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Shell banding pattern and chromosomal studies on the giant African snail, *Achatina fulica* (Bowdich) (Achatinidae: Gastropoda)

KEYWORDS: *Achatina fulica*, shell banding, chirality, chromosome.

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ABSTRACT *A pioneer report on the shell banding, chirality (shell coiling) and chromosomal studies on populations of the giant African snail, Achatina fulica (Bowdich) (Achatinidae: Gastropoda) occurring in Bengaluru region is detailed in this paper. Shell coiling in A. fulica was found to be dextral and significant variation in banding pattern within and between populations was observed. The chromosome number from well spread diakinesis and metaphase stages of meiosis in ovotestis is found to be n=30 and 2n=60, which is similar to earlier works, also the similarity in different stages is in agreement with the earlier reports.*

INTRODUCTION

The present paper deals with the shell banding, chirality and chromosomal studies on the giant African snail, *Achatina fulica* (Bowdich) (Achatinidae: Gastropoda). Shell markings of Achatinid land snails are in the form of streaks that run across the whorls and in common with many other pulmonates, the snails are often polymorphic for shell color and pattern. The presence of streaks on shell surface of *A. fulica* and *A.sylvatica* is associated with a dominant allele such that homozygous recessive individuals have unstreaked shells (Clarke et al., 1978; Allen, 1983, 1985). Unbanded/unstreaked strains of *A. fulica* are reported in Africa. Earlier studies on the species in this regard, involving variation in shell morphology in terms of size, shape and colour have been largely attributed to environmental conditions (Mead, 1961, Estenbenet et al., 2006). Fontanilla (2010) suggested that *A. fulica* should be designated under the subgenus Lissachatina based on conchological and anatomical features.

The coiled body plan of pulmonate snails allows for entire body dimorphism, with individuals coiled either clockwise (dextral: D) or Counter-clockwise (Sinistral-S). Coiling direction is normally determined by the maternal genotype at a single locus with genetic dominance of either the dextral or the sinistral allele (Murray and Clarke, 1976 and Freeman and Lundelivs, 1982; Van Batenberg and Gittenberger, 1996). Eco-genetic aspects of *A. fulica* occurring in the region are unavailable. Studies on chirality, shell banding pattern and chromosomal number of the snail were analyzed during the present work, to understand the impact of local environment and to ascertain the taxonomic status of the snail to the subspecies level.

Chromosomal studies of gastropod mollusc have increased since the reviews of Patterson (1969) and Thiriot-Quievreux (2003). The selection of a tissue for chromosomal preparations in molluscs is problematic because of the variation in mitotic index and presence of large number of very small chromosomes (Choudhary et al., 1992). The employment of air-drying Giemsa technique for chromosome preparation of molluscs by Prasad and Das (1978) and Goldman et al., (1980) are noteworthy. Besides the detailed studies on the chromosomes of new species, the reinvestigations of old data are warranted. Haploid chromosome numbers, n=30 of *A. fulica* in India are reported previously (Patterson, 1969; Natarajan, 1960; Choudhary and Mohapatra, 1991), but in the same species occurring in China, the haploid number is reported to be n=31 (Sun, 1995). Moreover, the karyotype of these two populations is found to be different. The population from India has a majority of metacentric chromosomes while that of China is found to possess a majority of telocentric chromosomes. Also within the Indian population there are differences in karyotypes (Natarajan, 1960; Choudhary and Mohapatra, 1991).

MATERIAL AND METHODS

Chirality

The Chirality of adult and juvenile shells as dextral (right-hand side) or sinistral (left-hand side) was based on visual examination of shells holding the apex pointing upwards and the facing the aperture.

Shell banding pattern variation

Twenty five snails were collected from the Botany Department garden, Bangalore University Campus. The living snails were scored for shell and mantle colour. The shell colour was either with merged streaks or the more typical streaks. The scoring of shell colour was made consistent by comparison with standard shells of each colour.

- Number of streaks on the first whorl
- Visual evaluation of streak intensity
- The distance between third and fourth streak on the first whorl.

Data on shell banding parameters of samples from the selected site were analyzed using ANOVA to test for significance of differences in means among samples. The significance level was set up at 5%.

Chromosomal studies

Specimens were collected from the Botany Department garden, Bangalore University, Jnana Bharathi Campus. Chromosome preparations were made by air drying technique (Venkatachaliah and Venu, 2002). Prior to dissection each living specimen was injected with 1 to 1.5 ml of 0.05% colchicine solution one previous night, by making a small puncture on the apex whorl below which is the lobed ovotestes embed-

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ded in the digestive gland. The next day of colchicine injection, the specimen was dissected and the ovotestes were dissected out for the cytological analysis. The cytological studies were conducted in the Centre for Applied Genetics, Department of Zoology, Bangalore University.

1. The ovotestes was homogenised with hypotonic solution (0.075M KCl) by thoroughly mincing until a fine cell suspension was obtained. The cells were re-suspended in 2-3 ml of hypotonic solution and left to stand for 45-60 minutes at room temperature. Then the cell suspension was centrifuged at 800-1000 rpm for 5 minutes. The supernatant liquid was discarded and a little amount of freshly prepared fixative (methanol and glacial acetic acid 3:1 v/v) was added and flushed with Pasteur pipette. Then centrifuged for 5 minutes again. The pellet was dissolved and fixed in a freshly prepared fixative. The fixative was added drop-by-drop and mixed. The cells in the fixative were allowed to stand for 10 minutes and then re-centrifuged for 2-3 times with fresh fixative, till the suspension was clear leaving about 0.5-1.0 ml of suspension to which few drops of the fixative was added making the volume to 5 ml. (The same was used for dropping and later stored for future use).
2. Using a Pasteur pipette 3-4 drops of the suspension was dropped over a pre-refrigerated clean slide held in an inclined angle. Excess liquid accumulated over the slide surface was removed by waiving and air-dried on a hot plate for a minute.
3. The air dried slides were immersed in coplin jar with 5% dilute Giemsa staining solution and stained for 30 minutes. The stained slides were rinsed briefly in distilled water and air dried over a hot plate.
4. Photomicrographs of well spread meiotic stages were taken with Orthoplan photomicroscope, Leitz model using Sony cyber-shot digital camera with 7x optical zoom and 14.1 mega pixcels.

RESULTS

Chirality

Shell coiling in *A. fulica* was found to be dextral only. Sinistral coiling of the shell is not reported in studies involving intensive field and laboratory work with hundreds of specimens of this species.

Banding pattern

Significant variation in banding pattern within and between populations was observed (Table 1, Fig. 1&2). A negative association (r=-0.088) was observed between band width and number of streaks. The banding pattern is highly variable among individuals from the same population of *A. fulica*. Colorless shells were not found or individuals (Var.albino) (Fig. 3). The variation involves the colour, the intensity, number and the width of bands. Bands darker than the background were observed during the field studies. Perhaps the intensity is directly related to shell thickness.

Table 1. Test for significance (t) for variation in streak width of first whorl.

Group	Mean	SD	t-value	df	p-value
3SW	5.34	3.89	-0.087	48	0.93^{NS}
4SW	5.44	4.20			

3SW=Third streak width, 4SW=Fourth streak width of the first whorl, ^{NS} =Not Significant.

Chromosomal studies

The chromosome number from well spread diakinesis and metaphase stages of meiosis in ovotestis (Fig. 4) , is found to be n=30 and 2n=60, which is similar to earlier works, also the similarity in different stages is in agreement with the earlier reports.

i. Leptotene stage (Fig. 5): The chromosomes appear diffused, slender thread like with stained granules, the chromomeres. They are irregularly shaped.
ii. Bouquet orientation (Fig. 6). Chromosomes have been observed, with chromosomes appearing thickened and beaded and clumped.
iii. Zygotene stage (Fig.7): The homologous chromosomes are paired with irregular outlines and beaded appearances. The chromosomes appear compact that the individual chromosomes are fairly distinguishable.
iv. Pachytene stage (Fig. 8): Short and thick paired homologues appear with beaded points of contact.
v. Diplotene stage (Fig. 9): Homologues separating and with few chiasmata are observed.
vi. Diakinesis stage (Fig. 10): The haploid number of n=30 has been confirmed with condensed and intensely stained chromosomes.
vii. Metaphase I (Fig. 11): Bivalents appear condensed and some appear as round dots from the polar view.
viii. Telophase I (Fig. 12): Chromosomes form two separate bundle and segregate separately with cytokinesis.

DISCUSSION

Chromosome number reported in the present study is in confirmation to earlier reports; further data on chromosomal banding pattern are to be carried out in addition to preliminary cytological studies to deal with detail morphology of the chromosomes. The amount of conchological data available with regard to *A.fulica* is a subject on its own merit. In spite of the fact that, many aspects of the conchological variation have been already studied, the present study is specific and first of its kind from the Indian scenario. The present ecological approach perhaps serves as a probe into the underlying phenomena like ecomorphology (ecophenotypy). These analyses need more work and larger sample sizes to fully confirm and describe the relationships of morphology to habitat preference and other life style performances.

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Fig 1. Streaked shell pattern blending with background

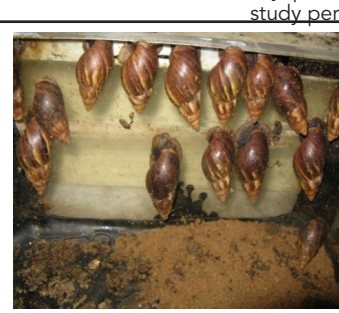

Fig 2. Variation in Streak/Banding pattern on shells.

Fig 3. *A.fulica* (albino) in a hotel in China during the first author's visit in 2008

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Fig 4. Ovotestis of *A.fulica* embedded in digestive gland.

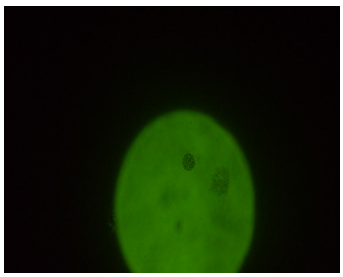

Fig 5. Leptotene

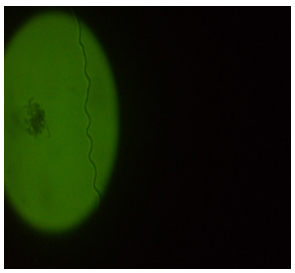

Fig 6. Bouquet Formation

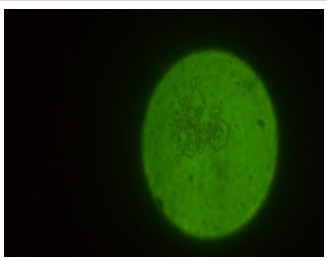

Fig 7. Zygotene

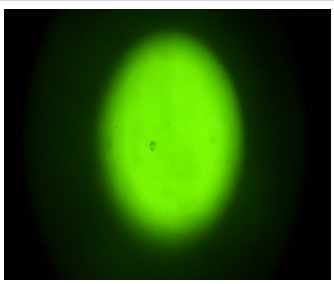

Fig 8. Pachytene

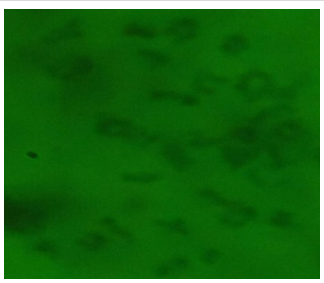

Fig 9. Diplotene

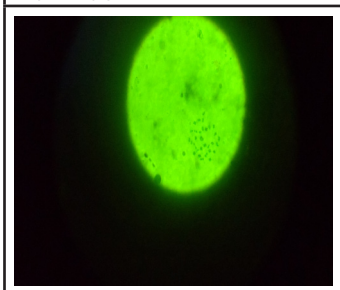

Fig 10. Diakinesis

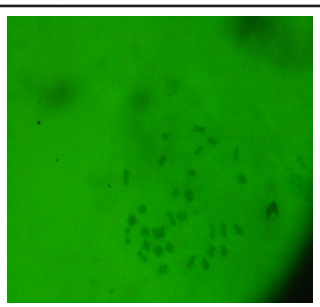

Fig 11. Metaphase I

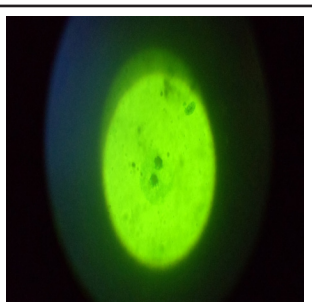

Fig 12. Telophase I

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