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Genetic Characterization of White-tailed Deer (*Odocoileus virginianus*) at the Centro de Vida Silvestre San Bartolome Yucatan, Mexico

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Authors' contributions

This work was carried out in collaboration among all authors. Author MPR designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors AMJ and VPS managed the analyses of the samples. Author SCJ performed the quantitative genetic analysis and managed the literature searches. All authors read and approved the final manuscript.

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Short Research Article

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ABSTRACT

Aims: Estimate the inbreeding rate, evaluate nucleotide divergences between deer in a captive population, and design a crossbreeding scheme to maintain genetic diversity.
Study design: A descriptive study was carried out to characterize the genetic divergence status of a captive population of deer, under uncontrolled mating conditions, without genealogical data.
Place and duration of Study: The deer under study were confined to the Centro de Vida Silvestre San Bartolomé in the locality of Tekax, Yucatan Mexico. The study was carried out from May 2006 to April 2007.
Methodology: Quantitative genetic models were applied to characterize the population of 39 adult deer (*Odocoileus virginianus*) confined in a 3 Ha pen. Blood samples were collected to apply the

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random amplification technique of polymorphic DNA fragments (RAPD). Two DNA primers were used in the RAPD to generate the DNA polymorphic band patterns. From the band pattern of each specimen, molecular analysis software was applied to estimate the nucleotide divergences between the analyzed units and, finally, dendrograms were generated using the UPGMA technique to group the animals according to their nucleotide divergences in the amount of nucleotides substituted per 100 bases. Groups of breeding animals were designed based on their divergences in the dendrograms.

Results: The effective population number was 19 and the expected inbreeding rate per generation was 0.0263 (2.63%). Eighteen specimens did not share DNA bands. The dendrograms of genetic divergences of each primer showed 11 specimens that appear in both dendrograms. The genetic divergences between the specimens were distributed from 0.086 to 2.62 substituted nucleotides in relation to 100 bases in both dendrograms. Breeding groups were designed for three generations with animals that have the greatest genetic divergence among them.

Conclusion: The RAPD used allowed the identification of deer that did not share bands within the analyzed population, while the other members presented different values of nucleotide divergences. From the highest values of nucleotide divergences, groups of reproducers were restructured to stop the increase in inbreeding, even when there are no genealogical data.

Keywords: White tailed-deer; Odocoileus virginianus; genetic variability; RAPD; inbreeding rate; nucleotide divergences.

1. INTRODUCTION

The conservation and sustainable use of wild fauna in Mexico has among its goals the conservation of the genetic diversity, as well as the protection, restoration and management of natural habitats, as main factors for the conservation and recovery of wild species [1].

The conservation of genetic variability is carried out at the level of populations in free and captive animals. This implies that to conserve a species, it is necessary to maintain the sufficient number of animals for them to reproduce, and that there is no risk of causing or increasing inbreeding in the local population; which can decrease fertility, reduction of offspring survival, which has as a consequence risk of loss of this population [2].

It is known that, in domestic species, inbreeding has a greater impact on reproductive function [3], a situation that is similar in wild fauna species, mainly in closed nuclei of reproduction, that is, in captive populations, where gene flow is reduced, due to the limitation of the entry of new animals continuously and therefore, generates genetic erosion [4].

On the other hand, there are techniques for the control of reproduction and therefore, for the controlled dissemination of genes in a close population. The most used technique is the registration of matings and the selection of studs, a situation that is frequently used in livestock [5]. However, in the reproduction of wild fauna and especially in that of native wild cervids, it is complicated, due to the nervous and elusive nature of the individuals of this species, because in many of the facilities where these specimens are confined there are no designed to carry the precise control of reproduction [6].

When genealogical records do not exist, biochemical and molecular biology techniques could be used to detect biochemical or genetic polymorphism, which allows obtaining polymorphic patterns of proteins or DNA chains, from which inferences can be made about the degree of similarity. Among the individuals that make up the colony, one of those techniques is the Random Amplification of Polymorphic DNA Fragments or RAPD [7]. This technique has been used successfully for the characterization of genetic relationships in cervids, such as *Cervus elaphus, Dama dama* and *Capreolus capreolus* [8], *Capreolus pygargus* [9], *Cervus nippon* [10] and Siberian *Capreolus pygargus* [11].

The objectives of this research were to estimate the expected inbreeding rate and determine the nucleotide divergences of a population of white-tailed deer confined in the San Bartolomé Wildlife Center [CVS] based on genetic analysis using RAPD, and designing a crossbreeding scheme to maintain the genetic diversity of the population.

2. MATERIAL AND METHODS

2.1 Study Site

The CVS is located at Km 3.5 of the Tekax-Tixmehuac highway, in the municipality of Tekax,

south of Yucatan Mexico. The climate is warm-subhumid, classification Awo (x´) (i´) g with average annual temperature of 26.6 °C and average annual rainfall of 1090.9 mm [12].

2.2 Population of Captive Deer

The study was carried out from May 2006 to April 2007 with 39 deer: 16 phenotypically healthy males and 23 adult females from CVS. The unidentified deer were provided numbered earrings to identify the specimen. They were kept in three pens of 1205 m^2 each, but in previous years they were confined to a single pen of 3 Ha. Reproduction was carried out without control and there were no records of matings or genealogy. Some genetic parameters were estimates assuming the random mating of adult animals. In addition, deer in the region were mated from October to May because they have seasonal reproduction behavior [13].

2.3 Effective Number and Inbreeding

Free mating occurs when there is no control of the animals and they reproduce randomly, as happened in CVS. The degree of inbreeding per generation, in this case, depends on the number of males and females and the male: female ratio due to their polygamous behavior. The estimation of the expected inbreeding rate per generation (ΔF) and the effective size (Ne) for a population with free mating in a closed reproduction nucleus, that is, without introducing new animals, was performed [3].

2.4 Physical and Chemical Containment of Deer and Blood Collection

The deer were physically contained by means of a closed mesh net, of soft thread, whose length was 6 m and width of 1.4 m. Once contained with the mesh net, deer were injected intramuscularly with a mixture of xylazine: ketamine (1.5 mg/kg : 4 mg/Kg). After deer sedation, 2 to 3 ml blood sample was extracted by puncture of the external jugular vein, with a sterile syringe. The blood samples were placed in a clean test tubes with sun anticoagulant (10% EDTA aqueous), carefully mixed and labeled to identify the samples.

The blood samples were kept refrigerated (8 °C) before being delivered to the laboratory for further processing. Blood samples were centrifuged at 3000 rpm for 10 min. The plasma was discarded and the red cell and leukocyte pack was mixed with 500 microliters (µl) of 3% acetic acid (1:20), to destroy the red cells. Samples were centrifuge again at 7,000 rpm for 10 min. Samples were decanted to obtain the leukocyte pellet that adhered to the bottom of the tube. The pellet was resuspended in 1 ml of PBS to be centrifuged again.

2.5 DNA Extraction and RAPD

Leukocyte DNA was extracted using the commercial QIAMP DNA blood mini kit (Quiagen, Cat. Q01-51104, USA), following the manufacturer's instructions. The amount of DNA obtained was sufficient (5 to 50 nanograms) for the study, using agarose gel electrophoresis. Subsequently, the protocol for Random Amplified Polimorphic DNA (RAPD) was carried out.

For the application of RAPD in the DNA extracts of deer, the commercial kit Ready-To-Go-RAPD Analysis Beads (Cat. 279502-01, Amersham Bioscience, UK) was used, in a reaction with 25 pmol of the initiator to be used, and 50 ng of the DNA sample. The method described by the manufacturer was followed, which briefly consists of placing the beads containing AmplifiTaq and Stoffel fragments at the bottom of the reaction tubes, adding 25 pMol of RAPD primer, 5 at 50 ng of the DNA template and distilled water. The contents were mixed slowly by vortexing or by pipetting for 1 min, then centrifuge the mixture briefly and cover the tubes with 50 µl of mineral oil. The samples were placed individually in a BIO-RAD MyCycler thermal cycler and the cycles were run with the following profile: 1 cycle at 95 C for 5 minutes, 45 cycles at 95 C for 1 min, 36 cycles at 0 C for 1 min and 72 cycles at 0 C for 2 min.

The RAPD working protocol was first executed to test six pairs of primers contained in the kit, to later decide to use the one that revealed the highest polymorphism. For this, blood samples from two white-tailed deer were used, and the first selection was made based on the results. The nucleotide sequence of the six primers is shown in Table 1.

For the analysis of polymorphic DNA fragments in gel, the protocol was: 2% ultrapure agarose gels were made, and sufficient agarose gel (20 cm) was poured into a chamber for electrophoresis, using 1X TEA or TBE buffer, containing 0.5 µg/µl of Ethidium Bromide and 1 µg/µl of 6x running buffer was added to 5 µl of each amplified sample to load the gel.

Afterwards, the samples were electrophoresed until the RAPD bands were separated, which were observed with Bromophenol blue, when the running front reached 2.5 cm from the edge. The run was carried out at 150 V for 3 hours. Finally, the gels developed in a polarized light lamp. Photographs were taken to subsequently determine the pattern of bands of polymorphic DNA fragments from each animal.

Table 1. Nucleotide sequence of the six primers tested by RAPD to evaluate nucleotide divergences in captive white-tailed deer at San Bartolome wildlife center in the municipality of Tekax, Yucatan, Mexico

Primer 1	5´ d(GGTGCGGGAA)3´
Primer 2	5´ d(GTTTCGCTCC)3´
Primer 3	5´d(GTAGACCCGT) 3´
Primer 4	5´d(AAGAGCCCGT) 3´
Primer 5	5´d(AACGCGCAAC) 3´
Primer 6	5´d(CCCGTCAGCA) 3´

From the band in the gel photographies, the distances traveled in millimeters of each band of the pattern obtained with each animal were measured. These data were entered into the SAF program [14]. In this first stage, the software eliminates from the analysis, individuals who did not share polymorphic fragments with others. In the second series of algorithms, the nucleotide divergences between the units analyzed are calculated using the models by [15], and finally a dendrogram was prepared using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) technique. The dendrogram groups the animals by their nucleotide divergences in number of substitutions per 100 bases.

3. RESULTS AND DISCUSSION

3.1 Inbreeding

Deer males have polygynous behavior, each male mating to three to four females [16] or 6 to 7 females under captive conditions [17]. Therefore, the ratio of 1:4 (M:H) was assumed given an effective number of 19 animals. The expected inbreeding rate was 0.0263 (2.63%) per generation; therefore, in five generations it would be expected a value of 0.1315 (13.15%). This annual inbreeding could be reduced by means of controlled mating schemes that avoid the crossing between siblings or parents and children. Given that in the San Bartolomé CVS there are 12 pens with a surface area of 1205 m^2 each, the surface measurements corresponding to each enclosure are adequate to house 10 to 20 adult white-tailed deer. According to 13, an adult white-tailed deer needs a minimum surface area of 25 m^2. If the deer population for each pen were 10 adult animals, then each deer would have 121 m^2, and if the number of deer were double, the living space available for each animal would be 61 m^2; therefore, the space was sufficient to house groups of deer with offspring.

If the decision were to keep the entire population in a single pen, with free mating management, it is suggested to increase the adult deer population to 90, which could be 15 males and 75 females, according to a 1:5 ratio (male: female). The expected inbreeding for this population, with free mating, would be then 0.01 (1.0%) per generation [18]. However, if the decision was to carry out controlled reproduction, then the inbreeding rate could be <1% per generation; once the animals with the greatest genetic divergence have been identified, according to the results of the RAPD.

3.2 Estimation of Nucleotide Divergences among Deer

Primers 5 and 6 were chosen, because they showed greater polymorphism; however, primer 5 showed slightly higher polymorphism than primer 6. The random amplification of polymorphic DNA fragments was useful to distinguish nucleotide divergences between deer, even to rule out those that do not share any band, such as was the case of 18 specimens, of which 13 females and 5 males did not appear in the dendrograms. On the other hand, there were 11 deer that did show genetic relationships among them, of these 7 females and 4 males appear in both dendrograms. These are the animals with identification numbers: 849, 848, 847, 846, 840, 837, 831, 850, 812, 836, 830. Two females and six males appear only in one dendrogram.

From the effective number of 19 animals, and under the assumption that the mating ratio in these specimens is 1: 4 male: females, then it is expected that only 4 dominant males will mate with 15 females; therefore, only four bucks and 15 dams will distribute their genetics to the offspring. Therefore, it could be assumed then that the 11 animals that appear in both dendrograms could be descendants of those animals. To test this hypothesis, the microsatellite technique should be

used with the DNA samples of these specimens [19].

Six males and seven females appear only in one dendrogram, which suggests that they were sired by different bucks than the four dominant ones. Assuming that the previous hypothesis is accepted, five males did not appear in any dendrogram, which suggests that they did not have the opportunity to mate and generate offspring. Thirteen females that do not appear in any dendrogram, which suggest that these came from other herds or that the RAPD was not able to identify their genetic linkage values with the rest of the animals. This technique has limitations such as the presence of artifact bands or "wrong" bands, the variability in the reproducibility of results and the comigration of bands, even the rare alleles in the populations studied with RAPD are not detected [20]. However, [20] report that the RAPD technique is capable of differentiating animals within closely related populations.

In the dendrogram of Fig. 1, which corresponds to primer 5, nucleotide substitution values are shown in relation to 100 bases. They were distributed between 0.156 to 2.62, while for the dendrogram of Fig. 2 corresponding to primer 6, the values nucleotide substitutions vary between 0.086 to 2.21; so the numerical differences are relatively small. In Fig. 1, it is also observed that two clearly separated clusters appear, one with substitution values of 2.31 and the other in 2.20, in addition a separate individual (836) appears that is at a substitution value of 2.62. In Fig. 2, primer 6, two clearly separated clusters also appear, one at a substitution distance of 1.10 and another at 1.34 and a separate individual (876) at a distance of 2.21. This shows that the trends of the nucleotide substitutions of the specimens in both dendrograms are in the range of 1 to 3 substitutions for every 100 nucleotides in integer values. Therefore, it allows assuming the feasibility to detect deer that show genetic relationships, as it has been reported by [21] in cattle, [22] and [23] for cervids in Asia and Europe. Based on the results of the dendrograms generated by the two primers, it is advisable to carry out controlled crosses for the next three generations, according to Tables 2, 3 and 4, to stop the increase in inbreeding.

Table 2. Scheme of controlled crossbreeding in a population of White-tailed deer in the first generation

641 Male	838 Male	647 Male	879 Male
837 Female	847 Female	840 Female	849 Female
877 Female	876 Female	848 Female	638 Female
827 Male	844 Male	833 Male	830 Male
831 Female	839 Female	804 Female	835 Female
834 Female	829 Female	888 Female	887 Female
828 Male	843 Male	850 Male	836 Male
851 Female	846 Female	637 Female	889 Female
878 Female	645 Female	638 Female	844 Female

Tabla 3. Scheme of controlled crossbreeding in a population of White-tailed deer in the second generation

879 Male	641 Male	838 Male	647 Male
837 Female	847 Female	840 Female	849 Female
877 Female	876 Female	848 Female	638 Female
830 Male	827 Male	844 Male	833 Male
831 Female	839 Female	804 Female	835 Female
834 Female	829 Female	888 Female	887 Female
836 Male	828 Male	843 Male	850 Male
851 Female	846 Female	637 Female	889 Female
878 Female	834 Female	846 Female	838 Female

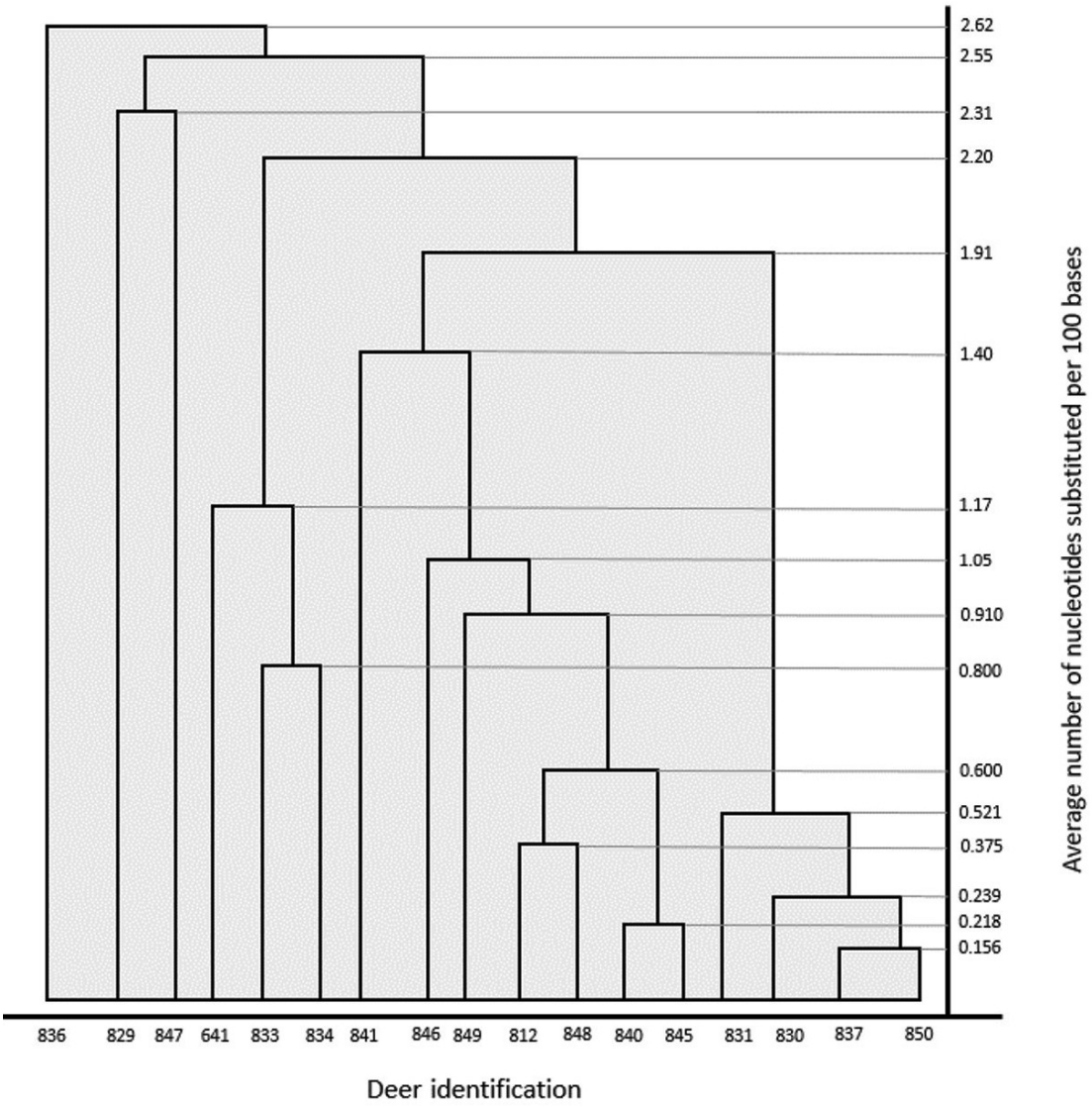

Fig. 1. Tree of genetic distances, between white-tailed deer, obtained with primer 5 whose sequence is 5´-d (AACGCGCAAC) -3´. The numerical scale on the horizontal line indicates the number of substitutions per 100 nucleotides, and the numbers on the vertical line indicate the identification of each animal

Table 4. Scheme of controlled crossbreeding in a population of White-tailed deer in the third generation

647 Male	879 Male	641 Male	838 Male
837 Female	847 Female	840 Female	849 Female
877 Female	876 Female	848 Female	638 Female
833 Male	830 Male	827 Male	844 Male
831 Female	839 Female	804 Female	835 Female
834 Female	829 Female	888 Female	887 Female
850 Male	836 Male	828 Male	843 Male
851 Female	846 Female	637 Female	889 Female
878 Female	878 Female	645 Female	828 female

3.3 Deer Mating Scheme

The mating scheme consists in that once each buck has been mated during the breeding season, it must be moved to the other pens, according to what is indicated in Tables 2, 3 and 4. The females are grouped with each male in each box. This procedure was carried out based on the results of Figs. 1 and 2. The groups with 1 male : 2 female ratio increased the effective number of the population, because there is an opportunity to mate a greater number of males with females that generate greater number of offspring per animal and in this way genetic variability is preserved [24]. In addition, genealogical and reproductive records must be generated in each generation to continue the controlled crosses subsequent generations.

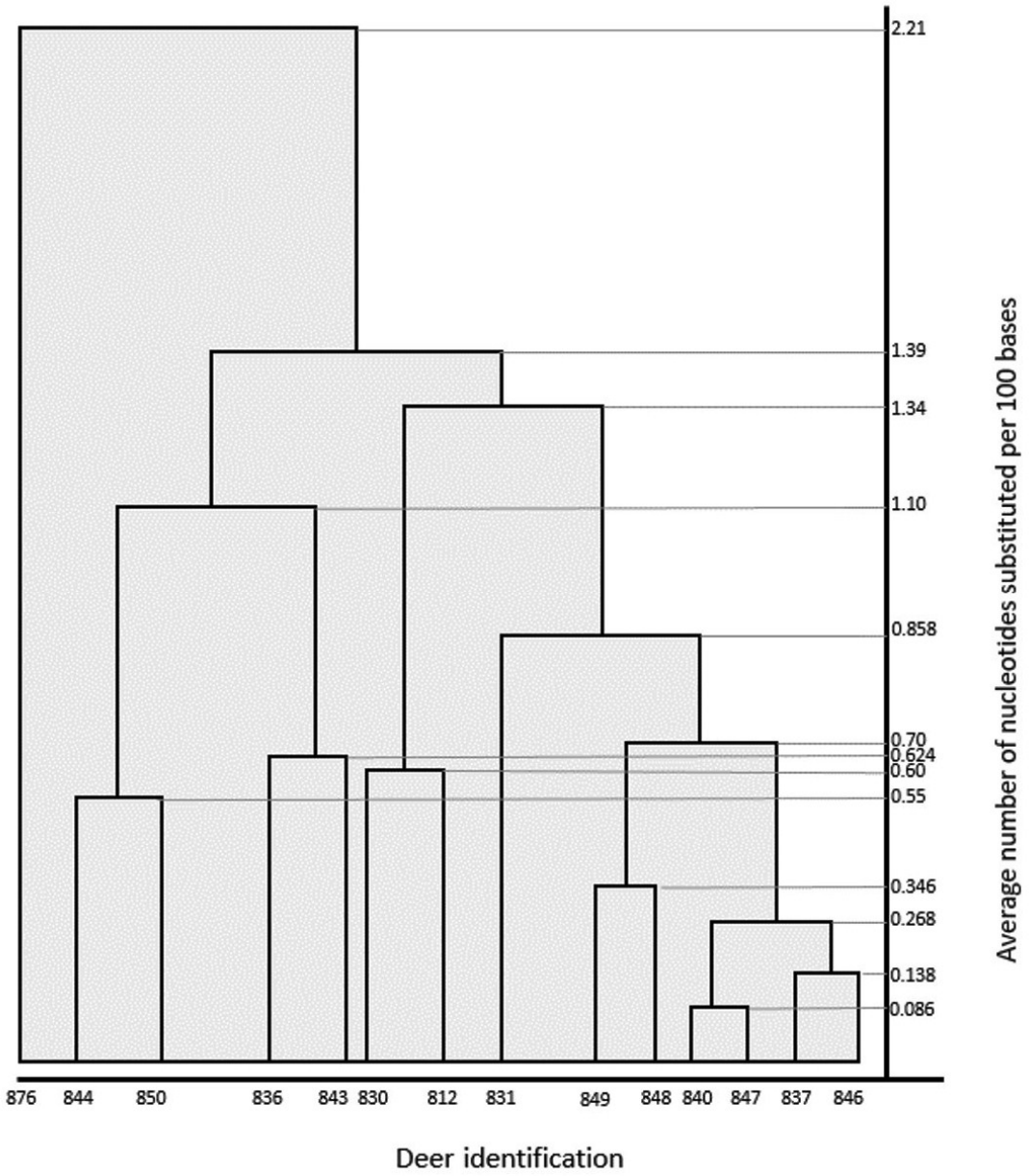

Fig. 2. Tree of genetic relationships between white-tailed deer, obtained with primer 6 whose sequence is 5´-d (CCCGTCAGCA) -3´. The numerical scale on the horizontal line indicates the number of substitutions per 100 nucleotides, and the numbers on the vertical line indicate the identifications of each animal

4. CONCLUSION

The RAPD used allowed to identify deer that do not share bands with the rest of the population evaluated as well as to identify those that showed nucleotide divergences with average values of 0.086 to 2.62 substitutions per 100 nucleotides. Based on the results, groups of reproducers were restructured, with the purpose of stopping the increase in inbreeding even in the absence of genealogical data

ETHICAL APPROVAL

Animal Ethic committee approval has been taken to carry out this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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