

Genetic Diversity in Slovak Pinzgau Cattle Based on Microsatellites



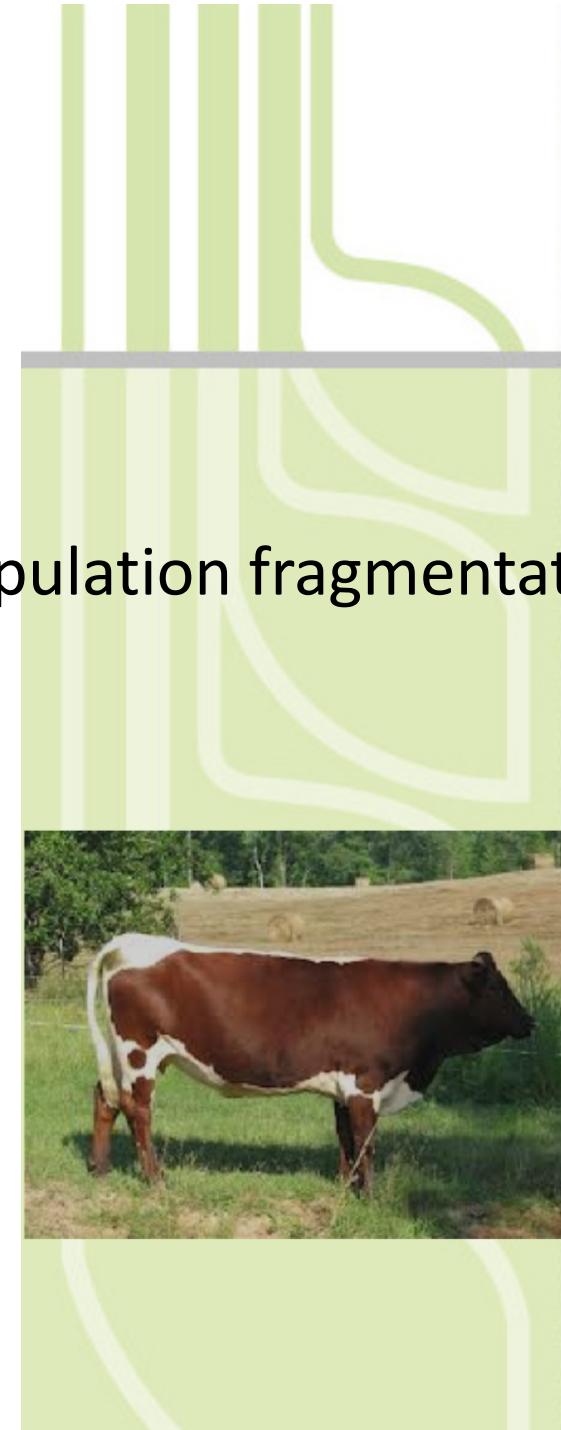
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Outline

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

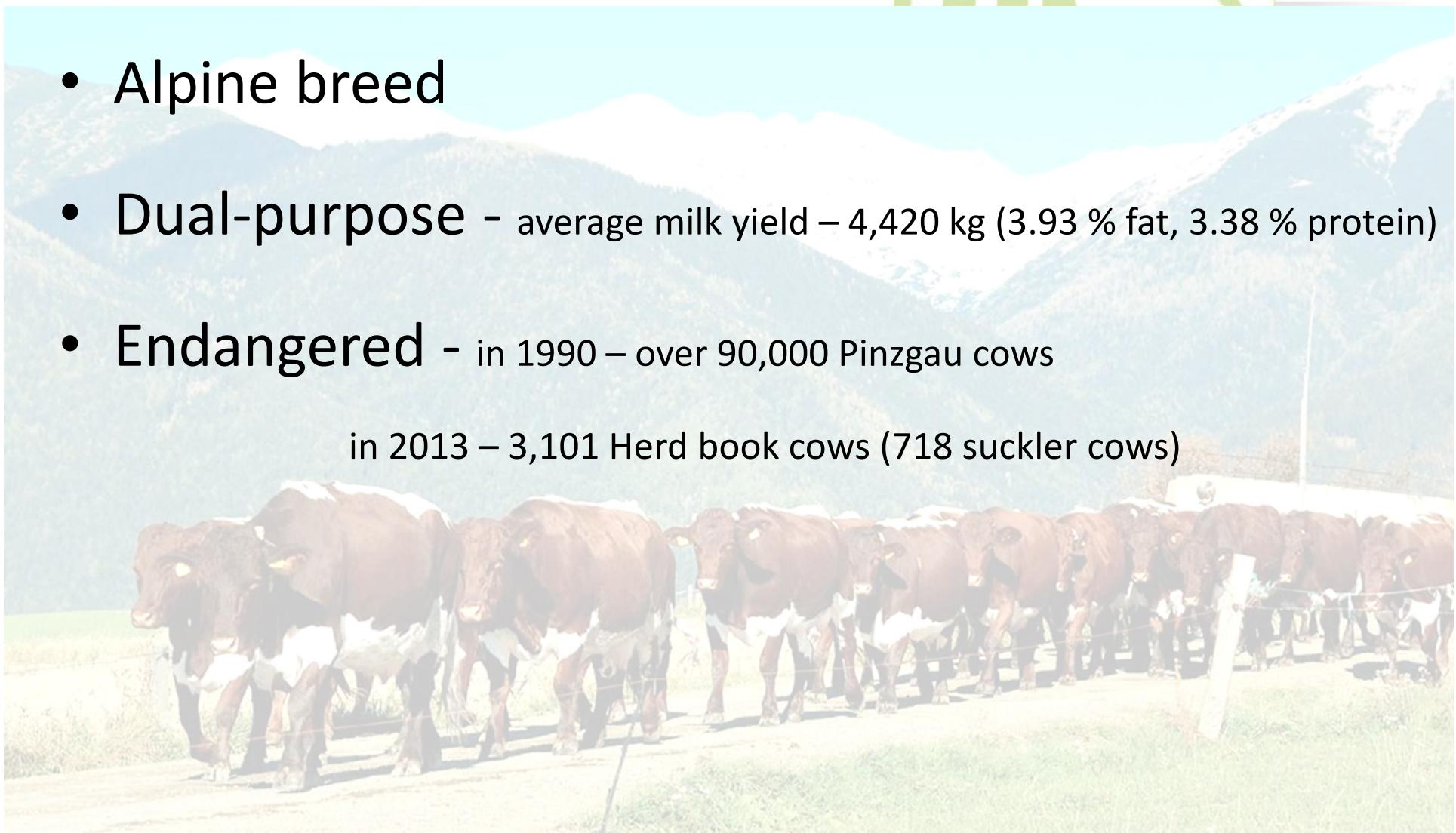


Pinzgau cattle



- 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

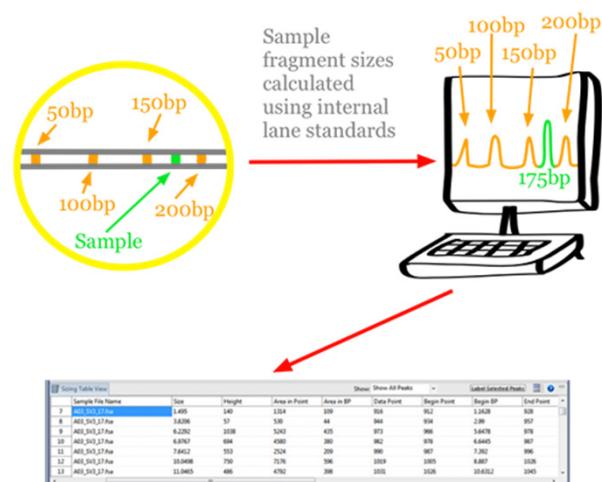
locus	label	primer sequence 5`- 3`	chromosome
CSRM60	NED	AAGATGTGATCCAAGAGAGAGGCA AGGACCAGATCGTGAAGGCATAG	10
CSSM66	NED	ACACAAATCCTTCTGCCAGCTGA AATTAAATGCACTGAGGGAGCTTGG	14
TGLA227	6FAM	CGAATTCCAAATCTGTTAATTGCT ACAGACAGAAACTCAATGAAAGCA	18
ETH3	VIC	GAACCTGCCTCTCCTGCATTGG ACTCTGCCTGTGGCCAAGTAGG	19
ILST006	NED	TGTCTGTATTCTGCTGTGG ACACGGAAGCGATCTAACG	7
TGLA122	PET	CCCTCCTCCAGGTAAATCAGC AATCACATGGCAAATAAGTACATAC	21
SPS115	6FAM	AAAGTGACACAACAGCTTCTCCAG AACGAGTGTCTAGTTGGCTGTG	15
BM1824	VIC	GAGCAAGGTGTTTTCCAATC CATTCTCCAATGCTTCCTTG	1



ABI PRISM 310 Genetic Analyser

- Fragmentation analysis

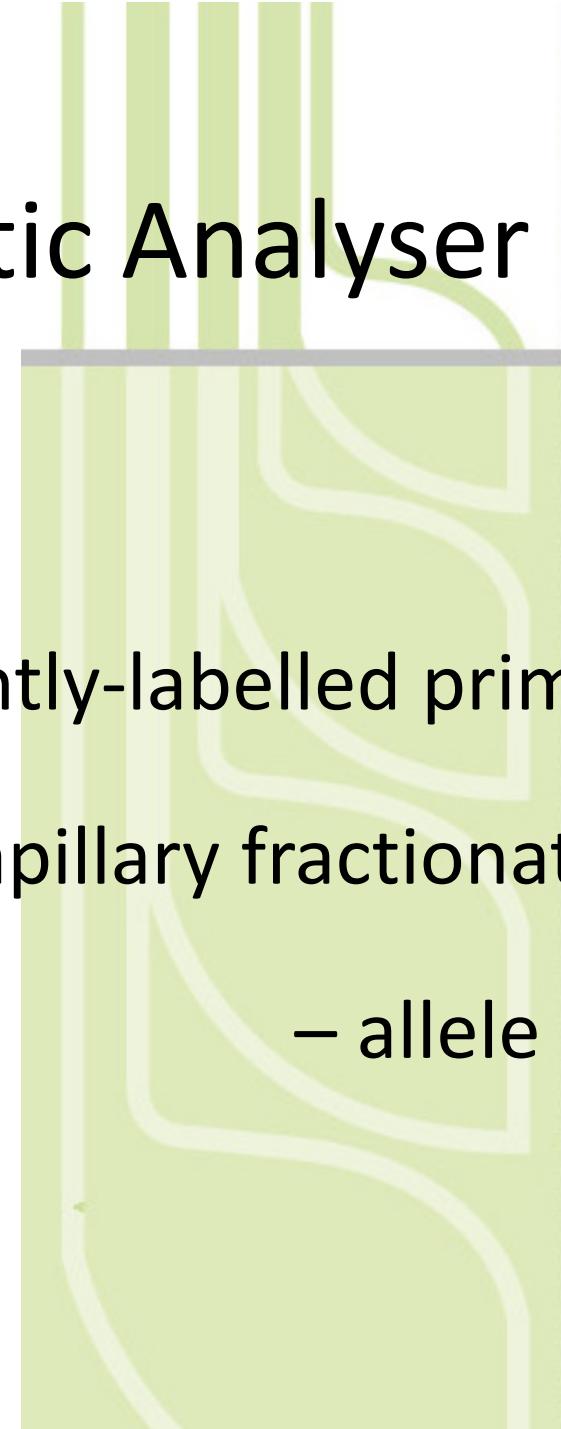
- fluorescently-labelled primers



Tabular data output for further analysis

- capillary fractionation

- allele size



PowerMarker

v3.25

- Genetic diversity parameters



number of alleles



number of genotypes per locus



expected heterozygosity



observed heterozygosity



PIC



F_{IS}

	MV_powermarker.lfrequency1Neil1972																		
	1	10	2	3	4	5	6	7	8	9	CARLO	GALMI	G02-001	KES-001	KOB-002	LESKO	LF-025	LUTE	LU*
1	0.0000	0.7710	0.0415	0.0378	0.0439	0.0464	0.0506	0.0326	0.0419	0.0494	0.4400	0.4547	0.4124	0.4456	0.4422	0.4424	0.4557	0.4	
10	0.7710	0.0000	0.7712	0.1554	0.7780	0.8038	0.7149	0.7641	0.2039	0.7881	0.4799	0.4814	0.4978	0.4788	0.5021	0.4731	0.4748	0.5039	0.4
3	0.0415	0.7712	0.0000	0.0448	0.0488	0.0411	0.0480	0.0518	0.0356	0.0416	0.4521	0.4337	0.4567	0.4367	0.4545	0.4483	0.4472	0.4519	0.4
3	0.0378	0.7654	0.0448	0.0000	0.0462	0.0228	0.0421	0.0489	0.0239	0.0467	0.4483	0.4373	0.4560	0.4213	0.4392	0.4473	0.4522	0.4469	0.4
4	0.0419	0.7805	0.0488	0.0462	0.0000	0.0375	0.0411	0.0473	0.0421	0.0425	0.4529	0.4482	0.4578	0.4427	0.4511	0.4414	0.4417	0.4621	0.4
8	0.0341	0.8088	0.0411	0.0228	0.0375	0.0000	0.0329	0.0368	0.0726	0.0385	0.4504	0.4414	0.4566	0.4395	0.4419	0.4485	0.4397	0.4479	0.4
5	0.0345	0.7515	0.0411	0.0251	0.0519	0.0000	0.0245	0.0249	0.0452	0.0484	0.4470	0.4762	0.4579	0.4410	0.4710	0.4484	0.4574	0.4454	0.4
9	0.0326	0.7999	0.0316	0.0299	0.0411	0.0716	0.0199	0.0116	0.0000	0.0312	0.4821	0.4873	0.5016	0.4894	0.5016	0.4887	0.4884	0.4978	0.4
9	0.0349	0.7683	0.0245	0.0487	0.0245	0.0585	0.0452	0.0501	0.0200	0.0467	0.4560	0.4555	0.4352	0.4411	0.4447	0.4417	0.4481	0.4457	0.4
CARLO	0.4494	0.1499	0.4511	0.4883	0.4529	0.4504	0.4484	0.4453	0.4821	0.4487	0.0000	0.3447	0.2111	0.2060	0.204	0.2048	0.2140	0.2059	0.2
GAUVE	0.4410	0.1474	0.4237	0.4273	0.4452	0.4414	0.4490	0.4479	0.4873	0.4690	0.1947	0.0000	0.2182	0.2059	0.2157	0.2069	0.1999	0.2397	0.1
G02-001	0.4547	0.4978	0.4567	0.4560	0.4578	0.4566	0.4582	0.4649	0.4598	0.4555	0.2111	0.0000	0.2162	0.2151	0.2155	0.2060	0.2244	0.2244	0.1
KES-001	0.4314	0.4783	0.4567	0.4513	0.4417	0.4595	0.4370	0.4400	0.4891	0.4332	0.2060	0.0255	0.2162	0.0000	0.1924	0.1894	0.2171	0.2117	0.2
KOB-002	0.4448	0.4211	0.4542	0.4193	0.4517	0.4412	0.4410	0.4405	0.5018	0.4444	0.2034	0.1527	0.2151	0.1924	0.0000	0.2077	0.2149	0.2099	0.1
LESKO	0.4498	0.4483	0.4492	0.4478	0.4614	0.4485	0.4510	0.4535	0.4887	0.4447	0.2048	0.2088	0.2155	0.1894	0.2077	0.0000	0.2094	0.2142	0.2
LF-025	0.4537	0.5059	0.4519	0.4469	0.4628	0.4479	0.4324	0.4454	0.4978	0.4485	0.2069	0.1997	0.2144	0.2117	0.2099	0.2142	0.2020	0.2000	0.2
LUTE	0.4492	0.4874	0.4475	0.4383	0.4519	0.4451	0.4482	0.4512	0.4884	0.4367	0.2048	0.1929	0.1880	0.2106	0.1841	0.2187	0.1954	0.2038	0.2
LU*	0.4557	0.4993	0.4576	0.4566	0.4513	0.4597	0.4587	0.4595	0.4210	0.4115	0.1946	0.1915	0.1915	0.1915	0.1915	0.2155	0.2158	0.2148	0.2
MENR	0.4428	0.4572	0.4527	0.4480	0.4597	0.4484	0.4551	0.4494	0.5063	0.4536	0.2033	0.1909	0.2153	0.2210	0.1953	0.2115	0.2077	0.2020	0.2
MCB-001	0.4759	0.4808	0.4709	0.4678	0.4859	0.4707	0.4573	0.4712	0.4775	0.4333	0.2119	0.1765	0.2170	0.1887	0.1942	0.2187	0.2039	0.1961	0.2
MF-001	0.4759	0.4759	0.4432	0.4322	0.4417	0.4597	0.4484	0.4474	0.4854	0.4417	0.2145	0.1948	0.2060	0.2171	0.2148	0.2094	0.2010	0.2057	0.2
MG-001	0.4444	0.4444	0.4460	0.4460	0.4455	0.4527	0.4541	0.4582	0.4907	0.4387	0.2181	0.2018	0.2170	0.2073	0.2098	0.2188	0.2168	0.2057	0.2
MUS-003	0.4468	0.4557	0.4500	0.4514	0.4632	0.4740	0.4719	0.4784	0.4615	0.4784	0.2079	0.2079	0.2079	0.2079	0.2079	0.2055	0.2067	0.2056	0.2
REM-002	0.4641	0.4778	0.4568	0.4559	0.4677	0.4660	0.4624	0.4658	0.4825	0.4531	0.2024	0.2059	0.2155	0.2111	0.2155	0.2186	0.2279	0.2179	0.2
SBA-001	0.4419	0.4681	0.4547	0.4581	0.4471	0.4494	0.4470	0.4477	0.4518	0.4299	0.2009	0.2118	0.2118	0.2118	0.2118	0.2142	0.2126	0.2186	0.2
Tauv+012	0.4478	0.4816	0.4661	0.4448	0.4614	0.4487	0.4509	0.5062	0.4748	0.2309	0.2310	0.2337	0.2200	0.2406	0.2320	0.2480	0.2454	0.2	
Tauv+013	0.4668	0.4809	0.4819	0.4626	0.4776	0.4712	0.4760	0.4740	0.5071	0.4716	0.2337	0.2330	0.2362	0.2267	0.2320	0.2294	0.2466	0.2512	0.2
Tauv+014	0.4665	0.4902	0.4708	0.4641	0.4727	0.4698	0.4731	0.4743	0.5041	0.4720	0.2286	0.2277	0.2204	0.2227	0.2348	0.2189	0.2518	0.2420	0.2
Tauv+020	0.4596	0.4917	0.4704	0.4641	0.4759	0.4660	0.4652	0.4638	0.4963	0.4873	0.2164	0.2247	0.2169	0.2183	0.2203	0.2198	0.2280	0.2229	0.2
Tauv+021	0.4445	0.4790	0.4520	0.4385	0.4487	0.4497	0.4461	0.4460	0.4932	0.4503	0.2162	0.2227	0.2175	0.2137	0.2287	0.2357	0.2378	0.2481	0.2
Tauv+022	0.4525	0.4905	0.4938	0.4894	0.4695	0.4642	0.4668	0.4622	0.5077	0.4522	0.2185	0.2228	0.2199	0.2199	0.2249	0.2482	0.2260	0.2467	0.2
Tauv+028	0.4513	0.5053	0.4912	0.4835	0.4633	0.4534	0.4595	0.4675	0.5222	0.4536	0.2270	0.2310	0.2382	0.2238	0.2339	0.2450	0.2480	0.2385	0.2
Tauv+310	0.4797	0.4817	0.4741	0.4761	0.4719	0.4640	0.4761	0.4741	0.4874	0.4704	0.2321	0.2376	0.2347	0.2246	0.2496	0.2551	0.2579	0.2278	0.2
Tauv+316	0.4480	0.4792	0.4568	0.4427	0.4593	0.4457	0.4536	0.4591	0.4945	0.4455	0.2355	0.2322	0.2287	0.2219	0.2389	0.2416	0.2415	0.2486	0.2
Tauv+318	0.4524	0.5030	0.4719	0.4488	0.4728	0.4537	0.4574	0.4607	0.5074	0.4540	0.2298	0.2322	0.2290	0.2292	0.2312	0.2387	0.2408	0.2387	0.2
Tauv+344	0.4507	0.4810	0.4647	0.4490	0.4610	0.4562	0.4597	0.4631	0.4940	0.4541	0.2186	0.2185	0.2195	0.2247	0.2333	0.2474	0.2399	0.2474	0.2
Tauv+345	0.4479	0.4905	0.4653	0.4647	0.4521	0.4540	0.4570	0.4593	0.5073	0.4516	0.2095	0.2095	0.2142	0.2195	0.2181	0.2279	0.2262	0.2328	0.2
Tauv+346	0.4507	0.5117	0.4973	0.4488	0.4605	0.4498	0.4556	0.4592	0.5197	0.4566	0.2092	0.2150	0.2150	0.2142	0.2195	0.2218	0.2279	0.2262	0.2
Tauv+353	0.4487	0.5053	0.4586	0.4487	0.4635	0.4531	0.4560	0.4541	0.5148	0.4540	0.2323	0.2334	0.2324	0.2324	0.2324	0.2473	0.2468	0.2468	0.2
Tauv+360	0.4550	0.4878	0.4745	0.4567	0.4761	0.4568	0.4627	0.4683	0.4999	0.4660	0.2394	0.2470	0.2361	0.2361	0.2356	0.2506	0.2506	0.2608	0.2
Tauv+361	0.4667	0.5015	0.4819	0.4553	0.4807	0.4714	0.4714	0.4714	0.5179	0.4714	0.2298	0.2470	0.2471	0.2471	0.2471	0.2577	0.2551	0.2551	0.2

- 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
Average	$34.241 \pm$	$10.46 \pm$	$0.7914 \pm$	$0.7984 \pm$	$0.7653 \pm$	$-0.0039 \pm$
± sd	3.6445	0.6609	0.016	0.0234	0.0182	0.0122

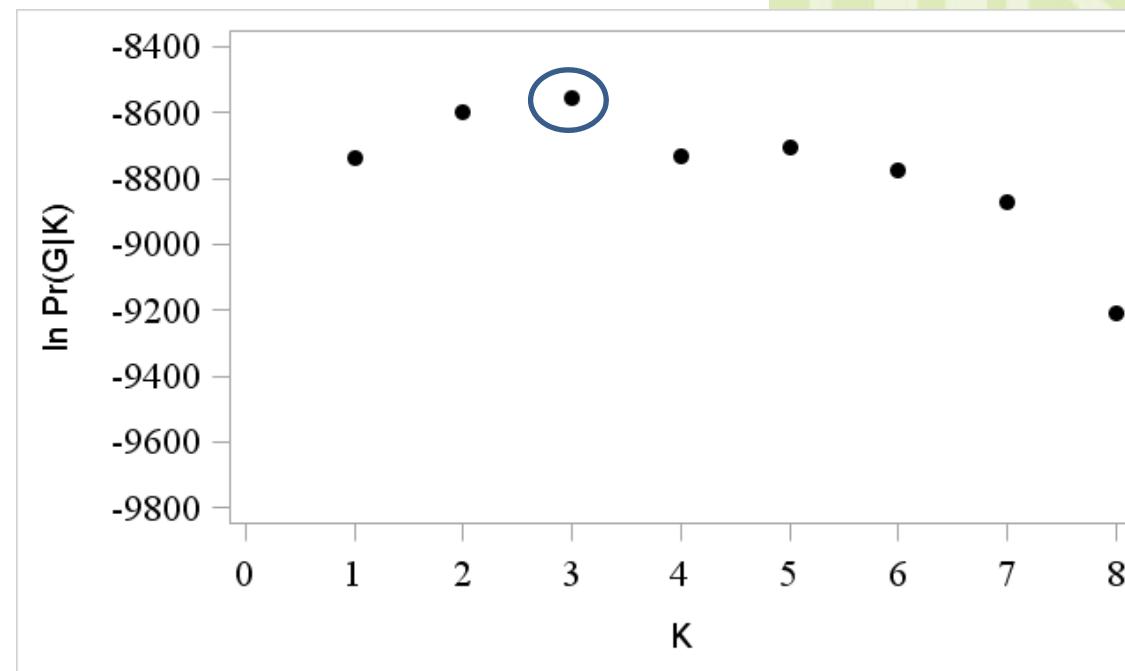
What are we looking for?

- **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)



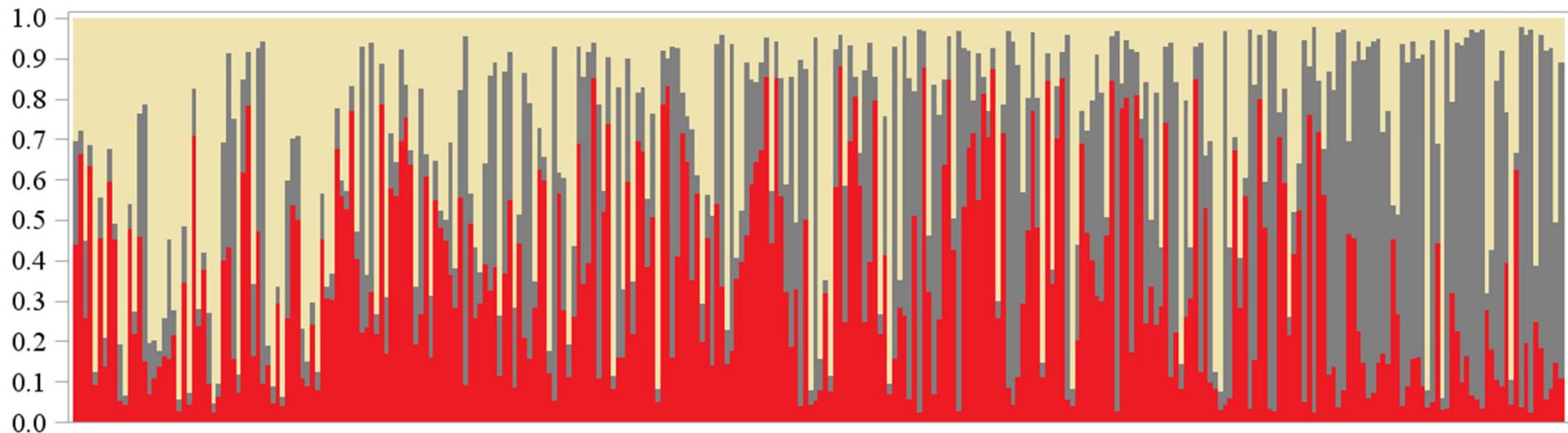
STRUCTURE

- 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

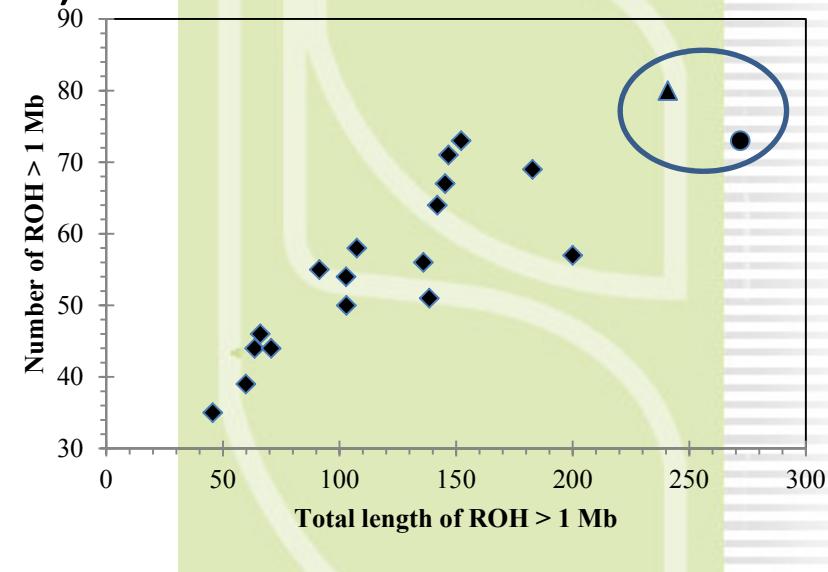
STRUCTURE $k = 3$



- 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
Ministerstva školstva, vedy, výskumu a športu SR
pre štrukturálne fondy EÚ



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demand-oriented project

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- INSEMAS
- Slovak biological services
- Slovak association of Pinzgau breeders





Thank you for your attention!