Genetic analysis of the Old Kladruber horse – an important genetic resource in the Czech Republic

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CZECH
UNIVERSITY
OF LIFE SCIENCES PRAGUE

Introduction

Old Kladruber is

- considered the original Czech breed
- was bred especially for ceremonial purposes and aristocracy
- baroque character of the breed has been conserved until now
- breed have unique characteristics and high cultural and historical value
- continually kept in the Czech Republic teritory for more than four hundred years.

History

- origin of this breed dates back to 1579
- objective was to produce horses for the Imperial Court of the Habsburgs
- Old Kladruber and Lipizzaner horse were bred on the same basis
  - Old Kladruber horses as carriage horses
  - Lipizzaner horses as riding horses
- the two Old Kladruber stallions were founders of classical Lipizzaner sire lines
- Lipizzaner stallion Favory founded the nonclassical sire line (1938)

History

- most significant bottleneck was detected in the first half of the 20th century
- near extinction of the breed occurred, mainly of the variety of black horses.
- Regeneration of the breed:
  - Lipizzian horse, Friesian horse, Orlov trotter, Nonius and others


GENESE
STAROŠPANĚLSKÉHO
A
STAROKLADRUBSKÉHO
KONĚ
VYSVĚTLIVKY:
MĚSTA
HŘEBČÍNY
GENESE ANDALUSKÉHO KONĚ
ROZŠÍŘENÍ LIPICKÉHO KONĚ
SEVERNÍ MOŘE
IRSKO
VELKÁ BRITÁNIE
ATLANSKÝ OCEÁN
KůŇ FRÝZSKÝ
1750
NDR
NSR
POLSKO
SSSR
ORLOVSKÝ KLUSÁK
1756
1974
1620
1560
1581
1675
1570
1540
1951 - 54
1958
KůŇ STAROKLADRUBSKÝ
ČSSR
VYSOKÁ ŠPANĚLSKÁ ŠKOLA 1735
TOPOLČIANKY 1921
RAKOUSKO
MAĎARSKO
RUMUNSKO
KůŇ LIPICKÝ
JUGOSLÁVIE
JADERSKÉ MOŘE
ČERNÉ MOŘE
BULHARSKO
ALBÁNIE
TURECKO
FRANCIE
ŠVÝCARSKO
ITÁLIE
PORTUGALSKO
ŠPANĚLSKO
KůŇ BERBERSKÝ + KůŇ IBERSKÝ
KůŇ ANDALUSKÝ
711
KůŇ ANDALUSKÝ
KůŇ APENINSKÝ
KůŇ STAROITALSKÝ
1540
NEAPOL
KORSIKA
SARDINIE
SICÍLIE
STŘEDOZEMNÍ MOŘE
MAROKO
ALŽÍRSKO
TUNISKO
KůŇ ARABSKÝ + KůŇ NUMIDSKÝ
KůŇ BERBERSKÝ
670
LIBYE
EGYPT
KůŇ ARABSKÝ
622

Characterization

- warm-blooded breed with a large frame of the heavy warmbloods
- typically baroque conformation until now
- two coat colour varieties
  - grey and black
  - both varieties are managed in one herd book
  - each variety is bred separately
  - no targeted mating between individuals of different variants


HH

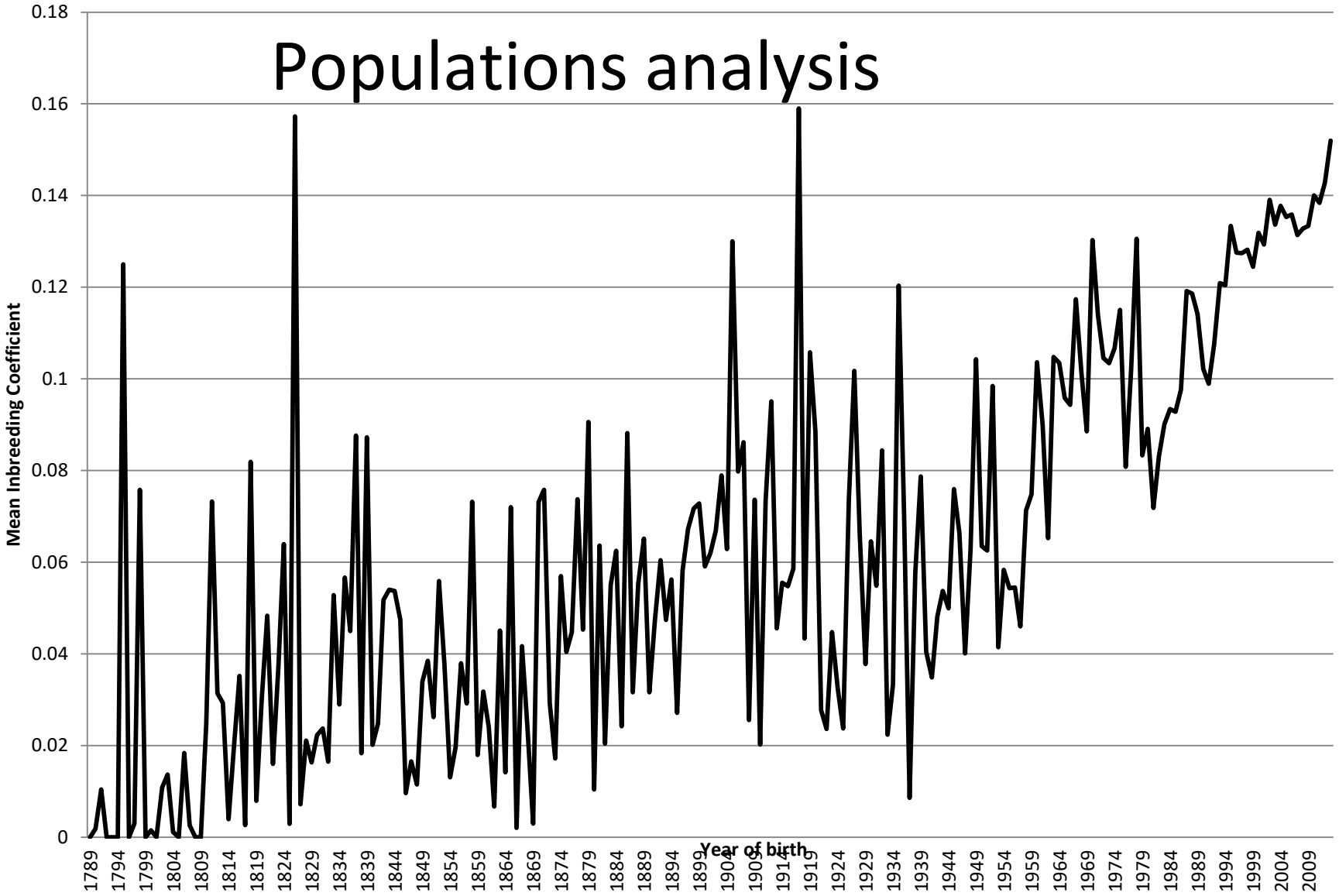
Grey (Kladruby n. Labem stud)

Black (Slatiňany stud)

- Population studies (inbreeding, effective population size, founders contribution…)

- Greying
- Melanoma occurence
- Vitiligo occurence
- Insect bite hypersensitivity

- Factors influencing coat colour (fading vs. non-fading)


Populations analysis
Mean Inbreeding Coefficient
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
Year of birth
1789
1794
1799
1804
1809
1814
1819
1824
1829
1834
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1844
1849
1854
1859
1864
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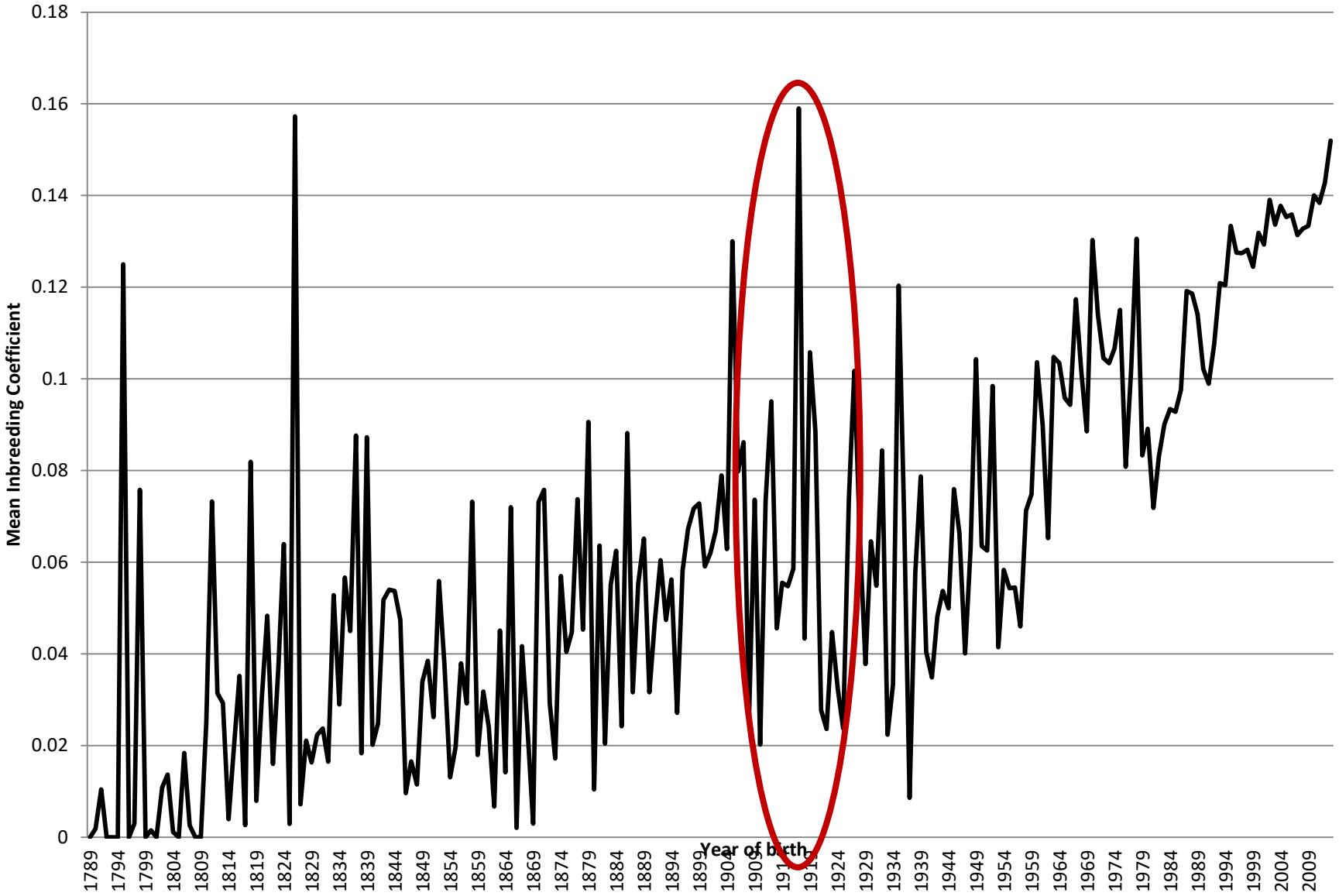
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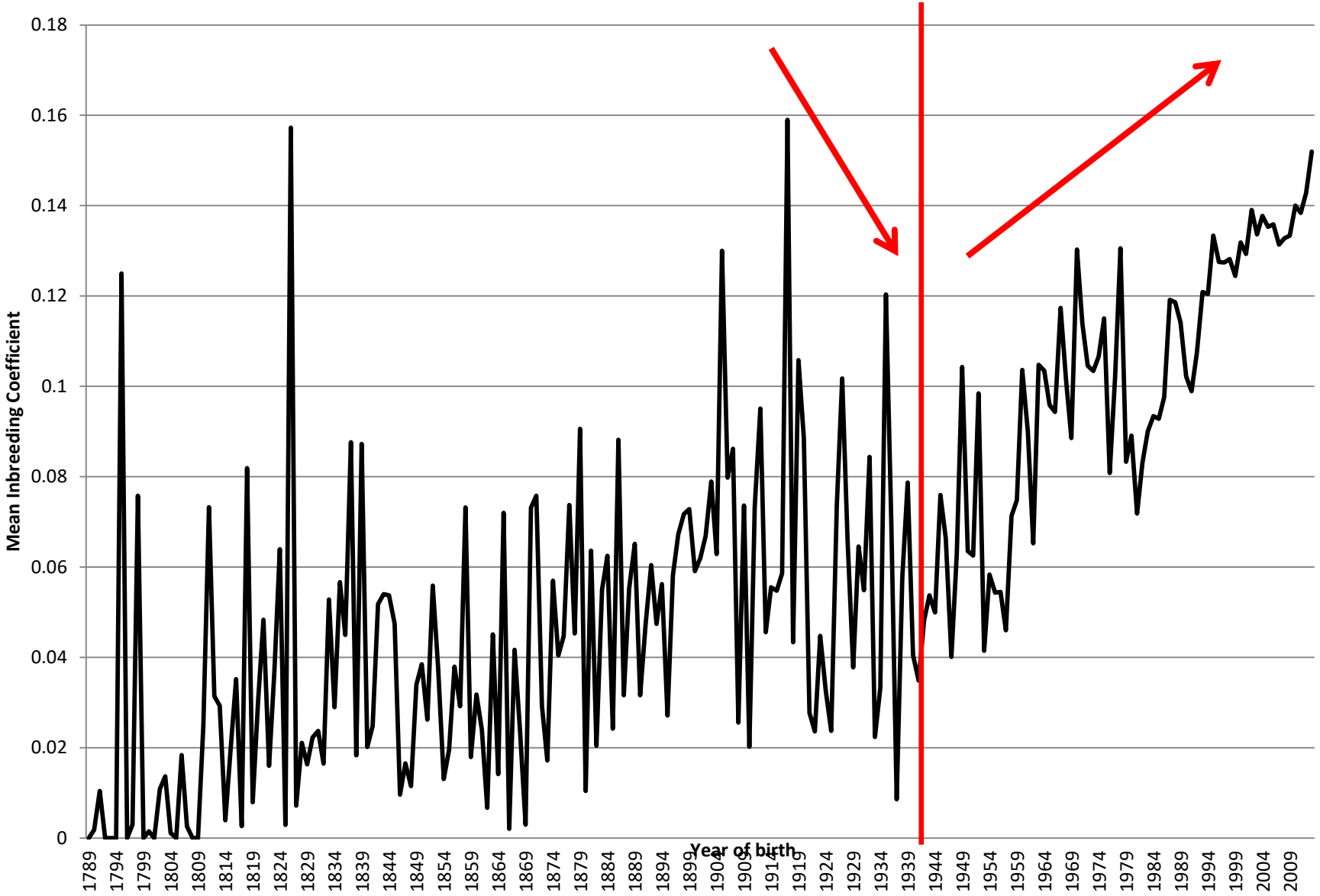
Year of birth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0.14
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0.1
0.08
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Year of birth
1789
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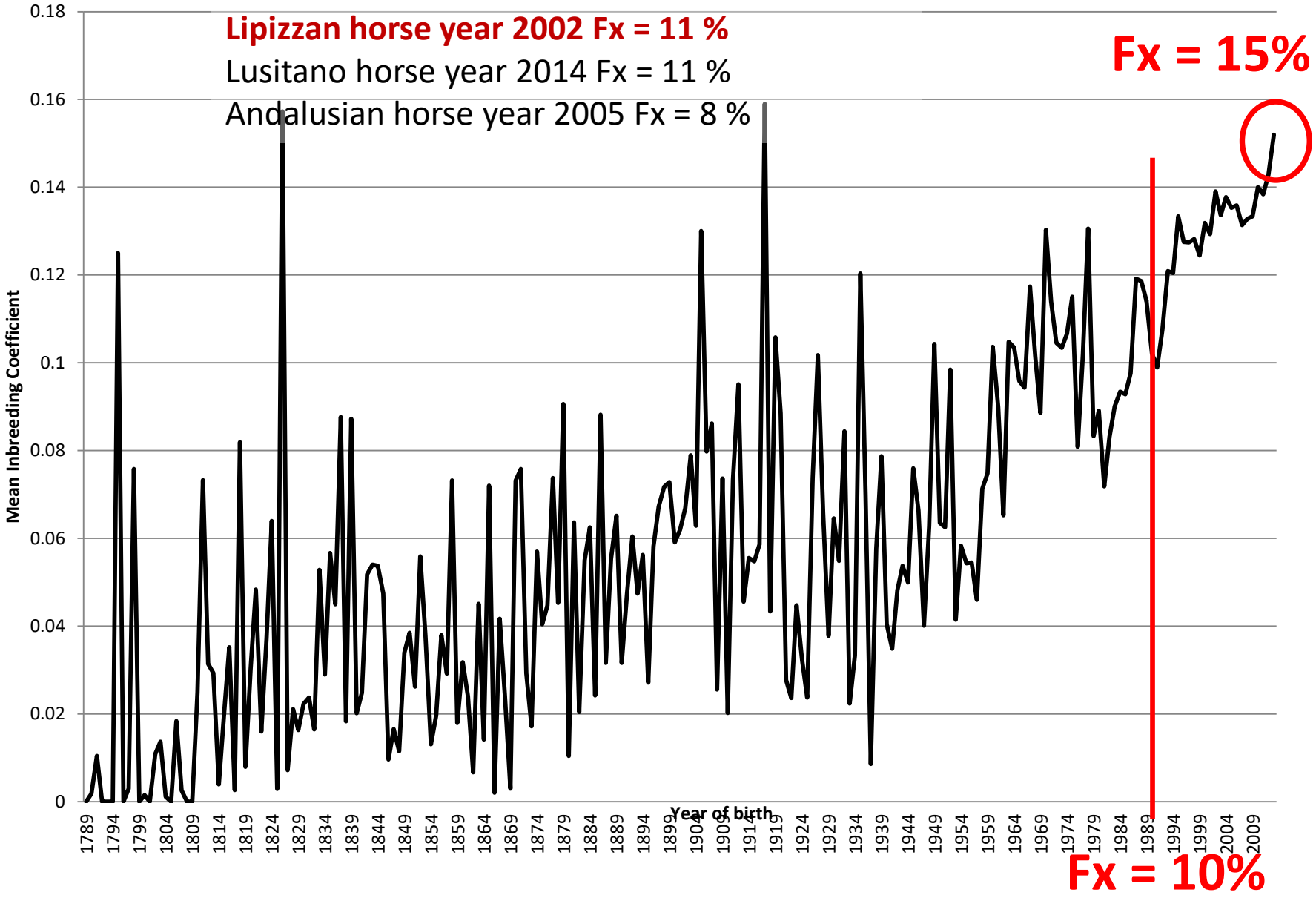
Lipizzan horse year 2002 Fx = 11 %
Lusitano horse year 2014 Fx = 11 %
Andalusian horse year 2005 Fx = 8 %
Fx = 15%
Fx = 10%
Mean Inbreeding Coefficient
Year of birth
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
1789
1794
1799
1804
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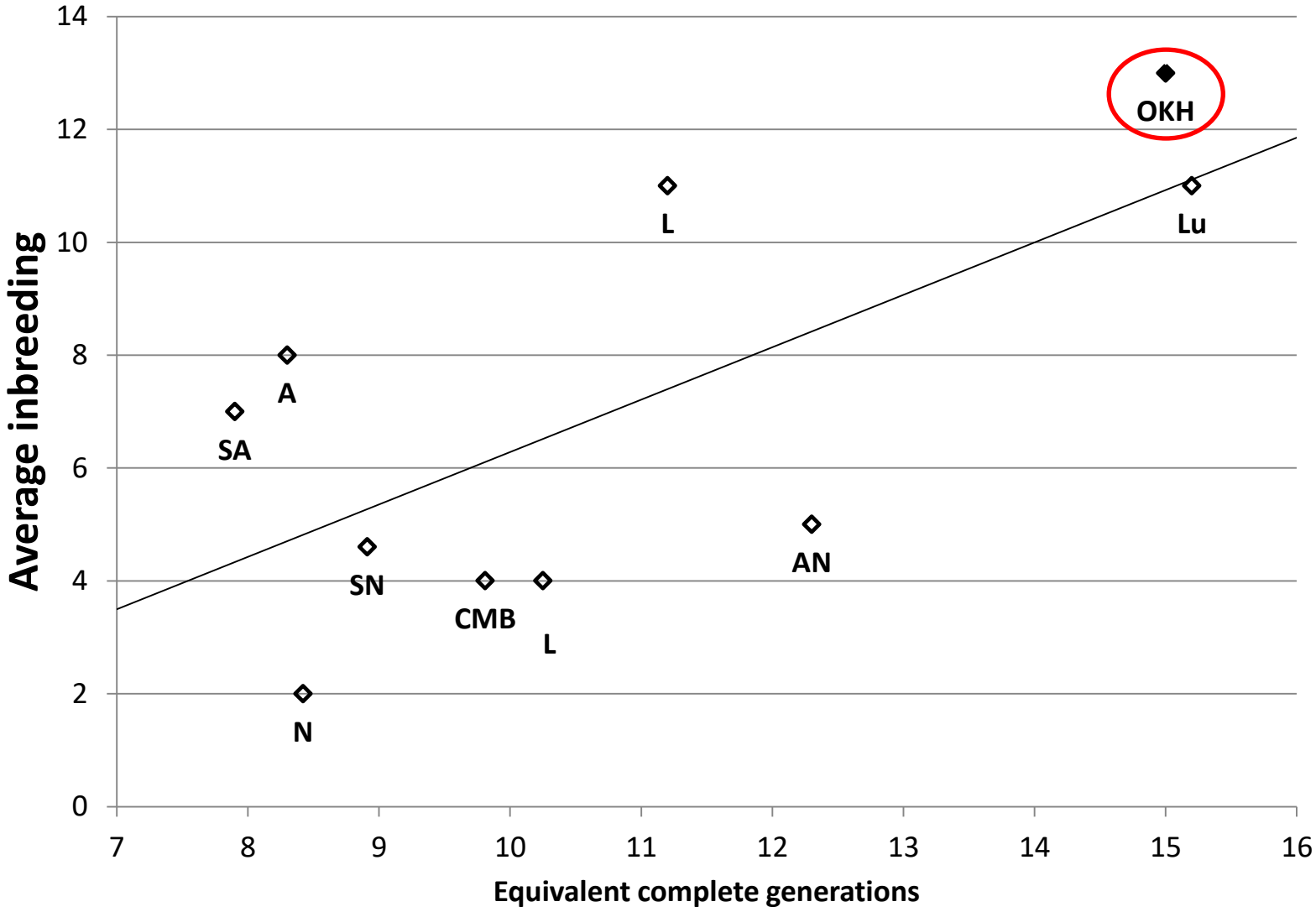
Average inbreeding
14
12
10
8
6
4
2
0
7
8
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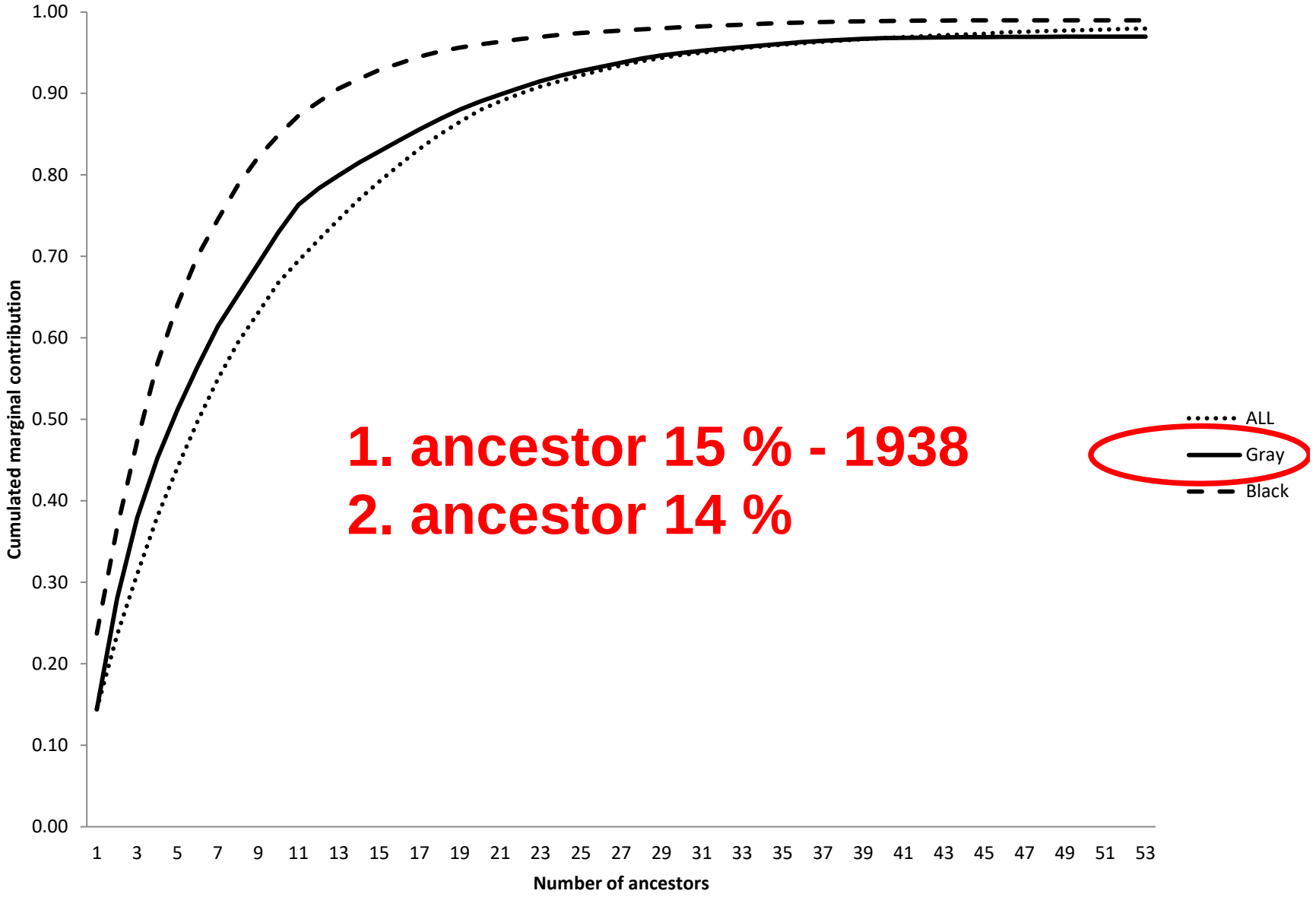
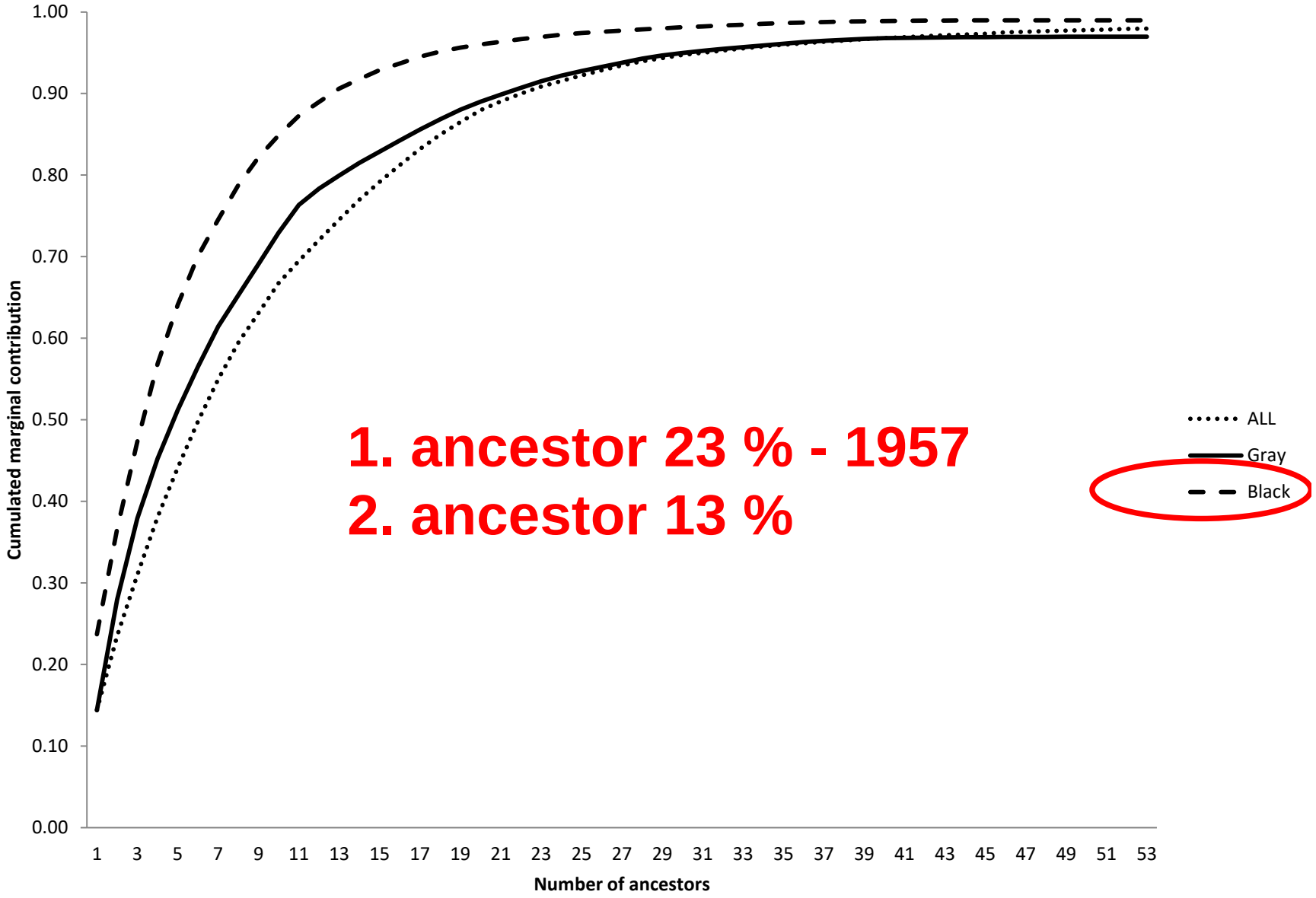
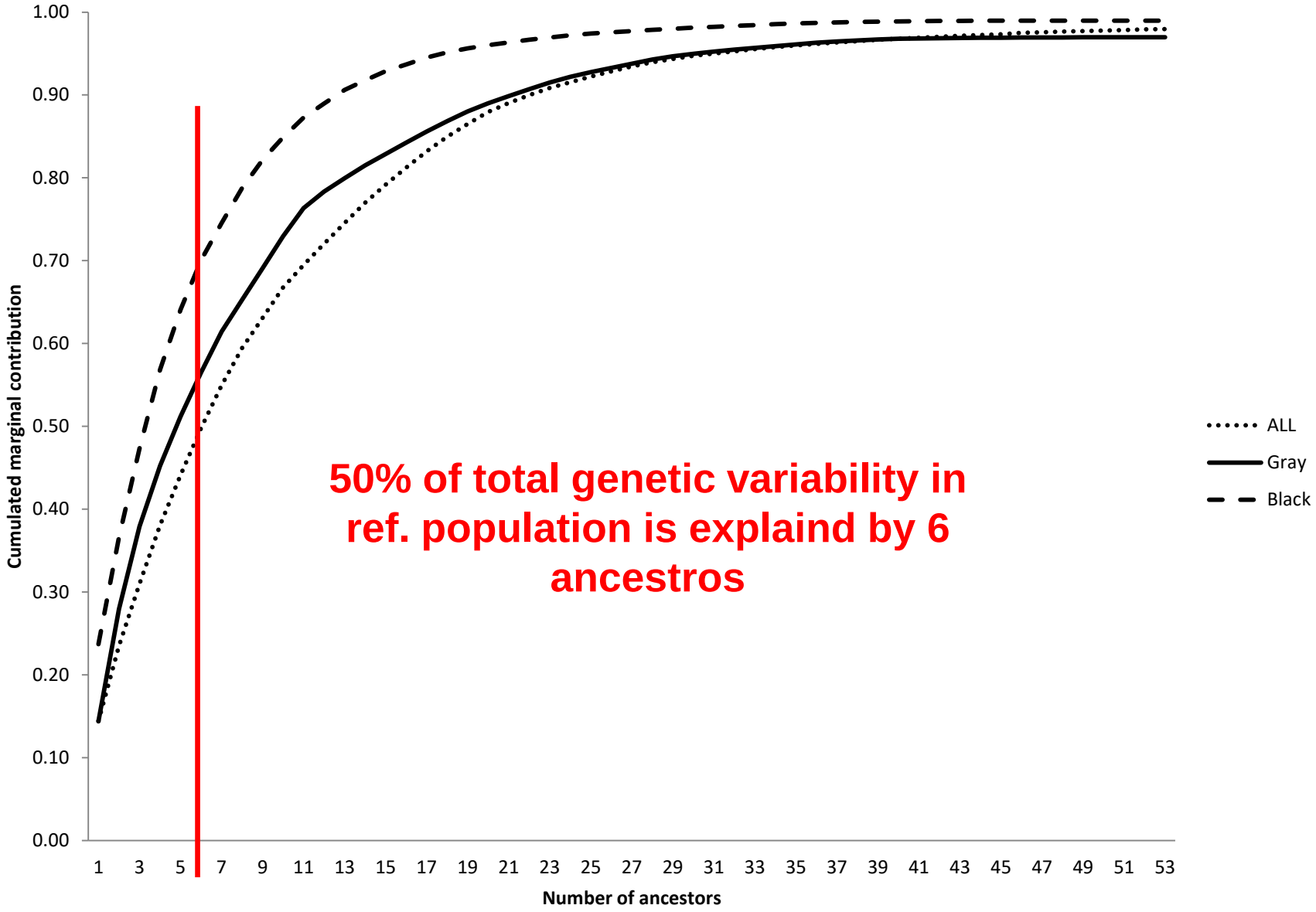
13
14
15
16
Equivalent complete generations
OKH
L
Lu
A
SA
SN
AN
CMB
L
N


1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
Cumulated marginal contribution
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53
Number of ancestors
1. ancestor 15 % - 1938
2. ancestor 14 %
ALL
Gray
Black


1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
Cumulated marginal contribution
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53
Number of ancestors
1. ancestor 23 % - 1957
2. ancestor 13 %
ALL
Gray
Black


1.00
0.90
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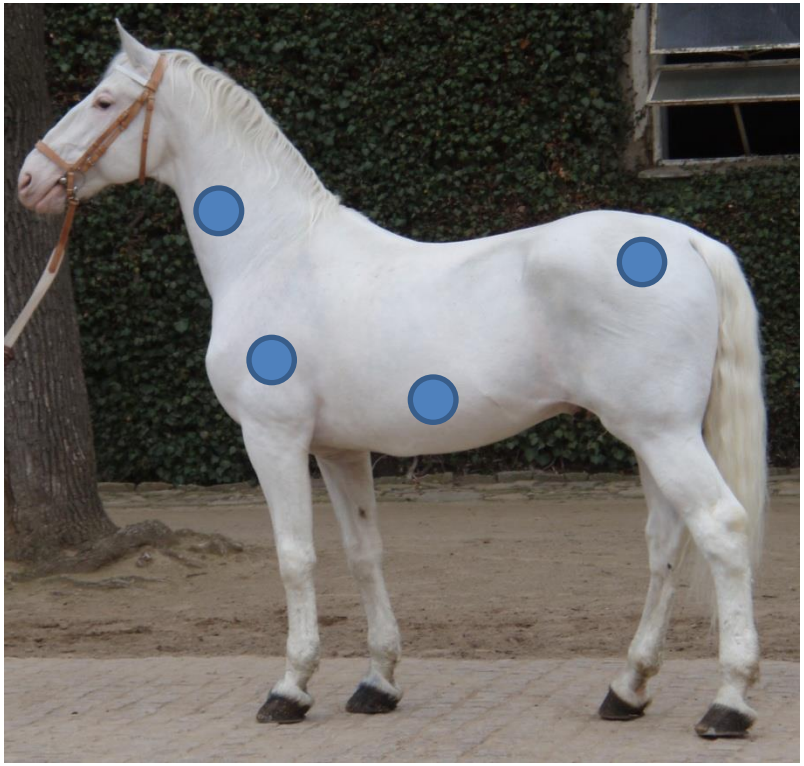
0.30
0.20
0.10
0.00
Cumulated marginal contribution
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53
Number of ancestors
ALL
Gray
Black
50% of total genetic variability in ref. population is explaind by 6 ancestros

	Ref. populace	bělouši	vraníci
Total number of founders (f)	1058	988	678
Effective number of founders (fe)	92.69	93.38	77.93
Effective number of ancestors (fa)	17.16	13.38	9.01
Founder genome equivalent (fge)	4.78	3.96	2.95
Ratio of effective number of founders to effective number of ancestors (fa/fe)	0.18	0.14	0.12
Ratio of effective number of founders to number of founders (fe/f)	0.09	0.09	0.12
Realized effective population size	**52.09**	**62.23**	**45.22**
Average inbreeding coefficient	**0.13**	**0.11**	**0.14**
Average relatedness coefficient	**0.21**	**0.25**	**0.34**
ΔF	**0.010**	**0.008**	**0.011**

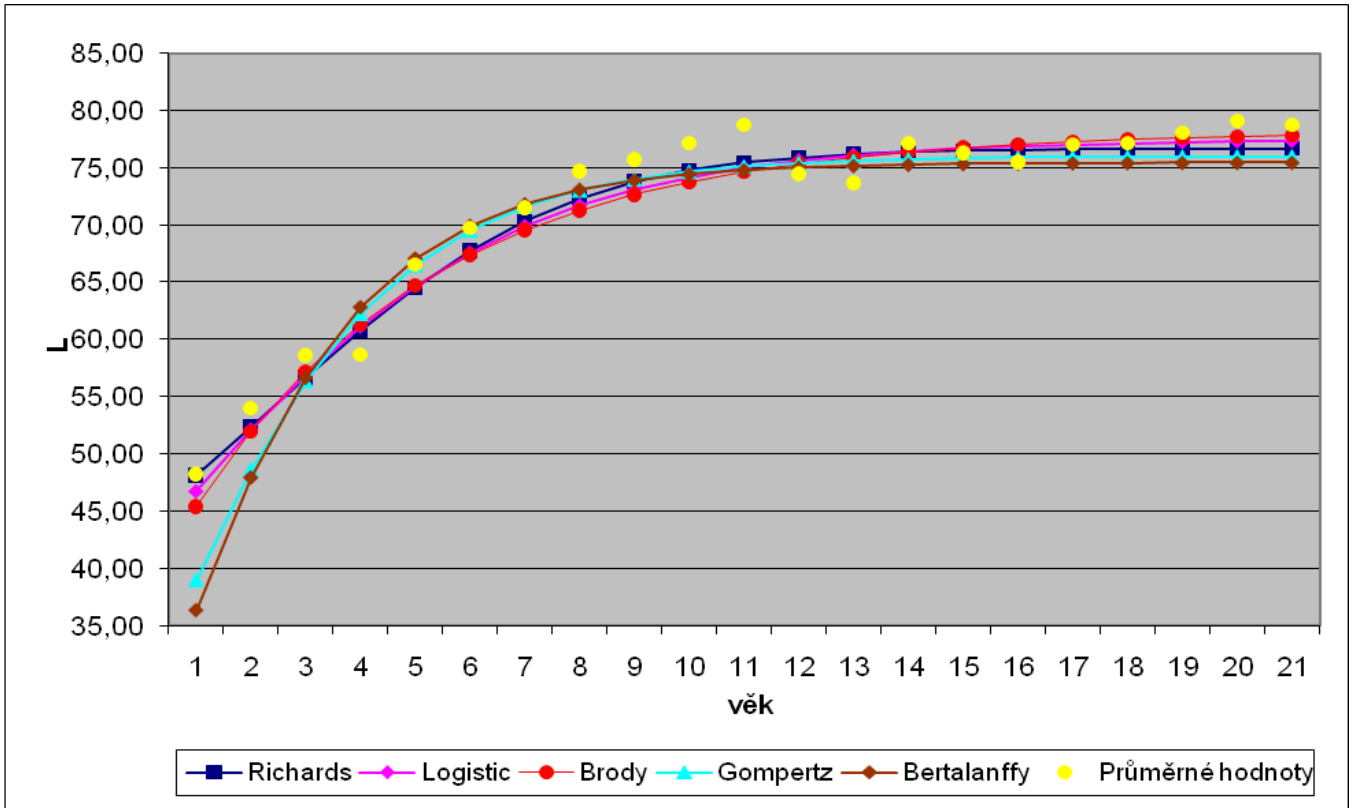
Greying



Minolta Spectrophotometr 2500d

- **L* - (0 = black, 100 = white)**
- a* - red +60 red; green -60
- b* - yellow +60; blue -60

Greying dynamics described by growth curves



$R^2 > 0,95$

Signicicant effects:

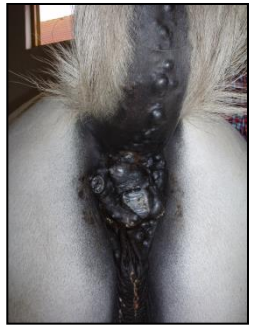
✓Age

✓Sex (higher level of greying in mares)

Melanoma occurence

Adspection according to *Sölkner et al. (2004) and Curik et al. (2013)*

Grade of melanoma	Description
0	Free of melanoma.
1	Early stages of plaque-type or nodule of Ø 0.5 cm.
2	Several nodules of Ø 0.5 cm or one nodus of Ø 2 cm.
3	One or several nodules of Ø 5 cm, or subcutaneous.
4	Extensive melanoma covered with skin, signs of destruction, metastasis.
5	Exophytic growth of tumours, vet surface, cachexia, disorders.

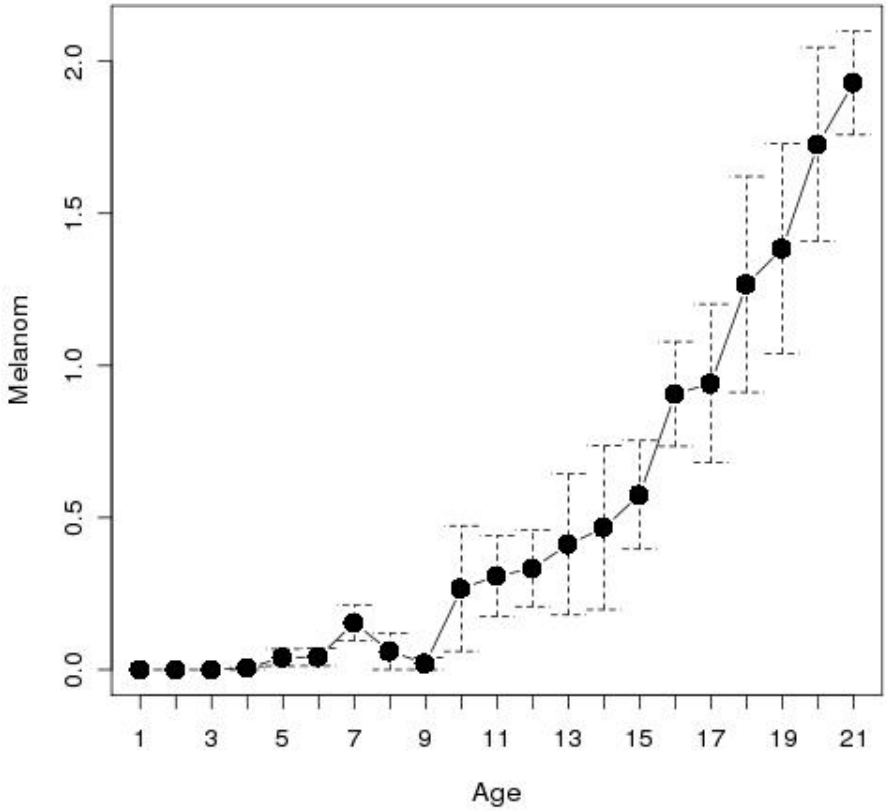


Melanoma occurence

14 % individuals,

33 % individuals older than 6 years,

68 % horses older than 15 years show melanoma

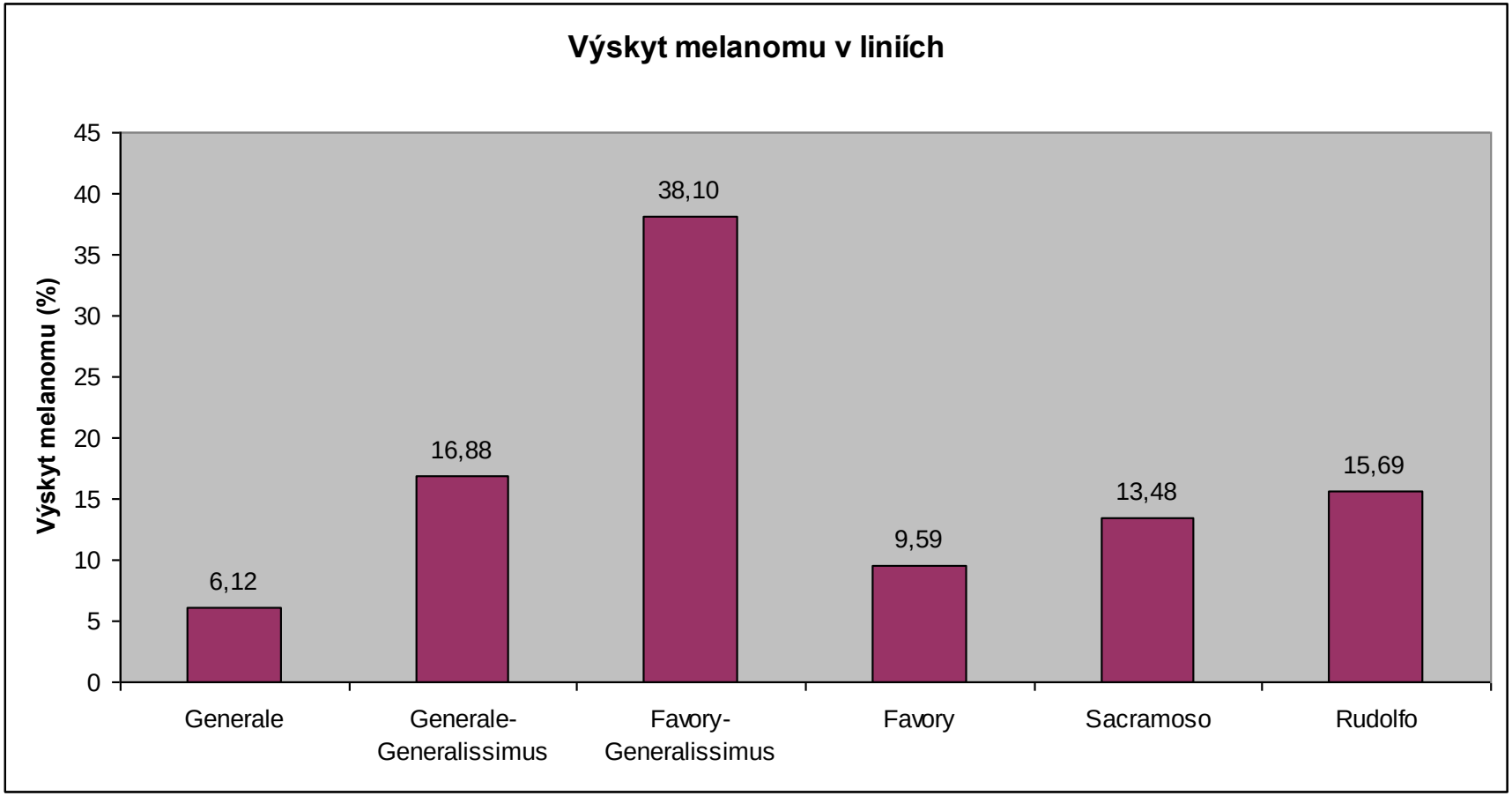


Significant effects:

✓Age

✓Line affiliation

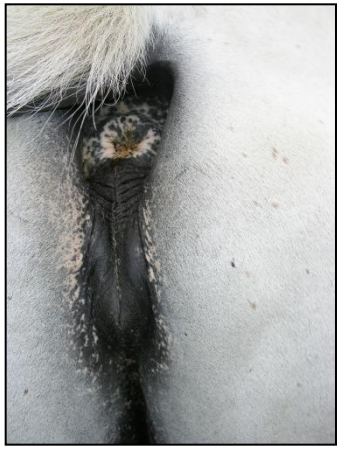
Melanoma occurence evaluated as
0 – NO;
1 – YES



Significant differences between lines ($\chi^2 = 40,9$; $p < 0,0001$)

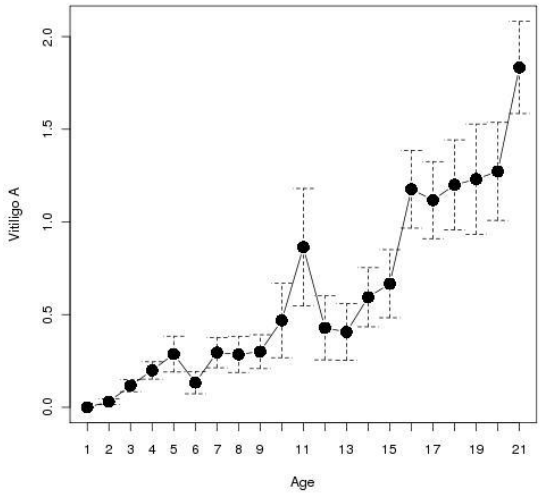
Vitiligo occurence

Separately scored at head part (vitiligo F) and back parts (vitiligo A)
According to *Sölkner et al. (2004) and Curik et al. (2013)*



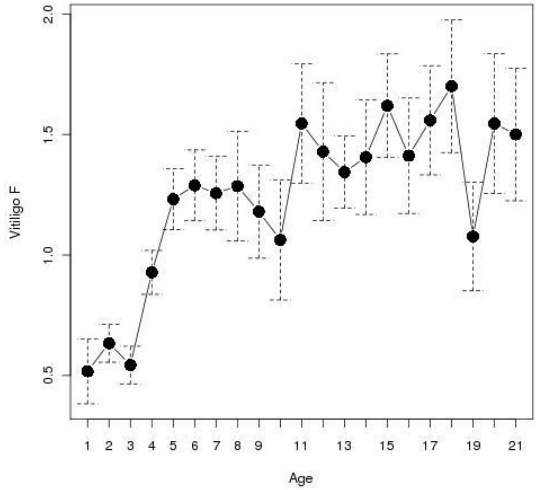
Vitiligo is quite common in Old Kladrubers, evaluated for possible correlation with melanoma.

Vitiligo A



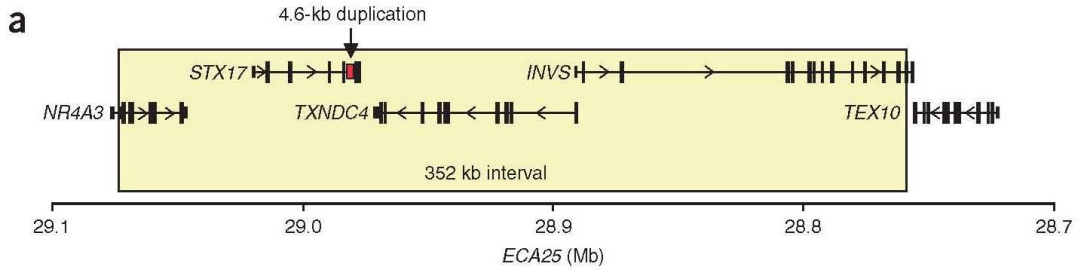
Significant effects:
- ✓Age
- ✓Line
- ✓Greying level (L*)

Vitiligo F



Significant effects:
- ✓Age
- ✓Line
- ✓Greying level (L*)
- ✓Sex
- ✓Year of evaluation
- ✓Stud

STX17 genotyping



(*Pielberg et al., 2008*)

50 breeding mares

Genotype	LS MEAN (SE) L*	MELANOMA	VITILA	VITILF
$G^G G^G$ *(74 %)*	**77,7832** (0,8032)	**0,8186** (0,1172)	**1,0162** (0,0856)	**1,6004** (0,1350)
$G^G G^g$ *(26 %)*	**67,0264** (1,1829)	**0,2226** (0,2135)	**0,3701** (0,1442)	**0,7230** (0,2273)

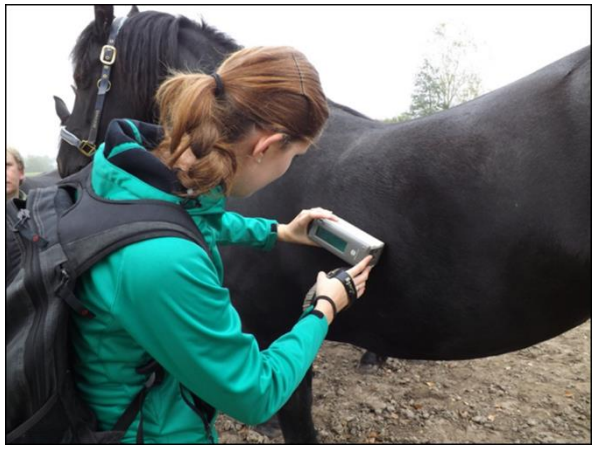
Significantly higher values in homozygote mares for all characteristics.

Genetic parameters

- **Phenotypic correlations** (under diagonal)
- **Genetic correlations** (above diagonal)
- **Heritability coefficients** (diagonal)

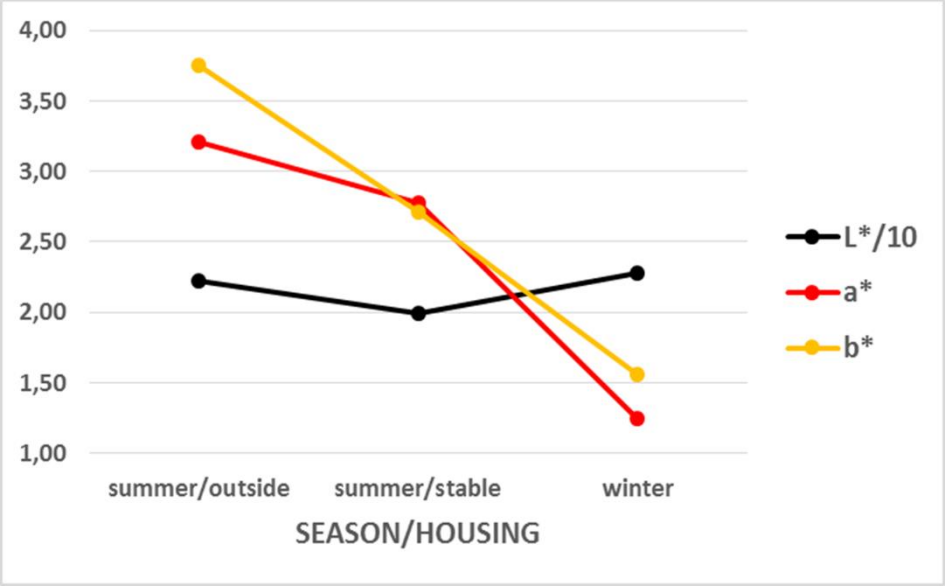
	L*	MELANOMA	VITILA	VITILF
L*	**0.5182**±0.0747	0.0247±0.2129	0.6727±0.1135	0.5332±0.1015
MELANOMA	0.2947	**0.0724**±0.0384	0.3774±0.1485	0.0113±0.1620
VITILA	0.3663	0.4060	**0.2019**±0.0534	0.5422±0.0763
VITILF	0.3725	0.1710	0.3103	**0.3459**±0.0644

Analysis of factors influencing black coat colour



Signicicant effects:

✓**Season/housing system**
(Fading due to sunlight in summer)
✓Age
✓Sex



Insect bite hypersensitivity (IBH)

- recurrent allergic skin disease
- most common allergic disease affecting horses worldwide
- starting impulse is a bite by midges of the *Culicoides* spp.
- main symptoms - itchiness, scaling, and hair loss

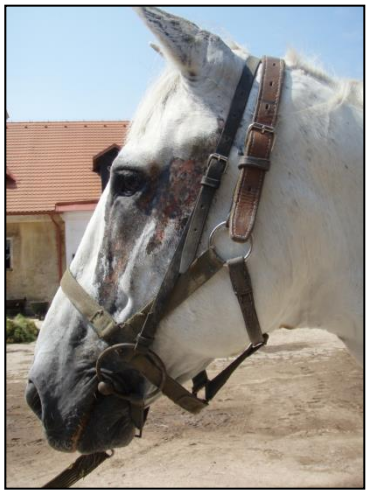
OCCURRENCE OF IBH

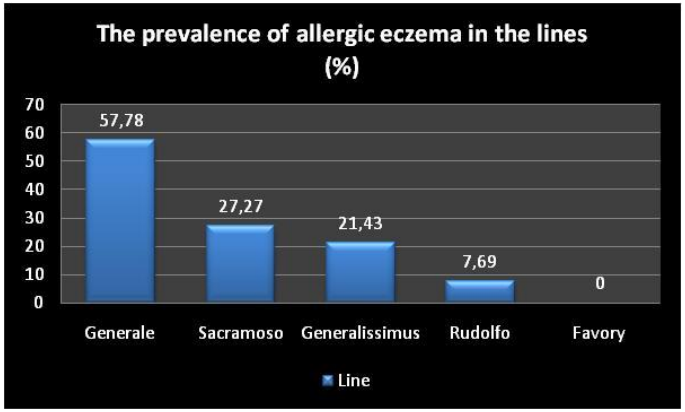
- Out of all horses examined in the stud (n=155), **30.97 % were affected with IBH**.

- The occurrence of IBH is significantly influenced by line origin of the horse.

- The extreme susceptibility for IBH showed offspring of the grey stallion Generale Proxima XLVIII by overall occurrence 81.58 %.

- The prevalence of IBH reached 91.7 % in offspring of Generale Proxima XLVIII with mares showing AE too.





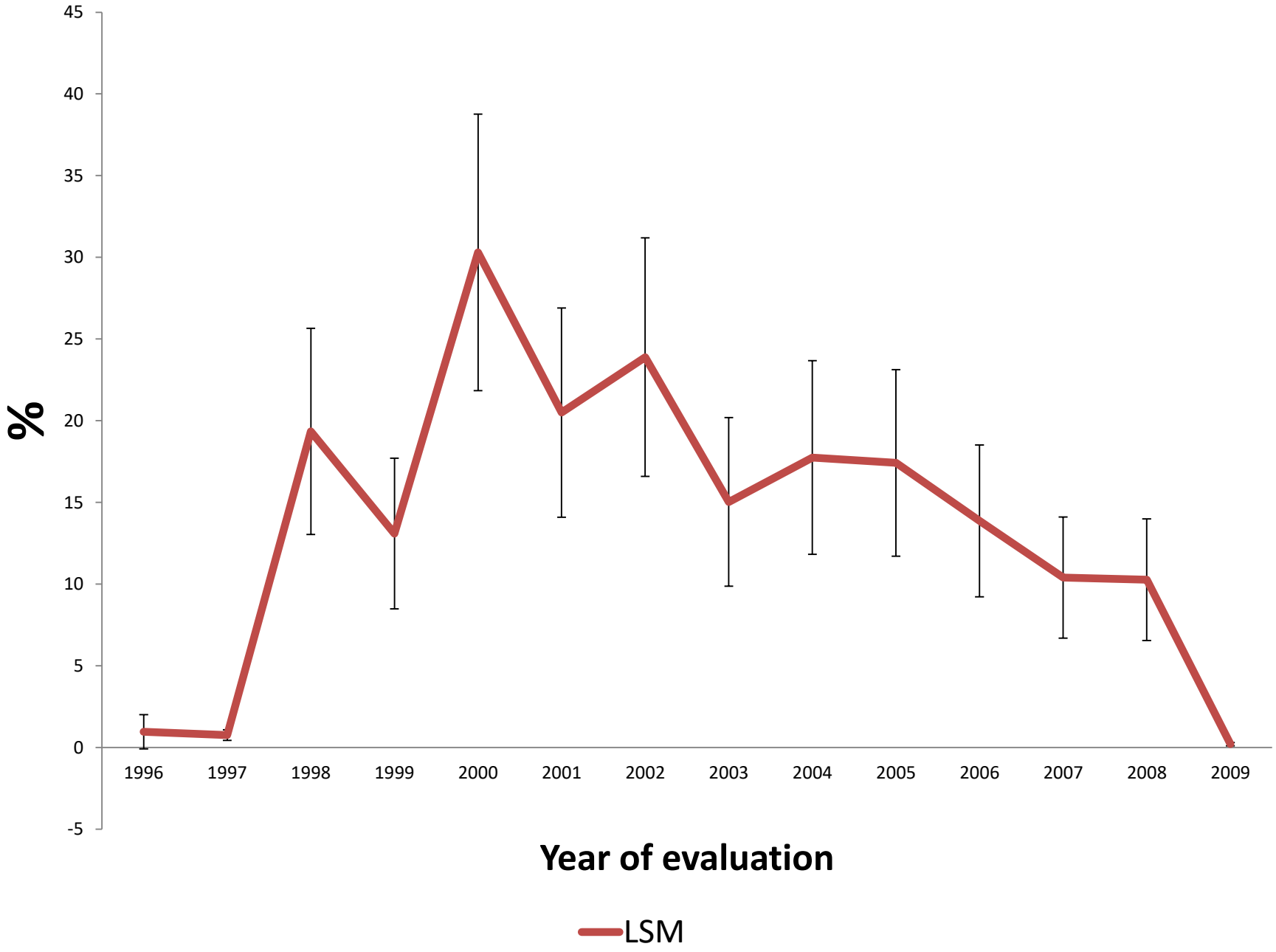
(Hofmanová et al., 2008)

Results

Effect		LSM	SE	*n*
Farm	Stud	22.76	2.77	1146
	Private	2.83	1.37	63

Around National Stud the conditions are convenient for the propagation of *Culicoides*.

		LSM	SE	n
Sex	Male	7.30	2.48	499
	Female	9.82	3.05	710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%
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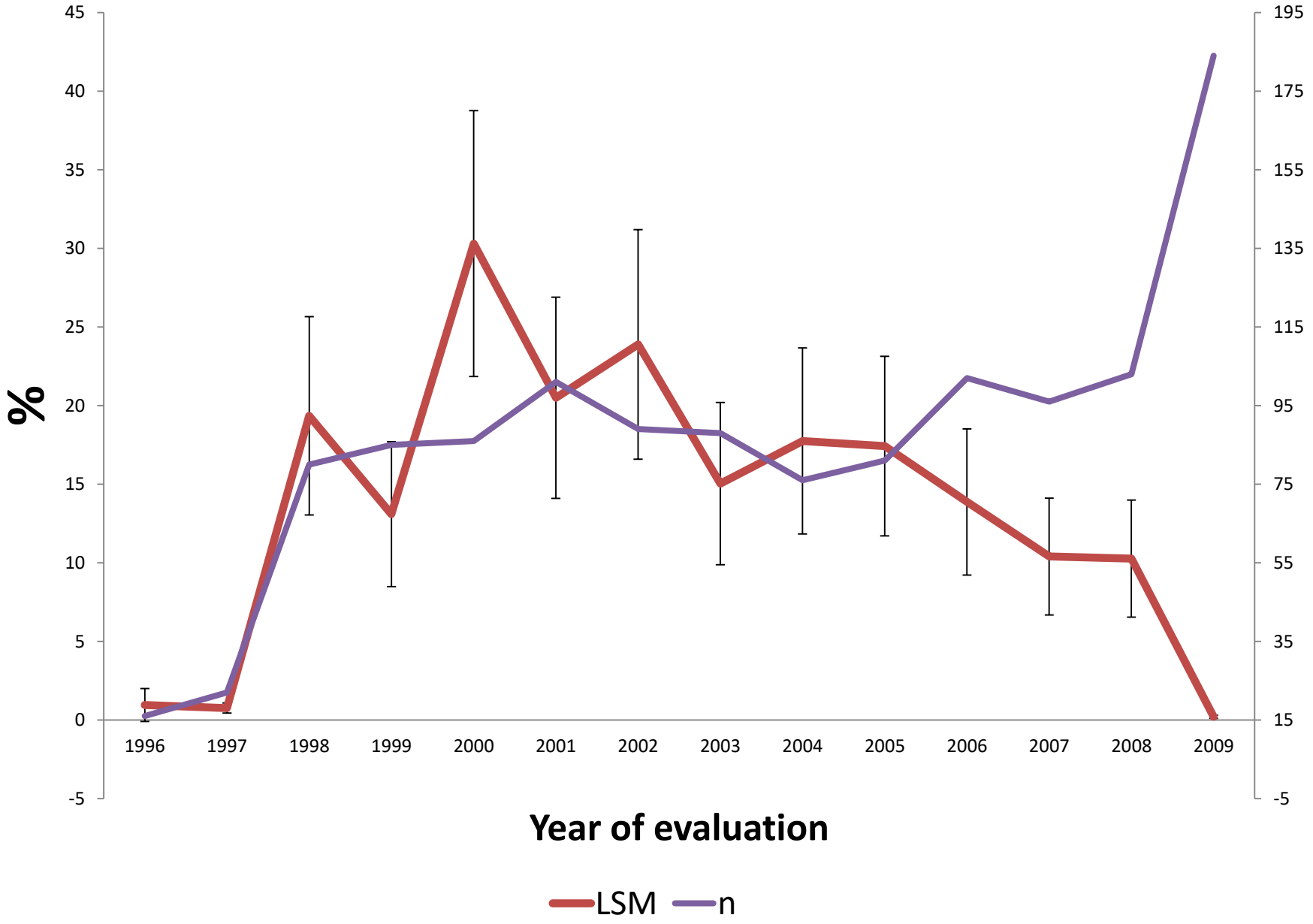
2007
2008
2009
Year of evaluation
LSM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Year of evaluation
LSM
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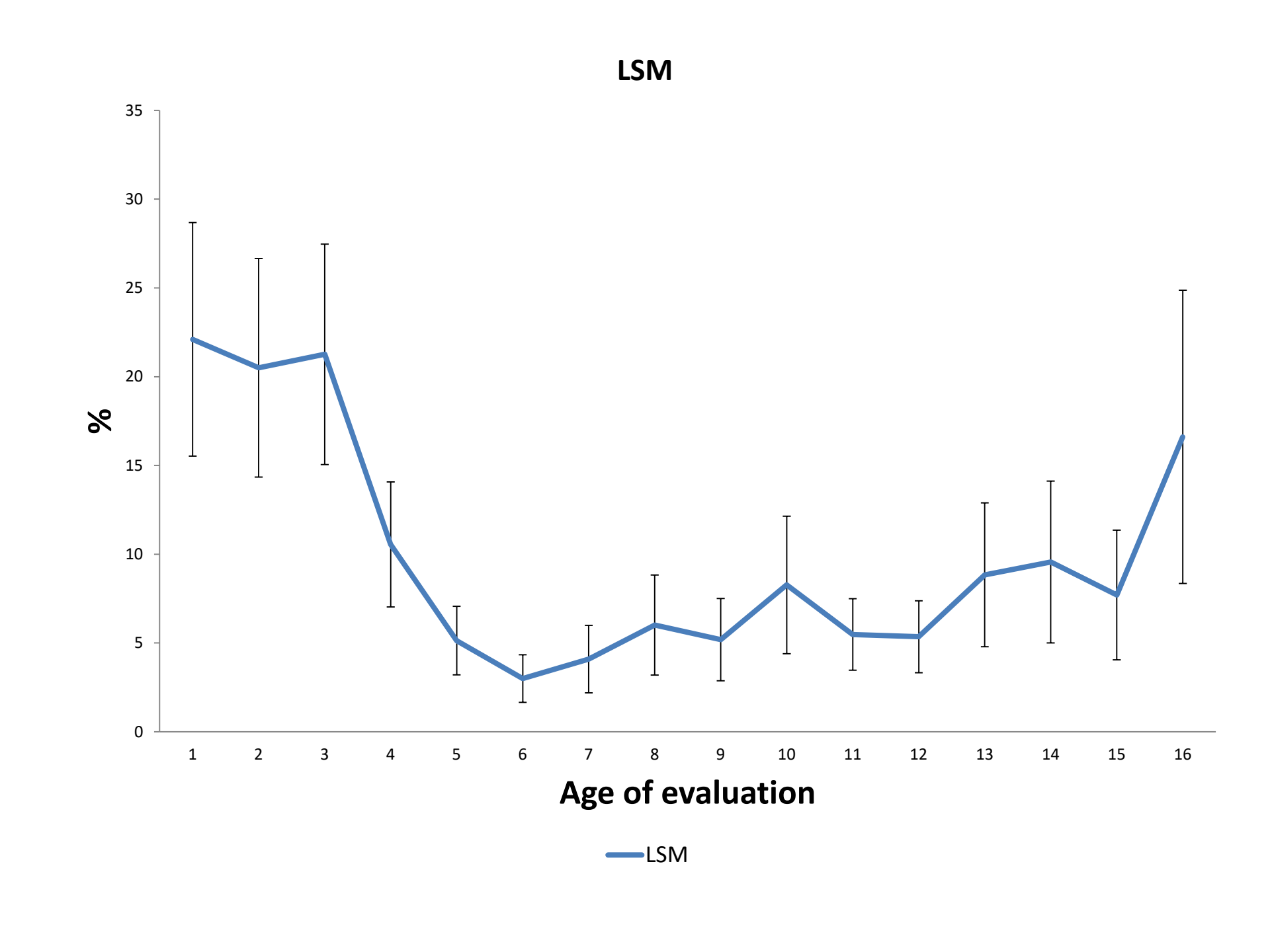
LSM
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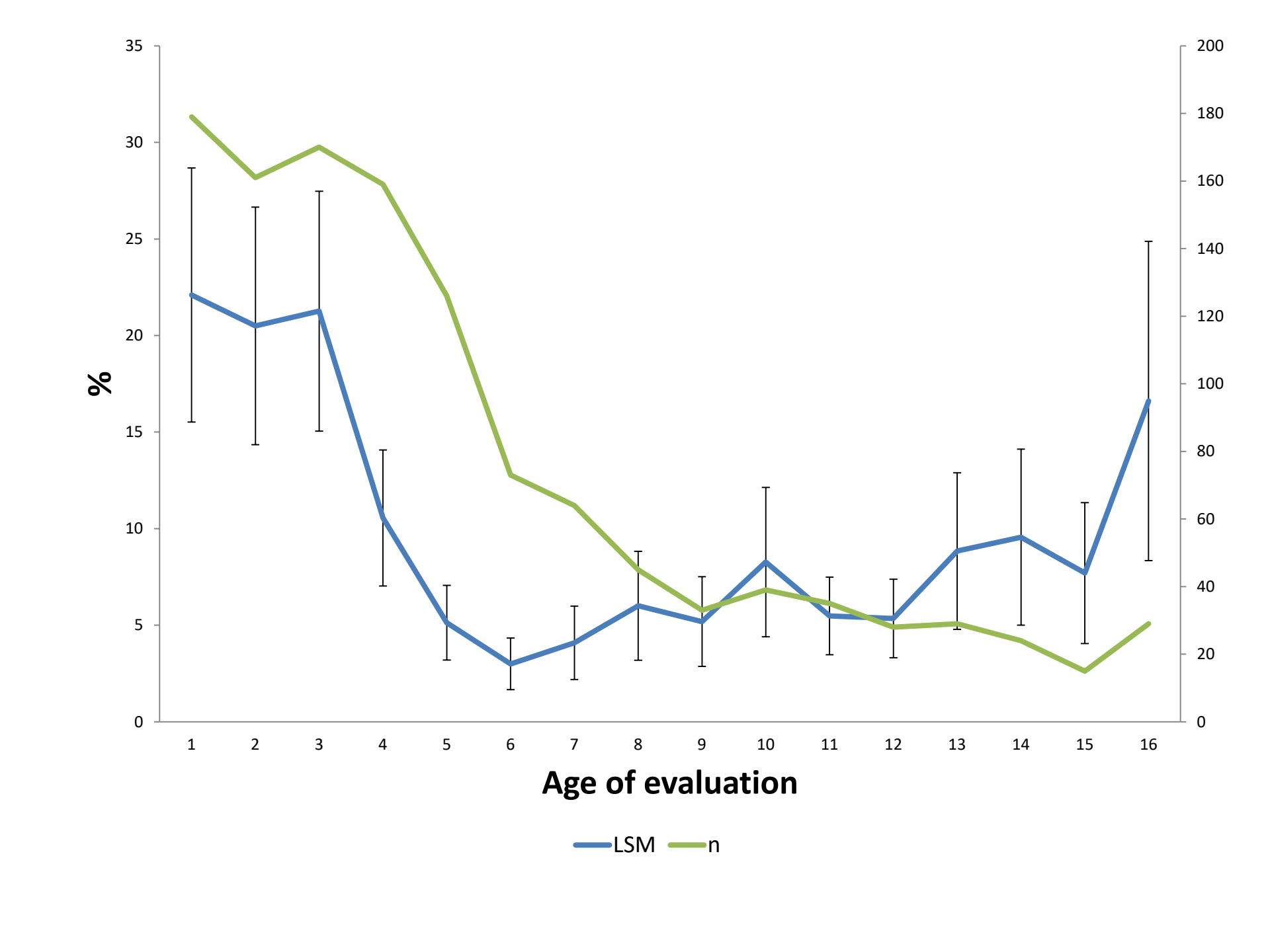
Age of evaluation
LSM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14
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16
Age of evaluation
LSM
n

	Model I	Model II	Model III
ΔAIC	0	30	10
$\sigma^2_{phenot.}$	8.641	8.164	8.331
h^2_a	**0.626 (0.116)**	**0.389 (0.089)**	**0.363 (0.098)**
h^2_m	**0.305 (0.141)**		**0.058 (0.071)**
r_{am}	**-0.769 (0.202)**		
r^2	0.636	0.615	0.623
e^2	0.364	0.385	0.377

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ΔAIC	0	30	10
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e^2	0.364	0.385	0.377

Vostrá-Vydrová, H., Vostrý, L., Hofmanová, B., Krupa, E., & Zavadilová, L. (2016). Pedigree analysis of the endangered Old Kladruber horse population. *Livestock Science*, *185*, 17-23.

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