

the experiment. The rate of infusion was selected to supply the liver with the normal rat plasma amino acid level every 15 min. Liver viability was checked by bile production and urea and glucose output in the perfusate. The oxygenation state of the liver was evaluated by the lactate/pyruvate ratio in the perfusion medium⁷. The perfusion was carried out at 37 °C for 4 h with a flow rate of 1.5 ml/g/min. When indicated, at the start of perfusion a portion of the median lobe (approximately 20% of the liver) was removed and rapidly frozen. The perfusion of lobectomized livers was continued for 2 or 4 h. At the end of perfusion all livers were washed with 30 ml of ice-cold 0.25 M sucrose, pH 7.5, and rapidly frozen. Livers rapidly removed from rats stunned and killed by decapitation were used as controls. RNA polymerase activities were determined using liver nuclei as previously described⁸. DNA was determined by the method of Burton⁹.

Results and discussion. The figure shows the time-course of RNA polymerase I and II activities in nuclei isolated from the liver portion removed at the start of perfusion and from the remaining liver perfused for 4 h. It can be seen that the 4-h perfusion of rat liver results in a net increase in the level of RNA polymerase I and II activities when compared to the polymerase levels of the liver portion removed at the start of perfusion.

The results shown in the table clearly demonstrate that the increase in polymerase activities during perfusion is not due to the initial removal of a liver portion since the RNA polymerase levels of the intact liver perfused for 4 h equal those of the initially lobectomized perfused liver. However, RNA polymerase levels in the liver portion removed at the start of perfusion are significantly below RNA polymerase levels in normal rat liver. These data could indicate that during the routine procedure for the isolation of rat liver there is a significant fall in RNA polymerase I and II activities which is followed by a rapid restoration to the level observed in control liver. As can be seen in the table, the recovery of RNA polymerase I and II activities during liver perfusion is rather rapid, appearing as early as 2 h after the start of perfusion. Moreover, this effect seems to

be related to liver viability. Actually we have observed that when liver perfusion was accompanied by any procedure which diminished the portal blood supply or elevated the lactate/pyruvate ratio in the perfusate the restoration of RNA polymerase activities did not occur.

The fall in RNA polymerase activity is concomitant with the slight but significant disaggregation of rat liver polysomes observed by others during routine surgery for the preparation of rat liver for perfusion^{3,10,11}. Nevertheless, while disaggregation of polysomes is counteracted by high levels of amino acids in the perfusing medium, we have observed that RNA polymerase restoration also takes place without amino acid addition to the perfusate (data not shown).

Clearly, the mechanism responsible for the variations of RNA polymerase activities reported above remains to be elucidated. However, the possibility that other enzyme activities may be affected in a similar fashion during rat liver isolation and perfusion cannot be excluded. In the light of this fact, when a liver sample is removed at the start of perfusion and used as a reference for the remaining tissue a comparison is being made between samples of the same liver which are in different functional states.

- 1 I. Bartosek, A. Guaitani and L.L. Miller, in: Isolated liver perfusion and its applications, p.3. Raven Press, New York 1973.
- 2 S. Watkins and M.G. Clark, Exp. Cell Res. 119, 111 (1979).
- 3 N. Fausto, Biochim. biophys. Acta 281, 543 (1972).
- 4 P.I. Christensson, G. Eriksson and U. Stenram, Cytobios 20, 199 (1978).
- 5 A. Cordone, E. Fugassa, G. Gallo and A. Voci, Boll. Soc. Biol. sper. 56, 295 (1980).
- 6 H. Schimassek and W. Gerok, Biochem. Z. 343, 407 (1965).
- 7 H. Schimassek, Life Sci. 1, 629 (1962).
- 8 A. Viarengo, A. Zoncheddu, M. Taningher and M. Orunesu, Endocrinology 97, 955 (1975).
- 9 K. Burton, Biochem. J. 62, 315 (1956).
- 10 L.S. Jefferson and A. Korner, Biochem. J. 111, 703 (1969).
- 11 E. McGown, A.G. Richardson, L.M. Henderson and P.B. Swan, J. Nutr. 103, 109 (1973).

Karyomorphology of two species of *Tor* (Pisces; Cyprinidae) with a high number of chromosomes

A.R. Khuda-Bukhsh¹

Department of Zoology, University of Kalyani, Kalyani-741235 (India), 27 January 1981

Summary. The karyotypes of *Tor khudree* and *Tor tor*, occurring in the Himalayan streams in India, are described. As reported earlier for *T. putitora*, the diploid complements in these 2 congeneric species also comprise 100 chromosomes. The modal number being $2n = 50$ in this family, *Tor* appears to be of tetraploid origin.

The occurrence of 100 chromosomes in the hillstream cyprinid *Tor putitora*² led me to examine cytologically some congeneric species of *Tor* to see if they also possessed a similarly high number of chromosomes. The present report embodies findings on the somatic chromosomes of 2 species of *Tor*, viz. *khudree* and *tor*.

Materials and methods. 7 adult specimens, 4 males and 3 females, of *Tor khudree* and 5 adult specimens, 3 males and 2 females, of *Tor tor* were captured respectively from the Tawi river off Jammu in the Jammu and Kashmir State and the Bhimtal lake in the Uttar Pradesh State in India. The colchicized specimens were processed for observation of the somatic and germinal chromosomes by employing the citrate flame-drying method described elsewhere³. The morphology of the chromosomes has been described following Levan et al.⁴. In the karyotypes (figs 1 and 2) the biarmed (meta-, submeta- and subtelo-centric=M, SM,

ST) and the rod-shaped (acrocentric=A) chromosomes have been arranged separately in decreasing order of lengths.

Results. As the preparations from kidney in *T. khudree* and gill materials in *T. tor* happened to yield better spreads, suitable for morphometrical analysis, kidney metaphase complements in the former and gill metaphase complements in the latter species were analyzed. The metaphase complements in the majority of cells examined in both *T. khudree* and *T. tor* had 100 chromosomes although the numbers varied between 96 and 104 in some others (table). Karyotypes of kidney metaphase complements of *T. khudree* (fig. 1) consisted of 50 pairs of chromosomes comprising 8 pairs of M (Nos. 5, 9, 10, 12, 13, 16, 20, 24), 14 pairs of SM (Nos. 1-4, 6-8, 11, 14, 17, 18, 21-23), 3 pairs of ST (Nos. 15, 19, 25) and 25 pairs of A (Nos. 26-50) chromosomes in both the sexes. Karyotypes of gill metaphase



Figure 1. Karyotype prepared from camera lucida drawings of a kidney metaphase plate of male *T. khudree*. M, SM, ST: metacentric, submetacentric and subtelocentric chromosomes; A: acrocentric chromosomes.



Figure 2. Karyotype prepared from a gill metaphase plate of male *T. tor*.

complements of *T. tor* (fig. 2) in both sexes contained 50 pairs of homomorphic chromosomes comprising 12 pairs of M (Nos. 5-7, 9-14, 17, 20, 26), 12 pairs of SM (Nos. 1-4, 8, 15, 18, 21-25), 3 pairs of ST (Nos. 16, 19, 27) and 23 pairs of A (Nos. 28-50) chromosomes. The chromosomes Nos. 6 and 8 in *T. khudree* and Nos. 23, 25 and 26 in *T. tor* had their centromeric indices in the borderline of 2 morphological entities. In the absence of heteromorphism in respect of any particular pair in the karyotype of either sex, and in the absence of any observable difference between karyotypes of 2 sexes, no sex element(s) could be recognized in any of the 2 species under study. The chromosomes from the longest to the shortest one measured between 2.5 μ m and 0.9 μ m in *T. khudree* and between 1.8 μ m and 0.6 μ m in *T. tor*. Unfortunately, the preparations from testis materials did not yield divisional stages of meiosis suitable for analysis, so that the haploid number could not be ascertained nor could the meiotic behaviour of chromosomes be studied in these 2 species.

Discussion. So far as the author is aware, cytological investigations on the 2 species of *Tor* under present study had not been carried out earlier. While *T. putitora* had $n=5$ M+12 SM+7 ST+26 A², *T. khudree* had $n=8$ M+14 SM+3 ST+25 A as against $n=12$ M+12 SM+3 ST+23 A in *T. tor*. This would indicate the involvement of pericentric inversions in 3 pairs of chromosomes more in *T. tor* than in *T. putitora* and 1 pair more than in *T. khudree*, apart from the difference in the lengths of chromosomes involved in pericentric inversions in the different species. Besides the genus *Tor*, 2 other cyprinid genera, *Carassius*

and *Cyprinus*, among some 56 genera cytologically studied so far⁵⁻⁹ are known to comprise species with a similarly high number of 100 ± 4 chromosomes. Ohno et al.¹⁰ studied the DNA content of *Carassius auratus* and *Cyprinus carpio* and obtained DNA values 50-52% that of placental mammals, as compared to 20-22% that of placental mammals in 2 other species of cyprinids with 50 chromosomes, the suggested modal number in this family^{8,9}. Therefore, Ohno et al.¹⁰ suggested that diploid-tetraploid relationships exist in this family. Polyploid origin was also conjectured in some catostomid fishes on the same basis¹¹. Correspondingly, tetraploid origin may also be suggested in *T. khudree* and *T. tor* as was done for *T. putitora*². This hypothesis should, however, be tested first by measurements of DNA content in the different species of *Tor*; the study of meiosis in them may provide information as to their possible allopolyploid origin.

Frequency distribution of chromosome numbers in different somatic spreads of *T. khudree* and *T. tor*

Species	Sex	Number of chromosomes									
		96	97	98	99	100	101	102	103	104	
<i>Tor khudree</i>	Male	1	1	3	5	14	2	1	-	1	
	Female	1	-	2	3	13	1	3	1	-	
<i>Tor tor</i>	Male	1	-	4	1	12	2	2	-	1	
	Female	-	1	4	2	15	3	3	-	2	

- 1 The author is greatly indebted to Prof. G.K. Manna, and Prof. A.K. Bose, Department of Zoology, University of Kalyani, for encouragements and laboratory facilities. Financial aid from the University of Kalyani is gratefully acknowledged. Sincere thanks are also due to Dr M.K. Jyoti and Miss Anu Agarwal of the Department of Biosciences, Jammu University for their cordiality and help in various ways during my stay at Jammu.
- 2 A.R. Khuda-Bukhsh, *Experientia* 36, 173 (1980).
- 3 A.R. Khuda-Bukhsh and G.K. Manna, *Indian Biol.* 8, 23 (1976).
- 4 A. Levan, K. Fredga and A.A. Sandberg, *Hereditas* 52, 201 (1964).
- 5 T.E. Denton, in: *Fish Chromosome Methodology*, p.166. Charles C. Thomas, Springfield, Illinois, 1973.
- 6 E.H. Park, *College Rev. College of Liberal Arts and Sciences, Seoul University*, 20, 346 (1974).
- 7 Y. Ojima, K. Ueno and M. Hayashi, *La Kromosomo* 11, 19 (1976).
- 8 G.K. Manna and A.R. Khuda-Bukhsh, *Nucleus* 20, 119 (1977).
- 9 G.K. Manna and A.R. Khuda-Bukhsh, *J. zool. Res.* 1, 34 (1977).
- 10 S. Ohno, J. Muramoto and L. Christian, *Chromosoma* 23, 1 (1967).
- 11 T. Uyeno and G.R. Smith, *Science* 175, 644 (1972).