*ISSN 2490-4392, Journal of Bioresources Valorization, 2016*
*Vol. 1 (1), pp (45-51), http://www.biolival.com/index.php/revue/archives-jvb*

Original Article

# Analysis of population genetic diversity in Algerian horse breeds

**Naima BERBER[*1], Souheil GAOUAR[2], Grégoire LEROY[3,4], Samia KDIDI[5,6] & Nadhira SAÏDI MEHTAR[1]**

[1]Laboratoire de Génétique Moléculaire et cellulaire, Université des sciences et de la technologie d'Oran, Mohamed Boudiaf-USTOMB, BP 1505 El M'naouer, Oran, Algérie.
[2]Département de biologie, Université de Tlemcen, Algérie.
[3]AgroParisTech, UMR1236 Génétique et Diversité Animales, 16 rue Claude Bernard F-75321 Paris, France.
[4]INRA, UMR1236 Génétique et Diversité Animales, 78352 Jouy-en-Josas, France.
[5]Livestock & Wildlife Laboratory, Arid Lands Institute, 4119 Medenine Tunisie.
[6]Laboratory of Genetics, Immunology and Human Pathology, Faculty of Sciences, Tunis-El Manar University, Tunisie.

***Auteur correspondant. E-mail: nima.berber@gmail.com***

*Mots clés :*
Diversité génétique
Races équines
Algérie
Marqueurs microsatellites
Programmes de conservation

***Résumé***

**Analyse de la diversité génétique de quelques races de chevaux Algériens.**

Dans la présente étude, une caractérisation génétique a été effectuée au niveau de cinq races équines algériennes (Barb, arabe-barbes, arabes, pur-sang et Trotteur français). Ces races de chevaux sont distinctes, non seulement par leur trait unique de performance et standards morphométriques, mais aussi leur adaptation aux différentes conditions agro-climatiques qui prévalent dans le pays. La diversité génétique des races de chevaux du monde entier ont été analysées par microsatellites, y compris les chevaux celtiques espagnols, diverses races européennes et asiatiques, races polonaises, races brésiliennes et races de chevaux indiens.

Cette recherche est la première application de caractérisation génétique des races équines en Algérie par des marqueurs moléculaires. Un total de 201 animaux ont été génotypés pour 11 marqueurs microsatellites. Les échantillons choisis ont été non apparentés et enregistrés dans le stud-book de la race.

Tous les cinq populations (Arabe-Barbe, arabes, barbes, pur-sang, et du Trotteur Français) avaient des valeurs d'hétérozygotie élevée (0,75, 0,71, 0,72, 0,71, et 0,69, respectivement). Ces valeurs sont parmi les valeurs d'hétérozygotie les plus élevés trouvées dans des études de caractérisation des populations équines du monde, utilisant des mêmes ou similaires loci. Trois différentes approches : distances génétiques, distances individuelles et de l'analyse factorielle des correspondances, ont été considérés pour étudier les liaisons génétiques dans les populations de chevaux.

Les données et les informations trouvées dans cette étude peuvent être utilisées dans l'organisation de programmes de conservation pour réduire la consanguinité et pour minimiser la perte de variabilité génétique

*Keywords:*
Genetic diversity
Horse breeds
Algeria
Microsatellite markers
Conservation programs

***Abstract***

In the present study, genetic analyses of diversity and differentiation of five Algerian horse breeds (Barb, Arab-Barb, Arabian, Thoroughbred and French Trotter) were performed. These breeds of horses are distinct not only because of their adaptation to different agro climatic conditions prevailing in the country, but also because they have unique performance traits. Genetic diversity of horse breeds around the world have been analyzed by microsatellites, including the Spanish Celtic breeds, various

European and Asian breeds, Polish breeds, Brazilian breeds, Portuguese breeds, French breeds and Indian horse breeds. However, the genetic relationships of horse populations in Algeria have not been investigated.
This research is the first applying molecular markers to characterize the horse breeds in Algeria. A total of 201 animals were genotyped for 11 microsatellite markers. We registered the individuals chosen in the breed's studbook and we avoided closely related animals.
All five populations (Arab-Barb, Arabian, Barb, Thoroughbred, and French Trotter) had high heterozygosity values (0.75, 0.71, 0.72, 0.71, and 0.69 respectively). These values are among the highest heterozygosity values reported for other horse populations using the same or similar loci. Three different approaches: genetic distances, individual distances and factorial correspondence analysis, were considered to study genetic relationships among the horse populations.
We can utilize the data and information found here in the organization of conservation programs planned to reduce inbreeding and to minimize loss of genetic variability.

## INTRODUCTION

Preserving biodiversity of indigenous species, especially of those of economic interest must represent a relevant aspect in the scientific research activity. Horses in Algeria have an economic, social and cultural importance. Equine population is evolving in recent years due to climatic and economic conditions, which makes that the number of horses is around one hundred miles of heads (Rahal, 2005).
The very great majority of these horses are commonly listed as Barb horses and Arab-Barb and these two breeds are originating in the Maghreb (Algeria, Morocco and Tunisia) (Benmerad, 2002). They are generally used to carry out traction and the agricultural work in rural medium, riding and leisure activities, notably the fantasia (traditional exhibition of horsemanship in the Maghreb). We also can add their growing use in equestrian sports: the Arabian, Thoroughbred and the French trotter breeds (Benmerad, 2002).
These populations of horses are distinct not only because of their adaptation to different agro-climatic conditions prevailing in the country, but also because they have unique performance traits. They are unequally distributed on the Algerian territory, the number of the horses as their breed, is indeed more significant in some areas than in others (Rahal, 2005).
Recently molecular techniques based on DNA markers have been developed to carry out studies of genetic variation. They have the attractive features that they can be highly automated, require only small amounts of biological samples, and the techniques involved are simpler and cheaper. Among molecular markers, microsatellites are considered suitable for evaluating breeds for genetic diversity (Takezaki and Nei, 1996).
Genetic diversity within and among horse breeds around the world has been analysed by microsatellites, including the Spanish Celtic breeds (Canon *et al*., 2000), Polish breeds (Zabek *et al*., 2005), Arabian breeds (Khanshour *et al*., 2013), Portuguese breeds (Luıs *et al*., 2007), French breeds (Leroy *et al*., 2009) and Indian horse breeds (Behl *et al*., 2007). However, the genetic relationships of horse populations in Algeria have not been investigated using microsatellites.
The present study aimed to characterize five horse populations and to investigate their genetic distinctiveness. We amplified eleven microsatellites in two multiplex PCR. We intend to evaluate not only the current diversity but also to know their relationship for the conservation of the genetic diversity in the context of biodiversity management programs.

## MATERIALS AND METHODS

### *Experimental animals*

Blood samples from 201 animals were collected from five horse breeds raised in Algeria (Figure1), the horse populations used in the present study included: 41 Barb, 55 Arab-Barb, 57 Arabian, 22 Thoroughbred, and 26 French Trotter (Table I). For the breeds where pedigree information was available, we selected unrelated individuals for at least three generations from different equine farms in the country.

### *DNA Extraction and microsatellite analysis*

We collected the blood samples (5–10 ml) from jugular vein of the animal in vacutainer tubes containing EDTA as anticoagulant. We extracted

total genomic DNA from whole blood using a routine salting out procedure of Miller *et al*. (1988).
In our study, we amplified eleven microsatellites in two multiplex using fuorescently-labelled primers. The first multiplex MP1 included microsatellites AHT4, AHT5, ASB2, HMS1, HMS3, HMS6, HMS7, HTG4, and HTG10. In addition, the second MP2 was composed of HTG6, VHL20 and HTG10.
We amplified the microsatellites by PCR by using marked with different fluorophores. The PCR reaction included 10 mM Tris-HCl Buffer, 0.25 units of Taq polymerase, 1.5 mM MgCl2, 250 µM dNTPs, 10 pM of primers and 200 ng of DNA. The PCR thermal profile started with an initial denaturation step at 95°C (15 min) followed by 30 cycles of 30 s at 94°C for DNA denaturation, 90 s for primer annealing at 58°C and 1 min at 72°C for primer extension followed by a final extension at 60 °C for 30 min. PCR products were analyzed by denaturing formamide using an automated ABI 3730 DNA sequencer (Applied biosystems, CA, USA). We analyzed the results using GENESCAN-LIZ 500 (Applied Biosystems, USA).


**Figure 1.** The five horse breeds raised in Algeria country.

**Table 1.** Population sampled size in Algeria

| Breed | Number of animals sampled | | |
|---|---|---|---|
| | Female | Male | Total |
| **Barb (BA)** | 22 | 19 | 41 |
| **Arab-Barb (AB)** | 21 | 34 | 55 |
| **Arabian (AR)** | 41 | 16 | 57 |
| **Pur Sang (PS) ( Thoroughbred)** | 16 | 6 | 22 |
| **French Trotter (TF)** | 15 | 11 | 26 |

***Statistical analysis***

Genotyping data of horse breeds was extracted from GENEMAPPER software 3.7. Genotypes of all loci were collected in an EXCEL file, and then converted to a computable file format through Excel microsatellite. Parameters of locus diversity were estimated for all microsatellite markers in all breeds using the Genetix 4.2 program (Belkhir *et al*., 2001), including: Allele frequencies and number of alleles per locus, observed heterozygosity ($H_o$) and expected heterozygosity ($H_e$), were calculated

across loci and populations. Wright's F-statistics for each locus were calculated using Weir and Cockerman's method with FSTAT (Goudet, 2001). A significance test on the estimates of Wright's F-statistics ($F_{IT}$, $F_{IS}$ and $F_{ST}$) for each microsatellite locus were obtained by constructing 95% and 99% confidence intervals based on the standard deviations estimated by jackknifing across populations using FSTAT.

Exact test was used to determine deviations from Hardy-Weinberg proportions and heterozygosity deficiency with the GENEPOP 4.0 software (Raymond *et al*., 1995) using exact tests and sequential Bonferonni correction.

PHYLIP 3.5 statistical package (Felsenstein, 1989) was used to calculate genetic distances, and to obtain bootstrap procedures and trees. Bootstraps' values were computed over 1000 replicates. The program TreeView (Page, 1996) was used to visualize the diagrams. The pairwise genetic distances between all individual animals were estimated by the logarithm of the proportions of shared alleles using the ape package for the R software (R Development Core Team, 2011). Finally, a factorial correspondence analysis was also performed using GENETIX.

## RESULTS

### *Genetic diversity*

All the equine microsatellites loci reported in this study amplified successfully, and they were also polymorphic in all breeds. A total number of 123 different alleles were detected across the 11 loci analyzed.

The average F statistics across loci were $F_{IT}$ = 0.070 ± 0.140, $F_{ST}$ = 0.050 ± 0.131, and $F_{IS}$ = 0.021 ± 0.092. The average PIC was 0.846 ± 0.070, and effective number of alleles $N_e$ was 6.86 ± 0.998 (Table 2). In addition, the average values of $F_{IS}$, $H_e$, and MNA across the 11 loci were estimated for each breed (Table 3), and all breeds showed relatively high heterozygosity. Among the populations studied the Arab-Barb horse (AB) had the highest $H_e$ and MNA values, and the French Trotter horse (TF) had the lowest.

**Table 2.** Genetic diversity across five horse populations for 11 microsatellite loci.

| Marker | FIS | FST | FIT | *Mean PIC* | Ne |
|---|---|---|---|---|---|
| **AHT4** | -0.038 | 0.049*** | 0.013** | 0.827 | 6.39 |
| **AHT5** | 0.078 | 0.061*** | 0.134*** | 0.832 | 6.31 |
| **ASB2** | 0.015* | 0.023*** | 0.038*** | 0.872 | 7.57 |
| **HMS1** | 0.067 | 0.026*** | 0.091*** | 0.773 | 5.32 |
| **HMS3** | 0.138 | 0.042*** | 0.174*** | 0.877 | 7.44 |
| **HMS6** | -0.048 | 0.015*** | -0.033 | 0.775 | 5.58 |
| **HMS7** | -0.007 | 0.037*** | 0.030*** | 0.875 | 6.48 |
| **HTG10** | -0.043 | 0.052*** | 0.011** | 0.881 | 8.55 |
| **HTG4** | 0.046* | 0.054*** | 0.097** | 0.799 | 5.26 |
| **HTG6** | 0.085 | 0.122*** | 0.196** | 0.792 | 4.47 |
| **VLH20** | -0.007 | 0.047*** | 0.0402** | 0.890 | 8.59 |
| **Average** | **0.021*** | **0.050***** | **0.070***** | **0.846** | **6.86** |

$F_{IS}$, total inbreeding estimate; $F_{ST}$, measure of population differentiation; $F_{IT}$ within-population inbreeding estimate. PIC, polymorphism information content. $N_e$, effective number of alleles.
Significance of F statistics*P < 0.05; **P < 0.01; ***P < 0.001.

**Table 3.** Genetic diversity measures in each breed.

| Breed | $F_{IS}$ | Mean $H_e$ | Mean $H_o$ | MNA |
|---|---|---|---|---|
| **AB** | 0.05698** | 0.750 | 0.712 | 7.857 |
| **AR** | 0.01810* | 0.717 | 0.703 | 6.428 |
| **BA** | -0.00226 | 0.725 | 0.740 | 7.642 |
| **PS** | 0.00236 | 0.713 | 0.723 | 6.071 |
| **TF** | 0.00009 | 0.690 | 0.700 | 5.714 |

$F_{IS}$, heterozygote deficiency coefficient; $H_e$, expected heterozygosity; $H_O$, observed heterozygosity; MNA, mean number of allele; Significance of F statistics *P < 0.05; **P < 0.01.

The overall F statistics values were determined after 10000 permutations. Levels of apparent breed differentiation were considerable, and the average $F_{ST}$ values over all loci and horse groups indicate that around 5% of the total genetic variation was attributed to significant differences between the horse breeds, with the remaining 95% corresponding to differences among individuals

(Table 2). Genetic differentiation among breeds was highly significant (P<0.01) for all loci. A significant excess of homozygotes across all breeds (P<0.05) were found for HTG4 and ASB2 loci. On average, breeds had a 2.1% (P <0.05) deficit of heterozygotes, whereas the total population had a 7% (P <0.01) deficit of heterozygotes.

The HWE was tested for all breed-locus combinations. Significant (P < 0.05) deviations from a HWE were observed for 6 (8.6%) of 70 breed-locus combinations. However, no significant (P > 0.05) deviation from a HWE was detected for either a single locus across all breeds or a single breed across all loci.

***Breeds relationship***

The Nei's ($D_{A)}$ and Reynolds' ($D_R$) genetic distances between pairs of populations are given Table 4. The result obtained with Nei's distance comparing five breeds ranged from 0.0366 between Arab-Barb and Barb to 0.3245 between Arabian and french trotter, showed a intermediare distance between Barb and Arabian (0.1871) horses, and between Arab Barb and Arabian (0.1403). Reynold's distance showed lower distance between Barb and the other breeds. A neighbour-joining tree was constructed on the basis of the DA genetic distances with relatively high bootstrap values (Figure 2).

**Table 4.** Reynolds' genetic distance below the diagonal and Nei's distance above the diagonal, among horse breed.

| Breed | AB | AR | BA | PS | TF |
|---|---|---|---|---|---|
| **AB** | - | 0.1403 | 0.0366 | 0.2735 | 0.2549 |
| **AR** | 0.0358 | - | 0.1871 | 0.2704 | 0.3245 |
| **BA** | 0.0013 | 0.0499 | - | 0.3176 | 0.2421 |
| **PS** | 0.0676 | 0.0747 | 0.0819 | - | 0.2255 |
| **TF** | 0.0635 | 0.0896 | 0.0625 | 0.0595 | - |

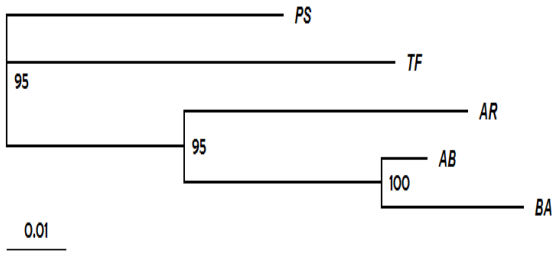


**Figure 2.** Phylogenetic tree showing the genetic relationships among the sampled horse breeds. The number at the nodes are values for 1000 bootstrap resampling of the 11 loci.

As expected, the Barb and Arab barb breeds clustered closely together and they closely related to Arabian, this could be explained by some influence from Arabian Breed in the original breed formation in more recent years.

Figure 3 shows an individual-animal-based neighbour-joining dendrogram built from the estimated distances between shared alleles among all the 201 individuals. The majority of animals within each population were closely assembled in discrete branches, but there were some exceptions. Few individuals from TF, PS and AR breeds misplaced in other breed's clusters, a high frequency of misplaced animals was seen among the AR horses. The AB and BA animals appeared admixed, and this result can be explained by the similar origin of those breeds.

The factorial correspondence analysis FCA strongly confirmed the genetic distinctiveness of the five horse breeds. Results of the 3-dimensional plot (Figure 4) clearly separated the native populations from the other breeds. Simultaneously, the Barb and Arab-Barb were clustered together.

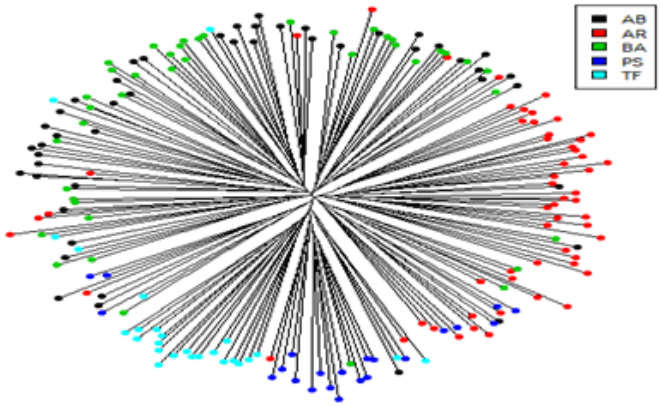


**Figure 3.** Neighbor-joining tree based on the pairwise genetic distances between all animals estimated by the logarithm of the proportions of shared alleles. Each tip represents a single animal, and breeds are distinguished by different colors as shown on the legend.

## DISCUSSION

In this study, we carried out the first genetic analysis of horse breeds in Algeria using DNA markers. In addition, we resolved the genetic relationships between these breeds, especially Barb and Arab-Barb horses.

The levels of differentiation among the five horse breeds analyzed in this study were similar to those previously found in Lipizzan horses (Curiket *et al*., 2003; Achmann *et al*., 2004), Spanish horses (Canon *et al*., 2000; Solis *et al*., 2005; Marletta *et al*., 2006),

German draught horses (Aberle *et al*., 2004), French horses (Glowatzki-Mullis *et al*., 2005) and Norwegian horses (Bjørnstad and Røed, 2002). The lower level of differentiation in this horse breeds is probably due to the more frequent utilization of crossbreeding in the farm animals in Algeria.

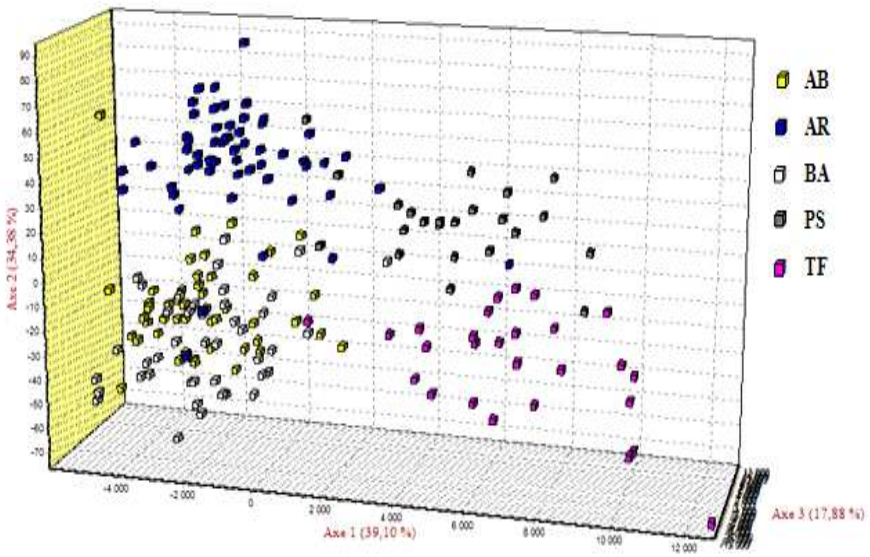


**Figure 4.** Factorial correspondence analysis of the 14 microsatellite loci analyzed in the five horse breeds. Each individual was plotted into 3-dimensional plot.

The high PIC values obtained for most of the markers suggest their usefulness in the evaluation of the biodiversity of native horse breeds. The heterozygosities, for all loci analyzed, were lower than expected (exception HMS6), which could be attributed to within-population inbreeding or by population subdivision (Wahlund's effects) (Arora and Bhatia, 2004).

The genetic structure of five horse breeds was investigated using two complementary methods, genetic distances and factorial correspondence analysis FCA. The native Algerian breeds were clearly differentiated from the others; these findings confirm the conservation of the genetic substratum originating from an ancient Iberian horse as well as the limited influence from any other breeds (Chaid Saoudi, 2006). However, The Barb and Arab-Barb populations clustered closely together, this was in agreement with the findings of previous studies reported by Ouragh et al., which showed that these populations were originally different but shared the same genetic using biochemical polymorphisms (Ouragh *et al*., 1994).

Finally, this study showed significant levels of genetic divergence between the five populations. These findings indicate the availability of horse microsatellite DNA markers in studying genetic diversity. Algerian breed were clearly differentiated from the others confirming the historical information reporting the genetic relation of these breeds. Therefore, our results support the decision to consider the breeds as the same population. For the both autochthonous breeds, the genetic parameters confirm the risk status due to the high level of inbreeding. Such information could help to guide the conservation strategies and to clarify the genetic components of crossbreed breeds

## ACKNOWLEDGEMENT

We would like to thank the partners who have provided samples: the Haras National CHAOUCHAOUA of Tiaret of and their groups for their help for collecting Samples. We are grateful to Sir. Benabdelmoumene S., Dr. Rahal K, Dr. Bouziane Z., Dr . Kébali A., Ms. Ginette A. for their kind collaboration. We further acknowledge the staff of Labogena laboratory INRA - Jouy en Josas for their expert help in the genetic typing.

## REFERENCES

Aberle K.S., Hamann H., Drögemüller C., Distl O. 2004. Genetic diversity in German draught horse breeds compared with a group of primitive, riding and wild horses by means of microsatellite DNA markers. *Animal Genetics*. 35: 270–277.

Achmann R., Curik I., Covc P., Kavar T., Bodo I., Habe F., Marti E., Sölkner J., Brem G. 2004. Microsatellite diversity, population subdivision and gene flow in the Lipizzan horse. *Animal Genetics*. 35: 285–292.

Arora R., Bhatia S. 2004. Genetic structure of Muzzafarnagri sheep based on microsatellite analysis. *Small Ruminant* Research. 54: 227–230.

Behl R., Behl J., Gupta N., Gupta S.C. 2007. Genetic relationships of five Indian horse breeds using microsatellite markers. *Animal*. 1: 483–488.

Belkhir K., Borsa P., Chikhi L., Raufaste N., Bonhomme F. 2001. GENETIX 4.04, Logiciel sous Windows TM pour la Genetique des Populations. Montpellier (France): Laboratoire Ge´nome, Populations, Interactions, CNRS UMR 5000, Universite´ de Montpellier II.

Benmerad A. 2002. Avant propos du Stud book algérien . Stud-book du cheval Barbe. 1: 3-4.

Bjørnstad G., Røed K.H. 2002. Evaluation of factors affecting individual assignment precision using microsatellite data from horse breeds and simulated breed crosses. *Animal Genetics*. 33: 264–70.

Canon J., Checa M.L., Carleos C., Vega-Pla J.L., Vallejo M., Dunner S. 2000. The genetic structure of Spanish Celtic horse breeds inferred from microsatellite data. *Animal Genetics*. 31: 39–48.

Chaid Saoudi Y. 2006. Les origines d'*Equus Caballus* en Algérie et les origines de la domestication. Revue de l'Organisation Mondiale du Cheval Barbe. 46: 52.

Curik I., Zechner P., Sölkner J., Achmann R., Bodo I., Dovc P., Kavar T., Marti E., Brem G. 2003. Inbreeding, microsatellite heterozygosity and morphological traits in Lipizzan horses. *Journal of Heredity*. 94: 125–32.

Felsenstein J. 1989. PHYLIP—Phylogeny Inference Package (Version 3.2). *Cladistics*. 5: 164–166.

Glowatzki-Mullis K.L., Muntwyler J., Pfister W., Marti E., Rieder S., Poncet P.A., Gaillard C. 2005. Genetic diversity among horse populations with a special focus on the Franches-Montagnes breed. *Animal Genetics*. 37: 33–9.

Goudet J. 2001. FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). [Internet]. Lausanne (Suisse): Universite´ de Lausanne; [cited 2011 October 1]. Available from: http://www2.unil.ch/ popgen/softwares/fstat.htm.

Khanshour A., Conant E., Juras R., Cothran E.G., 2013. Microsatellite analysis of genetic diversity and population structure of Arabian horse populations. *Journal of Heredity*. 3: 386–398.

Leroy G., Verrier E., Meriaux J.C., Ricard A., Danchin Burge C., Rognon X. 2009. Genetic diversity of a large set of horse breeds raised in France assessed by microsatellite polymorphism. *Genetics Selection Evolution*. 41: 13–21.

Luıs C., Juras R., Oom M.M., Cothran E.G., 2007. Genetic diversity and relationships of Portuguese and other horse breeds based on protein and microsatellite loci variation. *Animal Genetics*. 38: 20–27.

Marletta D., Tupsc-Yupanqui I., Bordonaro S., García D., Guastella A.M., Criscione A., Canon J., Dunner S. 2006. Analysis of genetic diversity and the determination of relationships among western Mediterranean horse breeds using microsatellites. *Journal of Animal Breeding and Genetics*. 123: 315–25.

Miller S.A., Dykes D.D., Polesky H.F. 1988. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Research*. 16: 1215.

Ouragh L., Meriaux J.C., Braun J.P. 1994. Genetic blood markers in Arabian, Barb and Arab-Barb horses in Morocco. *Animal Genetics*. 25: 45-47.

Page R.D.M. 1996. TreeView: an application to display phylogenetics trees on personal computer. *Cabios*. 12: 357-358.

R Development Core Team. 2011. R: A language and environment for statistical computing, R Foundation for Statistical Computing. Vienna, Austria.ISBN3-900051–07-0, URL http://www.R-project.org/.

Rahal K. 2005. Le barbe en Algérie, un acteur de développement durable des régions rurales. *Le monde hippique*. 48: 27-29.

Raymond M., Rousset F. 1995. GENEPOP (version 1.2). Population genetics software for exact tests and ecumenicism. *Journal of Heredity*. 86: 248-249.

Solis A., Jugo B.M., Mériaux J.C., Iriondo M., Mazón L.I., Aguirre A.I., Vicario A., Estomba A. 2005. Genetic diversity within and among four South European native horse breeds based on microsatellite DNA analysis: implications for conservation. *Journal of Heredity*. 96: 670–678.

Takezaki N., Nei M. 1996. Genetic distances and reconstruction of phylogenetic trees from microsatellite DNA. *Genetics*. 144: 389-399.

Zabek T., Nogaj A., Radko A., Nogaj J., Slota E. 2005. Genetic variation of Polish endangered Bilgoraj horses and two common horse breeds in microsatellite loci. Journal of Applied Genetics. 46: 299–305.