

Genomic data as a prerequisite for efficient conservation programme of the Czech Red cattle

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Abstract no 29760

The breed



Czech Red cattle

- low effectiveness
- prominent health traits
ancient origin, from Celtic shorthorn?
- speculative admixture of *B. primigenius*
- conserved from early 20'
- restored from 1 (50%) + 14 animals in 1987
- current population of 263 animals

- Program of Conservation and Utilisation of Farm Animal Genetic Resources

Czech Red cattle



Czech Red – additional data

Admixture from breeds:

- Polish Red
- Angler
- German Mountain Red cattle
- Czech Simmental cattle
- Ayrshire
- Red Holstein
- Piedmont cattle

Previous diversity studies

Čítek et al., Czech J. Anim. Sci. 51:429, 2006; Zaton-Dobrowolska et al., Anim. Sci. Pap. Rep. 25:45, 2007 - STRs and selected coding loci.

Čítek and Antes, Živočišná Výroba 41:49, 1996; Čítek et al., Czech J. Anim. Sci. 43:97, 1998; Čítek et al., Czech J. Anim. Sci. 46:433, 2001 - intravarietal milk protein polymorphism.

Hořín et al., Živočišná Výroba 42:533, 1997 - polymorphism in major histocompatibility complex class I.

Reference populations



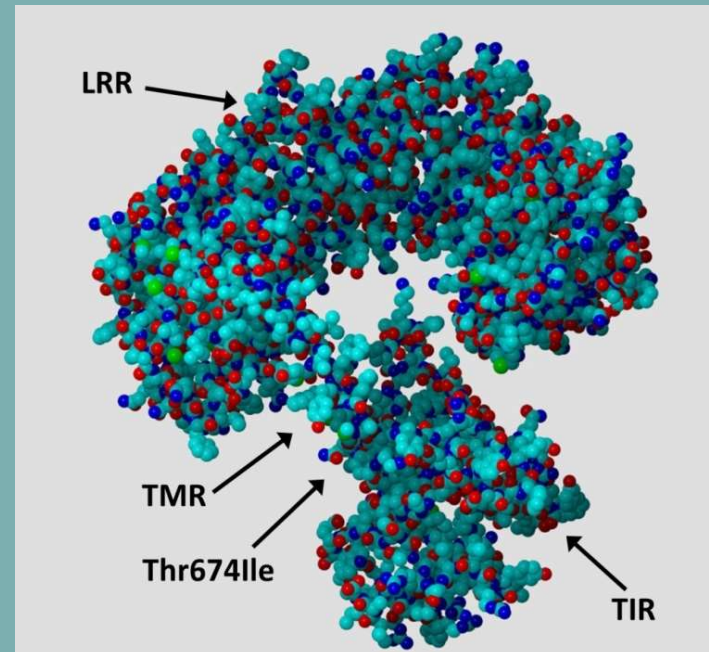
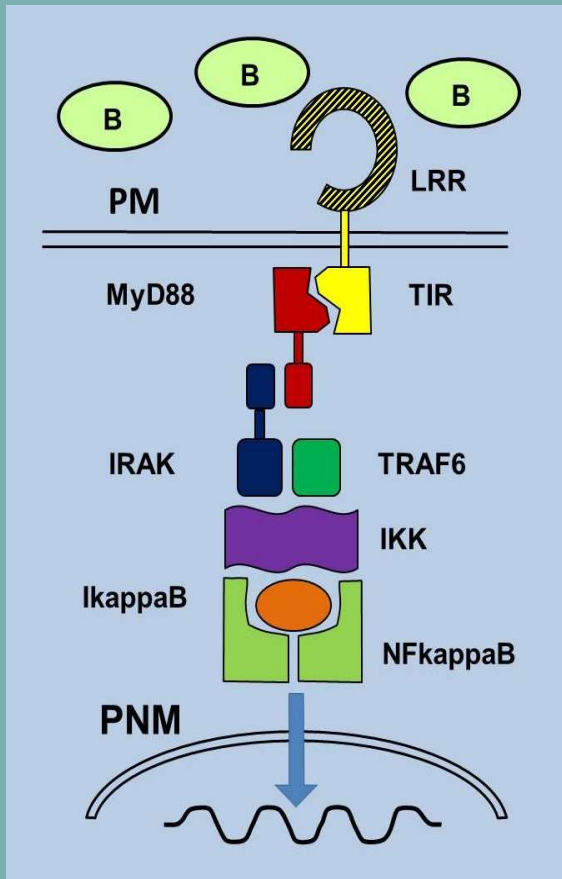
Czech Red Pied cattle
(Czech Simmental)

conserved x production populations

- origin in 19th century
- dual-purpose breed
- good effectiveness
- original type conserved in the population of 80 animals
- National Program of Conservation and Use of Farm Genetic Resources

Targeted resequencing of immunity genes

Toll-like receptor series 1 – 10
(*TLR* genes)

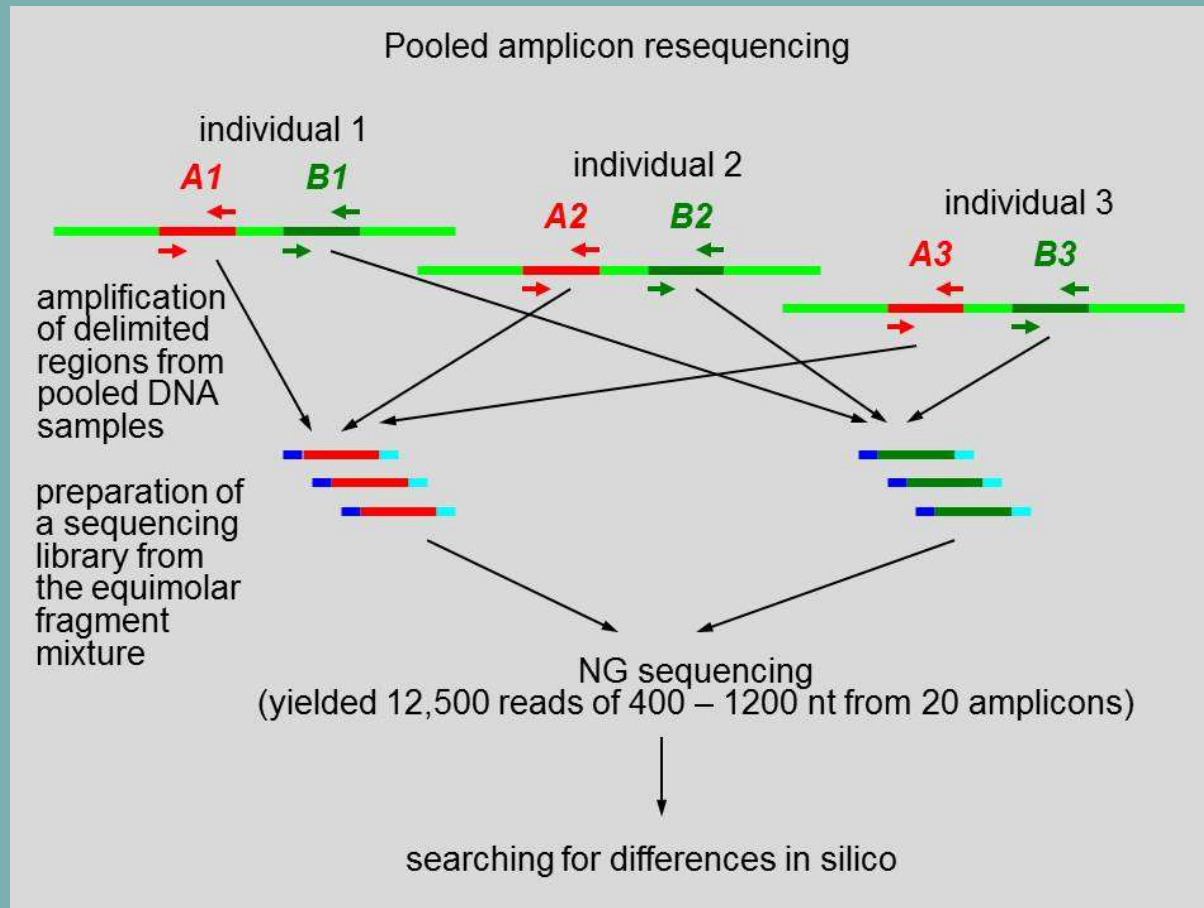


TLR gene diversification

Anti-bacterial TLRs	Recognised molecule	Candidate function in cattle
<i>TLR1</i>	lipopeptides of G+ bacteria	mycobacterial infection resistance, mastitis
<i>TLR2</i>	bacterial lipopeptides	mycobacterial infection resistance
<i>TLR4</i>	bacterial lipopolysaccharides	mastitis resistance, bovine respiratory disease complex
<i>TLR5</i>	flagellin	G- bacteria infections
<i>TLR6</i>	microbial lipopeptides	mastitis (QTL co-localisation)

Anti-viral TLRs	Recognised molecule	Candidate function in cattle
<i>TLR3</i>	dsRNA	QTL on chromosome 22
<i>TLR7</i>	ssRNA	viral infections
<i>TLR8</i>	ssRNA	viral infections
<i>TLR9</i>	CpG DNA	QTL on chromosome 27

Targeted resequencing of *TLR* genes with PacBio technology



Advantages of PacBio platform for resequencing:

- long reads enabling for direct phasing of present SNPs,
- easy bioinformatic processing.

Variants and haplotypes of antibacterial *TLRs*

Only 8 substitutions of 158 known in the coding sequences of *TLR4*.

But 18 haplotypes x 29 haplotypes in the world panel of 31 production breeds (Fisher et al., 2011), i.e. 62,1%.

Conclusion: Historical breed can be a source of diversity for resistance breeding.

Gene	Found SNPs/indels
<i>TLR1</i>	4/0
<i>TLR2</i>	14/0
<i>TLR4</i>	8/0
<i>TLR5</i>	30/1
<i>TLR6</i>	45/0
Total	101/1

TLR4 haplotypes

Found haplotype (gDNA 245, 610, 5087, 7999, 9422, 9787, 10307, 10310 , ref. AC000135.1)	Published haplotype (White et al. 2003)	Frekvency (%)	
		Czech Red	Czech Red Pied
Shared haplotypes			
G_C_A_G_C_C_T_T	A2	37.6	40.0
C_C_G_A_T_C_T_T	A5	27.1	28.3
G_C_G_G_C_C_T_T	A1	2.4	1.7
Haplotypes specific for CR			
C_C_G_A_C_C_T_T	A1	11.2	0.0
C_T_G_A_T_C_T_T	A5	8.8	3.3
G_C_A_A_C_C_T_T	A2	3.5	0.0
C_C_G_G_T_C_T_T	A5	1.8	0.0
G_T_G_A_T_C_T_T	A5	1.2	0.0
C_C_A_A_C_C_T_T	A2	0.6	0.0
G_C_A_G_C_C_T_G	-	0.6	0.0
G_C_G_G_C_C_T_G	-	0.6	0.0
C_C_A_G_C_C_T_T	A2	0.6	0.0
Haplotypes specific for CRP			
C_C_G_A_C_T_T_T	B1	1.8	13.3
C_C_A_A_T_C_T_T	-	1.2	3.3
G_C_G_A_T_C_T_T	A5	1.2	3.3
C_C_G_A_C_C_C_G	A4	0.0	3.3
C_C_A_A_C_T_T_T	-	0.0	1.7
G_C_A_A_T_C_T_T	-	0.0	1.7

Genome-wide sequencing

Mixed DNA sample from the population of 85 animals of the Czech Red cattle was sequenced with 60x coverage with X-ten technology.

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Comparative data from the Czech Simmental cattle (Czech Red Pied):

- 30 animals from genetic resources herd,
- 150 bulls from the production population..

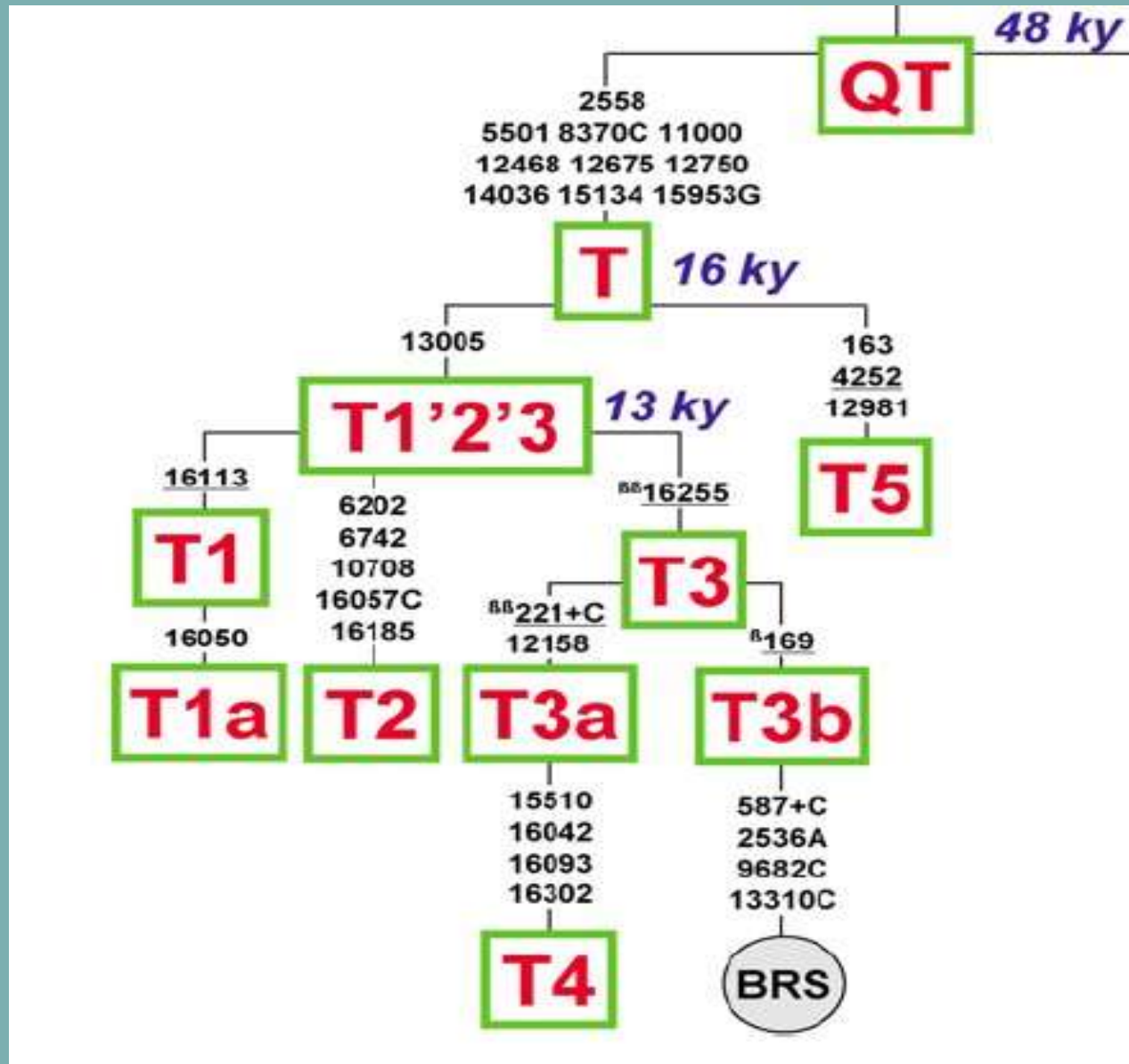
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gDNA of individual animals (2 Czech Red, 2 Czech Simmental) was sequenced at 20x coverage.

12.5 M polymorphisms detected with respect to the bovine reference sequence (Hereford).

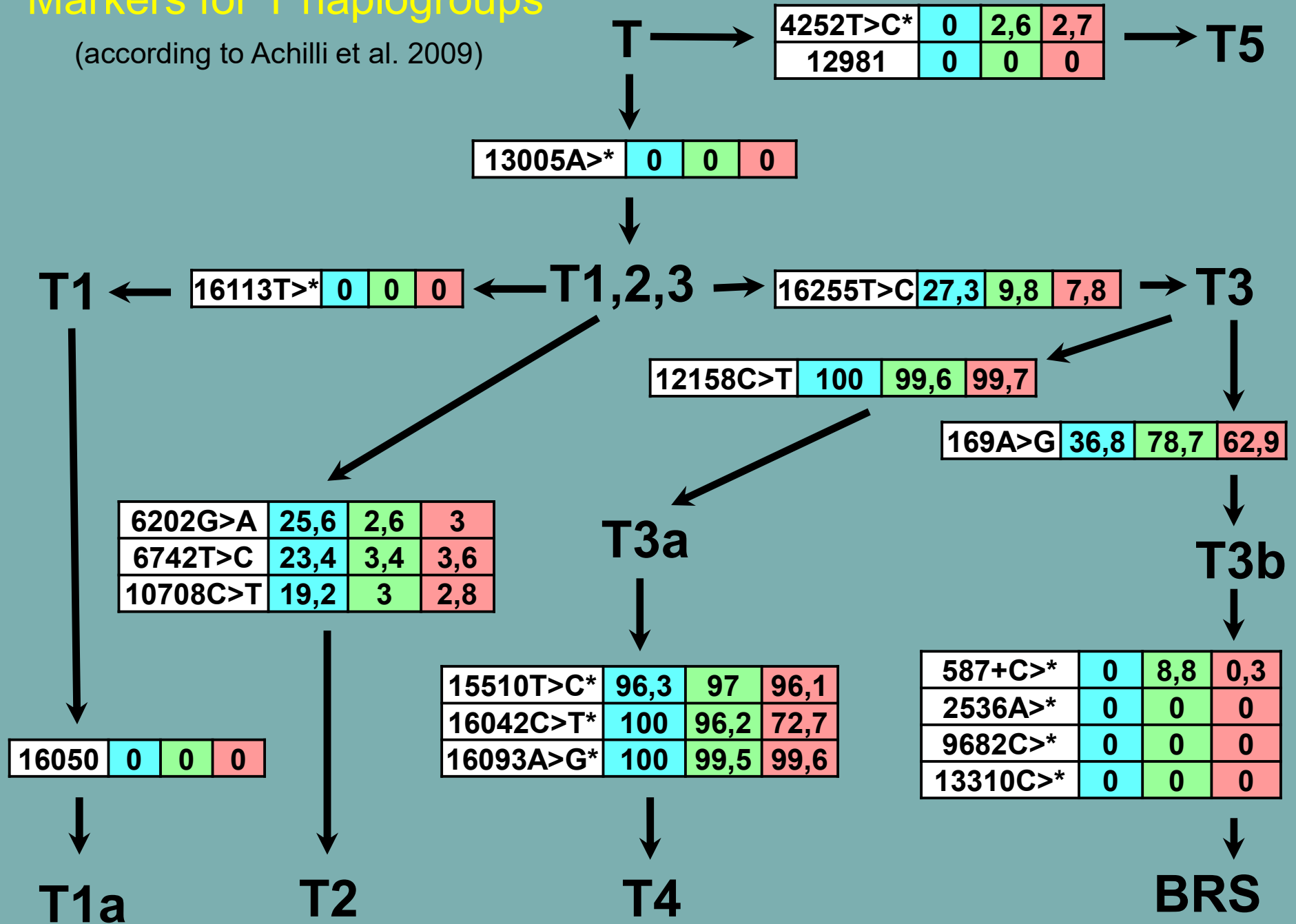
Markers for T haplogroups

(Achilli et al., PLoS ONE 4: e5753, 2009)



Markers for T haplogroups

(according to Achilli et al. 2009)



Bos primigenius mtDNA markers I.

	15793 (D-loop start)	16058	16074	16085	16121	16122	16231	16247	16264	16301
Ref. (V00654)	A	C	T	T	G	T	C	C	G	C
Bp (DQ12438)		T	C	C	G	C	T	T	A	C
Bp (CPC98)					R			C		T
PWA (JQ437479)									A	
CRP			C 40,0		A 8,3	C 16,7	T 13,0			T 8,3
CRP-GR				C 3,5			T 2,3	T 4,4	A 5,5	T 28,4
CR		T 20,8	C 4,2				T 23,1	T 7,2	A 4,7	T 4,5

DQ12438 – Achilli et al. 2008,
 CPC98 – Edwards et al. 2010,
 JQ437479 – Zeyland et al. 2016

CRP – Czech Red Pied cattle,
 CRP-GR – Czech Red Pied cattle – genetic resources,
 CR – Czech Red cattle

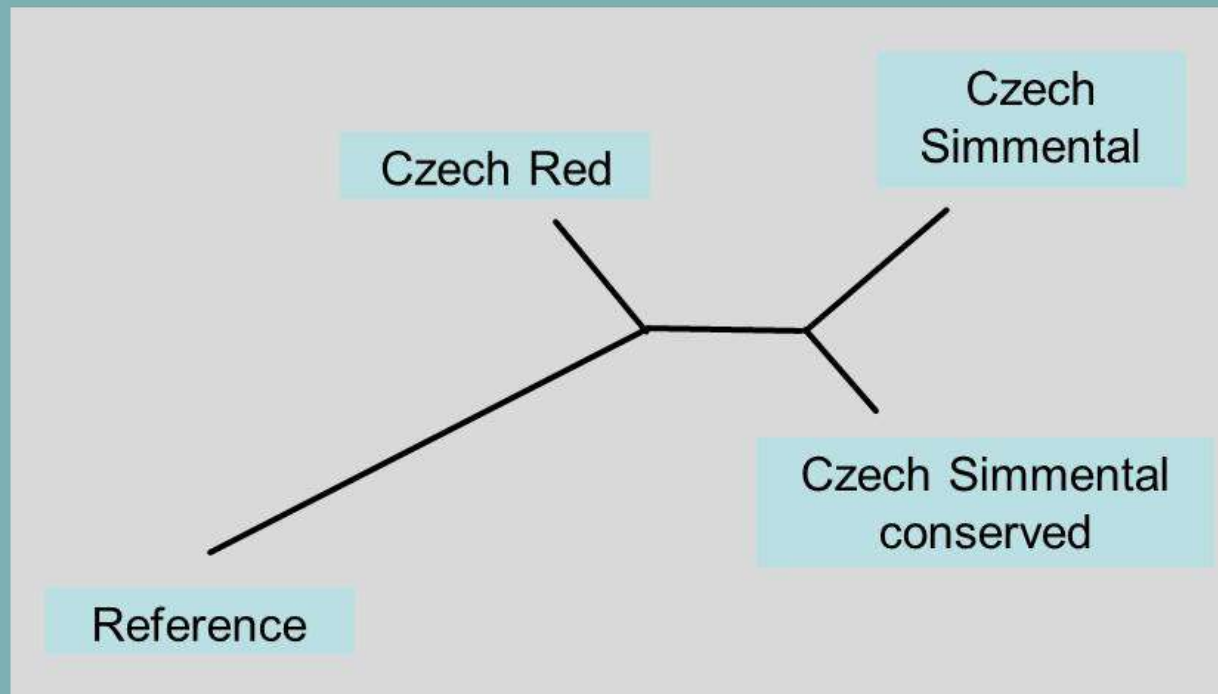
Bos primigenius mtDNA markers II.

Position	106	166	173	222	363 (D-loop end)	2145	12433	12525	15627
Ref. (V00654)	T	A	A	T	C	G	T	G	A
Bp (DQ12438)	C	G	G	C		G	T	G	A
Bp (CPC98)	T	A	A			A	C	A	G
Bp - PWA (JQ437479)									
CRP		G 20,0	G 21,1	C 25,0		A 44,3	A 55,1	A 42,3	
CRP-GR	C 15,8	G 3,9	G 7,2	C 7,4		A 4,3	C 3,8	A 4,7	
CR		G 4,0	G 13,4			A 4,6	C 3,6	A 3,9	G 2,3

DQ12438 – Achilli et al. 2008,
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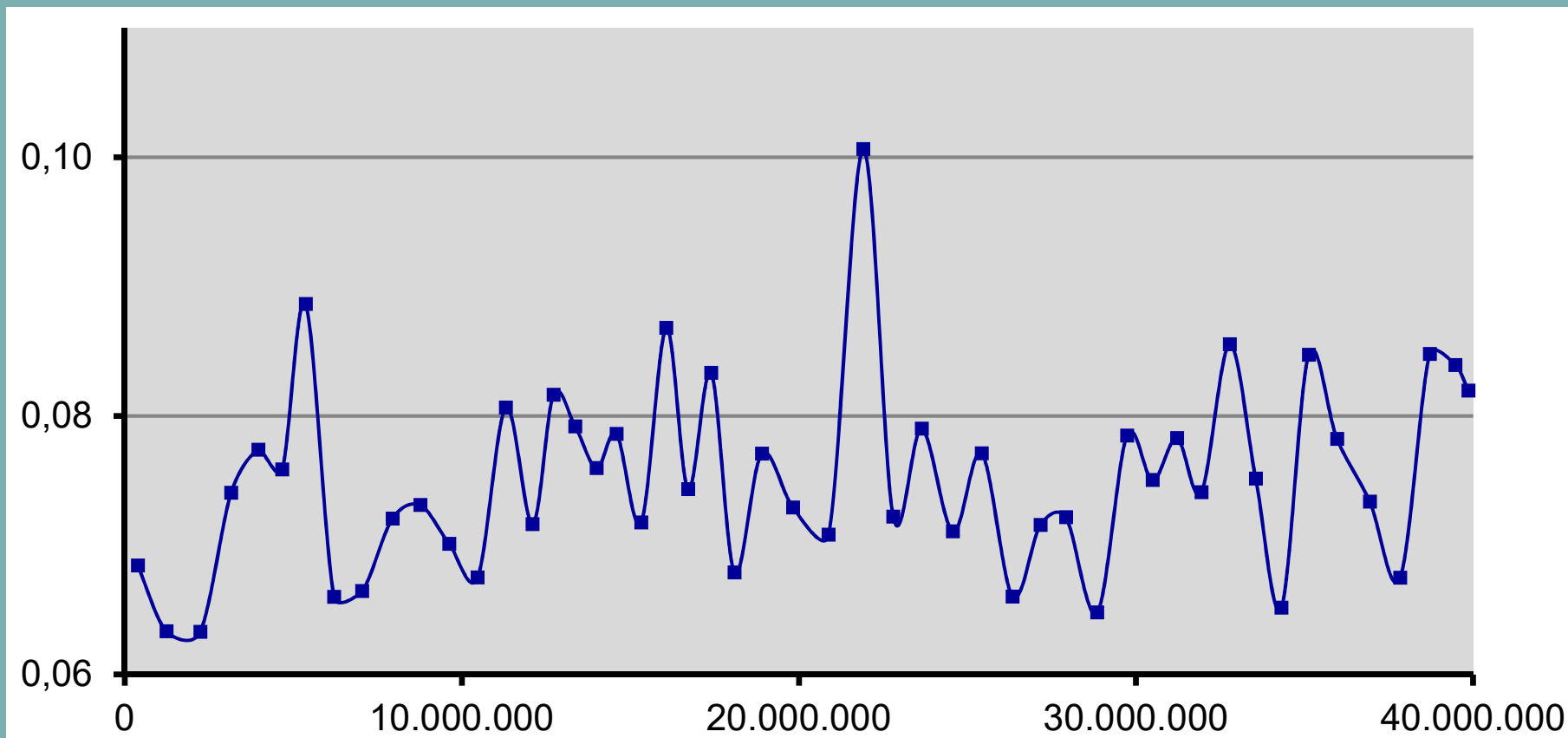
Genetic distance between populations derived from LC-WGS



Input: frequencies of all variants from chr1, metrics: Euclidean

Averaged distance along chr1 (1 – 40M nt)

Populations:
Czech Red vs. Czech Simmental
Pool-Seq data



Metrics: Euclidean, window: 800 000 nt, 492 807 polymorphisms

Next steps

- Completing the set of reference populations.
- Comparison of NGS statistics with less noisy microarray results.
- Elucidation of the haplotype structure of the current Czech Red population.
- Specification - if possible - of the markers of relevance to the conservation programme.