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Genetic Studies on Two Species of the Indian Carp *Labeo* & Their Fertile F_1 & F_2 Hybrids

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Karyotype analysis, haemoglobin profiles and muscle and serum protein patterns of the Indian carps *Labeo rohita* and *L. calbasu* together with those of their fertile F_1 hybrids '*Calbahu*' (*L. calbasu* ♀ × *L. rohita* ♂), '*Rosu*' (*L. rohita* ♀ × *L. calbasu* ♂) and F_2 '*Calbahu*' are presented. Study of mitotic metaphase spreads from kidney tissues revealed the modal diploid chromosome number to be 2n = 50 in all fishes with NF = 78 in *L. rohita*, NF = 82 in *L. calbasu* and NF = 80 in F_1 and F_2 hybrids. There was no indication of sex chromosomes in these fishes. On electrophoresis a new hybrid haemoglobin fraction besides those found in the parent species was noticed in the F_1 hybrid fishes. Presence of this new haemoglobin fraction had been confirmed by *in vitro* dissociation—recombination studies. Segregation of parent haemoglobins was seen in the F_2 '*Calbahu*'. The parent species showed species specific muscle protein patterns, the hybrids revealing an intermediate pattern. Similar studies were carried out on serum proteins also. The usefulness of the above parameters in hybridisation studies are discussed.

HYBRIDISATION with a view to combine the beneficial qualities of two breeds is gaining importance in fish culture. The genetic constitution of the parent breeds and their progeny play an important role in such studies. Often the hybrid fishes are identified by their morphological intermediacy between the parents. However, most of these morphological features of fishes have been shown to be environmentally plastic[1]. In view of this it is important to have certain genetic markers that have minimum of environmental distortion. Utter *et al.*[2] have reviewed the potentialitis and limitations of biochemical genetic studies of fish using electrophoretic methods. Information on the cytogenetic profile with a view to detect a possible marker chromosome in the hybrids can also prove to be very useful.

A comparative study on the chromosomes, haemoglobins and muscle and serum proteins of two species of the Indian carp of the genus *Labeo*, viz. *L. rohita* and *L. calbasu* and their fertile F_1 hybrids *'Calbahu'* (*L. calbasu* ♀ × *L. rohita* ♂), *'Rosu'* (*L. rohita* ♀ × *L. calbasu* ♂) and F_2 *'Calbahu'* has been attempted with a view to evaluate the usefulness of such a study in hybridization experiments. Paucity of information on the karyologial and biochemical data on Indian carps and their hybrids, with the exception of meagre data available for limited species[3-6] makes the importance of such a study obvious.

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Materials and Methods

The parent species were obtained from the Masunda lake near Bombay. The fertile F_1 and F_2 hybrids[7] were those reared and maintained in the experimental ponds of the Central Institute of Fisheries Education, Bombay.

Mitotic chromosome preparations were made, following essentially the same method as reported earlier[8], from 11 speciemens of *L. rohita* (7 females and 4 males), 9 of *L. calbahu* (6 females and 3 males), 4 of *'Calbahu'* (all females), 9 of *'Rosu'* (5 females and 4 males) and 12 of F_2 *'Calbasu'* (7 females and 5 males). Colchicine (0.06%—0.5 ml/100 g body wt) solution was given (i.m.) to the fish 3-5 hr prior to sacrifice and left in a well aerated aquarium. Following sacrifice, kidney tissues were collected and a homogeneous suspension was made in fish saline. After hypotonic treatment with 0.56% KCl and fixation in 3 : 1 methanol-acetic acid, chromosome preparations were made by the usual air-dry technique. Measurements of chromosomes were done and the karyotypes constructed following the methods of Reitalu[9] and Levan *et al.*[10] respectively. For karyotyping, chromosomes were arranged in groups of metacentrics (M,m), submetacentrics and subtelocentrics together (sm, st) and telocentrics (T, t) all in order of decreasing length.

Electrophoresis of haemoglobins on paper, cellulose acetate and starch gel were carried out as described earlier[6].

Muscle and serum protein studies were carried out on polyacrylamide gel disc electrophoresis according to the method of Davis[11], at 4°C employing 6%

acrylamide gel for muscle, 7% for serum, Tris-glycine buffer *p*H 7.8 and 3 mA current per tube/350 V. Duration of the run was 45 min. Photoelectric scans of the electropherograms were carried out using a scanner (Densicord Elec. densitometer 552–Filter No. 590, Photovolt Corporation, New York).

Results and Discussion

Chromosome studies — The frequency distribution of chromosome number and analysis of chromosomes in the parent species and the hybrids are summarized in Table 1. Fig. 1 shows the metaphase chromosome spread and the karyotype from a female *L. rohita*. The karyotype of the diploid complement in both sexes showed 50 chromosomes, consisting of 6 pairs of metacentrics (Nos 1–6), 4 of sub-metacentrics (Nos 8, 10, 11, 14), 4 of subtelocentrics (Nos 7, 9, 12, 13) and 11 of telocentrics (Nos 15–25). The arrangement of chromosomes was arbitrary especially in the case of 'sm' and 'st' groups, since these two types were not clearly distinguishable. The mean length of the longest and shortest chromosomes from 10 metaphases ranged between 2.04 ± 0.26 μm and 0.64 ± 0.14 μm Though the maximum difference between the two adjacent chromosomes was 0.47 μm (No. 7 and 8), in the rest it was never more than 0.20 μm. As the chromosomes were gradually seriated, size grouping was difficult. The relative percentage length of different chromosomes ranged between 7.44% and 2.33%. The longest pair of subtelocentrics (No. 7) could be demarcated as the marker for this species. Fig. 2 shows the metaphase spread and karyotype from a male *L. calbasu*. Male and female karyotypes showed, 2n = 50, consisting of 6 pairs of metacentrics (Nos 1–6), 6 of submetacentrics (Nos 8, 10, 11, 12, 15, 16), 4 of subtelocentrics (Nos 7, 9, 13, 14) and 9 of telocentrics (Nos 17–25). The longest subtelocentric of *L. calbasu* (No. 7) invariably showed a pattern suggestive of secondary constriction and hence this pair could be used as the marker for this species. The mean length of the longest and shortest chromosomes ranged between 2.44 ± 0.47 and 1.01 ± 0.25 μm. Maximum difference between the two adjacent chromosomes was 0.56 μm (No. 7 and 8) while in many cases hardly any difference was found (No 13 and 14; 19 and 20). The relative percentage length of chromosomes ranged between 6.95% and 2.88%. Figs. 3, 4, 5 present the metaphase chromosome spreads and karyotypes from a female *'Calbahu'*, male *'Rosu'* and a female F_2 *'Calbahu'* respectively, in which Nos 1–15 show the metacentrics, submetacentrics and subtelocentrics grouped together and Nos 16-25 the telocentrics, in the decreasing order of length. In *'Calbahu'* the mean length of the longest and shortest chromosomes ranged between 2.63 and 0.80 μm, in *'Rosu'* between 1.74 and 0.76 μm and in F_2 *'Calbahu'* between 2.5 and 1.01 μm. Although fairly good number of samples were investigated, in all these hybrids, due to condensation of chromosomes and unsatisfactory spreads proper measurements of arm lengths were not possible.

The modal diploid chromosome number reported here as 2n = 50 in *L. rohita* and *L. calbasu* is in agreement with the one reported earlier[3]. Incidentally, most of the cyprinids studied have a diploid chromosome number of 50 chromosomes about 20 of which are telocentrics, the rest metacentrics[12]. The F_1 and F_2 hybrids also showed a modal diploid chromosome number of 2n = 50. Karyotypically, however, all these fishes are distinguishable. *L. rohita* karyotype shows 14 biarmed and 11 uniarmed chromosomes with NF = 78, while *L. calbasu* shows 16 biarmed and 9 uniarmed chromosomes with NF = 82. Both *L. calbasu* and *L. rohita* are fresh water fishes with similar geographical distribution. They show several pairs of shared chromosomes especially the long subtelocentrics. As suggested by Manna and Prasad[13], the karyotype differences between the two species are probably due to pericentric inversions and unequal interchanges.

The hybrids showed NF = 80 which was consistent with parents of NF = 78 and NF = 82. The hybrids possess 15 biarmed chromosomes and 10 uniarmed chromosomes as against 14 and 11 in *L. rohita* and 16 and 9 in *L. calbasu*. Such composite hybrid complements composed of approximately haploid set of chromosomes from each parent species have been reported in certain minnow hybrids and fundulus hybrids[14,15]. Greenfield *et al.*[14] have suggested the use of the longest parental pair as the marker chromosome in the minnow hybrids studied. Such a marker chromosome could not be unequivocally demonstrated in the hybrids under study, since the parent chomosomes did not show any marked difference in their chromosomes structure or size. However, in some of the chromosome spreads of F_2 *'Calbahu'* a subtelocentric chromosome with a structure suggestive of secondary constriction as seen in *L. calbasu* was noticed (Fig. 5). A more exhaustive study in this direction would prove to be useful.

In the present study no definite indication of sex chromosomes was observed. Although scattered

TABLE 1 — FREQUENCY DISTRIBUTION OF DIPLOID CHROMOSOME NUMBER, NF AND TOTAL MEAN LENGTH IN HAPLOID SET IN *L. rohita, L. calbasu, 'Calbahu', 'Rosu'* AND F_2 *'Calbahu'*

Species	Chromosome No. of							Cell No. scored	% of cells with 2n=50 chromosome	NF	Total mean length in haploid set
	45	46	47	48	49	50	51				
L. rohita	—	3	—	17	7	197	1	225	87.5%	78	27.43
L. calbasu	—	2	3	6	15	206	—	232	88.8%	82	35.06
'Calbahu'	1	2	1	8	3	68	—	83	81.9%	80	25.68
'Rosu'	2	—	3	5	3	89	—	102	87.2%	80	26.03
F_2 *'Calbahu'*	1	2	1	4	—	97	—	105	90.2%	80	35.10

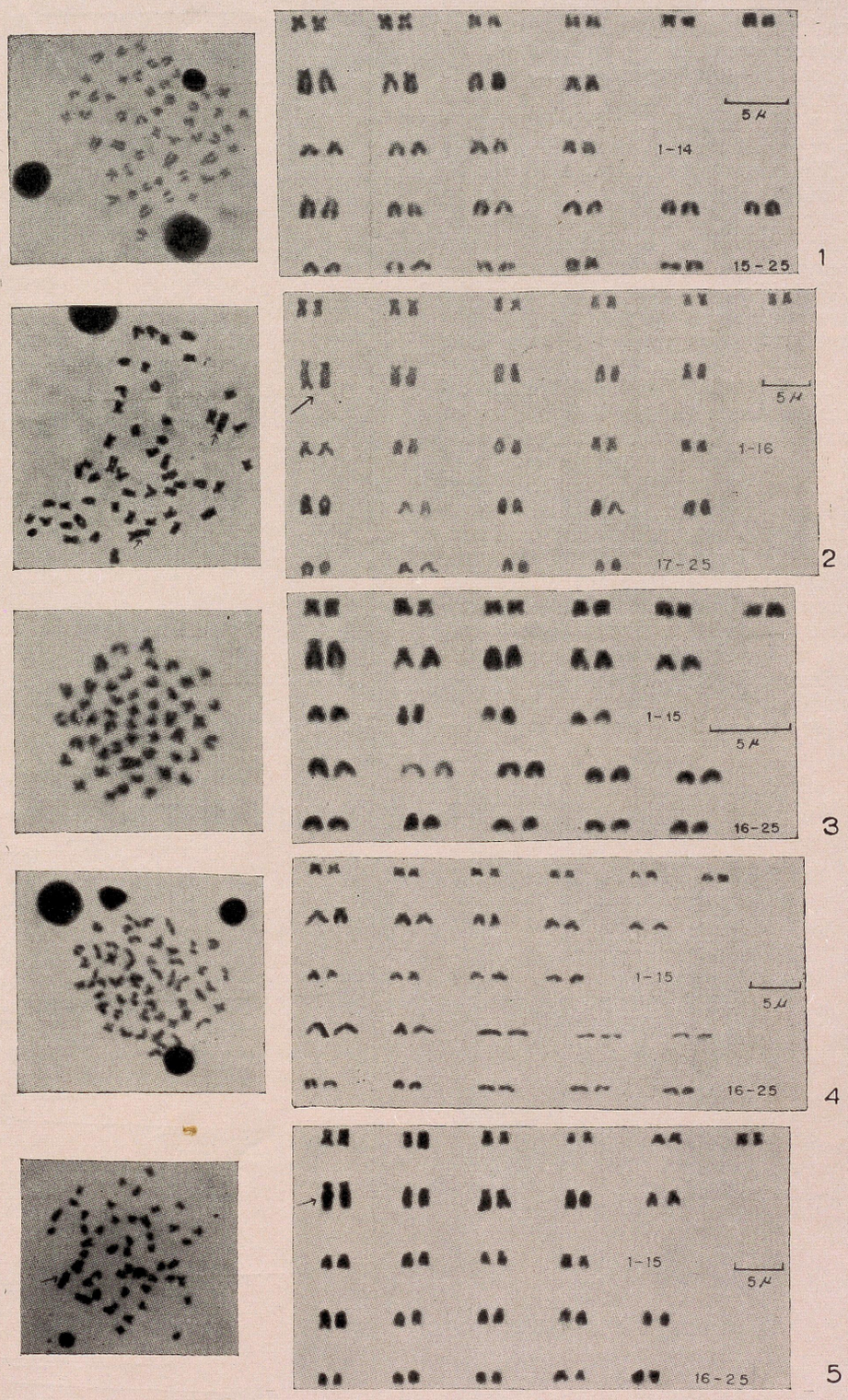

Fig.1 & 2 — Metaphase spread (2n=50) and its karyotype from a female *L. rohita* (1) and male *L. calbahu* (2) [Arrow indicates secondary construction]. Figs 3-5 — Metaphase spread (2n=50) and its karyotype from a female 'Calbahu' (3), male '*Rosu*' (4) and female F_2 'Calbahu' (5) [Arrow indicates secondary constriction]

reports of chromosomal sexual dimorphism in fishes are available, in the majority of the species studied, no definite sex chromosomes have been reported.

Haemoglobin studies — As reported earlier[6] the haemoglobin of *L. rohita* revealed only a single anodic fraction, while that of *L. calbasu* showed a fast moving major anodic fraction along with a minor fraction (8-10%) moving cathodically. Both the F_1 hybrids '*Rosu*' and '*Calbahu*' showed in addition to the presence of the parent haemoglobins, a new haemoglobin fraction intermediate in mobility between the parent haemoglobins. *In vitro* dissociation—recombination (hybridisation) studies following the method of Gammack *et al.*[16] confirmed the presence of the new hybrid fraction as has already been reported.[6].

Fig. 6 shows the F_2 '*Calbahu*' haemoglobin electrophoretic patterns on paper. Of the 12 specimens of F_2 '*Calbahu*' examined, 7 showed a pattern, indistinguishable from F_1 hybrid pattern and the remaining 5 showed one similar to that of *L. rohita*. Surprisingly, none of the specimens studied showed a characteristic *L. calbasu* pattern. The latter phenomenon cannot be convincingly explained, since the sampling is inadequate for a frequency distribution study, which alone can throw further light on this.

As pointed out by earlier workers[17], genetic recombination can bring about haemoglobin polymorphism in the subsequent generations of the hybrids. This assumption is based on the fact that a minimum of two loci are involved in the determination of the

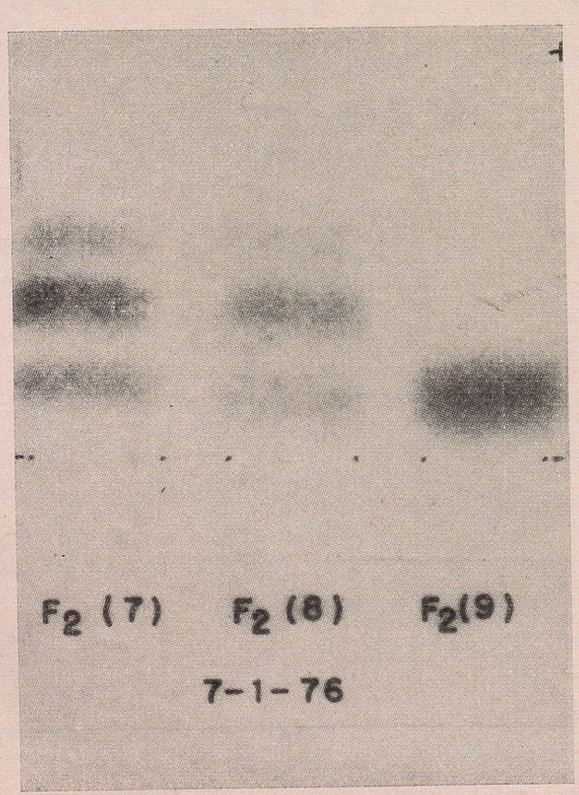

Fig. 6 — Paper electrophoresis of haemoglobins from 3 different F_2 '*Calbahu*' (unstained)

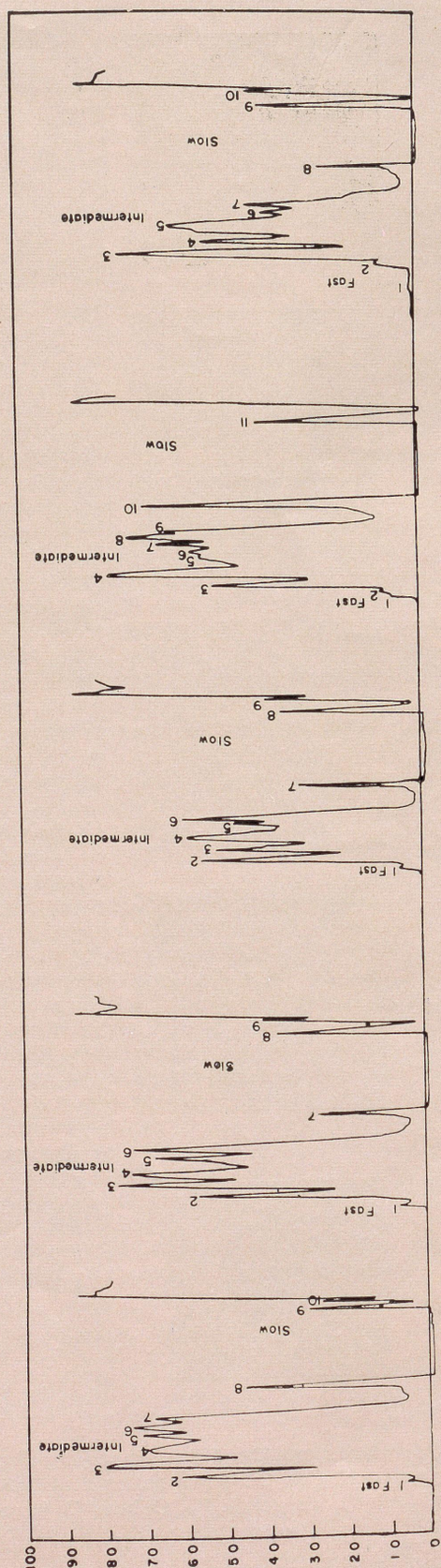

Fig. 7 — Densitometer trace of the acrylamide disc electrophoretic muscle protein patterns of *L. rohita*, *L. calbasu*, F_1 hybrids and F_2 '*Calbahu*'

haemoglobin in the parent species. Naturally one would expect the parent haemoglobins to be segregated in a definite ratio in the F_2 hybrids. However, the implication is ever with us that biochemial and anatomical complexity has functional significance. Since haemoglobin is a respiratory protein it seems logical to look for the significance of its heterogeneity in terms of respiratory function of the blood. In this connection it is pertinent to note that *L. rohita* as well as the F_1 and F_2 hybrids are pelagic in their habits unlike *L. calbasu* which is a bottom dweller. If so, selection might have favoured the haemoglobins of the former groups over the latter.

Muscle and serum protein studies — Densitometric tracings of the electrophoretic patterns of muscle proteins showed 3 distinct groups starting from the anodic end (Fig. 7). From the figure under reference it can be seen that *L. rohita* and *L. calbasu* show species specific pattern, whereas both the F_1 hybrids show an identical pattern which is intermediate between that of the two parents. A series of earlier studies, in particular by Tsuyuki *et al.*[18], have revealed the fact that muscle protein electrophoretic patterns have a very constant appearance and show species specificity with the probable exception of identical pattern in closely related species. An entirely different pattern obtained in F_2 *'Calbahu'* may be due to genetic recombination.

Incidentally, densitometric tracings of the serum protein patterns of the fishes under study also revealed species specificity in the case of the two parent speies, with the F_1 hybrids showing somewhat intermediate pattern between them, with the possible formation of a new fraction in *'Rosu'*.

In the light of all these findings it can be concluded that karyological and biochemical data can prove useful not only in pisciculture involving hybridization but also probably in the identification of natural hybrids especially since the gross morphological characters used for their identification can at times be misleading. It may, however, be pointed out here that chromosome studies employing epithelial cells from scales, fins, cornea, etc. either directly or by short term culture would have wider scope in systematic hybridization studies, since such a technique does not entail sacrificing the experimental animals. The advantage of haemoglobin studies in this respect can hardly be over emphasized, since it is least affected by environmental factors unlike some other proteins. Besides, only a small amount of blood is required for its electrophoretic studies. As far as the muscle and serum protein studies are concerned it can be said that muscle protein electrophoretic patterns have a very constant appearance. Though the latter may be true of serum proteins also, the influence of environmental and physiological factors on this cannot be over looked.

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