Chapter 7
Genetic Diversity of *Odocoileus virginianus veraecrucis* (Goldman & Kellog 1940) and Other's Subspecies in Mexico: Implications for Its Genetic Conservation

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1 Introduction

1.1 Evolution of Odocoileus virginianus

Cervids appeared in Asia at geological period known as Oligocene about 38 million years ago (MYA) and about 20 MYA colonized North America through the land bridge that connected current Alaska with northeast Siberia (Clément et al. 2006). Hassanin et al. (2012) determined that the Capreolinae family, which includes the *Odocoileus* genus, originated 10 MYA. Cap et al. (2002) indicated that the

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Odocoileus genus diversified approximately 3 MYA, but a fossil registry relating to the *Odocoileus* genus shows that it diverged in the Pleistocene, between approximately 2 MYA and 10,000 years ago (Kuznetsova et al. 2005; Groves and Grubb 2011). Pitra et al. (2004) estimated that *O. virginianus* appeared 6.87 MYA.

During the Pleistocene, the world climate cooled down and promoted the spreading of the ancestral *O. virginianus* towards South America, where it gradually became a member of specialized fauna with wide ecological plasticity (Merino and Vieira 2010). In the last 10,000 years, *O. virginianus* was free of competition or predators, and its population growth ratio kept increasing constantly (Halls 1981; Méndez 1984). Consequently, the species lineages colonized and established themselves in different ecosystems in the American Continent (Rees 1969), where each population is characterized by a likeness of morphological features as a result of the genotype-environment interaction (Kellogg 1956). This has allowed the recognition of subspecies (Halls 1984) and generating knowledge of the genetic diversity constitutes primordial information to understand its evolutionary history (Douzery and Randi 1997) and conserve its genetic diversity through the management of its populations (Ambriz-Morales et al. 2016).

In the American Continent, there are currently 38 subspecies of *O. virginianus*, from southern Canada in North America to Peru, Brazil, and Bolivia (Nowark 1991; Smith 1991). White-tailed deer (*Odocoileus virginianus*) is one of the 18 cervid species in Latin America (Weber and Gonzalez 2003; Gallina-Tessaro 2019), and it is the ungulate with the widest geographical distribution in Mexico where 14 species are recognized (Mandujano et al. 2010). Nevertheless, it is not easy to make a phenotypical distinction between most subspecies, as there is overlapping in their areas of natural distribution (Mandujano et al. 2010). In this sense, studies on the genetic diversity of the *O. virginianus* subspecies are basic to identify the genetic diversity parameters that distinguish each subspecies (Table 7.1; Logan-López et al. 2007; Calderón 2009; De la Rosa-Reyna et al. 2012; Ambriz-Morales et al. 2016).

1.2 Genetic Diversity Studies in Subspecies of O. virginianus

Studies have been carried out in different countries analysing the *D-loop* region of the *O. virginianus* mitochondrial DNA ($_{mt}$DNA). Carr et al. (1986) used $_{mt}$DNA restriction patterns to report hybridization of sympatric populations of *O. virginianus* and *O. hemionus* in Texas, USA; later corroborated by Bradley et al. (2003).

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Table 7.1 Summary of genetic diversity indicators described in *O. virginianus* subspecies. Sample size (n), number of identified haplotypes (H), haplotype diversity (*h*), nucleotide diversity (π), and the source of the report are shown. Nr indicates non reported values

Subspecies	n	H	*h*	π	Source
O. v. acapulcensis	8	8	1	0.12523	Ambriz-Morales et al. (2016)
	16	16	1.0 ± 0.0221	0.054 ± 0.028	Serna-Lagunes (2016)
O. v. carminis	2	Nr	Nr	Nr	Logan-López et al. (2007)
O. v. couesi	20	19	0.99474	0.12371	Hernández (2014)
	26	25	0.9969 ± 0.0117	0.176 ± 0.087	Serna-Lagunes (2016)
O. v. gymnotis	16	13	0.967	0.029	Moscarella et al. (2003)
O. v. goudi	4	4	1	0.005	Moscarella et al. (2003)
O. v. margaritae	6	6	1	0.016	Moscarella et al. (2003)
O. v. miquihuanensis	18	16	0.894	Nr	Molina (2002)
	4	Nr	Nr	Nr	Logan-López et al. (2007)
	4	4	1	0.019	Hernández (2014)
O. v. mexicanus	7	7	1	0.1102	Ambriz-Morales et al. (2016)
	8	7	0.9643 ± 0.0772	0.2773 ± 0.1521	Serna-Lagunes (2016)
	43	28	0.96678	0.04117	Hernández (2014)
O. v. nelsoni	14	12	0.9780 ± 0.0345	0.5551 ± 0.2838	Serna-Lagunes (2016)
O. v. oaxacensis	6	4	0.8 ± 0.1721	0.0021 ± 0.0019	Serna-Lagunes (2016)
O. v. sinaloae	3	3	Nr	0.533	Calderón (2009)
	42	32	0.97	0.0804	Ambriz-Morales et al. (2016)
	20	19	0.9947 ± 0.0178	0.3037 ± 0.1518	Serna-Lagunes (2016)
O. v. texanus	370	15	0.41	Nr	Purdue et al. (2000)
	29[a]	30[a]	0.978	Nr	Molina (2002)
	93	13	Nr	Nr	Logan-López et al. (2007)
	39	17	Nr	0.6 a 0.88	Calderón (2009)
O. v. toltecus	4	4	1.0 ± 0.1768	0.3863 ± 0.2536	Serna-Lagunes (2016)
O. v. thomasi	11	10	0.9818 ± 0.0463	0.5652 ± 0.2960	Serna-Lagunes (2016)
O. v. veraecrucis	3[a]	8[a]	1	Nr	Molina (2002)
	6	Nr	Nr	Nr	Logan-López et al. (2007)
	20	15	Nr	0.57 a 0.86	Calderón (2009)
	16	13	0.95 ± 0.05	0.06 ± 0.01	In this study
O. v. yucatanensis	16	14	Nr	0.71 a 0.86	Calderón (2009)

[a]There is a greater number of haplotypes than samples, due to the molecular technique used in the study, where each allele represented a haplotype, therefore, in one sample there could be one or more haplotypes

Ellsworth et al. (1994) investigated the biogeographic history of *O. virginianus* populations from the southeaster USA and found that the differentiation between the populations is due to the biogeographic isolation between the populations, while other studies infer that such differentiation derives from ecological and demographic effects (Darrell et al. 1994). A study evaluating the phylogeography of three subspecies of *O. virginianus* (*O. v. margaritae*, *O. v. goudotii*, and *O. v. gymnotis*) shows that *O. v. gymnotis* is polyphyletic, suggesting that each subspecies is an Evolutionarily Significant Unit (ESU) and therefore are subspecies subject to incorporation into a conservation program due to their genetic rarity (Moscarella et al. 2003).

The bibliographic analysis carried out by Mandujano (2004) reports 501 studies up to 2001, carried out on Cervid's distributed in Mexico, which deal with morphology, population density, eating habits and diet composition, incidence of diseases, and reproductive parameters. This analysis sheds light on the lack of studies on the topics of genetic diversity of *O. virginianus* and even more so, on the scarce knowledge of the genetic diversity relationships of distributed subspecies in Mexico. However, genetic diversity studies of *O. virginianus* have been conducted in the past two decades and address different subspecies in different geographic regions of Mexico (Castillo-Rodríguez et al. 2020).

In Mexico, studies have already been carried out on subspecies of *O. virginianus* based on the analysis of the *D-loop* region of the $_{mt}$DNA. The pioneering work was carried out by Molina (2002), who studied the subspecies *O. v. miquihuanensis*, *O. v. texanus*, and *O. v. veraecrucis* and reported 54 different haplotypes, suggesting great genetic diversity for each subspecies studied. A recent work (Calderón 2009), studied the subspecies *O. v. carminis*, *O. v. sinaloae*, *O. v. texanus*, *O. v. veraecrucis*, and *O. v. yucatanensis*. It does not report haplotypic diversity by subspecies, which is necessary if a genetic improvement program is implemented.

Logan-López et al. (2007) developed the first study to analyse the genetic variation of four subspecies: *O. v. texanus*, *O. v. carminis*, *O. v. veraecrucis*, and *O. v. miquihuanensis* in the states of Nuevo León, Coahuila and Tamaulipas, Mexico, in order to detect genetic introgression between subspecies resulting from translocation. In 105 samples of these deer they found shared haplotypes between subspecies, derived from hybridization between subspecies *O. v. texanus*, *O. v. veraecrucis*, and *O. v. miquihuanensis*, particularly in a habitat convergence zone. This induced changes in the genetic pool and affected the adaptation and speciation processes of each subspecies (Galindo-Leal and Weber 1994).

Ambriz (2010) analysed the genetic structure and variability of three subspecies: *O. v. sinaloae, O. v. mexicanus*, and *O. v. acapulcencis* distributed in four biogeographical regions (delimited by mountain ranges, vegetation and climates) of the Michoacán state, Mexico. The results show a differential genetic structure between subspecies due to geographic barriers and the environment that each subspecies inhabits. Subsequently, Ambriz (2012) sequenced the mitochondrial genome of four subspecies: *O. v. texanus, O. v. veraecrucis, O. v. sinaloe*, and *O. v. yucatanensis*, identifying phylogenetic separation between the subspecies of the southeast, north, centre, and south of the country (Ambriz-Morales et al. 2016). Hernández

(2014) analyzed the subspecies *O. v. mexicanus*, *O. v. couesi*, and *O. v. miquihuanensis* determining that these last two form a single phylogenetic clade, which is associated with the geographical proximity of these two subspecies.

The phylogenetic relationship tree showed a greater number of interspecific variations between *O. v. sinaloe* and *O. v. yucatanensis*, while the most related subspecies *O. v. texanus*, *O. v. veraecrucis*, and *O. v. yucatanensis* were grouped into a clade. Another study evidenced that the *O. v. yucatanensis*, *O. v. texanus*, *O. v. carminis*, *O. v. veraecrucis*, and *O. v. sinaloae* subspecies show a genetic-geographical association pattern (De la Rosa-Reyna et al. 2012).

For his part, Serna-Lagunes (2016) characterized the genetic diversity and structure, the genealogical and phylogenetic relationships of eight subspecies of *O. virginianus* distributed in Western Mexico: *O. v. acapulquensis, O. v. couesi, O. v. mexicanus, O. v. nelsoni, O. v. sinaloae, O. v. oaxaquensis, O. v. thomasi*, and *O. v. toltecus*. Gene genealogy showed that *O. v. acapulcensis* and *O. v. couesi* present ancestral haplotypes that originated haplotypes of *O. v. sinaloae*. No isolation pattern was detected by distance, this due to the limited geographic-gene flow between subspecies, so each subspecies must be considered an ESU and a Conservation Operational Unit (OCU).

This geographical-genetic diversity association of *O. virginianus* subspecies, it's a hypothesis of a possible indicator of a genetic structure associated with the geographical region where each *O. virginianus* subspecies is distributed (Moscarella et al. 2003). Hence the importance of their conservation since they are genetic reservoirs (Pisanty et al. 2016) that, after to their genetic characterization, could be used in gene restoration programs in populations (wild and/or captivity) with genetic erosion processes (Moscarella et al. 2003; Serna-Lagunes 2016).

1.3 The Management of O. virginianus in Mexico

The management of *O. virginianus* is carried out by Units for the Conservation, Management and Sustainable Use of Wildlife (UMAs) which regulates populations through habitat management (INE 2000). These UMAs aim to ensure that the annual harvest rate of deer is less than the annual population growth rate. However, there is no data to support the design of genetic conservation programs for the subspecies of *O. virginianus* in western Mexico (Castillo-Rodríguez et al. 2020). Such programs should include, for example, artificially restocking populations that have a genetic diversity deficit with genetically different individuals in order to improve the stability in the genetic structure.

Currently, UMAs operate as breeding foot production systems, gene-banks, examples of conservation and reproduction alternatives, environmental education and training (García-Marmolejo et al. 2008). This has led to research to generate optimal management strategies for ungulates under the UMA scheme (Escalante and Martínez-Meyer 2013). In the case of *O. virginianus*, there is already a White Tail Deer Management Plan for temperate and tropical zones, which proposes gen-

eral management actions *in-situ* and *ex-situ* and population studies are required to extract individuals (SEMARNAT 2014). Population report to SEMARNAT who rules the harvest rate of specimens in the UMA at established times (SEMARNAP 1997). To achieve the objectives of this system of production of wild fauna and flora, it is necessary to adequately manage the genetic component of populations in the long term of populations to improve productivity parameters (Castillo-Rodríguez et al. 2020). However, the management plans authorized for the white-tailed deer UMAs do not have genetic diversity conservation programs or genetic improvement plans that help to obtain specimens with phenotypic characteristics demanded by the hunting sector. To achieve this, it is important to characterize the genetic diversity of the populations and subspecies of *O. virginianus*, which will eventually allow making decisions for genetic management such as: the design of genetic improvement programs and the application of concepts of genetic conservation biology for the rescue of populations and/or subspecies identified with low genetic diversity.

Particularly, the *O. v. veraecrucis* subspecies is subject to different anthropic pressures, like cynegetic use (Weber 1993) and selective harvesting of specimens with cynegetic interesting features (Logan-López et al. 2006; Cienfuegos-Rivas et al. 2011), although it has not been included in the list of species at risk of the Mexican legislation and globally (IUCN red list), but in Mexico, Central America and South America most of the populations are declining, and most of the subspecies status are unknown (Gallina and Lopez-Arevalo 2016). Therefore, generating information on reproductive biology, its genetic diversity, and the status of its populations will help guide the level of risk found. This subspecies shows seasonal reproduction (Ahuja-Aguirre et al. 2017) and has a lower population density (4.2 ± 2.8 deer km^2) than other subspecies (Del Ángel and Mandujano 2017), while its habitat is constantly being fragmented (Gallina-Tessaro et al. 2007; Gallina et al. 2010; Delfín-Alfonso et al. 2009).

The objectives of this study are (a) to review of studies of the genetic diversity of *O. virginianus* subspecies, (b) to describe the genetic diversity, genetic structure, and phylogenetic relationships of the *D-loop* region of the $_{mt}$DNA of *O. virginianus* subspecies; information that could be incorporated into conservation programs to restore genetic processes in wild populations (Xiang-Dong et al. 2005). To achieve this last objective we described the phylogenetic relationships and compare the patterns of genetic diversity of the *D-loop* region between the subspecies of *O. virginianus* reported with distribution in Mexico, since the sequences of the *D-loop* region are often capable of providing information to study the genetic conservation status of a subspecies and determine the genetic limits between infraspecific taxa (Avise 2006, 2009) and this information can contribute to the restoration of populations, in decision-making in genetic improvement programs and consequently implement more effective genetic conservation strategies (Arif and Khan 2009). This study is an incentive for technicians and managers of subspecies of *O. virginianus* to genetically characterize their populations and for this information to be incorporated into improvement programs that guarantee the conservation of the genetic diversity of subspecies under the UMA scheme.

2 Material and Methods

2.1 Bibliometric Analysis

The review of the investigations carried out in Mexico on the genetic diversity of subspecies of *O. virginianus* was carried out by means of a bibliometric analysis (Ávila-Nájera et al. 2018) applied in the scientific databases available in the virtual library of the Universidad Veracruzana (BiVUV; https://www.uv.mx/bvirtual/). The collected information was synthesized and the genetic diversity values of the *O. virginianus* subspecies reported in the literature were analysed. This information was included in the introductory section of this chapter to describe the current state of the implications for conservation genetics in *O. virginianus* subspecies.

2.2 Description of the Deer Samples of O. v. veraecrucis

We studied 16 adult deer of the *O. v. veraecrucis* subspecies kept in captivity in the "El Pochote" UMA, located in the municipality of Ixtaczoquitlán, Veracruz, Mexico, authorized to handle this species (registry: SEMARNAT-UMA-IN-CR-0196-VER/18). The studied deer come from different geographical zones due to the interchange of specimens with other UMAs in order to avoid inbreeding problems. Thus, six deer come from the municipality of Las Vigas, four deer from the municipality of Tuxpan, three deer from the municipality of Paso de Ovejas, and three deer from the municipality of Ixtaczoquitlán, Veracruz, Mexico (Fig. 7.1). These places are within the geographical distribution area of *O. v. veraecrucis* (Mandujano et al. 2010).

Prior to selecting the sample and in order to guarantee that the studied deer belong to the *O. v. veraecrucis* subspecies, we used the values of the body characteristics of the deer under study and contrasted them against those reported in the literature for this subspecies (Logan-López et al. 2006). Those specimens that did not correspond morphologically were discarded from the sample. The deer were sedated, and 2 mL of blood were extracted from each one with a Vaccutainer® with EDTA as anticoagulant (Serna-Lagunes 2016). To do this, we considered the ethical norms for the treatment of each animal (Sikes et al. 2016).

2.3 DNA Extraction and Amplification of the D-Loop Region

The DNA was extracted with the Promega® DNA extraction kit. To verify the DNA quality an aliquot of 3μL of the DNA extraction was used, mixed with 1μL Diamond® (Promega®) and 1μL Green GoTaq (Promega®) buffer. The mix was run in agar gel at 1% under a horizontal electrophoresis camera at 90 V for 30 min.

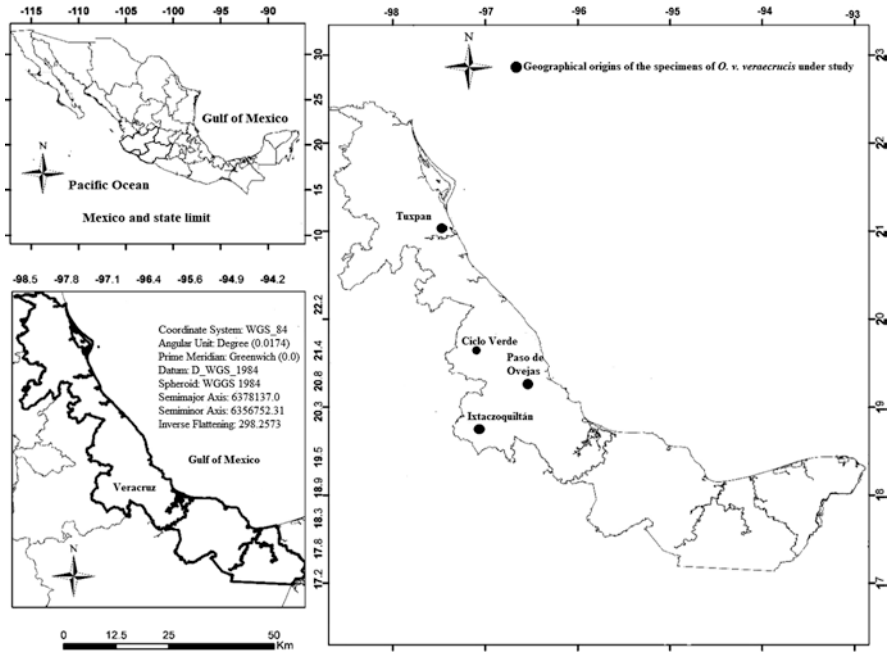

Fig. 7.1 Geographical origin of the *O. v. veraecrucis* specimens analysed in this study

To describe the genetic diversity of *O. virginianus* specimens we used the *D-loop* region of $_{mt}$DNA, since it represents a non-codifying genetic region and it is easy to obtain and analyze (Purdue et al. 2006); it has a simple genetic structure with no repetitions or presence of pseudogenes or introns, and an exclusively matrilineal transmission marker. Moreover, it has no genetic recombination or genetic fixes, and therefore tends to homoplasmy and a high nucleotide substitution ratio (Yamamoto 2001). In this sense, the *D-loop* region is genetically very variable and provides valuable information to infer relationships between individuals (Avise et al. 1987; Avise 2000, 2004).

The *D-loop* region of $_{mt}$DNA was amplified through PCR in triplicate in order to discard the origin of the variation being from PCR artifice. The PCR amplifications were done at a final volume of 25μL, containing 2.5μL ADN, 0.5μL primer (final concentration of 10μM) DL-F (5′-ATC GCC CAC TCT TTC CTC TT-3′), and 0.5μL primer DL-R (5′ TCA GTG CCT TGC TTT ATT GT-3′) developed for *Capreolus capreolus* (Tsaparis et al. 2019), 7.2μL PCR Master Mix 2× (25 mM Tris-HCl pH 9, 25 mM NaCl, 2.5 mM $MgCl_2$, 100μM of each nucleotide, 0.5 U *Taq* DNA polymerase, 0.05 mg mL BSA) Promega®, and 14.3μL nuclease-free water.

The amplification program through PCR of the *D-loop* region was done in a thermocycler (Axygen® MaxyGeneTM II Thermal Cycler) with the following sequence: polymerase activation at 95 °C for 3 min, denaturation at 95 °C for 30 s, followed by 31 cycles that included: 95 °C for 30 s (denaturation), 55 °C for 30 s

(alignment), and 72 °C for 30 s (polymeration). Finally, a conservation cycle was programmed at 4 °C. The amplification of the target region was verified using an aliquot of 3μL of the PCR product mixed with 0.5μL Diamond® and set in an agar gel at 1% to test the presence of a fragment between 400 and 550 base pairs (bp), based on a known molecular weight marker. The standard sequencing of nucleotides was done with an Applied Biosystems 3130 Genetic Analyzer sequencer, through the Sanger and Coulson technique (Sanger and Coulson 1975).

2.4 *Phylogenetic and Molecular Evolutionary Analyses of O. virginianus Subspecies*

The electropherograms of each sequence were edited manually with the Chromas v 2.1.1 software (Technelysium 2012), compared against the sequences deposited in the GenBank database (National Center for Biotechnology Information) to determine its identity and deposited in the GenBank (number accession GenBank: MH800299-MH800314). After that, the sequences were aligned with the Clustal W algorithm of Molecular Evolutionary Genetics Analysis Version X software (MEGA; Kumar et al. 2018).

Before building the phylograms (Hall 2013) in MEGA version X (Kumar et al. 2018), three sequences of the *D-loop* region of *O. virginianus* were downloaded (idGenBank: KX171760.1, KX171759, and KX171758.1) and used as external group (Heffelfinger 2011). Additionally, sequences from the *D-loop* region of other subspecies with geographic distribution in the West of Mexico, reported on the GenBank platform were obtained to compare the diversity and genetic structure with that found in *O. v. veraecrucis* in this study. The sequences used were (popset Genbank accession: 307088077), of which 7 sequences belonged of *O. v. mexicanus*, 32 sequences to *O. v. sinaloe*, and 8 from *O. v. acapulcencis* (Ambriz-Morales et al. 2016). In total, this study analysed a total of 66 sequences (including external group) of the *D-loop* region of four subspecies of *O. virginianus*.

Phylogenetic and molecular evolutionary analyses were conducted using MEGA version X (Kumar et al. 2018). Nucleotide substitution models were tested to describe the substitution pattern the best (Nei and Kumar 2000; Kumar et al. 2018) and substitution of nucleotide matrix in the control region of $_{mt}$DNA was calculate. The genetic distance measurements within and between subspecies were overall mean distance (d ± s.e.) (Tamura and Nei 1993), within group mean distance, between group mean distance, and net between group mean distance (Tamura et al. 2004). The measures of genetic diversity within and between subspecies were mean diversity within subspecies, mean diversity in entire subspecies, mean intersubspecies diversity and coefficient of differentiation (Nei and Kumar 2000; Tamura et al. 2004).

Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.3108)). There were a total of 377 positions in the final dataset.

Since the phylogram did not show a grouping structure by subspecies (see Results), the historical demographic structure for the set of sequences obtained from *O. virginianus* (n = 66 sequences) was studied to have an estimate of whether changes in population size promoted changes in nucleotide diversity. The analysis included a Mismatch distribution test under the model in a constant population that compares the observed frequencies with respect to the expected frequencies, where a population expansion is inferred if the distribution is unimodal and does not differ from the distribution expected (Rozas et al. 2017). This analysis was developed in DNA Sequence Polymorphism (DnaSP) software version 6.11 (Rozas et al. 2017).

Finally, to test the geographic-genetic association process of the subspecies of *O. virginianus* that have been inferred in the literature (Table 7.1), a haplotype network with Median Joining Network algorithm was built in the Population Analysis with Reticulate Trees software (PopArt) version 1.7 (Leigh and Bryant 2015). Network structures are used in population genetics to summarise the genetic diversity in a population (Leigh and Bryant 2015).

3 Results

The sequences had an average length of 395 bp and with a nucleotide composition mainly represented by a higher proportion of T (31.2%). In these sequences 333 identical nucleotide bases, 32 transitional pairs, and 23 transverse pairs were obtained (ratio = 1.38). The best substitution evolutionary model was Hasegawa-Kishino-Yano (HKY) + G (BIC = 10223.988425279). Substitution of nucleotide matrix in the control region of $_{mt}$DNA indicated up to 20 nucleotides between A and G (Table 7.2, data in parentheses).

Table 7.2 Estimates of Evolutionary Divergence over Sequence Pairs between Groups (below the diagonal) and standard deviation (above the diagonal) and substitution of nucleotide (in parentheses) of sequences of $_{mt}$DNA *D-loop* of *O. virginianus* subspecies

	To			
From	A	T	C	G
A	–	0.059 (6.15)	0.044 (4.57)	0.105 (11.85)
T	0.054 (5.65)	–	0.145 (12.21)	0.032 (3.32)
C	0.054 (5.65)	0.195 (16.43)	–	0.032 (3.32)
G	0.179 (20.1)	0.059 (6.15)	0.044 (4.57)	–

Table 7.3 Estimates of Net Evolutionary Divergence between Groups of Sequences (under the diagonal) and Estimates of Evolutionary Divergence over Sequence Pairs (above the diagonal) of *O. v.* subspecies in Mexico. References: 1. *O. v. mexicanus*, 2. *O. v. sinaloe*, 3. *O. v. acapulcensis*, 4. *O. v. outgroup*, 5. *O. v. veraecrucis*

	1	2	3	4	5
1		0.01 ± 0.00	0.04 ± 0.01	1.06 ± 0.38	0.02 ± 0.00
2	0.13 ± 0.02		0.04 ± 0.01	1.02 ± 0.37	0.02 ± 0.00
3	0.16 ± 0.02	0.15 ± 0.02		1.06 ± 0.40	0.05 ± 0.01
4	1.15 ± 0.03	1.09 ± 0.31	1.14 ± 0.34		1.04 ± 0.37
5	0.12 ± 0.02	0.10 ± 0.01	0.14 ± 0.02	1.10 ± 0.32	

The number of base substitutions per site from averaging over all sequence pairs and net evolutionary divergence between groups is shown in Table 7.3. Overall mean distance (d = 0.21 ± 0.04), within mean group distance (*O. v. mexicanus*: d = 0.13 ± 0.02; *O. v. sinaloe*: d = 0.1 ± 0.02; *O. v. acapulcensis*: d = 0.12 ± 0.02; *O. v. veraecrucis*: 0.08 ± 0.01).

The evolutionary history of *O. virginianus* subspecies inferred by using the Maximum Likelihood method and the Hasegawa-Kishino-Yano model showed two defined clades (Fig. 7.2). The phylogram with the highest log likelihood (−3869.46) with the percentage of trees in which the associated taxa clustered together next to the branches is shown. The clades grouped the sequences of the four subspecies indistinctly, the groupings show close genetic distances between haplotypes, which may be an indicator that the genetic diversity between subspecies is similar. This inference could reject the hypothesis of the genetic recognition of the subspecies that make up the group of *O. virginianus,* since the structure of the phylogram does not represent a genetic structure associated with the geographical distribution of the subspecies of *O. virginianus* (Fig. 7.2).

Expected values for constant population size of the sequence set of *O. virginianus* shows that nucleotide diversity ($\pi = 0.12694$), the average number of pairs of differences (k = 42.78; variance of k = 1988.74; observed CV of k = 1.0464), Raggedness statistic (r = 0.0017) mean absolute error (MAE = 0.9176) and Ramos-Onsins and Rozas (R^2 statistic = 0.0885), and parameters estimation (estimate of Theta initial = 44.113; estimate of Tau = 0.000) conform to a multimodal distribution. This is indicative of no population expansion and implies that the changes in the genetic diversity of *O. virginianus* are not due to population expansions (Fig. 7.3).

The 66 analysed sequences presented high values of haplotype diversity (H = 0.95), a moderate nucleotide diversity ($\pi = 0.176$), 235 segregating sites, and 220 parsimoniously informative sites. According to the results of the Tajima's test (D = 0.711) [(p(D ≥ 0.711317) = 0.48)] the sequences do not show departures of DNA polymorphisms from the neutral expectations. The frequencies of the polymorphisms do not represent evidence of selection, recombination, population subdivision, or changes in population size. The haplotype network does not show a structure that allows inferring any phylogeographic relationship between the

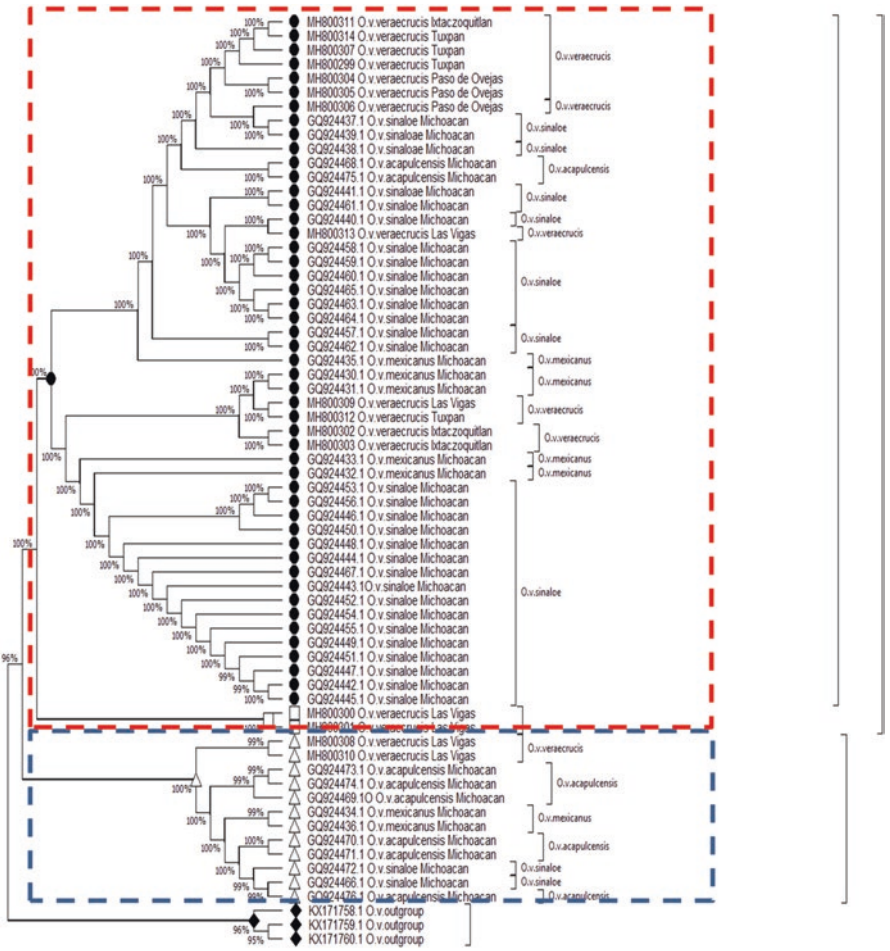

Fig. 7.2 Hypothesis of the phylogenetic relations between sequences of the *D-loop* region of $_{mt}$DNA of *O. virginianus* subspecies. The branches show the values obtained by Bootstrap based on maximum likelihood (ML)

subspecies of *O. virginanus* evaluated in this study (Fig. 7.4). The small sample size (n = 66) is another factor that limits the identification of a pattern of genetic-geographic association that supports the assumption of recognition of subspecies, which until now have been recognized by their morphometric, phenotypic, or delimited traits based on the correspondence of the type of vegetation and the range of geographic distribution.

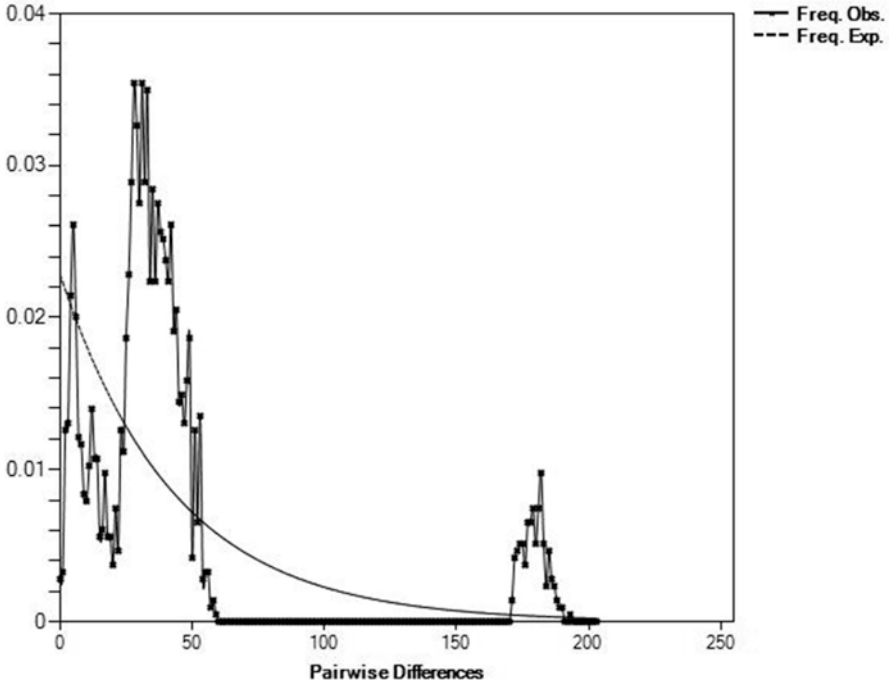

Fig. 7.3 Multimodal curve resulting from the *Mismatch distribution analysis*. The observed multimodal distribution does not indicate processes of population expansion that have modified the genetic diversity observed in *O. virginianus*

4 Discussion

In the bibliographic review carried out in this work, we found around ten research works related to the study of the genetic diversity of subspecies of *O. virginianus* in Mexico. This represents a challenge to increase knowledge on this and other topics related to the management of wild or captive populations and under different exploitation schemes in Mexico (Mandujano 2004). On the other hand, the information reported in the literature shows variations in the components of genetic diversity (haplotype diversity and nucleotide diversity) of each subspecies of *O. virginianus*. However, the low sizes of samples analysed prevent to reach more precise conclusions on the genetic differentiation of subspecies and to test genetic-geographic or phylogeographic hypotheses. It is important that technicians, wildlife managers, the Secretary of Environment and Natural Resources of Mexico and researchers specializing in studies of genetic diversity in cervids, articulate efforts to increase knowledge about the genomic, nuclear, and mitochondrial diversity of subspecies of *O. virginianus* that are under management in intensive UMA systems, in order to generate a pattern of white-tailed deer with outstanding genetic characteristics and that can be considered in a genetic improvement program. With this

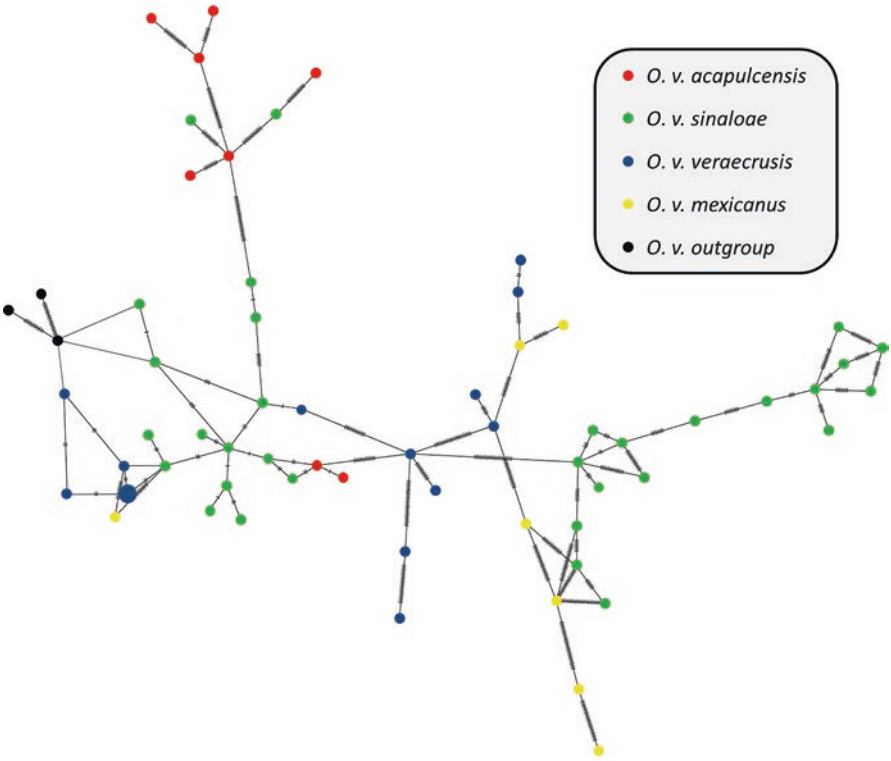

Fig. 7.4 Haplotype network of sequences of the *D-loop* region of the $_{mt}$DNA of four subspecies of *O. virginianus* with distribution in Mexico

information, the translocation of deer with desirable morphological characteristics to geographic regions where other subspecies are distributed would be avoided, since the information would be available on where to acquire stallions or even artificial insemination techniques can be chosen due to the different advantages that this represents.

The *D-loop* region of $_{mt}$DNA is often used in studies to describe genetic diversity due to its high level of polymorphism (Serna-Lagunes 2016). The haplotypic diversity (h) and moderate nucleotide diversity (π) values reported in the literature and recorded for subspecies of *O. virginianus* have been attributed to an increase in new mutations (Avise et al. 1984). These patterns of genetic diversity may be the result of the reproductive biology of *O. virginianus* and that the frequency of haplotypes in the population decreases. The haplotypes registered in the present study showed low frequency, which could be due to the reproductive biology of the species, as the females maintain philopatry and the males migrate great distances to mate with other females (Nelson and Mech 1992; Nelson 1993). This behaviour decreases the frequency in $_{mt}$DNA transference of *O. virginianus* (Purdue et al. 2000, 2006) and

mould the heterogeneity of the genetic flow (Nelson 1993) and the socio-genetic structure of the species (Mathews and Porter 1993).

The *h* and π values for the sequences of the four subspecies of *O. virginianus* studied as a single population, indicate that these genetic diversity parameters in this population are stable due to a long evolutionary history, or, it is the result of a genetic exchange between populations that were geographically isolated; females would colonize new types of vegetation, thereby dispersing new haplotypes due to cross-breeding between different lineages (Purdue et al. 2006). This result implies that the analyses of the genetic structure of *O. virginianus* carried out for the *D-loop* region, infer that the sample studied is composed of a single population without subdivisions (subspecies), while the observed genetic differences are represented by variations at the individual level between or within populations (Cronin et al. 1991).

Our results show that the studied sample of *O. virginianus* presents a genetic diversity that has not been modified by historical demographic processes such as population expansions (Rogers and Harpending 1992; Rogers et al. 1996). However, the pressure of clandestine hunting and legal hunting of the specie in Mexico (SEMARNAT 2013), its ecology and management (Gallina and Mandujano 2009), habitat fragmentation, poaching, consumption by local communities, and predators (Mandujano 2011; Gallina-Tessaro et al. 2019) are constant pressures on the populations of this species. This decreases the effective size of the mating population in wild populations (Mandujano and González-Zamora 2009), thus only a few lineages go on to the next generation while other lineages go extinct or are less frequent within the population (Rogers et al. 1996; Vázquez-Domínguez 2002). As expected for important game species, with poaching or commercial hunting, a similar effect was observed in the Tibetan antelope (*Pantholops hodgsonii*), a species at risk of extinction due to the loss of its genetic diversity (Xiang-Dong et al. 2005).

The phylogram showed two groups, the first consisting of eight sequences of *O. v. acapulcensis*, two of *O. v. sinaloae*, two of *O. v. veraecrucis*, and one of *O. v. mexicanus*. The other group was made up of the remaining 50 sequences where a polymorphic arrangement of subgroups is presented, showing a mix in the formation of the group between the different sequences, but without an arrangement that allows differentiating subspecies. Haplotypes are grouped as two monophyletic clades but they do not correspond to the subspecies studied, possibly because the sample studied corresponds to individuals with ancestral lineages that became established in the population and therefore there has not been enough evolutionary time to accumulate mutations that allow to genetically differentiating subpopulations (Cerritos 2007). Another fundamental aspect of the phylogram is that it also differentiates the phylogroups with a lower number of haplotypes. This means that haplotypes showed substitution rates of differential nucleotides and high levels of intraspecific polymorphism in the *D-loop* region, associated with an individual speciation process (Lunt et al. 1998). The branches of the phylograms showed a length over 95%, which indicated a similar divergence time between the haplotypes (Ruiz-García et al. 2007).

4.1 *Implications of Genetic Management of the Subspecies of O. virginianus in Mexico*

The deer of the subspecies of *O. virginianus* have socioeconomic, cultural, nutritional, and ecological importance; this species has been exploited since pre-Columbian times by providing meat, skin, bones, oil, fat, bait, pigments, medicinal, and natural properties aphrodisiac (Naranjo et al. 2010). The use of *O. virginianus* in UMA should be planned to avoid the unfavourable selective impact towards certain specimens of greater size and morphometry of antlers (characters of hunting interest) and to avoid the reduction of genetic diversity (Cienfuegos-Rivas et al. 2011).

Some biological conditions that this implies are morpho-physiological changes that limit their average longevity (Galindo-Leal and Weber 1998) because the successful mating of males with large antlers is correlated with the size of the individual and the structure of its antlers, a reflection of its genetic expression (Monteith et al. 2013). The loss of genetic diversity in subspecies of *O. virginianus* not only affects biological aspects such as the evolutionary potential to adapt to the environment (physical and biological) (Piñero et al. 2008a, b), but also affects the environmental services that the species generates for rural communities (Wright et al. 2000).

It is a priority to describe and conserve the genetic diversity of *O. v. veraecrucis* populations in captivity as a genetic reservoir to restore genetic erosion processes in wild populations. In the deer samples, we observed haplotypes that were frequently shared and haplotypes that were infrequent and which differ from the others by a few mutational changes (Cronin et al. 1991). This information shows unique genetic collections associated with specific geographical regions and could be useful to increase the frequency of rare genes in wild populations of the *O. v. veraecrucis* subspecies with problems of genetic erosion (Ellsworth et al. 1994).

The reduction of pressures on *O. virginianus* is necessary for its conservation (Crandall et al. 2000). Although we do not have information on the number, age, and sex of deer authorized for legal hunting in Mexico, we are aware of poaching of the subspecies *O. v. veraecrucis* in the centre of Veracruz, Mexico (Tlapaya and Gallina 2010). This is a threat to the genetic diversity of this subspecies in wildlife; therefore, studies of genetic diversity of populations of this subspecies in UMAs could generate a pool for use in genetic conservation programs.

5 Conclusions

The genetic diversity of the *O. v. veraecrucis* subspecies in the present study showed a genetic pattern characteristic of this subspecies, but different from other subspecies reported in the literature. A high number of unique haplotypes and low nucleotide diversity could be due to the geographical independence of the origin of deers and a low mutation rate, respectively. The genetic diversity of the deer in the El

Pochote UMA represents a valuable genetic reservoir to restore genetic processes in wild populations through a planned crossing program between deer with contrasting genetic patterns, previously genetically characterized, to conserve the genetic diversity of this subspecies.

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