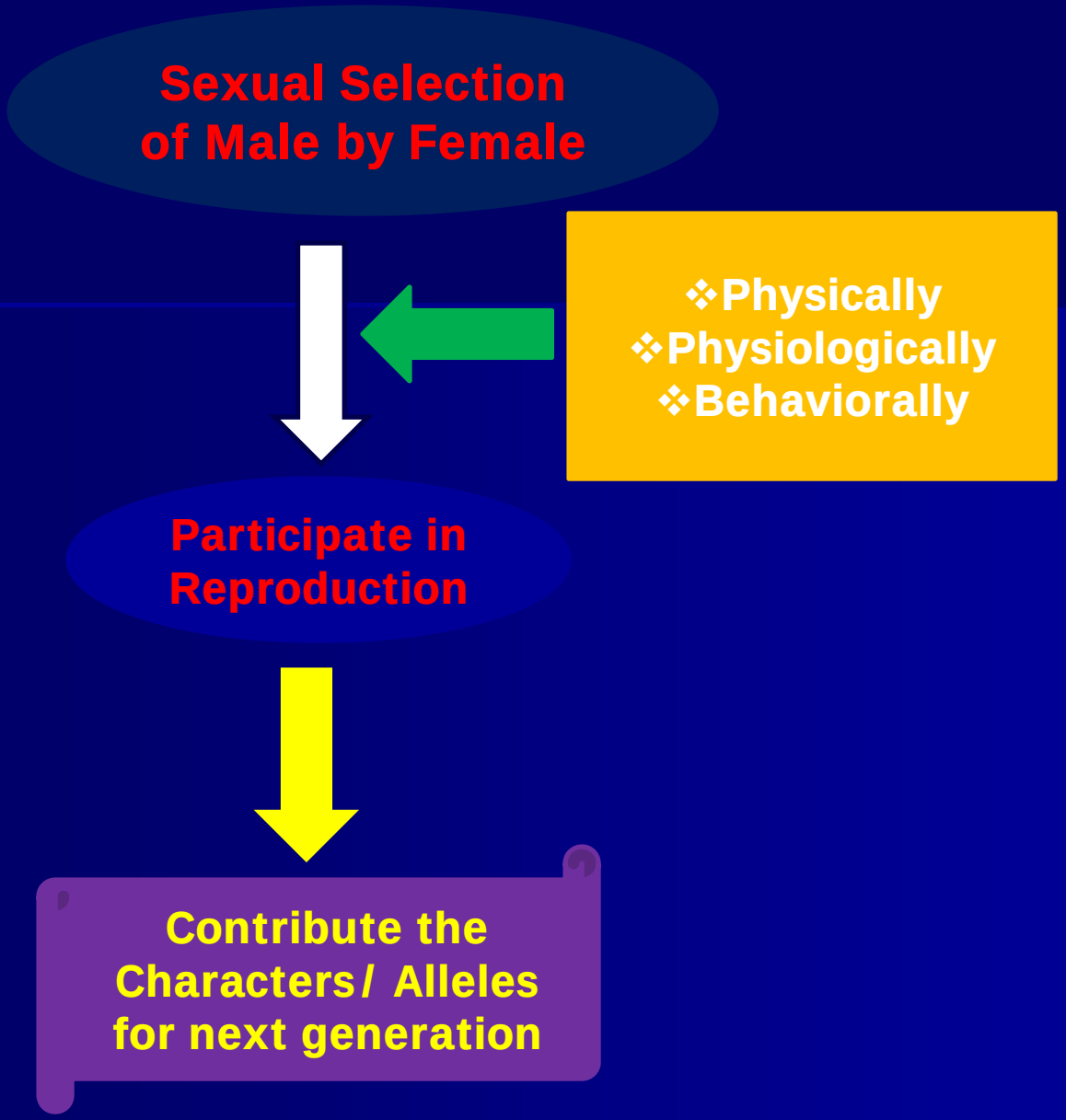
- how to preserve and manage biodiversity in a sustainable manner
- evolutionary processes, which are responsible for generating and maintaining genetic diversity within and among populations of marine and freshwater fishes.

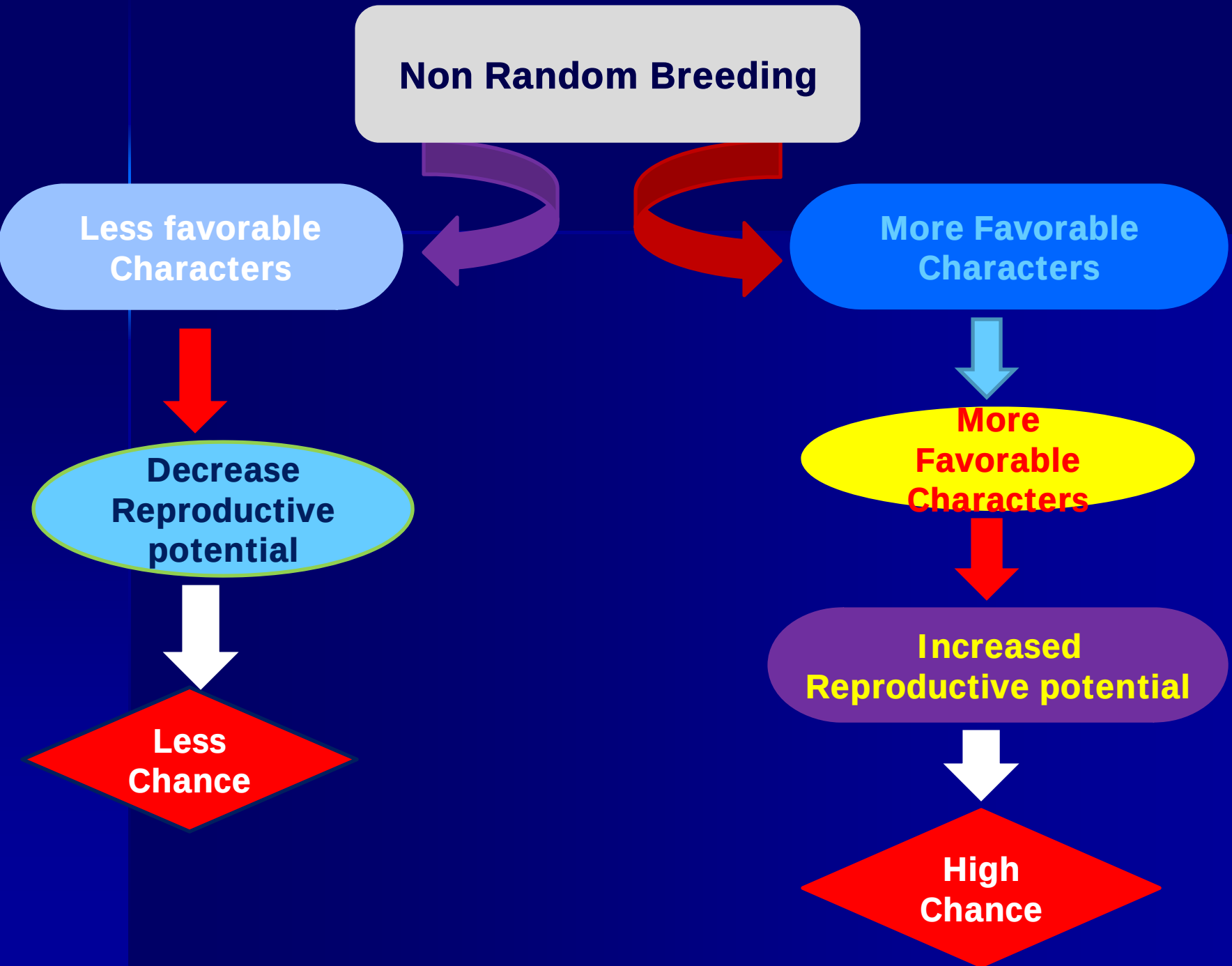
Why do we do research into Genetics Diversity?

Research into Genetics Diversity has five main themes:

- **Stock identification**
- **Genetic traceability and monitoring**
- **Local adaptation**
- **Genetic impact of climate change**
- **Genetic impacts of fisheries**

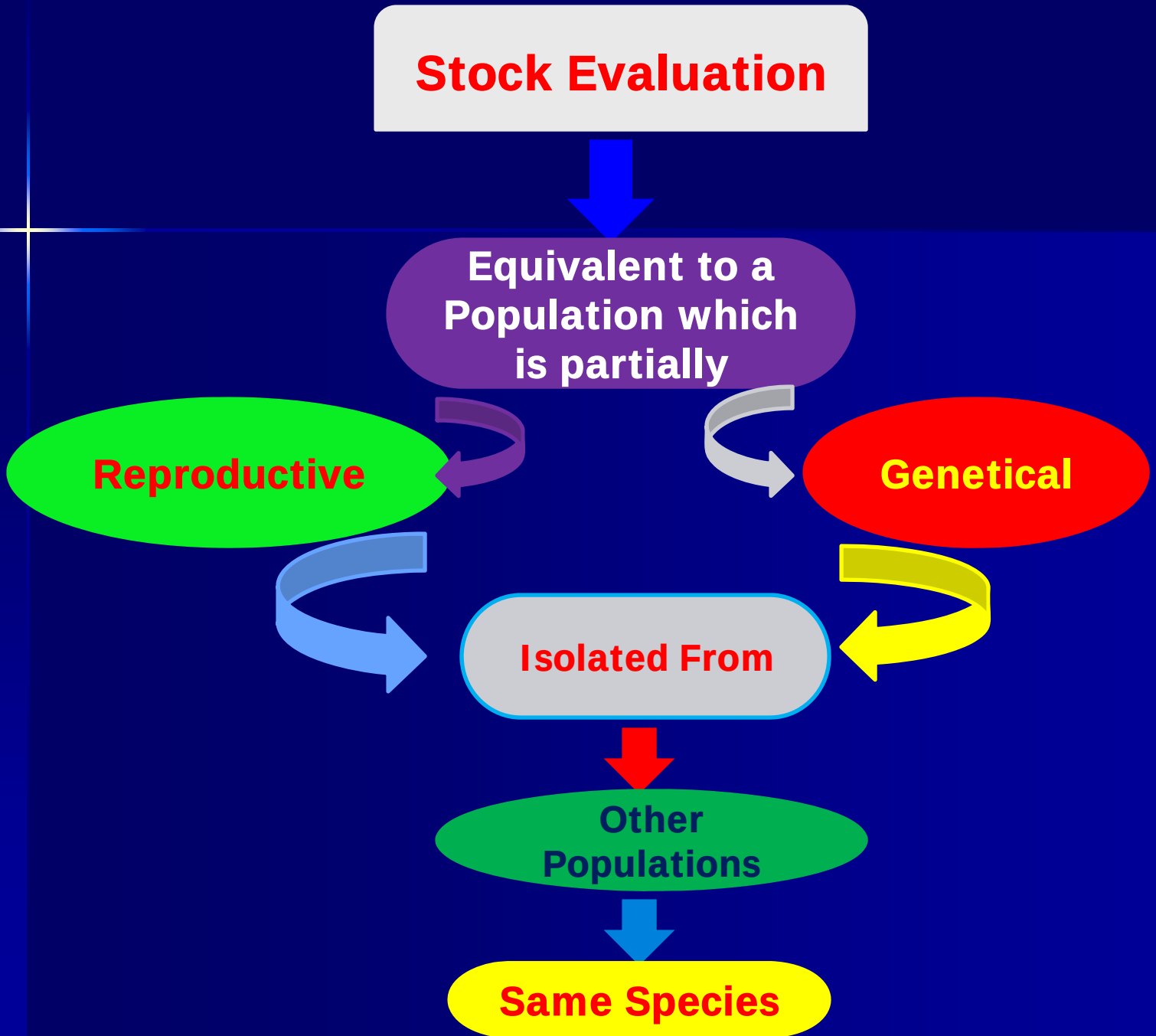

Source of Genetic variation
Crossing over
Independent segregation
Random Fertilization
Mutation
Natural selection
Gene Flow
Genetic load
Genetic-Drift
Sexual Recombination
Non – Random Breeding
❖Appearance of new alleles
❖Disappearance of alleles
❖ Change in genetic constitution
Genetic Reshuffling
Evolutionary Change
Stock Formation

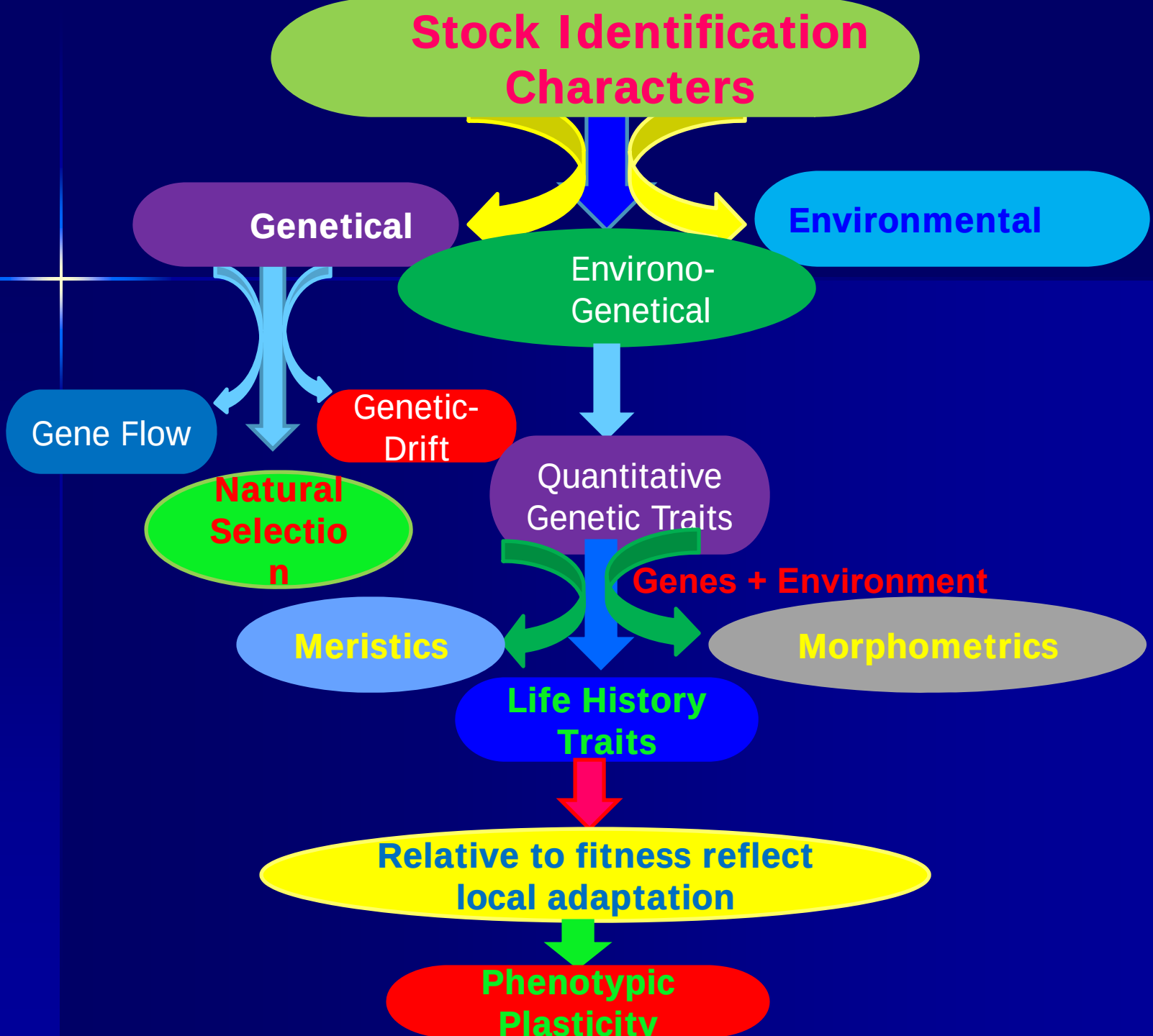

Sexual Selection
of Male by Female
❖Physically
❖Physiologically
❖Behaviorally
Participate in
Reproduction
Contribute the
Characters/ Alleles
for next generation


Non Random Breeding
Less favorable Characters
More Favorable Characters
Decrease Reproductive potential
More Favorable Characters
Less Chance
Increased Reproductive potential
High Chance

STOCK STRUCTURING?

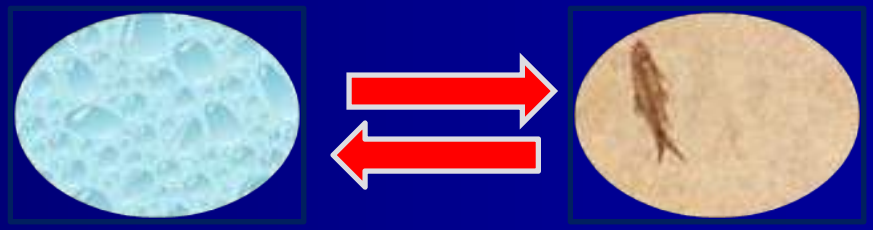
- Fishery Stock ?
- Phenotypic Stock ?
- Genetic Stock ?


Stock Evaluation
Equivalent to a Population which is partially
Reproductive
Genetical
Isolated From
Other Populations
Same Species

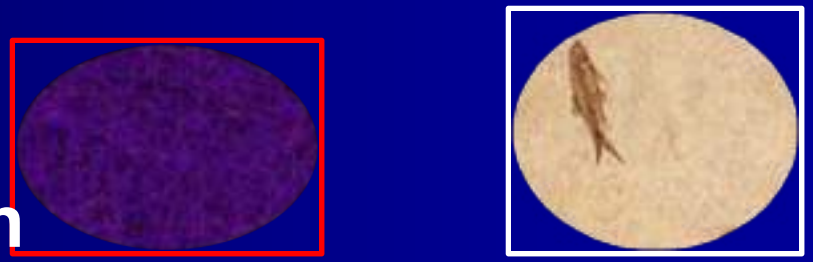

Stock Identification Characters
Genetical
Environmental
Environo-Genetical
Gene Flow
Genetic-Drift
Natural Selection
Quantitative Genetic Traits
Genes + Environment
Meristics
Morphometrics
Life History Traits
Relative to fitness reflect local adaptation
Phenotypic Plasticity

Panmixia

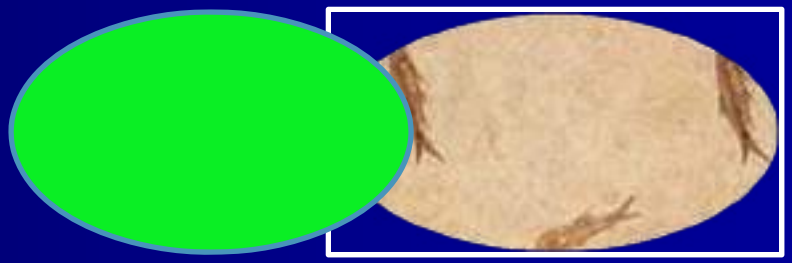
Weak Differentiation with Partial Isolation



Strong Differentiation with Complete Isolation

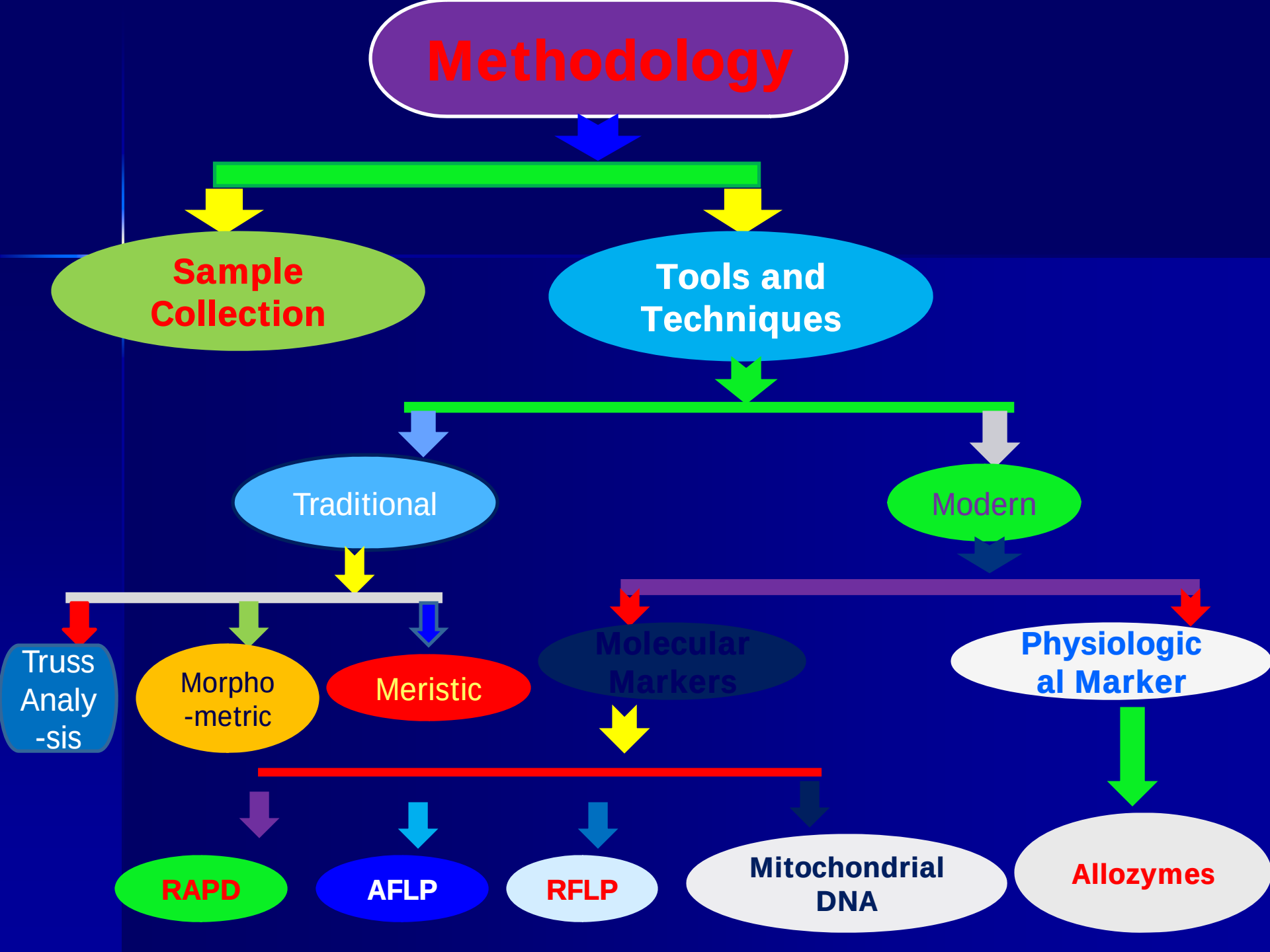


Geographic Reassociation after Isolation



- **Segregation and Characterization of various Stocks.**
- **Study of Growth Potential of Different Stocks.**
- **Use of Fast Growing Stock for the Cultivation/Stock Improvement Programme.**
- **Diversity is the cumulative effect of environment, selection and genetics of an individuals ontogeny.**
- **This cumulative interaction produces phenotypic differences within a species.**

- Altogether 28,500 fish species have been so far recorded from all over the world.
- Out of these, 22 hundred fish species are known to occur in different aquatic habitats of India.
- and out of this 79 species have been enlisted under different categories of threatened status.
- Conservation of fish genetic resources is basically important in protection of aquatic biodiversity.
- Preservation of aquatic diversity is imperative for sustainable management of fisheries resources.
- Fishes are considered to be important bio-indicators which reflect the wide range of environmental


Methodology
Sample Collection
Tools and Techniques
Traditional
Modern
Truss Analy -sis
Morpho -metric
Meristic
Molecular Markers
Physiologic al Marker
RAPD
AFLP
RFLP
Mitochondrial DNA
Allozymes

❖Traditional Techniques

Morphometrics:

- Now a days Morphometric analysis provides a powerful complement to genetic and environmental stock identification approaches.

Meristic Traits

- Meristic traits are the countable parameters.

Natural Markers

- Natural markers such as otolith etc.

Objective: Studies on Genetic Divergence

Segregation of Stocks

Morphometric and Meristic Studies

Study of a set of morphometric and meristic traits and computation of their ratios

Model species

Colisa lalia

- Inland water fish, distributed throughout India
- Seasonal breeder, breed during Monsoon
- Show intraspecific variations and DNA Polymorphism

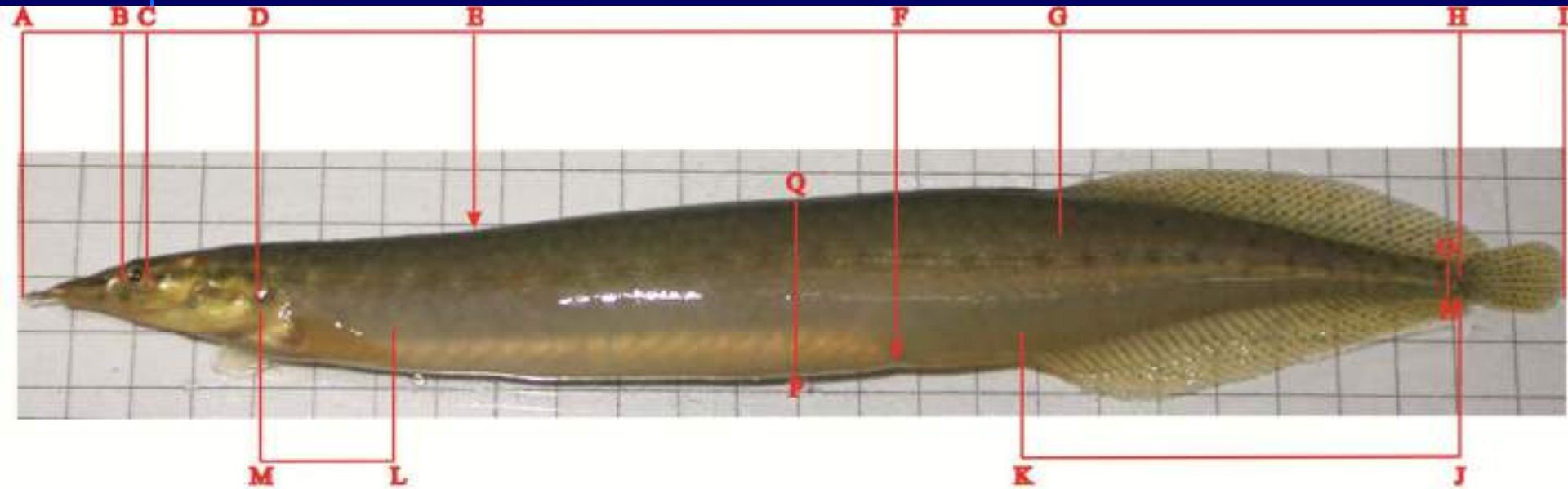
Model species

Macrognathus pancalus

Macrognathus aculeatus

- Inland water fish, distributed throughout India
- Seasonal breeders; breed during Monsoon
- Showed intraspecific variations and DNA Polymorphism

Various Morphometric Measurement

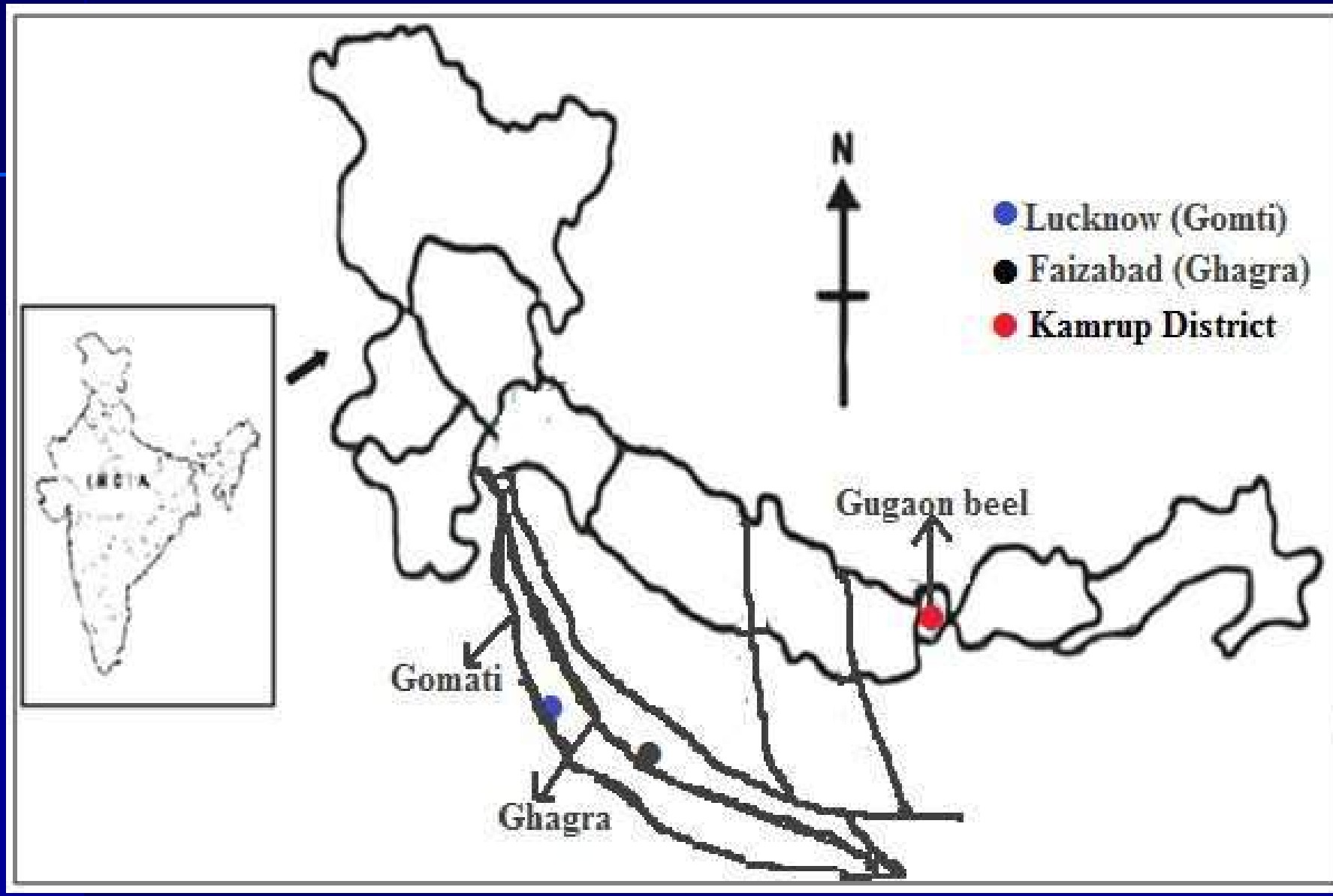


AB- Snout Length
BC- Eye Diameter
CD- Post Oribtal Length
AD- Head Length
AE- Predorsal Length

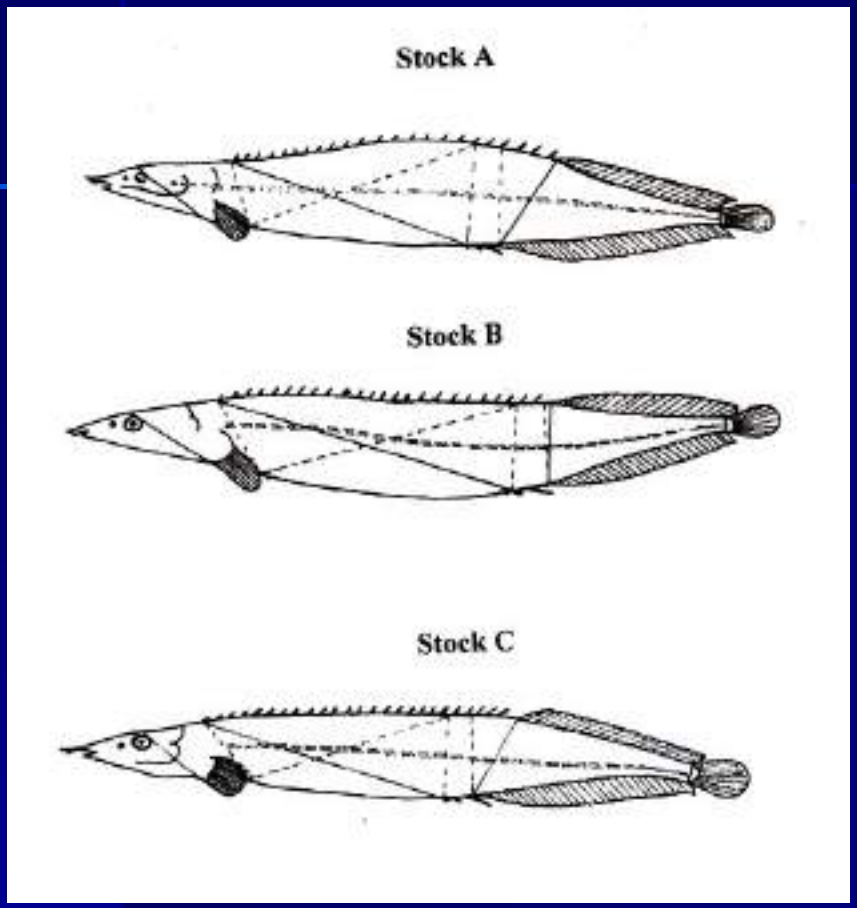
GH- post dorsal length
AF- Preanal Length
AH- Standard Length
AI- Total Length
HI- Length of Caudal Fin

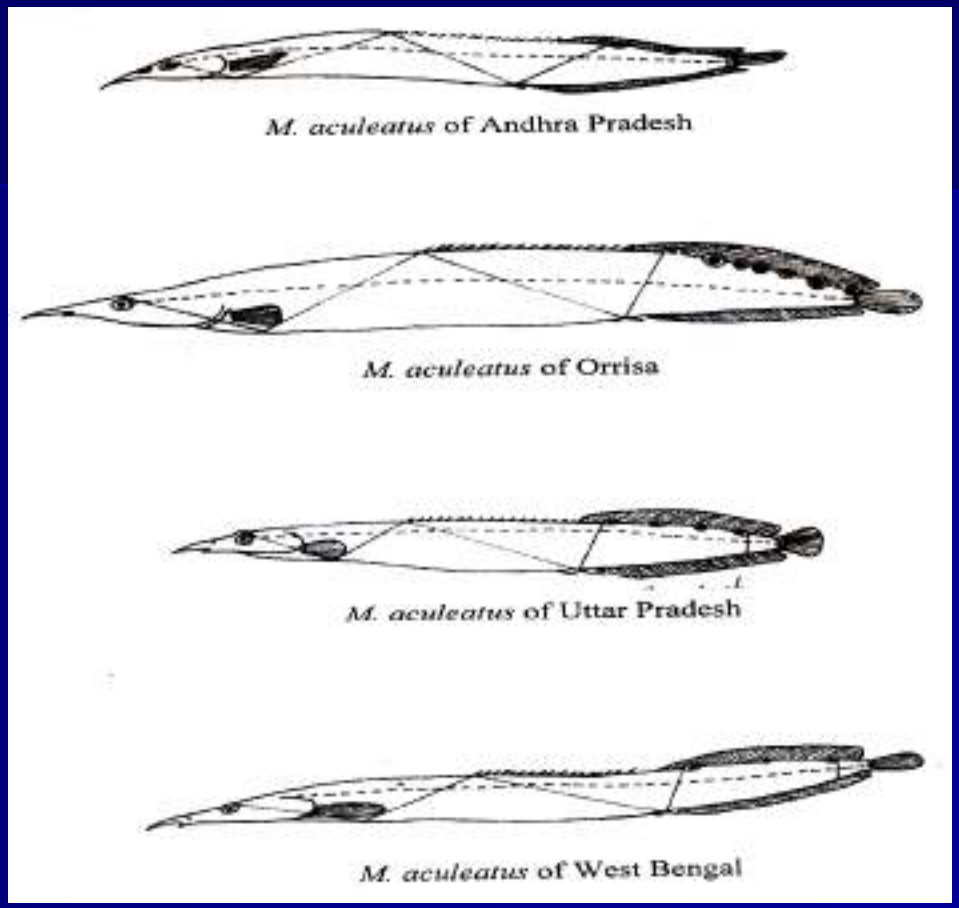
LM-Length of Pectoral Fin
NO-Depth of Caudal Peduncle
PQ- Body Depth
JK- Length of Anal Fin

Study Areas and Sampling Stations



Morphometric and meristic variations in spiny eel

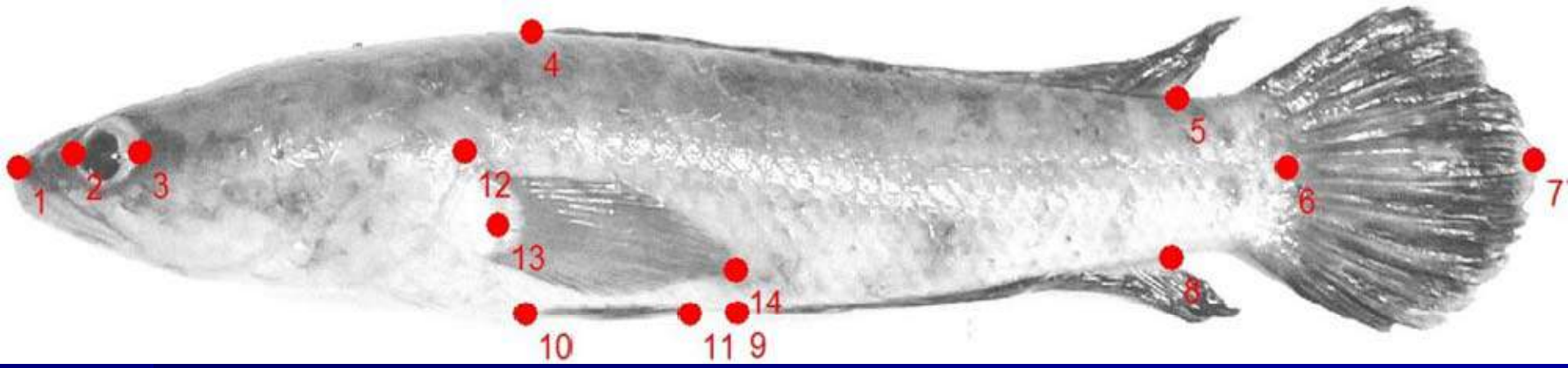




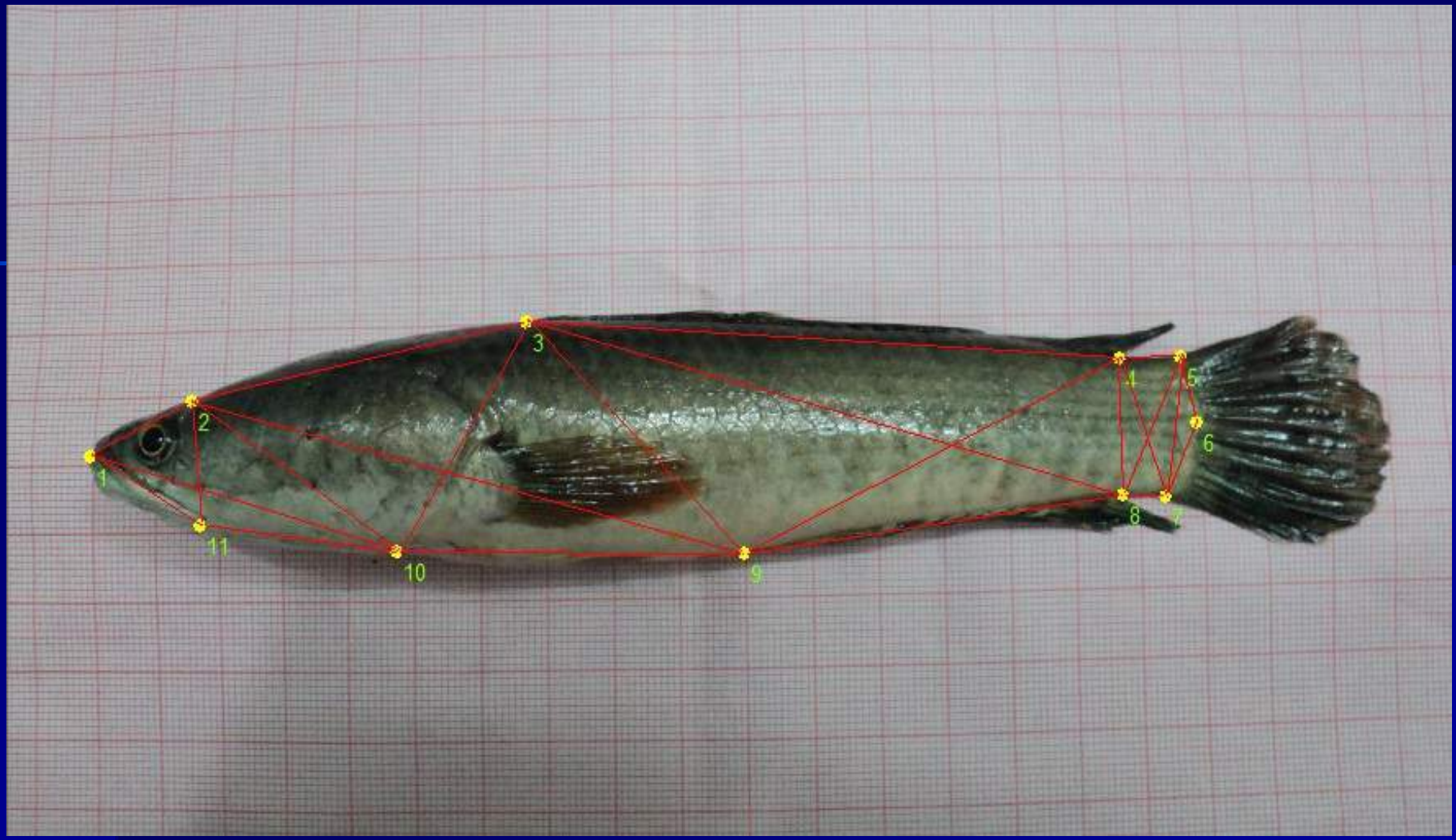
Macrognathus pancalus

Macrognathus aculeatus

MORPHOMETRIC MEASUREMENTS

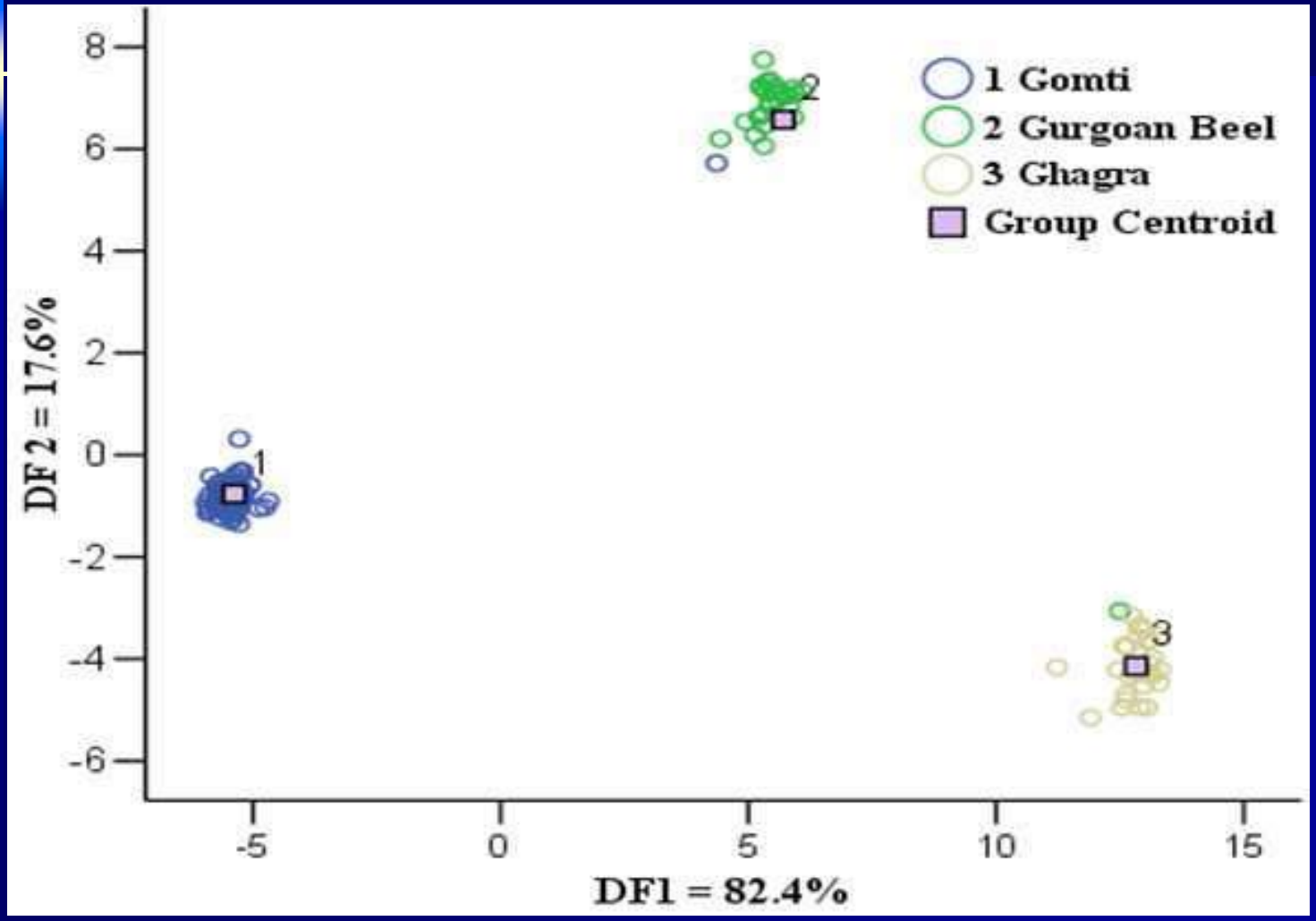


Measurements: total length (1–7), standard length (1–6), head length (1–12), snout length (1–2), pre dorsal length (1–4), pre pectoral length (1–13), pre pelvic length (1–10), pre anal length (1–9), dorsal fin length (4–5), pectoral fin length (13–14), pelvic fin length (10–11), anal fin length (8–9), caudal fin length (6–7), eye diameter (2–3)

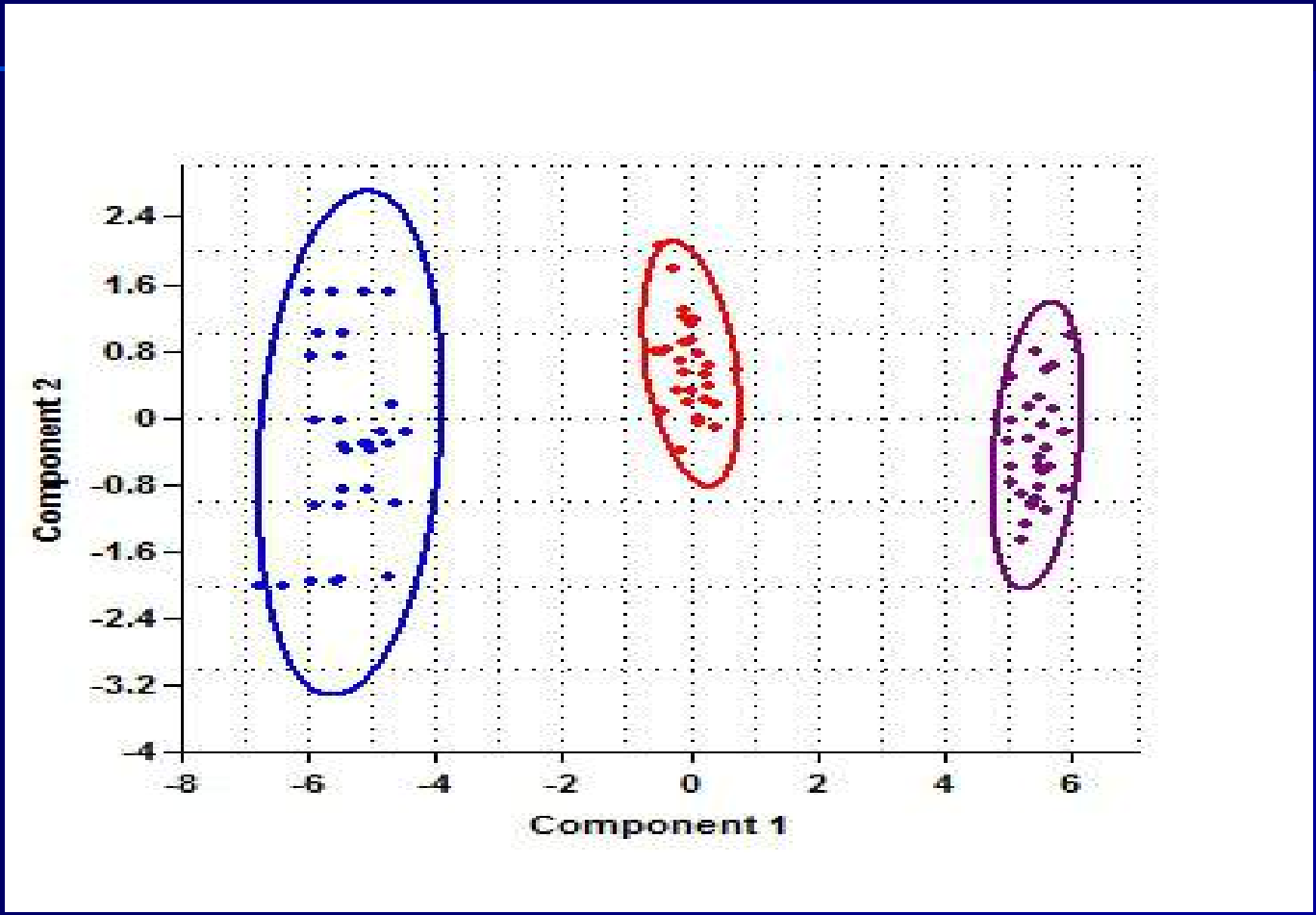


Photograph of *C. punctatus* digitized with Eleven Morphometric Landmarks for Truss Analysis.

Discriminant Function Plot of the Group Centroid for the Three Different Populations of Barred Spiny Eel, *M. pancalus*



All the 23 truss measurements were found to be highly significant ($p<0.001$) in one way ANOVA



Scatter plot of PC 1 on PC 2 showing 95% confidence ellipses of three populations of *C. punctatus*

Objective: Studies on Genetic Divergence

Variations at molecular level

Bar Coding/RFLP/RAPD

Isolation of Genomic DNA

DNA amplification with short oligonucleotide primers using PCR reaction.

Separation of amplified products using gel electrophoresis

Random Amplified Polymorphic DNA

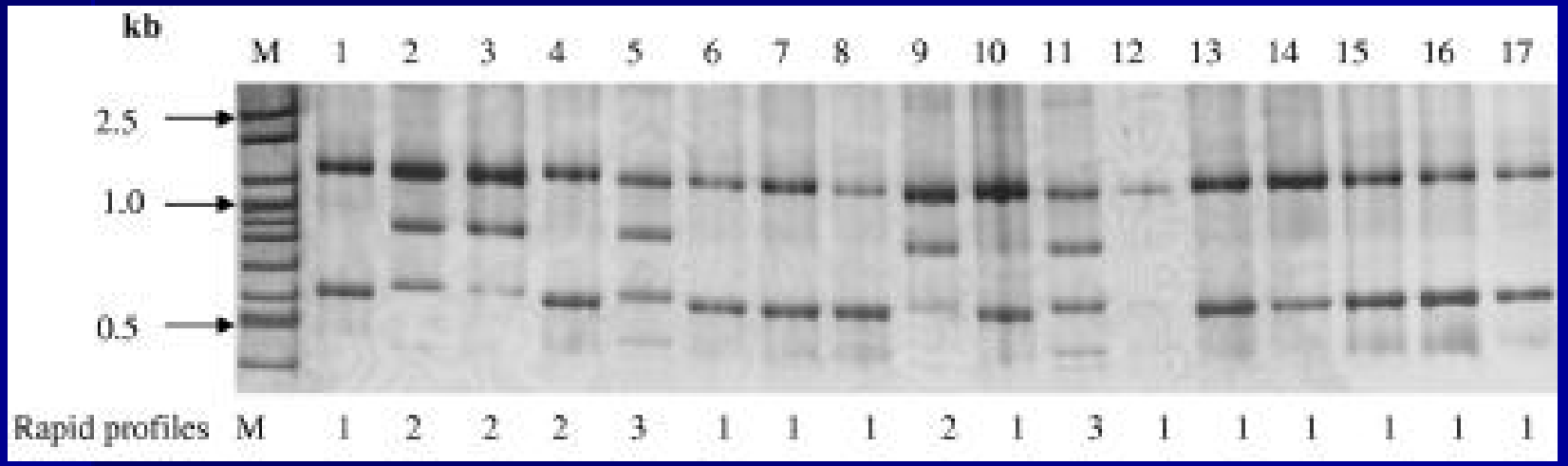
- Random primers were used
- The technique works with minute quantity of DNA .
- DNA polymorphism .
- The Random Amplified Polymorphic DNA (RAPD) has been successfully applied in taxonomic studies and for the stocks discrimination of marine and freshwater fishes.

Primer sequences and GC content percentage

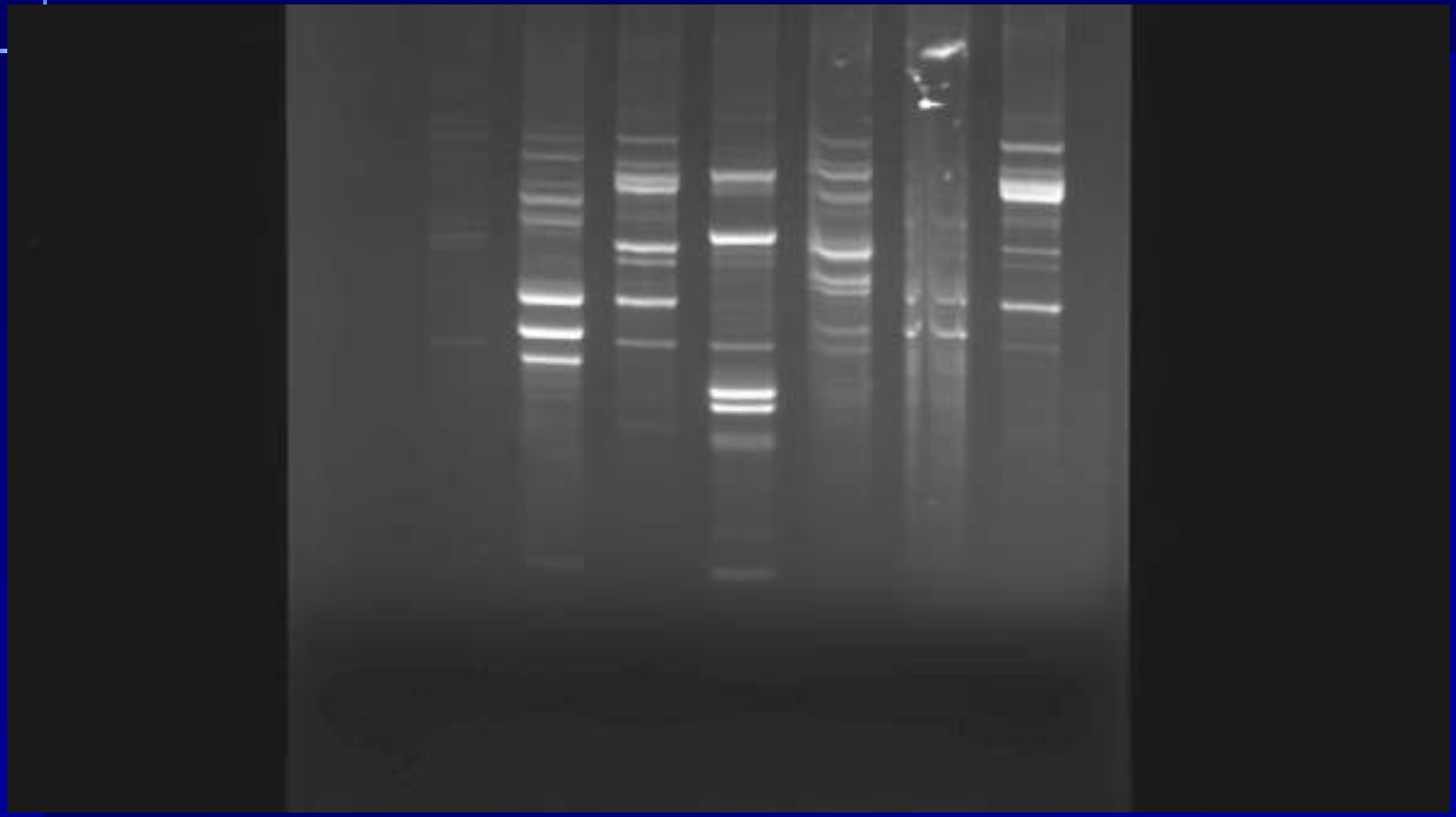
S.N.	Primer no	Primer Sequence	GC %
1	OPB-3	5’CATCCCCCT 3’	70
2	OPB-5	5’TGCGCCCTTC3’	70
3	OPB-7	5’GGTGACGCAG3’	70
4	OPB-8	5’GTCCACACGG3’	70
5	OPB-14	5’TCCGCTCTGG3’	70
6	OPB-15	5’GGAGGGTGTT3’	60

RAPD

Silver-stained polyacrylamide gel showing three distinct RAPD profiles generated by primer OPE15 for *Haemophilus ducreyi* isolates from Tanzania, Senegal, Thailand, Europe, and North America



Random amplified polymorphic DNA fragments pattern generated using OPB-1 primer (A) M3-M9 and (B) 10-16 are samples of M. *pancalus* from river Gomti



Random amplified polymorphic DNA fragment pattern generated using OPB-1primer. (A:1-10 and B: 11- 20 are samples of *M .pancalus* from river Ghaghara)

Monomorphic, Polymorphic and Unique Bands in the populations of *Colisa lalia* collected from Lucknow and Barabanki

Lucknow population						Barabanki population				
Primer No.	**M Bands**	**P Bands**	**U Bands**	**Total**	**P %**	**M Bands**	**P Bands**	**U Bands**	**Total**	**P %**
3	**12**	**13**	**1**	**26**	**50**	**12**	**28**	**0**	**40**	**70**
5	18	18	0	36	50	12	48	0	60	80
7	22	31	1	54	57.4	36	30	0	66	45.45
8	20	39	0	59	66.1	12	21	0	33	63.63
14	12	18	0	30	60	22	44	1	67	65.67
15	24	56	0	80	74.7	12	54	2	68	79.41
Total	108	175	2	285		106	225	3	334	

Patterns of polymorphism and monomorphism in *M.pancalus* (n=33) of rivers Gomti and Ghaghra of Gangetic basin.

S.No.	Polymorphism	Gomti		Ghaghra		
		Primer OPB-1	Primer OPB-3	Primer OPB-1	Primer OPB-3	Total Number of Bands
1.	Total no. of bands	67	95	102	102	366
2.	Total no. of polymorphic bands	09	32	21	09	71
3.	Total no. of monomorphic bands	58	63	81	93	295
4.	Polymorphism%	13.43	33.68	20.59	8.82	19.40
5.	Monomorphism%	86.57	66.32	79.41	91.18	80.60

Restriction Fragment length Polymorphism (RFLP)

- Restriction fragment length polymorphism, or RFLP (commonly pronounced “rif-lip”), is a technique that exploits variations in homologous DNA sequences.
- It refers to a difference between samples of homologous DNA molecules that come from differing locations of restriction enzymes.

Amplified Fragment Length Polymorphism (AFLP)

- It is the combination RFLP and RAPD.
- The technique is used for many types of genetic analysis such as molecular systematic, strain identification, genetic diversity, hybrid identification etc.
- Chong *et.al.* (2000) used AFLP for the analysis of five geographic populations of Malaysian catfish, *Mystus nemurus*.
- AFLP analysis were also used in the justification of sympatric speciation in cichlid fish, *Amphilophus sp* (M.Barluenga et al 2006 Nature).

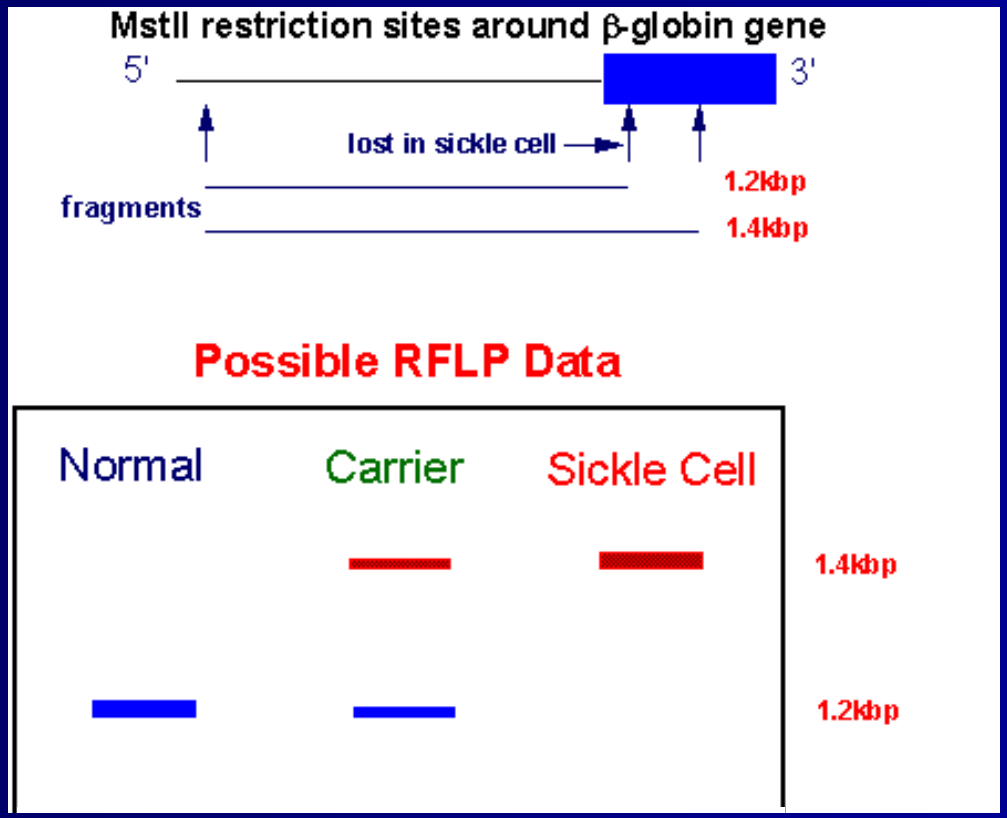
Restriction Fragment Length Polymorphisms (RFLPs)

- Consider two alleles having slightly different sequences

GAATTC GCATTC

CTTAAG CGTAAG

*Eco*RI will cut the first but not the second

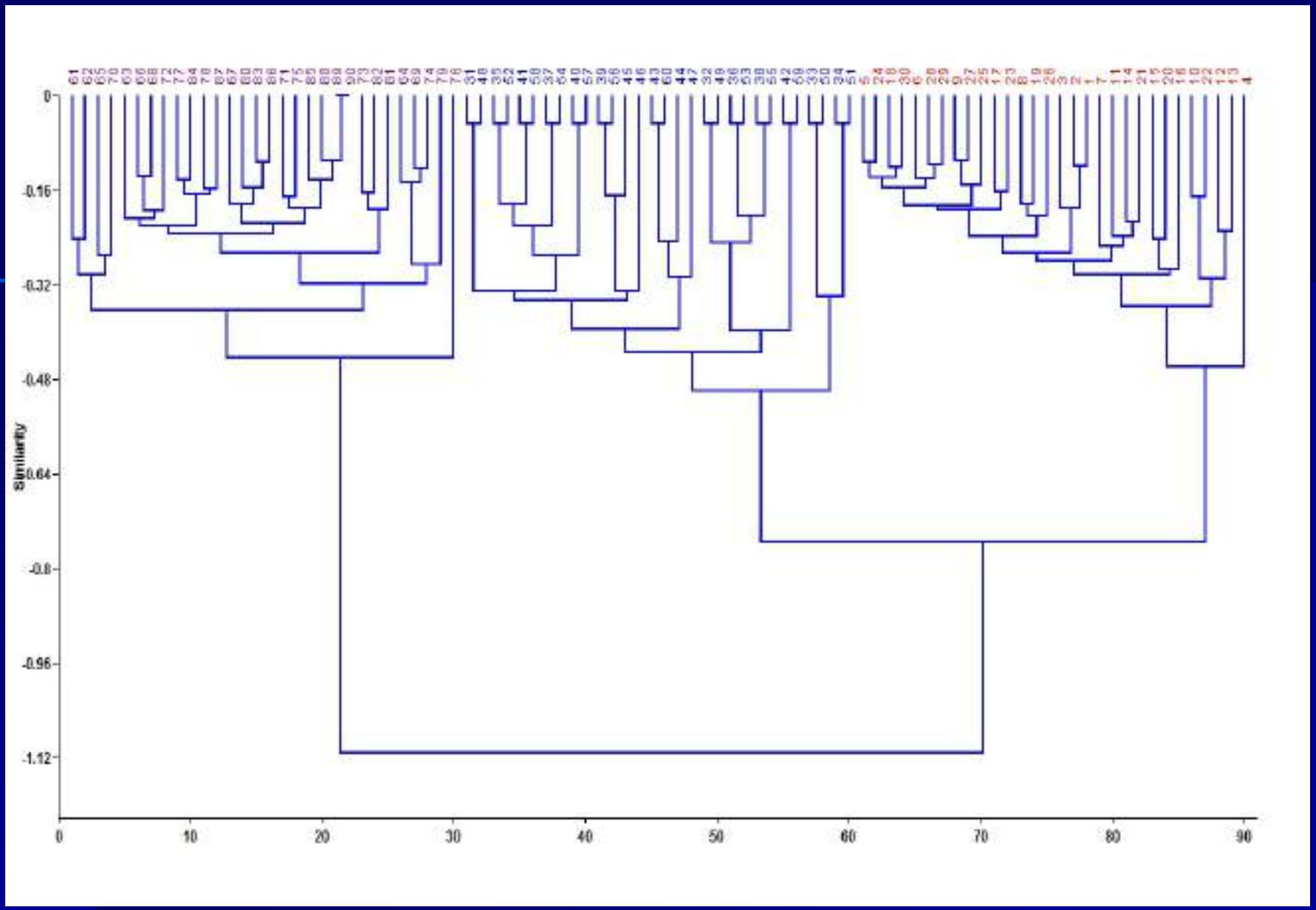


Mitochondrial DNA

- The mt DNA contains 13 genes and16,000 base pairs, which is maternally inherited.
- The variability in the size of mt DNA occurs within the species of fish, and is considered to be very effective molecule that can be used in order to find out the intraspecific variations in fishes.
- Now a days mitochondrial DNA is used in order to find out the difference at genetic levels.
- A mitochondrial mismatch analysis also indicates about the demographic expansion as reported in cichlid fish ,*Amphilophus citrinellus* (M.Barluenga et al 2006 Nature).

Allozymes

- Variant forms of an enzymes that are coded by different alleles at the same locus are called allozymes.
- Allozymes were also used in order to find out the spatial distribution of the genetic diversity in *Phaseolus lunatus* (Arsène Irié Zoro Bi et al 2007*Biotechnol. Agron. Soc. Environ).*
- The technique is used for many types of genetic analysis such as molecular systematic, strain identification, genetic diversity, hybrid identification.



Dendogram based on Cluster Analysis of all samples of the three populations of *C. punctatus*

Conclusion

- Conservation of genetic diversity of fishes is fundamentally important in protection of aquatic biodiversity.

- Preservation of aquatic diversity is imperative for sustainable management of fisheries resources.

- Sustainability is essential for security of food.

RESEARCH TEAM

BIPIN PATHAK

JYOTI VERMA

MADHU AWASTHI

ANKUR KASHYAP

QULSOOM NAZ

MINAKSHI SINGH

MOHD WASEEM

PRAGYA GUPTA

TARIQ MEHMOOD

SAIMA AMJAD

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Thank you