Some Observations on the Chromosomes of Certain Teleosts Using a Simple Method

KRISHNAJA, A. P. & REGE, M. S.

Department of Zoology, Institute of Science, Bombay 400 032

Manuscript received 25 *May* 1979; *revised manucript received* 22 *October* 1979

A simple and quick method for chromosome preparations (mitotic as well as meiotic) from various tissues such as scale, fin, gill, intestine, kidney and gonad of fishes is described. This method was employed in a screening programme to detect a species with a suitable karyotype to be used in a study on the mutagenic effects of industrial effluents released in water. *Boleophthalmus dussumieri, Gymnocorymbus ternetzi, Puntius tetrazona, Colisa fasciata* and *Tilapia mossambica* showed a modal diploid No. of 2n=46, 2n=50, 2n=50, 2n=48 and 2n=44 respectively. This is perhaps the first report on the shromosome complements of the former three species. In view of the fairly large sized all acrocentric chromosome, *Boleophthalmus dussumieri* could be a good material for mutagenic studies in fishes.

FISH as an *in vivo* model can be quite a promising system for screening the mutagenic effects of environmental pollutants, specially the various constituents of industrial effluents that are being dumped into our water bodies. In order to detect a species with a suitable karyotype for such studies, a number of fishes were analysed for their chromosome complements using a simple method. Their suitability for laboratory toxicity bioassays was also taken into consideration. The fishes studied included *Boleophthalmus dussumieri* (Family : *Gobiidae*), *Gymnocorymbus ternetzi* (Family : *Characidae*) *Puntius tetrazona* (Family Cyprinide) *Colisa fasciata* (Family : *Anabantidae*) and *Tilapia mossambica* (Family : Cichlidae).

The method followed was that of Kligerman and Bloom[1] with a few modifications. The fishes were given an intramuscular injection of 0.02% colchicine (1 ml–100 g body weight). After 5-6 hr, scale fin, gill, kidney, intestine and gonad were removed and placed separately in 0.56% KCl and cut into small pieces. After 25-30 min hypotonic solution was removed and tissues were fixed in 3:1 methanol-acetic acid. After 2 changes in the fixative of 30 min each, the tissues were stored in vials at 4°C and the slides subsequently prepared at convenience. A small piece of tissue was placed in a cavity slide and 2–3 drops of 50% acetic acid were added. The tissue was then titurated gently, for not more than one minute, to form a fine cell suspension. Using a

posteur pipette the cell suspension was expelled on to a clean slide, preheated to 50°C and quickly withdrawn back into the pipette, leaving a ring of cells approximately one cm diameter on the slide. In this way 2 or 3 rings were made on each slide. When dry, the slides were stained with Giemsa's stain (diluted with buffered distilled water 1:10) for 20–30 min, washed in tap water and air dried. Dried slides were cleared in xylene and mounted in DPX.

Metaphase spreads obtained by the method from various tissues of different fish species along with a meiotic stage are shown in Fig. 1. Unlike as in the conventional technique where the haemopioetic tissues like kidney or spleen are used, the present method is simpler and fairly good metaphases can be easily obtained from a number of tissues as can be noticed from the figure. Moreover the scanning of the slides beome easier since the spreds can be located on the periphery of the rings. As mentioned in our earlier paper[2], techniques employing scale and fin epithelium will have wider scope in systematic hybridisation studies, since that would not entail sacrificing of the experimental animal. In mutagenicity testing this method offers the possibility of cytogenetic monitoring in a number of different tissues after *in vivo* exposure.

Boleophthalmus dussumieri, Gymnocorymbus ternetzi, Puntius tetrazona, Colisa fasciata and *Tilapia mossambica* showed a modal diploid chromosome number of 2n = 46, 2n = 50, 2n = 50, 2n = 48 and 2n = 44 respectively. To our knowledge chromosome numbers of the former three species are being reported for the first time. The two large metacentrics that characterise many of the family characidae[3] are present in *Gymnocorymbus ternetzi* as well. Our observations on *Tilapia mossambica* and *Colisa fasciata* are in agreement with those reported earlier[4]. *Boleophthalmus dussumieri* could be a good material for *in vivo* studies, for the detection of chromosome aberrations after exposure to various pollutants, in view of its fairly long all acrocentric chromosomes with a 2n = 46.

References

1. KLIGERMAN, A. D. & BLOOM, S. E., *J. Fish. Res. Bd Can.*, 34 (1977), 266.
2. KRISHNAJA, A. P. & REGE, M. S., *Indian J. exp. Biol.*, 17 (1979), 233.
3. KIRBY, R. F., THOMPSON, K. W. & HUBBS, C., *Copeia*, 3 (1977), 578.
4. MANNA, G. K. & PRASAD, R., in *Proc. 1st all India Congr. Cytology and Genetics*, 1975, 235.

Fig. 1 — Metaphase spreads from [A, *Boleophthalmus dussumieri* 2n=46 (~~2130~~) tissue — fin, × 2130; B, *Gymnocorymbus ternetzi* 2n=50 tissue—gill, × 3100; C, *Puntius tetrazona* 2n=50 tiszue-scale, × 2950; D, *Colisa fasciata*, (~~3050X~~) tissue-kidney, × 3050; E, *Tilapia mossambica* (~~2210 X~~) tissue-intestine, × ~~2210~~, and F, The diakinesis stage-*Tilapia mossambica*,—testis, × 2460].

REGISTERED

Indian Journal of Experimental Biology
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Pub-3/4(EB- 1358)/79 Dated 11.2.80 1979

Dear Dr Rege

Ref. "Some observation ..... method".

Enclosed please find two sets of galley proofs of the above paper. The proofs may kindly be read very carefully and one set of duly corrected may be returned to us immediately.

No alterations of substance to the text, tables or legends will be acceptable.

The reprint order form enclosed herewith may also be returned alongwith the corrected proofs. *Non-receipt of form will be taken as implying that no reprints are needed.*

The paper is scheduled to appear in Mar 80 1979 issue of the Journal. Kindly note that if the corrected galley proofs are not mailed to us within two days after its receipt, it will not be possible for us to include the article in the said issue.

With compliments,

Prof. M. S. Rege
Deptt. of Zoology
Univ. of Science
Bombay

Yours sincerely,

Ashok Sen

Editor

Encl : as above

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Pub-3/4 (EB- 1358)/79 Dated 23. 10. 1979

Dear Dr Roge,

Ref : Paper entitled "Some observations on..... Chromosomes of certain Teleosts using a simple method."

The above ~~paper~~/short communication has been accepted for publication subject to necessary editorial modifications and shall be included in an early issue of the Indian Journal of Experimental Biology.

With best compliments,

Yours sincerely,

Rajinmathur
Editor

1451/79
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Kindly note the following :

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पत्रांक/Ref. No. Pub-3/4(EB-1358)/79 दिनांक/Dated August 7, 1979

Dr B.S. JANGI
M.Sc., Ph.D., F.Z.S.I.
Editor: IJEB & IJBB

Dr M.S. Rege
Department of Zoology
Institute of Science
Bombay 400032

My dear Dr Rege,

Thank you for your letter. I have looked into your file and would like to inform you that we will be in a position to accept your short communication provided that you omit some of the photomicrographs (which are out of focus and really flat) and send us the prints of others, freshly done on hard paper so that they show better contrast. Of late the journal has been taken to task for bad reproduction of halftones, and the Editorial Board has been insisting on our being highly selective. Keeping this in mind, you will, I hope, find some way out to reconcile with the situation.

I am likely to be in Bombay late in the second week of October and will not miss a trip to Santa Cruz. Congrats on assuming the headship of the department. Will write to you in due course about my programme. Hope you will not be moving out of Bombay.

With all best wishes, and kindest personal regards,

Yours sincerely

B.S. Jangi

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Ref. Pub-3/4(EB-1358)/79 Dated 6.8.79

Dear Dr Lege,

I acknowledge with thanks the receipt of your letter dated 3.8.79 resubmitting enclosing ~~revised~~ article entitled, "Some observations on ------ method.", for publication in the Journal. The publication of the article is under consideration.

A further communication will follow.

With compliments,

Yours sincerely,

Rajiv Wathur

Editor

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Department of Zoology,
Institute of Science,
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4 August 3, 1979

To

The Editor,
Indian Journal of Experimental Biology,
C.S.I.R.,
New Delhi.

Ref: Pub:3/4 (EB 1358)79 dated 30/7/79

Sir,

Thank you for the referees' report sent along with the manuscript. We do not completely agree with the referee's comments and the following are our explanations.

1. We have not given any karyotypes of the species mentioned but only given the chromosome spreads to show the usefullness of the method employed.

2. We prefer to send it to your journal and not to a Cytological journal since our main intention is not to report the karyoologically unknown species. The very purpose of this short paper is to highlight the possibility of cytogenetic monitoring in a no. of different tissues of fishes using a simple method. We have clearly indicated that the method followed was already described and we have used it with certain modifications.

3. In selecting the species Boleophthalmus dussumieri we carefully considered the availability of the species in large nos. for such a study, although we are aware of other species with better karyotypes (e.g. mudskipper, Black ghost knife fish etc.) which are not easily available in this part of the country. Considering the chromosome complement and such other factors we prefer to use this species for this work. We believe the photographs are clear enough for the purpose for which at it is intended in this paper (to show the spreads obtained from different tissues of different fishes). Reproduction of this photographs may not be bad as can be seen from one of our earlier papers published in your journal. (References given in the paper). Moreover the photograph of the meiotic stage gives clear details when compared to photographs published in other papers on fish chromosomes.

In view of the above explanations on the comments of your referee we request to reconsider the publication of this paper at an early date. Inspite of this if you still feel it cannot find a place in your esteemed journal we shall be obliged if you return the manuscript without undue delay.

Thanking you,

Yours faithfully,

(Dr. M.S. Rege)
Head of Zoology Department.

1 of 3

Registered letter

~~Botaea~~
~~Boleophthalmus~~
Boleophthalmus dussumieri

To

The Editor
Ind. Jour. of Experi. Biology
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New Delhi.

Ref. Publ: 3/4(EB/358)79 dtd 30/4/79

Sir, ~~With reference~~ With reference to the above

Thank you for the referee's report sent along with the manuscript. We do not completely agree with the referee's comments and the following are our explanations.

1. We have not given any karyotypes of the species mentioned but only given the chromosome spreads to show the usefulness of the method employed.

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Thanking you,

Yours faithfully,

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Pub-3/4 (EB- 1358)/79 Dated 30.7.1979

Dear Sir/Madam

Ref : Paper entitled, "Some observations - - - - - - - simple method"

Our referee is of opinion that the above paper is not suitable for publication in the journal. I am therefore returning the manuscript to you together with the referee's comments.
Your interest in our journal is greatly appreciated.

With compliments,

Encl : As above

Yours faithfully
Ashok Sen
Editor

Prof. M. S. Rege,
Deptt. of Zoology,
Instt. of Science
Bombay - 400 032

EB-1358

Referee's Report

The paper contains information on the karyotypes of 3 karyologically unknown species which deserves publication but preferably in a cytological journal. The other informations given in the paper are not particularly new, and fish with a better karyotype than Boleopthalmus dussumiari is known which can be used in studies on clastogenic effects of industrial effluents released in water. Photographs are not acceptable, because they are not in focus and also do not give any contrast. In view of the above, the paper is not suitable for publication in the Indian Journal of Experimental Biology.

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Hillside Road, New Delhi-110 012

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Ref. Pub-3/4(EB-1358)/ 79. Dated 25.5.79.

Dear Dr Rege,

I acknowledge with thanks the receipt of your letter dated 23.5.79. enclosing revised article entitled, "Some observations ---------- method." for publication in the Journal. The publication of the article is under consideration.

A further communication will follow.

With compliments,

Yours sincerely,

Ashok Sen

Editor

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Dr. M.S. Rege,
Associate Professor of Zoology,

Department of Zoology,
Institute of Science,
15, Madam Cama Road,
Bombay 400 032

May 23, 1979

To

The Editor
Indian Journal of Experimental Biology,
Hillside Road, New-Delhi. 110 012.

Sir,

I am sending herewith (in duplicate) a paper (shall communicate) entitled "Some observations on the chromosomes of certain teleosts using a simple method" along with one text figure. I shall thank you to kindly find a place in the early issue of your esteemed journal and oblige.

Thanking you,

Yours faithfully,

(Dr. M. S. Rege)

Encl: as above.

SOME OBSERVATIONS ON THE CHROMOSOMES OF CERTAIN TELEOSTS USING A SIMPLE METHOD

ABSTRACT

A simple and quick method for chromosome preparations (mitotic as well as meiotic) from various tissues, such as scale, fin, gill, intestine, kidney and gonad of fishes is described. This method was employed in a screening programme to detect a species with a suitable karyotype to be used in a study on the mutagenic effects of industrial effluents released in water . Boleophthalmus dussumieri, Gymnocorymbus ternetzi, Puntius tetrazona, Colisa fasciata and Tilapia mossambica showed a modal diploid no. of 2n=46, 2n=50, 2n=50, 2n=48 and 2n=44 respectively. This is perhaps the first report on the chromosome complements of the former three species. In view of the fairly large sized all acrocentric chromosomes, Boleophthalmus dussumieri could be a good material for mutagenic studies in fishes.

SOME OBSERVATIONS ON THE CHROMOSOMES OF CERTAIN TELEOSTS USING A SIMPLE METHOD

Krishnaja, A.P. and Rege, M.S.
Department of Zoology
Institute of Science
Bombay 400 032

Fish as an invivo modal can be quite a promising system for screening the mutagenic effects of environmental pollutants, specially the various constituents of industrial effluents that are being dumped into our water bodies. In order to detect a species with a suitable karyotype for such studies, a number of fishes were analysed for their chromosome complements using a simple method. Their suitability for laboratory toxicity bioassays was also taken into consideration. The fishes studied included Boleophtholamus dussumieri (Family: Gobiidae), Gymnocorymbus ternetzi (Family: Characidae), Colisa fasciata Puntius tetrazona (Family: Anabantidae), and Tilapia mossambica (Family: Cyprinidae). (cichlidae).

The method followed was that of Kligerman and Bloom[1] with a few modifications. The fishes were given an intramuscular injection of 0.02 % colchicine (1 ml - 100 gm body weight). After 5-6 hours, scale, fin, gill, kidney, intestine and gonad were removed and placed separately in 0.56 % KCL and cut into small pieces. After 25-30 minutes hypotonic solution was removed and tissues were fixed in 3:1 methanol-acetic acid. After 2 changes in the fixative of 30 minutes each, the tissues were stored in vials at 4°C and the slides subsequently prepared at convenience. A small piece of

tissue was placed in a cavity slide to which and 2-3 drops of 50 % acetic acid were added. The tissue was then titurated gently, for not more than one minute, to form a fine cell suspension. Using a pasteur pipette the cell suspension was expelled on to a clean slide, preheated to 50°C, and quickly withdrawn back into the pipette, leaving a ring of cells approximately one cm diameter on the slide. In this way 2 or 3 rings were made on each slide. When dry, the slides were stained with Giemsa's stain (diluted with buffered distilled water 1:10) for 20-30 minutes, washed in tap water and air dried. Dried slides were cleaned in xylene and mounted in DPX.

Metaphase spreads obtained by the method from various tissues of different fish species along with a meiotic stage are shown in Fig.1. Unlike as in the conventional technique where the haemopioetic tissues like kidney or spleen are used, the present method is simpler and fairly good metaphases can be easily obtained from a number of tissues as can be noticed from the figure. Moreover the scanning of the slides becomes easier since the spreads can be located on the periphery of the rings. As mentioned in our earlier paper[2], techniques employing scale and fin epithelium will have wider scope, in systematic hybridisation studies, since that would not entail sacrifing of the ~~experimental studies, since no~~ experimental animal. In mutagenicity testing this method offers the possibility of cytogenetic monitoring in a number of different tissues after in vivo exposure.

Boleophthalmus dussumieri, Gymnocorymbus ternetzi, Puntius tetrazona, Colisa fasciatus and Tilapia mossambica

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References

1. Kligerman, A.D. and Bloom, S.E.
J. Fish. Res. Bd. Can. 34 (1977) 266.

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Ind. J. Exp. Biol. 17 (1979).

3. Kirby, R.F., Thompson, K.W. and Hubbs, C.
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Proc. 1st all India Congr. Cytology and Genetics (1975) 235.

Indian Journal of Experimental Biology
Vol, 17, March 1979, pp. 253-257

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

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Central Institute of Fisheries Education, Bombay 400 058

and

M. S. REGE†
Department of Zoology, Institute of Science, Bombay 400 032

Manuscript received 20 *March* 1978; *revised manuscript received* 16 *December* 1978

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.

*Present Address: Department of Zoology, Institute of Science, Bombay 400032
†Reprint requests

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. calbasu* (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

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Species	Chromosome No. of							Cell No. scored	% of cells with 2n=50 chromosome	NF	Total mean length in haploid set
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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

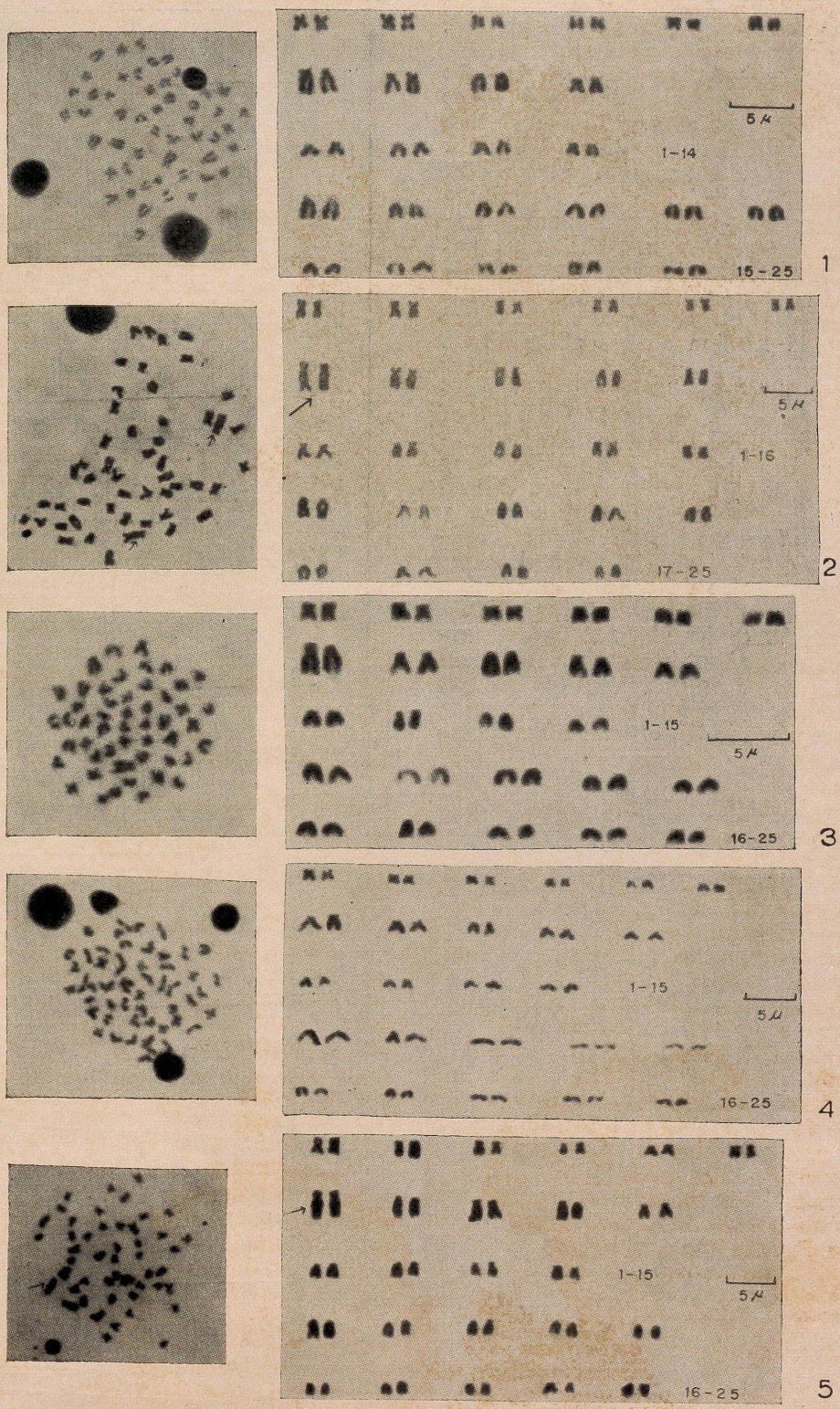

Fig.1 & 2 — Metaphase spread (2n=50) and its karyotype from a female *L. rohita* (1) and male *L. calbahu* (2) [Arrow indicates secondary constriction]. Figs 3-5 — Metaphase spread (2n=50) and its karyotype from a female 'Calbahu' (3), male '*Rosu*' (4) and female F_2 'Calbahu' (5) [Arrow indicates secondary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

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

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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

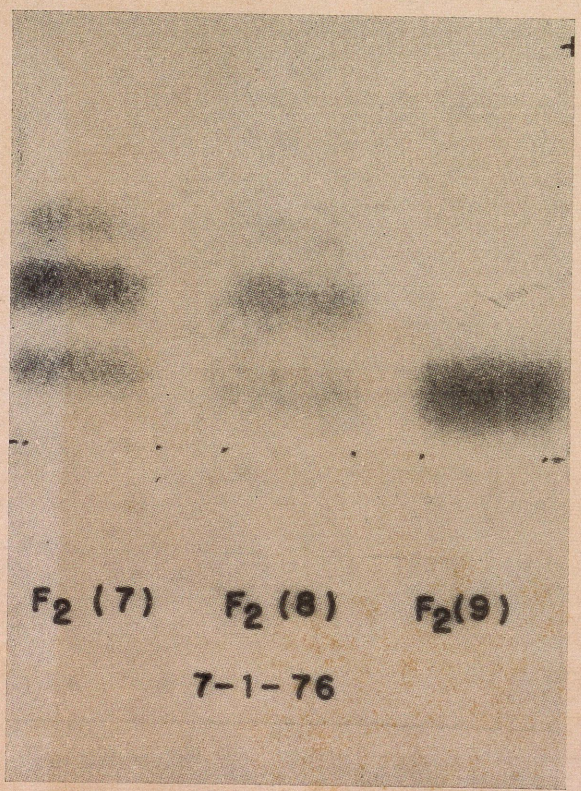

Fig. 6 — Paper electrophoresis of haemoglobins from 3 different F_2 '*Calbahu*' (unstained)

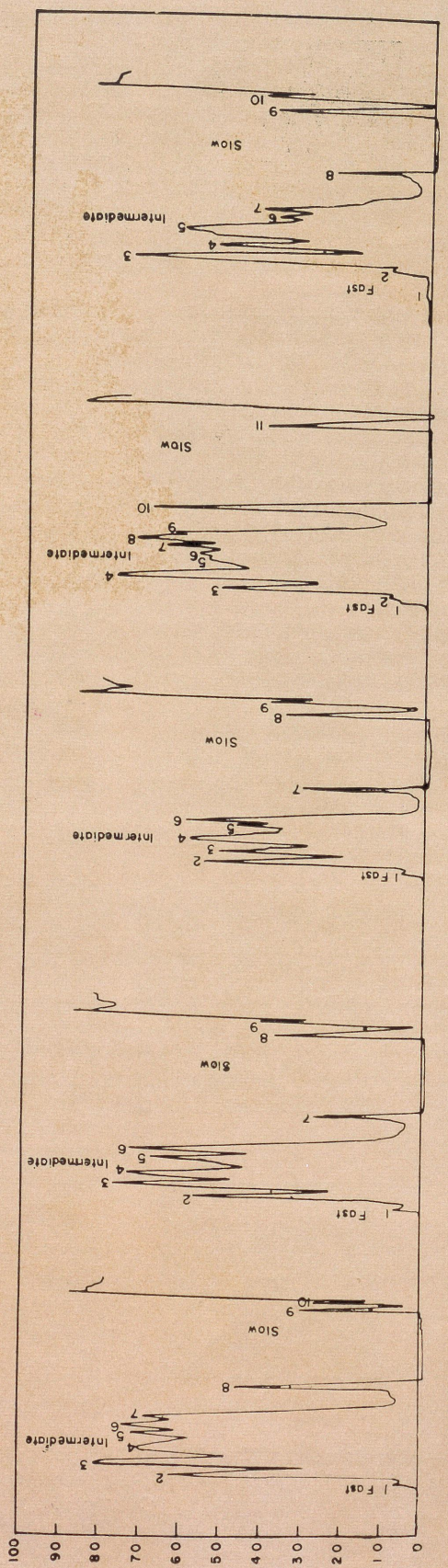

Fig. 7 — Densitometer trace of the acrylamide disc electrophoretic muscle protein patterns of *L. rohita*, *L. calbasu*, F_1 hybrids and F_2 '*Calbahu*'

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.

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Incidentally, densitometric tracings of the serum protein patterns of the fishes under study also revealed species specificity in the case of the two parent species, 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.

Acknowledgement

The authors express their sincere gratitude to Shri P. K. Sukumaran, Scientific Officer, Cancer Research Institute, Bombay, for his valuable help and suggestions throughout this investigation. One of us (APK) is grateful to ICAR, New Delhi for the award of a junior fellowship.

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

A. P. KRISHNAJA & M. S. REGE*
Department of Zoology, Institute of Science, Bombay 400 032

Manuscript received 20 *March* 1978; *revised manuscript received* 16 *December* 1978

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 model 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 chromosoes 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.

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*'

	Chromosome No. of							Cell No. scored	% of cells with 2n=50 chromosome	NF	Total mean length in haploid set
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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 evironmentally 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[3-5] makes the importance of such a study obvious.

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. calbasu* (6 females and 3 males), 4 of *'Calbahu'* (all females), 9 of *'Rosu'* (5 females and 4 males) and 12 of F_2 *'Calbahu'* (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).

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This is same as no. 3 & hence should be deleted with corresponding change in the numbers subsequent. Mistake is deeply regretted.

Fig. 1 & 2 — Metaphase spread (2n=50) and its karyotype from a female ~~(1) and male (2) *L. rohita*~~ [Arrow indicates secondary constriction] *L. rohita (1) and male L. calbasu (2)*

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]

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*'

It seems that from the manuscript Fig 8 is omitted. But if it is an inadvertent ommission then the caption should be as below

Fig. 8 Densitometer trace of the acrylamide disc electrophoretic serum protein patterns of L. rohita, L. calbasu, F1 hybrids and F2 'CALBAHU'.
1, a, b, c — prealbumins
2. Albumin
3-9. others like alpha, beta, gamma globulins etc.

INDIAN JOURNAL OF EXPERIMENTAL BIOLOGY
Publications & Information Directorate
CSIR, Hillside Road, New Delhi 110012.

Pub-3/4(EB- 275)/79 Dated 25 1 - 1979

Dear Dr Roge

Ref: Paper entitled, "Genetic studies ---- hybrid
_____"

The above paper/short communication has been accepted for publication subject to necessary editorial modifications and shall be including in an early issue of the Indian Journal of Experimental Biology.

With best compliments,

Yours sincerely,

(Ashok K. Sen)
Assistant Editor

Khullar

अन्तर्देशीय पत्र कार्ड

INLAND LETTER CARD

Prof. M.S. Rege
Deptt. of Zoology
Institute of Science
Bombay 400032

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INDIAN JOURNAL OF EXPERIMENTAL BIOLOGY
Publications & Information Directorate
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Pub-3/4(EB- 275)/78 Dated 16-12- 1978

Dear Dr Rege

I acknowledge with thanks the receipt of your letter dated 29.11.78 enclosing revised article entitled, " Genetic studies -- -- hybrid "
for publication in the journal. The publication of the article is under consideration.

A further communication will follow.

Sincerely yours

(ASHOK K. SEN)
Editor

N.B.: Please quote your reference number given above in future correspondence concerning the paper.

Khullar

अन्तर्देशीय पत्र कार्ड

INLAND LETTER CARD

Dr. M.S. Rege

Deptt. of Zoology

Institute of Science

Bombay 400032

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EB-275

Referee's report on revised Paper

The referee's contention that the section of the paper dealing with muscle and serum proteins needs to be drastically reduced still stands. The whole paragraph describing the band patterns of muscle proteins is purely qualitative and should be given as a legend to Fig. 7. Only relevant information indicating the nature of intermediate band pattern in hybrids needs to be retained.

As regards study of muscle proteins the last paragraph on page 8 and the first paragraph on page 9, in which the authors attempt to tentatively locate the serum proteins of fish, based on similarity in electrophoretic mobility with mammalian serum proteins need not be retained, since these paragraphs in no way increase the value of the paper. Moreover, the authors maintain that the studies on serum protein band patterns on polyacrylamide gel electrophoresis is of doubtful validity.

REGISTERED LETTER with A.D.

Dr. M. S. Rege,
Associate Professor of Zoology,

Department of Zoology
Institute of Science,
15, Madam Cama Road,
Bombay 400 032

November 29, 1978

To

The Editor,
Indian Journal of Experimental Biology,
New Delhi- 110012

Ref: Pub. 3/4 (EB- 275/78 dated 20-11-78.

Sir,

I am sending herewith the revised manuscript fully complying with the suggestions made by the referee. I shall thank you to kindly expedite the publication of this paper, since the original manuscript was submitted as early as on 13th March, 1978.

Thanking you,

Yours faithfully,

(Dr. M. S. Rege)

Registered Letter Post A/D

Dr.M.S.Rege,
Associate Professor of Zoology,

Department of Zoology,
Institute of Science,
15, Madam Cama Road,
Bombay 400 032

July 2, 1978

To,

The Editor,
Indian Journal of Experimental Biology,
Hillside Road, New-Delhi. 110 012.

Ref: Pub 3/4 (EB-275)/78 dated 20-11-78 (20-6-78)

Sir,

Thank you for your letter enclosing the referee's remarks on our paper entitled "Genetic studies----- F_1 and F_2 hybrids". The revised manuscript is submitted herewith after duly complying with some of the suggestions made by the referee. As regards a few others I would like to make the following clarifications.

The terms 'ROSU' and 'CALBAHU' referring to the hybrids between L. rohita (Rohu) and L. calbasu (Calbasu) as mentioned in the text are the terms referred to earlier and as such should be retained (Reference No.7,6 and 4).

As regards haemoglobin studies we have confirmed the presence of a new haemoglobin fraction by in vitro dissociation recombination (hybridisation) to which we have referred in our earlier paper. (Ref.6). However we have added a line mentioning this in the present paper also. Fig.No.9 in the original manuscript showing segregation of parent haemoglobins in the F has been retained. Since the purpose of this study was not structural analysis of these haemoglobins we have not resort to the techniques referred to by the referee. Paragraph on alkali denaturation studies has been deleted.

As regards referee's comment on muscle and serum proteins I may point out here that we have made the interpretations with caution and the relevant points have already been discussed in

P

the paper. Besides the description of the protein patterns is as brief as possible and as such ~~shall~~ should be retained as it is. It is felt that the Figures 12 and 13 in the original manuscript may be retained, since they show evidence of intermediate protein pattern in the hybrids.

Thanking you,

Yours faithfully,

(Dr. M.S. Rege)

Encl: as above.

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Pub-3/4(EB- 275)/78 Dated 20-11-1978

Dear Dr Rege

Ref: Paper entitled, " Genetic Studies ..... hybrids
"

Enclosed please find a copy of our referee's report on the above paper. A suitably modified and condensed paper may please be communicated, in duplicate, along with the original manuscript for consideration.

While submitting the revised manuscript, a note in duplicate should invariably be enclosed, answering referee's criticisms, one by one, indicating clearly which of the modifications suggested by the referee have been effected and giving reasons for not complying with such suggestions of the referee which have been considered unacceptable.

Non compliance with these instructions will unnecessarily delay consideration of the paper.

Yours sincerely,

K. Satyanarayana

Editor

Encl: as above

Dr. M. S. Rege
Dept of Zoology
Institute of Science
Bombay 400 032

SKK

Thank you for your letter enclosing the remarks from your referee on our paper titled "Genetic studies ... F_1 & F_2 hybrids". The revised manuscript is submitted herewith. Some of the suggestions of the referee have been duly considered however while appreciating the valuable suggestions we wish to point out the following answering referee's criticism and giving reasons for not fully complying with some of the suggestions therein.

(1) The terms 'ROSU' & 'CALBAHU' mentioned in the abstract/paper are to be retained as such, as they comprise the hybrids between *R. rotula* (Rohu) and *L. calbasu* (calbasu), as explained in the suggested earlier 7.6.4.

(2) The number of figures in chromosome studies are reduced as suggested. abstract/text are to be retained (they) seems different to earlier & is not should be retained coming in the next part, at the beginning itself

(3) In the right it is clearly mentioned that the preparation of our paper is to discribe genetic variability and among others the electrophoretically detectable

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haemoglobins as a reliable parameter in such studies. As described in our earlier paper[6], the presence of a new haemoglobin fraction found in the hybrids has already been confirmed by in vitro dissociation - recombination (hybridisation) studies by the method of Gammack et al[11]. Studies including urea starch gel, urea gel for peptide chain separation, peptide mapping and amino acid sequence studies for detecting point mutations and isoelectric focussing for detailed structural analysis are well established, for elucidating haemoglobin structure. But our aim was rather characterisation not structural analysis of these haemoglobins. As pointed out earlier our only purpose in this paper is to emphasise on the usefulness of a simple electrophoretic technique for the detection of haemoglobin variants, as an additional parameter for genetic studies wherever possible.

Information on the haemoglobin profile of Indian carps and their hybrids are scanty and that it would evince interest in workers engaged in the genetic studies of Indian fishes to include this among other parameters.

The paragraph on alkali-denaturation and the figures connected with it are deleted. as so Figure No. 6 alone with haemoglobins is retained inorder to stress the finding of segregation of parent haemoglobins.

Heterogenity in fish haemoglobins is a well known fact as we have already reported. Our findings in these two carps and their F1 hybrids highlights the importance of detection of haemoglobins as a reliable genetic marker.

Lastly it may be noted mentioned that interpretation of serum and muscle protein analysis by acrylamide gel had already been made with caution We feel that the last two figures may be retained since it shows clear cut evidence of intermediate pattern in the hybrids.

~~In the introduction it has been~~
~~mentioned that~~ The purpose of this paper, as
mentioned has been brought out in the introduction,
is to evaluate the usefulness of the study
of chromosome, haemoglobin, & muscle serum proteins
in hybridization experiments. Our studies has
revealed the usefulness of a simple electro-
phoretic for the detection of haemoglobin variation
in ~~fishes~~ & therefore justifies the exclusion of
this additional parameter for genetic studies in the
techniques of

Sir,

Thank you for your letter enclosing the referees remarks on our paper entitled "Genetic studies ... F1 and F2 hybrids". The revised manuscript is submitted herewith after duly complying with some of the suggestions made by the referee. As regards a few others I would like to make the following clarifications.

The terms 'ROSU' and 'CALBAHU' referring to the hybrids between L. rohita (Rohu) and L. calbasu (calbasu) as mentioned in the text are the terms referred to earlier and as such should be retained (References No. 7, 6 and 4).

As regards haemoglobin studies we have confirmed the presence of a new haemoglobin fraction by in vitro dissociation - recombination (hybridisation) studies, ~~[illegible]~~ to which we have referred

in our earlier paper. (Ref. b). However we have added a line mentioning this in the present paper also. Fig No. 9 in the original manuscript, showing segregation of parental haemoglobins in the F2 has been retained.

Since the purpose of this study was not structural analysis of these haemoglobins we have not restricted to the techniques required to by the referee. Paragraph on alkali denaturation studies has been deleted.

As regards referees comments on muscle and serum proteins, I may point out here that we have already made the interpretations with caution and the relevant points have already been discussed in the paper. Besides the description of the protein patterns is as brief as possible and as such should be retained. It is felt that the figures 12 and 13 in the original manuscript may be retained, since they show evidence of intermediate protein patterns in the hybrids.

EB-275

Referee's Report on the paper entitled 'Genetic studies on two species of the Indian Carp of the Genus Labeo and their fertile F_1 and F_2 hybrids'.

.....

I have gone through this paper and find it suitable for publication in Indian Journal of Experimental Biology but the following changes are necessary.

The authors in their paper have done a comparative study of Chromosomes, hemoglobins and muscle and serum proteins of two species of Indian Carps viz. Labeo rohita and Labeo calbasu and their F_1 hybrids CALBAHU (L. calbasu ♀ x L. rohita ♂) and ROSU (L. rohita ♀ x L. calbasu ♂) and also the F_2 CALBAHU. They have carried out these studies with the intention of locating genetic markers, which are not easily altered by environmental conditions, so as to be of use in identification of hybrids.

Karyotyping was done ~~with chromosome~~s from kidney tissue after injection of colchicine. Electrophoresis of hemoglobins was carried out on paper, cellulose acetate and starch gel. Muscle and serum proteins were studied by polyacrylamide gel electrophoresis.

Comparative study of chromosomes can be published as it is. It would be better to reduce the number of figures to a maximum of five. Studies on finding markers for hybrids based on net-electric charge of proteins needs to be drastically reduced into a para or two, giving the summary of what authors observe on electrophoresis. Electrophoretic pictures as well as densitometric tracings (fig. 9 through 13) should be deleted.

The resons for this are as follows:

1. Gross phenotypic expression of proteins without resolution of component proteins into separate identifyable entities are of no use as genetic markers in identifying hybrids. This should therefore eliminate emphasis on muscle and serum protein profile on polyacrylamide gels.

2. Since multiplicity of hemoglobins in teleost fishes is extensive, including Cyprinus carpio, proper methods of resolution should be applied if one wants to use it as a genetic marker. If column chromatographic procedures are not avaiable to the authors, isoelectric focussing on polyacrylamide gels could be done. Next subunit structures of the carp hemoglobins have to be elucidated on urea-starch gel electrophoresis to find out whether a new fraction appears in the hybrid as the authors claim.

3. The paragraph on alkali-denaturation studies of hemoglobins should be deleted. It should be noted that isoleucine is present in many animal hemoglobins including that of chicken.

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29-3-78

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13-3-78

Pub-3/4(EB- 275)/78 Dated 20-3-1978

Dear Dr Rege

Ref: Paper entitled, "Genetic studies ... F1 & F2 hybrid ..."

Enclosed please find a copy of our referee's report on the above paper. A suitably modified and condensed paper may please be communicated, in duplicate, along with the original manuscript for consideration.

While submitting the revised manuscript, a note in duplicate should invariably be enclosed, answering referee's criticisms, one by one, indicating clearly which of the modifications suggested by the referee have been effected and giving reasons for not complying with such suggestions of the referee which have been considered unacceptable.

Non compliance with these instructions will unnecessarily delay consideration of the paper.

Yours sincerely,

B.S. Jangi

Editor

Encl: as above

Sep-29 Reminder

Dr. M. S. Rege
Deptt. of Zoology
Institute of Science
15, Madam Cama Road
Bombay 32.

*SIKK

Registered Letter Post A/D

Dr. M.S. Rege,
Associate Professor of Zoology,

Department of Zoology,
Institute of Science,
15, Madam Cama Road,
Bombay 400 032

March 13, 1978

To

The Editor,
Indian Journal of Experimental Biology,
Hillside Road, New-Delhi. 110 012.

Sir,

I am sending herewith (in duplicate) a paper entitled "Genetic studies on two species of the Indian carp of the genus Labeo and their fertile F_1 and F_2 hybrids" along with 13 text figures. I shall thank you to kindly find a place in the early issue of your esteemed journal and oblige.

Thanking you,

Yours faithfully,

(Dr.M.S. Rege)

Encl: as above.

Sent on 16th March-78.

Abstract

Karyotype analysis, haemoglobin profiles and muscle and serum protein patterns of the Indian carps Labeo rohita and Labeo calbasu together with those of their fertile F_1 hybrids 'CALBAHU' (Labeo calbasu ♀ x Labeo rohita ♂), 'ROSU' (Labeo rohita ♀ x Labeo 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 Labeo rohita, NF=82 in Labeo 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.

GENETIC STUDIES ON TWO SPECIES OF THE INDIAN CARP OF THE GENUS LABEO AND THEIR FERTILE F_1 AND F_2 HYBRIDS.

Krishnaja, A.P. and Rege, M.S.*
Department of Zoology,
Institute of Science, Bombay 400 032

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. (1974)[2] have reviewed the potentialities 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.

In the present investigation a comparative study on the chromosomes, haemoglobins and muscle and serum proteins of two species of the Indian carp of the genus Labeo, viz. Labeo rohita and Labeo calbasu and their fertile F_1 hybrids 'CALBAHU' (L. calbasu ♀ x L. rohita ♂), 'ROSU' (L. rohita ♀ x L. calbasu ♂) and F_2 'CALBAHU' has been attempted with a view to evaluate the usefulness of such a study in hybridisation experiments. Paucity of information on the karyological and biochemical data on Indian

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* Reprint requests.

carps and their hybrids, with the exception of meagre data available for limited species[3,4,5,6] makes the importance of such a study obvious.

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 specimens of L. rohita (7 females and 4 males), 9 of L. calbasu (6 females and 3 males), 4 of 'CALBAHU' (all females), 9 of 'ROSU' (5 females and 4 males) and 12 of F_2 'CALBAHU' (7 females and 5 males). Intramuscular injection of colchicine (0.06 %-0.5 ml/100 g body wt) solution was given to the fish 3-5 hours 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 (1968)[9] and Levan et al. (1964)[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 (1964)[11], at 4°C employing 6 % acrylamide gel for muscle, 7 % for serum, Tris- glycine buffer pH 7.8 and 3 mA current per tube/ 350 V. Duration of the run was 45 minutes. Photoelectric scans of the electropherograms were carried out using a scanner (Densicord Elec. densitometer 552- Filter No.590, Photo volt 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 summarised in Table 1. Figure 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 $\mu \pm 0.26$ and $0.64 \mu \pm 0.14$. Though the maximum difference between the two adjacent chromosomes was 0.47 μ (No.7 and 8), in the rest it was

never more than 0.20 μ. 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. Figure 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 μ ± 0.47 and 1.01 μ ± 0.25. The maximum difference between the two adjacent chromosomes was 0.56 μ (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 %. Figures 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 μ and 0.80 μ, in 'ROSU' between 1.74 μ and 0.76 μ and in F_2 'CALBAHU' between 2.50 μ and 1.01 μ. 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[12]. Incidentally, most of the cyprinids studied have a diploid chromosome number of 50 chromosomes about 20 of which are telocentrics, the rest metacentrics[13]. 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 (1971)[14], 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[15,16]. Greenfield et al. (1973)[15] 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 chromosomes did not show any marked difference in their chromosome 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 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 by the authors[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.[17] confirmed the presence of the new hybrid fraction as has already been reported.

Figure 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[18], 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 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 biochemical 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 three distinct groups starting from the anodic end (Fig.7). The first group (I- fast) consists of either one or two faint bands towards the anode. The next one (II- intermediate) shows 5 to 7 thick bands and the last one (III- slow) 2-3 bands of varying intensity. Minute observation of the bands

however help in differentiating the species. In L. rohita, there is only 1 faint fast moving band in group I and 6 in group II as against 2 faint fast moving bands in group I and 5 in group II in L. calbasu. In group III both the species show 3 bands. The hybrids 'CALBAHU' and 'ROSU' show an identical pattern intermediate between that of the two parents in having only one faint band in group I, 5 in group II and 3 in group III. F_2 'CALBAHU' differs from the F_1 hybrids in having two bands in group I, 7 in group II and only 2 in group III.

It can thus be seen that L. rohita and L. calbasu show species specific pattern with regard to their muscle proteins. A series of earlier studies, in particular by Tsuyuki and co-workers[19], have revealed the fact that muscle protein electrophoretic patterns have a very constant appearance and show species specificity with the probable exception of identical patterns in closely related species. Besides, unlike the plasma proteins, muscle proteins have been shown to be quite independent of physiological factors, such as age, sex, maturation etc. The F_1 hybrids show an intermediate pattern between the parents without evidence of any new hybrid proteins. An entirely different pattern obtained in F_2 'CALBAHU' may be due to genetic recombination.

Figure 8 gives the densitometric tracings of the serum protein patterns of the fishes under study. A tentative identification of serum constituents is made here, based on the comparison with mammalian serum. Accordingly, fraction 2 which

is conspicuously present in all the fishes as a broad deeply stained band is considered as the major albumin fraction. Fractions 1a,b,c, appear to be pre-albumins and the rest 3-9 may include amongst others alpha, beta, gamma globulins etc.

From the figure under reference it can be seen that all the fishes show one prominent albumin band. In addition, L. rohita and 'ROSU' show 3 bands of pre-albumins (2 sharp thick and a faint third one), L. calbasu one, 'CALBAHU' two and F_2 'CALBAHU' none. As regards the rest of the protein components L. rohita shows 6, L. calbasu, 8, 'ROSU' 9, 'CALBAHU' 6 and F_2 'CALBAHU' 6. The pattern exhibited by the two parent species appears to be species specific, with the F_1 hybrids showing somewhat intermediate pattern between them, with the possible formation of a new fraction in 'ROSU'. F_2 'CALBAHU' surprisingly shows no prealbumin bands.

The serum proteins unlike muscle proteins are liable to be influenced by physiological and environmental factors. Such non- genetic factors are reported to involve qualitative as well as quantitative changes[20]. Besides, serum being a very complex mixture of proteins it resolves itself into a large number of components in stabilizing media like polyacrylamide, posing further problems regarding the accurate interpretation of the various fractions. So it is essential to identify the different fractions conclusively by methods such as binding of irons by transferrins and therefore unless all these factors are taken care of mere comparison of serum proteins as has been done above is of doubtful validity.

In the light of all these findings it can be concluded that karyological and biochemical data can prove useful not only in pisciculture involving hybridisation 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 hybridisation 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 appearence. 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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Fig. 1. Metaphase spread and its karyotype from a female L. rohita.

Fig. 2. Metaphase spread (2n=50) and its karyotype from a male L. calbasu.
(↗ suggestive of secondary constriction)

Fig. 3. Metaphase spread (2n=50) and its karyotype from a female 'CALBAHU'

Fig. 4. Metaphase spread (2n=50) and its karyotype from a male 'ROSU'

Fig. 5. Metaphase spread (2n=50) and its karyotype from a female F_2 'CALBAHU'. (↗ suggestive of secondary constriction)

Fig. 6. Paper electrophoresis of haemoglobins from three 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'

Fig. 8. Densitometer trace of the acrylamide disc electrophoretic serum protein patterns of L. rohita, L. calbasu, F_1 hybrids and F_2 'CALBAHU'

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

Fig. 8. Densitometer trace of the acrylamide disc electrophoretic serum protein patterns of L. rohita, L. calbasu, F_1 hybrids and F_2 'CALBAHU'

1. a, b, c - prealbumins
2. - albumin
3-9. others like alpha, beta, gamma globulins. etc.

continued from page No.7

Muscle and serum protein studies

Densitometric tracings of the electrophoretic patterns of muscle proteins showed three 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 and workers[19], 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 recombinations.

Incidentally densitometric tracings of the serum protein patterns (fig. 8) of the fishes under study also revealed species specificity in the case of the two parent species, with the F_1 hybrids showing somewhat intermediate pattern between them, with the possible formation of a new fraction in 'ROSU'.

continued on page. 10