

About OMICS Group

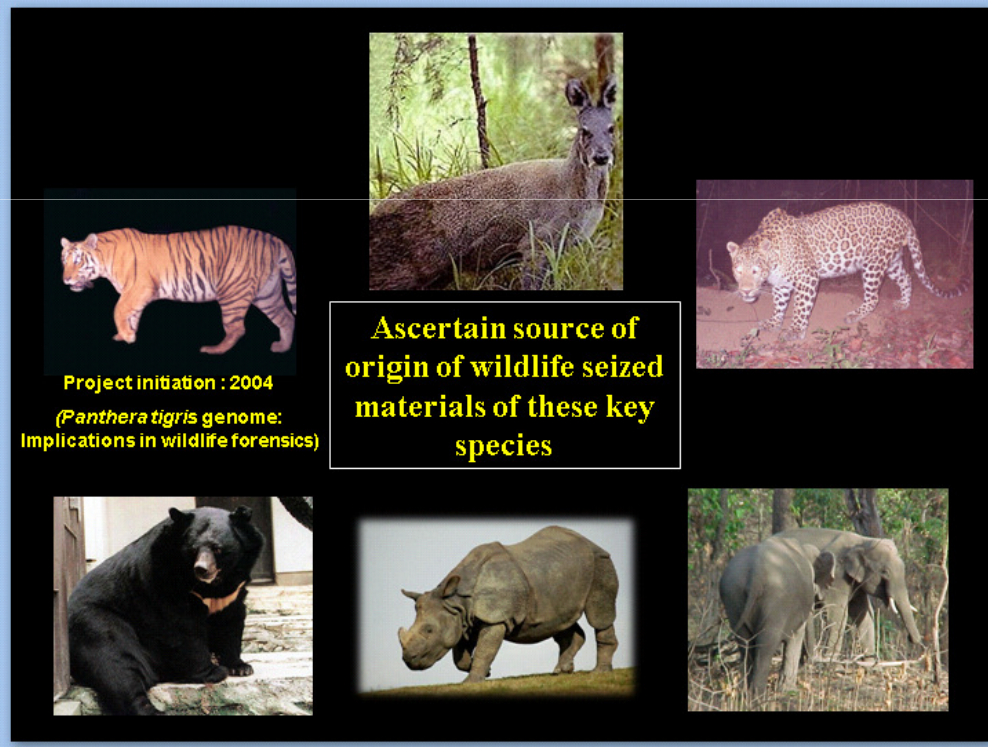
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Genetic structure of populations and its significance in forensics



Project initiation : 2004
(*Panthera tigris* genome: Implications in wildlife forensics)

Ascertain source of origin of wildlife seized materials of these key species

Genetic structure of tiger populations in India and its significance in forensics



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Tiger distribution



Habitat degradation

Poaching of tiger for its parts products

Anthropogenic factors

Developmental projects

Most of the tiger populations are in small size

One of the largest seizures in India



Why this study....?



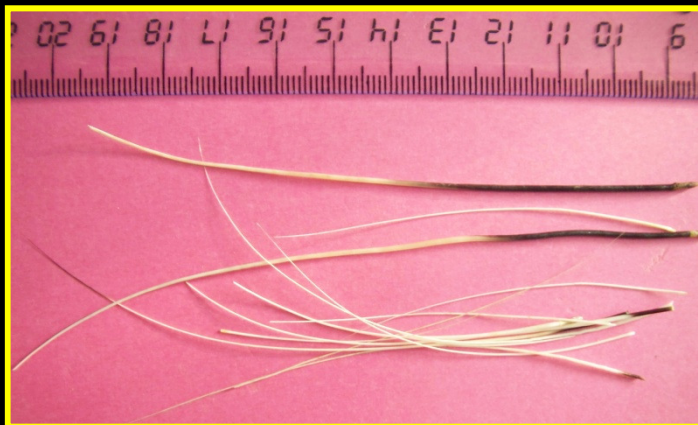
Claws



Bones



Whiskers

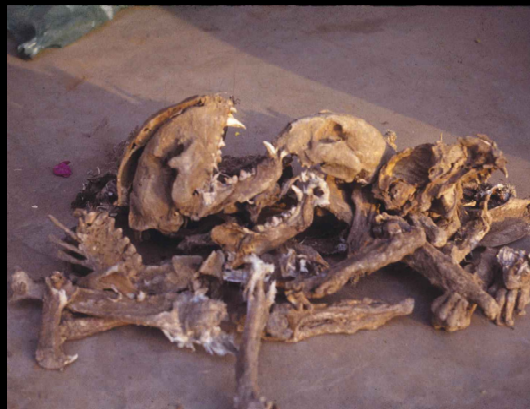


Canines

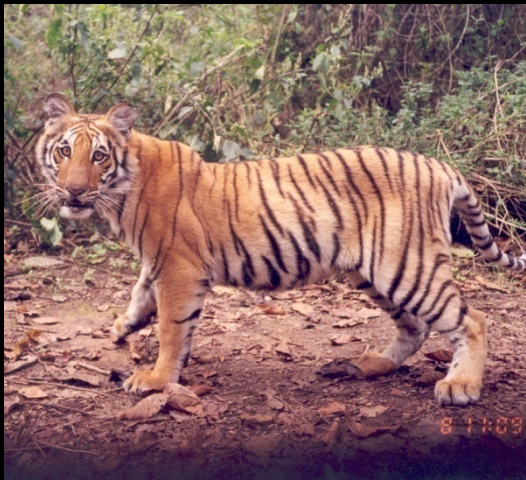


Why this study?

- Of different threats to tiger conservation, poaching is major conservation threats which can lead to local extinction as well as decline in tiger populations
- To strengthen scientific based management to minimize poaching of species, a genetic based population assignment has been evolved during last decade to determine which population is prone for poaching and to suggest appropriate effective measures for conservation e.g. African elephant, whales, mountain lion etc.

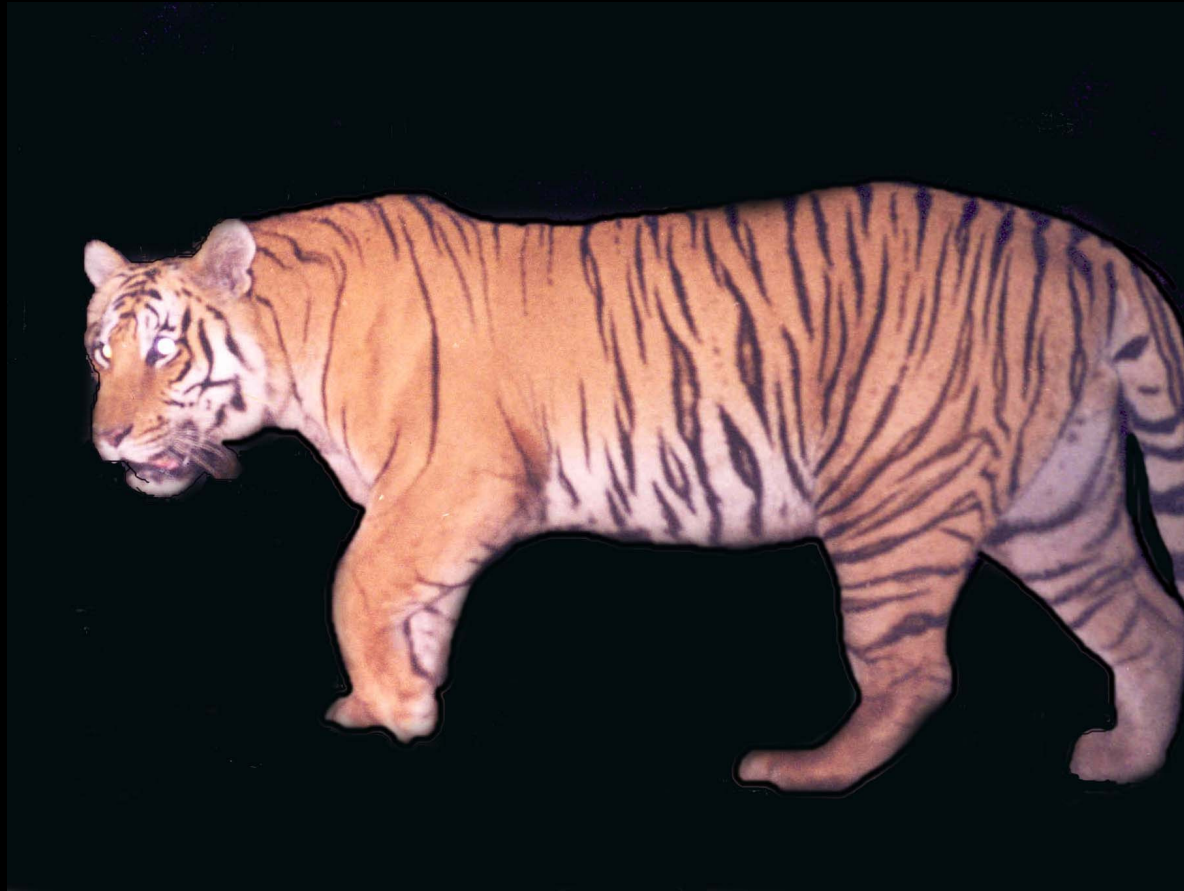


Objectives of the study



1. To develop & establish protocols for identification of tiger from various seizures in the form of skin, claws, whiskers and bones based on DNA techniques
2. To establish noninvasive genotyping of different populations of tigers in India
3. Determine source of origin of various tiger parts and products seized under wildlife offences, and
4. To provide genetic diversity information of different tiger populations in India

Population genetics and poaching prevention of tigers in India



1. Optimizing high throughput for fecal DNA
2. Detecting mitochondrial DNA haplotype in different tiger populations
3. Designing Multiplex PCR panels using highly polymorphic microsatellite loci
4. Determining genetic diversity & structuring in north, western and central India tiger populations of using a panel of highly polymorphic microsatellite loci
5. Assigning tiger seizures to source populations

DNA extraction

We optimized DNA extraction protocols for identification of tiger from various seizures viz. skin, claws, whiskers, bones and scats



Claws

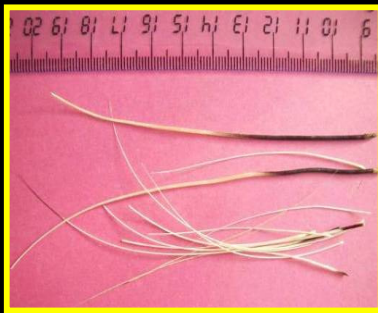


Skins



Bones

Whiskers



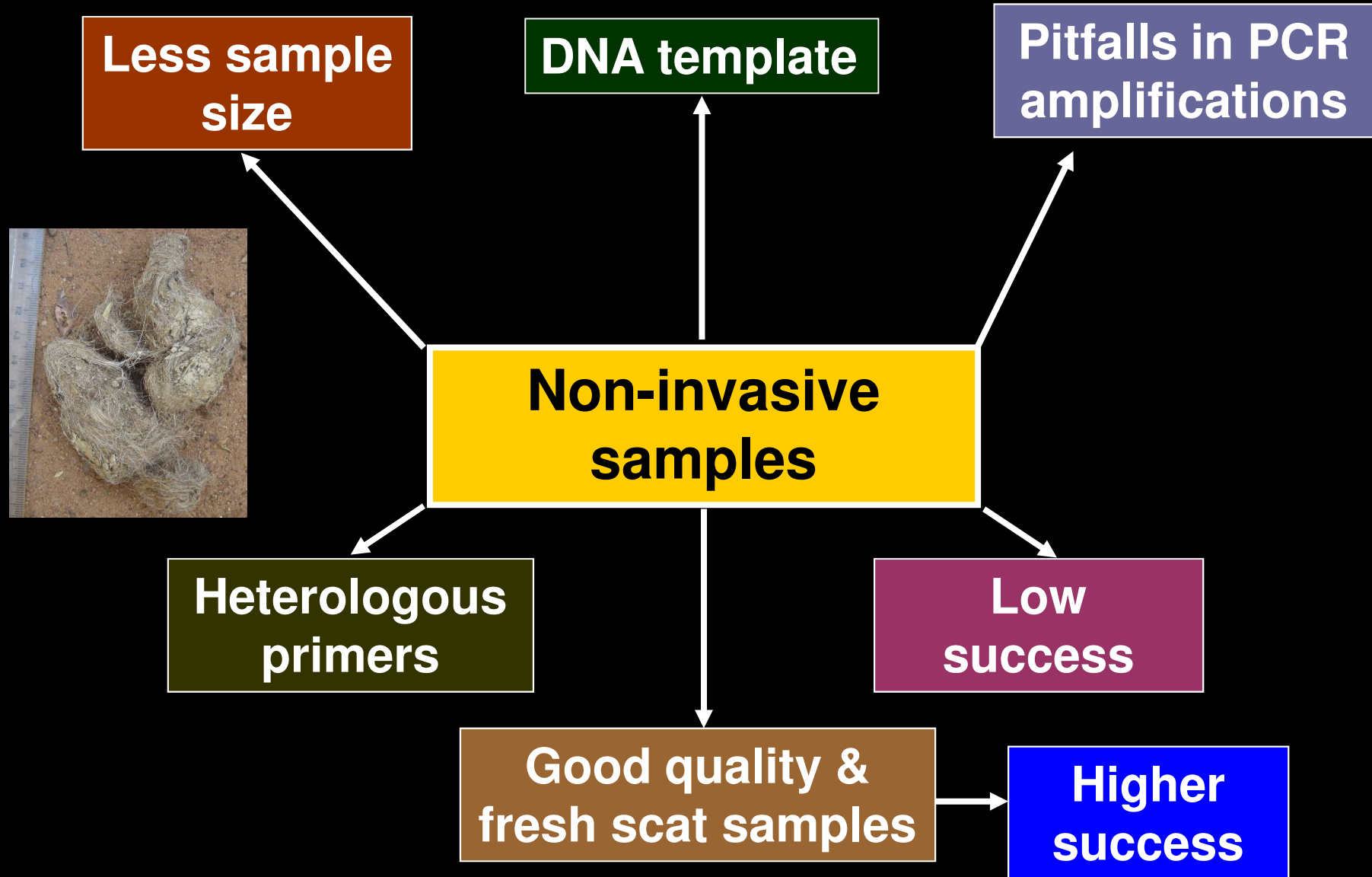
Scat

Canines



Limitations in Molecular Scatology

Molecular Scatology

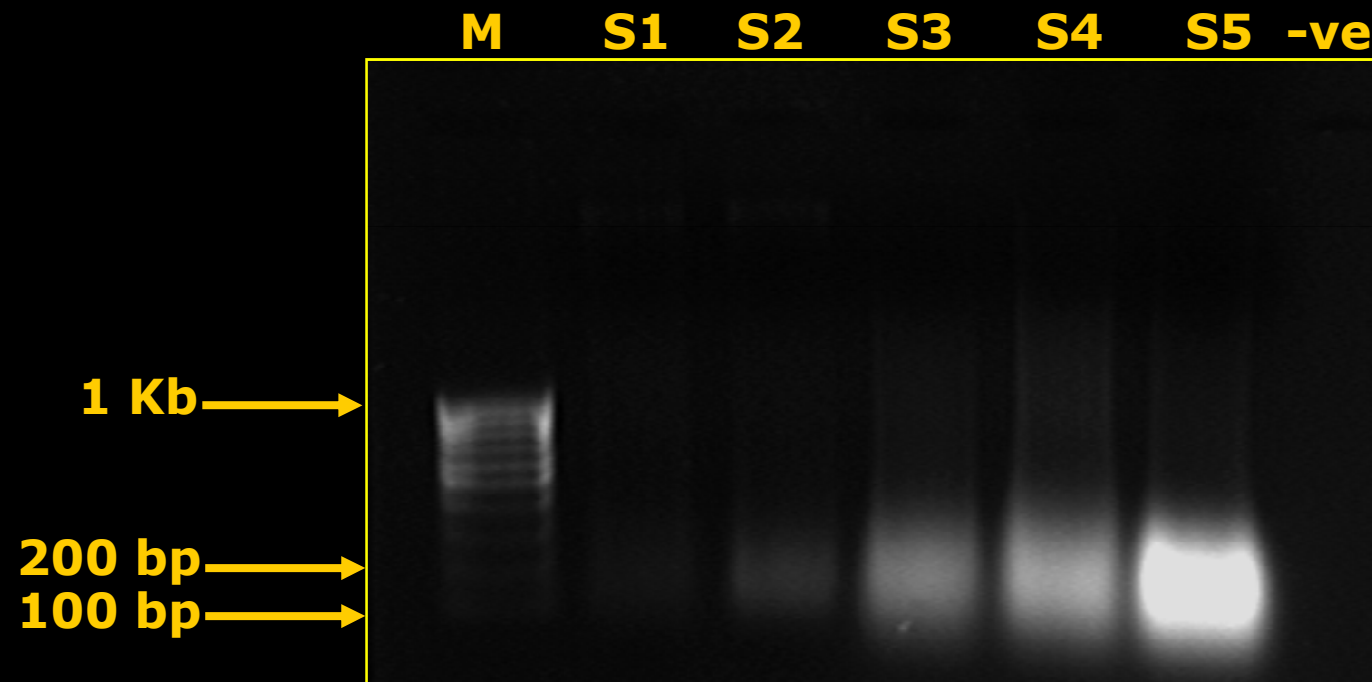


Success of study in using Molecular Scatology

- One of the most important aspect is to optimize protocols to identify species, sex and feasibility of using multilocus genotyping
- Success of any research study using non-invasive genetic samples depends on these optimizations and outputs

DNA extraction

- DNA was <200bp and highly degraded



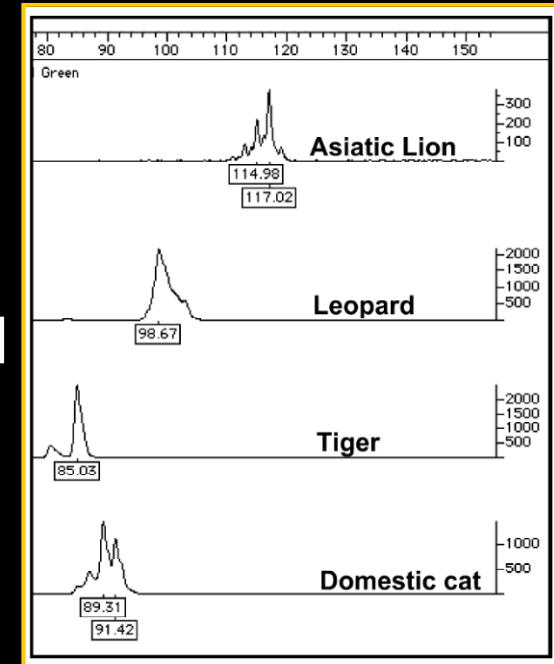
DNA extracted from different scat samples (S1-S5)

Species identification

2 methods were followed

Method 1.

Felid specific microsatellite loci was selected and species was identified on the basis of species specific allelic pattern (Singh *et al.*, 2004)



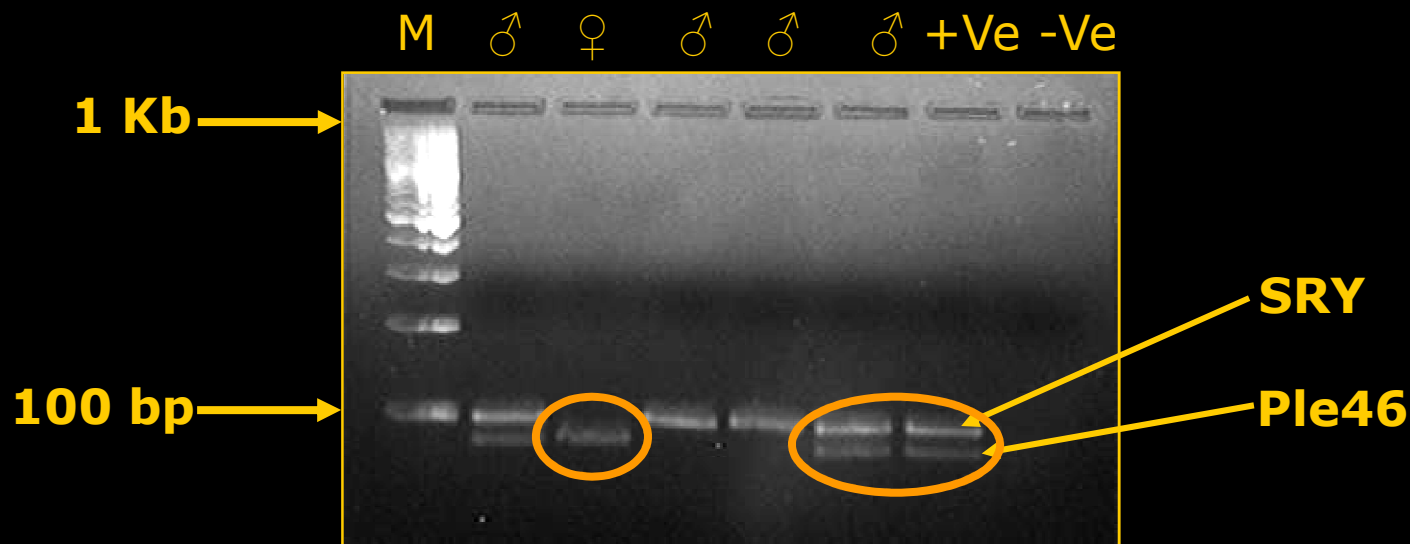
Method 2.

Species specific amplification of partial fragments of cytb gene (210 bp) and control region (250 bp) was amplified as **duplex PCR** from scat DNA. (Mukherjee *et al.*, 2007)



Sex identification

- Gender-identifying sequence tagged sites (STS) from the domestic cat Y chromosome SRY gene (Murphy *et al.*, 2000) was undertaken to identify sex.
- Primers for Ple46 were also taken in the same reaction with SRY primer



Duplex PCR amplification of SRY and Ple46 primers

Population genetic structure and tracking of tiger offences

1. Mitochondrial DNA (mtDNA) genes

2. Microsatellite DNA genotyping



Major classification of tiger populations of India

Based on Cytochrome Gene

✓ 1. gi 5835205 ref NC 001700.1 Felis catus	A	T	T	T	A	C	A	G	T	C	A	T	A	G	C	C	A	C	A
✓ 2. P.t.TIG3 c	G	T	T	T	A	C	G	G	T	C	A	T	G	G	C	T	A	C	A
✓ 3. P.t.TIG2 N	G	T	T	T	A	C	G	G	T	C	A	T	G	G	C	T	A	C	A
✓ 4. STR-1183 CYTB F OBERIEN E11 001 2010-07-	G	T	T	T	A	C	G	G	T	C	A	T	G	G	C	T	A	C	A
✓ 5. STR-159 CYTB R OBERIEN C11 003 2010-07-2	G	T	T	T	A	C	G	G	T	C	A	T	G	G	C	T	A	C	A
✓ 6. STR-160 CYTB R OBERIEN D11 004 2010-07-2	G	T	T	T	A	C	G	G	T	C	A	T	G	G	C	T	A	C	A
✓ 7. STR-108 CYTB F OBERIEN A10 001 2010-07-2	G	T	T	T	A	C	G	G	T	C	A	T	G	G	C	T	A	C	A
✓ 8. STR-158 CYTB F OBERIEN C10 003 2010-07-2	G	T	T	T	A	C	G	G	T	C	A	T	G	G	C	T	A	C	A
✓ 9. CI-TIG10 Sari	G	T	T	T	A	C	A	G	T	C	A	T	G	G	C	T	A	C	A
✓ 10. CI-TIG10Saris	G	T	T	T	A	C	A	G	T	C	A	T	G	G	C	T	A	C	A
✓ 11. CI-TIG10WII2	G	T	T	T	A	C	A	G	T	C	A	T	G	G	C	T	A	C	A
✓ 12. NE-TIG10 Damp	G	T	T	T	A	C	A	G	T	C	A	T	G	G	C	T	A	C	A
✓ 13. CI-TIG10 WII	G	T	T	T	A	C	A	G	T	C	A	T	G	G	C	T	A	C	A
✓ 14. CI-Tig10 RTR	G	T	T	T	A	C	A	G	T	C	A	T	G	G	C	T	A	C	A
✓ 15. CI-Panna 32 c	G	T	T	T	A	C	G	G	T	C	A	T	G	G	C	T	A	C	A
✓ 16. CI-Bandhwagar	G	T	T	T	A	C	G	G	T	C	A	T	G	G	C	T	A	C	A
✓ 17. CI-Khaga No.	G	T	T	T	A	C	G	G	T	C	A	T	G	G	C	T	A	C	A
✓ 18. NI-TIG7 C11 C	G	T	T	T	A	C	G	G	T	T	A	T	G	G	C	T	A	C	A
✓ 19. NI-corbett No	G	T	T	T	A	C	G	G	T	T	A	T	G	G	C	T	A	C	A
✓ 20. NI-RNP2 Conti	G	T	T	T	A	C	G	G	T	T	A	T	G	G	C	T	A	C	A

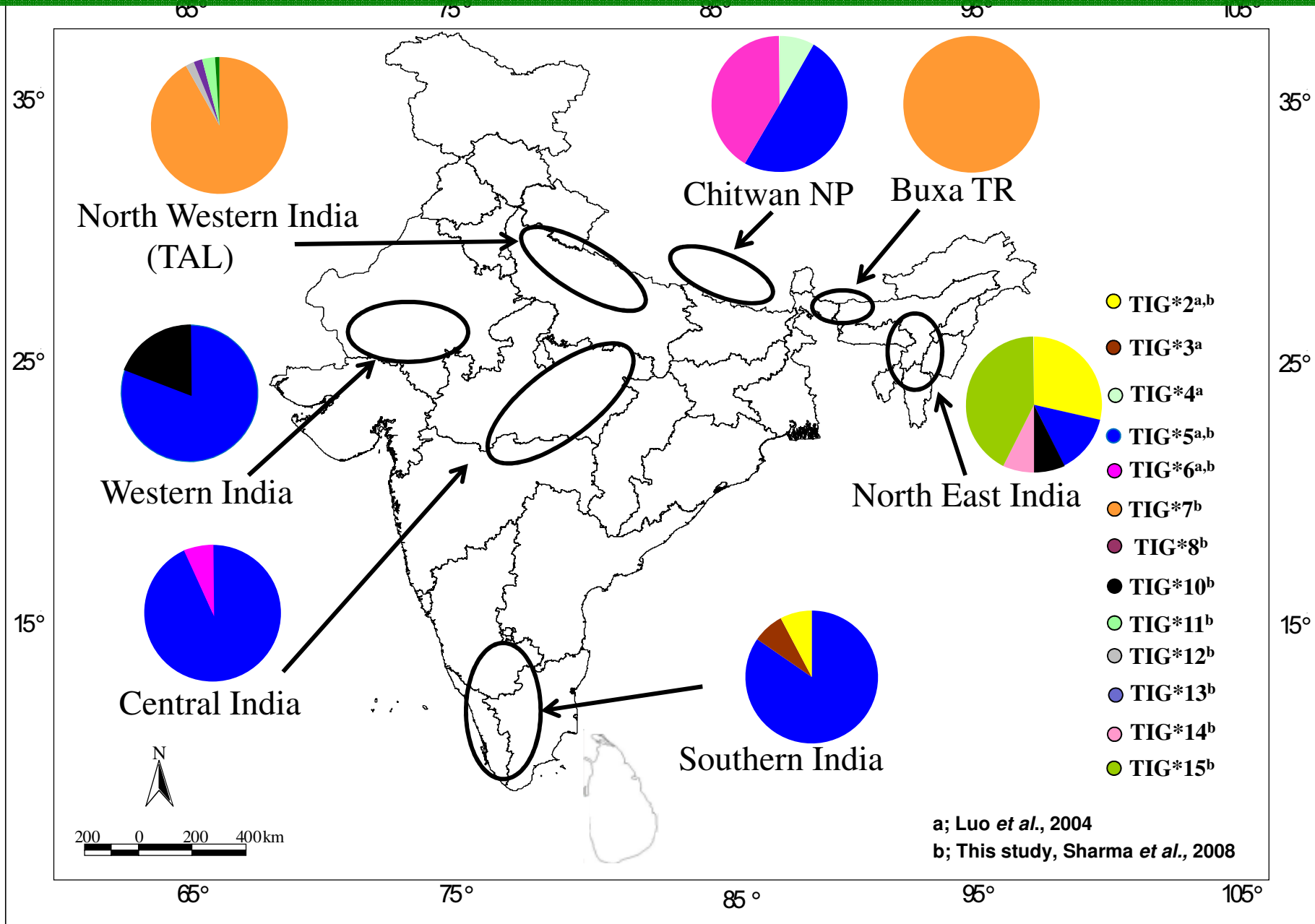
TIG5

Central
India

TIG7

Northern
India

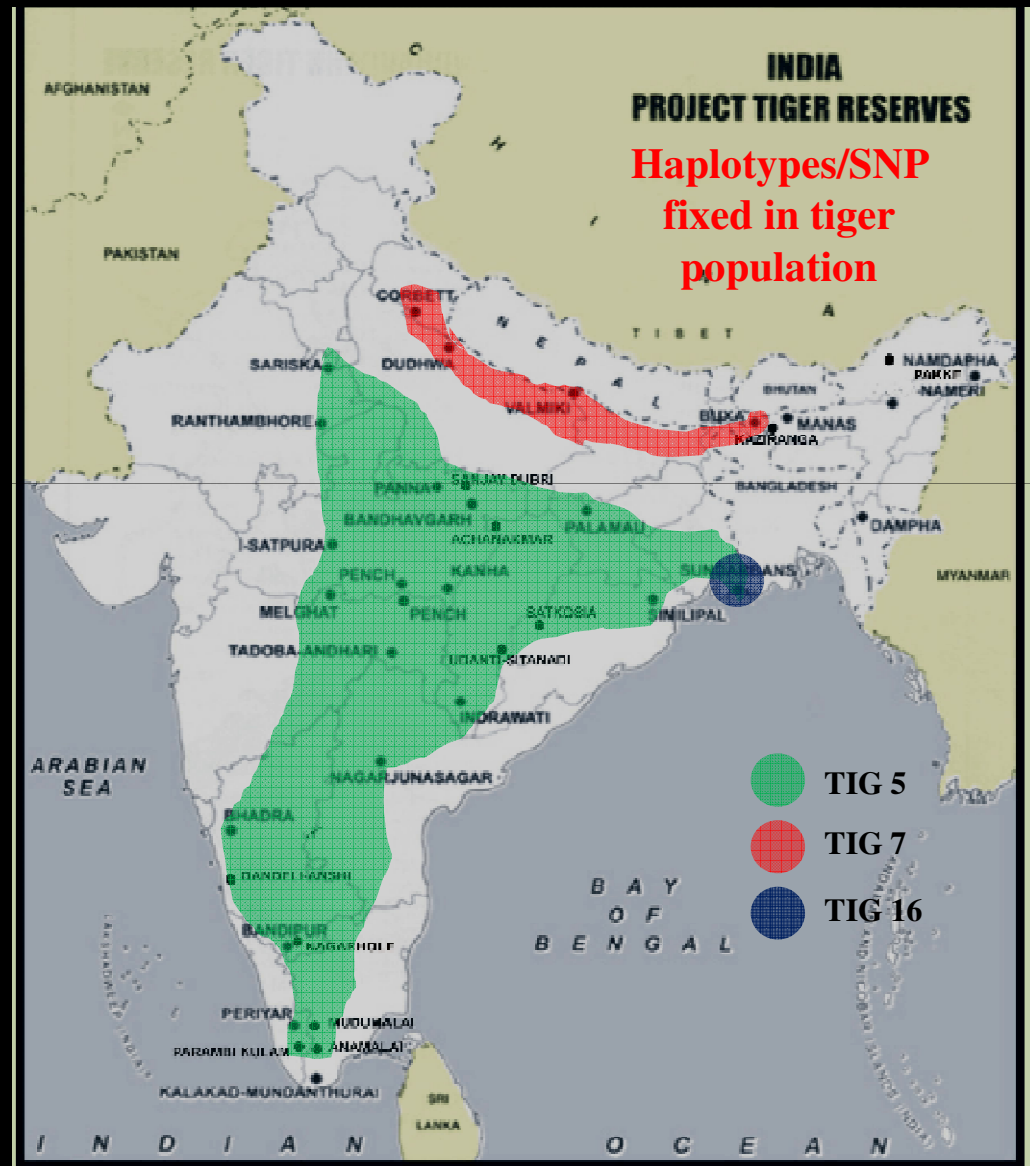
Observed haplotypes in tigers of Indian subcontinent based on four mtDNA genes



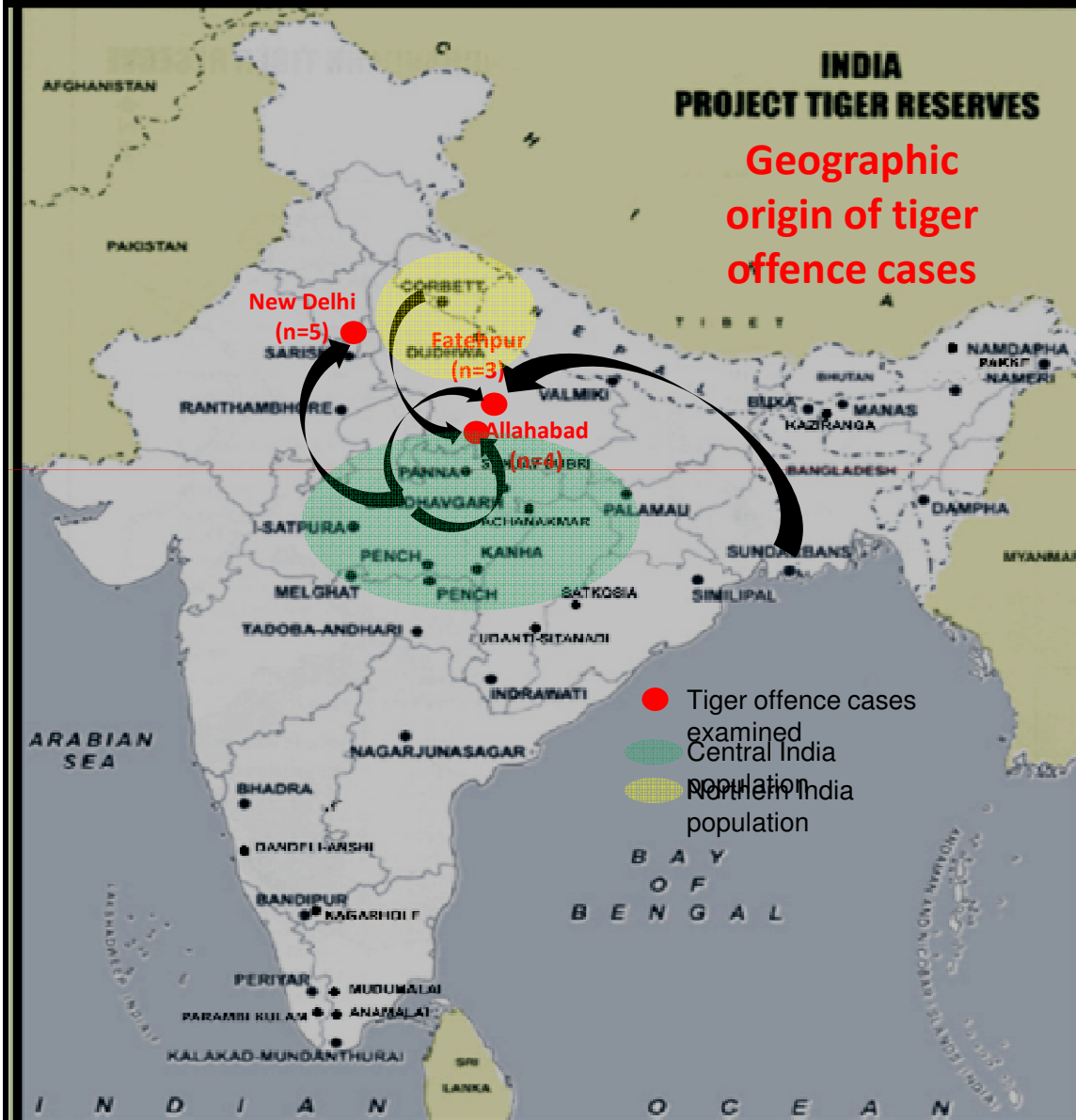
Summary

Based on observed mtDNA variations (haplotype/SNP) in different genes, it is possible to assign poaching case with 100 per cent certainty to the tiger population of Northern India, Peninsular India (including Central and Southern India), North East and Sunderbans.

Need to examine per cent occurrence of observed haplotype/SNP in each tiger population because present data are with few individuals from different populations.



Population assignment of tiger wildlife offences based on mtDNA



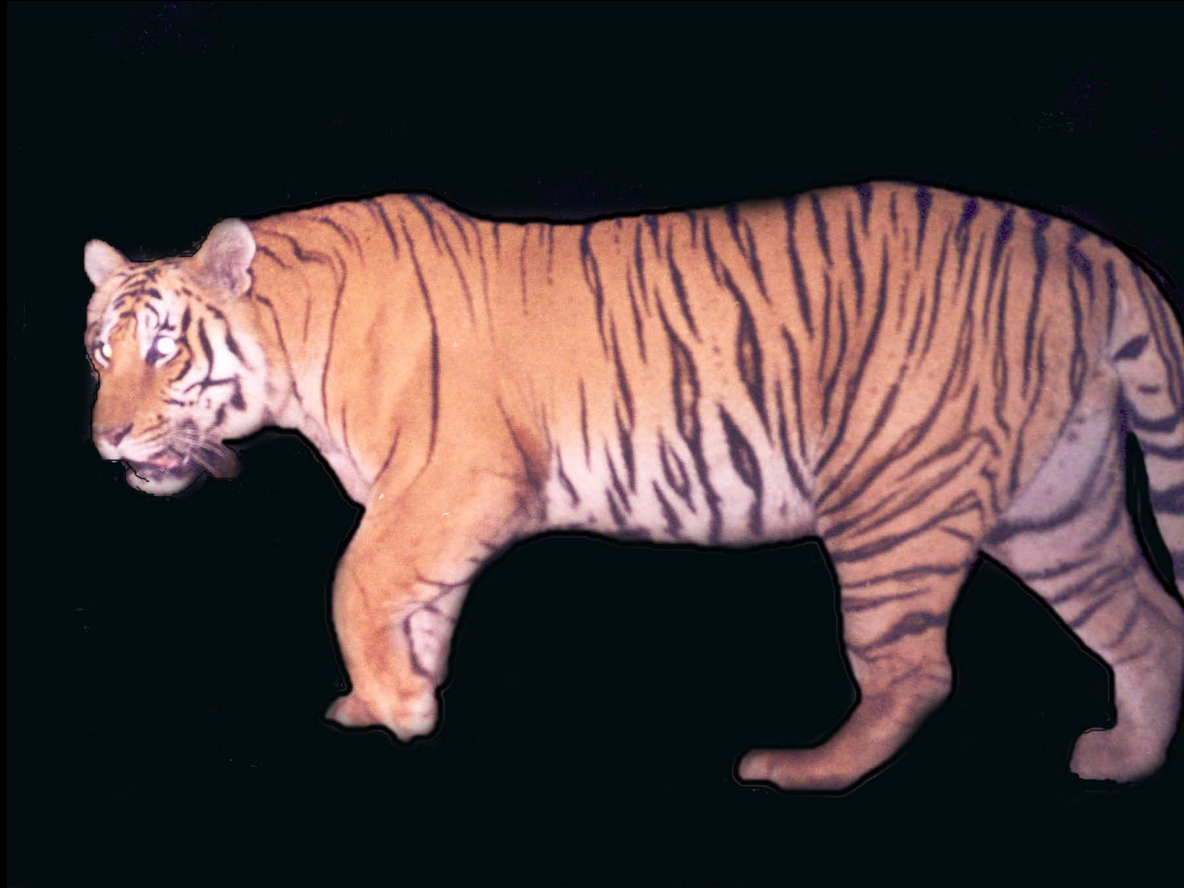
We examine genetic tracking of tiger seizures from New Delhi (n= 5), Allahabad (skins =4) and Fatehpur (skins = 3). Presence of tiger parts of Northern India and Sunderbans tiger populations in Central India indicates that places in Central India and New Delhi are probably major trading centers for wildlife parts.

Population genetic structure and tracking poaching

What is further needed to find frequency of observed haplotype/SNP in different tiger populations with more number of samples?

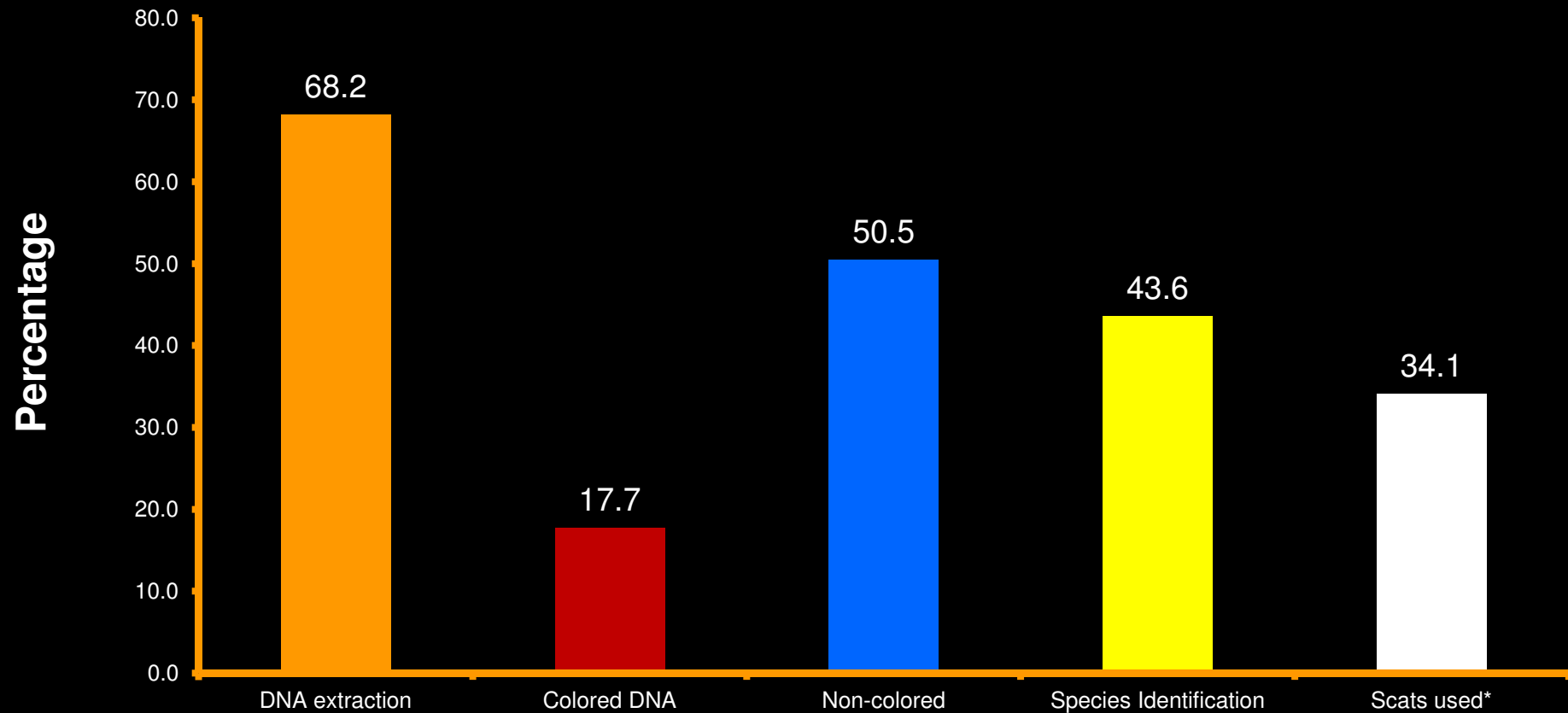


Population genetic structure using multilocus genotyping



Sample processing

(Total sampled collected n= 220)



*Minimum 5 loci data

Population genetic structure and assignment using multilocus genotyping

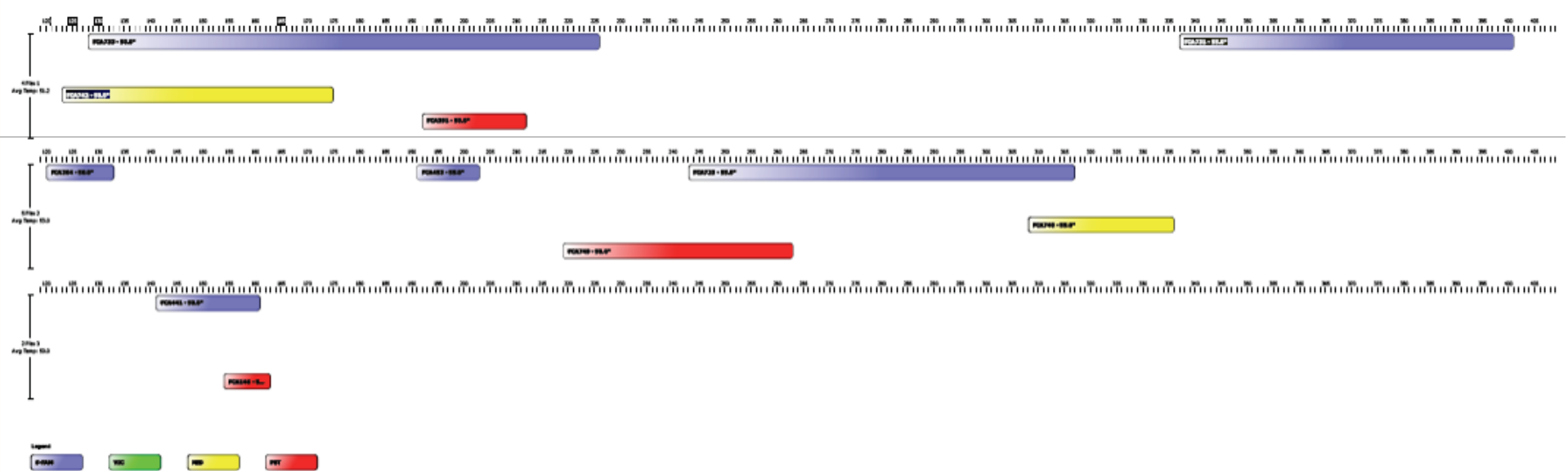
Method 2

- ❖ **60 microsatellite loci were screened and tested their applicability with scat DNA (n=50)**
- ❖ **Selected 23 highly polymorphic microsatellite loci (Mishra et al., unpublished)**

❑ **Attempted to design multiplex PCR panel to minimize the time**

Designing multiplex PCR panels -A need of high throughput”

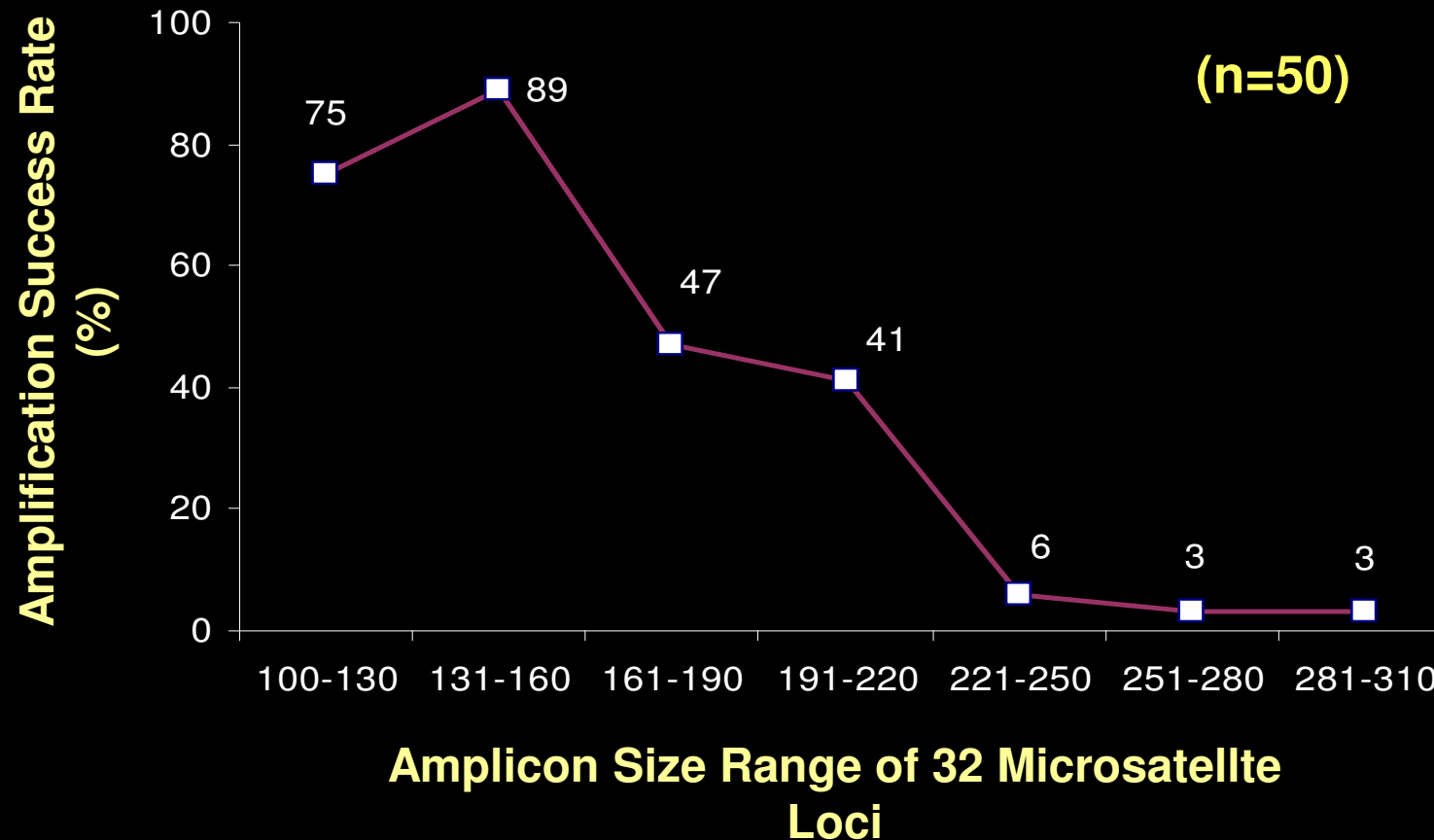
- 5 multiplex PCR panels were designed using **Multiplex manager software**



Different multiplex PCR panels generated by multiplex manager

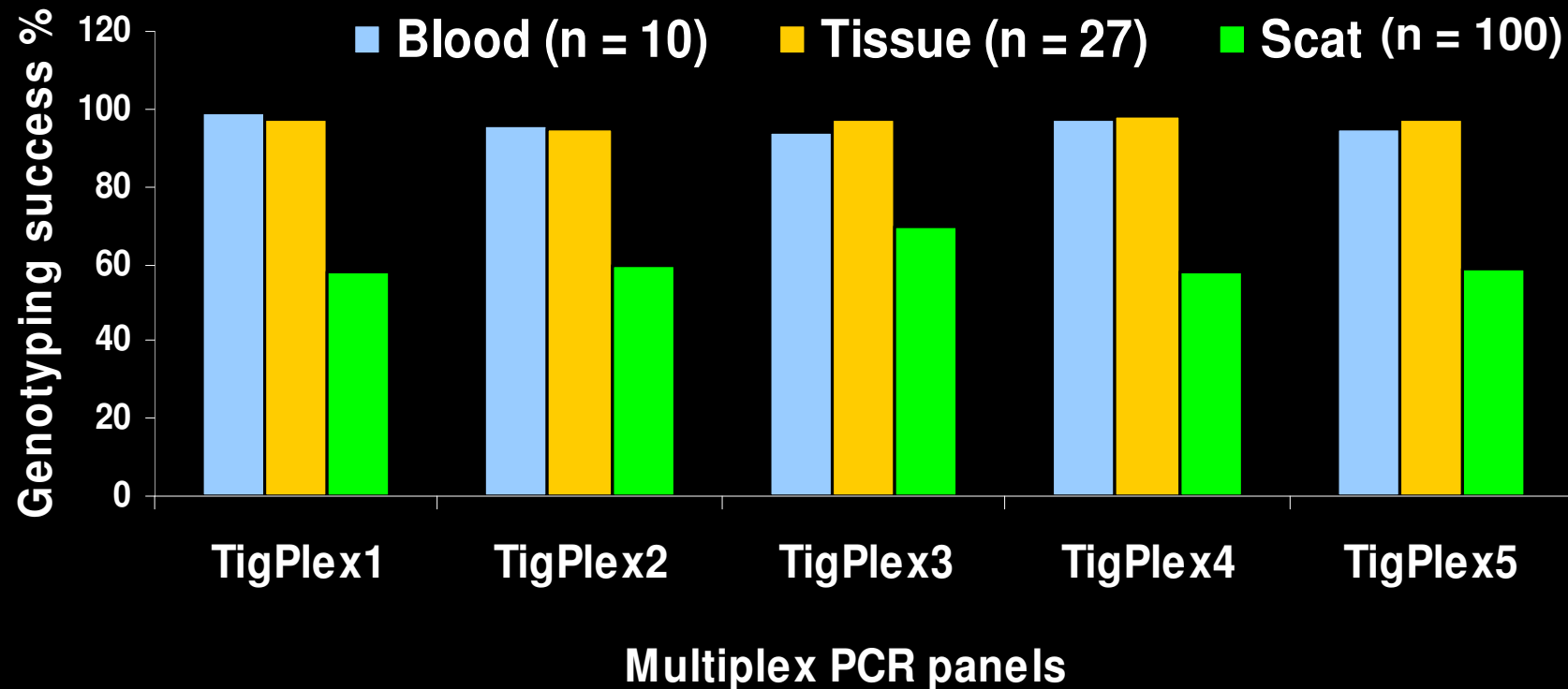
Multilocus non-invasive genotyping

Screening of microsatellite primers (n= 60)



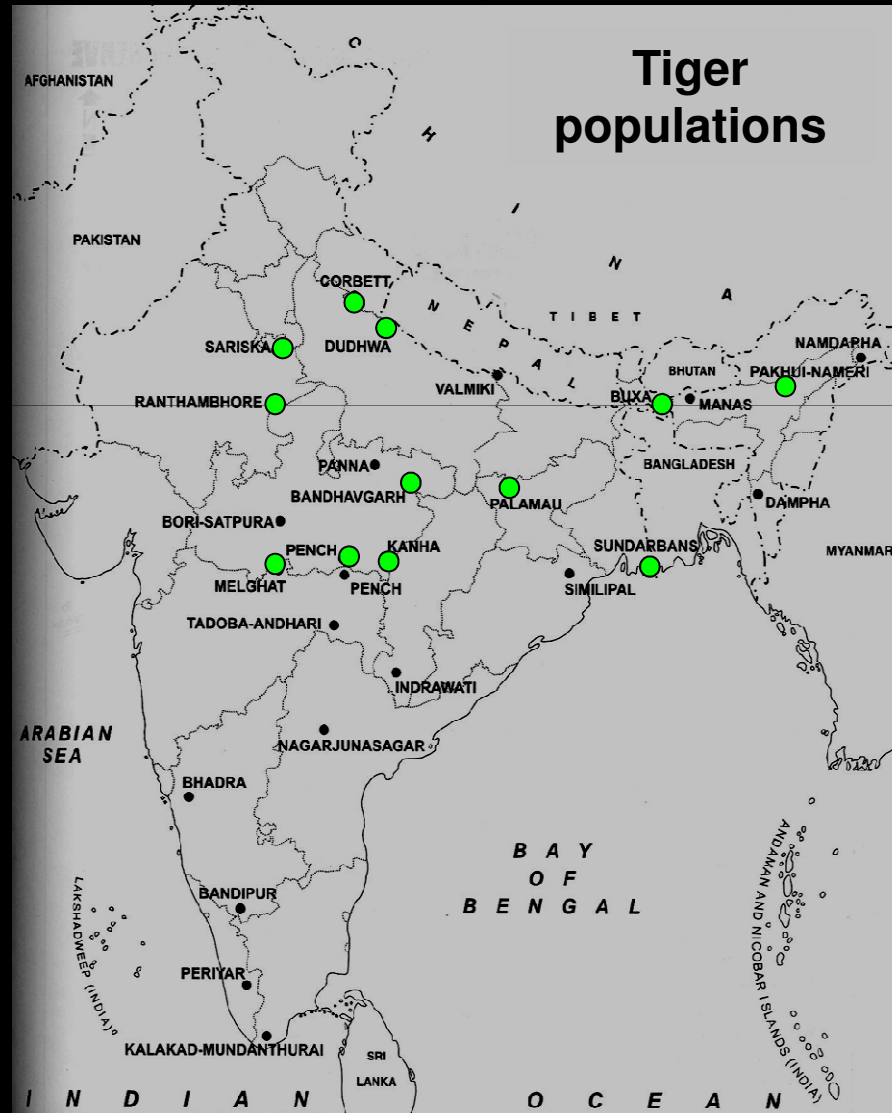
PCR amplification success rate with different amplicon size range of 32 msat loci using scat DNA

Designing multiplex PCR panels- “A need of high throughput”

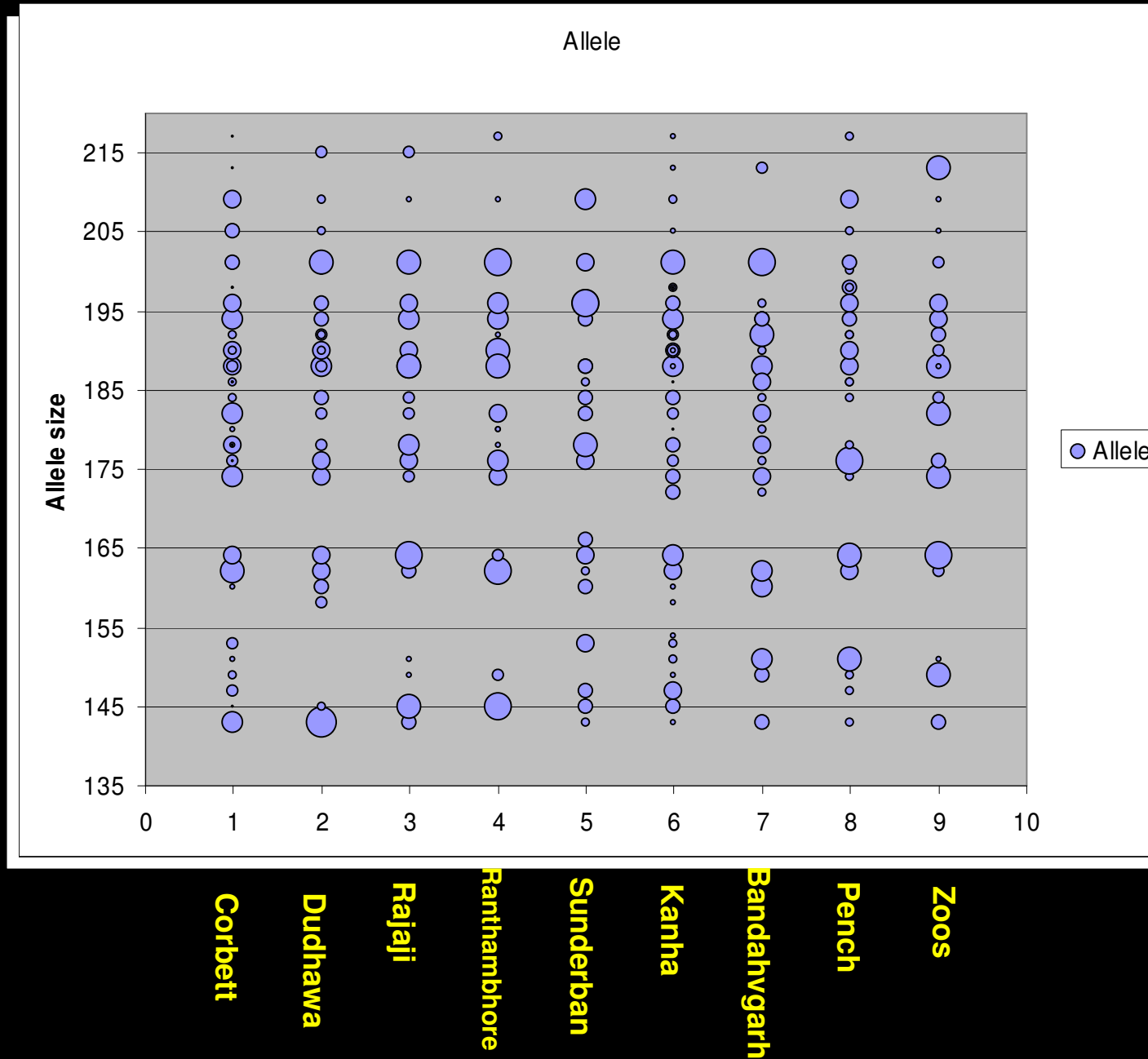


Genotyping success rate of each multiplex panel
with blood, tissue and scat DNA

Established multi locus genotyping (n=9 loci) of different tiger populations (n=12) in India



Allelic diversity of 12 tiger populations established



Gene flow among tiger populations of Terai Arc Landscape

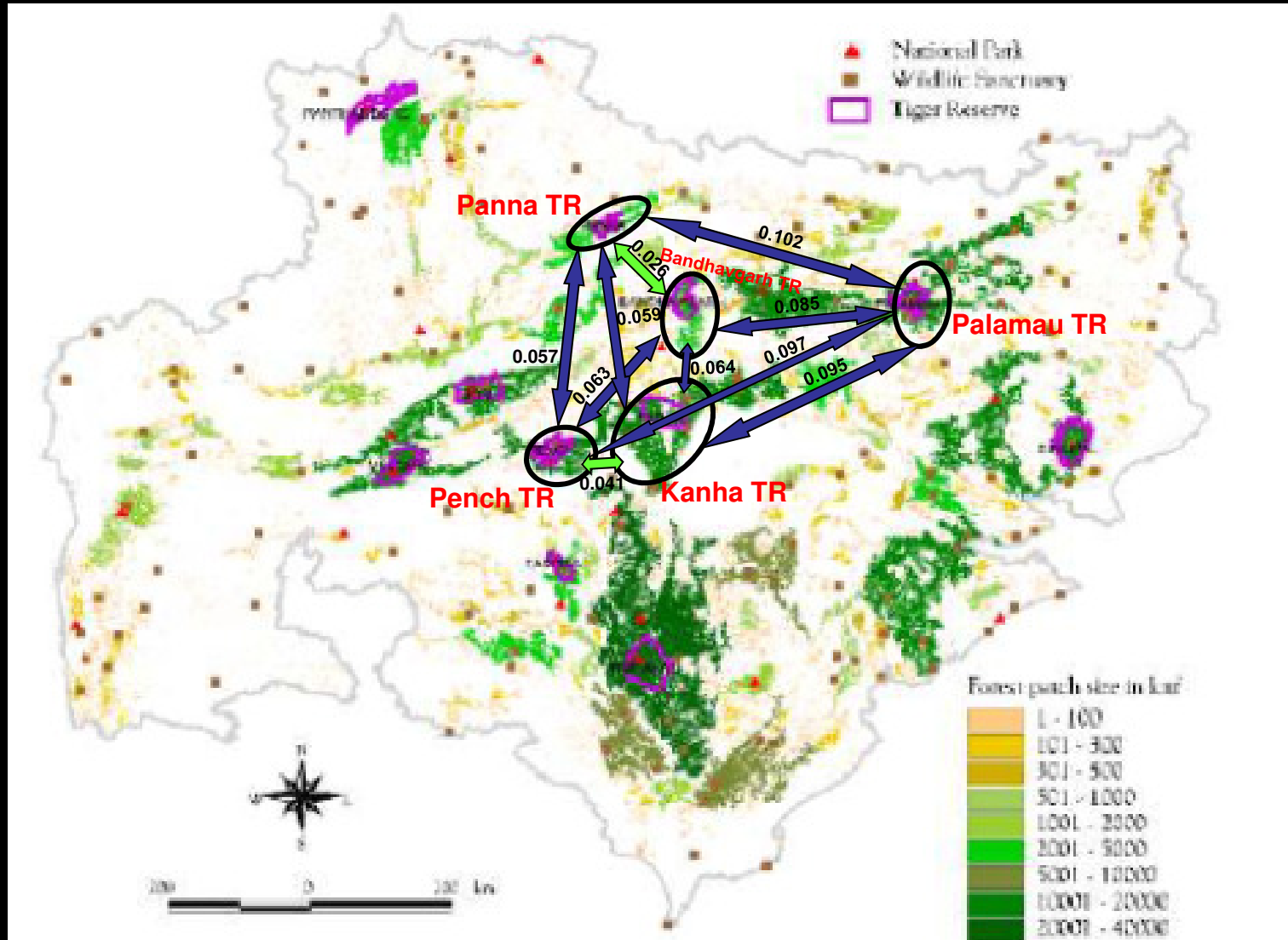


High gene flow,
 $F_{ST} = 0 - 0.04$

Moderate gene flow,
 $F_{ST} = 0.05 - 0.14$

Less or no gene
flow, $F_{ST} = >0.15$

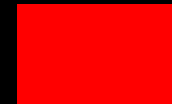
Gene flow (Pair wise population F_{ST}) between tiger populations of central India



High gene flow,
 $F_{ST} = 0 - 0.04$

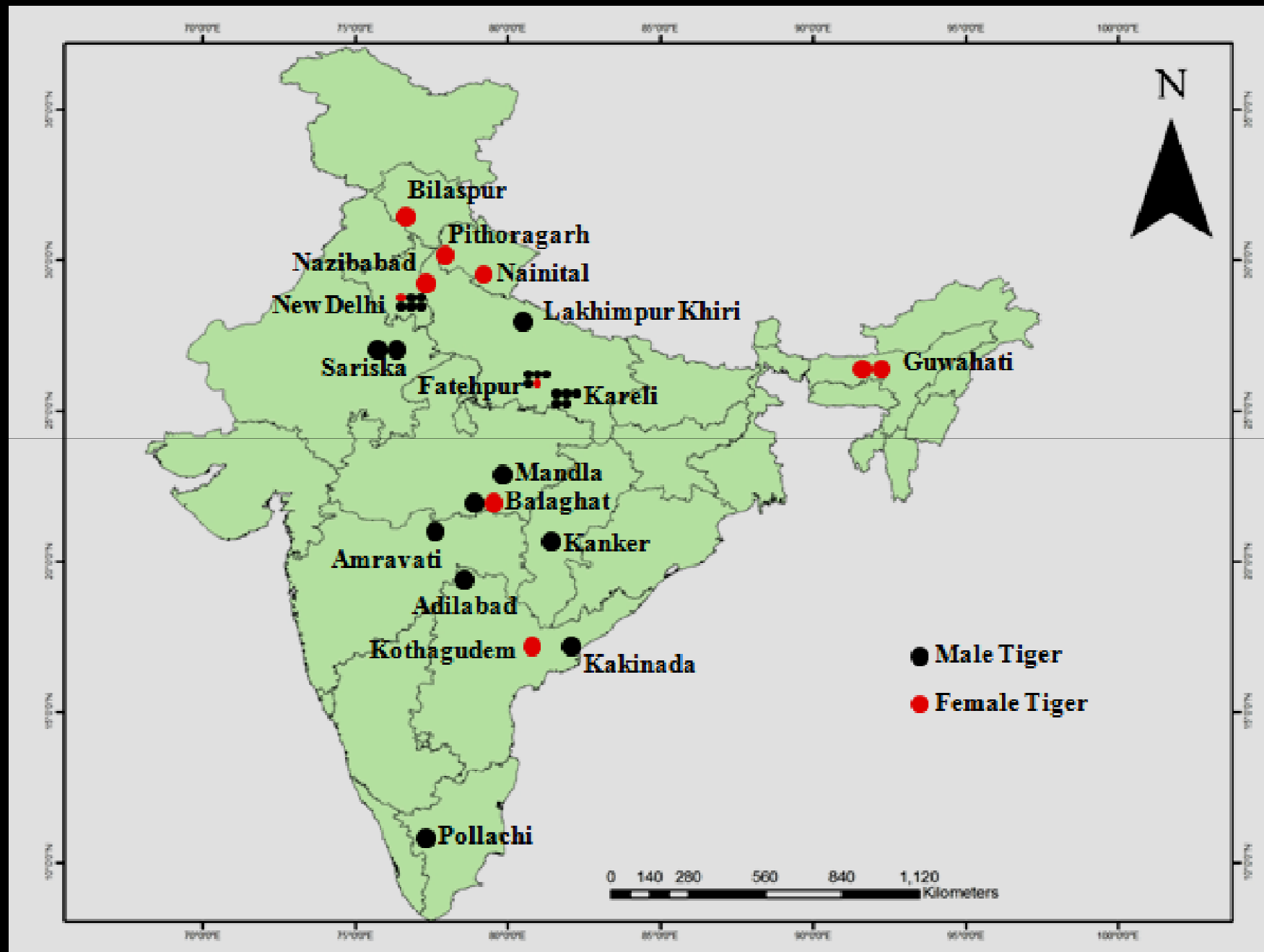


Moderate gene flow,
 $F_{ST} = 0.05 - 0.14$



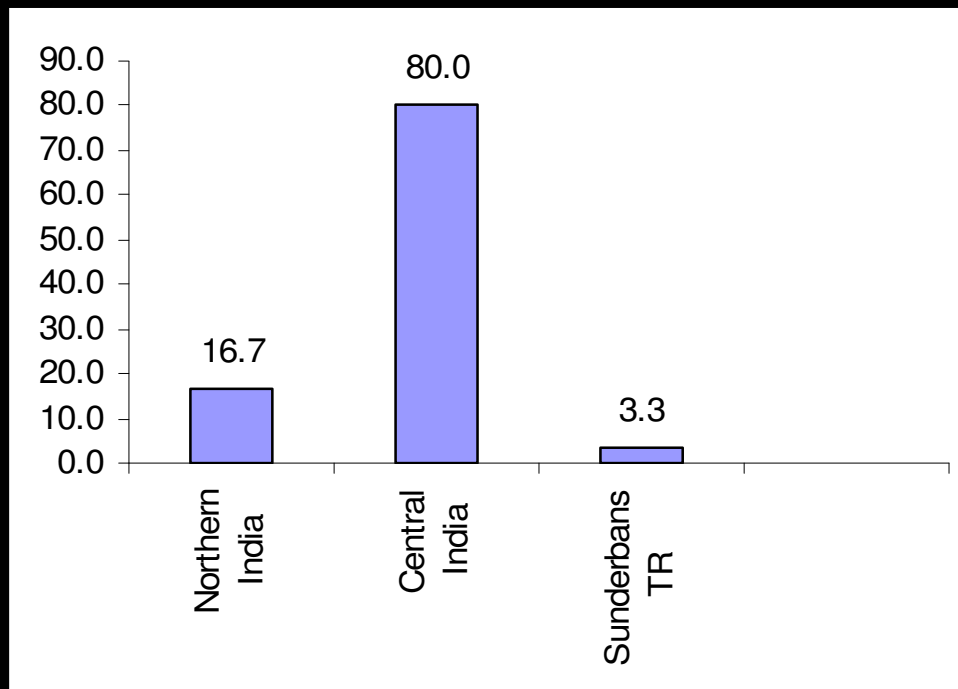
Less or no gene flow,
 $F_{ST} = >0.15$

Sex identification of tiger offences undertaken for analysis (n=34)

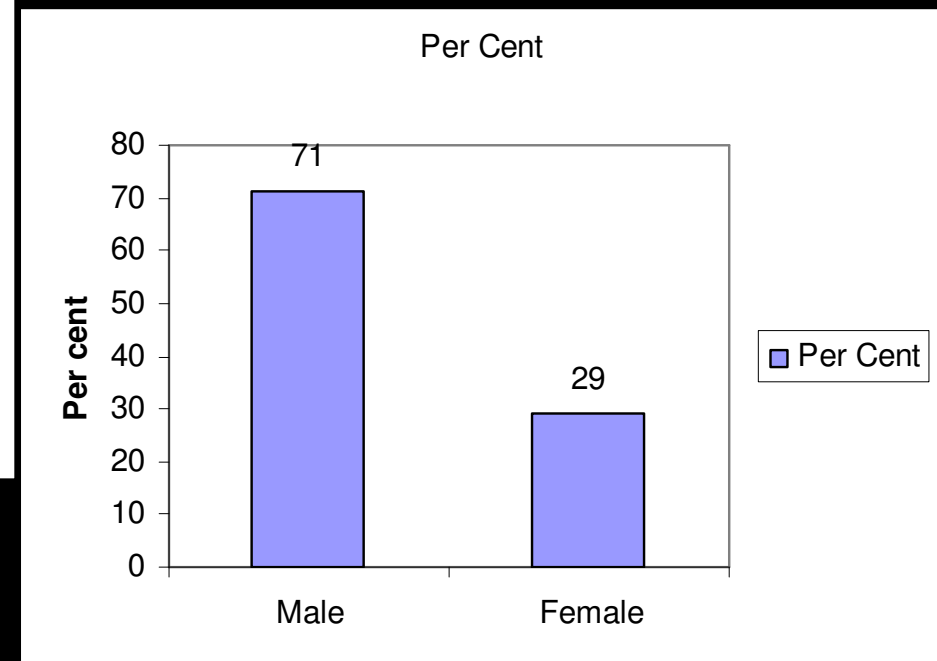


Distribution of tiger offence cases in relation to major zones and sex

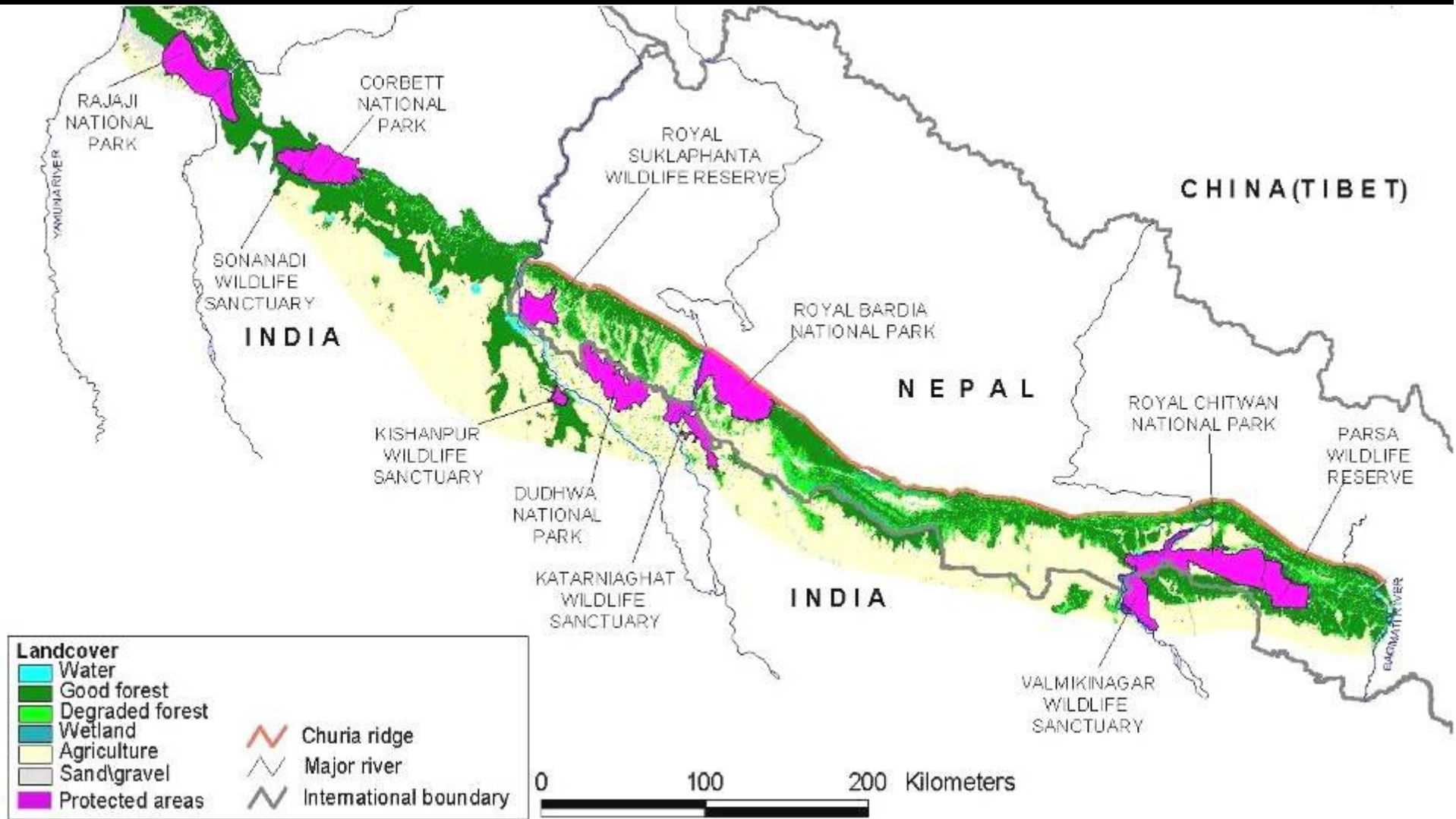
Percent tiger cases in relation to major zones based on observed major haplotypes (n=30)



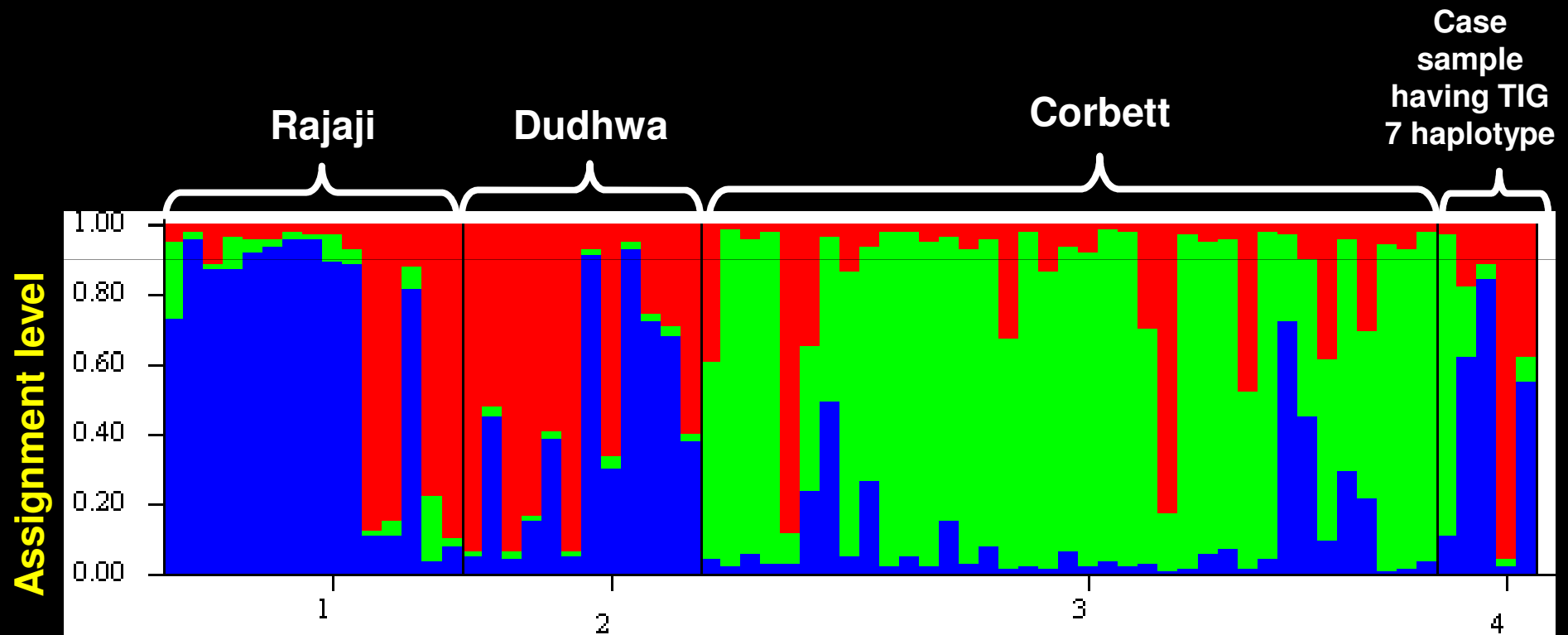
Per cent of tiger offences in relation to sex



Population assignment for tiger population of TAL using multi locus genotyping

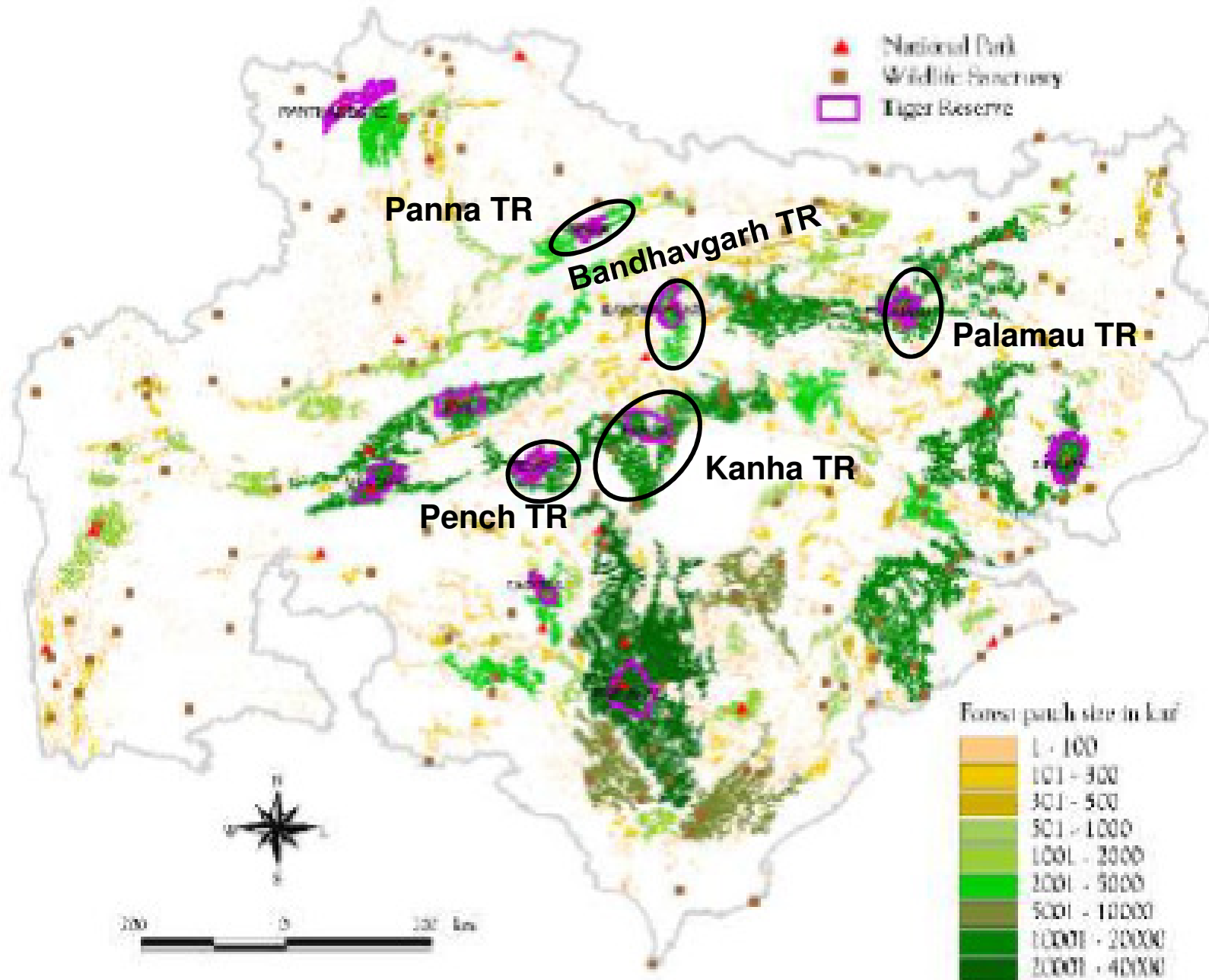


Population assignment of tiger seizures having TIG7 haplotype to tiger populations of Northern India using Structure 2.3.2
(Bayesian based using Marko Chain Monte Carlo)



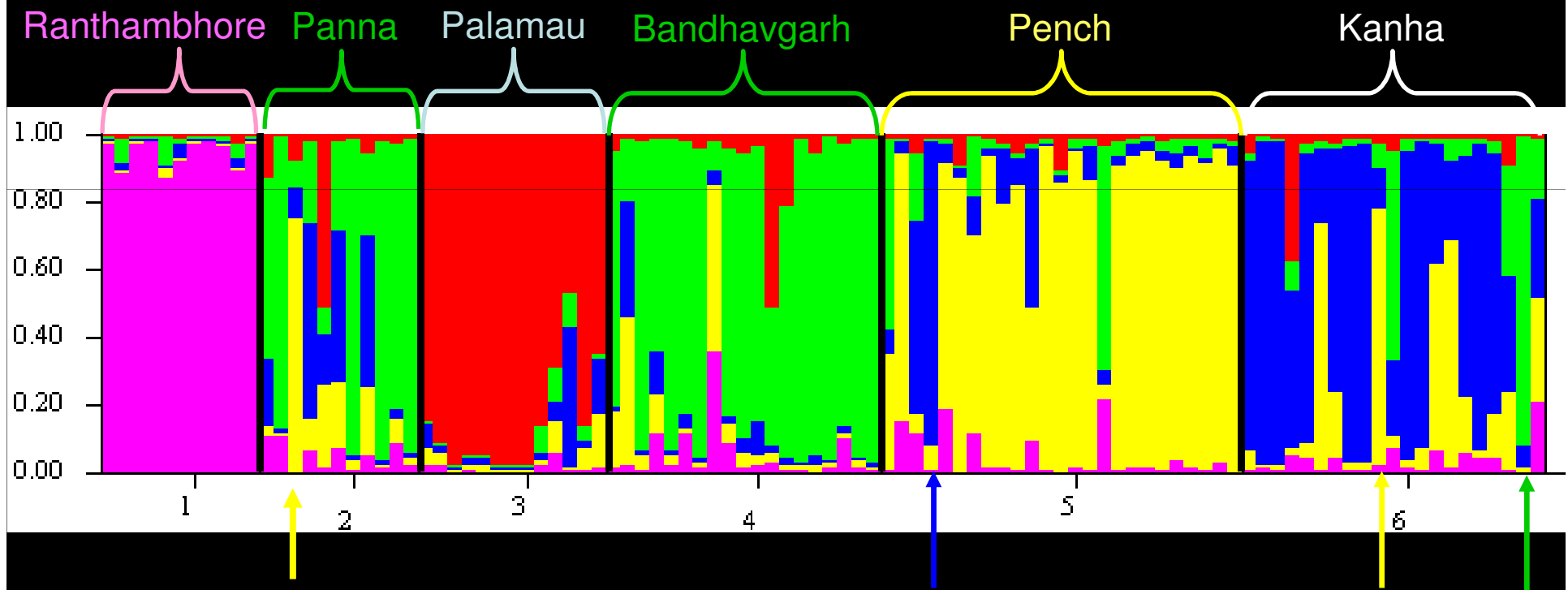
Wildlife offences = , Male= 3 ; Female= 2

Genetic structure of tiger populations of Central India



Structure 2.3.2 used to detect population genetic structure and assign individuals in central India

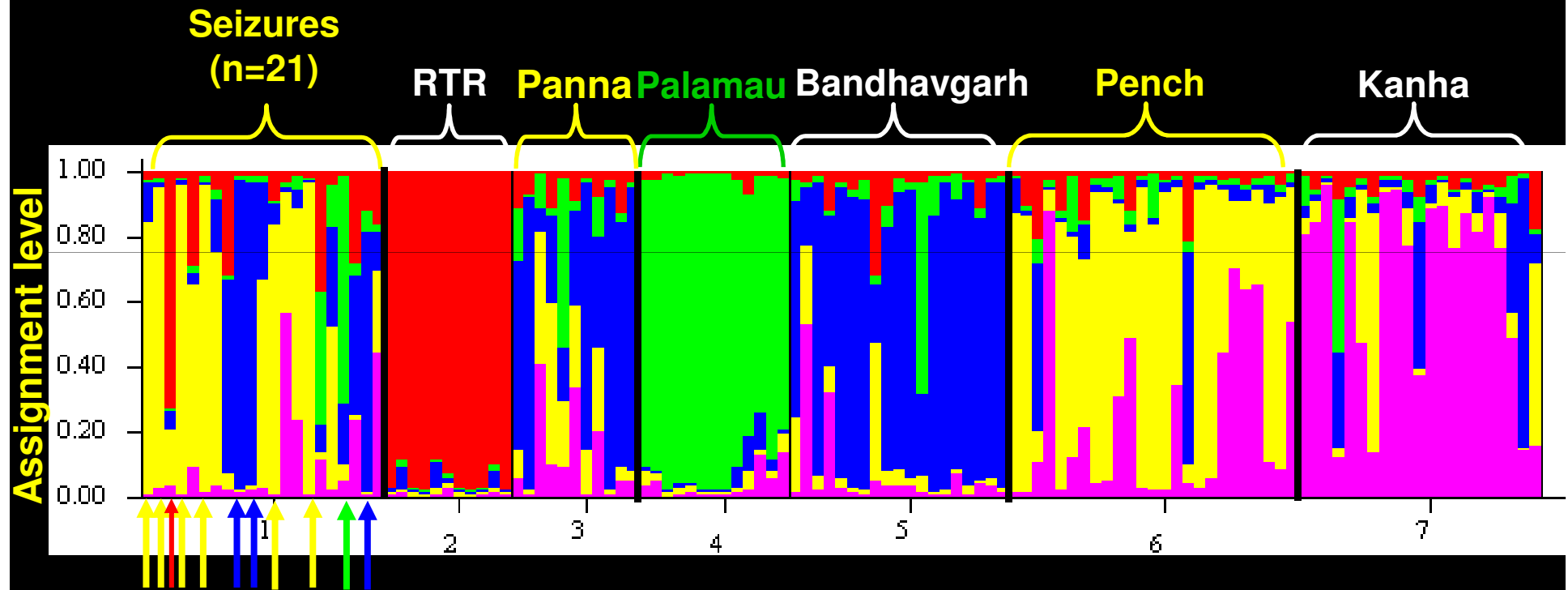
(Bayesian based using Marko Chain Monte Carlo)



Population assignment of samples selected for “Blind Test” to respective population using different software

Population	Selected samples (n)	Corrected assigned populations using different statistical software programs		
		GENETIX (n)	GENECLASS (n)	STRUCTURE (n)
Ranthambhore	3	3	2	3
Panna	3	3	0	0
Palamau	3	3	3	2
Bandhavgarh	3	2	2	0
Pench	3	2	2	2
Kanha	5	5	5	0

Structure 2.3.2 used to assign seizures to central India tiger population (Bayesian based using Marko Chain Monte Carlo)



Findings

- 1. Based on observed mtDNA variations (haplotype/SNP) in different genes, it is possible to assign poaching case with 100 per cent certainty to the tiger population of Northern India, Peninsular India (including Central and Southern India), North East and Sunderbans. Need to assess occurrence of population based SNP/haplotype**
- 2. Pair wise F_{st} value clearly Indicates that there is moderate gene flow between all the three populations in western most tiger population of Northern India .**
- 3. Assignment based on “Blind Test” of samples of known populations with the help of the multilocus genotyping data of 9 loci , clearly test the validity of assignment of individuals of Northern India tiger populations.**

Findings

4. Based on “Blind Test” undertaken using multi locus genotyping data for samples of known population origin, analysis indicates it is possible to assign individuals to Ranthambhore Tiger Reserve and Palamu TR with higher assignment value. Individuals with higher assignment value may be assigned to respective tiger populations of Central India whereas others may be poorly assigned having admixture.

Future Plan

Need to establish data for mtDNA haplotype/SNP at population level for identification.

A need to establish mtDNA and mutli locus genotyping data for other tiger population of southern India on standardized selected loci

A need to add data on more loci to enable us to get a better accuracy in assignment level of admixed individuals.



Thanks

15/5 302



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