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UNIVERSITÀ DEGLI STUDI DI PADOVA

Molecular Characterization of Italian Heavy Draught Horse (IHDH) using mtDNA and microsatellite markers

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Aim

Assessing genetic diveristy in the IHDH for efficient management decisions.

Molecular markers:
-microsatellite (23)
-mtDNA (Dloop)

Exploit:
-genetic variabilty
-molecular inbreeding
-population structure
-phylogenetic network

IHDH breed

Estabilshed in **1926** by the Italian government

Originated from crosses of **Norfolk-Breton stallions** with local derived Hackney, Percheron and Bretons mares.

Developed for agricutural draught works and artillery transport.
Nowadays it is manly selected for muscolarity and heavy draught works.

ASSOCIAZIONE NAZIONALE ALLEVATORI DEL CAVALLO AGRICOLO ITALIANO DA TPR

www.anacaitpr.it

Veneto: 785

Emilia R.: 594

Abruzzo: 850

Umbria: 1082

Lazio: 1814

Puglia: 291

Total: 5938

Samples

Blood spots on FTA® Cards of **130** unrelated animals:

90 IHDH:

- 34 from Vicenza
- 21 from Padova
- 35 from Rieti

19 IH (Italian Haflinger)

21 QH (Quarter Horse)

Methods

23 microsatellite:

Lex33, Aht4, Asb2, Asb23, Hms2, Hms3, Hms6, Hms7, Htg4, Htg6, Htg10, I18, Htg3, Tky341, Tky337, Vhl20, Tky301, Tky321, Cor69, Tky343, Um5, Cor22

DNA Purification and amplification in 5 multiplexes directly from FTA punches.

Pooled amplicons analysed on a Beckman Coulter CEQ 8000 and sized using Genetic Analysis Software v. 9.0

Statistical analysis:

MSA, Genepop 4.0, FSTAT 2.4.3, Molkin 3.0, Structure 2.3.1, BOA (R)

Methods

mtDNA Dloop:

304bp fragment within hypervariable domain I

IH: **15** sequences
IHDH: **22** sequences
QH: **17** sequences

DNA purification and amplification directly from FTA punches, sequenced in both directions using Beckman Coulter CEQ 8000 sequencer.

Phylogenetic analysis

eBioX, DNASP 5, Arlequin 3.11, Phylip

Molecular Characterization of IHDH

microsatellite RESULTS

202 alleles (**8.78** alleles/locus)
- **Tky343** -> **16** alleles
- **Htg7**, **Cor22** -> **5** alleles

W&C	**F_{ST}**	**F_{IT}**	**F_{IS}**
	0.073***	0.110***	0.040***

	Het Obs.	**Het Exp.**	**NMA**	**AMC**	**Fis (95% CI)**
IH	0.66 ± 0.18	0.69 ± 0.13	5.43	0.33	0.043 (-0.054 - 0.075)
IHDH	0.67 ± 0.15	0.71 ± 0.12	7.91	0.28	0.057 (0.023 - 0.080)
QH	0.77 ± 0.15	0.75 ± 0.07	5.74	0.27	-0.022 (-0.093 - -0.008)

W&C Pairwise **F_{ST}**

	IH	**IHDH**
IHDH	0.057	-
QH	0.097	0.083

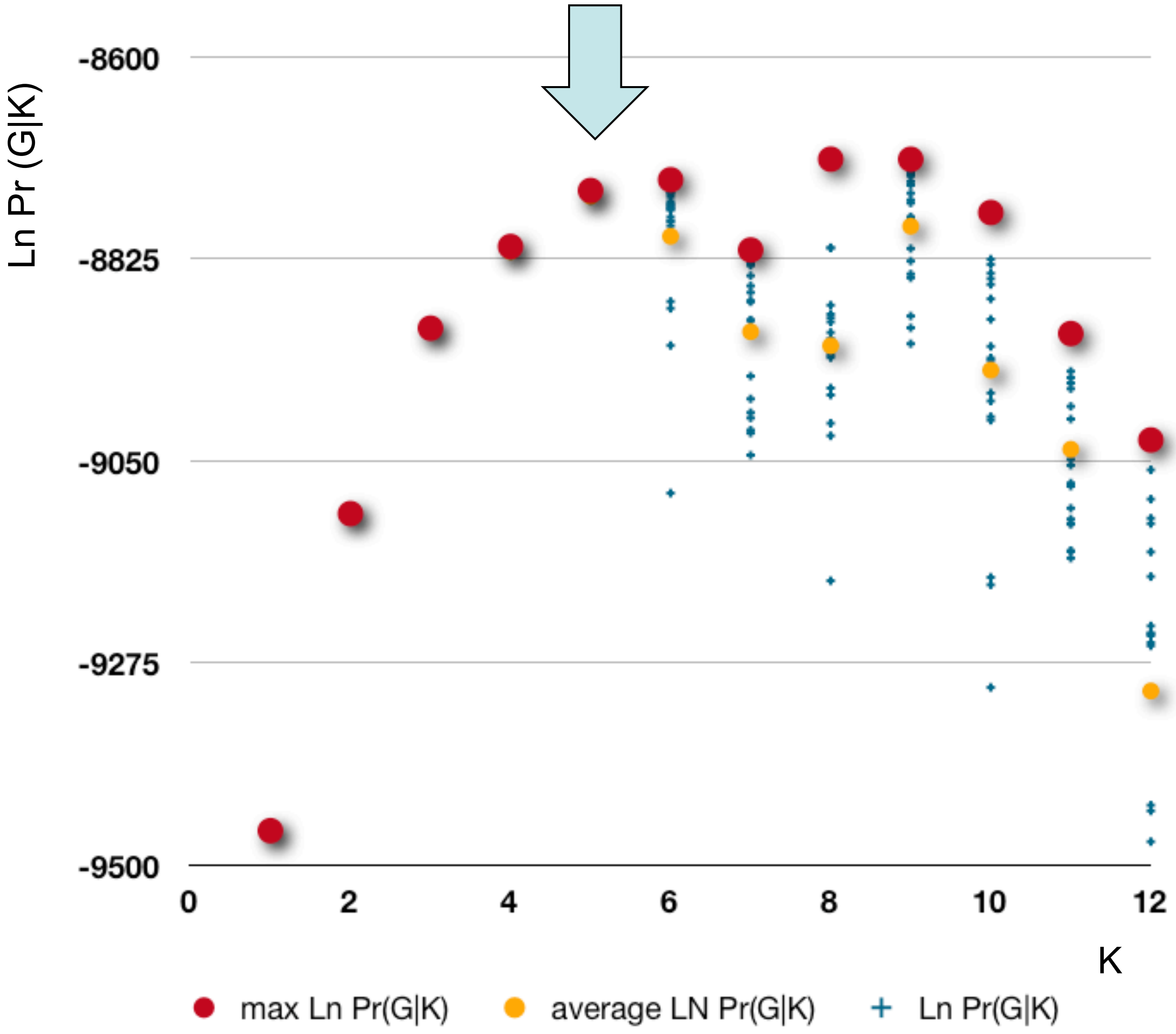
Molecular Characterization of IHDH

microsatellite RESULTS

STRUCTURE implements a model based clustering methodology to be used with multilocus genotype data to infer population structure and assign individuals to different populations

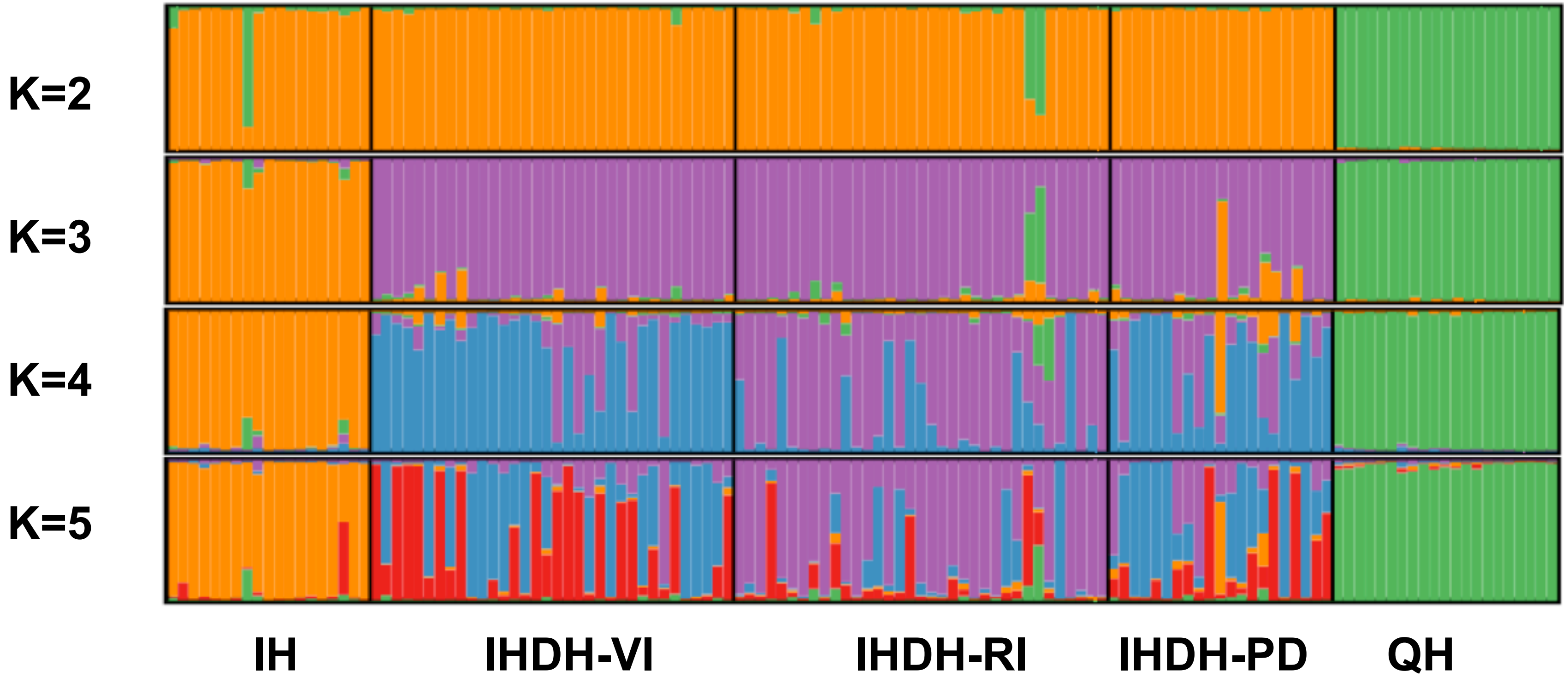
STRUCTURE
whole sample

Analysis parameters:
1<K<12
Admixture
Correlated Allele Frequencies
Burn-in: 20000
Reps: 250000
Replica: 20



microsatellite RESULTS

Graphical presentation of population structure (a priory defined 5 populations)



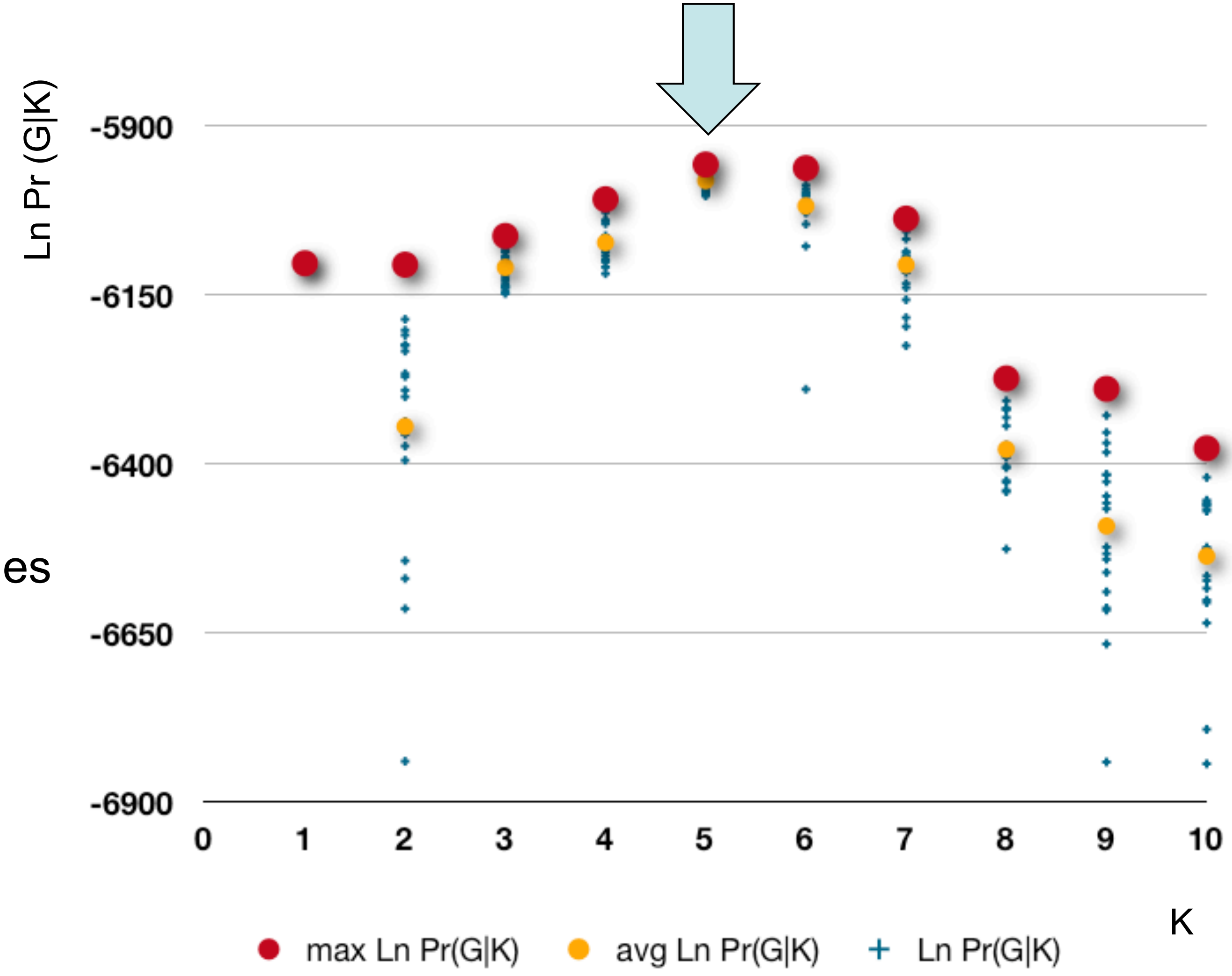
Each horse is represented by a single vertical line broken in K colour segments with lenghts proportional to the estimated membership of the inferred cluster

Molecular Characterization of IHDH

microsatellite RESULTS

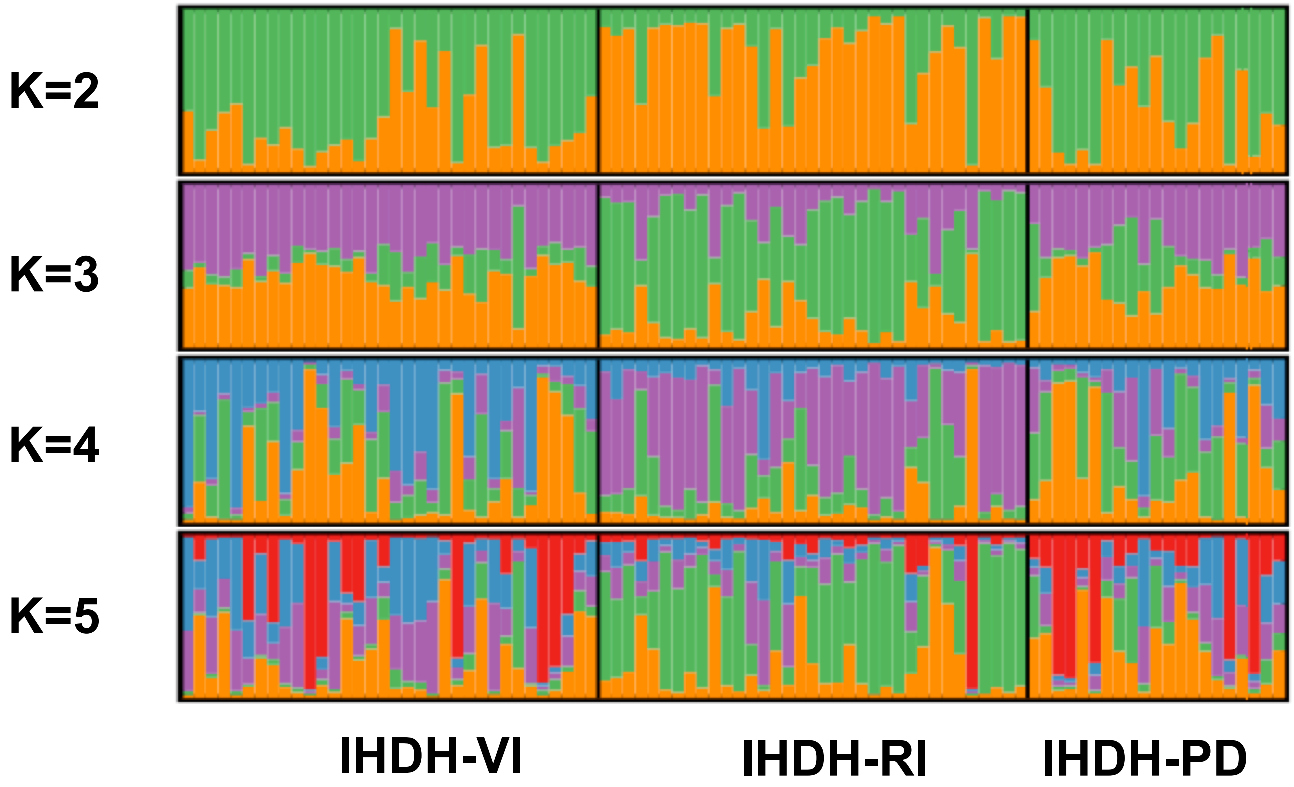
STRUCTURE
IHDH sample

Analysis parameters:
1<K<10
Admixture
Correlated Allele Frequencies
Burn-in: 100000
Reps: 1000000
Replica:20



microsatellite RESULTS

Graphical presentation of population structure (a priory defined 3 populations)



Each horse is represented by a single vertical line broken in K colour segments with lenghts proportional to the estimted membership of the inferred cluster

mtDNA RESULTS

56 sequences (15 IH, 22 IHDH and 17 QH)

29 polymorphic sites (304 bp fragment)

	N. hap.	**h**	**π**	**Φ_{ST}**
IH	9	0.933	0.021	0.025
IHDH	15	0.961	0.020	0.028
QH	9	0.890	0.018	0.035
all	27	0.962	0.020	0.029 n.s.

Shared haplotypes:

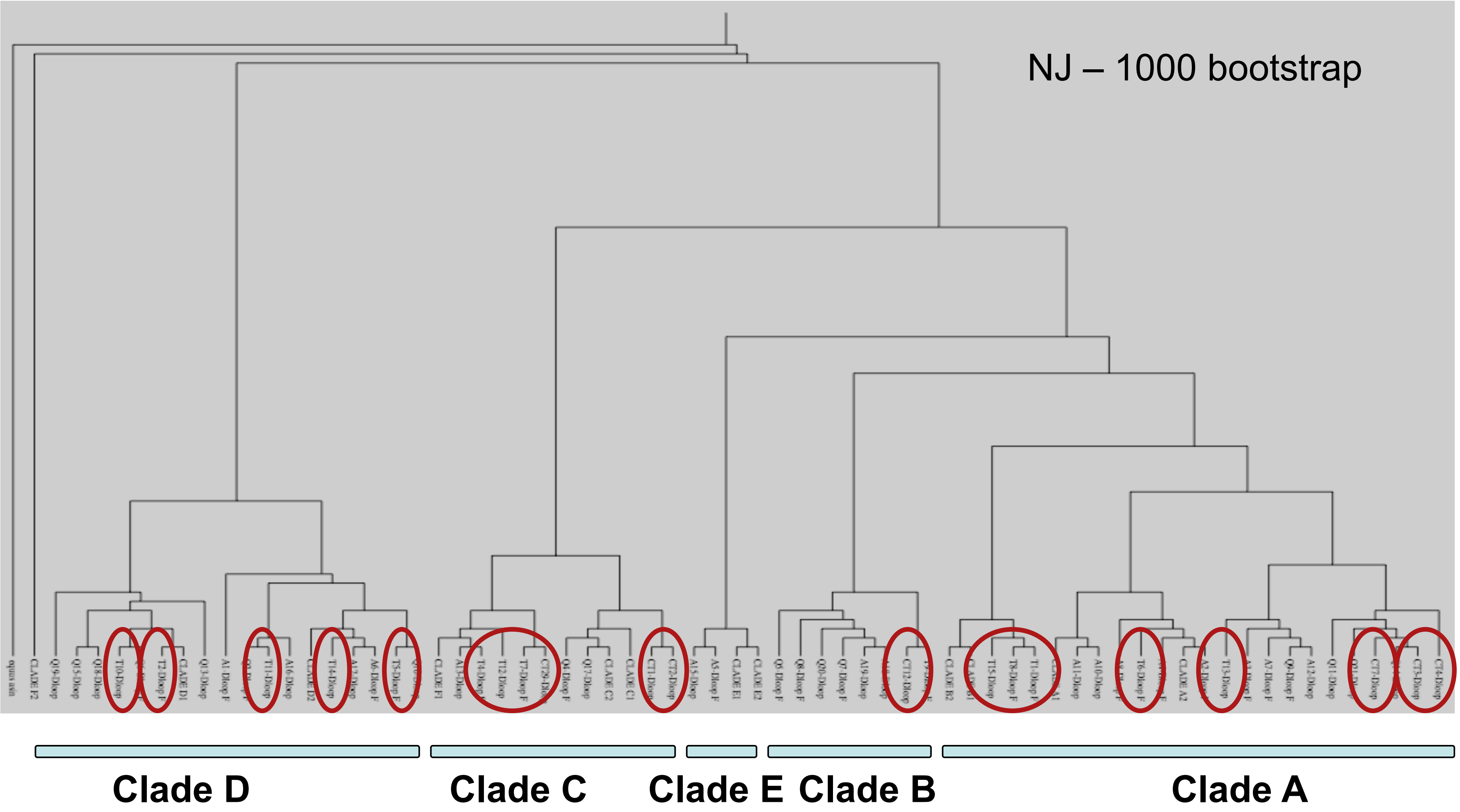
- IHDH vs IH: **5**
- IHDH vs QH: **3**
- IH vs QH: **1**

Pairwise Φ_{ST}

	IH	**IHDH**
IHDH	0.020	
QH	0.064	0.010

Molecular Characterization of IHDH

mtDNA RESULTS



Vila et al. (2001)

CONCLUSIONS

IHDH showed a **good genetic variability** compared to IH and QH, a very **moderate inbreeding level** and a **very limited molecular coancestry.**

Analysis of population structure indicates **presence of substructure** in **IHDH** probably due to limited interchange of genetic material among herds.

mtDNA analysis also show a **high genetic diversity** of **IHDH** breed probably due to the **recent mixed ancestry** of the breed. It also show **different maternal lineages** due to diverse provenance of female founders.

Acknowledgments

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Project Biotech I

… thank you.