CYTOGENETIC STUDY OF FOUR SPECIES OF LAND SNAILS OF THE FAMILY Achatinidae in South-Western Nigeria

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ABSTRACT

The chromosomal study of the four species of achatinid snails was carried out with the aim of determining their chromosome numbers as part of a preliminary attempt to understand the cytogenetics of land snails of Nigeria. The haploid chromosomes of various species of snails studied were obtained from their ovotestis while the meiotic metaphase chromosome of the four snail species were x= 28, x= 30, x= 27 and x= 24 for *Archachatina marginata* (Swanson, 1821) *Achatina achatina* (Linnaeus, 1758), *Achatina fulica* (Bowdich, 1822) and *Archachatina papyracea* (Pfeiffer, 1845) respectively. The meiotic metaphase chromosome numbers observed in species studied fell within the range reported for haploid chromosome numbers of the Stylommatophoran i.e. x=17 to x= 34. From this study, it is clear that chromosome number alone cannot be used to distinguish gastropod species but further studies employing banding techniques to study the karyotypes may provide better diagnostic criterion for separating the species.

Key Words: Cytogenetics, Achatinidae, Chromosomes, Taxonomy.

INTRODUCTION

Cytogenetics has been established as a significant tool which often provides relevant data that are used in taxonomy, identification of species and in understanding the mechanisms of speciation and evolution (Burch, 1968). Several rearrangements of the systematics of pulmonates have proved that their evolutionary relationships are largely unresolved (Solem, 1984). It is believed that cytogenetic studies may contribute valuable systematic characters which may be used to resolve some taxonomic problems in the group. However, literatures on karyotype analysis of molluscs are not abundant due to difficulties of obtaining mitotic fields with enough quality to carry out chromosome studies (Park *et al*., 1999).

There is a dearth of cytological information of the species of land snails in Nigeria. Until recently, two species of land snails *Archachatina marginata* and *Achatina* species have been described among the poorly understood members of the Achatinid snails in Nigeria with respect to their chromosomal cytology (Fagbuaro *et al*., 2002). The different species of snails belonging to the family Achatinidae are traditionally distinguished on the basis of the structure of the shell and reproductive anatomy (Bequaert, 1950; Mead,1950) while other workers had used banding and molecular methods more recently to characterize and separate different species of snails (Vitturi *et al*.,2005; Nomoto *et al*., 2001).

Skuza *et al*., 2009 reported that chromosomal knowledge is increasingly recognized as an important force in animal evolution. The purpose of this study, therefore, was to determine the chromosome numbers of four species of the family Achatinidae namely *Archachatina marginata, Archachatina papyracea, Achatina achatina* and *Achatina fulica*.

MATERIALS AND METHODS

Sampling Sites and Sample Collection:

Snail specimens used for this study were obtained from National Centre for Genetic Resources (NACGRAB), Ibadan, Oyo State with coordinates 07°23"N 003°54"E. The identification of the land snails was carried out according to Bequaert (1950) and Mead (1995).

Chromosome Preparation:

The snails were injected with 0.5ml of 0.075M KCl hypotonic solution that causes the cell to swell; this process spreads out the chromosomes so that they can be readily observed. Two hours later the snails were injected with 0.2ml of 0.02% colchicine to arrest the cells at metaphase.

Shells of adults of *Archachatina marginata*, *Achatina achatina, Achatina fulica, and Archachatina papyracea* were broken at the apex with the aid of an iron rod and the digestive gland was located to identify the ovotestes. Each ovotestis was removed and then put in specimen bottles containing freshly prepared fixative (3:1methanol: acetic acid) solution and left for 24 hours. Small pieces of ovotestis were cut with sharp razor blade and put in a mortar to which two drops of 40% acetic acid were added. The pieces of ovotestis were teased apart with fine mounted needles to enhance the

fixing of the cells. After this, one or two drops of the cell suspension were placed on pre-warmed slides and allowed to dry for an hour. The slides were then stained in formic acid-lactic acid-propionic acid (FLP) orcein for 75 minutes and rinsed several times under tap water to remove excess stain. The slides were left on a slide warmer overnight to dry after which they were examined under X10, X40 and X100 objectives of the microscope (Olympus model). Twenty slides were examined for each of the species following the method of Fagbuaro *et al.* (2002). Cells that were adjudged to contain well spread metaphase chromosomes were eye karyotyped and then photographed. The photography was done under oil immersion using a photo microscope (PW-BK5000T, PROWAY OPTICS, CHINA). The eye-karyotyping made it possible to identify homologous pairs more accurately and to differentiate artefacts, which could be easily confused with small chromosomes on the photograph.

RESULTS

Table 1 shows the number of cell spreads examined, the range of haploid numbers observed, the modal haploid and diploid chromosome numbers. Representative spreads for the species are shown in Plate 1. The diploid chromosome number for the species under study ranged from 2x = 44 to 2x = 60. A haploid chromosome complement of x = 28 (2x = 56) was found for *Archachatina marginata*. The chromosomes comprised metacentric and acrocentric. In *Achatina achatina* the haploid chromosome number was x = 30 (2x = 60). All the chromosomes were acrocentric. In both species sex chromosomes and polyploid cells were not observed. The diploid chromosome number of *Achatina fulica* was 2x = 56 from a haploid set of x = 28. The haploid sets of chromosomes from *Archachatina papyracea* were found to be 22 (2x = 44).

Table 1: The Number of Metaphase Cells, the Range of Haploid, and the Modal Haploid and Diploid Chromosome Numbers of Various Species Studied

Species	Number of Spreads	Range of Haploid Number	Modal Haploid Number	Modal Diploid Number
Archachatina marginata	200	22 - 28	28	56
Achatina achatina	195	22 - 28	28	56
Achatina fulica	205	20 - 26	26	54
Archachatina papyracea	200	20 - 22	22	44

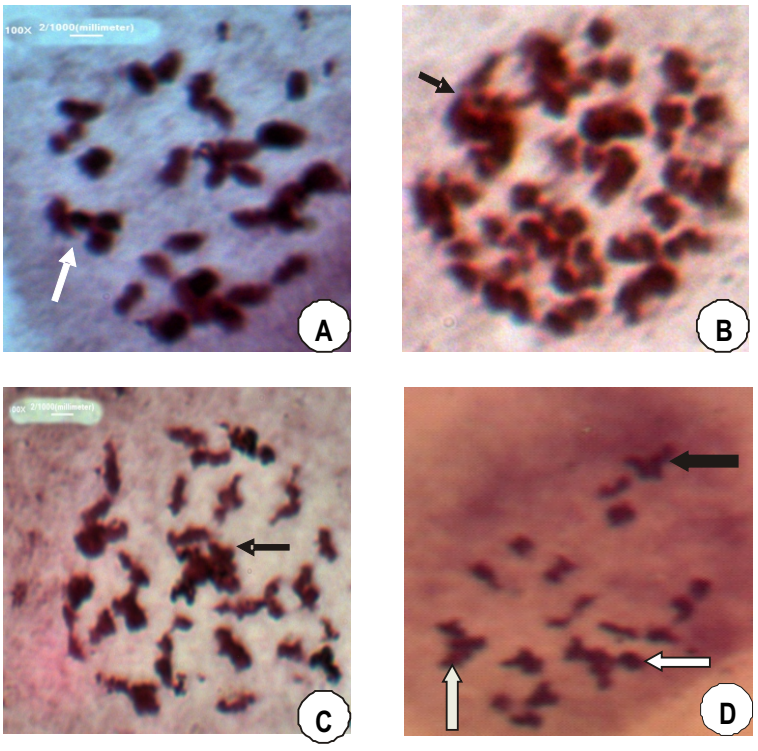

Plate 1 : Meiotic Metaphase Chromosome Spreads of the Species Studied.

A. *Archachatina margi*nata (2n = 56). The arrow shows a cluster of 4 bivalents

B. *Achatina achatina* (2n = 60). The arrow shows a cluster of 3 bivalents

C. *Archachatina papyracea* (2n = 44). The black arrow shows two chromosomes while each white arrow shows three chromosomes.

D. *Achatina fulica* (2n = 54). The arrow shows a cluster of 3 bivalents as in C.

DISCUSSION

Burch and Heard (1962) had reported that the haploid (n) chromosome number of eight species of land snails ranged between x = 17 to x = 34 while Burch (1964) reported that nearly all the stylommatophoran "pulmonates" had twenty or more pairs of chromosomes. Generally, in molluscs, the chromosome numbers vary from 5 pairs found in the land snail *Catinella rotundata* (Goud) of Hawaii (United States of America) to the 60 pairs recorded in the European, *Ancylus fluviatilis* (Mull) and the 72 pairs detected in the Ethiopian freshwater planorbid *Bulinus octoploidus* (Burch,1964). The results from this study showed a haploid (n) chromosome numbers of x = 28, x = 30, x = 28 and x = 22 in *Archachatina marginata, Achatina achatina, Achatina fulica* and *Archachatina papyracea* respectively.

The observed haploid chromosome numbers in this study suggested that the karyotypes were conservative as the haploid numbers of various species were constant in all the observed cells and there was also consistent uniformity in the morphology of chromosomes of the species studied. Most of the chromosomes of *Archachatina marginata and Achatina achatina* were large, maximally contracted and were made of metacentrics and acrocentrics while those of *Achatina sp.* were smaller and more of acrocentric types. The chromosomes of *Archachatina papyracea* were largely acrocentric. In all the species, sex chromosomes were not observed and polyploid cells were not found. The haploid chromosome number of *Achatina achatina* (x = 30) observed in this study was found to be different from the one determined by Fagbuaro *et al. (*2002) i.e. x = 22.

From this study it is clear that chromosome number alone cannot be used to distinguish gastropod species but further studies employing banding and molecular techniques may provide better diagnostic criteria for separating the species.

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