Chromosomal differentiations in the evolution of channid fishes – molecular genetic perspective

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Abstract — Chromosome complements, Ag-NORs and C-bands of Channa orientalis, Channa striatus and Channa punctatus (Channidae: Perciformes) were examined. C. orientalis has 2n = 52 (6 metacentrics + 20 submetacentrics + 8 subacrocentrics + 18 acrocentrics), one Ag-NOR per haploid set, 7-10 pairs of C-banded chromosomes and 26 synaptonemal complexes in pachytene nucleus. C. striatus has 2n = 40 (8 metacentrics + 10 submetacentrics + 22 acrocentrics), three Ag-NORs per haploid set, 5 pairs of C-banded chromosomes and 20 synaptonemal complexes in pachytene nucleus. C. punctatus has 2n = 32 (18 metacentrics + 14 submetacentrics), three Ag-NORs per haploid set, 6 to 12 pairs of C-banded chromosomes and 16 synaptonemal complexes in pachytene nucleus. Probable modes of chromosome differentiations and the probable effects of these differentiations on gene expression as cause of evolution of Channa species are discussed.

Key words: Channa, C-band, Silver-NOR, Synaptonemal complex

INTRODUCTION

Chromosomal differentiations help with the storage of genetic variability, with the establishment of isolating mechanisms and with protecting the gene pool against the influx of alien genes (Mayr 1969). Molecular basis of these evolution related functions of chromosomal changes can now be examined in the context of changes in gene expression brought by chromosomal changes since the effects of various genetic and epigenetic changes on gene expression are fairly understood. Robertsonian rearrangements, pericentric inversion and polyplody have been implicated for evolution of the Channid fishes (Rishi and Haobam 1990). These chromosomal changes are, however, inadequate to explain differentiation of these species because some of these species have similar chromosome number and closely similar chromosome morphology (Rishi and Haobam 1990; Nbfgr 1998). Moreover, karyotype evolution in fishes through pericentric inversion would require long-drawn cytological adaptations operated for long many years and through Robertsonian rearrangements would lead to too many complicated steps (Manna and Prasad 1977). It is important to examine other chromosomal changes than implicated changes mentioned above to gain more insight into the relationship between the chromosomal differentiation and evolution of Channa species as objective of this study and to understand evolution of fishes in general. We have examined chromosome complements and Ag-NOR in somatic and germ cells, and C-bands in somatic cells of Channa orientalis, C. striatus and C. punctatus and have observed differentiations in all the three chromosomal features. The recent knowledge of the effects of genetic and epigenetic changes on gene expression are discussed with respect to the observed chromosomal differentiations in the three Channa species as probable cause of their evolution.

MATERIALS AND METHODS

Channa orientalis, C. striatus and C. punctatus that were caught from various lakes of Manipur were purchased from fish sellers. The specimens were identified based on the morphological characters described by Nelson (1976). Mitotic metaphases were prepared from kidney cells of fishes of each species (3 males + 2 females). The fishes were given ip injection of 0.5% colchicine at the rate of 1 ml/100 gm bw. Slides were stained with Giemsa for chromosome morphology or follow-
ing the method of Sumner (1972) for C-banding or following the method of Goodpasture and Bloom (1975) for Ag-NOR. Synaptonemal complexes were prepared and stained from spermatocytes as described elsewhere (Sobita et al., 2004). Fifty selected nuclei were photographed each for chromosome morphology, C-band, Ag-NOR and Synaptonemal complexes for each species.

RESULTS

*C. orientalis.* Diploid chromosome number was 52 = 6 metacentrics + 20 submetacentrics + 8 subacrocentrics + 18 acrocentrics (Fig. 1a & b). Twenty-six synaptonemal complexes were present in each pachytene spermatocyte (Fig. 1c) corresponding to diploid chromosome number. Different numbers of chromosome expressed C-bands in different cells; in the 50 metaphases, the minimum and maximum numbers of C-band expressing chromosomes were seven and ten pairs respectively. C-bands were small and faint (Fig. 1d). One pair of Ag-NOR was present at the middle of p arm of metacentric pair No.1 (Fig. 1e). In pachytene spermatocytes, a large bivalent borne one Ag-NOR at the sub-central region (fig. 1c). Two nucleoli were present in somatic interphase nucleus indicating one Ag-NOR per haploid set (fig. 1e).

*C. striatus:* Diploid chromosome number was 40 = 8 metacentrics + 10 submetacentrics + 22 acrocentrics (fig. 2a & b). Each pachytene nucleus contained 20 synaptonemal complexes (fig. 2c). A maximum of five chromosome pairs expressed small and faint C-bands (fig. 2d). Two to six Ag-NORs were expressed in a somatic nucleus (fig. 2e). One to three Ag-NORs were present in a pachytene nucleus, two at the telomeric region of two bivalents and one at the middle of a bivalent (fig. 2c). The Ag-NOR bearing chromosomes were the largest metacentric No.1 and two medium acrocentrics.

*C. punctatus:* Diploid chromosome number was 32 = 18 metacentrics + 14 submetacentrics (fig. 3a & b). Each pachytene nucleus contained 16 synaptonemal complexes (fig. 3c). One or two bivalents with large Ag-NORs at terminal or sub-central region were present in a pachytene nucleus (fig. 3c, d & e) accounting for three pairs of Ag-NORs in a pachytene nucleus. In most of the somatic cells, a submetacentric chromosome pair borne strongly silver- positive NORs at the centromeric region (fig 3f). In other cells, a weakly silver-positive NOR was present at telomere of one to six acrocentric chromosomes (fig. 3g). Number

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![Fig. 1a & b. — Mitotic metaphase and karyotype from kidney cells of *C. orientalis.* Fig.1c. Pachytene nucleus with 26 synaptonemal complexes from spermatocytes of *C. orientalis* with one Ag-NOR in a bivalent (arrow). Fig.1d. C-banded (arrow) mitotic metaphase of *C. orientalis* Fig.1e. Mitotic metaphase of *C. orientalis* with a pair of Ag-NORs (arrow). An interphase nucleus of *C. orientalis* shows two nucleoli (arrow).](image-url)
of C-banded chromosomes per cell ranged between 6 and 12 (fig. 3h). The C-bands were large but faint.

**DISCUSSION**

Chromosome complements of *C. orientalis*, *C. striatus* and *C. punctatus* from different parts of India (Rishi and Haobam 1990; NBFGR 1998) are very similar with the chromosome complements of these species observed in the present study. The three species have different numbers of metacentrics, submetacentrics, subacrocentrics and acrocentrics. These chromosome differentiations can be most plausibly explained, though not cytogenetically proven, by polyploidy, Robertsonian rearrangements and pericentric inversions of acrocentric chromosomes of primitive fishes (Nogusa 1960; Post 1965; Nayyar 1966) as already postulated by (Rishi and Haobam 1990).

A reference to the karyotypes of five *Channa* species so far analyzed (Table 1) indicates that species with large number of chromosomes have higher number of acrocentric chromosomes. This observation supports the hypothesis of origin of biarmed chromosomes from acrocentric counterparts in *Channa* species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Karyotype</th>
<th>Reference</th>
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<tbody>
<tr>
<td><em>C. gachua</em></td>
<td>2n = 78 = 10m + 8s + 8st + 52t = 12m + 12 sm + 4st + 50t</td>
<td>NBFGR (1998)</td>
</tr>
<tr>
<td><em>C. orientalis</em></td>
<td>2n = 52 = 6m + 20sm + 8st + 18a</td>
<td>Present study</td>
</tr>
<tr>
<td><em>C. marulius</em></td>
<td>2n = 44 = 40m + 4t</td>
<td>NBFGR (1998)</td>
</tr>
<tr>
<td><em>C. striatus</em></td>
<td>2n = 40 = 8m + 2sm + 16st + 14t = 8m + 10sm + 22a</td>
<td>Present study</td>
</tr>
<tr>
<td><em>C. punctatus</em></td>
<td>2n = 34 = 16m + 14sm + 4a = 32 = 16m + 16sm = 16m + 14m + 2a = 18m + 14m</td>
<td>NBFGR (1998)</td>
</tr>
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**Pericentric inversion in Channa species evolution** - Pericentric inversion does not change chromosome number. Chromosome polymorphism in *Channa* species due to subtelocentric and acrocentric members evinces that pericentric inversion operated in the karyotype evolution of channid fishes. Current knowledge of molecular genetics can explain the molecular basis of speciation through pericentric inversion. Chromosome rearrangements that juxtapose the pericentric heterochromatin with euchromatin produce heritable position effect variegation (Grigliatti 1991; Reuter and Spierer 1992; Weiler and Wakimoto 1995; 1998). Such rearrangements can induce the variegated expression of genes that are euchromatic as well as genes that normally reside

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Fig. 2a & b. — Mitotic metaphase and karyotype from kidney cells of *C. striatus*. Fig. 2c. Pachytene nucleus with 20 synaptonemal complexes and 3 Ag-NORs (arrow) from spermatocytes of *C. striatus*. Fig. 2d. C-banded (arrow) mitotic metaphase of *C. striatus*. Fig. 2e. Mitotic metaphase of *C. striatus* with 3 pairs of Ag-NORs.
in the heterochromatin (Review: Weiler and Wakimoto 1995). Telomere also induces long range position effect affecting transcription of a variety of different genes (Gottschling et al. 1990). Major effects of pericentric inversion of chromosomes may, therefore, be heterochromatic position effect in the long arm and telomeric position effect in the short arm. Other effects may be mutational change in gene expression and to restrict the recombination of genetic loci by crossing over in the short arm, because of gene order reversion, and thereby to maintain in the population the inverted segment of the chromosome (a set of genes) conferring a selective advantage.

Centric fusion in Channa species evolution - Centric fusion (CF) or Robertsonian (Rb) translocation, the joining of two telo/acrocentric chromosomes at their centromere to form a metacentric, reduces chromosomes number. Decrease in chromosome number with concomitant increase in metacentric number among Channa species suggests that CF occurred during chromosomal evolution in this genus. Occurrence of C. punctatus with 2n = 34 = 16m + 14sm + 4a (NBFGR 1998) and 2n = 32= 18m + 14 sm (present study) shows that the four acrocentrics changed to two metacentrics thereby corroborating the above speculation. Up to now, no molecular mechanism for CF has been experimentally demonstrated. Evidence for minor satDNA − CENP − B protein complex to be the precise molecular substrate for CF (Garagna et al. 2001) does not envisage massive chromosomal reorganization during CF events. Different methodological approaches to show DNA loses in house mouse with as many as 18 Rb chromosomes have always failed (Redt et al. 1986). Molecular basis of the acquisition of reproductive

Fig. 3 a & b. — Mitotic metaphase and karyotype from kidney cells of C. punctatus. Fig. 3 c, d & e. Pachytene nuclei with 16 synaptonemal complexes and one Ag-NOR (arrow), two Ag-NORs (arrow) and one Ag-NOR (arrow) at different positions of the bivalents from spermatoocytes of C. punctatus. Fig. 3 f & g. Mitotic metaphases of C. punctatus with 1 pair and 3 pairs of Ag-NORs (arrow). Fig. 3 h. C-banded (arrow) metaphase from kidney cell of C. punctatus.
isolation and incipient speciation through CF remains elusive.

**Differentiation of constitutive heterochromatin in Channa species evolution** - In C. orientalis, C. striatus and C. punctatus C-bands are expressed in 38%, 25% and 30% of the chromosome complements respectively. The C-bands are inconspicuous in C. orientalis and C. striatus, while they are comparatively larger and denser in C. punctatus. Differentiation of centromeric heterochromatin apparently occurred in Channa fishes although a definite proof with molecular biological techniques is needed. Increase in centromeric heterochromatin may occur in one category of karyotype evolution (Hsu and Arrighi 1971) or diminution of centromeric heterochromatin may occur in another category of karyotype evolution (Yosida 1973). Quantitative centromeric heterochromatin changes observed in three Channa species in the present study, although similar data for other Channa species are not available, could produce change in the expression of neighbouring genes as already described for pericentric inversion. C-bands apparently contain no gene (Saito and Laemmli, 1993), and hence no mutational effect is expected from quantitative C-band change. Effect of heterochromatin on expression of neighbouring genes is inheritable (Weiler and Wakimoto 1995) and hence C-band changes might contribute to evolution of Channa species.

**Differentiation of NOR in Channa species evolution** - One pair of NOR in C. orientalis and three pairs of NOR in C. striatus and C. punctatus indicate NOR division by chromosome rearrangements or DNA magnification. These mechanisms can adjust rDNA redundancy under selection pressure for a changed phenotype (Ritossa et al. 1973). This effect may be due to disturbance of transcribing sequence by the inserted NOR similar to the effect of transposon on gene expression. The ribosomal genes are organised into tandem arrays in the heterochromatin in multicellular organisms. Some regions of heterochromatin contain actively transcribed gene which require proximity to heterochromatin for their normal expression (Review: Weiler and Wakimoto 1995). Chromosome rearrangements that separate these genes from large blocks of heterochromatin result in their variegated expression. NOR differentiation might be an effective process in the evolution of Channa species.

**Centromere repositioning in Channa species evolution** - Similarity in number but dissimilarity in morphology of chromosome complements within a Channa species (table1) cannot be fully explained by pericentric inversion alone. These observations can be rather attributed to centromere repositioning via neocentromere emergence as demonstrated for primates (Review: Wong and Choo 2001). Many questions on the molecular basis of karyotype evolution leading to speciation brought by centromere repositioning, however, remain unanswered (Wong and Choo 2001).

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**REFERENCES**


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