Genomic information a tool for Assessment of Genetic diversity in Mithun

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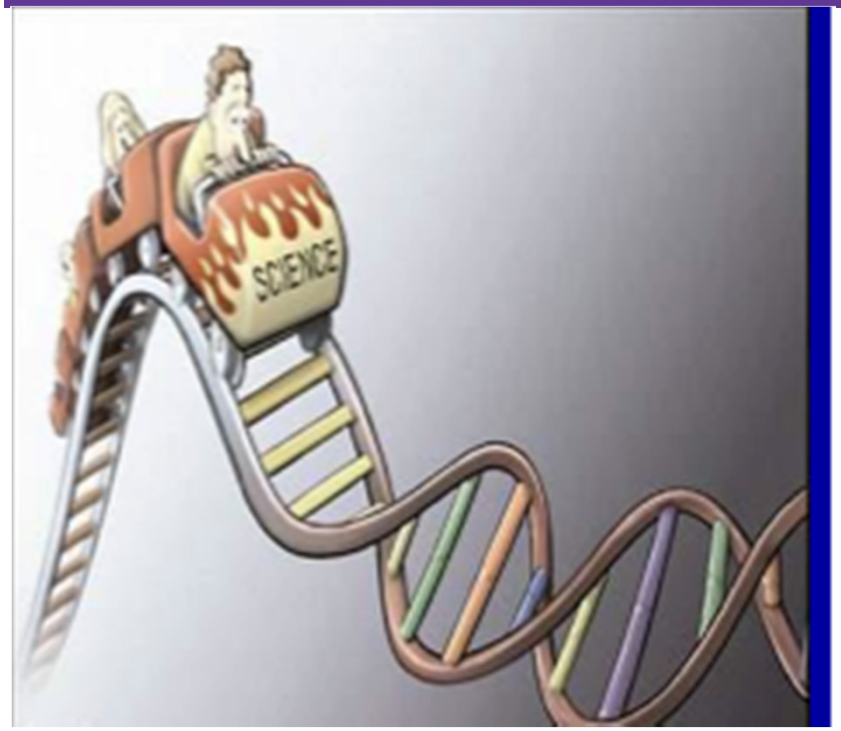
This I believe in genetics:



Discovery can be a nuisance

Replication is science,

Implementation matters

“I don't know what I may seem to the world, but as to myself, I seem to have been only like a boy playing on the sea-shore and diverting myself in now and then finding a smoother pebble or a prettier shell than ordinary, whilst the great ocean of truth lay all undiscovered before me” –

Issac Newton (1642-1727)

NRC on Mithun (ICAR)

Pebbles and Ocean

- The Mithun research has been able to find so many beautiful pebbles and shells. Works on genetics, nutrition, product and value addition.

- Present day research now desires to leave the pebbles and shells at the shore and go deep to search for prettier jewels at the ocean floor. This is the field of genome selection

Introduction

- Mithun (*Bos frontalis*), a ruminant species belonging to family *bovidae, a*ssumed to be the domesticated form of wild gaur *(Bos gaurus)*

- Indigenous to the eastern Himalayas and has been referred to as ‘sacrificial ox’ of the Naga Tribes of NEH.

Strains of mithun

Four strains –

Arunachal,

Nagaland,

Mizoram and

Manipur

Nagaland strain

Arunachal strain

Manipur strain

Mizoram strain

Population distribution of Mithun in India

State	1987	1997	2003	2007
Arunachal Pradesh	98540 (74%)	124194 (70.21%)	192000 (69.06%)	**218931 (82.89%)**
Nagaland	13496 (10.12%)	33445 (18.90%)	40000 (14.39%)	33244 (12.59%)
Manipur	19895 (15%)	16660 (9.42%)	20000 (7.19%)	10024 (3.79%)
Jammu & Kashmir	-	-	24,000 (8.63 %)	-
Mizoram	1435 (1%)	2594 (1.47%)	2000 (0.73%)	1939 (0.73%)
Total	**133366**	**176893**	**278000**	**264138**

Prospects and Challenges

- The world population will reach 9 billion people by 2030.
- The traditional livestock farming will face greatest challenges in fulfilling the growing food demand with fewer resources, (FAO, 2006).
- Increase in global meat consumption of 68% and in global milk consumption of 57% by 2030 (Steinfeld and Gerber, 2010).
- Needs to apply scientific advancements (innovations) and general improvement of animal to fill the productivity gaps with reduce costs and limit environmental impacts (Capper, 2011).
- New advances --- whole genome sequencing – cattle & swine
  --- to improve accuracy of genomic selection or
  --- mapping new QTL (Meuwissen & Goddard, 2010)

Why Genomics ??

- Traditionally, phenotypic & pedigree information

  genetic evaluations (EBV)

- The biggest (r) evolution -- emergence & application of genomics in livestock breeding.

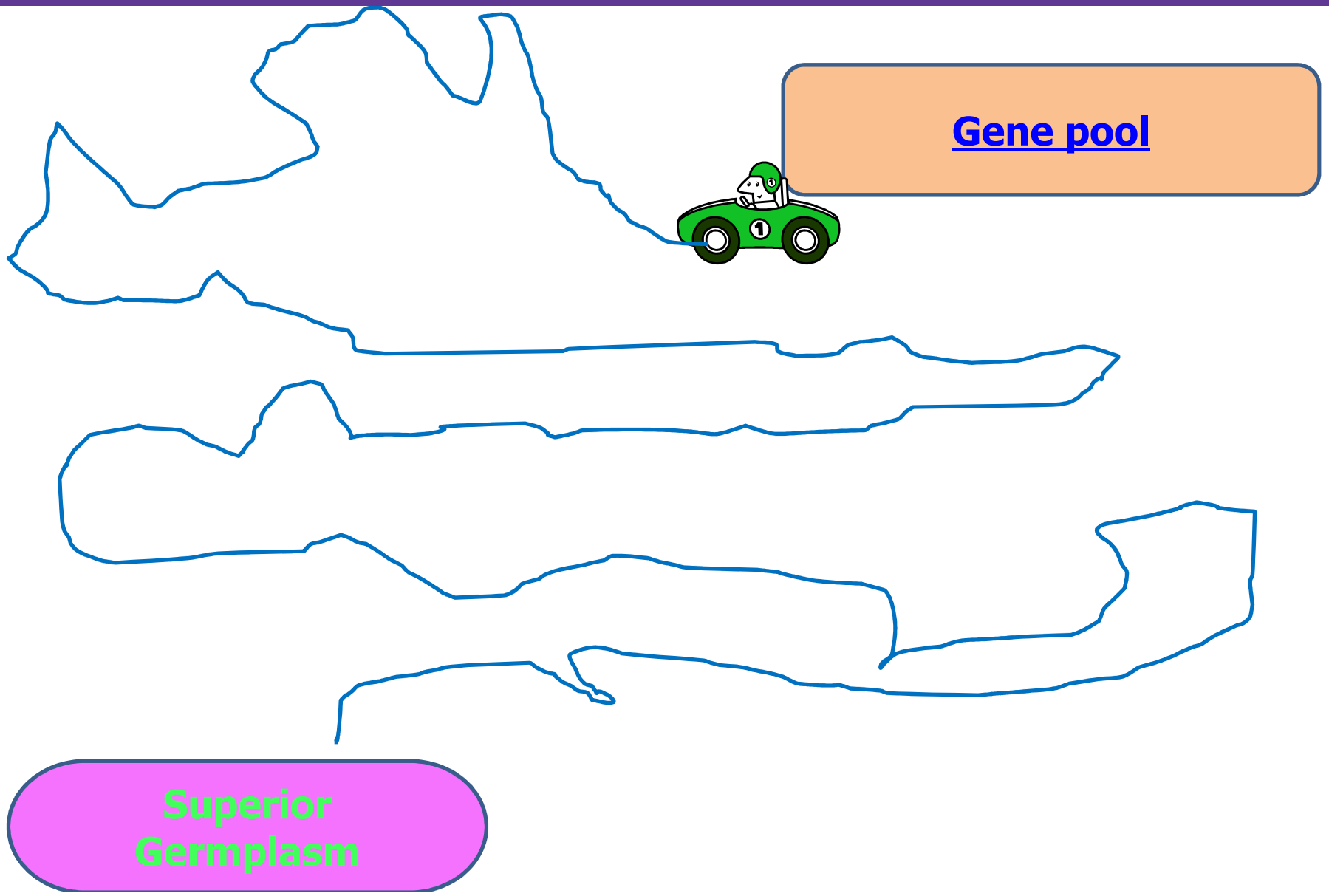
- For breeders, genomics = ↑ efficiency & productivity

  ---for consumers = ↑ security & quality of animal products.

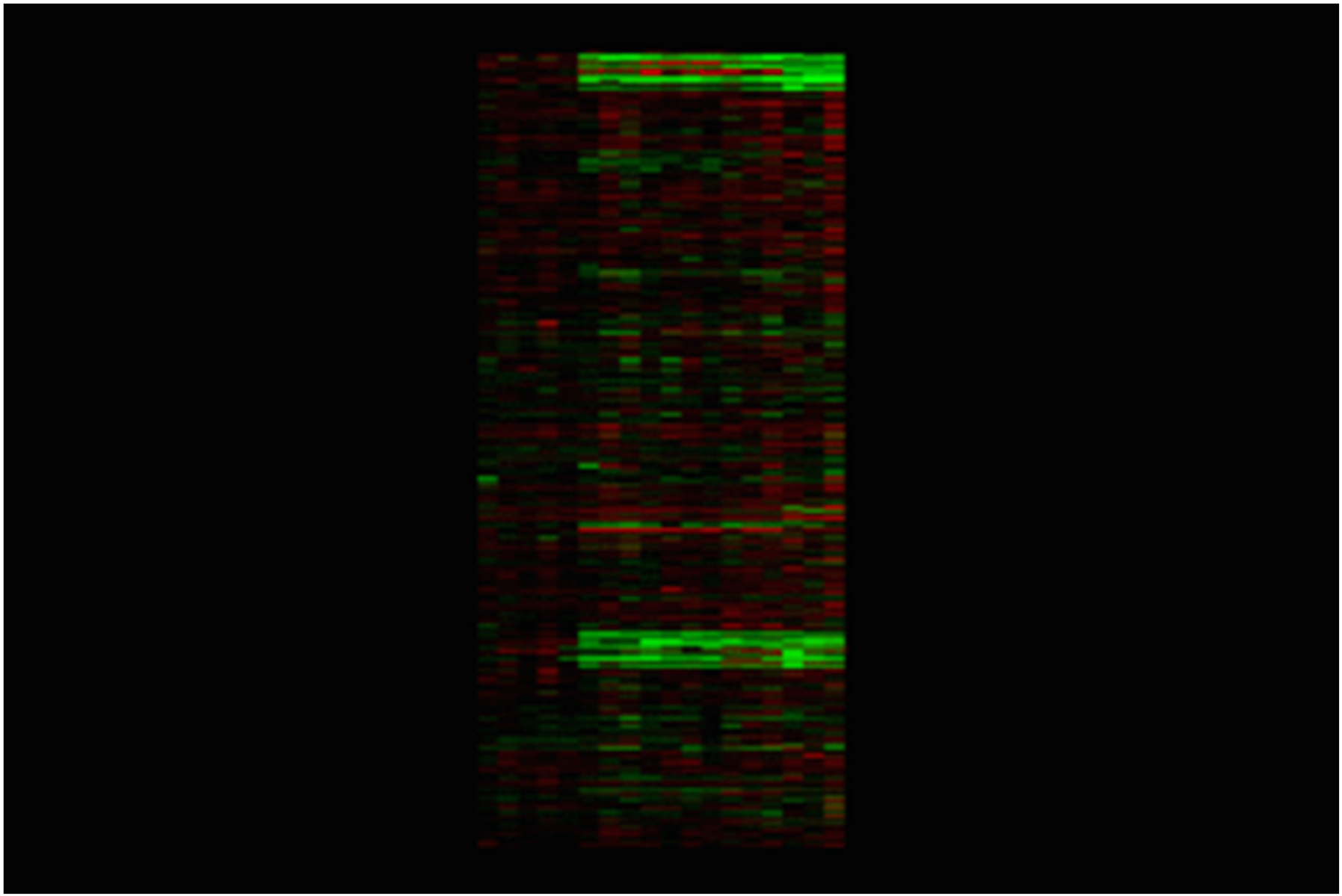
- Genomics proposes ---- improve selection accuracy
  ↓ costs, generation intervals, and

  exploiting new sources of polymorphisms

A route developed and taken by breeders: From gene pool to superior germplasm



A Walk Through Our Genome



-- All regions of the genome are not created equal

Overview

- Why genomics important for the genetic improvement of mithun?
- Organization of mithun genome
- Tools in the toolbox
  - SNP markers
  - High density assays
- How we use the tools to detect and exploit genetic variation
  - Linkage Disequilibrium
  - Linkage
  - Genome Selection (GS)

1. Why genomics for the genetic improvement of mithun ? ?

- Mithuns are reared traditionally in forest, no proper r trait and no pedigree information.

- Mithun meat is better than cattle and buffalo in terms of quality and quantity.

- Efficient convertor of forages and tree leaves into meat and milk, --- and is considered a future dairy and meat animal in the scenario of climate change and scarcity of nutrition.

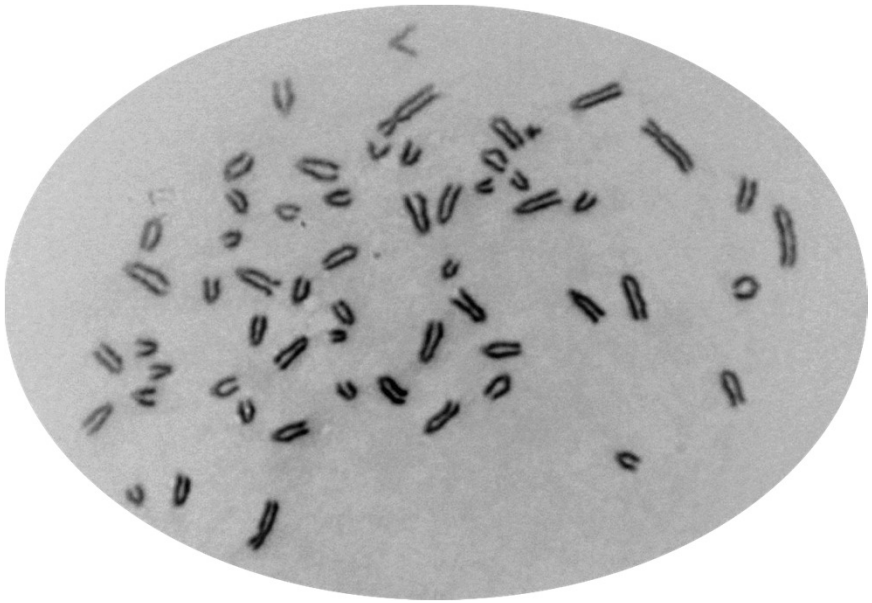
- To assess the genetic worth of mithun is the bottleneck in the genetic improvement and selection in this species.

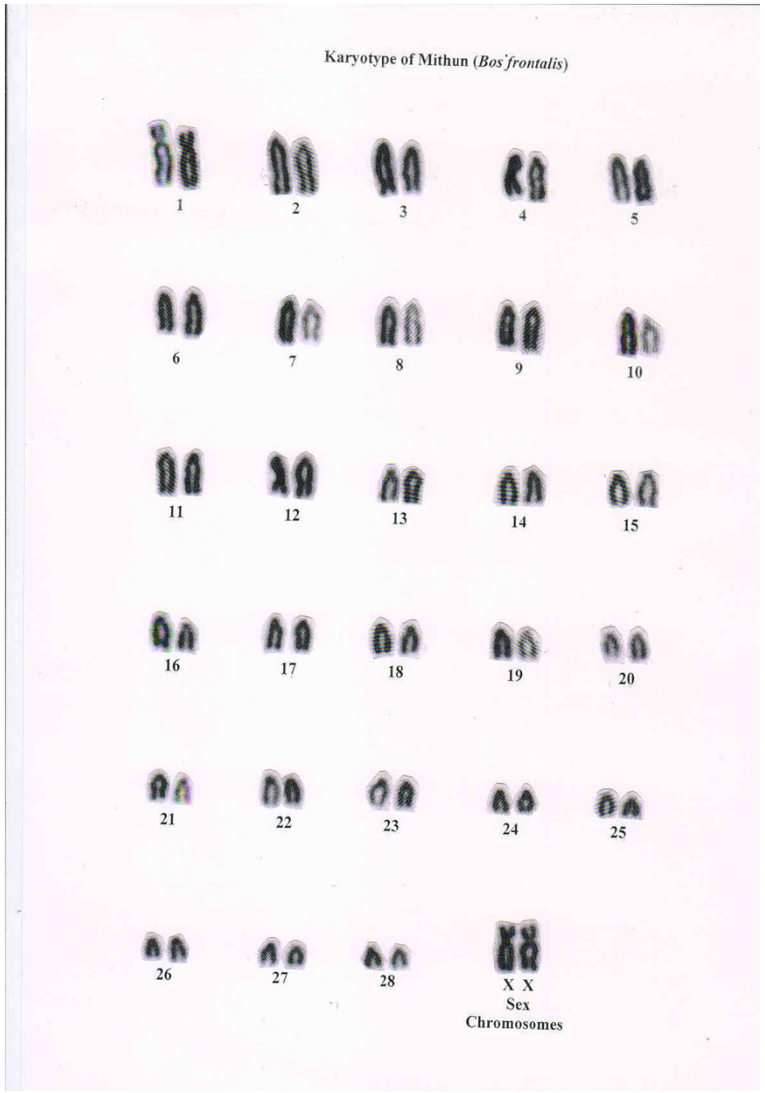
- A genome-wide association study involves high-throughput genotyping platforms for rapidly scanning markers across whole genome and to find genetic variations associated with a particular phenotype.

- The main aim to conduct Genomic study in Mithun, is to identify chromosome regions that harbor the gene(s) that contribute to the phenotypic variation.

- The identified SNPs with high effects can be selected to obtain more accurate breeding values for effecting genomic selection in Mithun where no phenotypic observations are available.

2. Organization of Mithun Genome

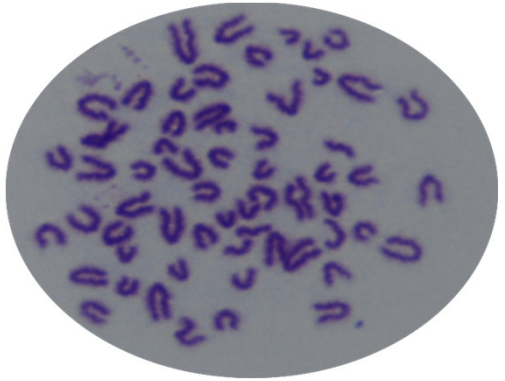




The Biology Assures Variation in Progeny

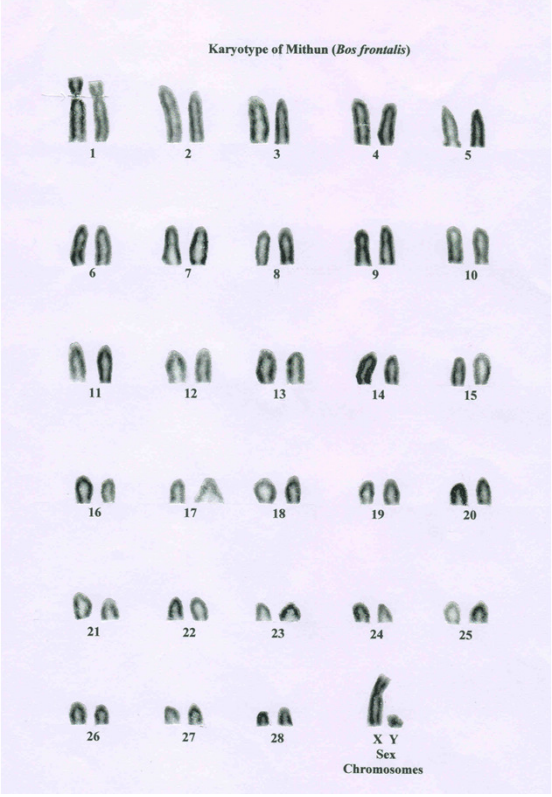
Mithun have 29 pairs of chromosomes

- 28 autosomes, 1 sex determining
- Diploid (2 copies of each chromosome)
- ~3.5 Billion base pairs



Meiotic cell division forms gametes

- Eggs and sperm are haploid
- 1 chromosome from each pair; random
- Recombination or cross-over events

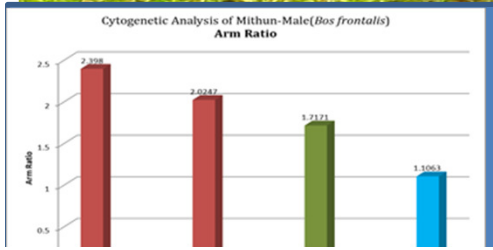


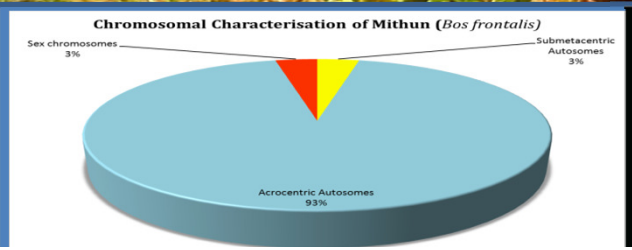
Fertilization restores diploid chromosome count

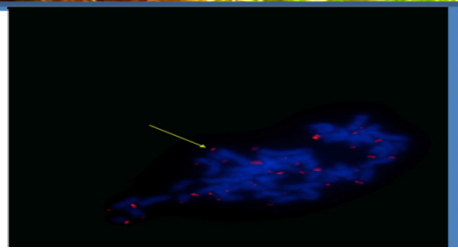
Two copies of each gene

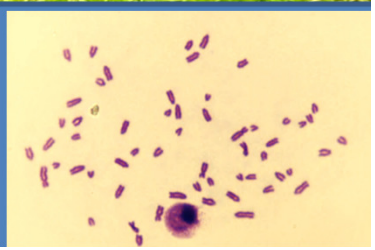
- Alternate forms are called allele

MITHUN CYTOGENETICS:
From chromosome Shape to Chromosome Mapping









3. Tools available - Genomics Tool Box

- **SNP Markers**
- **High density assays**

Single Nucleotide Polymorphism (SNP) DNA Marker Example

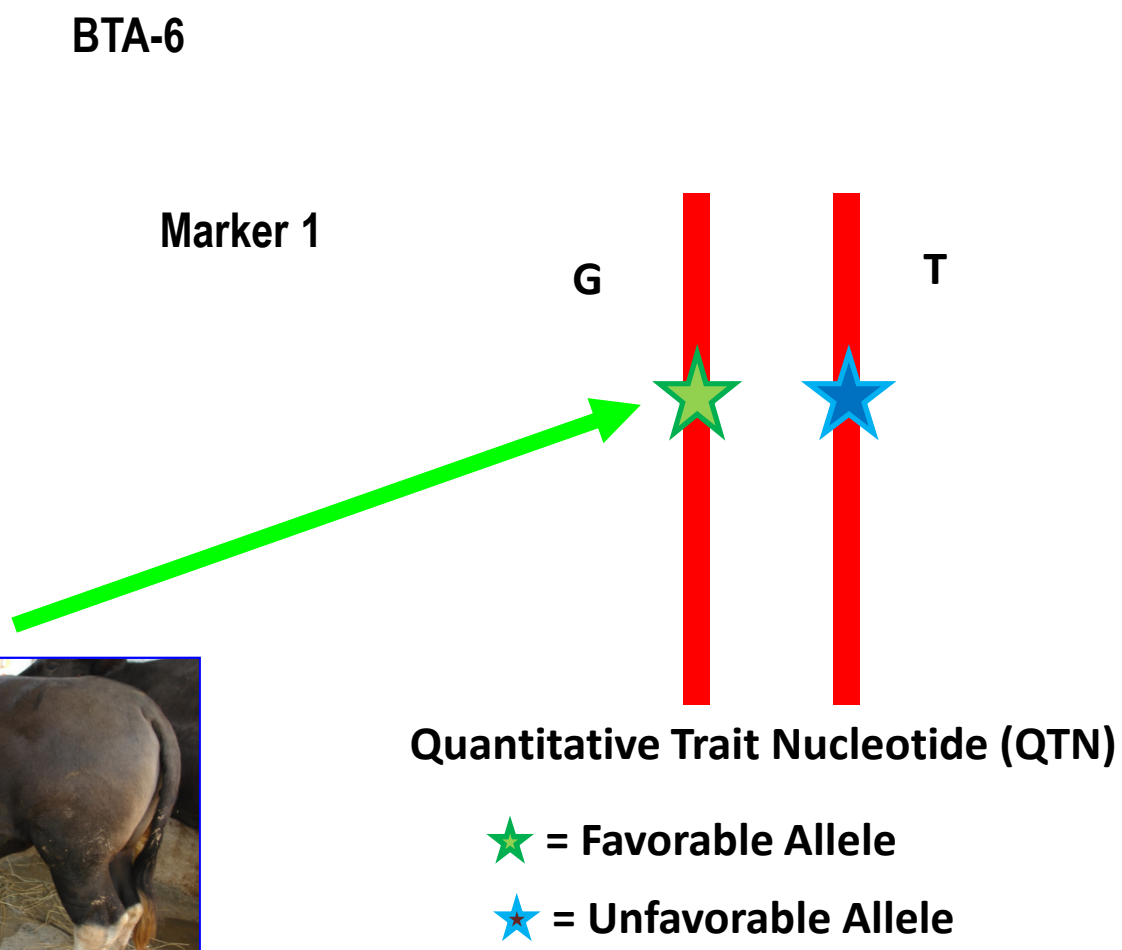
G/T SNP

1 BTA-6 …ATCGTAGATATTGGCC…
…TAGCATCTATAACCGG…

2 BTA-6 …ATCGTATATATTGGCC…
…TAGCATATATAACCGG…

- **Mutation may be in exon (coding sequence; possibly causal) or in intron (non-coding sequence) of gene**

SNPs and QTNs



What a Marker Test Tells You:

Marker 1

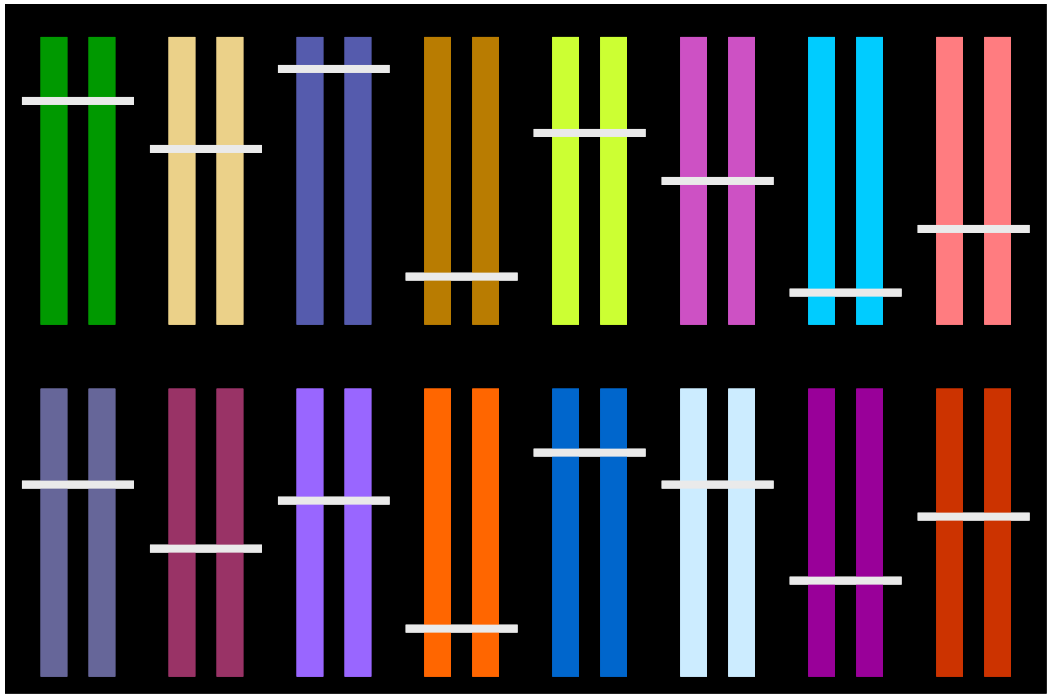
But What About These Genes?

I have found one more also !!

How many ? ?

What an EPD Tells You:

Cumulative effect of all genes and their interactions on a trait.



A Question of Resolution

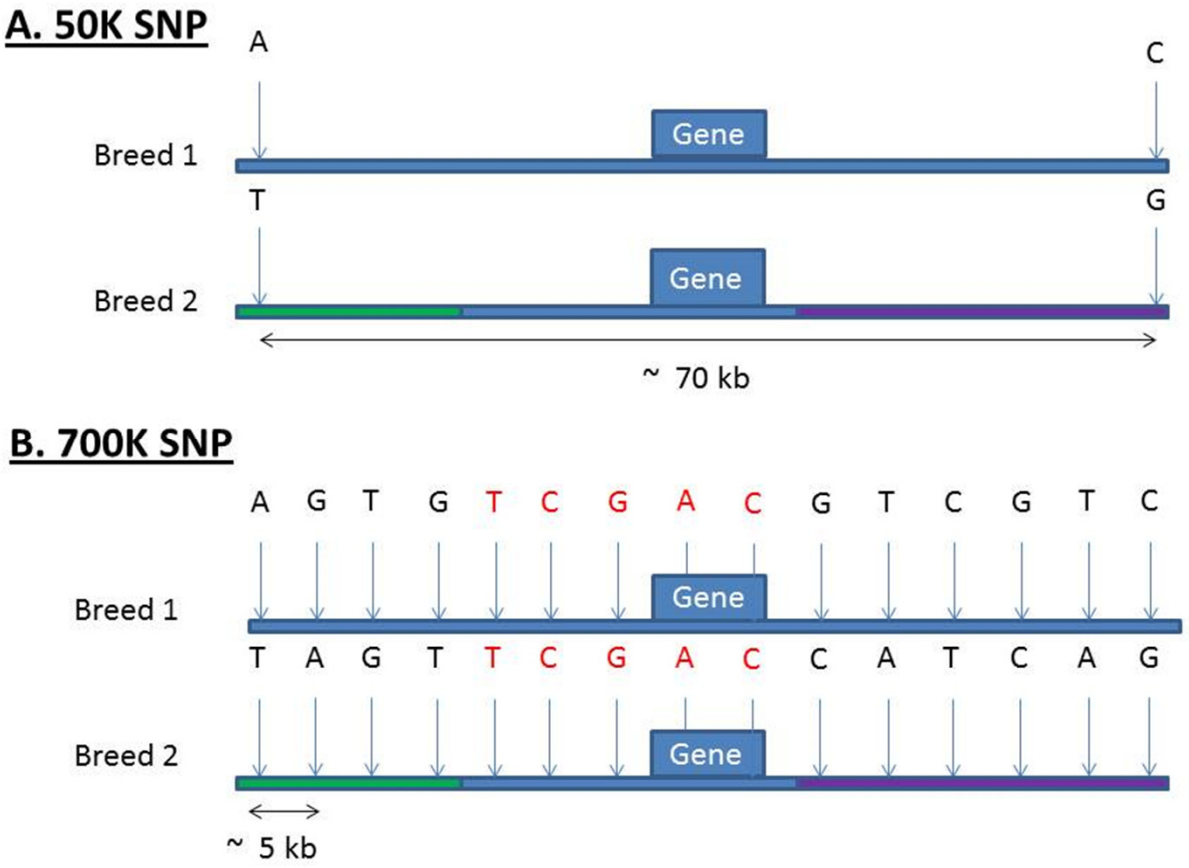
- Mithun Genome as distance
  New Delhi to Dimapur~2,500 Km.

- 3K panel is equivalent to
  marker every == 100Km.

- 50K panel is
  marker every == Km.

- 700K panel is
  marker every == meter.

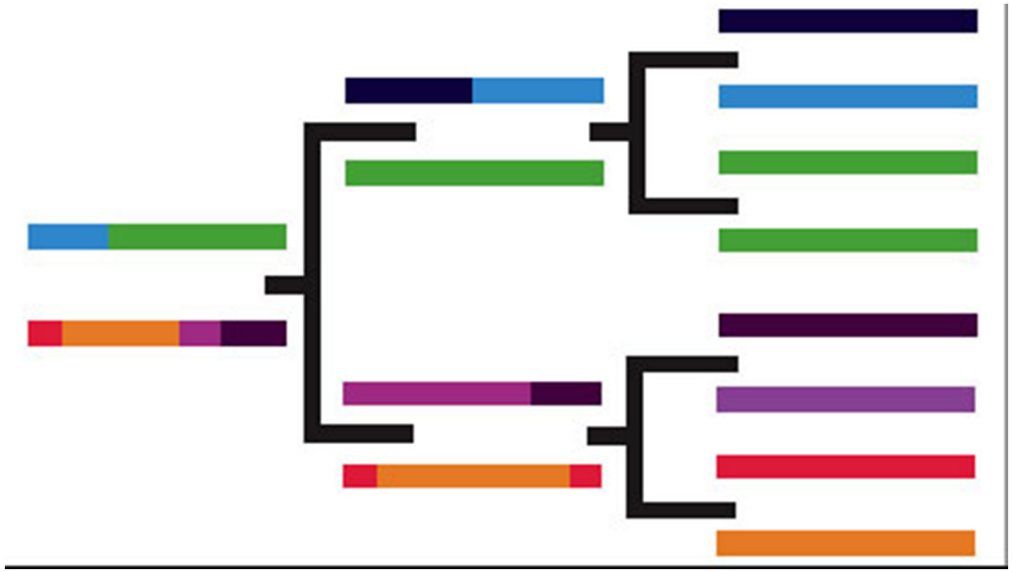
Research Transition for Higher Density Panels



Mithun Genome ~3.5 Billion bp; 3K SNP/1.17 Mb

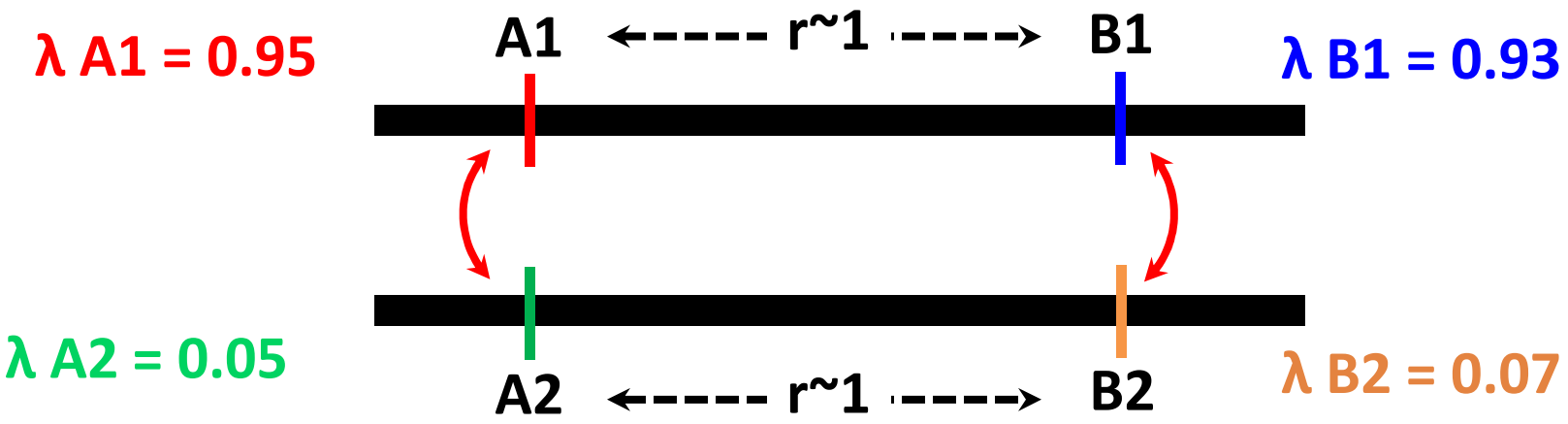
Linkage

- The tendency of certain loci to be inherited together
- Loci that are close to each other on chromosome tend to stay together during meiosis.
- Crossing over (recombination) breaks up linkage.

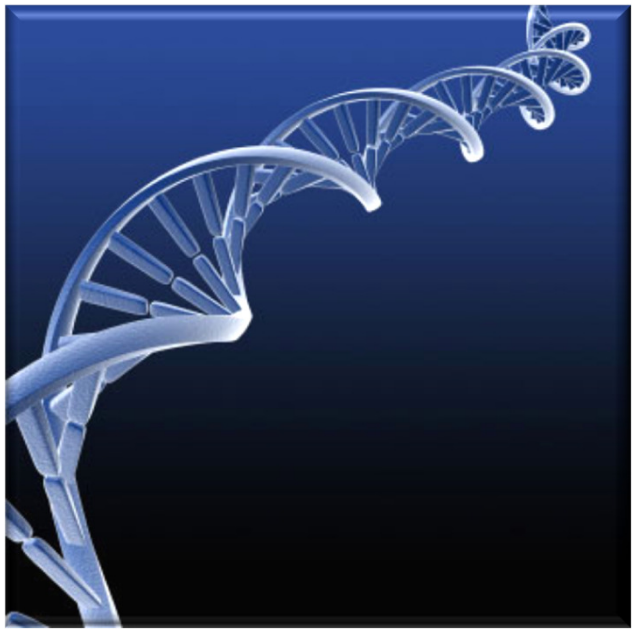


Linkage Disequilibrium (LD)

- LD is the non-random association of alleles (markers) at two or more loci.
- LD describes the ability of SNP at one locus to act as surrogates for SNP at another locus
- Think correlation…ability of SNP at locus 1 to predict SNP at locus 2…
  0 = independent, 1 = dependency



How do we use these tools?



Genome Wide Association Study (GWAS)

- Find associations
  a subset of markers (from a panel of markers)
  with variation in a trait (s)

- Variety of methods available

- With 50K panel not all markers –
  associated with a trait(s), in fact many are not…

- Estimation of Genomic Breeding Value (GBV)
  ---- Genomic Selection (GS)

Developing infrastructures and sign posts for providing directions








A variety of approaches (cars)

- **MAS: MARKER-ASSISTED SELECTION**
  - Animals are selected for one or more (up to 8-10) alleles

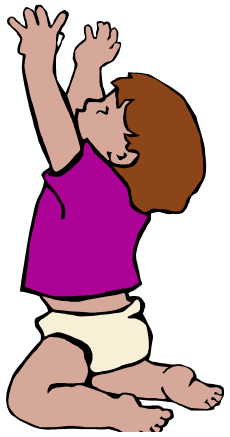
- **MABC: MARKER-ASSISTED BACKCROSSING**
  - **One or more (up to 6-8) donor alleles are transferred to an elite line**

- **MARS: MARKER-ASSISTED RECURRENT SELECTION**
  - **Selection for several (up to 20-30) mapped QTLs relies on index (genetic) values computed for each individual based on its haplotype at target QTLs**

- **GWS: GENOME-WIDE SELECTION**
  - **Selection of genome-wide several loci that confer tolerance/resistance/ superiority to traits of interest using GEBVs based on genome-wide marker profiling**

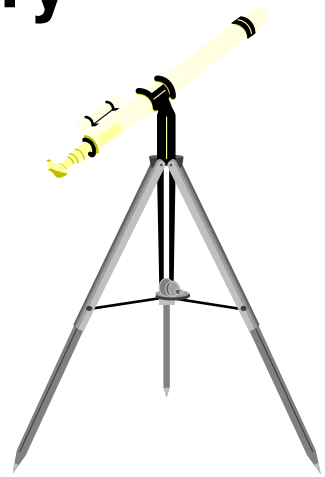
Genome wide selection

- Relatively young technology



- Widely adopted

- Mainly used in gene discovery



Objective

- To characterize the mithun population structure for determining the genetic diversity within population.
- To validate the Bovine SNP770K HD Bead Chip and to determine the level of polymorphism and allele frequency distribution in Mithun population.

Methodology

Study Population

1. Farm stock

Parents, F_1, F_3 and F_4 populations maintained at the NRC on Mithun farm with phenotypic data records. This are especially needed for association studies.

2. Field stock

Blood samples collected all around. Needed for population study

Summary Selected diverse Animals maintained at the farm

Source	Unrelated individuals	Parents	F1	F2	F3	F4
Arunachal	3	1	7	13	6	
Manipur	4		4	12	4	
Mizoram	2	2	13	12		
Nagaland	15	5	16	19	12	5
Total	24	8	40	56	22	5
Grand Total		**16**	**80**	**112**	**44**	**10**

Source	Number related individuals	Number of Families
Arunachal	23	3
Manipur	25	2
Mizoram	28	3
Nagaland	62	10
Total	**138**	

Source	Number of unrelated individuals from field	Total
Dam-geat grand offsprings	29	52
Dam-geat grand offsprings	5	30
Dam-geat grand offsprings	61	89
Dam-geat grand offsprings	36	98
		269

Work Plan

- To characterize the mithun population structure for determining the genetic diversity within the mithun population.

➢ Selection of mithuns from four different strains and recording of phenotypic parameters related to growth.

➢ Estimation of statistical parameters to determine genetic diversity.

➢ Isolation of DNA and assessment of quality and concentration.

➢ Generation of genotypic data using 770K HD bovine Bead Chip.

➢ Identification of clusters using appropriate statistical software and method.

- To validate the Bovine SNP770K HD Bead Chip and to determine the level of polymorphism and minor allele frequency distribution in Mithun population.

- Genotype information and quality control of the genotypic data viz exclusion of sample duplication based on Identity by state (IBS) pruning of genotypic data, estimation of minor allele frequency, SNP call rates and final testing for Hardy- Weinberg Equilibrium.

- Statistical parameters were generated for dependent traits.

QC Steps (1)

- Check genotype gender
- Filter Mendelian inhertance (family-based)
- Check for relatedness...

QC Steps (2)

- SNPs that fail Hardy-Weinberg
  - Suppose a SNP with alleles A and B has allele frequency of p. If random matting, then
    - AA has frequency p*p
    - AB has frequency 2*p*(1-p)
    - BB has frequency (1-p)*(1-p)

QC Steps (3)

- SNPs with low call rate (e.g., <97%) **(GCR)**
  - Proportion of SNPs actually called by software
  - If it's low, the clusters aren't well defined, artifacts
- with low minor allele frequency **(MAF**< 0.05 **)**?
- SNPs / Individuals who have too much **missing data**

Quality Control Report of DNA for Infinium Genotyping

Dr.Anupama – NRCM – Bovine Samples – 16-11-13

Basic diversity indices across population based on 127,432 SNPS

	N	H_{ob} ±SD	H_{ex} ±SD	Inbreeding f	IBS±SD	% SNPS not in HWE (P ≤ 0.05)	% Markers with MAF≥0.05
Arunachal		0.2995±0.0442	0.2675±0.1690	0.1047±0.1321	0.8320±0.0254	0.8537±0.0030	100
Manipur		0.2665±0.0171	0.2452±0.1833	0.1858±0.0526	0.9334±0.0574	0.7151±0.0037	100
Mizoram		0.2872±0.0356	0.2567±0.1711	0.1612±0.1037	0.8130±0.0207	0.7182±0.0035	100
Nagaland		0.2629±0.0400	0.2589±0.2629	0.0865±0.1389	0.8567±0.0285	1.1698±0.0014	85.4

Global and pairwise fixation indices for mithun strains using 127,432 SNPs

	Arunachal	Manipur	Mizoram	Nagaland
Arunachal	-	0.0658	0.0512	0.0566
Manipur	-	-	0.0808	0.0496
Mizoram	-	-	-	0.0561
Nagaland	-	-	-	-
F_{IS}	0.8705			
F_{IT}	0.8778			
F_{ST}	0.0563			

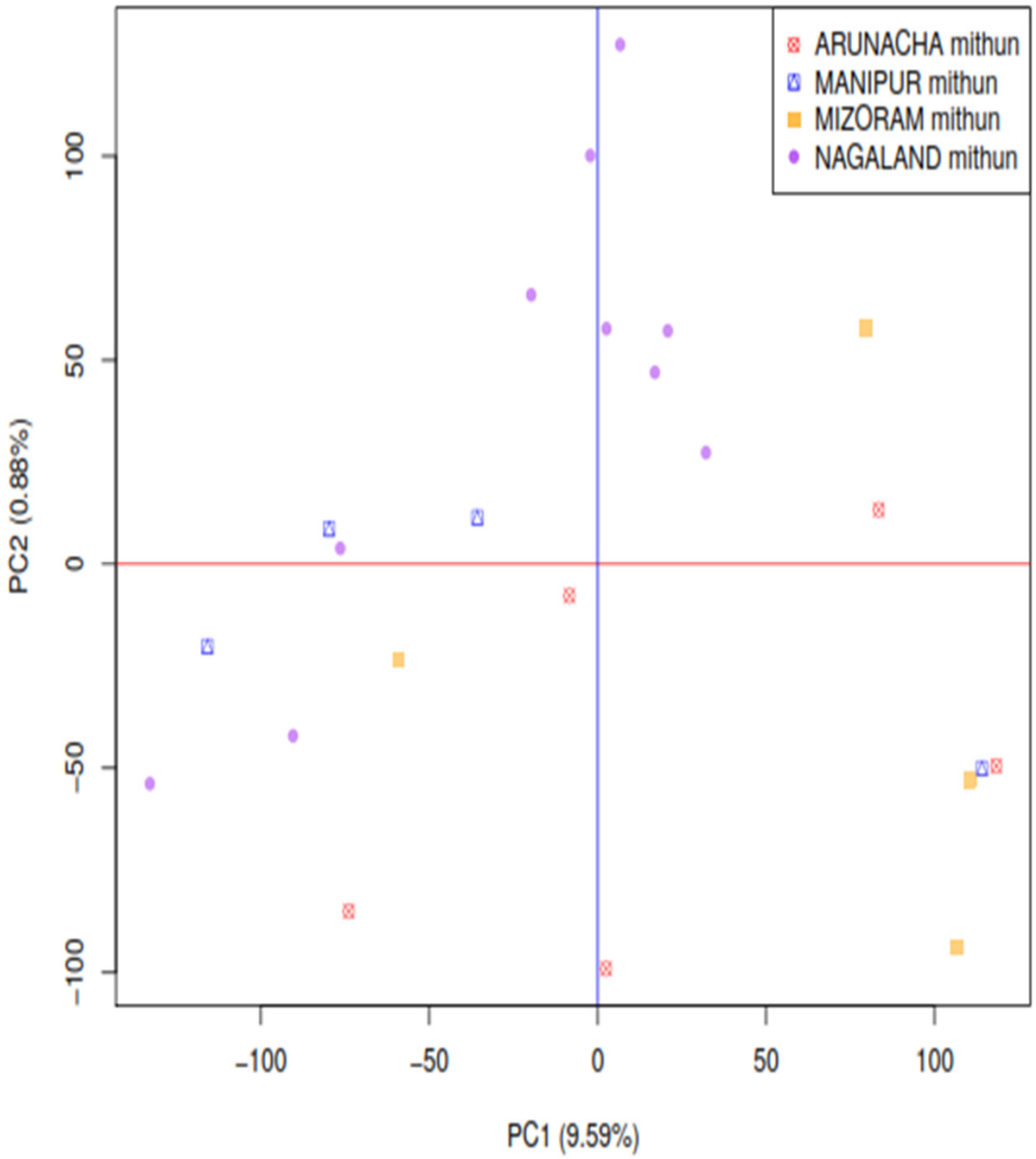


Figure 1. Population stratification of mithun strain based on PCA across 127,432 SNPs. PC1 showing north-south orientation, while PC2 shows an east west orientation.

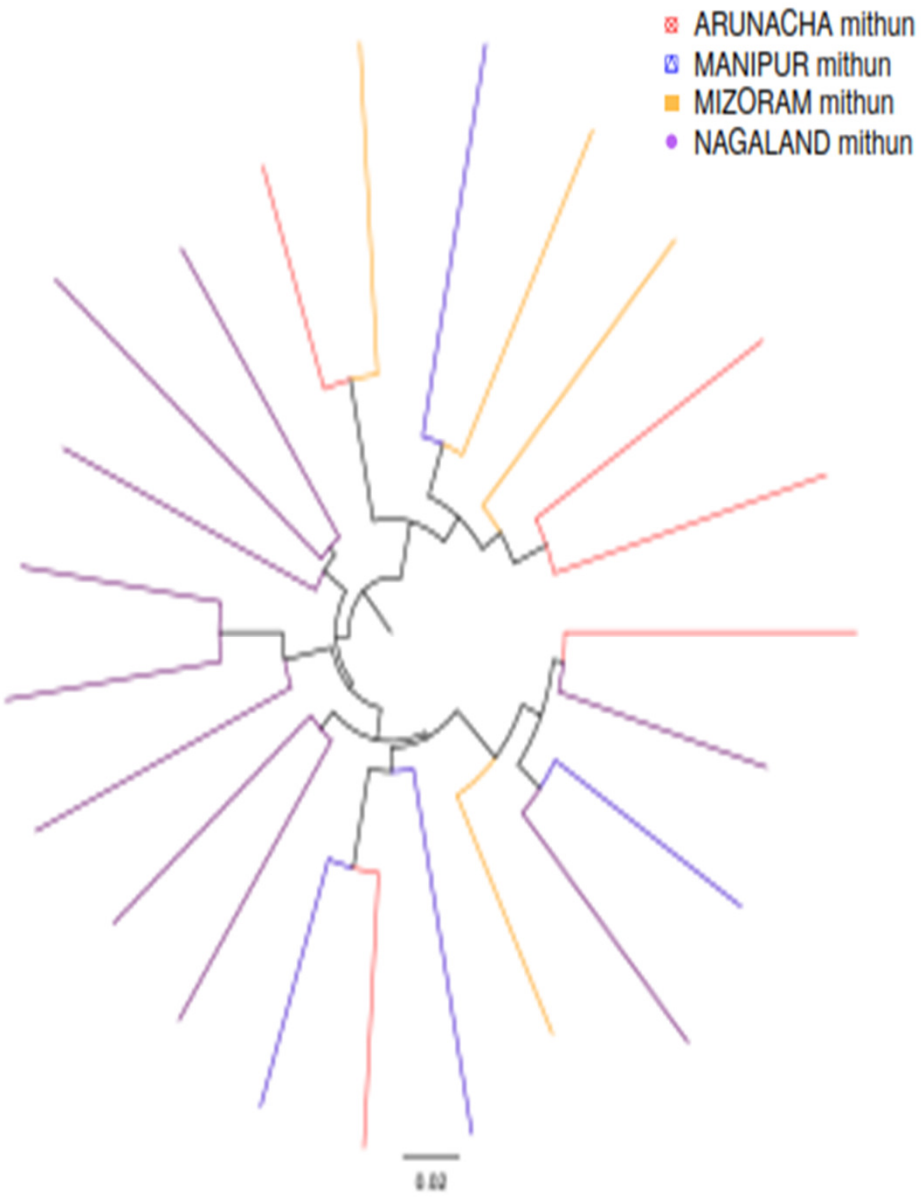


Figure 2. Neighbour-joining tree reconstructed using MEGA 5.2 software from 127,432 SNPs among strains of mithun populations.

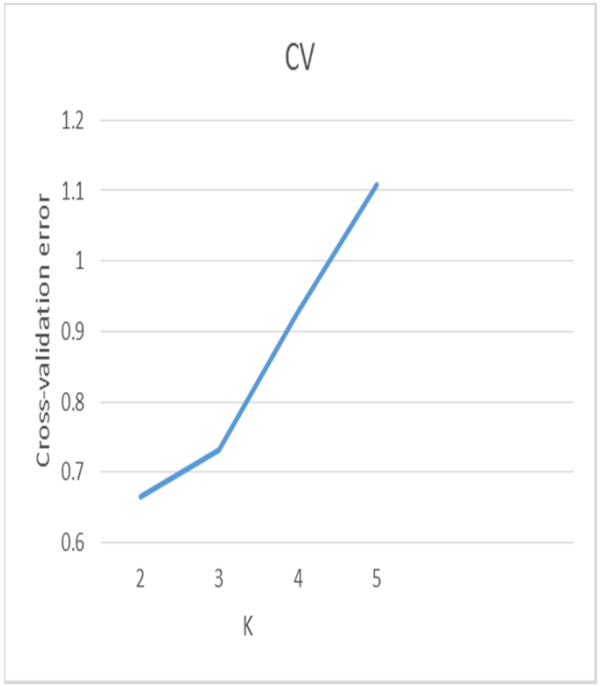


Figure 3. Plot of cross validation error for *k* for the population structure analysis.

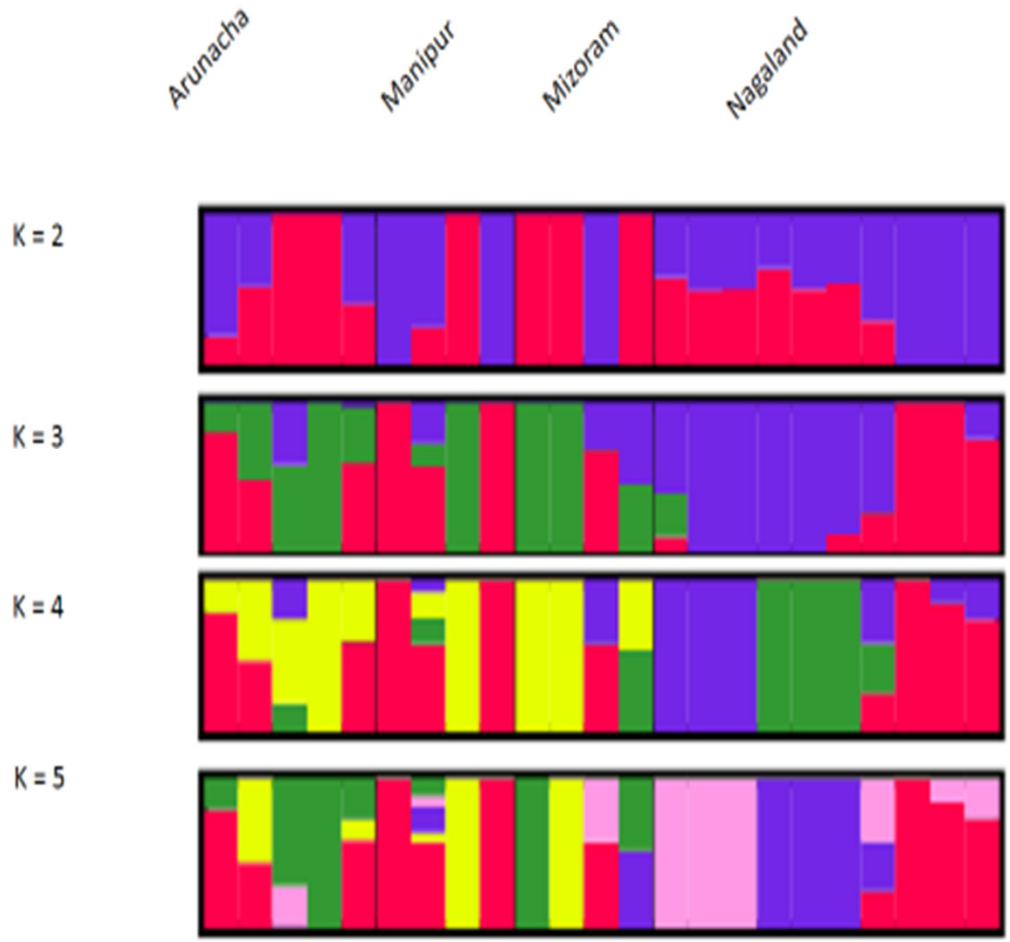
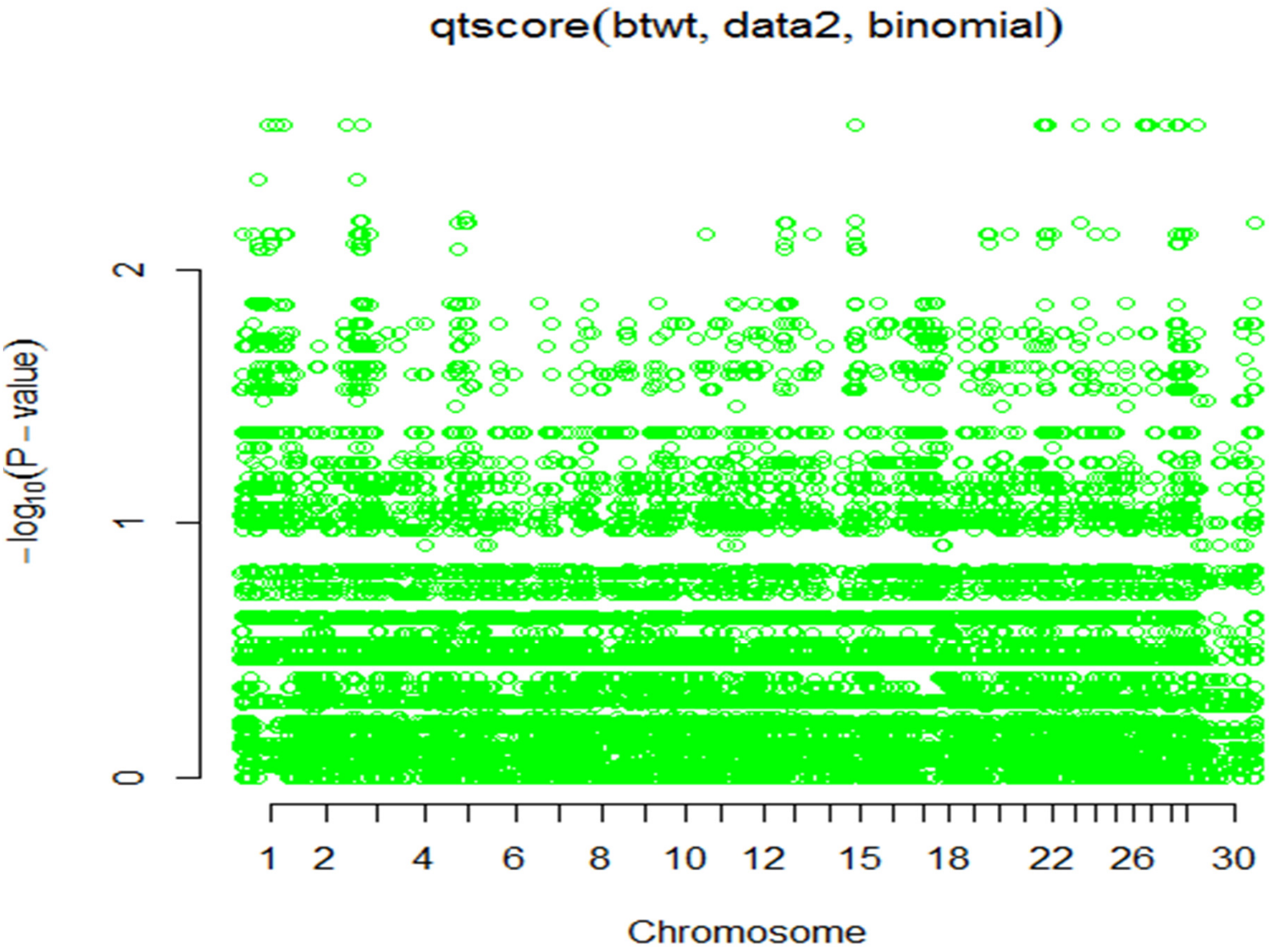


Figure 4. Estimated Population Structure using 127, 432 SNPs generated by ADMIX for *K*=2-5


qtscore(btwt, data2, binomial)
−log10(P−value)
0
1
2
1
2
4
6
8
10
12
15
18
22
26
30
Chromosome

Conclusions

In this study the use of the Illumina 770k HD Bead chip has not being successful enough to infer using it as a tool for selection purpose in mithun improvement program.

Inclusion of mithun genome in future design will aid such purpose.

The mithun population show little divergence from each other based on the polymorphic loci.

There was no substructure inferred. Non sub structure can either be due to the difference in the chip used or geographical distance of the mithun population is not enough to label them as separate populations.

Recommendation

This preliminary study being the first of its kind on mithun has been able to show that 80% of the SNPs on the array have MAF < 0.05, so approximately 20% are polymorphic (1/5).

For the purpose of conservatory program via utilization or ex-situ conservation of the mithun it is recommended before genome wide SNP analysis can be achieved a whole-genome re-sequence of a pool of 10 individuals (1 library) on Illumina HiSeq2000 is carried out. Map reads could then be done to UMD3.1 genome, or try **de novo assembly**.

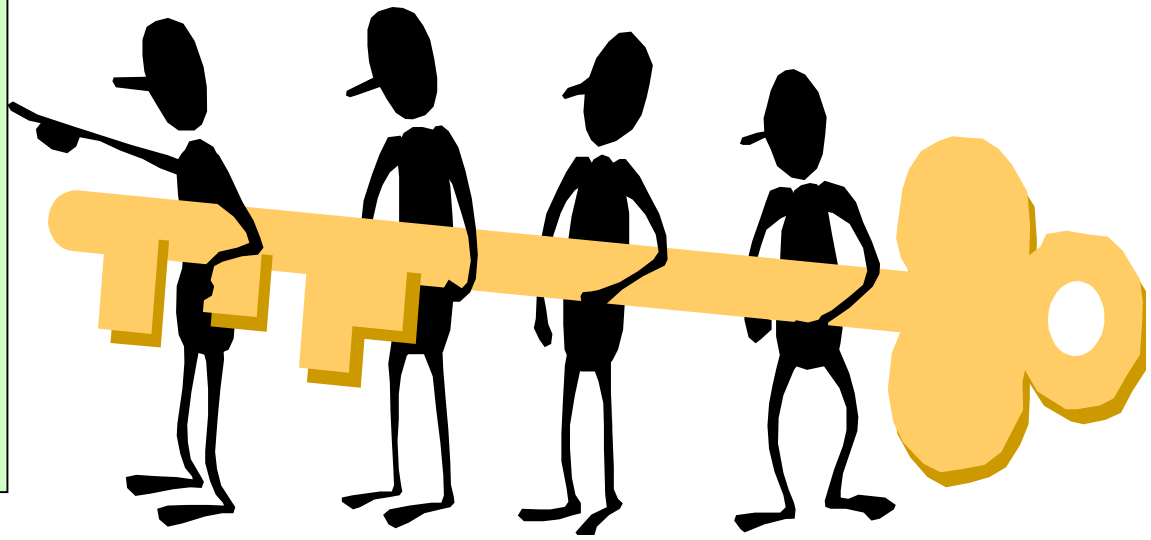
Possible outcomes

- **Breeder-friendly genome database of mithun.**

Only as team
we can be successful!

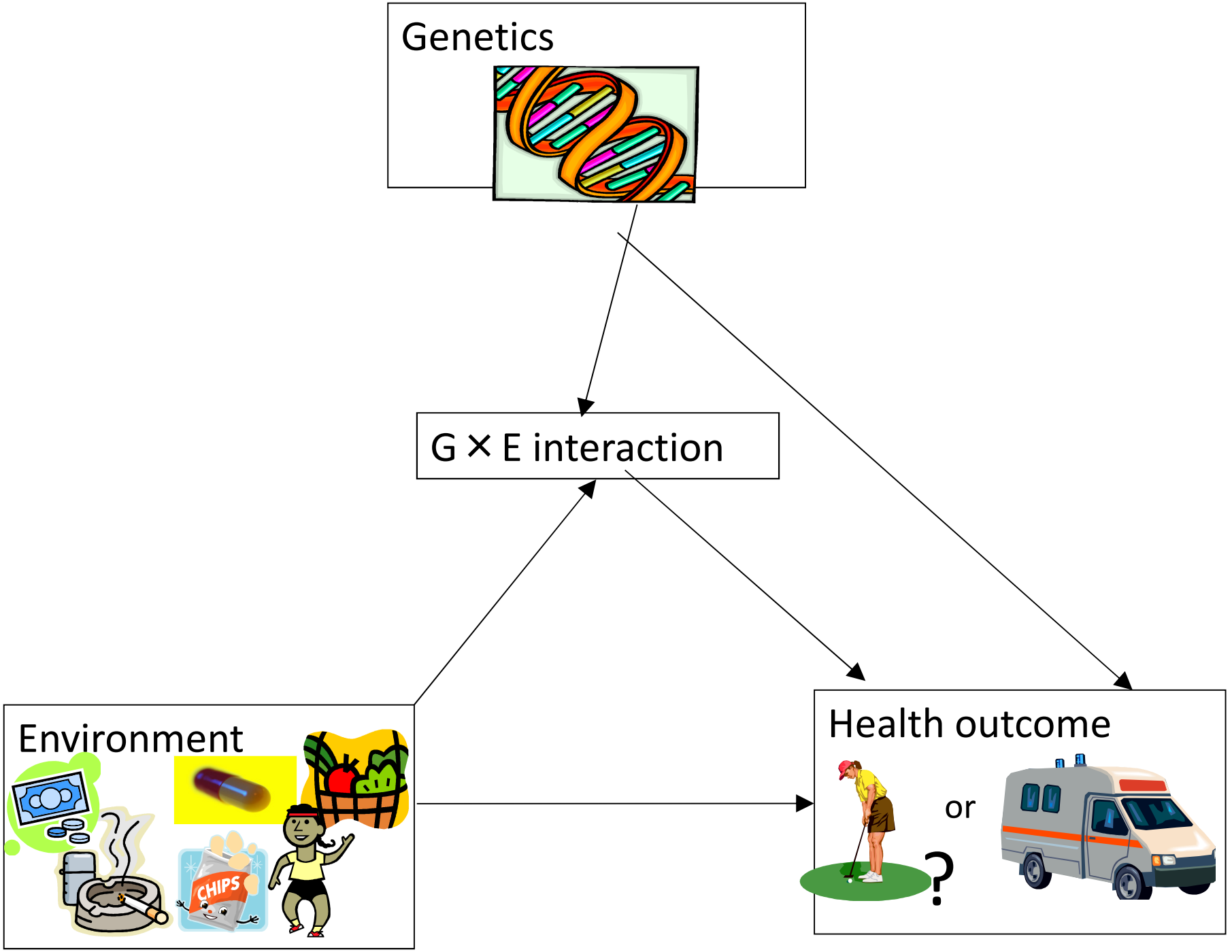
We are key to the success!

Anupama Mukherjee;
Sabyasachi Mukherjee;
A.O.Adebambo;
Imusosang Longkumer;
Moonmoon Mech
and
C.Rajkhowa



Thank you !!

Investing in science : Securing our future


Genetics
G × E interaction
Environment
CHIPS
Health outcome
or
?