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White-tailed deer sex identification from faecal DNA and pellet morphometry by neural network and fuzzy logic analyses

Identificación del sexo de venado cola-blanca por ADN fecal y morfometría de los pellets mediante análisis de redes neuronales y lógica difusa

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Abstract

Knowing the sex of white-tailed deer (*Odocoileus virginianus*) individuals can provide information to set harvesting rates and management activities. Therefore, the aim of this study is to identify the sex through classification function by using faecal pellet morphometry. Faeces were collected for 12 months in Durango, Mexico; their morphometric variables were measured, the faecal DNA was extracted, and the SRY gene marker was amplified to identify sex. A neural network and fuzzy logic sex classification functions were obtained. The outputs were validated with the SRY gene results. Data from adults in the winter were used to obtain the classification functions. Classification functions could accurately classify sex in 94.4% with neural networks and 86.9% with fuzzy logic. The neural network classified more accurately the sex of adult white-tailed deer studied in winter with the faecal pellets morphometry than with the fuzzy logic analysis. This technique can be a tool for non-invasive studies and monitoring of populations.

Keywords: Faecal DNA; faecal morphometry; sex classification function; SRY gene; white-tailed deer.

Resumen

Conocer el sexo del venado cola blanca (*Odocoileus virginianus*) puede proporcionar información para establecer tasas de aprovechamiento y actividades de manejo. El objetivo de este estudio es identificar el sexo mediante la creación de funciones de clasificación para sexo obtenidas mediante morfometría de pellets fecales. Se colectaron heces durante 12 meses en Durango, México, a los cuales se les midieron sus variables morfométricas, extrajo ADN y amplificó el marcador genético SRY para identificar el sexo. Luego, se obtuvo una función de clasificación de sexo con redes neuronales y lógica difusa. Los resultados fueron validados con el gen SRY. Se utilizaron datos de adultos en invierno para obtener las funciones de clasificación. Se clasificó con precisión el sexo en 94.4% con redes neuronales y 86.9% con lógica difusa. Las redes neuronales clasificaron con mayor precisión el sexo del venado cola blanca con morfometría de pellets fecales de adultos en invierno que con lógica difusa. Esta técnica puede ser una herramienta para el estudio y monitoreo no invasivo de las poblaciones bajo manejo.

Palabras clave: ADN fecal; morfometría fecal; función de clasificación de sexo; gen SRY; venado cola-blanca.

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Introduction

White-tailed deer (*Odocoileus virginianus*) is one of the most important wildlife species in Mexico due to the number of animals that are hunted annually (Clemente-Sánchez, F., Cessa-Reyes, V., Cortez-Romero, C., Tarango-Arámbula, L. A., & Arenas-Baez, 2015). Established regulations for the harvest of white-tailed deer by institutions in North America usually allow different numbers of animals to be harvested depending upon their sex, having a preference for males (Lindsay & Belant, 2008; Riley *et al.*, 2003). It is necessary to obtain information about the number of females and males inhabiting an area to facilitate compliance with applicable laws and to estimate harvesting rates more accurately to avoid the hunting pressure over the males (Lindsay & Belant, 2008). Sex determination in wild populations is also essential for understanding the structure and dynamics of the population, habitat use, behaviour, and the mating system (Morden, Weladji, Ropstad, Dahl & Holand, 2011; Shaw, Wilson & White, 2003). Historically, faeces have been used as indexes of animal abundance (Kobelkowsky-Sosa *et al.*, 2001; Lounsberry *et al.*, 2015), a source for animal identification (Brinkman *et al*., 2010), and as a means for determining diet (Homolka, Heroldová & Bartoš, 2008), parasite infestation (Myers, Foreyt, Talcott, Evermann & Chang, 2015), and hormone metabolites (McCoy & Ditchkoff, 2012), which indicate physiological stress in the animals and their reproductive status.

Some non-molecular methods for sex identification that are used for several species of deer are difficult to carry out and are not entirely reliable; for example, direct observation of the genital region (Morden *et al*., 2011), analysis of the hormone levels in the blood, and pelvic morphology have been used for several species of deer. These techniques cannot always be readily used in free-living animals (Shaw *et al.*, 2003) since the animals need to be captured, which is problematic or harmful (Han, Lee, Cho, Oh & Oh, 2009). For these reasons, non-invasive monitoring techniques, such as deoxyribonucleic acid (DNA) faecal extraction or pellet morphometry, have been used (Morden *et al*., 2011).

Using DNA extracted from epithelial cells shed from the intestinal lining present in faeces, it is possible to obtain an individual animal's DNA and to genotype the animal (Huber, Bruns & Arnold, 2002 2003; Tolleson, Randel, Stuth & Neuendorff, 2005). Since faeces can be easily collected without disturbing any species, genotyping using DNA obtained from faeces has become a promising tool in studies of behaviour, ecology, and wildlife management (Huber *et al*., 2003). The determination of sex from DNA extracted from the faeces of wildlife species has become more feasible recently (Han *et al.*, 2009; Yamauchi *et al.*, 2000) due to the amplification of specific fragments of DNA associated with a sex chromosome, the SRY gene (Bryja & Konečný, 2003; Han *et al*., 2009; Yamauchi *et al*., 2000), with PCR. The SRY gene produces a transcription factor of a high mobility group (HMG) that recognises specific DNA sequences and binds to them. This process begins with the embryonic differentiation of testicles. Therefore, it is a gene of males, and it is present in a single copy in the non-recombinant region of the Y chromosome in most mammals (Bryja & Konečný, 2003).

Information obtained from faecal groups can be used to estimate ecological parameters in mammals, and this information can be an excellent tool for researchers, particularly if information about the sex and age of the animals can be obtained from the faeces (Sánchez-Rojas, Gallina & Equihua, 2004). Several studies have been able to identify age using faecal pellet morphometry of white-tailed deer (Ezcurra & Gallina, 1981), reindeer (*Rangifer tarandus platyrhynchus*) (Morden *et al*., 2011), and Sonoran pronghorn (*Antilocapra americana sonoriensis*) (Woodruff, Johnson & Waits, 2016). Additionally, the sex of moose (*Alces alces*) (MacCracken & Van Ballenberghe, 1987) and mule deer (*Odocoileus hemionus*) (Sánchez-Rojas *et al*., 2004) was determined by using the morphometry of faecal pellets. To determine the age and sex of deer populations based on the features of faecal pellets has significant implications for the management, use, and conservation of species, especially when the animals are difficult to track and see (Sánchez-Rojas *et al*., 2004).

This work aimed to generate classification functions using neural networks and fuzzy logic approaches to predict animal sex from faecal pellet morphometry in white-tailed deer. Sex was used as the dependent variable and faecal pellet morphometry as the independent variable. Results obtained from the amplification of faecal DNA using the SRY gene marker were contrasted to obtain the percentage of correct classifications.

Materials and Methods

Study area

Faecal groups of white-tailed deer were collected (there is no presence of other cervids in the area) in two management units for wildlife conservation (UMA) (Salvador Allende and Molinillos), which are located in the municipality of Durango, Durango, Mexico. The Salvador Allende UMA is located at 24° 71'- 24° 05' north latitude and 104° 51'-104° 56' west longitude. The elevation ranged from 2200 m to 2680 m, and the area was 3200 ha. The UMA had a temperate, semi-cold and mild, semi-humid climate. The main vegetation was pine-oak (*Pinus sp. -Quercus sp.*) forests, pine (*Pinus sp.*) forests, and oak (*Quercus sp.*) forests (González, González & Márquez, 2007). The Molinillos UMA is located at 23° 36'- 23° 39' north latitude and 104° 59'- 105°06' west longitude. The elevation ranged from 2000 m to 2680 m, and it encompassed a 300 ha area. This UMA had a temperate, semi-humid and mild, semi-cold climate, and the main types of vegetation were pine (*Pinus sp.*) forests, pine-oak (*Pinus sp.-Quercus sp.*) forests, oak-pine (*Quercus sp. – Pinus sp.*) forests, oak (*Quercus sp.*) forests, and grassland (Gramineae) (Rosales & Villanueva 2014).

Sample collection and the measurement of the faecal pellets

The faecal groups were collected every two weeks for 12 months in each UMA. For the Salvador Allende UMA, the sampling period was from March 1st 2015 to March 31st 2016, and for the Molinillos UMA from October 1st 2015 to October 31st 2016. The sampling period covered all seasons: spring (21 March-21 June), summer (22 June-22 September), autumn (23 September-21 December), and winter (22 December-20 March). By counting the faecal groups in previous surveys, areas with the most activity of white-tailed deer for each UMA were identified. Two random transects with a length of 1000 m were tracked on every sampling visit. Fresh faecal groups were collected. Fresh faecal groups were recognized by the softness and moisture of the outer mucus of the pellets. When there was rain on the day of or the day before the collection, sampling was avoided in order to identify fresh faecal groups easily. Twenty to thirty pellets from the top of every individual faecal group were placed in 50 ml Falcon™ (Corning Life Sciences C352070) tubes with 96% ethanol. The remainder of the pellets was placed in plastic bags. Faecal groups were identified with the name of the UMA, date, and geolocation. Then, 10% of the faecal pellets (20-35 pellets) of each group kept in plastic bags were measured with a digital calliper and placed them in a freezer at -20 °C. The measured variables were the width (W = maximum width of the pellet), length (L = maximum length of the pellet), radius ($R = \frac{W}{2}$), volume ($V = \pi R^2 L$), and length/width proportion ($\frac{L}{W}$) (Sánchez-Rojas *et al*., 2004). To prevent the morphometric measured variables of the animals from the different age and sex groups from overlapping and, thus, obstructing a separation between the sexes, samples were grouped by age and analysed by every variable. Based on the calculation of the mean volume of the faecal pellets and the method of probability for the graphical analysis of the polymodal frequency distributions created by Harding (1949) and used by Ezcurra & Gallina (1981) and Kobelkowsky-Sosa *et al*. (2001), faecal groups were assigned to an age category: 0 mm^3 to 290 mm^3 as fawns, 291 mm^3 to 605 mm^3 as yearlings, and 605 mm^3 and greater as adults.

DNA extraction

DNA was extracted from two to four pellets (depending on the pellet size) maintained in 96% ethanol. First, faecal pellets were left to dry for 45 minutes at room temperature in sterilised Petri dishes. Once the pellets were dried, they were placed in sterile 1.5 ml microcentrifuge tubes, and then 1 ml of extraction buffer (2% CTAB, 100 mM TRIS, pH 8, 20 mM EDTA, pH 8, 1.4 M NaCl and 1% PVP) and 5 µL of 2-mercaptoethanol per tube were added. The tubes were vortexed at 3400 rpm for 10 seconds and placed in digestion for one hour at 65 °C. After one hour, 50 µL of proteinase K were added and the tubes were placed at 65 °C to digest for one more hour. Next, to separate the solid and liquid phases, the samples were centrifuged for 15 minutes at 13 200 rpm. The supernatant was transferred to new sterile tubes and centrifuged again for 15 minutes at 13 200 rpm to remove any solid remaining impurities. This new supernatant was transferred to new tubes and 400 µL of chloroform-isoamyl alcohol (24:1) were added. Then, the tubes were vortexed at 3400 rpm for 10 seconds and centrifuged for six minutes at 13 200 rpm. The liquid on the top was separated into new tubes. To these new tubes, 300 µL ammonium acetate (3 M, pH 5.2) and 600 µL of isopropanol were added. The tubes were left overnight at -20 °C. Then, the tubes were left at room temperature for five minutes and centrifuged for 15 minutes at 13 200 rpm. The supernatant was discarded leaving a DNA pellet at the bottom of the tube. The DNA pellet was washed by adding 500 µL of 80% ethanol, vortexing it for 10 seconds at 3400 rpm and centrifuging it for six minutes at 13 200 rpm. After that, the alcohol was discarded, and the pellet was washed again with 500 µL of 80% ethanol. Finally, the remaining alcohol was left to evaporate at room temperature. The DNA was eluted with 55 µL of molecular biology grade water (Cellgro Corning® 46000). The DNA purity and quantity in ηg/µL were obtained by using a Nanodrop® 2000 spectrophotometer. With the use of ultraviolet light, the DNA integrity was observed on a 1% agarose gel stained with ethidium bromide.

SRY gene marker amplification

The SRY gene marker was amplified with the primer set SRY forward: CAT CTT GTC TGT GTG TCG TG and SRY reverse: CGG GTA GTG TCG TTT GTC TA (Lounsberry *et al*., 2015). Two adult male and two female white-tailed deer tissue samples, legally harvested from the Salvador Allende UMA, were used as positive controls. The final concentration for the PCR reaction was 100 ηg/µL-150 ηg/µL DNA, 2.5 mM $MgCl_2$, 0.5 mM dNTPs, and 0.6 pM of each PCR primer and 0.750 units of Taq DNA polymerase. DNA amplification was carried under a thermal profile of 15 minutes at 95 °C of initial denaturation, 35 cycles of 30 seconds at 94 °C, 1.30 minutes at 58 °C, 1 minute at 72 °C, and, finally, 10 minutes at 72 °C of final elongation. Every time the PCR reactions were carried out, one DNA sample from male deer tissue was included as a positive control, and water as a negative control. PCR products were observed on a 1% agarose gel stained with ethidium bromide; a molecular weight marker and PCR product from the positive and negative control samples were added to each of the gels. To ensure the results, the DNA extractions, the PCR reactions, and the observations of the gel of all the samples were performed twice to verify their accuracy. Then, the samples that did not show amplification on the gels were categorised as females and the amplified samples (one band of ~200 base pairs [bp]) as males. The samples that showed diffuse bands were not included in the study. Some molecular sex identifications were corroborated with the visual records that were observed in the field.

Statistical analysis

An analysis of variance (Anova) with a random factorial experimental design (3 × 4 × 2) was performed. The factors were age, season, and sex for all the morphometric variables to find significant differences ($\alpha = 0.05$). PROC GLM in the SAS 9.2 was used (SAS Institute, Cary, N.C., 2008). The null statistical hypothesis was that there were not significant differences in the main and interaction effects. The cases where there were

significant differences in the morphometric variables were used for the sex classification functions performance; in this case, they were just in the season-sex-age interaction.

A neural network analysis was performed to classify the faecal groups according to sex based on the measured morphometric characteristics (width, length, radius, volume, and length/width). A neural network is an accurate and powerful technique for classification, since it can store patterns and repeated information to give a solution or classification (Chairez, Gurrola, García & Echavarría, 2008). The neural network of this study included one hidden layer and neurons N_h under the following model (Chairez *et al*., 2008):

$$Y_k=f_k\left(\alpha_k+\sum_{j\to k} w_{jk}f_j\left(\alpha_j+\sum_{i\to j} w_{ij}x_{i,}\right)\right)$$

where Y_k is the dependent variable (Y_k; 1 = male, 0 = female), and x_i are the values of the morphometric variables. The weights w_{ij} and w_{jk} and the constant α_j and α_k were estimated by using the back-propagation algorithm. The activation function was sigmoidal in the inner layer and the output:

$$f(x)=\frac{e^x}{1+e^x}$$

The neural network was performed using the Statistical Package for the Social Sciences (SPSS) 18 programme (SPSS Inc., 2009).

To classify the faecal groups with fuzzy logic, the membership values for each morphometric variable were found; these values are the mean values that most differentiate the males and females in the width, length, radius, volume, and length/width variables. Then, the variables were coded into a scale in binary form as 1 and 0, where 1 represents a male and 0 represents a female. Each logical combination of values of the independent variables is represented as one row of a truth table. When the truth table is constructed, each row is assigned an output value (1 or 0 for the dependent variable) based on the score of the cases that share the combination of input values. The fuzzy logic analysis was performed using the fsQCA software (Ragin, Drass & Davey, 2006). Then, the results of both classification methods were compared with DNA results to obtain a classification percentage.

Results

Faecal DNA from 398 fresh faecal groups collected in both UMA were extracted. By amplifying the SRY gene marker, 385 faecal groups were assigned to one sex, achieving 96.7% success in the PCR analysis. By calculating the faecal pellets volume, 31 faecal groups were assigned as fawns, 241 as yearlings, and 113 as adults; at the same time, 219 faecal groups were classified as female and 166 were classified as male (table 1).

Table 1. Classification of age and sex of the white-tailed deer faecal groups collected in Durango, Mexico, from March 2015 to October 2016, based on SRY gene marker (sex) amplification and the morphometry (age) of faecal pellets.

	Fawns		Yearlings		Adults		
Season	F	M	F	M	F	M	Total
Spring	11	3	37	18	12	11	92
Summer	2	1	14	21	2	9	49
Autumn	4	4	53	36	23	33	153
Winter	5	1	39	23	18	5	91
Total	22	9	143	98	55	58	385

Source: Author's own elaboration.

Differences among seasons were found; autumn and summer showed higher values than winter and spring in width and radius ($F_{3, 1148} = 3.8787$, $p < 0.009$ and $F_{3, 1148} = 3.8787$, $p < 0.0099$, respectively). The length/width had higher values in winter and spring than in autumn and summer ($F_{3, 1148} = 3.99$, $p < 0.0088$). Regarding the interactive effects, it was only found differences between sex in width, radius ($F_{6, 1148} = 33.9$, $p = 0.013$), and length/width ($F_{6, 1148} = 33.95$, $p = 0.0111$) in the data from the adult white-tailed deer faeces collected in the winter. Since the statistical differences were only found in the interaction sex-age-season, specifically in faecal groups from adults collected in winter, the data from 23 faecal groups of adults collected in the winter were used to perform the sex classification functions.

The variable width showed the lowest (8.59%) coefficient of variation, and volume presented the highest value (22.10%). As age increased, the coefficient of variation values in all the morphometric variables increased (table 2); however, homoscedasticity assumption was not violated.

Table 2. Mean values and coefficient of variation (CV) of the morphometric variables of the white-tailed deer faecal pellets collected in Durango, Mexico, from March 2015 to October 2016, sorted by age, sex, and season.

			Variables									
Age	**Season**	**Sex**	**Width (mm)**	**CV (%)**	**Length (mm)**	**CV (%)**	**Radius (mm)**	**CV (%)**	**Volume (mm^3)**	**CV (%)**	**Length /width**	**CV (%)**
Fawns	Spring	F	5.79	10.36	9.42	10.83	2.89	10.38	249.4	21.01	1.65	15.76
		M	5.75	4.52	10.04	7.07	2.87	4.53	261.41	11.73	1.75	7.43
	Summer	F	6.33	3	9.33	6.22	3.17	3.15	294.14	9.37	1.47	6.12
		M	5.8	5.52	9.61	12.07	2.9	5.52	252.12	9.24	1.67	16.17
	Autumn	F	5.66	8.3	9.11	11.53	2.83	8.13	230.21	18.73	1.62	14.81
		M	5.65	4.25	9.22	10.2	2.82	4.26	232.53	15.8	1.63	9.2
	Winter	F	5.79	6.22	9.43	15.38	2.9	6.21	250.94	21.87	1.63	14.11
		M	6.3	3.17	9.47	20.17	3.15	3.17	293.9	17.05	1.51	22.52
Yearlings	Spring	F	6.86	9.18	11.64	14.78	3.43	9.04	434.29	24.71	1.71	16.96
		M	6.77	10.04	12.21	11.88	3.39	10.03	445.66	24.42	1.82	15.93
	Summer	F	6.99	10.16	11.62	14.72	3.5	10.29	446.45	20.53	1.69	21.89
		M	7.13	8.27	11.36	12.76	3.56	8.15	458.74	22.93	1.6	13.13

	Autumn	F	7.1	9.01	11.57	15.21	3.55	9.01	460.56	22.67	1.64	18.29
		M	7.06	8.92	11.66	13.98	3.53	9.07	459.53	22.03	1.67	16.17
	Winter	F	6.86	8.75	12.16	16.94	3.43	8.75	452.34	23.08	1.79	19.55
		M	7.01	8.56	12.37	13.82	3.5	8.57	479.53	20.32	1.78	17.42
Adults	Spring	F	8.11	5.43	13.53	9.16	4.06	5.42	702.3	15.61	1.67	10.18
		M	8.23	16.16	14.35	15.4	4.11	16.06	809.45	58.92	1.76	14.2
	Summer	F	9.17	16.36	13.53	11.38	4.58	16.38	896.21	29.44	1.53	23.53
		M	8.92	8.86	13.49	14.46	4.46	8.97	856.51	27.48	1.52	14.47
	Autumn	F	8.46	12.29	14.09	13.13	4.23	12.29	803.95	30.13	1.69	16.57
		M	8.45	11.6	13.51	14.51	4.22	11.61	760.85	25.86	1.63	20.86
	Winter	F	7.81	8.96	14.65	10.58	3.91	8.95	708.05	21.31	1.89	14.29
		M	8.32	8.29	13.74	13.83	4.16	8.17	744.18	16.22	1.67	17.37

Source: Author's own elaboration.

For the neural network analysis, it was tested from two to 40 neurons in the hidden layer, and the model that included 22 neurons showed a lowest percentage error (5%). The weights, w_{ij} and w_{jk}, and the constants, α_j and α_k, were calculated, organised, and substituted in the model for neural network classification (table 4), a predicted classification rate of 94.4% was obtained by correctly assigning four of the five faecal groups of the males and all of the faecal groups of the females. Through fuzzy logic analysis, a predicted classification rate of 86.9% was obtained by correctly assigning four of the five males and 16 of the 18 females (table 3).

Table 3. Observed sex by amplification of the SRY gene marker from faecal DNA against the predicted sex generated by neural networks and fuzzy logic classification functions using the faecal morphometry of the white-tailed deer faecal groups, collected from March 2015 to October 2016 in Durango, Mexico.

	Neural network			Fuzzy logic		
	Predicted sex		Correct	Predicted sex		Correct
Observed sex	M	F	classification (%)	M	F	classification (%)
Male	4	1	80.0	4	1	80.0
Female	0	18	100.0	2	16	88.8
General			94.4			86.9

Source: Author's own elaboration.

The membership values to convert the real variables to binary form to use in fuzzy logic were as follows: width ≥ 8.2 mm = 1 (male), length ≥ 15.2 mm = 0 (female), radius ≥ 4.1 mm = 1 (male), volume ≥ 730 mm^3 = 1 (male) and length/width ≥ 1.8 = 0 (female).

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Table 4. Neural network weights, hidden layer (width, length, radius, volume, and length/with) and output layer (sex = 0 and sex = 1).

W_{ij}		W_1	W_2	W_3	W_4	W_5	W_6	W_7	W_8	W_9	W_{10}	W_{11}	W_{12}	W_{13}	W_{14}	W_{15}	W_{16}	W_{17}	W_{18}	W_{19}	W_{20}	W_{21}	W_{22}
Width		0.334	3.419	0.015	0.185	-0.028	-0.148	0.025	-0.599	-0.75	-0.16	-2.57	0.013	-1.173	0.099	-1.864	0.449	-6.549	1.074	-1.424	0.108	0.167	0.414
Length		0.438	0.75	-0.071	0.219	-0.678	-1.882	1.23	5.396	0.837	-0.217	4.701	1.296	-0.89	0.542	1.159	-0.554	2.987	-0.9	-0.563	0.191	0.749	-0.351
Radius		0.669	2.377	-0.027	0.296	0.19	0.178	0.131	0.039	-0.583	0.091	-2.26	0.213	-1.174	0.121	-1.148	-0.176	-6.647	1.468	-1.099	0.041	0.577	-0.592
Volume		0.289	3.317	-0.637	-0.189	0.752	-0.691	0.652	3.527	-0.326	0.608	0.39	0.706	-0.327	1.507	-1.72	0.493	-3.783	0.526	-1.428	1.051	1.519	-1.067
Length/ width		-0.345	-1.41	0.903	-0.003	0.029	-1.509	0.712	2.892	0.842	-0.807	4.53	-0.12	0.249	0.311	1.939	-0.484	4.306	-1.52	0.523	-0.451	0.153	-0.409
	α_j	-0.614	2.425	0.299	-0.394	0.341	-0.979	-0.839	7.115	-0.056	0.348	4.078	-0.918	-0.513	-1.59	3.868	0.199	4.606	0.153	-0.434	-0.841	-2.14	0.897
W_{jk}	α_k	W_1	W_2	W_3	W_4	W_5	W_6	W_7	W_8	W_9	W_{10}	W_{11}	W_{12}	W_{13}	W_{14}	W_{15}	W_{16}	W_{17}	W_{18}	W_{19}	W_{20}	W_{21}	W_{22}
Sex=0	0.065	-0.412	4.566	0.741	-0.008	0.143	1.074	-1.144	-5.213	1.046	-0.027	5.179	-0.627	-0.26	-1.707	4.373	-0.809	-8.031	-2.33	-0.665	-0.605	-1.516	1.13
Sex =1	-0.664	0.646	-4.489	-0.79	0.109	0.712	-1.628	1.248	5.136	-1.376	0.747	-5.4	0.95	0.406	0.906	-4.362	0.119	7.973	2.186	0.693	0.915	1.892	-0.692

Source: Author's own elaboration.

Discussion

The use of faecal DNA for sex identification of individuals is a feasible and non-invasive tool to generate information of great importance for the management of wildlife species (Lindsay & Belant, 2008; Shaw *et al*., 2003). Unlike other studies, which have relied on the amplification of two or more genes (Huber *et al*., 2002; Lindsay & Belant, 2008; Shaw *et al*., 2003; Yamauchi *et al*., 2000), the amplification of the SRY gene marker to identify sex in the faecal groups was used. Since the SRY gene is exclusive to males, it generates enough information with a single pair of PCR primers. Lindsay & Belant (2008) observed that in white-tailed deer, with only the amplification of this gene, the sex of individuals can be identified from DNA in faeces. The lack of PCR product for the SRY gene marker is not the result of a failed reaction, but rather the absence of the Y chromosome (Huber *et al*., 2002).

In this study, sex classification by faecal pellet morphometry was successful only with adult faecal groups collected in winter. The classification functions were able to correctly classify a high percentage of the adult faecal groups into sex by using morphometric variables from the faecal pellets. However, the neural network classification function was more accurate than the fuzzy logic classification function. In the classification performed by Sánchez-Rojas *et al*. (2004) for mule deer using mean stepwise discriminant analysis with the faecal pellet morphometry variables, adult males were correctly assigned in 100% of the cases, and adult females were assigned in 91.66%. By using fuzzy logic, they were able to classify 100% of the males and 75% of the females. MacCracken & Van Ballenberghe (1987) successfully identified the sex of moose faecal groups using a stepwise discriminant analysis in 92% of the males and 91% of the females. Finally, Yu-Chun *et al.* (2008) correctly classified faecal groups from eld's deer (*Cervus eldi hainanus*) in 76.17% of males and 42.22% of females by discriminant analysis; also, they classified 19.49% of males and 20.02% of females by fuzzy analysis.

The variables that mostly contributed to the differentiation of the winter faecal groups of adult females and males collected with neural networks, in order of importance, were width, radius, length, length/width, and volume. Regarding the fuzzy logic analysis, the most critical variables to determine sex in the faecal groups were width and the length/width; and the less important variables were radius, length, and length/width. MacCracken & Van Ballenberghe (1987) classified the sex of moose by faecal morphometry with the variables volume and width-width$_2$ by using a stepwise discriminant analysis. Sánchez-Rojas *et al*. (2004) found that the best variables to classify sex in mule deer were width and volume in faeces by fuzzy logic and discriminant analysis. Morden *et al*. (2011) classified sex in reindeer with the combination of variables width, length, and depth in faeces using a discriminant analysis.

Differences between females and males were found only in the width, radius, and length/width variables in winter for adults were found in this study. Likewise, Sánchez-Rojas *et al*. (2004) found differences between age and sex only in the width and volume variables in the faecal pellets from mule deer in all the seasons they sampled. The morphometric method is based on the assumption that the measures of the faecal pellets depend on the body size of the individual, and this, in turn, depends on age and sex (Camargo-Sanabria & Mandujano, 2009). MacCracken & Van Ballenberghe (1987) suggested that the pellet morphometry technique would work best in species that exhibit strong sexual dimorphism. White-tailed deer is a dimorphic species; this dimorphism could be more evident in winter, since this is when the breeding season occurs and the production of testosterone increases (Clemente-Sánchez *et al*., 2015). Males have well-developed antlers, flared necks, and an accumulation of body fat to maintain themselves during the winter and breeding season (Dostaler, Quillet, Therrien & Côte, 2011; Galindo-Leal & Weber, 1998). Sánchez-Rojas *et al*. (2004) attributed the success of their sex classification in the faecal groups of mule deer to the high degree of sexual dimorphism in the species. Since the faecal pellet morphometry technique has shown good results in species with marked sexual dimorphism, Camargo-Sanabria & Mandujano (2009) suggested that faecal pellet morphometry was not successful in their study, because white-tailed deer living in the tropical areas shows less sexual dimorphism than those in the north of Mexico, and they did not consider the seasons of the year or fawns in their classification function.

Additionally, seasonal vegetation changes in North America are well known. In Northern Mexico, in the summer or rainy season, the available vegetation becomes more abundant, and its quality increases. Meanwhile, in the dry season or winter, the number of forage species available decreases, and the quality of the plant species is reduced (Dostaler *et al*. 2011). Barboza & Bowyer (2000) mentioned that increases in the size of the digestive tract are associated with seasonal vegetation changes in female white-tailed deer and with pregnancy and lactation in domestic ruminants. However, the digestive morphology may not change in males of dimorphic species. These vegetation changes are reflected directly in the defecation rate and faecal pellet size (Ball, 2010; Clemente *et al*., 2005; Tolleson *et al*., 2005). Alvarez (1994) found seasonal differences in the morphology of the pellets of red deer (*Cervus elaphus*) and fallow deer (*Dama dama*). Morden *et al*. (2011) also found these differences between seasons, comparing the dimensions of the faecal pellets of reindeer. Camargo-Sanabria & Mandujano (2009) commented that faecal pellets in summer can be larger and present considerably more variation in their morphometry due to the change in diet resulting from the change in vegetation. In this study, faeces of the adults in the summer had the largest volume and width (table 2). With respect to the coefficients of variation, all the morphometric variables ranged from 9% to 35% (table 2); meanwhile, Sánchez-Rojas *et al*. (2004) obtained CV of 5% to 23%. This indicated small or medium variance between the individual pellet groups. It was observed that, as the age increased, the CV was higher. The morphometric variable with the highest CV value in this study was volume. Sánchez-Rojas *et al*. (2004) found highest CV values in the same variable.

Regarding age groups, the average of the morphometric variables was always higher in the adults than in the yearlings and fawns. This assumption agrees with the findings by MacCracken & Van

Ballenberghe (1987) for moose, Camargo-Sanabria & Mandujano (2009) for white-tailed deer, Morden *et al*. (2011) for reindeer, Woodruff *et al*. (2016) for Sonoran pronghorn, and Hanya *et al*. (2017) for sika deer. Sánchez-Rojas *et al*. (2004) found that in mule deer, the faecal pellets belonging to the yearlings were more rounded, and the length/width proportion was lower in the yearlings than in the adults, but no significant differences were observed. It was found in this work that, as age increased, the length/width proportion decreased, and the faecal pellets became more rounded in the males than in the females (table 2).

Conclusions

Based on the statistical analysis results, differences between females and males in faecal morphometric variables were found only in faeces from adults collected in winter. This was caused due to the influence of the season over vegetation and the changes in diet, at the same time, over faecal pellets size mostly in the summer. Moreover, the pellet size was influenced for body size given by the age and sex of the animals. Then, the technique of faecal pellet morphometry to classify sex in faecal groups could be applied in adults surveyed in winter. Both classification functions, neural network and fuzzy logic, showed high percentage of successfully classification of faecal groups. However, the neural network function was even more accurate (94.4%) than the fuzzy logic (86.9%). Neural network sex classification function can serve as a tool to identify sex from faecal groups without disturbing or endangering the populations of white-tailed deer.

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