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# Inbreeding, Microsatellite Heterozygosity, and Morphological Traits in Lipizzan Horses

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#### **Abstract**

While the negative effects of inbreeding and reduced heterozygosity on fecundity and survival are well established, only a few investigations have been carried out concerning their influence on morphological traits. This topic is of particular interest for a small and closed population such as the Lipizzan horse. Thus, 27 morphological traits were measured in 360 Lipizzan mares and were regressed on the individual inbreeding coefficients, as well as on the individual heterozygosity and mean squared distances (mean  $d^2$ ) between microsatellite alleles within an individual. Both individual heterozygosity and mean  $d^2$  were based on 17 microsatellite loci dispersed over 14 chromosomes. The results obtained by multivariate analysis reveal significant effects of stud (P < .0001), age at measurement (P < .0001), and mean  $d^2$  (P = .0143). In univariate analyses, significant associations were obtained between length of pastern-hindlimbs and inbreeding coefficient (P < .01), length of cannons-hindlimb and mean  $d^2$  (P < .01), and length of neck and mean  $d^2$  (P < .001). After adjustment of single-test P values for multiple tests (Hochberg's step-up Bonferroni method), only the association of the length of neck and mean  $d^2$  remained significant (P = .0213). Thus, no overall large effects of inbreeding, microsatellite heterozygosity, and mean  $d^2$  on morphological traits were observed in the Lipizzan horse.

Severe negative effects of inbreeding—that is, inbreeding depression—on traits that are closely related to fitness (fecundity and survival) are well documented in wild (Crnokrak and Roff 1999; Lacy et al. 1993), laboratory (Festing 1979; Wright 1977), and domestic animals (Pirchner 1985). On the other hand, the effects of inbreeding on traits that are less closely related to fitness, such as morphological traits, have been studied in only a few investigations.

As stated by Falconer and MacKay (1996), fitness traits show a reduction on inbreeding, whereas traits less related to fitness (i.e., morphological traits) show little or no change. This widely appreciated but rarely systematically tested statement is indirectly supported by several hypotheses offered in classical quantitative genetics textbooks. First, the theoretical explanation considers that inbreeding depression is a consequence of dominant gene effects (partial dominance, dominance, or overdominance), and thus traits with a higher dominance component are more sensitive to inbreeding depression (Falconer and MacKay 1996; Lynch and Walsh 1997). Second, experimental evidence indicates that

life-history traits have lower narrow sense heritabilities (additive to phenotype variance ratio) than morphological traits (Falconer and McKay 1996; Hartl and Clark 1997; Mousseau and Roff 1987) and relatively larger nonadditive genetic components (Fuerst and Sölkner 1994; Gengler et al. 1998).

Recently, DeRose and Roff (1999) analyzed data from the literature and provided evidence that morphological traits show slight inbreeding depression, although less severely than life-history traits (i.e., fitness-related traits). All 15 populations, with respect to the analysis of morphological traits, included in the study of DeRose and Roff (1999) belonged to nondomestic animal populations. While in domestic animals the number of unreported nonsignificant results is unknown, there are few reports presenting evidence of inbreeding depression on morphological traits (Gandini et al. 1992; Smith et al. 1998; Von Krosigk and Lush 1958; Wiener et al. 1992). Whether inbreeding affects morphological traits is important for the population of Lipizzan horses. Breeding is practiced in a small and closed population with census numbers ranging between 2,000 and 3,000 purebred horses, and the mating of related individuals is unavoidable. Whether bred for show and parade at the Spanish Riding School in Vienna (original purpose) or as carriage horses at the highest performance level, morphological traits are very important in the breeding. In the relevant literature, the only direct evidence related to the effects of inbreeding on morphological traits is found in the Haflinger horse (Gandini et al. 1992), where significant negative effects of inbreeding were reported for height at withers and circumference of chest. In the same study, nonsignificant effects of inbreeding on circumference of the cannons were reported. The results from the study of Klemetsdal (1998), who found a negative effect of inbreeding on the racing performance of Norwegian cold-blooded trotters, may be taken as indirect evidence of a negative effect of inbreeding on morphological traits.

Least-squares linear regression of individual performance on the pedigree inbreeding coefficient is a common method applied to livestock populations. The use of molecular markers—that is, the establishment of an association between individual heterozygosity and traits under study—is a common approach used in biological research when pedigree information is not available (Allendorf and Leary 1986; Lynch and Walsh 1997; Mitton 1993). Recently Coulsen et al. (1998) proposed a new measure, known as mean  $d^2$ , which is calculated from the squared difference in the number of repeat units between two alleles at a microsatellite locus, averaged over all typed loci. Under the assumption that microsatellites evolve under the stepwise mutation model (Goldstein et al. 1995; Valdes et al. 1993), mean  $d^2$  focuses on events deeper in the individual's ancestry than can be obtained by individual heterozygosity or pedigree information.

The data from the first experimental studies (Coltman et al. 1998; Coulsen et al. 1998) demonstrated that mean  $d^2$  provides a more powerful predictor of inbreeding depression and/or heterosis on juvenile survival traits than individual

heterozygosity (see also Pemberton et al. 1999). Based on their analyses of a wolf population with known inbreeding level and founder sources, Hedrick et al. (2001) questioned the usefulness of mean  $d^2$  to detect inbreeding and outbreeding and to identify biologically important associations with fitness-related traits and suggested that theoretical model ing is needed to determine the power and statistical properties of mean  $d^2$ .

Recently, using a theoretical approach, Tsitrone et al. (2001) concluded that individual heterozygosity outperforms mean  $d^2$  in assessing microsatellite genotype/fitness correlations in all but a few circumstances. However, as the microsatellite mutation process is not understood completely, it is still unclear to what extent mean  $d^2$  is a reliable measure, and the answer to this question will have to be validated further through experimental work.

While inbreeding coefficient is a relative measure related to the defined base population (see Materials and Methods), both individual heterozygosity and mean  $d^2$  are absolute measures and are related to the inbreeding—outbreeding continuum. For that reason, any significant associations between individual heterozygosity or mean  $d^2$  and studied traits might also be a consequence of heterosis as a phenomenon antithetical, although not completely (for the explanation, see Lynch and Walsh 1997), to inbreeding depression.

The aim of the present study was to analyze whether inbreeding affects morphological traits in Lipizzan horses. In addition to pedigree information, we used molecular markers and examined the association between individual heterozygosity as well as mean  $d^2$  and morphological traits.

#### **Materials and Methods**

The analyzed data set included 360 breeding Lipizzan mares from the following seven national state studs: Beclean-Romania (24), Fagaras-Romania (76), Djakovo-Croatia (38), Lipica-Slovenia (39), Piber-Austria (78), Szilvásvárd-Hungary (68), and Topol'cianky-Slovakia (37).

#### Inbreeding Coefficient and Pedigree Structure

Individual inbreeding coefficients (F) were calculated from the pedigree data file consisting of 3867 horses by the tabular method using the algorithm of Van Raden (1992). We also calculated inbreeding coefficients with restricted pedigree information. It should be noted that the inbreeding coefficient is a relative measure of inbreeding referring to a defined base population of unrelated individuals. When the inbreeding coefficient is calculated from the pedigree, it refers to the founding animals that are assumed to be unrelated. Accordingly, the length of the pedigree and missing pedigree information (completeness) are factors that should be reported along with inbreeding coefficients (for more details about this subject, see Baumung and Sölkner 2001; MacCluer et al. 1983; Van Raden 1992). In this article we quantified the length of the pedigree as the percentage of ancestors known in each ascending generation and pedigree

Locus	No. of typed mares	No. of alleles	Size range (bp)	Mean individual heterozygosity	Mean size difference (bp)	Chromosome location
AHT4	359	7	144–159	0.694	5.813	24
AHT5	349	6	127-138	0.799	4.387	6
AHT21	358	7	195-211	0.508	4.570	10
HMS1	360	5	174-184	0.547	3.256	15
HMS2	359	8	217-236	0.691	3.532	10
HMS6	358	6	157-168	0.665	4.673	4
HMS7	357	7	171-186	0.756	4.538	1
HMS8	360	5	205-221	0.647	3.522	19
HTG4	322	6	127-137	0.640	3.019	9
HTG6	359	6	78–97	0.535	6.638	15
HTG7	359	3	118-126	0.643	3.721	4
HTG10	357	10	86-108	0.672	4.462	21
LEX053	339	5	122-132	0.788	3.215	23
UCDEQ405	333	7	247-274	0.598	5.538	25
UCDEQ437	354	9	165-191	0.613	5.689	3
UCDEQ505	360	10	175-195	0.728	4.878	16

86-105

**Table 1.** Microsatellite loci investigated, number of typed mares, number of alleles, size range, mean individual heterozygosity, mean size difference, and chromosome location

completeness as the complete generation equivalent (CGE) (see equation 1),

360

VHL20

$$CGE = \sum_{i=1}^{m} \frac{n_i}{2^i} \tag{1}$$

where m is the maximal (successive) number of ancestral generations,  $n_i$  is the number of horses in the pedigree in generation i, and  $2^i$  is the number of horses that would be found in a complete pedigree in that generation. Both measures were already used in studies associated with variables related to the pedigree structure (e.g., Boichard et al. 1997; Moureaux et al. 1996; Sölkner et al. 1998).

#### Microsatellites, Heterozygosity, and Mean $d^2$

Individual mares (360) were typed from blood samples at 17 dinucleotide repeat microsatellite loci-AHT4, AHT5, AHT21, HMS1, HMS2, HMS6, HMS7, HMS8, HTG4, HTG6, HTG7, HTG10, LEX053, UCDEQ405, UC-DEQ437, UCDEQ505, and VHL20—dispersed over 14 different chromosomes (Table 1). Polymerase chain reaction (PCR) amplification products were separated on a ABI PRISM 310 Genetic Analyzer (PE Applied Biosystems Division), applying standard loading and electrophoresis conditions (Wenz et al. 1998). The separation medium was ready-to-use polymer POP4 (PE Applied Biosystems Division). Allele sizes were determined after processing the raw data with GeneScan 2.0 and Genotyper 2.1 software (PE Applied Biosystems Division). Allele designations refer to the center value (calculated as the weighted average) of similarsize alleles (within the range of 1 base pair, bp) rounded to the next integer as suggested by the "add category" submenu of Genotyper 2.1. Therefore alleles did not necessarily differ in size by multiples of 2 bp, which would be expected if the number of dinucleotide repeats is the variable factor. The genotyping was successful at 17, 16, 15, 14, and 11 microsatellite loci for 276, 56, 26, 1, and 1 mares, respectively.

Individual heterozygosity was calculated as the number of loci at which the mare was heterozygous, divided by the total number of loci at which a mare was scored. Mean  $d^2$  was calculated for each mare according to equation 2,

5.344

mean 
$$d^2 = \frac{1}{n} \sum_{i=1}^{n} (a_i - b_i)^2$$
 (2)

30

in which  $a_i$  and  $b_i$  refer to the lengths (in bp) of each allele at a locus i, and n is the total number of loci at which a mare was scored (see Coltman et al. 1998). Thus calculated mean  $d^2$  is proportional to the mean  $d^2$  expressed in repeat units.

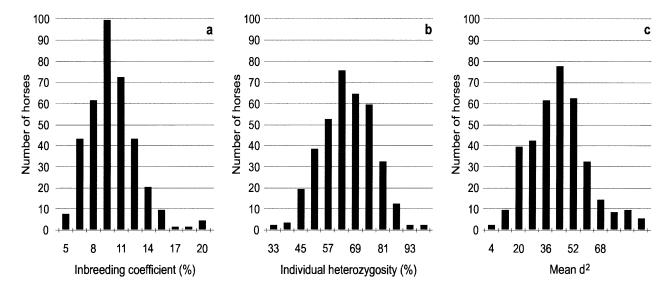
#### Morphological Traits

0.781

All 360 mares were measured for 27 morphological traits related to head and neck (length of head, width of head, and length of neck), body (height at withers, height of back, height of rump, body length, length of forequarters, length of barrel, length of rear quarter, depth of chest, width of chest, width of hips, width of thurls, length of shoulder, and circumference of chest), and legs (circumference of cannon bone-forelimb, circumference of cannon bone-hindlimb, length of upper arm 1, length of upper arm 2, length of forearm, length of cannons-forelimb, length of pasternforelimb, length of thigh, length of second thigh, length of cannons-hindlimb, and length of pastern-hindlimb). Definition of traits, description of the measuring procedures, and parameter estimates (heritability and repeatability) are described in more detail in Zechner et al. (2001). Since the Lipizzan is a late-maturing breed, only mares ≥4 years old were measured.

#### Statistical Analysis

Multivariate analysis of variance (MANOVA) was used to assess the effects of inbreeding, heterozygosity, and mean  $d^2$  on 27 morphological traits. We started with a model



**Figure 1.** Frequency distribution of inbreeding coefficients (mean = 10.3%, std = 3%), individual heterozygosity (mean = 67%, std = 11%), and mean  $d^2$  (mean = 43.45, std = 16.91) for 360 Lipizzan mares.

including stud (Beclean-Romania, ..., Topol'cianky-Slovakia) as a fixed effect, age at measurement (4, ..., 23 years) as a covariable, and a linear regressor (inbreeding coefficient, individual heterozygosity, or mean  $d^2$ ) and all two-way interactions between those terms. Then, from higher- to lower-order terms, the independent variables having the highest P values were omitted from the model if they were nonsignificant. Lacy and Horner (1996) applied the same modeling strategy when considering the effects of inbreeding on skeletal developments in Rattus. In addition to MAN-OVA, we also performed univariate analyses to identify single traits that have contributed to the overall multivariate effects. Since many of the tests (27) were performed per hypothesis (inbreeding coefficient, individual heterozygosity, or mean  $d^2$ ), P values were adjusted according to Hochberg's step-up Bonferroni method (Hochberg 1988). All analyses were performed with the SAS statistical package (SAS 1999). As expected from previous knowledge, all morphological traits were approximately normally distributed and were therefore not transformed prior to statistical analysis.

#### **Results and Discussion**

#### Inbreeding Level

The frequency distribution of inbreeding coefficients is shown in Figure 1, and pedigree structure (percentage of known ancestors and CGE) and inbreeding levels calculated by restricted pedigree information are presented in Table 2. In absolute numbers, the obtained mean inbreeding level (F = 10.30%) was somewhat higher in the Lipizzan than in the Swedish standardbred trotter (F = 2.26%; see Ström 1982), the North American standardbred (F = 8.99%; see MacCluer et al. 1983), the Norwegian trotter (F = 5.83%; see Klemetsdal 1993), the Italian Haflinger (F = 6.59%; see

Gandini et al. 1992), and five horse breeds raised in France (Arab, F = 7.10%; Anglo-Arab, F = 2.90%; Selle Français, F = 2.40%; thoroughbred, F = 2.60%; and Trotteur Français, F = 5.20%; see Moureaux et al. 1996), but slightly lower than the inbreeding level obtained from a sample of 59 thoroughbred mares (F = 12.5%; see Mahon and Cunningham 1982). However, the level of inbreeding is influenced very much by pedigree length and completeness, and only the studies of MacCluer et al. (1983) and Mahon and Cunningham (1982) are comparable in this respect. When compared with pedigrees of about the same depth and completeness, the inbreeding level apparent in Lipizzan horses is similar to that obtained in other studies. Very close inbreeding has been avoided, and only a small percentage of mares has become inbred due to a common ancestor in the second (grandparent [1.1%]) or third (great-grandparent [11.4%]) generation.

#### Individual Heterozygosity and Mean $d^2$

Descriptive statistics and frequency distributions of the individual heterozygosity and mean  $d^2$  are shown in Figure 1. Coltman et al. (1998) and Slate et al. (2000) used transformed (standardized) heterozygosity and mean  $d^2$  in order to reduce the influence of highly polymorphic loci and to normalize the data. In the study in question, values were not transformed (standardized) prior to analysis. First, all loci contributed similarly to the overall heterozygosity and mean  $d^2$  (Table 1), and second, both variables were approximately normally distributed (Figure 1). Mean individual heterozygosity and mean  $d^2$  were 67% and 43.16 (in bp<sup>2</sup>), respectively. As expected, there was a positive correlation (r = 0.406, P < .0001) between the two variables. A nonsignificant correlation was observed between inbreeding coefficient and individual heterozygosity (r = -0.034, P = .526), as well

	Inbreedi	ng coefficient (%	)			
Generation	Mean Minimum		Maximum	Complete generation equivalent	Percentage of known ancestors	
2	0.14	0	12.50	2.00	100	
5	1.96	0	13.7	4.99	100	
8	3.97	0.05	15.20	7.89	94	
11	5.78	0.58	16.21	10.57	86	
14	7.72	1.99	18.05	12.90	73	

14.53

15.07

Table 2. Inbreeding coefficient, complete generation equivalent, and percentage of known ancestors by generation

19.86

20.49

as between inbreeding coefficient and mean  $d^2$  (r = 0.017, P = .754). In a study of red deer, Pemberton et al. (1999) observed a similar correlation coefficient between those two variables (r = 0.387, P < .001). Working on the same data, Coulson et al. (1998) did not observe a significant relationship between individual heterozygosity and mean  $d^2$  with the pedigree inbreeding coefficient. Contrary to these results, in a study by Hedrick et al. (2001), the inbreeding coefficient, calculated from shallow pedigrees, explained a large amount of variance in heterozygosity ( $R^2 = 0.521$ , P < .001) and a somewhat lower amount of variance in mean  $d^2$  ( $R^2 = 0.201$ , P < .013).

3.90

4.50

9.36

10.13

17

20

## Effects of Inbreeding and Microsatellite Heterozygosity on Morphological Traits

The results of the MANOVA are presented in Table 3. In all three models, with the exception of two terms (stud and age at measurement) that were highly significant (P < .0001) in all models, only the effect of mean  $d^2$  was significant (P = .014), while the effects of inbreeding and individual heterozygosity, as well as all two-way interactions, were not significant (P > .050). The results of univariate analyses are shown in Table 4. Of 27 analyses, a significant effect of the inbreeding coefficient (P = .004) was present only for the length of pastern-hindlimbs. The significant estimate showed an increase of 3.5% from the mean per 10% of inbreeding. However, when adjusted for multiple tests, the effect of inbreeding on the length of pastern-hindlimbs did not remain significant (P = .108). No single significant estimate was obtained when individual heterozygosity was taken as an

independent variable. When mean  $d^2$  was considered as a variable in the hypothesis, significant associations were obtained only with length of cannons-hindlimb (P = 0.003) and length of neck (P = .001). Both estimates were positive, showing an increase of 3.8% and 6.0% from the mean per 100 mean  $d^2$  units, respectively. After adjustment of exact P values for multiple tests, only the relationship of the length of neck and mean  $d^2$  remained significant (P = .021). For the traits significantly associated with mean  $d^2$  (length of cannons-hindlimb and length of neck) in the univariate analyses, we also performed locus-specific tests to analyze if the significant associations are the result of the single locus contribution or a consequence of the genome-wide effect. Two univariate analyses were performed per trait and locus. First, mean  $d^2$  from a single locus were fitted in the model. Second, 17 mean  $d^2$  variables (each based on 16 loci) were constructed by subsequent dropping of only one microsatellite locus at a time and then fitted in the model. When fitted individually, only microsatellite locus UCDEQ437 was significantly associated with analyzed traits (Table 5). However, mean  $d^2$  associations remained significant after dropping any one locus, suggesting that other loci were also contributing to the association between mean  $d^2$  and length of cannons-hindlimb and length of neck.

42

8

### **Conclusion**

Pedigree completeness, depth of the pedigree, and pedigree errors are among the factors known to influence the estimation of inbreeding effects. Although the length (percentage of known ancestors) and completeness (CGE

**Table 3.** Results of MANOVA for the effects of inbreeding, heterozygosity, mean  $d^2$ , age, stud, and all two-way interactions on the 27 morphological traits of 360 mares

Inbreeding		Heterozygosity	Mean d <sup>2</sup>		
Term	P	Term	P	Term	Р
Stud	.0001	Stud	.0001	Stud	.0001
Age	.0001	Age	.0001	Age	.0001
Inbreeding (a)	.7334	Heterozygosity (b)	.6286	Mean $d^2$ (c)	.0144
Stud × age	.0625	Stud × age	.0934	Stud × age	.1807
Inbreeding × stud	.0504	Heterozygosity × stud	.8151	Mean $d^2 \times$ stud	.0661
Inbreeding × age	.1481	Heterozygosity × age	.0577	Mean $d^2 \times age$	.6877

Significance in the MANOVA (P) based on Wilks' criterion for calculation of exact F and was determined by successive deletion testing. Linear regression slopes associated with (a) pedigree inbreeding coefficient, (b) individual heterozygosity, and (c) mean  $d^2$ .

**Table 4.** Effects of inbreeding, heterozygosity, and mean  $d^2$  on morphological traits of 360 mares after removing effects of stud and age

			Inbreeding (a)		Heterozygosity (b)		Mean d <sup>2</sup> (c)	
Trait	Mean	Std	Effect	SE	Effect	SE	Effect	SE
Head and neck								
Length of head	59.52	1.79	1.2905	3.6928	0.3517	0.7860	-0.0046	0.0052
With of head-lower jaw	15.38	0.90	-0.6748	1.8313	-0.4466	0.3892	0.0006	0.0026
Length of neck	73.75	5.59	6.8757	9.3093	3.4243	1.9747	0.0439***	0.0130
Body measurements								
Height at withers	154.44	3.82	6.0765	7.8528	1.5957	1.6771	0.0023	0.0111
Height of back	144.26	3.79	0.0616	7.4351	1.5701	1.5804	0.0019	0.0105
Height of rump	152.88	3.93	1.2905	3.6928	0.8896	1.6490	0.0005	0.0110
Body length	160.91	4.62	7.8864	9.2701	-0.6527	1.9750	0.0002	0.0131
Length of forequarters	38.42	2.90	2.8444	5.1079	1.4398	1.0851	0.0036	0.0072
Length of barrel	73.52	4.01	1.2398	7.8248	-1.0764	1.6650	0.0114	0.0111
Length of hindquarters	49.06	2.73	2.4025	5.1079	-1.0325	1.0513	-0.0093	0.0070
Depth of chest	73.08	2.50	5.7083	4.9960	0.0432	1.0655	-0.0051	0.0071
Width of chest	41.06	2.94	3.0936	5.3783	-0.2750	1.1450	-0.0029	0.0076
Width of hips	53.76	2.50	1.9033	4.8466	-1.0422	1.0304	-0.0038	0.0069
Width of thurls	51.25	2.61	-2.6643	4.9876	-0.4350	1.0620	-0.0057	0.0071
Length of shoulder	58.02	2.42	5.9905	4.8116	0.1315	1.0265	-0.0080	0.0068
Circumference of chest	187.63	7.45	3.1223	13.1657	-1.2692	2.8020	-0.0250	0.0186
Legs								
Circumference of cannon bone-forelimb	19.69	0.85	0.6272	1.4840	-0.0430	0.3160	-0.0008	0.0020
Circumference of cannon bone-hindlimb	22.13	0.97	1.8279	1.7710	-0.0737	0.3776	0.0027	0.0025
Length of upper arm one	30.67	1.85	-2.3494	3.6002	-0.2581	0.7667	0.0022	0.0051
Length of upper arm two	37.45	1.52	-0.9048	2.9491	0.3402	0.6276	0.0030	0.0042
Length of forearm	39.40	1.43	-2.0994	2.9678	-0.2555	0.6320	0.0062	0.0042
Length of cannons-forelimb	24.21	1.14	2.1339	2.1398	-0.4021	0.4556	0.0023	0.0030
Length of pastern-forelimb	14.57	0.78	1.5923	1.6650	0.2550	0.3546	0.0016	0.0024
Length of thigh	41.09	1.96	3.1341	4.1890	-1.1747	0.8901	-0.0031	0.0059
Length of second thigh	38.11	2.16	-2.3881	4.2778	-0.4566	0.9107	-0.0101	0.0060
Length of cannons-hindlimb	28.64	1.23	0.7931	2.6198	0.2198	0.5576	0.0108**	0.0037
Length of pastern-hindlimb	14.05	0.80	5.0281**	1.7358	-0.1495	0.3738	0.0006	0.0025

Single-test significance level; \*P < .05, \*\*P < .01, and \*\*\*P < .001. Linear regression slopes associated with (a) pedigree inbreeding coefficient, (b) individual heterozygosity, and (c) mean  $d^2$ . Bold numbers indicate significance level of P < .05 obtained after adjustment for multiple tests based on Hochberg's step-up Bonferroni method.

= 15.12) of the pedigree data used in this study are among the best in domestic animals (compare the percentage of known ancestors and CGE values obtained in this study with values from Boichard et al. 1997, Moureaux et al. 1996, and Sölkner et al. 1998), we do not know to what extent it has influenced the estimation of inbreeding effects. However, there was a significant and high correlation (r = 0.821, P < .001) between the inbreeding coefficient based on all pedigree information and the inbreeding coefficient calculated from the five-generation pedigree (almost complete pedigrees, CGE = 4.99).

In addition, we performed MANOVAs where the inbreeding coefficient was based on five generations and found no significant association between inbreeding and morphological traits (Wilks'  $\Lambda=0.906,\,P=.188$ ). While pedigree errors have occurred—according to Dovc et al. (2001) approximately 8% of the horses were assigned to an incorrect maternal line (based on mitochondrial DNA)—these would have no influence on the estimates based on molecular data. However, we also did not find any significant

association between individual heterozygosity and morphological traits, while only two significant associations where found for mean  $d^2$ . Thus the main conclusions drawn from the results based on pedigree information are in agreement with conclusions made from the molecular data, although the correlations between estimated effects based on inbreeding coefficient and individual heterozygosity (r = 0.260, P =.187), as well as on inbreeding coefficient and mean  $d^2$  (r =0.170, P = .395) were not significant. On the other hand, there was a highly significant correlation between estimated effects obtained from individual heterozygosity and mean  $d^2$ (r = 0.680, P < .0001). However, mean  $d^2$  explained variation in length of cannons-hindlimb and in length of neck that was not detected by individual heterozygosity. Considering this fact, our study supports Coulson et al.'s (1998) concept that mean  $d^2$  might be a practical tool for studying the consequences of inbreeding and/or heterosis.

The presence of the artificial selection and breeding decisions that have been made by Lipizzan horse breeders over the last three centuries might have an influence on the

**Table 5.** Influence of  $d^2$  measured individually for each locus on the length of neck and length of cannons-hindlimb

	Length of	neck	Length of cannons-hindlimb		
Locus	P (EWL)	P (SLE)	P (EWL)	P (SLE)	
AHT4	.0009	.3790	.0064	.2232	
AHT5	.0009	.6771	.0032	.8599	
AHT21	.0016	.1713	.0031	.6201	
HMS1	.0006	.3799	.0037	.6020	
HMS2	.0004	.5509	.0024	.8070	
HMS6	.0011	.4750	.0028	.7597	
HMS7	.0010	.4795	.0021	.5196	
HMS8	.0009	.6813	.0047	.4353	
HTG4	.0013	.2342	.0034	.4945	
HTG7	.0006	.7823	.0025	.4999	
HTG10	.0007	.9132	.0022	.7970	
<i>LEX053</i>	.0012	.0614	.0040	.9985	
UCDEQ405	.0015	.3635	.0086	.3380	
UCDEQ437	.0037	.0200	.0136	.0367	
UCDEQ505	.0013	.3048	.0050	.3888	
VHL20	.0006	.8627	.0040	.5157	

EWL is related to the effects of mean  $d^2$  without a defined single locus, while SLE is related to the single-locus  $d^2$  effects.

genetic diversity of the population as well as on the estimation of inbreeding effects. (For the influence of selection on the estimation of inbreeding depression, see Curik et al. 2001). However, we are unable to quantify the impact of those factors. For example, we do not know to what extent breeders excluded horses with extreme morphological values, and also, the genetic architecture of the morphological traits (number of genes involved, presence of epistasis, etc.) is still not known. The evidence obtained from mtDNA (Kavar et al. 1999) and microsatellite (Achmann et al. 2001) data is concordant with the fact that, despite the relatively low effective population size, the Lipizzan horse has maintained a level of genetic diversity comparable to that of other horse breeds or domestic animal species.

Cothran et al. (1984) argued that the avoidance of close inbreeding by standardbred breeders might explain the absence of a strong inbreeding effect on reproductive performance. It is possible that the negative effects of inbreeding come mainly from close inbreeding, as demonstrated by Wiener et al. (1992), which was not the case in this study, where only a very small percentage of mares have become inbred due to a common ancestor in the first three generations. The explanation is that remote inbreeding allows for selection to remove deleterious alleles and to purge some of the negative consequences of inbreeding. However, beyond speculations, our data provided no evidence of effects of inbreeding and heterozygosity on morphological data.

There are three theoretical explanations why morphological traits did not exhibit strong inbreeding depression (see DeRose and Roff 1999). First, there was simply no dominance variance in morphological traits. Second, morphological traits possessed dominance variance, but the dominance was not directional. Third, there was directional

dominance associated with morphological traits, but the amount of dominance variance was negligible. A large body of evidence obtained from crossbreeding experiments on various species of domestic animals indicates that significant heterosis effects are more often found for life-history traits than for morphological traits (for extensive reviews see Hohenboken 1985 and Sheridan 1981). In addition, estimation of variance components on the same set of horses indicated moderate to high narrow sense heritability for most of the traits considered (Zechner et al. 2001). The results of this study are therefore in agreement with the hypothesis that additive effects are the main factors responsible for the inheritance of morphological traits.

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