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Characterization of Wolfdog Hybrids by Molecular Genetic Methods

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Biodiversity

A variability of genofond of freely living animals in original populations as well as in breeds reared by people represents one of the keys factors which is responsible for an adaptability and vitality of a whole population.

Genetic markers

Both morphological and anatomic markers typical for a particular zoology species are used in evaluating of variability degree assessing. In these days, a lot of genetic markers, which are not liable to outer environs, are being used.

Czechoslovakian and Saarloos Wolfdogs

The breeds of Saarloos Wolfdog and Czechoslovakian Woolfdog, which are recognized by FCI are originated from a breeding of a German Shepherd Dog and a Eurasian wolf. The origin of Czechoslovakian Wolfdog breed is dated in 30ies of 20 century.

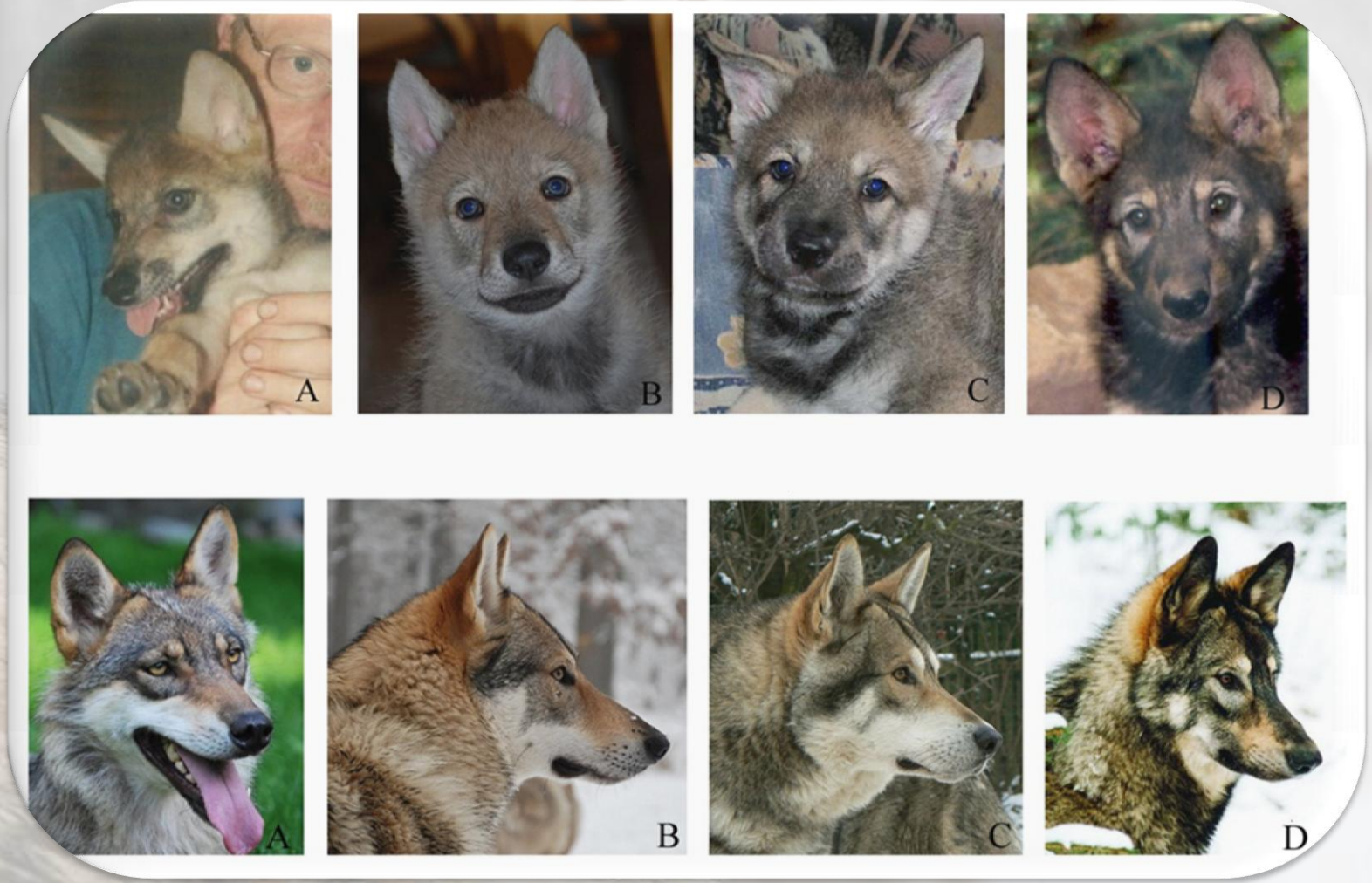
Czechoslovakian Wolfdog

Saarloos Wolfdog

Evaluated individuals

- 55 males and 65 females of Czechoslovakian Wolfdog
- 6 males and 16 females of Saarloos Wolfdog
- 2 Eurasian wolf males and 8 Eurasian wolf females
- 1 male and two female crossbreeds of F_1 generation originated from a crossing between a wolf female and a German Shepherd Dog male
- 1 female of F_2 generation (F_1 female (wolf female x German Shepherd Dog male) x Czechoslovakian Wolfdog male) and one male (F_1 male (wolf x Saarloos female) x Saarloos female
- 2 male offsprings of F_3 generation, which has come from the crossing of the male from F_2 generation (described above) and Saarloos Wolfdog female
- 14 males and 39 German Shepherd Dog females

Evaluated individuals

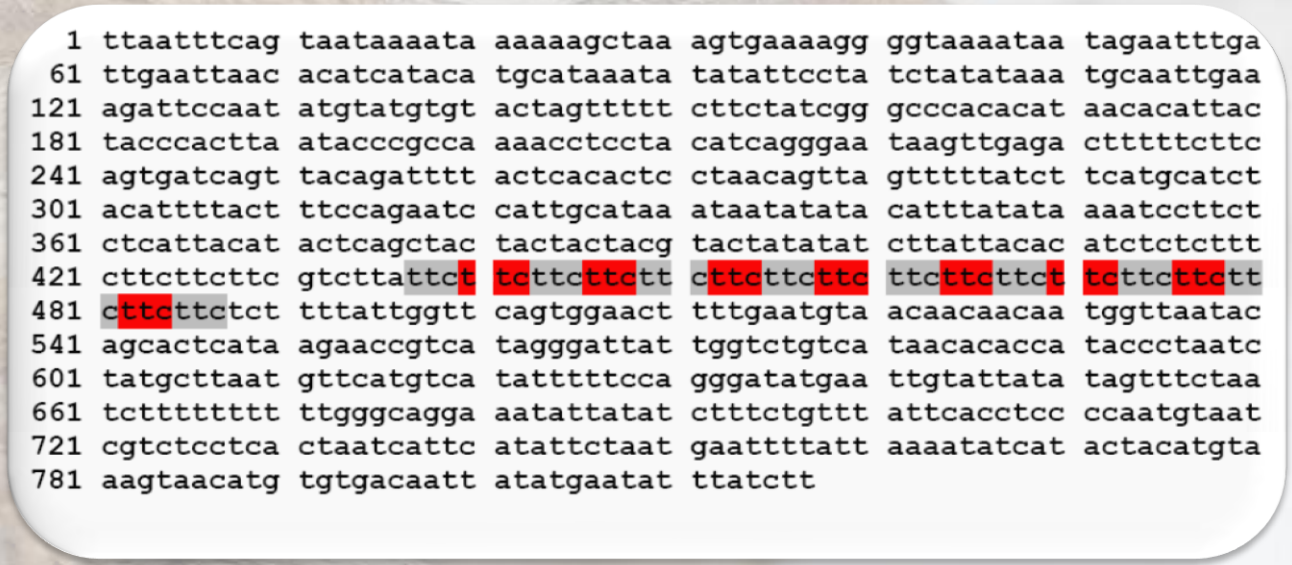


Canis lupus Czechoslovakian Wolfdog Saarloos Wolfdog F_1 hybrid

Variability of SSR markers of gonozome Y

Simple Sequence Repeats (SSR) are mostly noncoding part of genome which are usable for population genetics analyses.

SSR are codominant, highly polymorphic, abundant and uniformly dispersed in genomes.



Variability of SSR markers of gonozome Y

WORLD OF THE CZECHOSLOVAKIAN WOLFDOGS

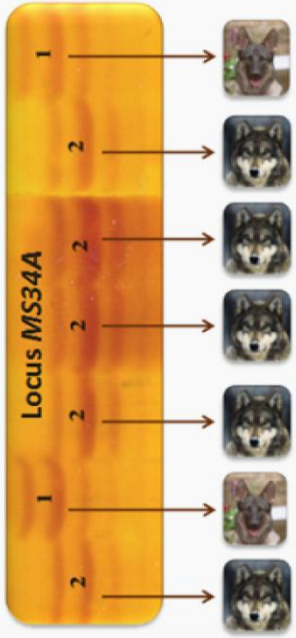
Saarlooswolfdog
Saarlooswolfhond

DNA izolation

Polymerase Chain Reaction

Electrophoresis

1. Saarloos Wofdog male
2. Czechoslovakian Wofdog male
3. Czechoslovakian Wofdog male
4. Czechoslovakian Wofdog male
5. Czechoslovakian Wofdog male
6. Saarloos Wofdog male
7. Czechoslovakian Wolfdog male



dog origin of gonozome Y
wolf origin of gonozome Y
wolf origin of gonozome Y
wolf origin of gonozome Y
wolf origin of gonozome Y
dog origin of gonozome Y
wolf origin of gonozome Y

Variability of SSR markers of gonozome Y

Canis lupus

Czechoslovakian Wolfdog

- SSR markers (*MS34A*, *MS34B*, *MS41A*) located in heterologic part of Y gonozome were used to identify male crossbreeds.
- The experiments were based on a hypothesis supposing a SSR variability between wolf and dog Y gonozomes.
- This hypothesis was confirmed by results of our experiments.

Variability of SSR markers of gonozome Y

- Each of three SSR markers has showed only 2 polymorphic alleles. One of these alleles was highly specific to wolf gonozome Y and the second one was specific to gonozome Y of dog origin.
- These results have perfectly fit to evaluations of all Czechoslovakian Wolfdog and Saarloos Wolfdog pedigrees.
- 64.3 % of Czechoslovakian Wolfdog males have its origin derived from F_1 generation of crossing of wolf male and German Shepherd Dog female.
- No wolf allele was found in males of Saarloos Wolfdog.

Variability of SSR markers of gonozome Y

- These results correspond to the fact that only wolf females participated in a Saarloos Wolfdog breeding.
- The wolf type SSR allele was identified in each of F_1, F_2 and F_3 male crossbreeds.

Saarloos Wolfdog

F_1 hybrid

Agouti-grey coat pigmentation

- Agouti-grey colour is characteristic for wolves.
- The same coat colouring is also characteristic for both Czechoslovakian Wolfdog and Saarloos Wolfdog representatives.

Coat colour variability between two sisters of F_1 wolfdog hybrid Canadian wolf female and male of German Shepherd Dog

Bos-brown coat pigmentation

The bos-brown coat colouring, which is also presented in Saarloos Wolfdog breed besides the rare white colouring, is caused by a recessive mutation in *TYRP1* locus.

bos-brown

agouti-grey

bos-brown

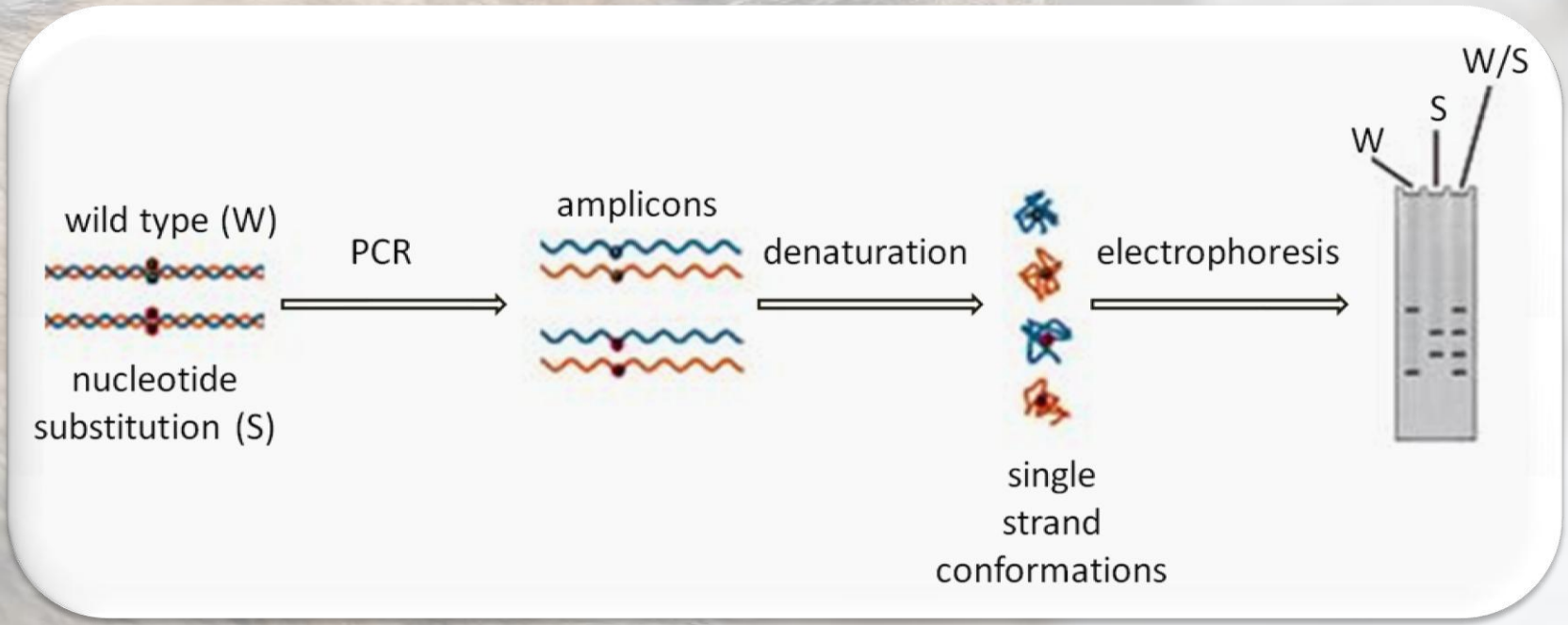
bos-brown

Three known mutations in gene *TYRP1*

- SCHUMTZ *et al*. (2002) was focused on analysis of *TYRP1* gene and 3 types of point mutations were found in this locus.
- The first mutation causes a premature STOP codon formation in exon 5.
- The second mutation causes a deletion in a triplet encoding proline, which is also located in exon 5.
- The third known mutation is in exon 2, where 121T>C causes a replacing of serine for cysteine.

Principles of PCR-SSCP method

Method Single Strand Conformation Polymorphisms of Polymerase Chain Reaction (PCR-SSCP) amplicons is suitable for detection of mutations based on nucleotide substitutions.



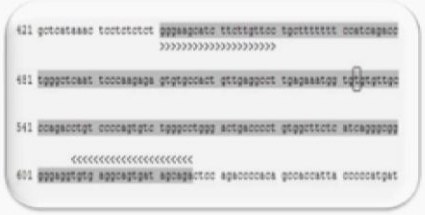
PCR-SSCP detection of mutation in exon 2

Grey-agouti pigmentation of *Canis lupus*

Grey-agouti pigmentation of Czechoslovakian Wolfdog

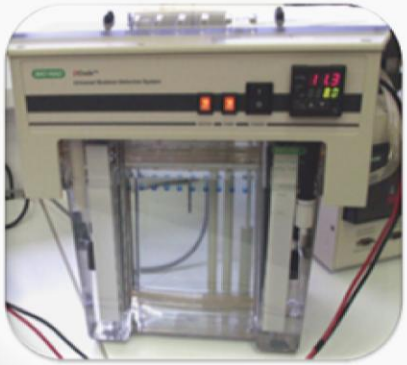
Bos-brown pigmentation of Saarloos Wolfdog

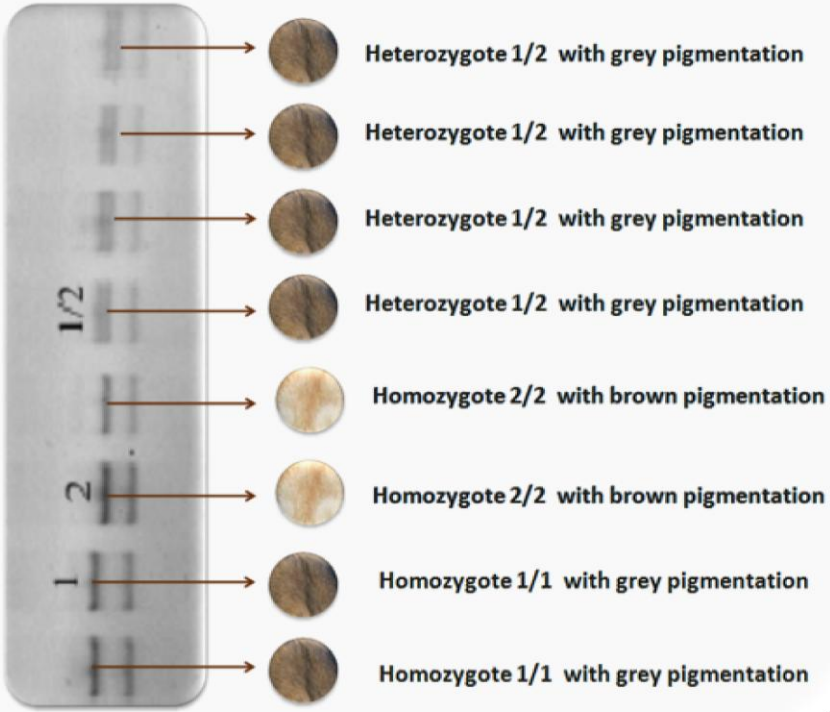
PCR primers position in canine sequence XM_ 531934.2



BIO-RAD

SSCP *analysis*





PCR-SSCP detection of mutation in exon 2

- Allele 1 was discovered in all of Czechoslovakian Wolfdogs, Saarloos Wolfdogs, wolfdogs hybrids and wild wolves with agouti-grey pigmentation.
- Homozygous constitution 1/1 was always found in wolves and Czechoslovakian Wolfdogs.
- This result corresponds to the fact that brown pigmentation has never been detected in wolves and Czechoslovakian Wolfdogs.
- The heterozygous constitution 1/2 was found in Saarloos Wolfdogs with grey agouti pigmentation and also in hybrids of wolf and bos-brown Saarloos Wolfdog female.

PCR-SSCP detection of mutation in exon 2

- Homozygous constitution 2/2 was found only in all of bos-brown Saarloos Wolfdogs and in 1 individual of F_3 wolfdog hybrid.
- This hybrid has its origin in crossing of 2/2 homozygous brown Saarloos Wolfdog female and 1/2 heterozygous grey wolfdog F_2 hybrid male.
- The second puppy from this crossing had grey agouti pigmentation and heterozygous constitution 1/2.
- These results confirm monogenic heredity of brown coat pigmentation according to Mendel´s laws.

Conclusions

- The origin of Y gonozome in Czechoslovakian Wolfdog was characterized by SSR analysis of *MS34A*, *MS34B* and *MS41A* loci.
- 64.3% of Czechoslovakian Wolfdog males had Y gonozome of wolf origin and 35.7% had Y gonozome of dog origin.
- All of Saarloos Wolfdog males had only Y gonozome of dog origin.
- Analysis of F_2 and F_3 wolfdog hybrids of an unknown wolf and a Saarloos Wofdog female showed that the unknown wolf had the identical allele of *MS34B* locus as dogs.



Conclusions

- Molecular analysis of *TYRP1* locus confirmed the absence of three known mutation in wolves and Czechoslovakian Wolfdogs which is connected to agouti grey coat pigmentation.
- A new PCR-SSCP marker was developed for detection of S42C mutation in exon 2 of *TYRP1* locus which is typical only for bos-brown Saarloos Wolfdog.

Acknowledgements

The collective of authors would like to thank to Ing. Monika Soukupová, Helena Hubáčková, Cornelia Keizer, Magda Andrlová, Jiří Andrle, František Hrach and to collective of co-workers in the German Shepherd Dogs Breeding Station of Policy of the Czech Republic in Prackovice.

The research was supported by projects 1312/3133 GA FAPPZ CULS Prague and MSM 6046070901.