

# Genetic Diversity in Slovak Pinzgau Cattle Based on Microsatellites



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# Outline

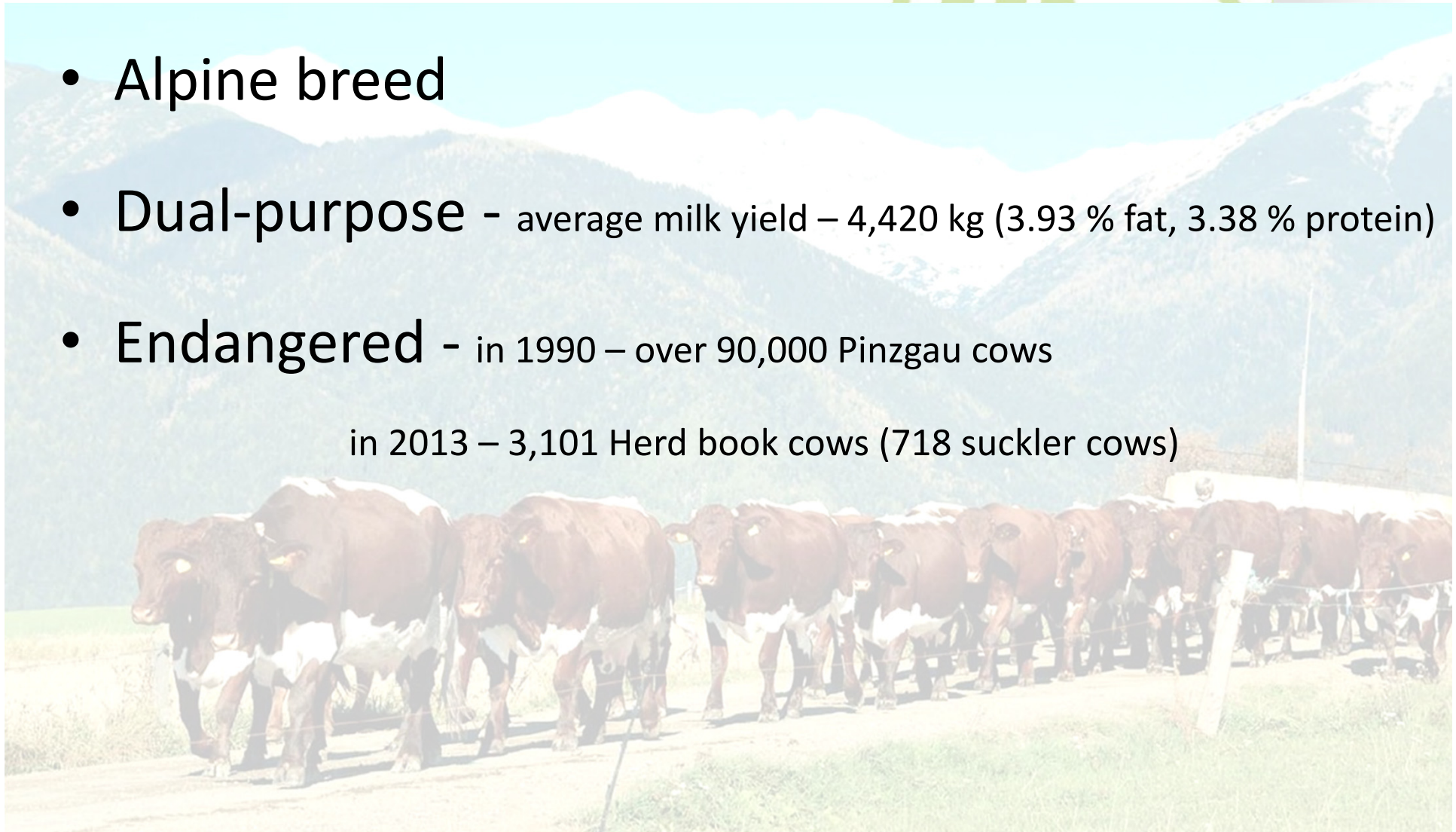
- Pinzgau cattle
- Information on diversity and population fragmentation
- Aim of the study
- Material and methods
- Results
- Further investigation



# Pinzgau cattle

- Alpine breed
- Dual-purpose - average milk yield – 4,420 kg (3.93 % fat, 3.38 % protein)
- Endangered - in 1990 – over 90,000 Pinzgau cows

in 2013 – 3,101 Herd book cows (718 suckler cows)



# Information on diversity and population structure

- Loss of genetic diversity within breeds
  - Decrease of effective size of population
  - Increase of inbreeding
  - Increase of homozygosity
- Population fragmentation
  - Origin
  - Herd/ farm
  - Alleles
- Molecular DNA markers
  - Microsatellites

# Aim of the study

- To evaluate the genetic diversity of Slovak Pinzgau cattle
- To analyze genetic structure of population without prior information





# Material and methods



- DNA isolation

  - 302 Slovak Pinzgau

- PCR

  - 8 microsatellites recommended by FAO / ISAG

locus	label	primer sequence 5' - 3'	chromosome
<b>CSRM60</b>	NED	AAGATGTGATCCAAGAGAGAGGCA AGGACCAGATCGTAAAAGGCATAG	10
<b>CSSM66</b>	NED	ACACAAATCCTTTCTGCCAGCTGA AATTTAATGCACTGAGGAGCTTGG	14
<b>TGLA227</b>	6FAM	CGAATTCCAAATCTGTTAATTTGCT ACAGACAGAACTCAATGAAAGCA	18
<b>ETH3</b>	VIC	GAACCTGCCTCTCCTGCATTGG ACTCTGCCTGTGGCCAAGTAGG	19
<b>ILST006</b>	NED	TGTCTGTATTTCTGCTGTGG ACACGGAAGCGATCTAAACG	7
<b>TGLA122</b>	PET	CCCTCCTCCAGGTAAATCAGC AATCACATGGCAAATAAGTACATAC	21
<b>SPS115</b>	6FAM	AAAGTGACACAACAGCTTCTCCAG AACGAGTGTCTAGTTTGGCTGTG	15
<b>BM1824</b>	VIC	GAGCAAGGTGTTTTCCAATC CATTCTCCAAGTCTTCCTTG	1

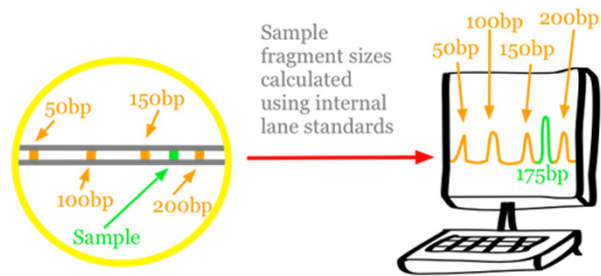
# ABI PRISM 310 Genetic Analyser

- Fragmentation analysis

– fluorescently-labelled primers

– capillary fractionation

– allele size



Sample File Name	Size	Height	Area in Point	Area in BP	Data Point	Begin Point	Begin BP	End Point
7_ABI_310_27.Run	1465	140	1184	100	85	852	11628	938
8_ABI_310_27.Run	32306	57	510	44	94	94	280	937
9_ABI_310_27.Run	62262	3338	5243	415	101	101	5478	978
10_ABI_310_27.Run	63787	684	4280	280	82	82	6845	857
11_ABI_310_27.Run	75612	153	2224	200	96	97	7362	986
12_ABI_310_27.Run	104688	750	7176	366	103	103	8487	1026
13_ABI_310_27.Run	113685	486	4792	388	103	103	10412	1045

Tabular data output for further analysis

# PowerMarker

v3.25

- Genetic diversity parameters



number of alleles



number of genotypes per locus



expected heterozygosity



observed heterozygosity



PIC



$F_{IS}$

	1	10	2	3	4	5	6	7	8	9	CARLO	GALMI	GOZ-001	KES-001	KOB-002	LESKO	LF-025	LOITEL
1	0.0000	0.7710	0.0415	0.0378	0.0439	0.0041	0.0464	0.0506	0.8026	0.0419	0.4494	0.4400	0.4547	0.4314	0.4458	0.4422	0.4434	0.4537
10	0.7710	0.0000	0.7712	0.7654	0.7780	0.8038	0.7649	0.7661	0.0099	0.7680	0.4799	0.4874	0.4978	0.4788	0.5021	0.4732	0.4746	0.5039
2	0.0415	0.7712	0.0000	0.0448	0.0488	0.0401	0.0480	0.0518	0.8356	0.0435	0.4521	0.4537	0.4567	0.4453	0.4483	0.4432	0.4419	0.4419
3	0.0378	0.7654	0.0448	0.0000	0.0462	0.0000	0.0431	0.0489	0.8299	0.0467	0.4483	0.4373	0.4560	0.4313	0.4393	0.4473	0.4322	0.4489
4	0.0439	0.7780	0.0488	0.0462	0.0000	0.0000	0.0375	0.0421	0.0473	0.8421	0.0425	0.4520	0.4452	0.4578	0.4447	0.4414	0.4417	0.4628
5	0.0041	0.8038	0.0401	0.0228	0.0375	0.0000	0.0329	0.0396	0.8726	0.0395	0.4504	0.4414	0.4566	0.4493	0.4453	0.4485	0.4367	0.4479
6	0.0464	0.7649	0.0480	0.0473	0.0000	0.0431	0.0329	0.0300	0.0252	0.8299	0.0452	0.4494	0.4470	0.4582	0.4410	0.4510	0.4484	0.4574
7	0.0506	0.7661	0.0518	0.0473	0.0329	0.0431	0.0000	0.0000	0.0000	0.0000	0.0502	0.4493	0.4573	0.4549	0.4400	0.4415	0.4525	0.4454
8	0.8026	0.7680	0.8356	0.8299	0.8421	0.8421	0.8421	0.8421	0.8421	0.8421	0.4482	0.4478	0.4478	0.4481	0.4481	0.4481	0.4481	0.4478
9	0.0419	0.7683	0.0435	0.0467	0.0425	0.0385	0.0473	0.0501	0.8312	0.0000	0.4487	0.4560	0.4555	0.4332	0.4441	0.4447	0.4417	0.4481
CARLO	0.4494	0.4799	0.4521	0.4483	0.4529	0.4504	0.4484	0.4453	0.4521	0.4487	0.0000	0.1947	0.2111	0.2060	0.2034	0.2048	0.2140	0.2059
GALMI	0.4400	0.4874	0.4327	0.4373	0.4452	0.4444	0.4430	0.4373	0.4873	0.4380	0.1947	0.0000	0.2182	0.2055	0.1927	0.2028	0.1995	0.1997
GOZ-001	0.4547	0.4978	0.4567	0.4560	0.4578	0.4566	0.4582	0.4649	0.5036	0.4655	0.2111	0.2182	0.0000	0.2162	0.2151	0.2155	0.2060	0.2244
KES-001	0.4314	0.4788	0.4367	0.4313	0.4437	0.4395	0.4370	0.4400	0.4891	0.4332	0.2060	0.2055	0.0000	0.1924	0.1894	0.1864	0.2171	0.2117
KOB-002	0.4458	0.4921	0.4542	0.4592	0.4527	0.4432	0.4410	0.4405	0.5018	0.4441	0.2024	0.2127	0.2151	0.1924	0.2000	0.2077	0.2149	0.2099
LESKO	0.4422	0.4434	0.4453	0.4473	0.4462	0.4445	0.4430	0.4455	0.4887	0.4447	0.2048	0.2069	0.2155	0.1894	0.2077	0.2000	0.2094	0.2142
LF-025	0.4419	0.4419	0.4419	0.4419	0.4419	0.4419	0.4419	0.4419	0.4419	0.4419	0.2048	0.2069	0.2155	0.1894	0.2077	0.2000	0.2094	0.2142
LOITEL	0.4537	0.4537	0.4537	0.4537	0.4537	0.4537	0.4537	0.4537	0.4537	0.4537	0.2048	0.2069	0.2155	0.1894	0.2077	0.2000	0.2094	0.2142

- 1000 bootstrap replicates

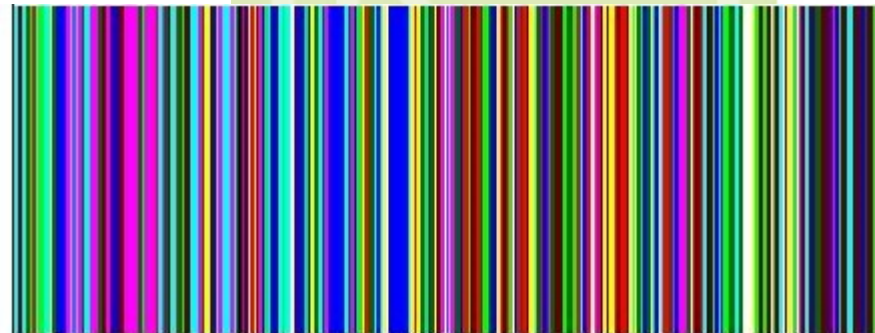


# Basic observations

Marker	$N_G$	$N_A$	$H_e$	$H_O$	PIC	$F_{IS}$
TGLA122	47	14	0.7747	0.745	0.7447	0.0399
TGLA227	45	12	0.8394	0.8675	0.8207	-0.0318
CSSM66	47	12	0.8598	0.8874	0.8438	-0.0304
CSRM60	32	10	0.8019	0.8344	0.7767	-0.039
ILST006	27	10	0.7732	0.7781	0.7396	-0.0048
SPS115	24	9	0.714	0.7053	0.6836	0.0139
ETH3	31	9	0.8257	0.8543	0.8022	-0.033
BM1824	20	8	0.7474	0.7086	0.7108	0.0535
<b>Average</b>	$34.241 \pm$	$10.46 \pm$	$0.7914 \pm$	$0.7984 \pm$	$0.7653 \pm$	$-0.0039 \pm$
<b><math>\pm</math> sd</b>	3.6445	0.6609	0.016	0.0234	0.0182	0.0122

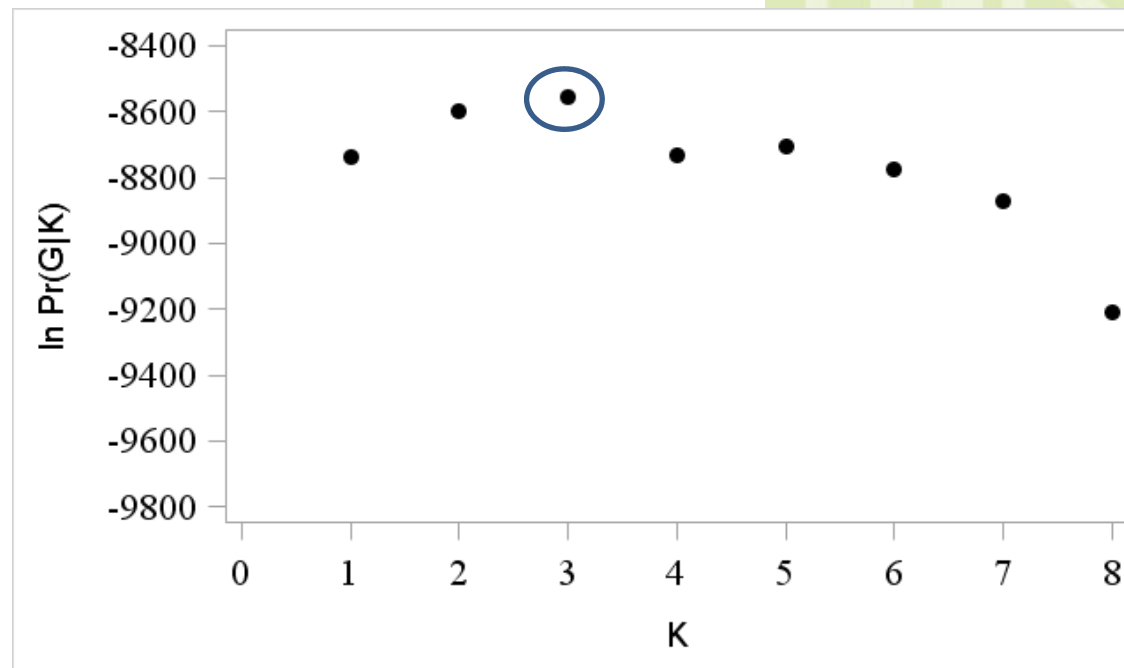
# What are we looking for?

- **structure of population**
  - Bayesian approach
  - without any a priori information
- BAPS – simple algorithm
  - overestimation of no. of clusters (17)  
(more sensitive to low no. of microsatellites)



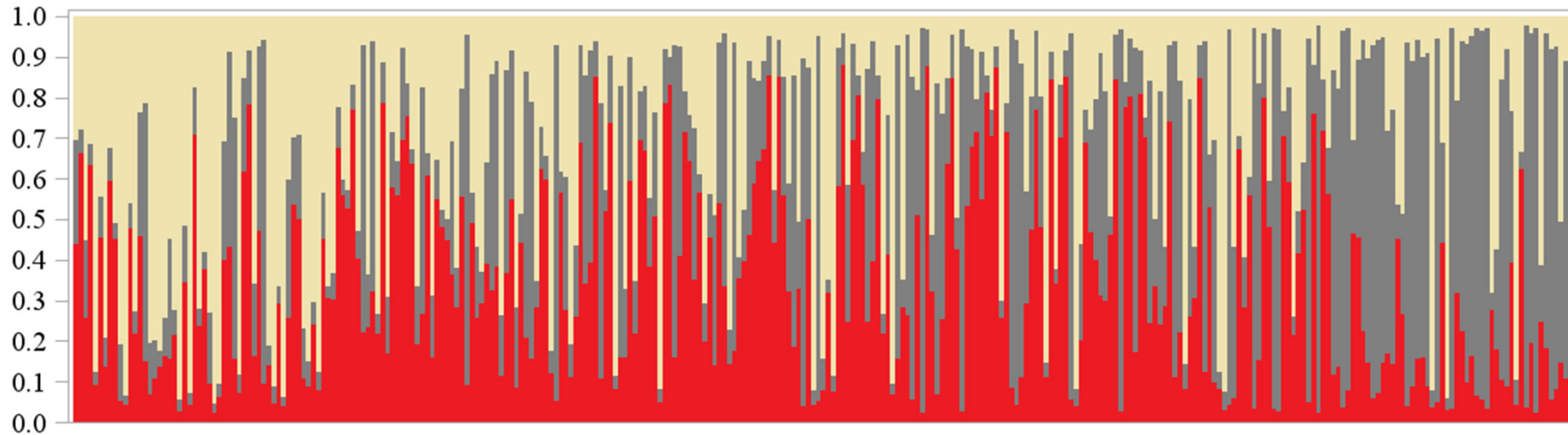
# STRUCTURE – longer computation time

- more accurate results
- subjective interpretation



- $\ln \Pr(G|K)$  values presented as a function of the number of clusters
- The largest  $\ln \Pr(G|K)$  values within each  $K$  (among 10 runs) presented

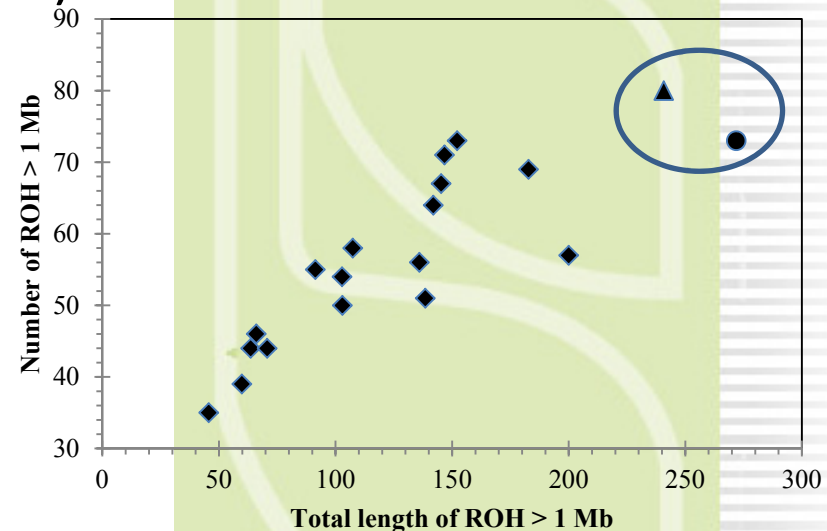
# STRUCTURE $k = 3$



- population structure – without a priori information about subpopulations
- each cow is represented by a single vertical line broken into  $k$  color segments, with lengths proportional to the estimated membership of the inferred cluster.

# Further investigation

- What is the population structure based on?
  - genealogical information – paternal lines???
- ROH – run of homozygosity
  - molecular inbreeding coefficients
  - total length (in Mb) and number of ROH





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**Európska únia**  
Európsky sociálny fond



Agentúra  
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- INSEMAS
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**Thank you for your attention!**

