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RESEARCH ARTICLE

Genetic Variability Among Arabian Horses in Turkey

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Summary

The genetic variability within the Arabian horses and its relationship between three different national studs in Turkey was evaluated using 16 loci (five of blood groups and eleven protein polymorphisms) analyzed in 4055 Arabian horses. The *F*IT (0.019) and *F*ST (0.034) values estimated for over all loci were positive, while *F*IS (-0.015) value was negative. The differences among population were found to be statistically significant (P<0.001). The estimated *F*ST for all loci indicated that 3.4% of total genetic variation was originated from the differences among population, whereas 96.7% of genetic variation was originated from the differences among individuals. Genetic diversity computed as *F*ST (0.034) is found to be statistically significant among populations (P<0.001). The gene flows occurred between populations within each generation was ranged from 4.47 for Karacabey and Private Farms and to 16.42 for Çifteler and Sultansuyu studs. The estimated mean gene flow was 7.1 for each generation among populations. According to dendogram, horses in Çifteler and Sultansuyu are forming a group and then Karacabey studs as well as national farms are joining into this group. In conclusion, there is indicated considerably high gene flow among national studs, especially between Çifteler and Sultansuyu. The reason for genetic diversity between horse population in private farms and three national studs may be due to the low genetic flow from these three national studs to private farms.

Keywords: *Arabian horse, Cluster analysis, F-statistics, Genetic distance*

Türkiye'deki Arap Atları Arasındaki Genetik Farklılıklar

Özet

Türkiye'de farklı haralarda ve halkelinde yetiştirilen toplam 4067 Arap atı arasındaki genetik farklılıklar ve genetik ilişkiler 16 lokus (5 kan grubu ve 11 protein lokusu) yardımıyla incelendi. *F*IT (0.019) ve *F*ST (0.034) değerleri pozitif, *F*IS değeri ise negatif (-0.015) tahmin edilmiştir. Populasyonlar arasındaki bu farklılıklar önemli bulunmuştur (P<0.001). Tüm lokuslar üzerinden hesaplanan *F*ST değerleri bölgesel farklılıklardan kaynaklanan toplam genetik varyasyonun %3.4, bireyler arası farklılıkların ise %96.7 düzeyinde olduğunu yansıtmaktadır. Populasyonlar arasındaki genetik farklılığı gösteren *F*ST (0.034) önemli bulunmuştur (P<0.001). Her jenerasyon populasyonlar arasında meydana gelen gen akışı 4.48 (Karacabey-Özel çiftlikler) ile 16.72 (Çifteler-Sultansuyu) arasında değişmekte ve ortalama gen akışı ise 7.10 olarak hesaplanmıştır. Kümele analizine göre Çifteler ve Sultansuyu haraları bir küme oluşturmakta, daha sonra bu kümeye sırasıyla Karacabey ve özel çiftlikler katılmaktadır. Sonuç olarak, devlet haraları, özellikle Çifteler ve Sultansuyu arasında etkin bir gen akışının olduğu görülmektedir. Özel çiftlikler ve üç devlet harasındaki at populasyonları arasındaki genetik farklılığın nedeni haralar ile özel çiftlikler arasındaki düşük genetik göçten kaynaklanabilir.

Anahtar sözcükler: *Arap atları, F-istatistikleri, Genetik uzaklık, Kümeleme analizi*

INTRODUCTION

Historical records indicated that Arabian horses have been inhabited to Arabian Peninsula and its surrounding areas around 2000 B.C. The best Arabian horses were brought and bred in Anatolia when Turks began to conquer and rule Anatolia during Turkish States, including Seljuq and Ottoman Empire era. The Arabian Horses have been bred, raised and their pedigree record has carefully been kept in Republic of Turkey since 1925 [1]. For the animal husbandry Arabian horses,

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63 stallion and 161 mares were purchased from various locations in Anatolia and were imported from Syria, Iraq, Jordan and Saudi Arabia between 1925 and 1936 [2].

Turkish Arabian horses have been breeding in national studs such as Karacabey, Sultansuyu and Çifteler and private enterprises. The pedigrees records of Turkish Arabian horses including national and private studs have carefully been kept under the supervision of Ministry of Agriculture since 1925. Arabian horses in Turkey have been demonstrating a great deal of genetic variability among different studs and private farms. To assess intra- and interbreed genetic differences in horse breeds, allelic variability analysis at genetic system including red blood cell antigen and plasma protein loci have extensively been used. The number and frequencies of alleles at different loci can be used to determine the genetic profile of a breed and to distinguish between individuals, populations and breeds [3]. Also, microsatellites, minisatellites, mitochondrial DNA (mt-DNA), random amplification of polymorphic DNA (RAPD) and restriction fragment length polymorphism (RFLP) have recently started being employed to determine the genetic variability in many different species.

The *F*-statistics have proven to be a very useful tool for elucidating the pattern and determining genetic variation within and among natural populations in animals and plants. The *F*-statistic models described by Wright [4] are generally accepted for determining the relative breeding situation or the estimation of selection models in the subpopulations related to the polymorphic alleles in a population. The F_{ST} is the correlation between two gametes randomly selected from each subpopulation related to the whole breeds, while the F_{IS} and F_{IT} are used to determine the correlation between two coupling gametes randomly selected from a subpopulation and from all the populations, and relate them to the deviation from panmixia in each subpopulation as well as in whole population [5]. Therefore, F_{IT}, F_{IS} and F_{ST} parameters are used. Although genetic variability in Arabian horses and genetic relatedness of Arabian horses between different horse populations has been studied [3,6,7], the genetic variability in Turkish Arabian has not been studied.

This study was therefore conducted to determine the genetic variability in Turkish Arabian horses in different national studs and private farms by using allelic variability analysis on red blood cell antigen and plasma protein loci.

MATERIAL and METHODS

A total of 4055 Arabian horses from three different national studs and private farms were used *(Table 1)*. Blood samples were collected to confirm the accuracy of pedigree analysis and transferred to genetics laboratories at Etlik Central Research Institute, Ministry of Agriculture from 1992 to 2004. The protein polymorphism in 5 blood groups (A, C, D, P, Q) and 11 protein loci: Albumin *(ALB)*, *A*1*B*-glycoprotein (*A*1*B*), protease inhibitor *(PI)*, carbonic anhydrase *(CA)*, catalase *(CAT)*, serum carboxylesterase *(ES)*, vitamin D binding protein *(GC)*, haemoglobin-alpha (HBA), 6-phosphogluconate dehydrogenase *(PGD)*, phosphoglucomutase *(PGM)*, transferrin *(TF)* were determined by using the techniques such as standard serological tests and electrophoresis.

Table 1. *Sample sizes according to the growth region of Arabian horses*
Tablo 1. *Arap atlarının yetiştirildikleri yerlere göre örnek büyüklükleri*

Population sizes	Breeder
1640	Çifteler National Stud, Eskisehir
1186	Karacabey National Stud, Bursa
1141	Sultansuyu National Stud, Malatya
88	Private Farms

Gene frequencies in the loci containing co-dominant alleles were estimated by gene counting. Mean heterozygosity in each locus (h_i) for every population were calculated at all loci *(H)*, and the standard deviation for heterozygosity (SD_h) were estimated to minimize the sampling error [8]. Significance in the differences between mean heterozygosity values were tested by using t-test.

When inbreeding or selection was performed in a population, changing occurs in the Hardy-Weinberg proportions, which is in favor of homozygosities called fixation index [4]. The *F*-statistics were calculated as described by Weir and Cockerham [9]. To determine the statistical significance, χ^2 tests were used [10]. The effective number of individual exchange between populations per generation (N_em) was computed with $N_em = (1 - F_{ST})/4F_{ST}$ [8,11]. Mean genetic distance among the Arabian horses was calculated by using the data from 16 polymorphic

loci as the method developed by Nei [12]. The dendrograms for breeds were drawn by using the matrix of genetic distance values according to the unweighted pair-group method (UPGMA) [12], clustering in numerical taxonomy [13]. All computations for statistical analysis were performed using the Tools for Population Genetic Analysis (TPFGA) program [14].

RESULTS

Heterozygosity indexes in loci, the average heterozygosity and the standard deviation in three national studs and private farms are shown in *Table 2*. The estimated mean heterozygosity values *(H)* and the standard deviations were 0.450±0.054, 0.436±0.053, 0.464±0.048 and 0.387±0.065 in Çifteler, Karacabey, Sultansuyu studs and private farms, respectively. The differences among mean heterozygosity values were not significant (P>0.05).

Table 2. *Heterozygosity indexes in loci (h_i), the average heterozygosity and standard deviation ($H \pm SD_h$) in populations*
Tablo 2. *Populasyonlardaki lokuslardaki heterozigotluk indeksleri (h_i), ortalama heterozigotluk ve standart sapmaları ($H \pm SD_h$)*

Locus	Çifteler	Karacabey	Sultansuyu	Private Farms
	h_i	h_i	h_i	h_i
A	0.746	0.746	0.761	0.747
C	0.492	0.492	0.473	0.485
D	0.438	0.438	0.488	0.587
P	0.370	0.370	0.533	0.370
Q	0.599	0.599	0.560	0.413
CA	0.487	0.487	0.462	0.480
A1B	0.102	0.102	0.054	0.149
ALB	0.489	0.489	0.490	0.503
PI	0.595	0.595	0.548	0.631
PGD	0.173	0.173	0.362	-
PGM	0.502	0.502	0.481	-
ES	0.066	0.066	0.205	-
GC	0.308	0.308	0.247	0.512
CAT	0.467	0.467	0.449	0.438
HBA	0.558	0.558	0.531	0.130
TF	0.814	0.814	0.783	0.755
$H \pm SD_h$	0.450±0.054	0.436±0.053	0.464±0.048	0.387±0.065

The effective number of individual exchange among populations per generation and the estimated *F*-values for each locus as well as for all loci were given in *Table 3*. F_{IS} and F_{IT} values estimated for each locus were positive in *A1B*, *PGD, PGM, GC, CAT, HBA,* and *TF* loci while they were negative in other loci. On the other hand, F_{ST} values were positive in all loci. The differences among population were found to be statistically significant (P<0.001). The estimated F_{ST} for all loci indicated that 3.4% of total genetic variation was originated from the differences among population, whereas 96.7% of genetic variation was originated from the differences among individuals. Genetic variability was significantly different in all loci except *CAT, C, PGA* and *CA* (P<0.01). Negative values obtained for F_{IS} showed that the heterozygote animals in each population have a better chance for breeding than that of homozygote animals. This refers to a decrease in the homozygosity level with a rate of 1.5%. Genetic diversity computed as F_{ST} = 0.034 is found to be statistically significant among populations (P<0.001). Pair comparisons of F_{ST} values for populations are given in *Table 4*. As shown in *Table 4*, the value of genetic differences between Çifteler and Sultansuyu is 1.5%, while it is 5.3% in Karacabey and private farms. Individuals' movements between populations for every generation were indicated in diagonal of *Table 4*. The N_em values between populations ranged from 4.47 for Karacabey and Private Farms and to 16.42 for Çifteler and Sultansuyu studs. The mean gene flow occurred for each generation among populations was estimated about 7.10 *(Table 3)*. Genetic distances were calculated and drawn UPGMA dendogram *(Figure 1)* as described by Nei [12]. According to dendogram, Çifteler and Sultansuyu are forming a group and then, Karacabey studs and national farms joining into this group.

Table 3. *F-statistic values and the effective number of individuals exchanged between populations per generation (N_em)*
Tablo 3. *F-istatistik değerleri ve her jenerasyon populasyonlar arasında göç eden ortalama birey sayısı (N_em)*

Locus	$F_{IS} = f$	$F_{IT} = F$	$F_{ST} = \theta$	N_em
A	– 0.294 ***	– 0.284 ***	0.008 ***	
C	– 0.536 ***	– 0.531 ***	0.003	
D	– 0.087 ***	– 0.077 ***	0.010 ***	
P	– 0.179 ***	– 0.178 ***	0.001	
Q	– 0.193 ***	– 0.174 ***	0.016 ***	
CA	– 0.660 ***	– 0.655 ***	0.003	
A1B	0.174 ***	0.186 ***	0.015 ***	
ALB	– 0.045 ***	– 0.042 ***	0.003 *	
PI	– 0.225 ***	– 0.216 ***	0.008 *	
PGD	0.201 ***	0.234 ***	0.041	
PGM	0.932 ***	0.949 ***	0.249 ***	
ES	– 0.045 ***	– 0.025 **	0.020 *	
GC	0.517 ***	0.524 ***	0.013 **	
CAT	0.170 ***	0.170 ***	0.000	
HBA	0.624 ***	0.638 ***	0.035 ***	
TF	0.104 ***	0.189 ***	0.087 ***	
Mean estimates	– 0.015 ***	0.019 ***	0.034 ***	7.10

f, within-population inbreeding estimate; F, total inbreeding estimate; θ, measure of population differentiation.
** P<0.05, ** P<0.01, *** P<0.001*

Table 4. *F_{ST} estimates (below the diagonal) and gene flow N_em (above the diagonal) between pairs of horse population in Turkey*
Tablo 4. *Türkiye'deki at populasyonları arasındaki F_{ST} tahminleri (dik üçgen) ve gen göçleri (N_em) (ters dik üçgen)*

Breeder	Çifteler	Karacabey	Sultansuyu	Private Farms
Çifteler		8.68	16.42	5.43
Karacabey	0.028		5.85	4.47
Sultansuyu	0.015	0.041		4.75
Private farms	0.044	0.053	0.050	

Analyzed loci for four populations showed that the F_{IS}, F_{IT} and F_{ST} values were different than zero. The F_{IS} value was found to be negative (-0.015), while F_{IT} and F_{ST} were found to be positive as 0.019 and 0.034, respectively. The negative values of FIS may indicate that the rate of heterozygote genotype in populations is higher than the expected rate of heterozygosity in Hardy-Weinberg equation. This could be explained that the selective factors had been in favor of hetero-zygote individuals and inbreeding had carefully been avoided.

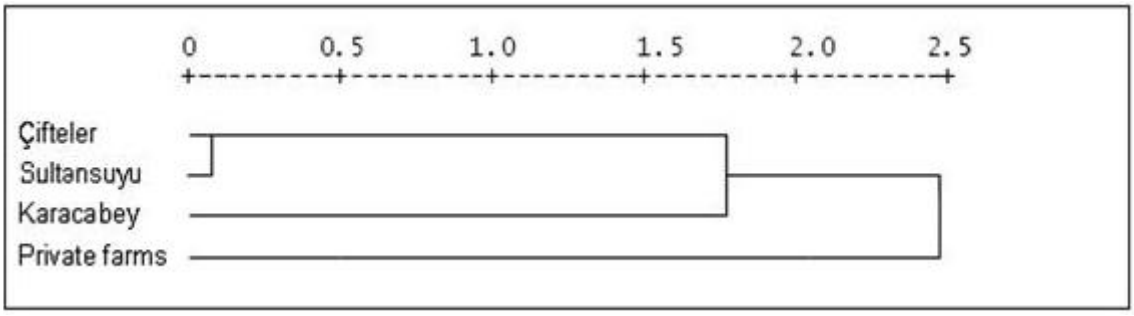

Figure 1. Dendogram of the genetic distance matrix computed by the UPGMA method
Şekil 1. UPGMA metodu ile çizilmiş kümeleme analizi

DISCUSSION

Mean heterozygosity level in investigated population was ranged from 0.387 to 0.464. According to this estimated mean heterozygosity level, it could be inferred that the genetic variation among horses is quite high. This inference is also supported by the negative values of F_{IS}.

In this study, the estimated *H* values is smaller than that of Spanish Celtic [15] and Argentina Creole horse breeds [16], whereas it is higher than that of Arabian [6], Czech warm-blooded horse, Trakehner horse, Moravian warm-blood horse [17], Great Basin feral [3] and Cheju native as well as Cheju racing horses [18]. However, the values of *H* found in this study for Turkish Arabian horses is similar to the reported values of *H* for Arab-Barb, the English horses [16], Barb [6] and Uruguayan Creole [7].

The difference between the estimated mean heterozygosity levels is not significant (P>0.05). In some cases, the difference between the estimated heterozygosity levels for population is not statistically significant and this could be interpreted that populations have the same level genetic variation but the genetic variation in populations could not be determined in terms of the estimated mean heterozygosity level.

Turkish Arabian horses are raised in national studs and their pedigrees record are carefully kept in under the supervision of Ministry of Agriculture, therefore, *F*-statistics calculated in this study is reflecting the true values, which inbreeding in Arabian horses have been strictly avoided.

The estimated F_{IS} in Turkish Arabian horses appeared to be higher than other animals. For instance, the value of F_{IS} have been reported -0.156 for Argentine Creole and Thoroughbreds horse breeds [16], 0.076 for European cattle breeds [5] and 0.085 for Turkish dog breeds [19], 0.11 and 0.07 for Arabian and Morgan horses [20], respectively. On the other hand, this value is similar that has been reported the value (0.015) for Spanish Celtic horses [15].

Close relative crossing might cause a decrease in heterozygosity. Furthermore, a decrease in the heterozygosity level could be due to the selective advantages of different alleles in different loci or different selection criteria for different alleles [21]. In this study, however, calculated F_{IS} values were either negative or very low close to zero. This strongly confirmed that the relative cross-breeding among Turkish Arabian horses is at the very low level and it appeared that there was no selective

advantage for any allele in particular loci or there was a high rate of gene flow in a population.

The population substructure within the breed or in breeding units, more or less large and more or less isolated could be a logical explanation to understand the high deficit of heterozygote observed in some loci (Wahlund's effect) [5].

The F_{IT} values were positive in all loci and they were significant (P<0.001). These provide advantages that if there is an increase in the frequency of homozygote genotypes in population at the subpopulation level, then possible selection factors on these loci could be detected.

As mentioned above, mean genetic diversity among population is 3.4% and it is statically significant (P<0.001). It could be said that 96.4% genetic variation is due to the differences among individuals, while 3.4% genetic variation is due to the differences among populations. Furthermore, F_{ST} values estimated for Turkish Arabian horses in this study were extremely low in comparison to the other breeds and species. For example, F_{ST} values have been reported 0.088 for human [22], 0.099 for Spanish dog breeds [23], 0.068 for Western European cattle breeds [5], 0.078 for Spanish Celtic horse breeds [15], 0.065 for Black Forest horse breeds [24], 0.109 for Argentine Creole breeds [16], 0.078 for Spanish horses and 0.088 for horse breeds in the USA [25], 0.170 for Switzerland goat breeds [26], 0.049 for Turkish Brown Cattle Breeds [21] and 0.160 for Turkish dog breeds [19].

Mean gene flow among population ranged from 4.47 (Karacabey-Private Farms) to 16.42 (Çifteler-Sultansuyu) *(Table 4)*. Mean gene flow for each generation was 7.10 *(Table 3)*. Gene flow plays a very important role for a genetic uniformity in populations located in a close geographic proximity. In this study, it is shown that there is effective gene flow in only three national studs. This could be due to the exchange of breeder stallion between studs or due to the purchasing of high performance racing horses as breeders from other studs. In the case of N_em >1, gene flow causes a decrease in the genetic diversity [27]. In this study, the estimated low F_{ST} could be originated by gene flow among population in three national studs.

Genetic distances were calculated and drawn UPGMA dendogram *(Figure 1)* as described by Nei [12]. According to dendogram, Çifteler and Sultansuyu are forming a group and then, Karacabey studs and national farms joining into this group. The reason for a large genetic distance between horse population in private farms and three national studs may be due to the low or non genetic flow from these three national studs to private farms.

In conclusion, genetic diversity of Turkish Arabian horses is constituted by different genotypes in different private farms and national studs. Furthermore, Turkish Arabian horse populations show heterogenic structure even in the same studs or private farms that the investigated loci constituted distinct allelic structures and there is considerably high gene flow among national studs, especially between Çifteler and Sultansuyu. This suggests that there has been stallion or mare exchange among studs. Thus, heterozygosis has been preserved without stallion or mare import from abroad. This indicated that heterogenic structure of Turkish Arabian horse population will be conserved for many years ahead. Furthermore, this study shows that in order to determine the genetic structure of a population, polymorphic biochemical methods in addition to other methods could be used and the level of inbreeding could be determined by using heterozygosity values along with *F*-statistics and the relationship between populations could be assessed by determining the individual movement for each generation or grouping analysis method.

REFERENCES

1. Arpacık R: At yetiştiriciliği. Şahin Matbaası, Ankara, 1-15, 1996.

2. Girgin ÖF: Türkiye Safkan Arap Atı soy kütüğü. Türkiye Jokey Klübü Yayınları, Vol. 1, İstanbul, Turkey, 1995.

3. Bowling AT: Population genetics of Great Basin feral horses. *Anim Genet,* 25, 67-74, 1994.

4. Wright S: The theory of gene frequencies. Evolution and the genetics of populations. University of Chicago Press, Vol. 4, 1978.

5. Jordana J, Alexandrino P, Beja-pereira A, Bessa I, Cañon J, Carretero Y, Dunner S, Laloe D, Moazami-Goudarzi K, Sanchez A, Ferrand N: Genetic structure of eighteen local south European beef cattle breeds by comparative *F*-statistics analysis. *J Anim Breed Genet,* 120, 1-15, 2003.

6. Ouragh L, Meriaux JC, Braun JP: Genetic blood markers in Arabian, Barb and Arab-Barb horses in Morocco. *Anim Genet,* 25, 45-47, 1994.

7. Kelly L, Postiglioni A, De Andres DF, Vega-Pla JL, Gagliardi R, Biagetti R, Franco J: Genetic characterization of

the Uruguayan Creole horse and analysis of relationships among horse breeds. *Res Vet Sci,* 72, 69-73, 2002.

8. Nei M: Molecular evolutionary genetics. Columbia University Press, New York, 1987.

9. Weir BS, Cockerham CC: Estimating *F*-statistics for the analysis of population structure. *Evolution*, 38, 1358-1370, 1984.

10. Nei M, Chesser RK: Estimation of fixation indices and gene diversities. *Ann Hum Genet,* 47, 253-259, 1983.

11. Hartl DL, Clark AG: Principles of population genetics. Sinaur Associates, Sunderland, Massachusetts, 1989.

12. Nei M: Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*, 89, 583-590, 1978.

13. Sneath PHA, Sokal RR: Numerical Taxonomy: The principle and practice of numerical classification. **In,** Kenedy D, Park RB (Eds): Taxonomy structure. 134-230, W.H. Freeman, San Francisco, 1973.

14. Miller MP: TFPGA (Tools for Population Genetic Analysis). Ver. 1.3., Department of Biological Sciences, Northern Arizona University, 1998.

15. Cañon J, Checa ML, Carleos C, Vega-Pla JL, Vallejo M, Dunner S: The genetic structure of Spanish Celtic horse breeds inferred from microsatellite data. *Anim Genet,* 31, 39-48, 2000.

16. Diaz S, Dulout FN, Peral-Garcia P: Greater genetic variability in Argentine Creole than in Thoroughbred horses based on serum protein polymorphisms. *Genet Mol Res,* 3, 261-265, 2002.

17. Jiskrová I, Glasnák V, Misař D: The use of blood protein polymorphism for determining the genetic distance between the Moravian warm-blooded horse and the Czech warm-blooded and Trakehner horses. *Czech J Anim Sci*, 47, 98-105, 2002.

18. Shin JA, Yang YH, Kim HS, Yun YM, Lee KK: Genetic polymorphism of the serum proteins of horses in Jeju. *J Vet Sci*, 34, 255-263, 2002.

19. Erdoğan M, Özbeyaz C: Investigation of Blood Protein Polymorphism and Estimation of Genetic Distances in Some Dog Breeds in Turkey. *Turk J Vet Anim Sci,* 28, 583-590, 2004.

20. Behara AMP, Collins DT, Cothran EG, Gibson JP: Genetic relationships between horse breeds based on microsatellite data: Applications for livestock conservation. http://cgil.uoguelph.ca/PUB/6wcgalp/6wcBehara.pdf. *Accessed*: 08.06.2005.

21. Özbeyaz C, Yıldız MA, Çamdeviren H: Türkiye'de yetiştirilen farklı Esmer sığır sürüleri arasındaki genetik ilişkiler. *Turk J Vet Anim Sci,* 25, 453-461, 2001.

22. Nei M, Roychoudhury AK: Genetic relationship and evolution of human races. *Evol Biol,* 14, 1-59, 1982.

23. Jordana J, Piedrafita J, Sanchez A, Puig P: Comparative F-statistics analysis of the genetic structure of ten Spanish dog breeds. *J Hered,* 83, 367-374, 1992.

24. Aberle KS: Untersuchung der Verwandtschaftsverhältnisse, Inzucht und genetischen Distanzen bei den deutschen Kaltblutpferderassen. Hannover, Deutschland, 2003.

25. Rodríguez-Gallardo PP, Aguilar Sánchez P, Vega Plá JL, de Andrés Cara DF: Blood group and protein polymorphism gene frequencies for the Andalusian horse breed. A comparison with four American horse breeds. *Arch Zootec,* 41, 433-442, 1992.

26. Saitbekova N, Gaillard C, Obexer-Ruff G, Dolf G: Genetic diversity in Swiss goat breeds based on microsatellite analysis. *Anim Genet,* 30, 36-41, 1999.

27. Trexler JC: Hierarchical organization of genetic variation in the Sailfin Molly, Poecilia latipinna (Pisces: Poeciliidae). *Evolution,* 42, 995-1005, 1988.