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Chromosomal Studies on Rabbits from Ilorin, Nigeria

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ABSTRACT: Knowledge of the chromosome morphology of a species is valuable in detecting chromosomal abnormalities, taxonomy and evolution. Despite this, chromosomal studies on rabbits is scarce in Nigeria. This study presents a description of the chromosome morphology of *Oryctolagus cuniculus* from Ilorin, Nigeria. Two male and one female samples received intraperitoneally 0.01% colchicine (1ml/100g body weight). The chromosomes were investigated by examining the metaphase chromosome spreads obtained from the bone marrow of the femur and humerus. 60 metaphase spreads were examined and the diploid number of chromosomes obtained ranged from 42-44 with percentage occurrence of 67.5% for 2n = 44. The karyotypic formula was found to be 2n = 24m + 2sm + 18t. Chromosomal aberrations were not observed during scoring. These results contribute to the karyotypic data on *O. cuniculus.*

Keywords: *Oryctolagus cuniculus*, rabbits, chromosome morphology, metaphase, Nigeria

Introduction

Oryctolagus cuniculus, the only species in its genus, belongs to the family Leporidae and order Lagomorpha (Wilson and Reeder, 1993). All domestic rabbits descended from O. cuniculus. Originally, O. cuniculus was restricted to the Iberian Peninsula, the Mediterranean area of France and northwest Africa but now is widely distributed across in all continents (except Antarctic) and over 800 islands (Parker, 1990; Flux and Fullagar 1992; Wilson and Reeder, 1993; Callou 1995). Its successful geographical expansion has been attributed to the independent processes of translocation of wild rabbits and domestication (Zeuner, 1963; Ferrand and Bran, 2007). O cuniculus is present in Nigeria in the wild (ISSG, 2011). In sub-Saharan Africa, Nigeria is a major producer of domesticated rabbits (FAO, 1997) where it is reared for meat.

The routine determination of the chromosome morphology of rabbits is of considerable interest as the karyotype could be utilized as a tool for identification. Also, knowledge of the karyotype is valuable in studies of detecting chromosomal abnormalities. Chromosome studies have great significance in taxonomy and evolution (Schroder and Loo, 1979). There is great diversity in the karyotypes of rabbit species, even within the same genus. Therefore, studies on the chromosomes of rabbit species will shed light on the phylogeny of this group considering lack of morphological differentiation during their evolution (Schroder and Loo, 1979).

Following the initial determination of the chromosome of *O. cuniculus* by Painter (1926), several authors have carried further studies on chromosomes of rabbit. Sarkar *et al.* (1962) undertook the study of the karyology of *O. cuniculus* from corneal tissues. Using three different banding techniques, Hageltorn and Gustavsson (1979) described the chromosome morphology of *O. cuniculus*. By applying lymphocyte culture technique, Paul *et al* (2004) investigated the karyotype of New Zealand white rabbits. Arslan (2010) examined the karyotype *Lepus europaeus* from Turkey using the bone marrows of colchicine-treated animals.

In Nigeria, several works have been carried out on domestic rabbits covering areas such as their parasitic condition (Biu and Nwosu, 1998; Ola-Fadunsin *et al*., 2018; 2019), breeding and production (Mailafia *et al*., 2010; Baruwa, 2014; Oluwatusin, 2014) and genetic variation (Omotoso *et al*., 2019). Despite the importance of

chromosome morphology, such information *O. cuniculus* is scarce in Nigeria. In this present study, we described the chromosome morphology of *O. cuniculus* from Ilorin, Nigeria.

Materials and Methods

Sampling: The rabbits (*O. cuniculus*) were caught from the bush at Ita-Elepa, Asa Dam road, Ilorin, Kwara State, Nigeria (Latitude 8.45°N and Longitude 4.57°E). Two male and one female (Plate 1) were caught using an improvised metallic cage-like trap and brought to the laboratory of Department of Zoology, University of Ilorin, Nigeria for chromosomal preparation. The specimens weighed between 100 and 150 g.

Plate 1: *Oryctolagus cuniculus* collected from Ita-Elepa, Ilorin.

Chromosomal preparation: Chromosome preparation was done according to Baker *et al*. (1982). Each specimen was injected intraperitoneally with 0**.**01% of colchicine (1ml/100g body weight). The colchicine was used to arrest cell division at metaphase stage. Prior to this, the rabbit was injected with 3ml of yeast concentration and was allowed to stay for two days before administering colchicine (Lee and Elder, 1980). The rabbits were then sacrificed after 2 hours by cervical dislocation with the humerus and femur bones taken out. The cells in the bone marrow of the femur and humerus were flushed using hypotonic solution of 0.56% KCl. This helped to increase the volume of the cells. Using a Pasteur pipette, the tissues and solutions were transferred into a centrifuge tube and incubated by holding the tube in the hands for 45 minutes.

The cell suspension was then centrifuged for 2 minutes at 1500 rpm. After that, the supernatant was removed. Fixation was carried out by adding a cold mixture of freshly prepared Carnoy's fixative (3:1 methanol: acetic acid v/v) solution. This helped to preserve the internal structure of the cells. Thereafter, it was centrifuged again for 2 minutes at 1500 rpm and the supernatant was removed. Refixation was then carried out twice as above. The cell suspension was spread by a Pasteur pipette on clean slides held vertically on a paper and the excess was allowed to dry off. Six slides were prepared for each specimen.

The slides were stained in 2% Giemsa stain and left for 10 minutes. The stained slides were gently rinsed over a sink with distilled water and air-dried and viewed under light microscope with a magnification of x1000. For each bone marrow cells, 5 good metaphase spreads were studied and analyzed with the images of the good spreads taken with an 8 megapixel camera for clear observation of the chromosomes.

Data analysis: Chromosomes were classified according to the nomenclature of Levan *et al.* (1964) into four classes; metacentric, submetacentric, acrocentric and telocentric. Metacentric chromosomes have the centromere in the center, such that both sections are of equal length. The arm ratio is between 1.0 and 1.7. Submetacentric chromosomes have the centromere slightly offset from the center leading to a slight asymmetry in the length of the two sections. The arm ratio is between 1.7 and 3.0. Acrocentric chromosomes have a centromere which is severely offset from the center leading to one very long and one very short section. The arm ratio is 7.0 and above. Telocentric chromosomes have the centromere at the very end of the chromosome. The arm ratio is ∞. KaroType was used to determine the length of chromosomal arms and idiogram (Altınordu *et al*., 2016).

Results

Sixty chromosome metaphase spreads for *O. cuniculus* were examined. The metaphase chromosomes obtained from the bone marrow cells are shown in Figures 1 and 2.
Table 1 presents the percentage occurrence of each diploid number of chromosomes obtained from bone marrow cells with 2n=44 having 65%. The idiogram and arm ratio and types of centromeres of *O. cuniculus* are represented in Figure 3 and Table 2 respectively.
A karyotypic formula of 2n = 24m + 2sm + 18t was obtained. The sex chromosomes could not be distinguished.

Table 1: Percentage occurrence of diploid chromosome numbers of bone marrow cells of *Oryctolagus cuniculus* from Ilorin, Nigeria

Specimen	**2n=42**	**2n=43**	**2n=44**
Male 1	3	5	12
Male 2	5	-	15
Female	8	-	12
Total	16	5	39
Percentage	26.7%	8.3%	65%

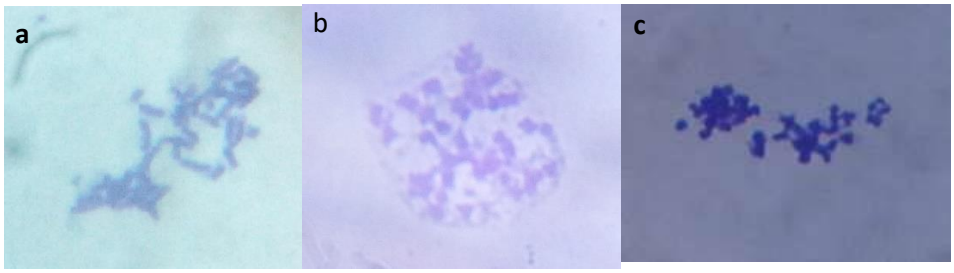

Figure 1: Metaphase chromosomes of *Oryctolagus cuniculus* from Ilorin, Nigeria. 1a and 1b: Male; 1c: Female.

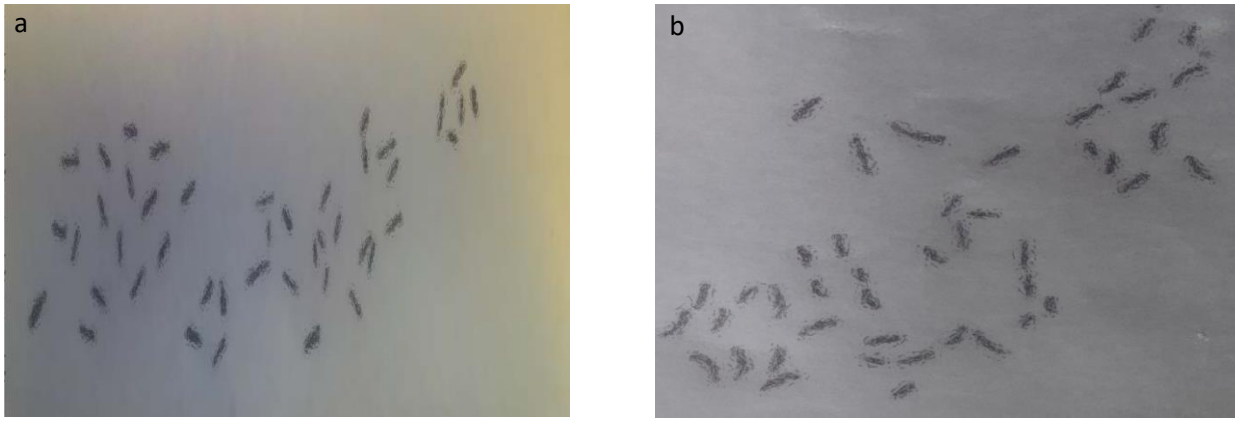

Figure 2: Metaphase chromosomes of *Oryctolagus cuniculus* from Ilorin, Nigeria (Traced). 1a: Female; 1b: Male

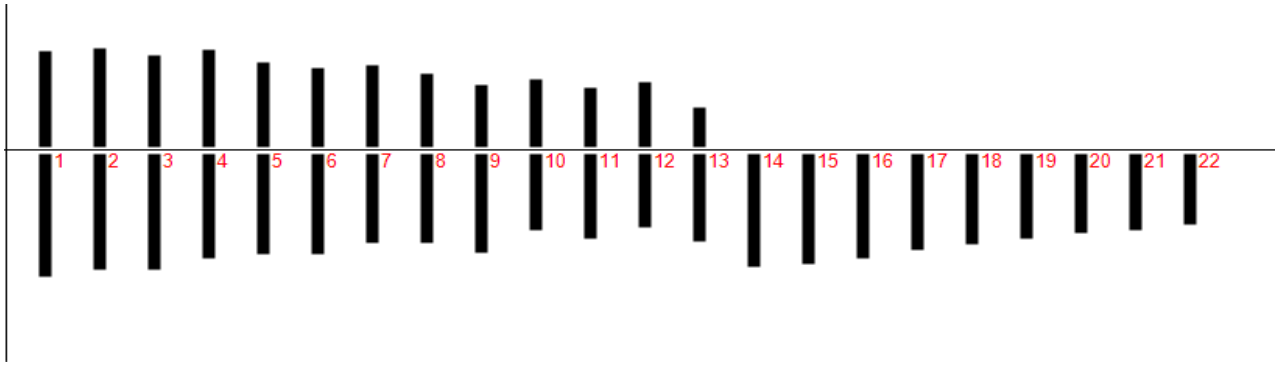

Figure 3: An idiogram of *Oryctolagus cuniculus* constructed on the basis of chromosome numbers and the position of centromere

Table 2: Arm ratios and types of centromeres of *Oryctolagus cuniculus*

Chromosome number	Long arm (L) %	Short arm (S) %	L+S	Arm ratio (L/S)	Type of chromosome
1	3.98	3.13	7.11	1.27	M
2	3.74	3.24	6.98	1.15	M
3	3.8	2.97	6.77	1.28	M
4	3.36	3.16	6.52	1.07	M
5	3.26	2.75	6.01	1.19	M
6	3.24	2.55	5.79	1.27	M
7	2.89	2.66	5.55	1.09	M
8	2.88	2.38	5.26	1.21	M
9	3.22	2.03	5.25	1.59	M
10	2.47	2.21	4.68	1.12	M
11	2.76	1.9	4.67	1.45	M
12	2.4	2.15	4.55	1.12	M
13	2.83	1.27	4.10	2.24	Sm
14	3.67	0	3.67	∞	T
15	3.56	0	3.56	∞	T
16	3.34	0	3.34	∞	T
17	3.1	0	3.10	∞	T
18	2.96	0	2.96	∞	T
19	2.76	0	2.76	∞	T
20	2.59	0	2.59	∞	T
21	2.48	0	2.48	∞	T
22	2.3	0	2.30	∞	T

Discussion

The metaphase chromosomes of *O. cuniculus* were prepared from the bone marrow cells gotten of the femur and humerus. The bone marrow cells gave good metaphase spread. Previous studies have indicated the suitability of chromosomal preparation from bone marrows. Arslan (2010) obtained good metaphase preparations from the bone marrows in *Lepus europaeus* in Turkey. Using the bone marrow is advantageous in saving time and elaborate materials need in culturing leucocytes obtained from blood.

In the present study, a modal diploid number of *O. cuniculus* gotten from Kwara State, Nigeria, was found to be 2n=44. Similarly, Ray and Williams (1966) using leucocyte culture in White New Zealand rabbits *(O. cuniculus*), reported a diploid chromosome number of 44. Sarkar *et al* (1962) determined a chromosome number of 44 from the cells of the cornea and lungs of the same specimens of rabbits. From New Zealand white rabbits, a diploid chromosome complement of 44 was observed by Paul *et al.* (2004).

The chromosomes of *O. cuniculus* were metacentric, submetacentric and telocentric following this karyotypic formula of 2n = 24m + 2sm + 18t. There has been discrepancies in the karyotypic formula of *O. cuniculus*. For example, Paul *et al*. (2004) observed that chromosomes 1 to 6 were metacentric, 7 to 11 submetacentric, 12 to 17 subtelocentric and 18 to 21 acrocentric. Ray and Williams (1966) arranged the chromosomes of *O. cuniculus* into seven groups based on morphological similarities. According to them, chromosomes 1 – 4 are large metacentrics and submetacentrics, 5-8 are large with subterminal centromere, 9 – 10 medium-sized with subterminal centromere, 11 –15 medium-sized submetacentrics, 16 – 17 medium-sized acrocentrics, 18 – 19 small metacentrics and 20 – 21 Small acrocentrics with distinctly visible satellites.

These discrepancies could be attributed to technical differences in chromosome morphological description and arrangement of diploid pairs (Hageltorn and Gustavsson, 1979 and Arslan, 2010) or indicate possible

geographic diversity in the karyotype of *O. cuniculus* as similarly observed by Arslan (2010) in *Lepus europaeus*.

The sex chromosomes were not identifiable. Melander (1956), in the same vein, could not distinguish the sex chromosomes in the work conducted. This might have been due to clumping nature of rabbit chromosomes. We recommend the use of molecular cytogenetic tools for accurate morphological description of the chromosomes of *O. cuniculus*.

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