

## MATING STRATEGY IN THE ARAB HORSE POPULATION OVER 30 YEARS IN ALGERIA

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The objective of this research was to describe the variation in breeding practices in the population of Arabian horses in Algeria in relation to the population size drop observed in the 2000s, due to an increase in the cost of food and a change in interest in racing, related to the black decade. As a result, the breeders had to face two opposite constraints: enhancing reproduction to counter the population decline and preventing the loss of genetic diversity. The aim of the study was to evaluate the genetic consequences of the decisions taken over a period of 30 years, starting from 1988 to 2018. The study was based on the analyses of the stud-book and on 11 microsatellite markers in a group of 943 horses, distributed into 13 age classes. Between 2004 and 2008, the breeders purchased foreign sires and dams, allowing a relative stability in unbiased heterozygosity of about 71%, due to the high genetic distances between foreign and local horses. As these importations stopped from 2009 on, there was a decrease in allele numbers of about 20%. Moreover, from 2010 on, we observe an excess of inbreeding and an increase in genetic drift relative to the starting population, due to population size decline. From the analysis of genetic distances between local individuals, it seems that the only constraint for sires and dams allowed to mate was a null coefficient of inbreeding.

**Keywords:** Arabian horse, genetic distance, heterozygosity, microsatellite markers, population decline

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## INTRODUCTION

In the case of horses, good management practice consists in different methods limiting inbreeding and maximizing the effective population size ( $N_e$ ), in order to avoid concerns in individual productivity, and even fertility and mortality [1]. Indeed, when the population size decreases, a genetic drift can be predicted [2], an increase in inbreeding and a more probable decrease in heterozygosity and mean allele numbers [3]. Different tools can be used to help in decision making. One common method involves analyzing pedigree information from stud books, which can provide data on thousands of individuals [4-5]. It allows calculating the inbreeding coefficient, average relatedness and effective number of founders, among others on a period spanning up to a century [6]. With the advent of new technologies implying DNA, with microsatellite markers [7-12], mtDNA D-loop sequencing [13], or SNP [14-15], a fewer number of generations could be analyzed, nevertheless allowing to quantify more accurately the polymorphism of populations and address the “real” degree of genetic divergence between individuals [16].

Selection for different purposes (e.g. endurance, speed or beauty) is a critical point in horse breeding. The genomic selection applied to breeding programs of livestock has brought a spectacular genetic progress [17]. However, this methodology, based on the best performance of parents before mating, i.e. on the additive effect that can be inherited to the offspring, can lead to an increasing rate of inbreeding and a decrease in genetic variance.

Different studies based on pedigree analysis [18], microsatellite markers [9], and SNPs [19] evidenced a relative low heterozygosity in the Arab breed mainly due to inbreeding. As a result, there is a risk of recessive genetic disorders [20]. It should be pointed out that there is variability among its different populations, and several authors noted the weak level of heterozygosity in the Straight Egyptian population [12,21]. In the case of Algeria, the Chaouchaoua national stud farm is a renowned horse breeding farm located on a high plateau, around 1000 meters above sea level and 5 km away from Tiaret, the capital of Wilaya. It covers an area of 922 ha, of which 876 ha is dedicated to agriculture (details in Supplementary Text). It was established in 1877 by the French Ministry of War, under the name “Jumenterie de Tiaret”, with the aim of producing horses for the army using the Barbe, Arabian-Barbe, Arabian breeds, and Draft Horses. With the Black Decade (1992–2002), racetracks received negative views. Moreover, in the 2000s, the cost of basic necessities for horse breeding (e.g., barley, fodder and straw) skyrocketed, sometimes increased up to ten times the original price in just ten years. These factors have contributed to a decrease in the prestige of the stud farm of Chaouchaoua, and a decline in the population of Arabian horses in Algeria. As of 2018, there were only 208 horses, with slightly over half being of Arabian horse breed, and approximately 40 foals auctioned off annually [22].

Facing the population drop of Algerian Arabian horses that occurred in the early 2000s, several concerns had to be considered at the same time by the breeders: on the

genetic point of view, an expected progressive decrease of diversity and growing rate of inbreeding [23-24], and on an economic point of view, a decrease in foaling. Two non-exclusive decisions could be taken. If they preferred to favour foal production by multiplying the mating involving local stallions and mares, this would lead to enhance the kinship and thus a high rate of inbreeding inside the next generation [25]. On the contrary, if they wanted to maintain the genetic diversity, the best solutions were to import foreign adults of both sexes or to assess the complementation of parents to be mated through genomic mating [26]. In the last decades, the only information available to Algerian breeders was the pedigree data and the relatedness deduced from the stud book. It should be noted that the population genetics of horses is still a topic to be explored in Algeria, and very few works have been devoted to it.

The questions we wanted to address in this work were: (i) explore the variation in several genetic parameters before and after the population drop, (ii) assess the effect of foreign adults in limiting the population decrease and degradation of genetic diversity, (iii) understand the choice of mating in absence of foreign animals.

## **MATERIAL AND METHODS**

### **Genetic parameters deduced from microsatellite data**

The majority of the samples were obtained from the Chaouchaoua Stud in Tiaret, and the rest came from 14 other provinces in Northern Algeria. The stud-book was open in 1972, but the genotyping parentage verification tests begun in 2021, to fulfil the WAHO (World Arabian Horse Organization) rules. The blood samples were sent to LABOGENA DNA experts (Jouy-en-Josas) to extract the DNA, obtain microsatellite data and perform parentage tests. Their results were analyzed to conduct this work. The study protocol was approved by the USTHB-Ethical committee of animal experimentation (Université des Sciences et Techniques Houari Boumediene, Algeria), with the certificate code of ethics CEEA-USTHB-03-2023/11113. It concerned 943 Arabian horses born between 1984 and 2018 with 14 microsatellite markers: HTG6, VHL20, HTG10, HTG4, AHT5, AHT4, HMS3, HMS6, HMS7, HMS1, ASB2, ASB17, ASB23, HMS2. Results of the ASB17, ASB23, and HMS2 microsatellites failed for 809 individuals, leading us to use the first 11 microsatellites. Of the 943 individuals, 2 showed uninterpretable genotypes and 14 had no documentation for the birth date. Of the remaining 927 births, 277 parents could not be validated by microsatellites because of death at the moment of the sampling.

To understand the changes in genetic parameters due to the reduction of population size and the introduction of foreign adults, we divided the population into 13 classes of two consecutive years of birth, most often (Table 1). In the extreme parts of the period where too few foals were genotyped, the classes were extended to three years. The size of each age class varied between 17 and 176 individuals.

**Table 1.** Foal classes by years of birth

Category of foal births	Year range	Number of genotyped foals	Category of foal births	Year range	Number of genotyped foals
Pop84	1984–1989	17	Pop102	2002–2003	96
Pop90	1990–1991	17	Pop104	2004–2005	146
Pop92	1992–1993	25	Pop106	2006–2007	164
Pop94	1994–1995	30	Pop108	2008–2009	176
Pop96	1996–1997	35	Pop110	2010–2012	35
Pop98	1998–1999	56	Pop213	2013–2015	14
Pop100	2000–2001	79	Pop216	2016–2018	29

The genotypes were recorded in formats compatible with Genetix 4.05 [27] and Arlequin 3.5 [28]. To describe the variation in genetic diversity of the successive age classes, we calculated the Nei's unbiased heterozygosity ( $H_{ub}$ ) [29]. To estimate the variation in inbreeding for each age class, we computed the Wright's fixation index  $F_{IS}$ , according to Weir and Cockerham [30] per locus. To assess the differentiation between populations due to the effect of the reduction of population size and the gene flow linked to the introduction of foreign adults, we calculated by means of  $F_{ST}$  using Arlequin 3.5. We verified that the mating decided by the breeders were in conformity to HWE (Hardy-Weinberg Equilibrium) by an exact test using a Markov chain, implemented in the same software.

### Genetic and reproductive parameters of local and foreign horses

To describe the variation in the overall population size over the study period, we considered the Algerian Arabian horse stud book (1970–2010) and the data provided by parentage tests of foals born in the period 1984–2018. The population size in the early 1980s was not known accurately as most horses were not registered in the stud book. Moreover, some horses were sold or passed away before they could undergo genotyping.

To assess the contribution of foreign individuals to the genetic diversity of the population, we considered separately the subpopulations of foreign and local horses. We considered as foreign individuals those born from at least one foreign parent (France, Belgium, Sweden, Great Britain, Poland, Egypt, and Russia). To compare their offspring production, we calculated the yearly foaling of mares (offspring number over the period between first and last foaling) and the significance in their differences by Kruskal-Wallis tests using PAST version 2.17 [31]. Then, to compare the divergence between the two subpopulations, we calculated the genetic distances following Cavalli-Sforza and Edwards [32] method using Arlequin 3.5, between all the combinations of foreign, local, mares, and stallions. The differences in the mean distances between

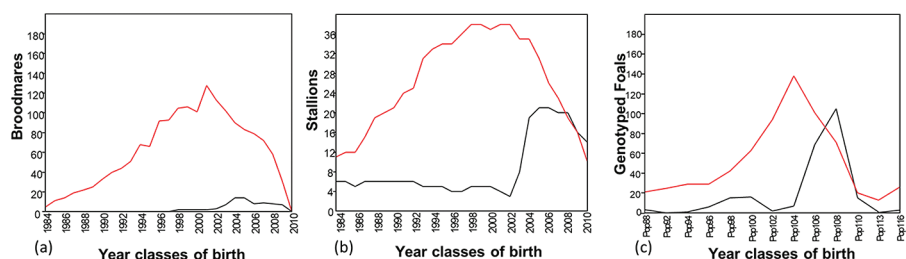
each category of individuals were assessed with ANOVA followed by Tukey's pairwise comparisons, using PAST version 2.17.

To overcome the predicted reduction of genetic diversity due to the reduction size of the adult population, it was important to understand how the breeders decided to choose the individuals allowed in the reproduction process. First, we calculated the inbreeding coefficient of every foal using kinship information using Pedigree.tools version 0.2 written in R [33]. Second, in the case of lack of available foreign adults, we hypothesized that the breeders tried to enhance the divergence between the members of each couple, leading us to calculate the genetic distances between the parents to test whether (i) these divergences differed from random, using a one-sample t test against hypothetical value implemented in PAST version 2.17 (here the observed genetic distance compared to all the distances between the considered mare and every stallion) and (ii) there were variations in the degree of parentage over time.

## RESULTS

### Variation in population size

There was an increasing number in the registrations of local horses in the stud-book from 1970 on, until a peak of 128 and 42 reached around 2001, for mares and stallions, respectively (Figure 1A-B). The importation of foreign animals was limited until 2000, mostly for mares, but we observe a peak between 2004 and 2006 with around 14 and 44 for mares and stallions, making 8.8% and 37.5% of the whole population, respectively. As for foals, there was also a peak of genotyping reached in 2004–2005 and 2008–2009 for the local and foreign animals, respectively (Figure 1C). In summary, the increase in population number until around 2006 reflects the progressive registration in the stud-book or genotyping of animals, in contrast to the following years where we observe a real drop in the population.

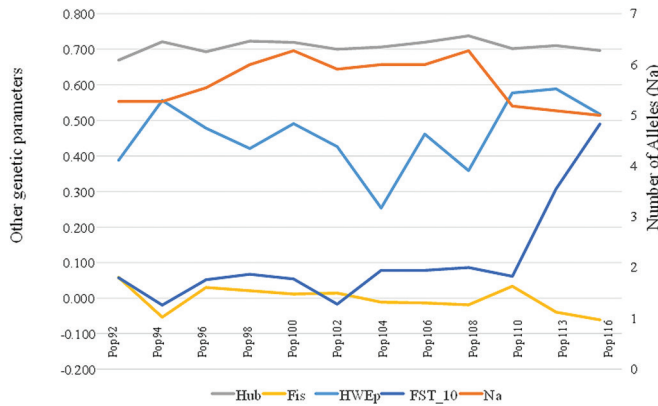


**Figure 1.** Variations in the number of registered mares (a) and stallions (b) in the Algerian stud-book, and of genotyped foals (c). Red: local animals; black: foreign animals; Pop92 and so on: number of genotyped foals born in 1992 and 1993. The figure is made using PAST 2.17.

### Genetic Data Analyses of the whole population

As indicated previously, these data were considered by age classes of foals. If the data of all the loci are gathered, no deviation from random mating (HWE) was recorded in any age class. If we consider now the deviations locus by locus, we retrieved significant deviations for three loci in Pop106 (VHL20, HTG10, HMS7), for two loci in Pop106 (VHL20, HMS1), and one locus for Pop100 (AHT4), Pop102 (HMS3), and Pop116 (VHL20). Taken together, VHL20 was the most frequent locus showing a deviation.

The mean number of alleles ( $N_a$ ) per age class was  $5.63 \pm 0.456$  and varied between 5 (Pop116) and 6.27 (Pop100 and Pop108; (Figure 2). This parameter increased until 1998 but suddenly declined from 2008 on. Variations in unbiased heterozygosity ( $H_{ub}$ ) were relatively parallel to those observed in allele number, but less markedly (Pearson correlation between  $N_a$  and  $H_{ub}$ ,  $\rho = 0.054$ ). Their mean value was 0.71.



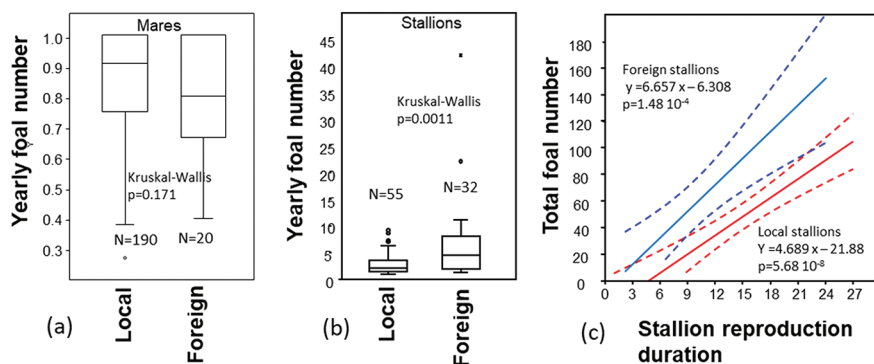
**Figure 2.** Variations in genetic parameters. The different classes of foal births during 2-3 years are symbolized by Pop92 to Pop116 (Pop 92 = births between 1992 and 1993). Abbreviations. **Na:** mean allele numbers; **Hub:** unbiased heterozygosity; **Fis:** mean  $F_{IS}$ ; **HWEp:** p-value of HWE; **FST 10:** divergence from the start population (values multiplied by 10 for readability). The figure was made using Excel from Microsoft.

The inbreeding measured by mean  $F_{IS}$  coefficient was  $-0.005 \pm 0.036$  and an excess of inbreeding was found in populations Pop94, Pop113 and Pop116, with the values  $-0.053$ ,  $-0.039$  and  $-0.061$ , respectively. Using the information of kinship, Pedigree tools returned inbreeding values of zero for all the foals, except one, with 0.125 value.

The calculated divergence from the starting population showed a gradual elevation with time, with a sharp increase in slope from Pop113 on, supported by significant differences in Pop108, Pop113 and Pop116, with p-values of 0.036, 0.027 and  $< 0.0001$ , respectively.

### Reproductive and genetic parameters of local and foreign horses

Besides the introduction itself of foreign stallions and mares cited above, it can be asked whether their offspring was greater than that of local adults. Regarding the mares, we noticed no difference in the yearly production of foals by the local and foreign ones (Kruskal-Wallis test,  $p=0.171$ ), with medians between 0.8 and 0.9 (Figure 3A). In contrast, the foreign stallions showed a significantly higher yearly production of foals than the local ones (Figure 3B), with medians of 4.46 and 2.17, respectively. Along the reproduction period of each stallion, there was an increasing cumulated number of foals with a higher slope for the foreign than for the local stallions (Figure 3C).

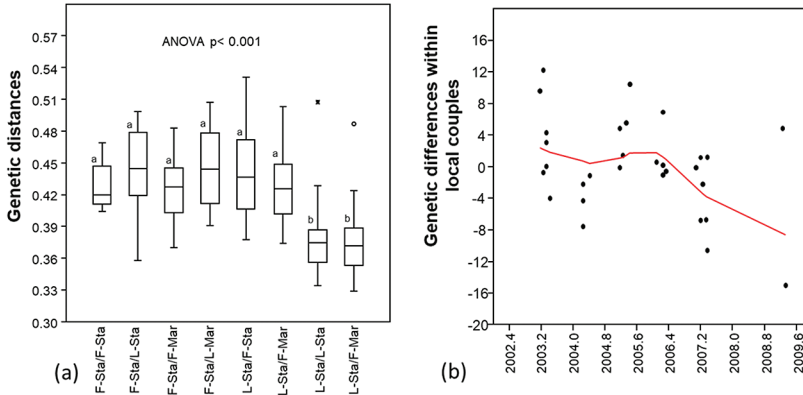


**Figure 3.** Foal production by local and foreign stallions and mares. **(a)** yearly offspring by local and foreign mares; **(b)** yearly offspring by stallions; **(c)** total foal production by stallions according to reproduction duration, in years. The regression equations, the p-values, and the 95% confidence limits are given for local (in blue,  $N = 55$ ) and foreign stallions (in red,  $N = 32$ ); in the major part of the regression curves, there was no overlap between the 95% confidence intervals. The figure as made using PAST 2.17.

The analysis of the mean genetic distances in each combination of local and foreign mares and stallions revealed that there were two situations differing highly significantly (ANOVA,  $p < 0.0001$ ): a group with higher genetic distances containing foreign stallions associated to local or other foreign adults, and a group with lower distances composed of local stallions or local mares with other local stallions (Figure 4A). The same analysis conducted on local and foreign mares lead to similar results (Supplementary Figure).

Inside the subpopulation of local individuals, we observed that among the 30 couples where information was available in the period 2002-2009, 12 did not show a distance significantly different from random, 8 and 9 having distances significantly higher and lower, respectively. However, as illustrated in Figure 4B, we observed a relative steady state before 2006, and a sudden drop in the divergence afterward. The decreasing tendency was confirmed by a linear model ( $y = -1.2807x + 2568.8$ ) with a significant p-value at 0.037.





**Figure 4.** Genetic distances between the reproductive stallions and mares. **(a)** mean distances between foreign and local stallions versus foreign or local stallions and mares. **F-Stal**=foreign stallions; **L-Stal**=local stallions; **F-Mar**=foreign mares; **L-Mar**=local mares; to compare the different couple types, the different letters a and b design significant differences given by ANOVA and Tukey's pairwise comparisons. **(b)** genetic differences between each couple member of local mares and stallions between 2004 and 2009. The decimals of the years correspond to proportion of the date. For example, the first of April corresponds to 0.33. The red curve was obtained by Loess smoothing. The linear tendency ( $y = -1.2807x + 2568.8$ ) is significant, with  $p = 0.037$ . The figure was made using PAST 2.17.

## DISCUSSION

As mentioned in the introduction, the Arab horse population shows a low amount of heterozygosity relatively to most other horse breeds. The genetic diversity of Arabian horses of Algeria measured by unbiased heterozygosity ( $0.701 \pm 0.02$ ) was close to that recorded previously in Algeria [11], Syria [34], Desert Bred and Egyptian Straight [12] and Romania [35]. There was a slight deficiency of heterozygotes in the Arabian horse population of the present work ( $-0.005 \pm 0.036$ ) relatively to Desert Bred and Egyptian Straight [12] and Algeria [11]. It was difficult to compare the allele number between different populations due to different sets of microsatellite markers. These data support the importance acquired by the Algerian stud farms in the diffusion of remarkable Arabian horses in many countries over the world.

There are many breeds of horses considered as endangered at risk or endangered, due to their small population size [36]. However, these populations can maintain a genetic diversity in certain cases, when the proportion of brooding mares is sufficient [37], when the population has undergone a bottleneck in the past [38], or when there is a probable avoidance of mating between closely related individuals [39]. Facing the population drop of the Arabian horses around 2000-2004, it is interesting to understand what were the decisions taken by the breeders to counter the expected degradation in genetic parameters and to limit the fall in the population.



The importation of foreign horses in 2004-2008 had temporally positive consequences on demography and genetic parameters. The contribution of foreign sires in foaling was valuable, as they could father double foals per year than local sires. Given that they represented more than 30% of sires, the foaling was multiplied by 1.23 during a few years. In contrast, the effect of foreign mares was null as their number was less than 10% and their foaling was identical to local ones.

As regard the effect of foreign breeding animals in enhancing genetic diversity, this introduction was a success on heterozygosity values, explained by greater genetic distances between foreign animals and themselves or local animals than within local animals [40]. In spite of these globally encouraging results, the purchase of foreign horses could not be maintained, maybe in reason of their considerable cost [41]. Following the end of the importation of foreign adults, the population drop was dramatic. First, the benefic role of foreign adults in increasing allele number and heterozygosity and decreasing the Wright's fixation index was very temporary and progressively vanished after 2008. More problematic was the genetic drift that suddenly increased from the same date, that could be due, according to the theory, to the reduction of population size [42].

If the use of foreign animals is set apart, it can be questioned whether the breeders took appropriate decisions for the choice of mating within local horses. They did not have information about the genetic diversity and inbreeding in the 2000s, since the genotyping results were only available in 2021, i.e. more than ten years after the population drop. With the only pedigree knowledge, they worked correctly as almost no inbreeding was detected by our pedigree analyses. But did they try to mate distantly related animals, as recommended [43]? Our results between 2002 and 2009 seem to indicate that before 2006, they did not pay attention to the coefficient of kinship [21], as the positive deviations of distance from random between dams and sires were as numerous as the negative ones. Afterward, it could be that the mating between distantly related animals were preferred, in order to preserve heterozygosity level.

## **CONCLUSION**

This work is one of the few devoted to the population genetics of North African horses. The history of the recent decline of the Arabian horse population in Algeria outlined a number of clearly cited explanations. The use of microsatellite markers allowed to understand the genetic changes that occurred during these years. The efforts of the breeders to avoid the reduction of the good level of genetic diversity, including the purchase of foreign breeding adults, could not resist due to lack of resources. The impact of this sad story is dramatic for one of the oldest Arabian centers of Arabian horse breeding stud in Africa, in spite of the valuable efforts of the breeders. We hope that solutions will be found soon to meet the challenge of rebuilding a successful series of stud farms in Algeria.

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## Authors' contributions

NB and FB contributed significantly to hypothesis generation, methodology, and data curation. ARB provided essential pedigree and racing data, contributing substantially to data curation. AB offered valuable documentation support. DP involved drafting the manuscript, data analysis, and supervision. All authors have actively participated in the study execution, reviewed, and endorsed the final manuscript.

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## Conflict of interest

The authors declare that they have no conflicts of interest with respect to the research, authorship, and/or publication of this article.

## Statement of Informed Consent

The owner understood procedure and agrees that results related to investigation or treatment of their companion animals, could be published in Scientific Journal Acta Veterinaria-Beograd.

## Supplementary information

Supplementary text. History of Algerian Arab horse population.

Supplementary figure. Genetic distances between the reproductive stallions and mares


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## **STRATEGIJA PRIPUSTA U ALŽIRU U POPULACIJI APAPSKIH KONJA TOKOM 30 GODINA**

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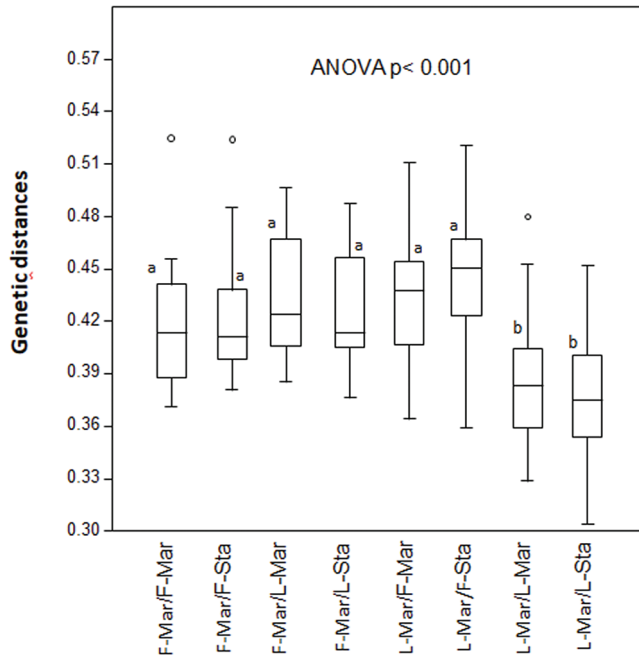
Cilj ovog istraživanja bio je da opiše varijacije u praksi uzgoja u populaciji arapskih konja u Alžiru u odnosu na pad populacije uočen 2000-ih, usled povećanja cene hrane i promene interesovanja za trke, vezano za crnu deceniju. Kao rezultat toga, uzgajivači su morali da se suoče sa dva suprotna ograničenja: poboljšanje reprodukcije kako bi se suprotstavilo opadanju populacije i sprečavanje gubitka genetske raznovrsnosti. Cilj studije je bio da se procene genetske posledice odluka donetih u periodu od 30 godina, počev od 1988. do 2018. godine. Studija je zasnovana na analizama matičnih knjiga i na 11 mikrosatelitskih markera u grupi od 943 konja, raspoređenih u 13 starosnih grupa. Između 2004. i 2008. godine, uzgajivači su kupovali strane pastuve i kobile, omogućavajući relativnu stabilnost nepristrasne heterozigotnosti od oko 71%, zbog velike genetske udaljenosti između stranih i lokalnih konja. Kako su ovi uvozi prestali od 2009. godine, došlo je do smanjenja broja alela za oko 20%. Štaviše, od 2010. godine, primećujemo višak inbreedinga i povećanje genetskog drifta u odnosu na početnu populaciju, usled smanjenja veličine populacije. Iz analize genetske udaljenosti između lokalnih jedinki, čini se da je jedino ograničenje za pastuve i kobile kojima je dozvoljeno da se pare bio nulti koeficijent inbridinga.

## Supplementary text

### HISTORY OF ALGERIAN ARAB HORSE POPULATION

The introduction of the Arabian horse breed in Algeria goes back to the Islamic Conquests in the seventh century (Geoffroy Saint-Hilaire, 1919). In the early 20<sup>th</sup> century, the French Ministry of War initiated missions to purchase oriental Arabian stallions and broodmares to improve the local Arabian breed. Thus, several well-known dam lines and sire lines have been introduced into the Algerian breeding program, including Wadha (female, bay, born in 1874) and Cherifa (female, bay, born in 1869), both imported from Syria in 1875; Nimrin (female, chestnut, born in 1894) imported in 1898; Goutta (male, bay, born in 1884) imported in 1889 from the oriental country; Venture (male, bay, born in 1889) imported from Lebanon in 1895, etc. The progeny of these imported dams and sires' lines have significantly improved the breeding program not only in Algeria but also in France, Poland, Russia, and the Maghreb. This led to thousands of foals of different breeds being born annually (AFP, 2018).

Several dams and sire families were famous around the world, such as Horse racing used to be extremely popular until the 1970s. During this time, the Chaouchaoua stud farm had a significant impact as it supplied approximately 90% of the Arabian horses in the country. In fact, 35% of the bets placed by bettors were used to finance the equine sector, both for races within France and Algeria. In 1977, the government banned betting on foreign horse races, which had a significant impact on the funding of the national stud. To compensate, the stud began relying on the sale of foals, fillies, mares, and stallions for 40% of its resources, while the remaining 60% came from agricultural production. In 1984, Algeria joined the World Arabian Horse Organization (WAHO) and had a special stud book for the Arabian horse, fulfilling all WAHO rules and regulations, including systematic parentage control using the approved technique (blood typing and after microsatellite analysis).



**Supplementary figure 1.** Genetic distances between the reproductive stallions and mares. Mean distances between foreign and local mares versus foreign or local stallions and mares. **F-Stal**=foreign stallions; **L-Stal**=local stallions; **F-mar**=foreign mares; **L-mar**=local mares; to compare the different couple types, the different letters a and b design significant differences given by ANOVA and Tukey's pairwise comparisons. The figure was made using PAST 2.17.