SCALE PATTERN AND SCALE COUNTING METHODS IN RELATION TO CERTAIN TROUT AND OTHER SALMONIDS

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INTRODUCTION

THE scales of salmonid fishes conform to a general type in structure and arrangement. Within the family, considerable variations occur in the number of scales present on the body. Such differences have been used rather extensively in characterizing genera, species, and smaller units. The purpose of the present paper is to discuss certain features in the development of the scale pattern and to consider the significance of different scale-counting methods in attempting to distinguish between certain species and populations.

MATERIAL

Most of the material was obtained during the course of experimental work at the Cowichan Lake Hatchery, Vancouver Island, and consisted of various populations of *Salmo clarkii* Rich., *S. gairdneri* Rich., and *S. trutta* L., both "wild" and hatchery-raised specimens being used. A few additional observations were made on Pacific salmon (*Oncorhynchus tschawytscha* Walb., *O. kisutch* Walb., and *O. gorbuscha* Walb.) and chars (*Salvelinus fontinalis* Mitch. and *S. malma spectabilis* Gir.).

TECHNIQUE

In small fish it is difficult or impossible to appreciate the number and arrangement of the scales or scale papillae on the intact individual. The skin can readily be removed, however, from fish which have been fixed in formalin and the following procedure was adopted for small individuals. After wiping the mucus from the surface of the body, incisions are made with a sharp blade along the back, belly, and around the gill region of one side. The skin is then stripped off from head to tail (Fig. 1), placed on a slide and scraped on both sides with a safety-razor blade. This removes the underlying pigment and the scales. After a brief staining in methylene blue the wet skin is dried with a cloth and wiped from tail to head. This causes the scale pockets or scale papillae to stand out with great clearness. The removal and preparation of a skin in this manner usually require less than five minutes.

Development of Scale Pattern

In the species examined by the writer (*S. clarkii, S. gairdneri, S. trutta, S. fontinalis, O. tschawytscha, O. kisutch*) the first scale papillae to appear occur beneath the sense organs of the lateral line. At or about the time of hatching these sense organs are present as a single series along the side of the trunk and tail. Throughout most of the length of the body each is situated just behind a myoseptum. At the posterior end of the series, however, the myomeres are indistinct or not yet established. After a more or less lengthy period some of the sense organs divide, giving off a neuromast which lies dorsad to the original organ and may become widely displaced from it. About the same time or somewhat later new organs arise along the line of the original series, alternating with the original members. Thus the number of sense organs in strict lateral line series becomes doubled.

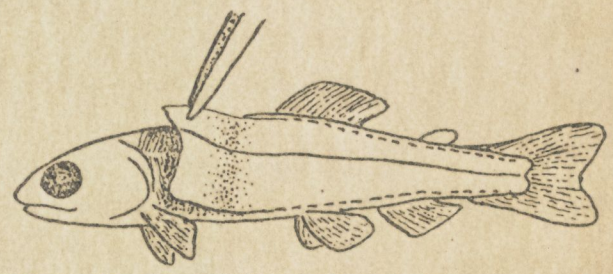

Figure 1.—Young salmonid, showing method of removing skin.

No scale papillae are evident until these developments have taken place. Papillae then appear beneath the organs of the completed lateral line series, those which arise beneath the newer, interstitial organs being frequently smaller than the others for a considerable time. Alternation of larger and smaller papillae was observed by Elson (1939) in the speckled trout, although the association with sense organs was not indicated. Formation of papillae spreads dorsally and ventrally from the lateral line by means of oblique lines of papilla-forming cells extending from the centres of development on the lateral line (Neave, 1936; Elson, 1939). In the posterior body region of *Salmo* each lateral line papilla commonly gives rise to one dorsal and one ventral oblique outgrowth. In *Salvelinus*, however, and to a varying extent in the anterior region of *Salmo*, the outgrowths may be double from their starting point and/or may branch later, thus increasing the number of oblique scale rows. Extinction of oblique lines may also occur but since this is less common than branching, the number of scales in horizontal rows is usually greater

(sometimes very much greater) at levels above and below the lateral line than on the line itself.

At the extreme caudal end of the body, scale formation both on and outside the lateral line is considerably delayed.

Metamerism

As indicated above, the original scale papillae of the lateral line, in the species discussed, are distributed with a high degree of metameric regularity throughout at least the greater part of the body. The typical relationship in position between sense organs, papillae, and myosepta is shown in Fig. 2. From the data supplied by Foerster and Pritchard (1935) regarding number of lateral line scales and vertebrae in *Oncorhynchus*, it is evident that the ratio of two scales per metamere is maintained, at least approximately, in *O. tschawytscha*, *O. kisutch*, and

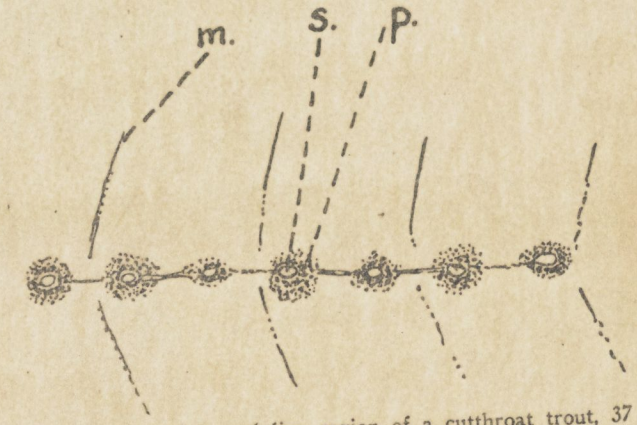

Figure 2.—Portion of lateral line region of a cutthroat trout, 37 mm. long, showing relation between sense organs (s), scale papillae (p), and myoseptum (m) × 48.

O. nerka of a somewhat more advanced age. In the pink salmon (*O. gorbuscha*), however, the number of scales is greatly in excess of this ratio (average number of scales 175.2, average number of vertebrae 66). After examining a few small pink salmon fingerlings the present writer believes that the first scale papillae show the same distribution as in other species but that subsequently papillae develop between the primary members of the lateral line series, as well as dorsad and ventrad to the latter. This development can perhaps be correlated with the comparatively large size attained by this species before scale formation begins, resulting in a wider spacing between the sense organs and thus leaving room for the establishment of papillae. (Spatial considerations also

appear to be of importance in the establishment of papillae in certain cases of regeneration (Neave, 1940).) Appearances suggestive of similar interpolation are occasionally seen on the lateral line of trout.

The scales which develop above and below the lateral line appear to be quite independent of the metamerism of the body.

Methods of Scale Counting

In attempting to compare the scale patterns of different fish, it is customary to count the number of scales between certain points on the body. Scale-counting methods in current use in North America include:

(*a*) Counting of scales in the lateral line series from the gill aperture to the "end of the vertebral column" (Foerster and Pritchard, 1935) or "base of the tail-fin rays" (Needham, 1938).

(*b*) Counting of scales in the longitudinal row immediately dorsad to the lateral line series. This method is recommended by Foerster and Pritchard (l.c.) and Clemens (1935) for Pacific salmon.

(*c*) Counting of oblique rows of scales between the points indicated above (Dymond, 1932; Mottley, 1934). In this method the count is not necessarily made at the same horizontal level throughout the body. On average, the level is usually considerably above the lateral line.

From the remarks made under "Development of the scale pattern" it would seem that a fourth method might be found in an estimate of the degree of branching exhibited by the oblique scale rows, as distinct from the total number of scales at a given level of the body. This could be expressed as:

(*d*) Counting the number of oblique scale rows corresponding to a given number of lateral line scales.

Application of Methods

As a means of identifying individual fish, lateral line counts do not provide a clear-cut distinction between the various species of Pacific salmon, although the pink salmon can nearly always be separated from the four other species in this manner (Foerster and Pritchard, 1935).

Lateral line counts made by the present writer on various samples of trout are present in Table I. From these data it is evident that while the brown trout (*S. trutta*) examined are almost completely segregated by this means, lateral line counts afford very little basis for assigning individuals to the other specific and intraspecific groups represented. It may be added that a few specimens of *Salvelinus fontinalis* and *S. malma spectabilis* also yielded counts well within the range shown by *Salmo clarkii* and *S. gairdneri,* namely 124 to 129.

TABLE I

FREQUENCY OF SCALE COUNTS MADE ON THE LATERAL LINE

Salmo clarkii (1) = cutthroats reared at Cowichan Lake Hatchery.
(2) = cutthroats reared at Veitch Creek Hatchery, Vancouver Island.

Salmo gairdneri (1) = steelheads reared at Cowichan Lake Hatchery.
(2) = sea-run steelheads, caught in Cowichan River.
(3) = non-sea-run rainbow trout, caught in Cowichan River.
(4) = Kamloops trout, hatched and reared at Cowichan Lake Hatchery.

Salmo trutta = brown trout caught in Cowichan River and tributaries.

No. of scales	Salmo clarkii		Salmo gairdneri				Salmo trutta
	(1)	(2)	(1)	(2)	(3)	(4)	
105	–	–	–	–	–	–	1
106	–	–	–	–	–	–	–
107	–	–	–	–	–	–	–
108	–	–	–	–	–	–	–
109	–	–	–	–	–	–	2
110	–	–	–	–	–	–	5
111	–	–	–	–	–	–	–
112	–	–	–	–	–	–	6
113	–	–	–	–	–	–	1
114	–	–	–	–	2	–	8
115	–	–	–	–	1	–	1
116	2	2	–	–	2	–	1
117	1	5	–	–	3	–	–
118	4	6	–	–	7	–	–
119	2	3	1	3	10	–	–
120	5	6	2	2	15	–	–
121	5	–	3	4	10	1	–
122	9	6	5	8	5	2	–
123	2	–	4	10	3	–	–
124	5	–	16	14	3	4	–
125	2	1	8	3	–	5	–
126	3	1	11	5	–	5	–
127	2	–	4	2	–	2	–
128	6	–	10	–	–	4	–
129	–	–	2	1	–	–	–
130	1	–	3	–	–	2	–
131	–	–	1	–	–	–	–
132	–	–	–	–	–	–	–
133	1	–	–	–	–	–	–
No. of fish	50	30	70	52	61	25	25
Mean	122.8	119.5	125.2	123.3	119.7	125.7	112.0

TABLE II

FREQUENCY OF COUNTS OF OBLIQUE SCALE ROWS ABOVE LATERAL LINE

No. of scales	Salmo clarkii		Salmo gairdneri				Salmo trutta
	(1)	(2)	(1)	(2)	(3)	(4)	
115	–	–	–	–	1	–	–
116	–	–	–	–	1	–	1
117	–	–	–	–	3	–	2
118	–	–	–	–	1	–	–
119	–	–	–	–	9	–	–
120	–	–	–	–	3	–	–
121	–	–	–	–	10	–	4
122	–	2	–	–	4	–	2
123	–	2	2	2	5	–	1
124	–	–	3	–	10	–	2
125	–	–	1	1	5	–	2
126	–	1	3	4	2	–	3
127	–	1	3	5	2	–	–
128	–	1	5	2	2	–	–
129	–	–	9	5	1	–	–
130	–	–	5	2	2	1	2
131	–	–	5	4	–	–	3
132	–	–	4	3	–	3	–
133	–	1	3	3	–	–	1
134	–	1	5	6	–	1	1
135	–	2	5	1	–	2	–
136	–	2	4	5	–	–	1
137	–	1	6	1	–	–	–
138	–	2	2	4	–	2	–
139	–	–	2	2	–	–	–
140	–	2	1	–	–	2	–
141	–	3	–	1	–	–	–
142	–	1	1	–	–	1	–
143	–	2	1	–	–	2	–
144	–	1	–	–	–	1	–
145	–	–	–	–	–	–	–
146	2	1	–	–	–	1	–
147	–	1	–	–	–	1	–
148	–	–	–	–	–	–	–
149	3	–	–	–	–	2	–
150	–	–	–	–	–	1	–
151	1	–	–	–	–	2	–
152	3	–	–	–	–	–	–
153	1	2	–	–	–	1	–
154	1	1	–	–	–	–	–
155	2	–	–	–	–	2	–
156	4	–	–	–	–	–	–
157	–	–	–	–	–	–	–
158	2	–	–	–	–	–	–

TABLE II (*Continued*)

No. of scales	Salmo clarkii (1)	Salmo clarkii (2)	Salmo gairdneri (1)	Salmo gairdneri (2)	Salmo gairdneri (3)	Salmo gairdneri (4)	Salmo trutta
159	4	–	–	1	–	–	–
160	1	–	–	–	–	–	–
161	2	–	–	–	–	–	–
162	5	–	–	–	–	–	–
163	1	–	–	–	–	–	–
164	3	–	–	–	–	–	–
165	3	–	–	–	–	–	–
166	2	–	–	–	–	–	–
167	1	–	–	–	–	–	–
168	2	–	–	–	–	–	–
169	2	–	–	–	–	–	–
170	–	–	–	–	–	–	–
171	2	–	–	–	–	–	–
172	1	–	–	–	–	–	–
173	–	–	–	–	–	–	–
174	–	–	–	–	–	–	–
175	–	–	–	–	–	–	–
176	1	–	–	–	–	–	–
177	1	–	–	–	–	–	–
No. of fish	50	30	70	52	61	25	25
Mean	160.4	137.4	131.5	132.23	122.3	142.6	125.3

While certain recent authors have indicated that a high number of scales "on the lateral line" is characteristic of *Salvelinus* and *Salmo clarkii*, it is apparent that the figures quoted by them do not in fact refer to this scale row, but to counts made at a different level of the body. As shown in the table, the average lateral line scale count of cutthroats (*S. clarkii*) from Cowichan Lake Hatchery is actually lower than that of steelheads (*S. gairdneri*) from the same source.

Although of very limited value as a criterion for the indentification of species, lateral line scale counts, used in a statistical manner, will sometimes serve to demonstrate differences between intraspecific populations. For example, in Table I there are significant differences of the mean value between rainbow and Kamloops trout and between rainbow and steelhead.

The possibility of finding a more satisfactory criterion at some other level of the body would obviously depend on the occurrence of specific differences in the degree of departure from the lateral line condition in the development of the scale pattern above or below the line. Foerster and Pritchard (1935) have shown that in the pink salmon the increase in

TABLE III

FREQUENCY OF COUNTS REPRESENTING NUMBER OF OBLIQUE SCALE ROWS CORRESPONDING TO FIRST SIXTY SCALES OF LATERAL LINE

No. of scales	Salmo clarkii (1)	Salmo clarkii (2)	Salmo gairdneri (1)	Salmo gairdneri (2)	Salmo gairdneri (3)	Salmo gairdneri (4)	Salmo trutta
60	–	–	1	–	3	–	–
61	–	–	4	2	9	–	–
62	–	–	4	1	10	–	–
63	–	1	3	5	8	–	–
64	–	1	4	2	14	–	–
65	–	1	10	4	10	–	3
66	–	1	6	4	3	–	–
67	–	–	4	3	2	1	1
68	–	–	7	7	1	1	2
69	–	2	3	6	1	3	1
70	–	–	4	5	–	–	1
71	–	–	7	1	–	3	5
72	–	1	6	3	–	3	–
73	–	3	2	3	–	3	1
74	–	–	4	2	–	2	3
75	–	1	–	2	–	4	4
76	–	2	1	1	–	2	1
77	–	4	–	–	–	1	3
78	–	2	–	–	–	1	2
79	1	3	–	–	–	3	1
80	1	1	–	1	–	2	–
81	2	1	–	–	–	3	–
82	2	2	–	–	–	–	1
83	4	1	–	–	–	–	1
84	2	–	–	–	–	–	–
85	4	1	–	–	–	1	–
86	–	–	–	–	–	1	1
87	5	1	–	–	–	–	–
88	4	–	–	–	–	–	–
89	8	1	–	–	–	–	–
90	6	–	–	–	–	–	–
91	4	–	–	–	–	–	–
92	4	–	–	–	–	–	–
93	4	–	–	–	–	–	–
94	3	–	–	–	–	–	–
95	2	–	–	–	–	–	–
96	3	–	–	–	–	–	–
97	–	–	–	–	–	–	–
98	1	–	–	–	–	–	–
99	–	–	–	–	–	–	–
100	–	–	–	–	–	–	–
101	1	–	–	–	–	–	–
No. of fish	61	30	70	52	61	34	31
Mean	89.0	76.1	67.4	68.3	63.4	75.2	73.5

the number of scales in the first row dorsad to the lateral line is so much greater than in the other species of *Oncorhynchus* that a clear separation of this species can be made. The writer has not applied this method extensively to trout, partly because the scales next to the lateral line are often irregular in disposition and doubt frequently arises as to whether a scale should be considered as belonging to a particular horizontal row. Observations on cutthroats and steelheads suggest that this procedure is somewhat less convenient than the following, although leading to rather similar results.

With references to the third method of scale counting (in the genus *Salmo*) Mottley (1937) says: "In western North America, however, it has become the established custom to count the number of oblique parallel rows of scales running in a downward and backward direction from the dorsum to the lateral line; the series begins just behind the head and terminates at the end of the vertebral column at the posterior reference point of the standard length measurement." He states further, "It has been the practice of the writer to make the count about ten or fifteen rows above the lateral line in the anterior part of the series, and beyond the adipose fin to continue the count at a level from five to ten rows above the lateral line." It is probable that considerable latitude exists in the practices of different systematists. While it would appear at first sight that this method is less precise than confining the count to a particular longitudinal row, it avoids to a considerable extent the difficulty mentioned in the previous method.

From Table II it can be seen that the counting of oblique scale rows does not provide a reliable means of distinguishing between any of the three species concerned. It does, however, provide a clear distinction between the cutthroats and steelheads reared at Cowichan Lake Hatchery. A complete or nearly complete separation is also apparent between this particular series of cutthroats and the brown trout and between the imported Kamloops and local rainbow trout. Significant differences of the means are also found in making certain other comparisons between these series.

The practice of counting oblique scale rows throughout the length of the body has certain disadvantages in common with the procedure discussed previously. (1) The caudal region of the series frequently permits of some latitude in interpretation both because of difficulty in determining the posterior reference point and because of irregularities in the oblique scale rows. (2) Since the posterior scales are late in developing, counts cannot be made on very small fingerlings. (3) These methods fail to take account of scale pattern, as distinct from mere

number of scales. For example, they fail to distinguish between a trout which yields a high scale count through virtue of possessing a large number of metameres, and one in which the high count is due to more frequent branching of the oblique scale lines.

In order to obviate or diminish these difficulties, the writer has at times practised a method of counting the oblique scale rows corresponding to a portion of the lateral line. The procedure has been to count sixty scales along the lateral line, beginning at the anterior end, and then to count the oblique rows back to the head, beginning with the row corresponding to the sixtieth lateral line scale. In general the count was made about a quarter or a third of the distance from the lateral line to the dorsum. In the case of very small fish in which scale development has not advanced far, it may be necessary to descend to the first or second row above the lateral line at the extreme anterior end. In the writer's experience this has not seriously affected the results.

Table III shows the results of applying this method to the material previously considered (together with a few additional fish which were not sufficiently developed for making scale counts involving the caudal end of the body). In general the groupings obtained are similar to those which result from counting the total number of oblique rows, though the shift in position of the brown trout relative to the steelhead shows the greater amount of branching which takes place in the anterior region of the former species. There are indications, however, that the procedure may at times be more discriminating than the one previously considered, in addition to the advantages of its applicability to smaller fish and, in the opinion of the writer, the somewhat greater precision attained in counting. The cutthroats tabulated as from Veitch Creek include individuals from the brood years of 1937 and 1939. Taken separately, the 1937 fish yielded the following individual scale counts:

Total number of oblique rows	132, 135, 136, 137, 138, 138, 138, 140, 140, 141, 141, 143, 144, 145, 146, 153, 154, 156.
Oblique rows related to first 60 lateral line scales	75, 77, 75, 79, 79, 79, 79, 81, 78, 77, 82, 82, 80, 84, 83, 87, 89, 85.

According to Dymond (1932) the number of oblique scale rows in the steelhead or rainbow trout of British Columbia waters varies from 124 to 146 and in the coastal cutthroat from 143 to 180. Steelheads and cutthroats reared at Cowichan Lake Hatchery showed very similar ranges of variation, namely 123 to 143 and 146 to 177, respectively. The "first 60" counts for these hatchery fish were: steelheads, 60 to 76; cutthroats,

79 to 101. According to these three standards, the allocation of these Veitch Creek cutthroats would be as follows:

	Cutthroat	Intermediate	Steelhead
Total oblique rows (Dymond)	3	4	11
Total oblique rows (hatchery)	4	2	12
Oblique rows to 1st 60 l.l scales (hatchery)	13	3	2

It would thus appear that in this particular series of fish the reduction in the total number of scales had not entailed an equivalent reduction of oblique scale rows in the anterior region.

Discussion

It is evident that in the genus *Salmo* the range of variation in number of scales is least on the lateral line. When the various groups examined are combined as species, an extreme range of 18 scales is apparent both for *S. clarkii* (116 to 133) and *S. gairdneri* (114 to 131). This presumably represents a variation of about nine metameres in the architecture of the body. The smaller series of *S. trutta* showed a range of variation of 12 scales.

At a higher level on the body the range is much greater, amounting to 56 scales in *S. clarkii* (122 to 177), 45 scales in S. *gairdneri* (115 to 159) and 21 scales in *S. trutta* (116 to 136). In individual fish of all these species, the total number of oblique rows always equalled or exceeded the number of lateral line scales except in a very few *S. gairdneri,* in which counts of one or two scales less were recorded. A slight deficiency in oblique rows is more often apparent, however, if attention is confined to the posterior half of the body, where the individual scales are larger.

While, therefore, the number of lateral line scales appears to fix an approximate *minimum* figure for the number of oblique rows, the actual number of the latter which develop may exceed this minimum to a small or large degree. Determination of the number of oblique rows apparently takes place at a different time from, or under the influence of other factors, than determination of the number of lateral line scales. Thus, the series of Kamloops trout show an average lateral line count almost identical with that of hatchery-reared steelheads, whereas the average "oblique rows" counts differ by 11 scales. An aberrant sea-run steelhead yielded a count of 159 oblique rows, about 27 rows higher than the average of its group, although its lateral line count of 126 was less than one above the mean.

In comparing species, some degree of correlation can be found between the number of oblique rows and the size of the fish when these

rows are developing. In *S. trutta and S. gairdneri,* which usually possess fewer rows, these begin to develop (in individuals examined by the writer) when the fish is about 26 to 30 mm. long (standard length), whereas in the more finely-scaled *S. clarkii* and *Salvelinus fontinalis* the same degree of development is deferred until a length of 32 to 39 mm. has been reached. The similar distinction between the pink salmon and other species of *Oncorhynchus* has already been mentioned. Indeed the difference between species in the degree of scale development at equivalent sizes is sometimes a helpful indication in identifying very small trout.

It is evident from the samples discussed that while one or more methods of scale counting may serve to separate *S. clarkii* and *S. gairdneri* belonging to certain populations or in a given locality, no very useful distinction of this sort can be drawn between these species in general at the present time, even if *S.g. kamloops* is eliminated from consideration. Attention may be drawn to the much lower scale counts recorded for cutthroats than are usually credited to this species. In each species significant average differences may occur between the scale counts of different populations.

With regard to the influences which determine variations in number of scales, external conditions, including temperature, no doubt play a part (Mottley, 1934). There is, however, strong evidence (Neave, MS) that hereditary factors are concerned in the production of the scale pattern in certain populations of *S. gairdneri.*

Summary

The development of the scale pattern in certain species of *Oncorhynchus, Salmo* and *Salvelinus* is described and discussed.

Scale development begins after establishment of the series of lateral line sense organs, under each of which a scale papilla develops.

These papillae tend to be metamerically distributed. Departures from the metameric condition are due, in some cases at least, to interpolation.

At levels dorsad to the lateral line the scales depart more or less widely from the metameric condition. Counts made at such levels are usually higher than lateral line counts, the increase in number of scales being mainly or entirely on the anterior part of the body.

Variations in number of scales are less extreme on the lateral line than at higher levels of the body.

The lateral line scales and non-lateral line scales may vary in number independently.

Considerable differences between the mean scale counts of different intraspecific populations occur.

Lateral line counts will usually serve to distinguish individual brown trout from individual cutthroats and steelheads.

Counts made at higher levels do not provide a sound general basis for the specific identification of any of these three species, though clear distinctions may occur between certain populations.

Certain advantages are claimed for a counting method involving the enumeration of oblique scale rows corresponding to a given number of lateral line scales but not involving the posterior part of the body.

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FISH EVOLUTION IN LAKE NYASA

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Fryer's consideration of evolution in Lake Nyasa (1959) illuminates many points in the evolution of lake fishes and marks a great step forward in the study of endemic lake faunas. However, it also brings up some points which have not yet been clearly understood.

While I agree that the more easily perceived specializations of endemic species flocks in large lakes appear to be largely trophic in nature, some specializations almost certainly are not trophic. We are barely at the beginning of an understanding of the astounding diversity of fish adaptions and specializations, many of which are non-trophic and may be scarcely if at all indicated by external morphology or stomach-content analysis. Even observations of the living fish, unless made intensively for 24-hour periods throughout the year, may not uncover some important specializations. For example, we may cite much recent work on fish sounds and communication, species differences in the electric field with which mormyrids surround themselves, the mucous "sleeping capsules" of parrot fishes, the precision with which certain species select an exact type of substrate for breeding sites, the marvelous but differing ability of fishes to detect excessively dilute chemical traces, etc. Specializations of these or other non-trophic types may go completely unrecognized for a long time, even in relatively well studied fishes. In other words, we know very little about the real daily and seasonal life of most fishes, and to assume that the specializations of any considerable number of species of fishes are mostly trophic, or mostly anything else, is seldom fully warranted. In my Lanao paper (Myers, 1960) I refrained from speculation in regard to the kinds of specializations exhibited by endemic lake fishes, preferring to refer only to the uniqueness of known morphological characters.

For much the same reason, I must urge caution in the acceptance of Fryer's thesis that fish habitats in Nyasa are not diverse. When I read my Lanao paper (Myers, 1960) at the London Zoological Congress (including the aphorism that biologists are, unfortunately, not aquatic vertebrates), E. B. Worthington remarked that diving with an aqua-lung in Lake Tanganyika had given him a new conception of the diversity of Tanganyika fish habitats (see Myers, 1959: 152). To say that any subaquatic area forms a very uniform fish habitat on the basis of brief observation, even when aided by the common types of limnological sampling, is, I believe, not wholly justified *from the viewpoint of the fishes concerned.* All we know about the ecology of most lake species is what they often eat, where and how they are usually caught, and some gross information on the general type and time of breeding. Their ecology may involve a great deal more than this.

Fryer's observation that plankton feeding and the shoaling (schooling) habit go hand-in-hand in Nyasa cichlids is most interesting but it is so much to be expected that only the absence of this correlation would be remarkable. Except for minute fishes to which plankters are large prey, and for large plankton-feeders such as *Polyodon* and *Cetorhinus,* I know of no fish anywhere which strains out plankton with fine gillrakers that does not habitually school. Even *Polyodon* and *Cetorhinus* school at times for certain purposes, possibly not connected with feeding. The schools of basking-sharks off the Irish coast and elsewhere are well known.

Fryer's suggestion that the schooling behavior of some Nyasa cichlids may be a sort of behavioral neoteny is even more interesting, but cannot be proved until a good deal more work has been done. Investigation of schooling behavior has been carried out on relatively few species of fishes and there are apparently notable differences in such behavior as developed in different fish groups. The schooling of herring-like fishes and of various Cyprinidae, for example, is different in many ways. My colleague, Dr. Stanley Weitzman, has reminded me that there is seasonal and temperature-controlled schooling in centrarchid fishes, which are in many ways similar to cichlids, but this may not be true of Lake Nyasa cichlids. However, if the schooling of adults is "behavioral neoteny" among Nyasa cichlids, one or more important changes in the behavioral patterns of cichlids may be involved.

Certain basic patterns in the breeding behavior of cichlids are rather rigidly fixed, and appear to be less plastic than any morphological characters within the family. A large part of the elaborate courting, spawning, and parental-care ritual of many and presumably all Cichlidae is stereotyped to a remarkable degree (Baerends and Baerends-van Roon, 1950). I have twice had a female of the West African *Pelmatochromis kribensis* mate and spawn with a male of the Central American *Cichlasoma octofasciatum* in one of my own aquaria, indicating that even in a cross-mating of these distantly related and strikingly different forms,

the complex chain of behavioral releasers and responses inherent in cichlid breeding behavior could proceed to successful spawning. Moreover, the fertilized hybrid eggs were guarded until the cessation of embryonic development 36 to 48 hours later. In fact, the principal dichotomy of cichlid breeding behavior is between the nesting and the oral-brooding forms, the evolutionary relationship between which I have already pointed out (Myers, 1937).

Apparently all young cichlids school, whether they be oral brooders or not; I have personally observed this behavior in perhaps 30 species from America and Africa. However, it is now well known that the schooling of young cichlids, both nesters and oral brooders, is strongly oriented toward the body of one or both parent fish (Noble and Curtis, 1935; Baerends and Baerends-van Roon, 1950). At a certain point, after the young have been schooling with the parents for some time (and taking refuge in the mouth in oral brooders), both the relationship and the schooling definitely break up, and the young scatter. Perhaps this is due to the loss of high nuptial coloration in the parents. The scattering has happened with all the cichlids that I have studied, and it appears to occur generally, in both nesters and oral brooders. It seems possible that orientation toward the parents might become definitely prolonged in some species, but if so we would expect to find some schools composed of adults and smaller young. The finding of such heterogeneous shoals would definitely be indicative of behavioral neoteny, but if so, this would break a general rule of fish schooling or shoaling—that individual schools of fish are almost invariably composed of individuals of approximately the same size. I doubt that the Nyasa cichlid schools are of this heterogeneous type. On the other hand, if the schooling young lose parent-orientation *yet do not scatter,* an apparently rigidly developed behavioral pattern of cichlids would be broken. It would be interesting to know what really happens during the growth of these schooling, plankton-feeding, Nyasa forms. Until we do, Fryer's suggestion of behavioral neoteny must remain merely a suggestion, albeit a most interesting one.

Fryer's emphasis on the permanence of the lake environment, in contradistinction to the impermanence of the stream environment, as a factor in the extreme specialization of Nyasa cichlids, is a highly debatable point. That stream environments, and indeed, most lake environments, are usually transient, in comparison to the environments of older lakes, is true in one sense and not in another. Perhaps a better statement of ecological difference would be that lake habitats are seldom periodically disturbed (save in the shallows of fluctuating lakes), while those of streams usually are. Stream environments are often permanent, even in the geological sense, but are usually periodically disturbed, or even moved upstream or downstream, by floods and freshets. But the fishes, even very small species, survive these periodic disturbances, and, if displaced, return to the accustomed habitat. I have observed a small school of minnows (*Dionda episcopa*) in a small, disconnected, sandy pool in a Texas stream, seen the stream rise at that point to a swift torrent 12 feet deep and 200 feet wide after a desert rain, and returned two weeks later to see the same school in the same disconnected pool, including at least one identical individual seen before and recognizable by a fin-defect. Many North American fluviatile species are very narrowly adapted to sandbars, the gravel of riffles, or other distinctive biotopes, which, in country of low relief, may be exceedingly permanent features of the streams, in the geological sense. Moreover, the general fluviatile habitat of the larger tropical continental lowlands is usually accepted as one of the most permanent on earth.

That some of the narrow food and habitat specializations of Nyasa fishes is due in part to the lesser disturbance of lake habitats may be true, but Fryer's attempt to bolster this theory by a reference to the lack of environmental specialization amongst British freshwater fishes is most unfortunate. In fact, his statement that "freshwater fishes are, in general, relatively unspecialized" is simply not generally true except in what we might call atypical fish faunas. If he had compared the specializations of Nyasa cichlids with those of the fishes of the Zambesi River, from which much or all of the Nyasa fish fauna must have come, his comparison would have had some weight. That it is quite off the point is easily shown.

The fresh-water fish faunas of western Europe, Siberia, most of Canada, Alaska, Patagonia, and Chile are all depauperate, that of Britain especially so. Amongst the members of any such fish fauna, trophic and habitat specializations are almost invariably broad. In the far richer temperate fresh-water fish faunas (e.g., of north-central China, the rivers of Ohio or Virginia, or the vicinity of Buenos Aires) a river is usually inhabited by five or ten times as many species as those of a comparable British or Canadian stream, and many narrow habitat and trophic specializations are obvious. In tropical continental streams, the number of species is often or usually much larger than in any known lake fauna, and narrow trophic and habitat specializations are the rule. There are over 300 species of fishes in the Ganges Basin, probably over 200 in the Niger and 200 in the Nile (outside its headwater lakes), and perhaps over 500 in the Congo system (outside

Lake Tanganyika). In a minor South American river, the Essequibo in British Guiana, the fish-fauna probably totals between 250 and 300 species. Eigenmann got between 70 and 90 species in a single haul of a 100-foot seine in the Essequibo, and collected 60 species from a forest trickle too small to be called a brook (Eigenmann, 1912). After many years of study of the Amazonian fish-fauna, I estimate the number of fish species that must eventually be found to occur within a 20-mile radius of Manáus, in the lowlands of the middle Amazon, to be almost certainly in excess of 700. While I am unable to cite even approximate figures for the Zambesi and Shiré Rivers, from which Nyasa was populated, I do know that the Zambesi fish fauna is large, poorly explored, and poorly studied.

In fact, when the ecology of the fishes of the larger continental streams of tropical Africa, Asia, and America is carefully investigated, it seems certain that narrow specialization will be as clearly evident as it is amongst the endemic fishes of Nyasa and other large lakes. But the specializations will be found to be distributed through the species of many groups and not concentrated mostly in one dominant family, as they are in the larger endemic lake faunas (Myers, 1960).

One of the most promising future lines of attack on the problems of fish evolution in Nyasa would appear to be a careful investigation and comparison of the entire fish-fauna and its ecology with that of the Zambesi River, from which the lake was populated. The same may be said of Lake Tanganyika and the Upper Congo. Such a comparison ought to be exceedingly illuminating, and would provide a much better basis for theorizing than we now have.

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Serology in the Study of Fish Subpopulations.

Seminar by Steve Mathews, presented fall semester, 1961.

Abstract

In every animal group adequately investigated intraspecific differences in erythrocyte antigens have been found and there is good evidence that such differences are genetically controlled.

Fish are no exception. Cushing(1956) showed that different species of tunas have differing antigenic compositions, and Hildeman(1956) found that individual blood types in goldfish are genetically determined.

According to Dobzhansky races are subpopulations of a species which can be distinguished by different frequencies of variable alleles. Thus a way of distinguishing races is to find genetically controlled variable characters and determine whether frequencies of these variations between isolated subpopulations are significantly different.

Ridgway, Cushing, and Durall(1958) and Ridgway and Klontz(1960) have shown that erythrocyte antigens in at least two species of Pacific salmon may be useful variable characters in distinguishing geographically isolated races. Significantly differing frequencies of occurence of blood types in Sockeye salmon were shown from isolated Mendelian sub-populations.

Methods used to determine erythrocyte antigen variation in fishes include 1) immunization, utilizing various animals as the antibody producers, 2) isoimmunization, using the species to be tested as the antibody former, and 3) agglutiation, relying on the naturally occurring hemagglutinins or antibodies which agglutinate red blood cells.

Serology in racial studies is a pioneer field with some obvious limitations. It is not the answer to the taxonomist, but it may become a further tool to be used in conjunction with morphologic and behavioral considerations.

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EVOLUTIONARY GENETICS OF CAVE-DWELLING FISHES OF THE GENUS *ASTYANAX*

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Received July 13, 1971

Attempts to understand evolutionary processes in cave organisms have been largely limited to speculation on the causes of loss of photoreceptor organs and pigmentation, increase in size and complexity of tactile sensory structures, and certain modifications in physiology (see review in Barr, 1968). Little is known of the population genetics of cave organisms or of the genetic changes accompanying the transformation of epigean (surface-dwelling) forms to troglobites (obligate cavernicoles). Yet genetic information is essential to the confident development of theories of troglobite evolution. For this reason, we have compared the genic character and degree of variability in troglobitic and epigean populations of the characid fish *Astyanax mexicanus* in Mexico.

Students of cave biology have generally accepted the thesis that the ancestors of many troglobites entered caves as troglophiles (facultative cavernicoles) before the end of the Pleistocene, and became isolated with the local extinction of surface populations as a result of climatic changes associated with glaciation (Barr, 1968). If this is true, many troglobites have been living in an environment of darkness, silence, nearly constant temperature, and relative biotic simplicity for periods ranging from 10,000 to 2,000,000 years. And if cave environments are in fact relatively stable and uniform temporally and spatially, according to both the niche width and the gene flow hypotheses (see discussion in Soulé, 1971), troglobites would be expected to maintain relatively low levels of genetic variability. But alternative reasoning yields a different expectation: assuming that few organisms are able to adapt to cave life, the relatively depauperate nature of cave faunas should permit troglobites to expand their niches and exploit resources that would under normal circumstances be utilized by two or more species (ecological release). In this case, one might expect relatively high levels of genetic variability to be maintained.

With regard to genetic variability in troglobites, two hypotheses are current. Barr (1968) suggests that there is an initial loss of genetic variability in cave populations at the time of separation from epigean populations, due to reductions in population size. And he further visualizes "genetic revolutions" (Mayr, 1954, 1963) occurring in the small founder populations isolated in caves, followed by a reconstruc-

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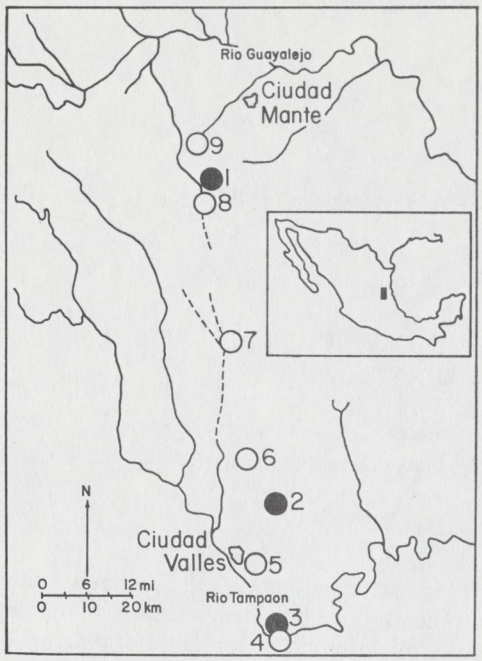

FIG. 1. Sample localities for *Astyanax mexicanus* in northeastern Mexico. Localities numbered according to Table 1. Open circles are surface localities; closed circles are caves.

tion of the epigenotype as the troglobite gradually regains normal levels of genetic variability. However, Poulson and White (1969) believe that low levels of genetic variability are consistently maintained as a result of strong stabilizing selection in the cave environment.

Considering the generally small size of cave populations of many organisms, particularly vertebrates, it seems probable that stochastic processes play an important role in the evolution of cave populations, yet they have received little attention from students of cave biology. Culver's (1970) discussion of chance factors affecting the diversity of species numbers in caves brings to mind possibilities of stochastic processes influencing the composition of gene pools as well. In particular, genetic drift may be expected to reduce variability in many cave populations.

Employing electrophoretic techniques to detect allelic variation at structural gene loci encoding proteins, we have sought to determine (1) whether troglobitic populations of *Astyanax* are indeed less variable genetically than populations of closely related surface forms; (2) if cave populations are relatively monomorphic, whether the decrease in variation is best attributed to environmental stability or to drift; and (3) the degree of genetic modification involved in the evolution of troglobitic forms from their epigean ancestors. Additionally, this research has yielded the first estimate of the degree of genic variability in populations of a fish, based on a random sample of structural gene loci.

THE *ASTYANAX* POPULATIONS

Collecting Localities

Information on collecting localities and samples is presented in Table 1 and Figure 1. A total of 136 fish was collected in three caves in the area around Ciudad Valles, San Luis Potosi, and Ciudad Mante, Tamaulipas, Mexico. These and other caves in the area and their inhabitants have been described by several authors (see Breder, 1942; Sadoglu, 1956; McKenzie, 1965; Russell and McKenzie, 1965; and Russell and Raines, 1967). The three caves are located on a line extending north from the Rio Tampaon, one of the largest rivers in the area.

Chica Cave, the type locality of "*Anoptichthys jordani*" (Hubbs and Innes, 1936), is about one-half mile from the Rio Tampaon and contains fish showing the full range of variation from eyeless and unpigmented to fully eyed and darkly pigmented. This linear cave is 750 feet long and contains several travertine pools, some of which are connected; the largest is about 50 feet long, 30 feet wide, and 10 feet deep.

Los Sabinos Cave is 15 miles north of Chica Cave. The fish in this cave, which were originally described as "*Anoptichthys hubbsi*" (Alvarez, 1947), are uniformly eyeless and unpigmented. Speleologists have mapped an extensive network of 3000

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Phosphoglucose Isomerase Gene Duplication in the Bony Fishes: An Evolutionary History

John C. Avise[1,2] and G. Barrie Kitto[3]

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Electrophoretic patterns of phosphoglucose isomerase (PGI) in bony fishes provide strong evidence for a model of genetic control by two independent structural gene loci, most likely resulting from a gene duplication. This model is confirmed by a comparison of certain kinetic and molecular properties of the PGI homodimers (PGI-1 and PGI-2) isolated from extracts of the teleost Astyanax mexicanus. *In addition, in most higher teleosts examined, the PGI enzymes show a regular pattern of tissue distribution, with PGI-2 predominant in muscle, the heterodimer often strongest in the heart, and PGI-1 predominant in liver and other organs. An examination of 53 species of bony fishes belonging to 38 families indicates a widespread occurrence of duplicate PGI loci and an early origin of the gene duplication, perhaps in the Leptolepiformes. The apparent presence of three PGI loci in trout and goldfish exemplifies how new loci can be incorporated into the genome through polyploidization.*

INTRODUCTION

Almost all vertebrates and invertebrates thus far studied possess only a single phosphoglucose isomerase (PGI) gene locus: man (Detter *et al.*, 1968), deer (Ramsey *et al.*, 1972), rodents (Carter and Parr, 1967; Selander

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et al., 1969; DeLorenzo and Ruddle, 1969; Johnson and Selander, 1971; Johnson *et al.*, 1972), birds (Nottebohm and Selander, 1972), lizards (Webster *et al.*, 1972), frogs (Ralin *et al.*, 1972), horseshoe crabs (Selander *et al.*, 1970), fiddler crabs (Selander *et al.*, 1972), and certain insects (Avise and Selander, 1972; Ramsey and Avise, unpublished).

Yoshida and Carter (1969) observed multiple electrophoretic bands of phosphoglucose isomerase, representing at least two structurally different isozymes, in rabbit hemolysates and muscle extracts. However, the number of genetic loci controlling PGI was not determined since all specimens showed identical patterns on the gels. Similarly, the genetic basis of three isozymes observed by Nakagawa and Noltmann (1967) in brewers and bakers yeast was not determined.

Avise and Selander (1972) describe allozymic patterns of phosphoglucose isomerase in a characid fish (*Astyanax mexicanus*) that are consistent with a model of control by two independent structural gene loci, most likely resulting from a gene duplication. In this paper, we present the evidence on which this conclusion is based, including an analysis of certain kinetic and molecular properties of the PGI homodimers in *A. mexicanus*. On the basis of a study of the tissue distribution and occurrence of multiple PGI loci in a wide variety of bony fishes (Osteichthyes), we propose a hypothesis of the time of origin of the gene duplication and the course of the divergent specialization of the homodimeric and heterodimeric enzyme forms.

MATERIALS AND METHODS

Specimens of *A. mexicanus* used in the extraction of PGI for characterization were collected in Cueva del Pachon, near Ciudad Mante, Tamaulipas, Mexico (see Avise and Selander, 1972). Other species were collected in Texas and Massachusetts or purchased from commercial dealers. All fish were stored on dry ice immediately after capture.

Fructose 6-phosphate (F6P), glucose 6-phosphate dehydrogenase (G6PDH), triphosphopyridine nucleotide (NADP), phenazine methosulfate (PMS), MTT tetrazolium (MTT), 6-phosphogluconic acid, and EDTA disodium salt were obtained from Sigma Chemical Company, phosphoenol pyruvic acid from Calbiochem, adenosine 5′-triphosphate from Pabst Laboratories Biochemical, and Electrostarch lot 171 from Otto Hiller, Madison, Wisconsin. Other chemicals were of reagent grade.

Starch Gel Electrophoresis

Extracts for electrophoresis were prepared by homogenizing the whole animal or tissue in a glass tissue grinder with an equivalent volume of buffer

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BIOCHEMICAL GENETICS OF SUNFISH. II. GENIC SIMILARITY BETWEEN HYBRIDIZING SPECIES

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Recent improvements in electrophoretic and biochemical staining techniques have allowed a quantification of levels of genic similarity between various taxa and have stimulated renewed interest in the role of speciation in converting intraspecific to interspecific variation (Lewontin 1967). Mutationists in the early 1900s claimed that certain types of single mutations could give rise to new species. However, an examination of the tremendous amount of variability in interspecific F_2 hybrids soon showed that even closely related species could differ at a large number of genetic loci (Harland 1936; Sumner 1932; Darlington 1940; Timofeeff-Ressovsky 1940). Electrophoretic studies have generally supported the latter observation. Populations belonging to closely related species usually show differences in allelic composition at a far higher percentage of genetic loci than conspecific populations. Studies on sibling species of *Drosophila* (Hubby and Throckmorton 1965; Nair et al. 1971; Ayala et al. 1970), *Peromyscus* (Smith, Selander, and Johnson, in preparation), kangaroo rats (*Dipodomys*) (Johnson and Selander 1971), and cotton rats (*Sigmodon*) (Johnson et al. 1972) indicate that even sibling species show major differences at between 20% and 50% or more of their genetic loci and that "in spite of their morphological and ecological similarity and their evolutionary affinity these species have very different gene pools" (Ayala et al. 1970). These and similar studies provide a base with which genic similarities between other species may be compared.

In this study, we examine protein differences in a group of fishes whose propensity to hybridize is well known. Natural interspecific hybridization is more common in fishes than in any other group of vertebrates (Lagler et al. 1962), and no group of fishes is more renowned for its ability to hybridize (with the possible exception of certain cyprinids) than are the centrarchids and particularly species of *Lepomis* (Birdsong and Yerger 1967). Theoretically the 11 species of *Lepomis* can produce 55 F_1 interspecific hybrid types, and of these, at least 21 have been found in nature (Childers 1967). Artificially raised hybrids are frequently fertile (West 1970), and Hubbs (1955) has noted that introgressive hybridization may occasionally occur in nature.

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We examined electrophoretic variation in proteins encoded by 18 loci in 10 of the 11 species of *Lepomis* (sunfish family Centrarchidae). Our primary objective is to determine whether ability and propensity for *Lepomis* species to hybridize are indicative of close similarity at the protein level, relative to previously published estimates of genic similarities between other nonhybridizing congeners. Since introgressive hybridization between *Lepomis* species is thought to be rare in nature, if it occurs at all, we clearly cannot test whether introgressive hybridization causes an increase in genic similarity between congenerics. We can examine whether close genic similarity is a necessary corollary of hybridizing ability.

Second, we apply data on protein variation within and among species of *Lepomis* to problems of systematics in the genus. A dendrogram based on a cluster analysis of genic similarity coefficients among *Lepomis* species is compared to published relationships of sunfish based on the acousticolateralis system, general morphology, karyotypes, and hybridization success.

MATERIAL AND METHODS

The Family Centrarchidae

The sunfish family Centrarchidae represents a natural assemblage of fishes which most likely originated in the Mississippi River Basin (Branson and Moore 1962) very early in the Cenozoic (Schlaikjer 1937). Although the fossil record indicates that centrarchids occupied an extensive range including most of North America during the Miocene and Pliocene (Schlaikjer 1937; Miller 1958), they are now largely restricted to the eastern and central United States. Of the nine genera within Centrarchidae, *Lepomis* has the most species.

Species of *Lepomis* were originally described on the basis of external morphology. Adults of all 11 species are easily distinguished and generally remain morphologically distinct throughout their respective ranges. Studies on premating and postmating isolating mechanisms (West 1970; Gerald 1971; Keenleyside 1967; Clark and Keenleyside 1967; Merriner 1971*a*, 1971*b*; Childers 1967; Whitt et al. 1973) confirm that biological species concepts fully agree with the species limits described by morphologists for *Lepomis*. With the possible exception of the *L. marginatus–L. megalotis* complex (see discussion), there is no doubt that members of *Lepomis* are true biological species, despite their ready ability to form F_1 hybrids.

Analyses

Results of this study are based on analyses of variability in proteins in 1,099 specimens of 10 species of *Lepomis* (table 1). In a previous study (Avise and Smith 1974) we examined patterns of geographic variation in 2,415 bluegills (*L. macrochirus*) from seven southern states. Because genetic similarity values were high between all bluegill populations, three bluegill

GENE FREQUENCY COMPARISONS BETWEEN SUNFISH (CENTRARCHIDAE) POPULATIONS AT VARIOUS STAGES OF EVOLUTIONARY DIVERGENCE

JOHN C. AVISE AND MICHAEL H. SMITH

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[Mysid = Shrimp]

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May 16, 1979

- Pend Oreille
- Flathead
- Priest
- Cour d'Alem

Mysid Research Group
Communication #3

Enclosed is the new MRG membership list. As you can see, the group is large and quite diverse in interests. Please check your name and address for accuracy. Also, it would be nice to include an area of interest for each person. This will aide in the identification of other group members who may be pursuing similar lines of research.

The organizers of the ASLO Stoney Brook meeting in June apparently did not feel our request for a mysid symposium deserved a response. I did not find out for sure that there would be no symposium until I received the program announcement in March. They never even acknowledged receipt of the request. I had hoped to be able to announce a time and a place for an MRG meeting in this letter, but our request for this was also ignored. Al Beeton has agreed to arrange a meeting after he arrives at Stoney Brook. Contact him or watch the bulletin boards for details.

Because of my impending move to the University of Texas Marine Science Laboratory at Port Aransas, I do not believe I will be able to go to Stoney Brook. I would, however,suggest the group make a concerted effort to explore the possibilities of scheduling a symposium sometime during the next year, possibly at the 1980 ASLO or SIL meeting. Last year at Victoria, I discussed the need for a better understanding of mysid biology and how we hoped to organize a mysid symposium with Dr. Stevenson, the editor of the Journal of the Fisheries Research Board of Canada. He indicated that JFRBC would be very interested in publishing the proceedings of sych a symposium, subject of course to technical review. I believe this presents us with a unique opportunity to significantly advance current knowledge of mysid biology. I hope we can take advantage of it.

Notes:

-- Dr. Mihai Bacescu has produced numerous papers on the zoogeography and ecology of tropical mysids and is willing to send reprints to anyone who is interested.

-- Dr. John Mauchline has just completed a large review entitled "The Biology of Mysids" which will be published in "Advances in Marine Biology" (F.S. Russell and C.M. Yonge, eds.). It should come out in the autumn of this year.

Have a good summer,

Mark

Mark Morgan

Encl.

ms P.S. Let me know if you are interested

Mysid Research Group - 1979

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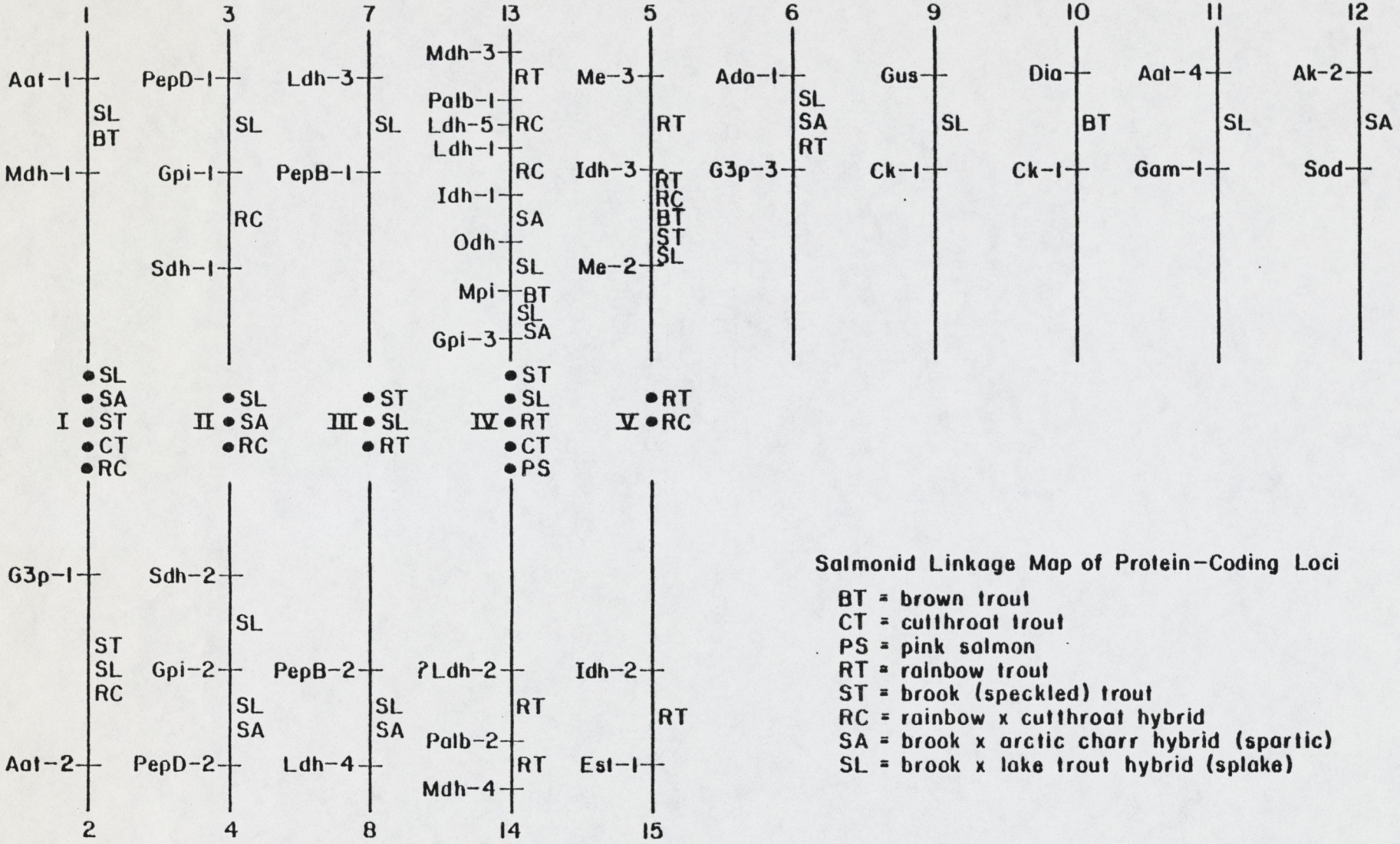
genetic maps 1984

A COMPILATION
OF LINKAGE
AND RESTRICTION MAPS
OF GENETICALLY
STUDIED ORGANISMS
Volume 3

Stephen J. O'Brien, EDITOR

Laboratory of Viral Carcinogenesis
National Cancer Institute

COLD SPRING HARBOR LABORATORY
1984



Salmonid Linkage Map of Protein-Coding Loci

BT = brown trout
CT = cutthroat trout
PS = pink salmon
RT = rainbow trout
ST = brook (speckled) trout
RC = rainbow x cutthroat hybrid
SA = brook x arctic charr hybrid (spartic)
SL = brook x lake trout hybrid (splake)

Figure 1. Classical linkage groups (Arabic numerals) and pseudolinkage groups (Roman numerals) in salmonid species and hybrids shown in legend.

LINKAGE AND PSEUDOLINKAGE GROUPS IN SALMONID FISHES (TROUT, CHARR, SALMON)

December, 1983

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A few individuals in a few laboratories have examined joint segregation of isozyme loci to establish linkage groups in a limited number of species and fertile hybrids of the tetraploid derivative salmonid fishes. The trouts studied have been cutthroat (Salmo clarki; 2n=64, 66, or 68), rainbow (S. gairdneri; 2n=58-62) plus hybrids between these species, and brown (S. trutta; 2n=80). The charrs studied include brook or speckled trout (Salvelinus fontinalis; 2n=84), lake trout (S. namaycush; 2n=84), and arctic charr (S. alpinus; 2n=80) plus hybrids among these species. Limited studies of salmon include pink salmon (Oncorhynchus gorbuscha; 2n=52) and Atlantic salmon (Salmo salar; 2n=54-60). Tentative assignment of loci, relative to one another, to chromosomes of this group of fishes is shown in Figure 1. The assignments are based on published data from other labs and on published and unpublished results from our laboratory, as shown in Tables I and II.

Fifteen classical linkage groups have been established among salmonid species and hybrids; since the linkage relationships appear to be highly conserved among species, these are grouped and shown in Table I and in Figure 1. Note that recombination values are generally greater in females than in males and that members of duplicated loci (relics of tetraploidization) belong to different classical linkage groups. Correct order of linked loci is difficult to determine when data are available only from male parents (for example, Idh-1, Ldh-1 and Ldh-5 data from male rainbow x cutthroat hybrids). Presumably, this is because of structural constraints imposed on crossing over by multivalent pairing involving metacentric (centrically fused) chromosomes (16). Gene-centromere mapping in females (13) promises more accurate placement of loci.

However, in hybridized genomes (strain or species hybrids) duplicated loci, as well as diploidized single loci, belonging to different linkage groups, often show an exceptional pattern of nonrandom assortment (pseudolinkage) in males. That is, nonparental (recombinant) gametes are formed in excess of parental ones. Five such pseudolinkage groups have been found; since they also are conserved among species, each is grouped and shown in Table II and in Figure 1. Pseudolinkage has been proposed (16) to be based on facultative multivalent pairing of metacentric chromosome arms with homoeologous arms of other chromosomes in male salmonids, as contrasted to strictly bivalent pairing in females (3, 5, 16).

The fifteen linkage groups and five pseudolinkage groups assume the map configurations shown in Figure 1. Species and hybrids examined (symbols in lower right of figure) to establish linked loci are indicated in the regions between loci; those of males exhibiting pseudolinkage are placed aside dots as associating homoeologous linkage groups. While both types of linkage relations appear to be highly conserved among species, the loci Ck-1 and Dia were tightly linked in Salmo trutta but randomly assorted in Salvelinus hybrids. Such results are not unexpected since all salmonids have relatively the same chromosome arm numbers but quite different 2n numbers, reflecting different numbers of centric fusions during chromosomal diploidization.

Table I. Classical Linkage Groups (Chromosomes) in Salmonidae.

	Linkage Group	$\hat{r}$ (2SE)	Sex	Species	Reference
1.	Aat-1 - Mdh - (1,2)				
	Aat-1 with Mdh-(1,2)	.04 (.02)	♂	Splake	May et al. 1980; unpublished
	Aat-1 with Mdh-(1,2)	.34 (.06)	♀	Splake	unpublished
	Aat-1 with Mdh-(1,2)	.03 (.02)	♂	Brown	Taggert 1981
	Aat-1 with Mdh-(1,2)	.20 (.11)	♀	Brown	Taggert 1981
2.	Aat-2 - G3p-1				
	Aat-2 with G3p-1	.11 (.04)	♂	Brook	May et al. 1980 Wright et al. 1980
	Aat-2 with G3p-1	.09 (.04)	♂	Splake	May et al. 1980; unpublished
	Aat-2 with G3p-1	∿.5 (random)	♀	Splake	unpublished
	Aat-2 with G3p-1	.20 (.05)	♂	Cutbow	unpublished
3.	Gpi-1 - PepD-1 - Sdh-1				
	Gpi-1 with PepD-1	.04 (.02)	♂	Splake	Hollister et al.
	Gpi-1 with PepD-1	.05 (.07)	♀	Splake	Hollister et al.
	PepD-1 with Sdh-1*	.02 (.02)	♂	Cutbow	unpublished
4.	Gpi-2 - PepD-2 - Sdh-2**				
	Gpi-2 with PepD-2	.04 (.03)	♂	Splake	Hollister et al.
	Gpi-2 with PepD-2	.00 (.05)	♀	Splake	Hollister et al.
	Gpi-2 with Sdh-2	.04 (.02)	♂	Splake	Hollister et al.
	Gpi-2 with Sdh-2	.11 (.08)	♀	Splake	Hollister et al.
	Gpi-2 with Sdh-2	.11 (.10)	♀	Sparctic	unpublished
	PepD-2 with Sdh-2	.01 (.01)	♂	Splake	Hollister et al.
	PepD-2 with Sdh-2	.13 (.03)	♀	Splake	Hollister et al.
5.	Idh-3 - Me-2 - Me-(3,4)				
	Idh-3 with Me-2	.04 (.01)	♂	Splake	Stoneking et al. 1979 May et al. 1980
	Idh-3 with Me-2	.08 (.03)	♀	Splake	May et al. 1980
	Idh-3 with Me-2	.02 (.04)	♂	Brook	unpublished
	Idh-3 with Me-2	.10 (.07)	♀	Brown	unpublished
	Idh-3 with Me-2	.02 (.02)	♂	Rainbow	May et al. 1982
	Idh-3 with Me-2	.06 (.04)	♂	Cutbow	unpublished
	Idh-3 with Me-(3,4)	.00 (.05)	♂	Rainbow	unpublished
6.	Ada-1 - G3p-3				
	Ada-1 with G3p-3	.01 (.01)	♂	Splake	May et al. 1980; unpublished
	Ada-1 with G3p-3	.15 (.06)	♀	Splake	May et al. 1980
	Ada-1 with G3p-3	.03 (.03)	♂	Sparctic	unpublished
	Ada-1 with G3p-3	.12 (.11)	♀	Sparctic	unpublished
	Ada-1 with G3p-3	.04 (.02)	♂	Rainbow	May et al. 1982; unpublished
7.	Ldh-3 - PepB-1				
	Ldh-3 with PepB-1	.00 (.05)	♂	Splake	unpublished
	Ldh-3 with PepB-1	.08 (.09)	♀	Splake	unpublished
8.	Ldh-4 - PepB-2				
	Ldh-4 with PepB-2	.06 (.05)	♂	Splake	unpublished
	Ldh-4 with PepB-2	.00 (.05)	♀	Splake	unpublished
	Ldh-4 with PepB-2	.04 (.03)	♂	Sparctic	unpublished
	Ldh-4 with PepB-2	.02 (.02)	♀	Sparctic	unpublished
9.	Gus - Ck-1				
	Gus with Ck-1	.09 (.07)	♂	Splake	May et al. 1980
	Gus with Ck-1	.20 (.07)	♀	Splake	May et al. 1980

Table I. Continued.

	Linkage Group	$\hat{r}$	(2SE)	Sex	Species	Reference
10.	Dia - Ck-(1,2)					
	Dia with Ck-(1,2)	.00	(.06)	♂	Brown	unpublished
	Dia with Ck-1	∿.5	(random)	♂	Splake	May et al. 1980; unpublished
	Dia with Ck-1	∿.5	(random)	♀+♂	Sparctic	unpublished
	Dia with Ck-2	∿.5	(random)	♂	Splake	unpublished
11.	Aat-4 - Gam-1					
	Aat-4 with Gam-1	.09	(.08)	♂	Splake	May 1980
12.	Ak-2 - Sod					
	Ak-2 with Sod	.32	(.08)	♂	Sparctic	unpublished
	Ak-2 with Sod	.08	(.05)	♀	Sparctic	unpublished
13.	Mdh-3 - Palb-1 - Idh-1 - Ldh-1 - Odh - Mpi - Gpi-3					
	Mdh-3 with Palb-1	.02	(.03)	♂	Rainbow	unpublished
	Mdh-3 with Palb-1	.03	(.04)	♀	Rainbow	unpublished
	Palb-1 with Idh-1	.08	(.04)	♂	Cutbow	unpublished
	Palb-1 with Ldh-1	.07	(.05)	♂	Cutbow	unpublished
	Idh-1 with Ldh-1	.01	(.01)	♂	Cutbow	unpublished
	Mpi with Gpi-3	.03	(.06)	♂	Brown	unpublished
	Mpi with Gpi-3	.10	(.07)	♂	Sparctic	unpublished
	Mpi with Gpi-3	.01	(.01)	♂	Splake	May et al. 1980; unpublished
	Mpi with Gpi-3	.30	(.10)	♀	Splake	May et al. 1980
	Mpi with Odh	.19	(.09)	♀	Splake	May et al. 1980
	Odh with Gpi-3	∿.5	(random)	♀	Splake	May et al. 1980
	Mpi with Ldh-1	.01	(.02)	♂	Sparctic	unpublished
	Gpi-3 with Ldh-1	.06	(.03)	♂	Sparctic	unpublished
	Gpi-3 with Ldh-1	∿.5	(random)	♀	Sparctic	unpublished
	Mdh-3 with Mpi	∿.5	(random)	♀	Splake	May et al. 1980
	Gpi-3 with Ldh-5	∿.5	(random)	♂	Brown	Taggert 1981
	Ldh-1 with Ldh-5	.00	(.02)	♂	Cutbow	unpublished
	Ldh-5 wtih Mdh-3	.05	(.03)	♂	Rainbow	unpublished
	Ldh-5 with Palb-1	.02	(.03)	♂	Rainbow	unpublished
14.	Mdh-4 - Palb-2					
	Mdh-4 with Palb-2	.03	(.04)	♂	Rainbow	unpublished
15.	Idh-2 - Est-1					
	Idh-2 with Est-1	.09	(.05)	♀	Rainbow	Thorgaard et al. 1983

*Arbitrary locus numbers; could be PepD-2 with Sdh-2.

**Only one Sdh locus is expressed in Salvelinus (arbitrarily designated Sdh-2).

Table II. Pseudolinkages in Salmonidae.

Pseudolinkage	$\hat{r}$ (2SE) in ♂♂[1]	Sexes[2] Tested	Species	Reference
I. Linkage Group 1 Pseudolinked with Linkage Group 2				
Aat-1 with G3p-1	.79 (.04)	♀ + ♂	Splake	May et al. 1980; unpublished
Aat-1 with G3p-1	.68 (.11)	♀ + ♂	Sparctic	unpublished
Aat-1 with G3p-1	.69 (.08)	♀ + ♂	Brook	Wright et al. 1980
Aat-1 with G3p-1	.60 (.06)	♂	Cutbow	unpublished
G3p-1 with Mdh-1	.74 (.03)	♀ + ♂	Splake	May et al. 1980; unpublished
G3p-1 with Mdh-1	.61 (.07)	♀ + ♂	Sparctic	unpublished
Aat-1 with Aat-2	.86 (.08)	♀ + ♂	Splake	May et al. 1980; unpublished
Aat-1 with Aat-2	.72 (.06)	♀ + ♂	Brook	Wright et al. 1980
Aat-1 with Aat-2	.92 (.06)	♂	Sparctic	unpublished
Aat-1 with Aat-2	.82 (.05)	♀ + ♂	Cutthroat	Allendorf & Utter 1976 Wright et al. 1982
Aat-1 with Aat-2	.85 (.02)	♀ + ♂	Cutbow	Wright et al. 1982; unpublished
Aat-2 with Mdh-1	.71 (.10)	♀ + ♂	Splake	May et al. 1980; unpublished
II. Linkage Group 3 Pseudolinked with Linkage Group 4				
PepD-1 with PepD-2	.86 (.04)	♀ + ♂	Splake	Hollister et al.
PepD-1 with Gpi-2	.83 (.05)	♀ + ♂	Splake	Hollister et al.
PepD-1 with Sdh-2	.83 (.05)	♀ + ♂	Splake	Hollister et al.
PepD-1 with Sdh-2	.96 (.03)	♀ + ♂	Sparctic	unpublished
PepD-1 with Sdh-2	.84 (.08)	♂	Cutbow	unpublished
Sdh-1 with Sdh-2	.84 (.08)	♂	Cutbow	unpublished
III. Linkage Group 7 Pseudolinked with Linkage Group 8				
Ldh-3 with Ldh-4	.80 (.02)	♀ + ♂	Splake	Morrison 1970; Davisson et al. 1973; May et al. 1980
Ldh-3 with Ldh-4	.52 (.05)	♀ + ♂	Brook	Davisson et al. 1973
Ldh-3 with Ldh-4	.59 (.03)	♀ + ♂	Rainbow	Wright et al. 1975
IV. Linkage Group 13 Pseudolinked with Linkage Group 14				
Mdh-3 with Mdh-4	.53 (.08)	♂	Brook	May et al. 1979
Mdh-3 with Mdh-4	.89 (.02)	♀ + ♂	Splake	May et al. 1979, 1980
Mdh-3 with Mdh-4	.73 (.05)	♂	Rainbow	May et al. 1982; unpublished
Mdh-3 with Mdh-4	.90 (.08)	♂	Pink Salmon	Aspinwall 1974
Mdh-4 with Ldh-1	.56 (.08)	♂	Cutthroat	unpublished
Palb-1 with Palb-2	.59 (.10)	♀ + ♂	Rainbow	unpublished
V. Linkage Group 5 Pseudolinked with Linkage Group 15				
Idh-2 with Idh-3	.67 (.11)	♀ + ♂	Rainbow	May et al. 1982; unpublished
Idh-2 with Idh-3	.84 (.06)	♂	Cutbow	unpublished
Idh-2 with Me-2	.65 (.11)	♂	Rainbow	May et al. 1982; unpublished
Idh-2 with Me-2	.83 (.05)	♂	Cutbow	unpublished

[1] Values for species hybrids were calculated from F_1's only.

[2] All females tested showed random assortment.

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Linkage associations in hybridized *Salvelinus* genomes

The duplicate loci encoding pepidase-D and glucosephosphate isomerase and the unduplicated sorbitol dehydrogenase locus

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ABSTRACT: Electrophoresis was performed on parents and progeny of 21 families of tetraploid derivative *Salvelinus* species. Variable phenotypes were shown for the duplicate loci encoding the enzyme peptidase D in lake (*S. namaycush*) and brook trout (*S. fontinalis*) and in the fertile hybrid (splake) between them. Pairwise examinations of joint segregation between the duplicated *PepD* loci and 23 other biochemical loci were performed. Nonrandom assortment was found among progeny of parents doubly heterozygous for the *PepD-1* and *PepD-2* loci, the duplicate loci encoding glucosephosphate isomerase (*Gpi-1* and *Gpi-2*) and the single locus encoding sorbitol dehydrogenase (*Sdh*). Two classical linkage groups were found among the uniquely marked loci; *PepD-1* with *Gpi-1* and *PepD-2* with *Gpi-2* with *Sdh*. Pseudolinkage among loci in the two different groups was found in males. The results are shown to fit a chromosomal model involving preferential tetravalent pairing of homoeologous chromosomes. The linkage of *PepD* and *Gpi* loci is consistent with that reported in a variety of vertebrate species and indicates the conservation of this linkage group over taxa separated by at least 300 million years of evolution.

OF THOSE FAMILIES belonging to the suborder Salmonoidea, trout and salmon (family Salmonidae), whitefish (Coregonidae), and graylings (Thymallidae) show extensive evidence for being derivatives of a tetraploid lineage[34]. Ohno[30–32] suggested that this tetraploidization was a relatively recent event (50 million years ago) and transpired after the one occurring early (500 million years ago) in the evolution of vertebrates. The evidence supporting the tetraploid origin of the salmonids is that their DNA content per cell and their chromosome arm number are twice that of their close diploid relatives, the smelts (family Osmeridae). Further, a great amount of gene duplication has been reported in salmonoid fish[5,10,22] compared to their diploid counterparts; however, disomic modes of inheritance were earlier considered to be the norm for duplicated loci[4,5,8,10,45].

It has been reported that at least 50 percent of the ancestral gene duplication has been lost in the salmonids[3,5,22]. This genic diploidization is considered to be due to the silencing (by deletions, mutations, regulators, etc.) of redundant copies of loci subsequent to their duplication[32]. The primary means of achieving chromosomal diploidization of the tetraploid salmonid genome appears to have been Robertsonian fusions of acrocentric chromosomes[34]. Evidence for this view includes reports of intraspecific Robertsonian polymorphisms[12,13,33,42,43], Robertsonian polymorphisms among cells within individuals[8,33,36,37,43], but relatively constant arm numbers with highly variable $2n$ numbers among salmonids[1,8,12,13,15,18,29,33,37,39,43].

As a result of centric fusions, one would expect a high frequency of observed linkage associations. If fusions of homologous chromosomes occurred, then one would expect duplicated genes to be linked more often than if fusions of nonhomologous chromosomes occurred. Failure to show classical linkage between the members of a number of duplicate loci supports the hypothesis of nonhomologous fusions[2,8,11,23–26,28,41,45–47].

Further evidence for the occurrence of Robertsonian fusions is the formation of multivalents in males of all salmonid

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species[8,12,17,33,46,47]; the types of multivalents found in some salmonids (rod tetravalents consisting of two metacentric and two acrocentric chromosomes) further supports the view that nonhomologous fusions occurred[17,29,47].

The formation of multivalents, along with reports of finding unexpected progeny types in very low frequencies among progeny of heterozygous males (apparently due to chromatid segregation or double reduction)[4,7,26,46], is believed to reflect "residual tetrasomy"[24,47]. This residual tetrasomy would prevent duplicate loci located distal from the centromere from becoming fixed for different alleles; fixation could occur if only disomic inheritance were occurring. Since certain duplicated loci, e.g., *Aat-(1,2)*, *Mdh-(3,4)*, and *Palb-(1,2)*, still are not fixed, but rather share alleles, the phenomenon of residual tetrasomy seems to be fairly extensive. Therefore, it appears that salmonid genomes are still in the process of evolution with respect to both chromosomal and genic diploidization.

Another genetic phenomenon, a form of residual tetrasomy, that gives further evidence of the continued evolution of salmonid genomes is the occurrence of pseudolinkage. Pseudolinkage in trout is characterized by an aberrant pattern of nonrandom assortment of pairs of loci in males with the nonparental progeny types being in excess of parental ones; sibling females show random assortment of these same loci[8,24–26,28,45–47]. The phenomenon appears to be due to multivalent pairing of homoeologous chromosome arms in males[46,47].

The present paper reports instances of nonrandom assortment, including cases of both classical linkage and pseudolinkage, among the duplicated loci that code for forms of the enzymes glucosephosphate isomerase (GPI-1,2; EC 5.3.1.9) and peptidase-D (PEPD-1,2; EC 3.4.13.9) and for a single locus coding for a form of sorbitol dehydrogenase (SDH; EC 1.1.1.14). The inheritance of the respective gene loci, *Gpi-(1,2)*, *Pep D-(1,2)*, and *Sdh*, in brook trout (*Salvelinus fontinalis*), lake trout (*S. namaycush*), and the fertile hybrid (splake) between these two species supports and extends the views on behavior and evolution of the salmonid genomes cited above. Linkage of the uniquely marked members of the duplicated loci for GPI/PEP-D probably represents a case of fish-mammal linkage conservation.

Materials and Methods

Single-pair matings involving lake, brook, splake and recurrent backcrosses of splakes and brooks were made in the fall of 1980, 1981, and 1982. Sources of the parents of each family are listed in Table I. The matings made in 1980 resulted in progeny designated as *A* lots; those made in 1981 and 1982 are designated *B* and *C* lots, respectively. Ripe fish were stripped of their gametes that were stored at 5°C until fish were typed electrophoretically for isozyme phenotypes. Gametes were then combined within 12 hours, based on electrophoretic results, with the parents. Progeny were reared in the Pennsylvania Fish Commission's Upper Spring Creek Hatchery and were typed electrophoretically in the spring of 1981, 1982, and 1983.

Tissue extractions, horizontal starch gel procedures, and staining recipes used for all isozymes except PEP-D were described by May et al.[23]. Eye extracts gave the best resolution for PEP-D when the gel buffer system described by Markert and Faulhaber[19] was used. An electric potential of 250°C (maximum of 75 mA) was applied across chilled gels for 3½ to 4 hours. The stain buffer used was the gel buffer described by Ridgway et al.[35]. The staining mixture, consisting of 10 ml stain buffer, 50 mg phenylalanyl-proline, 20 mg peroxidase, 10 mg amino acid oxidase, and 5 mg O-dianisidine was adapted from Harris and Hopkinson[14]. This mixture was combined with 10 ml of 2 percent agar maintained at 60°C, and poured over the gel slice[6].

The nomenclature of isozymes and gene loci follows that used by May et al.[23] with such modifications as those proposed by May[22]. While there are a number of specific and nonspecific peptidases, the form studied here, proline dipeptidase or peptidase-D, is specific for dipeptides having proline or hydroxyproline as their carboxyterminal amino acid[14].

The statistical treatment of the data for testcross and single backcross families was described by May et al.[23], as adopted from Mather[20]; the heterogeneity chi-square values were calculated according to those of Mather[20].

Results

Genetic variation of PEP-D, GPI, and SDH in Salvelinus

Electrophoresis of lake trout eye extracts revealed a single three-banded phenotype for PEP-D in two individuals examined. All brook trout showed a single-banded phenotype, the band migrating anodally from those of lake trout. The genotypes, confirmed by inheritance results, are designated as follows: brook trout = *AAAA*; lake trout = *A′A′A″A″*; and splakes = *AA′AA″*. The *AAA′A′* or *AAA″A″* genotypes were never found in F_1 splakes. Also, when a recurrent backcross or advanced generation individual of genotype *AA′A′A″* was crossed to *AAAA* brook trout, all progeny types were either *AAAA′* or *AAA′A″*, never *AAA′A′* or *AAAA″*. Thus the two loci were uniquely marked in the hybridized splake genomes examined in this study; the *A′* allele was found only at one PEP-D locus (arbitrarily assigned as the *PepD-1* locus) and the *A″* allele only at the *PepD-2* locus. The PEP-D phenotypes are shown in Figure 1.

Banding patterns for the dimeric GPI isozymes in brook trout were illustrated by May et al.[23]. All lake trout produced banding patterns identical to those of the most common brook trout homozygote. While three loci code for forms of this enzyme, the duplicate loci of interest in this study (*Gpi-1,2*) are the ones that produce a fixed three-banded pattern in all homozygotes. Therefore, these duplicate loci appear fixed and do not share alleles. Different brook trout sources contributed the variant alleles for *Gpi-1* (the variant homodimer migrates to the same position as the common GPI-2 homodimer) and for *Gpi-2* (variant homodimer migrates cathodally to

Table I. Sources of the parents of each family used in this study

Family	Source of female	Source of male
A14	ST* × Splake†	ST
A27	ST × BSRS‡	ST × BSRS
A44	ST × Splake	ST × Splake
A56	ST	ST × Splake
A74	Splake × ST	ST
A77	ST	ST × Splake
A78	ST	ST × Splake
A79	ST	Splake
A83	ST	Splake × ST
A94	ST	ST × Splake
A115	ST	Splake
B2	ST	Splake
B73	ST	(ST × BSRS) F_2
B114	ST	ST × Splake
B123	Splake	ST
B124	Splake	ST
B125	ST × Splake	ST
B126	ST × Splake	ST
B127	ST × Splake	ST
B129	ST × Splake	ST
B132	Splake	ST
C10	ST	(ST × BSRS)F_2
C54	ST	ST × Splake
C64	ST	(ST × BSRS)F_2

* Brook (or speckled) trout

† F_1 hybrid between brook trout male and lake trout female

‡ Benner Spring Research Synthetic, a population derived from multiple crosses of lake, splake, and first backcrossed fish with intercrossed brook trout inbred lines

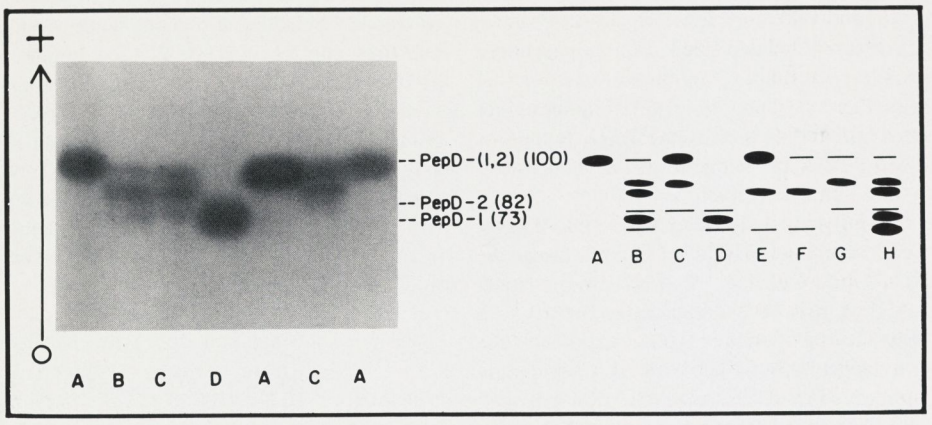

FIGURE 1 PepD phenotypes of brook, lake, splake, and splake backcrosses. The observed position of the homodimeric protein product specified by each allele is indicated on the right, along with a diagrammatic representation of all possible banding patterns. Allelic designations are as follows: A = PepD (100), A' = PepD (73), A'' = PepD (82). Genotypes: A—brook trout, ($AAAA$); B—splake, ($AAA'A''$); C—splake backcross, ($AAAA''$); D—lake trout, ($A'A'A''A''$); E—splake backcross, ($AAAA'$); F—splake backcross, ($AAA'A'$); G—splake backcross, ($AAA''A''$); H—splake backcross, ($AA'A''$).

that of the common GPI-1 homodimer). Therefore, the doubly heterozygous parents for joint segregation studies of these two loci had to be derived from intercrosses of the two resultant splake phenotypes.

SDH-1 is a tetrameric enzyme[9,21]. Liver extracts of brook trout characteristically show only a single band that migrates anodally to that in the single-banded lake trout phenotype. All F_1 splakes exhibit five bands of symmetrical staining intensities; backcrosses to brook trout gave two progeny phenotypes in a 1:1 ratio while F_2 splake progeny showed three phenotypes in 1:2:1 proportions. These results led May et al.[23] to conclude that SDH is coded for by a single locus in *Salvelinus*; the three phenotypes also were illustrated in that paper. In contrast, SDH is coded for by two loci in *Oncorhynchus*[21] and in *Salmo*[9].

Single-locus segregation

Only one family (B-124) showed statistically significant deviations from the expected 1:1 for single-locus segregations—for *Sdh* and for *PepD-2*. Considering the fact that 93 chi-square values were calculated for segregation at single loci, it is not unreasonable that two deviations significant at the 0.05 level would occur by chance alone. Morover, four other loci in family B-124 segregated 1:1, while in all other families segregation for *Sdh* or *PepD-2* showed ratios consistent with Mendelian expectations.

Joint segregation of isozyme loci

Since the present study is the first report of variation for the duplicate *PepD* loci in salmonids, the two loci were tested for joint segregation and also with a number of other isozyme loci. Nonrandom assortment was found between *PepD-1* and *PepD-2*, and the *Gpi-1*, *Gpi-2* and *Sdh* loci. Indeed, pairwise examinations for all combinations of these five loci revealed a single linkage grouping consisting of cases of both classical linkage and pseudolinkage.

The *PepD-1* and *PepD-2* loci were shown to be pseudolinked in four males tested while two females showed random assortment for these two loci. The average fraction of nonparental type progeny was 0.855 from the four male heterozygotes, but 0.510 from the two females. Details of the analyses of parents and progeny are presented in Table II.

In contrast to the pseudolinkage results shown for *PepD-1/PepD-2* in Table II, detailed analyses of parents and progeny are shown in Table III revealing the classical linkage of the *PepD-2* and *Sdh* loci. The four male parents, listed as the first four families in Table III exhibited an average recombination value of 0.01, while the five females showed a significantly higher mean value of 0.13.

The nonparental values for the above two comparisons and for those of the eight others possible among the five loci are summarized, for each of the families in which they were made, in Table IV. The mean nonparental values for all comparisons in all families, together with the statistically nonsignificant heterogeneity chi-square values justify combining the several families (see Table V.)

Table II. Joint segregation of *PepD-1* with *PepD-2* in four male heterozygotes and two female heterozygotes. All hybrids were *AB/A'B'*; therefore, the first and fourth columns of progeny represent parental types and the second and third columns represent nonparentals

		Parents		Progeny								
Family	sex	*PepD-1*	*PepD-2*	*AA BB*	*AA' BB*	*AA BB'*	*AA' BB'*	χ^2_A	χ^2_B	χ^2_{Joint}	*r*	*N*
A79	F	*AA*	*BB*	6	36	30	6	0.46	0.46	36.45	0.850	80
	M	*AA'*	*BB'*									
A115	F	*AA*	*BB*	3	35	35	7	0.20	0.20	45.00	0.875	80
	M	*AA'*	*BB'*									
B2	F	*AA*	*BB*	9	31	33	7	0.20	0.00	28.80	0.800	80
	M	*AA'*	*BB'*									
C54	F	*AA*	*BB*	7	38	50	4	2.56	0.82	59.90	0.889	99
	M	*AA'*	*BB'*									
B123	F	*AA'*	*BB'*	36	32	40	29	1.64	0.01	0.36	0.526	137
	M	*AA*	*BB*									
B132	F	*AA'*	*BB'*	12	9	10	9	0.40	0.10	0.10	0.475	40
	M	*AA*	*BB*									

The *PepD-1* and *Gpi-1* loci were found to be classically linked in eight backcross families studied. The seven male parents showed a mean recombination value of 0.046, but the values ranged from 0 to 0.13; the one female tested gave a recombination value of 0.051.

The *PepD-1* and *Gpi-2* loci were pseudolinked in three full-sib /F_1 splake males, with a mean nonparental value of 0.827. In the two male parents of families C-10 and C-64, the nonparental values are not significantly different from the random assortment found in the one informative female parent tested. However, the two males were advanced generation splake backcrosses and the results indicate that pseudolinkage breaks down in advanced generations of these hybrids; this result is consistent with that reported for *Ldh* pseudolinkage by Davisson et al.[8].

Only one family, C-54, provided information about the association between *PepD-2* and *Gpi-1*; the male parent showed a nonparental value of 0.889, indicating pseudolinkage. It is of interest that this same male showed an identical pseudolinkage intensity (0.889 nonparentals) for the *PepD-1* and *PepD-2* loci and no recombinants (very tight linkage) between *PepD-1* and *Gpi-1*.

The *PepD-2* locus showed tight classical linkage to *Gpi-2* in three male and one female informative parents tested. Two of the males showed recombination values (0.041 and 0.097) much higher than the zero level of recombinants in the female; over 200 progeny of the latter were tested. (Usually, the higher recombination values are shown in females.)

The *Gpi-1* and *Gpi-2* loci were shown clearly to be pseudolinked in the two advanced generation splake sibling male parents of families C-10 and C-64. Both families exhibited significant departures from random assortment, with nonparental values of 0.647 and 0.638, respectively.

Family C-10 also revealed pseudolinkage, with a nonparental value of 0.657, between the *Gpi-1* and *Sdh* loci. Note that this nonparental value is almost identical to that (0.647) between the *Gpi-1* and *Gpi-2* loci; as shown in the last column of Table IV, this same male parent showed no recombination between *Gpi-2* and *Sdh*. (Based on the model shown in Discussion, the two pseudolinkage values might be expected to be very similar.)

Pairwise examinations in one female and in four males (only three of which are shown in Table IV) confirmed an earlier report[24] that *Gpi-2* and *Sdh* are classically linked. The mean nonparental value for males is 0.045 while this value is 0.105 in the female.

Pairwise examination of *PepD-1* and of *PepD-2* with the following loci revealed random assortment in all families tested: *Aat-(1,2)*, *Ada*, *Adh*, *CK-1*, *Dia*, *G3p-1*, *Gpi-3*, *Gus*, *Idh-3*, *Ldh-3*, *Ldh-4*, *Mdh-1*, *Mdh-(3,4)*, *Me-1*, *Me-2*, *Mpi*, *Pgm-2*, and *Sod*. The linkage relations of the latter isozyme loci were summarized by Wright et al.[47].

Discussion

The five loci involved in this study were found to belong to one linkage grouping based on classical linkage and pseudolinkage results, but they can actually be divided into two classical linkage groups. Since the members of the duplicated *PepD* and *Gpi* loci were uniquely marked, *PepD-1* and *Gpi-1* can be assigned to one such linkage group and *PepD-2*, *Gpi-2*, and *Sdh* to the other. Based on the recombination values in males shown in Tables IV and V, the two chromosome maps would appear as follows:

PepD-1—4—*Gpi-1* and

PepD-2—4—*Gpi-2*—4.5—*Sdh*

The results in columns 1, 3, and 4 of Table IV reveal independent assortment in all three cases where females were dually heterozygous for loci in different linkage groups. On the other hand, pseudolinkage resulted for all possible combinations involving loci in the two groups when males were heterozygous for them (columns 1, 3, 4, 5, 8, and 9 in Table IV). The only exception to the latter is the breakdown of pseudolinkage of *PepD-1* with *Gpi-2* or *Sdh* in the two advanced generation hybrid male parents of families C-10 and C-64. (It is of interest to record here a consistency from some unpublished results from our laboratory with another *Salvelinus* hybrid, *S. fontinalis* × *S. alpinus*, involving some of these same loci. A female hybrid showed linkage of *Gpi-2* with *Sdh* but random assortment for *Gpi-2* with *PepD-1* and for *PepD-1* with *Sdh*. A sibling male hybrid showed pseudolinkage of *PepD-1* and *Sdh*.)

Thus, the results recorded above are con-

Table III. Joint segregation of *PepD-2* with *Sdh* in four male heterozygotes and five female heterozygotes. All hybrids were *AB/A′B′*. Therefore, the first and fourth progeny columns represent parental combinations and the second and third columns represent nonparentals

		Parents		Progeny								
Family	sex	*PepD-2*	*Sdh*	*AA BB*	*AA′ BB*	*AA BB′*	*AA′ BB′*	χ^2_A	χ^2_B	χ^2_{Joint}	*r*	*N*
A56	F	*AA*	*BB*	31	0	0	43	2.85	1.95	74.00	0.000	74
	M	*AA′*	*BB′*									
A83	F	*AA*	*BB*	28	1	1	36	0.94	1.66	58.24	0.030	66
	M	*AA′*	*BB′*									
A94	F	*AA*	*BB*	31	0	0	45	2.58	2.85	76.00	0.000	76
	M	*AA′*	*BB′*									
B114	F	*AA*	*BB*	25	0	1	22	0.08	0.08	44.08	0.021	48
	M	*AA′*	*BB′*									
B124	F	*AA′*	*BB′*	21	3	6	43	5.26	9.72	41.44	0.123	73
	M	*AA*	*BB*									
B125	F	*AA′*	*BB′*	30	3	4	34	0.01	0.22	45.76	0.099	71
	M	*AA*	*BB*									
B126	F	*AA′*	*BB′*	27	4	6	35	1.85	1.66	37.56	0.139	72
	M	*AA*	*BB*									
B127	F	*AA′*	*BB′*	21	5	4	30	1.92	1.25	29.40	0.150	60
	M	*AA*	*BB*									
B129	F	*AA′*	*BB′*	39	3	7	30	1.80	0.32	44.06	0.127	79
	M	*AA*	*BB*									

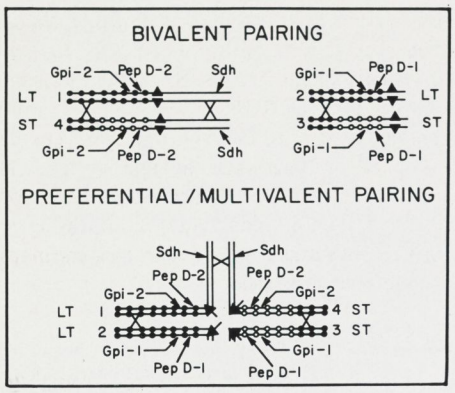

FIGURE 2 Diagrammatic representation of homoeologous bivalent pairing and of preferential pairing of metacentric chromosome arms with acrocentric chromosomes, in splakes. Solid versus open circles indicate varying homoeologous chromosome arm regions. Small arrows indicate positions of loci, larger ones the positions of centromeres. Chromosomes labeled 1 and 2 are assumed to come from the lake-trout parent (LT), and those labeled 3 and 4 come from the brook-trout parent (ST). Alternate separation of chromosomes as indicated by direction of the larger arrows would result in pseudolinkage.

sistent with those recorded previously for the pseudolinkage phenomenon (summarized by Wright et al.[47]). That is, cases of pseudolinkage involve nonsyntenic genes and are manifested only in males; they always involve duplicate genes but nonduplicated genes can be carried along; they are more frequent when males that show them possess genomes from diverse sources (as in the splakes used here), and thus they often break down in generations succeeding the F_1. A model was first proposed by Wright et al. in 1980[46] and extended in 1983[47] to explain the mechanisms of pseudolinkage and it can explain the results presented here. Moreover, the latter supports and extends the former. The model was based on such data as was reported above and on meiotic pairing of chromosomes in salmonid fishes. An adaptation of the model is depicted in the diagramatic representation in Figure 2 of the kinds of chromosomal pairing found in splake hybrids with gene locations assigned on the basis of data shown in Tables IV and V.

The chromosomal basis of the model is that only bivalent pairing occurs in *Salvelinus* females, but variable amounts of bivalent and multivalent pairing occur in males[8,17]. The multivalents always consist of two metacentric and two acrocentric chromosomes, indicating that one arm of each of a pair of metacentrics pairs with two homeologous acrocentrics. All metacentric chromosomes must have resulted from nonhomologous centric fusions since from four to eight rod tetravalents appear in splake spermatocytes[47]. From two to seven tetravalents occur in spermatocytes of brook trout[17]; therefore, there is preferential multivalent pairing in splake males as shown in Figure 2. One might expect that in splake males the chromosomes inherited from the lake trout (LT) would show greater affinity for one another and so would those contributed by the brook, or speckled, trout (ST). These affinities should break down among recombinant chromosomes in advanced generation splakes. The preferential pairing is depicted in Figure 2 by labeling as 1 and 2 the chro-

Table V. Among-family heterogeneity of testcross progeny frequencies and combined-family recombination fraction estimates from joint segregation analyses of *PepD-1*, *PepD-2*, *Gpi-1*, *Gpi-2*, and *Sdh*

Pairs loci tested	Sex tested	No. families tested	χ^2_{het} (*df*)	P_{het}	Combined families N	$\hat{r}$	2 SE ($\hat{r}$)
PepD-1/PepD-2	F	2	0.32 (1)	>0.50	177	0.510	0.075
	M	4	0.65 (3)	>0.80	337	0.855	0.038
PepD-1/Gpi-1	M	7	2.25 (6)	>0.80	359	0.039	0.020
PepD-1/Gpi-2	M	3	1.16 (2)	>0.50	225	0.827	0.050
	M*	2	0.48 (1)	>0.40	238	0.546	0.065
PepD-1/Sdh	F	2	0.20 (1)	>0.60	166	0.482	0.078
	M	3	0.58 (2)	>0.70	214	0.827	0.052
PepD-2/Gpi-2	M	3	1.41 (2)	>0.40	225	0.040	0.013
PepD-2/Sdh	F	7	0.80 (6)	>0.99	521	0.130	0.029
	M	7	0.50 (6)	>0.99	478	0.010	0.009
Gpi-1/Gpi-2	M*	2	0.01 (1)	>0.90	115	0.643	0.089
Gpi-2/Sdh	M	4	3.30 (3)	>0.30	312	0.045	0.023

* These are male parents of families C-10 and C-64; they resulted from recurrent backcrosses and exhibited a breakdown of pseudolinkage

Table IV. Nonparental frequencies among testcross progeny from joint segregation analyses of the five loci involved in the linkage associations

Families	Pairs of loci marked *PepD-1 PepD-2*	*PepD-1 Gpi-1*	*PepD-1 Gpi-2*	*PepD-1 Sdh*	*PepD-2 Gpi-1*	*PepD-2 Gpi-2*	*PepD-2 Sdh*	*Gpi-1 Gpi-2*	*Gpi-1 Sdh*	*Gpi-2 Sdh*
Female heterozygous										
B-123	0.526	—	0.500	0.492	—	0.000	0.119	—	—	0.105
B-132	0.475	—	—	0.450	—	—	0.175	—	—	—
A-14	—	0.051	—	—	—	—	—	—	—	—
Male heterozygous										
A-79	0.850	—	0.792	0.813	—	0.097	0.040	—	—	0.129
A-115	0.875	—	0.875	0.866	—	0.000	0.000	—	—	—
B-2	0.800	—	0.808	0.806	—	0.041	0.000	—	—	0.030
A-77	—	0.132	—	—	—	—	—	—	—	—
B-73	—	0.000	—	—	—	—	—	—	—	—
C-10	—	0.044	0.565	0.574	—	—	—	0.647	0.657	0.000
C-54	0.889	0.000	—	—	0.889	—	—	—	—	—
C-64	—	0.042	0.520	—	—	—	—	0.638	—	—

mosomes coming from the LT parent and by labeling as 3 and 4 those from the ST parent. Wright et al.[47] have supported the view that primarily alternate separation of chromosomes occurs from the tetravalent. This would lead to the preponderance of nonparental combinations of marked loci as seen in pseudolinkage. Most, if not all, parental combinations would result from bivalent pairing in these males.

The placement of the five loci on the chromosomes shown in Figure 2 is somewhat arbitrary in view of the variety of recombination values seen. Since PEP-D in the two lake trout appeared as a single three-banded type and *Gpi-1, 2* appeared to be fixed duplicate loci in brook trout, we have tentatively placed these loci close to the centromere. No allelic exchange has been revealed between the *PepD-1* and *PepD-2* loci nor between *Gpi-1* and *Gpi-2* in the inheritance studies reported herein. In multivalents, crossing over would be strongly inhibited and therefore prevent exchange of alleles between the members of duplicate loci near the centromere. The single *Sdh* locus shows linkage with *Gpi-2* and *PepD-2*; they are arbitrarily placed on the metacentric chromosome with *Sdh* in a different arm and distal enough to permit crossovers between it and the centromere in multivalents. An alternative site for the *Sdh* locus is possible; it could be located distally in the same arm as *Gpi-2* and *PepD-2* and therefore this linkage group could be on either the metacentric or the acrocentric chromosome. In the assignment as shown in Figure 2, *Gpi* is placed distally from *PepD* on the basis of the higher recombination values between *Gpi-2* and *Sdh* in males (Table V); in females *PepD-2* showed the greater recombination value with *Sdh* (Table IV). If *Sdh* is placed at the other end of the metacentric, the positions of *Gpi* and *PepD* loci would be reversed. The latter positions are supported if there has been fish-mammal conservation of the *Gpi-PepD* linkage group (see below); in mice the single *Gpi* locus is closer to the centromere of acrocentric chromosome 7 than the single *PepD* locus[38]; however, the single *Sdh* locus is on acrocentric chromosome 2 in mice[38].

Placement of the *Sdh* locus provides interesting speculation about the basis for the loss of its duplicate gene expression in *Salvelinus* since in *Salmo* species there are duplicate *Sdh* loci[9,21]. If *Sdh* is on the same chromosome arm as *Gpi-2* and *PepD-2*, on either the acrocentric or the metacentric, loss of expression (diploidization) of one locus could be due to its deletion, regulation, or null status (pseudogene). However, if it be on the different arm of the metacentric as shown in Figure 2, then loss of expression could be from the loss of an acrocentric pair of chromosomes in *Salvelinus*. Support for this argument is found in the fact that all *Salvelinus* species have 100 total arms ($NF = 100$)[8,17,29]. However, in the *Salmo* species native to western North America, e.g., *Salmo gairdneri* and *S. clarki*, $NF = 104$[12,13,33,42,43]. While most authors report the brown trout, *Salmo trutta*, to have $NF = 100$[29], Zenzes and Voiculescu[48] consider the *NF* to be 102. Further support for this argument is provided by some unpublished results from our laboratory whereby in *Salmo gairdneri* × *S. clarki* hybrids one *PepD* locus is linked to *Sdh-1*, but pseudolinked to *Sdh-2*. Although the orthology of loci in the two genera has not been established, these results could be accommodated by adding a centrically fused pair of acrocentrics to the two acrocentrics in the multivalent shown in Figure 2 to produce a radial-4 multivalent configuration for *Salmo*. This added pair of acrocentrics could have been lost in *Salvelinus* and result in the loss of the *Sdh* locus carried thereon.

Ohno[30,31] has argued that since Robertsonian fusions and inversions have been the two main forces operating during the evolution of vertebrate karyotypes, the original linkage relationships between various structural genes should have been conserved to "a surprising extent" by diverse descendants of a common ancestor. Evidence for this view has been seen in linkage conservations between a great majority of the placental mammals (see review by Stallings and Siciliano[40]). It also has been shown that both classical linkage and pseudolinkage are conserved among genera of the salmonids[22,47].

Lalley et al.[16] reported that the linkage group *PepD-Gpi* was conserved in man and mouse after 80 million years of divergent evolution. This linkage group is on chromosome 19 in man[27] and located very close to the centromere of chromosome 7 in the mouse[38]. Wright et al.[44] have shown that these two loci also are on the same chromosome in frogs. A most dramatic conservation of linkage groups throughout evolutionary history is seen in the linkage of *PepD* and *Gpi* in both *Salvelinus* and mammals. These taxa are separated by at least 300 million years of evolution[31] and are probably the most widely divergent groups so far reported to have a common linkage group.

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BIOCHEMICAL GENETICS OF SUNFISH*
III. GENETIC SUBDIVISION OF FISH POPULATIONS INHABITING HEATED WATERS

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ABSTRACT

Electrophoretic techniques were used to examine allele frequencies at polymorphic genetic loci in populations of largemouth bass (*Micropterus salmoides*), mosquito fish (*Gambusia affinis*), and bluegill (*Lepomis macrochirus*) for evidence of genetic changes associated with their inhabiting waters receiving thermal effluent from nuclear reactors. Comparisons were made between populations inhabiting natural bodies and those living in waters receiving thermal effluent. There were no clear effects of thermal effluent on allele or genotype frequency at any of the observed loci. However, there is significant regional genetic heterogeneity, and two species showed significant differences in allele frequencies between lentic and lotic environments.

Because our increasing requirements for energy frequently place heavy demands on the environment, a pressing need exists for information on biological effects associated with energy production. In the United States there are 34 operable power reactors and 138 in various stages of planning and construction (Atomic Energy Commission, Technical Information Center, 1973). Thermal effluent resulting from the use of large quantities of water for cooling power plants introduces new selection pressures into aquatic communities. Heated effluent may directly stress some organisms or it may act to alter temperature-dependent factors, e.g., dissolved-oxygen content, embryo development, or biological rhythms (Hubbs, 1966; Hubbs, Baird, and Gerald, 1967; and Wilson and Hubbs, 1972). Indirect effects may include alterations of food-chain relationships or

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other organismal interactions. Populations that are not eliminated from a community as a result of increased water temperature may be expected to show genetic responses to the new selection pressures.

For our study about 20 loci were electrophoretically examined in each of three species of fish, *Lepomis macrochirus* (bluegill), *Micropterus salmoides* (largemouth bass), and *Gambusia affinis* (mosquito fish), for evidence of genetic changes associated with their inhabiting waters receiving thermal effluent. Contrasts were made between populations in running and standing waters that presently receive effluent, waters that received heated effluent in the past, and waters that have maintained ambient temperatures.

There are several reasons why we might expect allelic frequency in aquatic poikilotherms to be altered by changing water temperatures. The biochemical effects of temperature on enzyme activity and function are well documented (Hochachka and Somero, 1968; and Somero, 1969). In addition, Holland et al. (1973) have shown that fish from heated waters show significantly higher thermal tolerances after acclimation, indicating a genetic response. Koehn (1970) observed correlations between allele frequencies and latitude in natural fish populations and demonstrated maximum enzyme activities at higher temperatures for the allele more prevalent in the warmer southern region. Koehn, Perez, and Merritt (1971) found that heterozygotes in *Notropis stramineus* had an advantage at higher environmental temperatures, whereas homozygotes had an advantage at lower temperatures. Johnson (1971) found that fish with different lactate dehydrogenase (LDH) genotypes had different thermal tolerances and that allele frequencies for LDH varied seasonally in response to ambient-temperature fluctuations. The primary objective of this study is to ascertain whether or not allele frequency changes due to thermal effluent from nuclear reactors can be detected in fish populations.

Normal patterns of subdivision of natural fish populations may prove to be a complicating factor. Avise and Smith (1974) have shown that within a drainage system there exists significant genetic heterogeneity between populations of bluegill but that populations within any single lake or reservoir generally appear homogeneous in allele frequency. In this paper we supply additional information about the problem of subdivision of natural fish populations.

STUDY AREA

Our study was conducted on the Atomic Energy Commission Savannah River Plant (SRP) site near Aiken, S. C. Because the nuclear production reactors on the site require large quantities of cooling water, several bodies of water on the plant area receive thermal effluent. The Par Pond system, constructed in 1958 as a source of reactor cooling water, consists of three major cooling ponds, Pond B, Pond C, and Par Pond (Fig. 1). Pond B is an 81-ha impoundment and has not received effluent since 1964. Most of Pond C, a 67-ha pond, contains

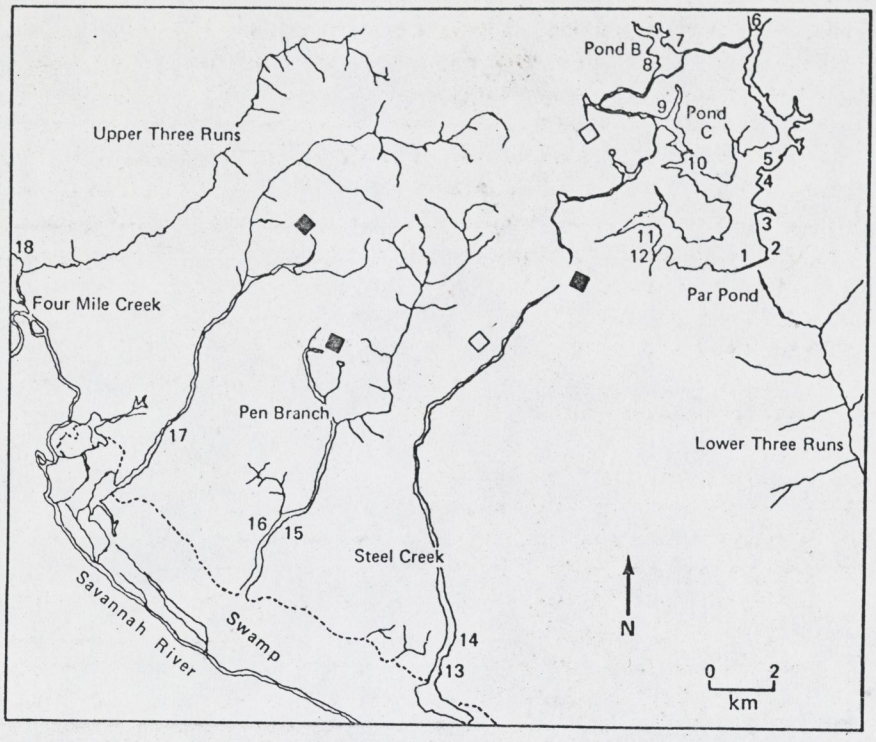

Fig. 1 Major aquatic habitats on the Savannah River Plant area. Numbers indicate the sampling locations referred to in Table 1. ■, active reactors. • □, reactors no longer in operation.

extremely hot water (50°C+) except for peripheral areas fed by springs, where temperatures are sublethal to some fish species. Water flows from Pond C into Par Pond, a 1120-ha impoundment. Surface-water temperatures at the point of thermal input into Par Pond gradually decrease to ambient near the outflow. The surface-water temperature during winter months remains as high as 34°C in the area where the heated water enters Par Pond (Gibbons, 1970). There is apparently no interchange of fish between Pond C and Par Pond. In addition to these lakes, four streams were examined. Steel Creek represents a post-thermal area that until 5 years ago received thermal effluent but now maintains normal temperature. Upper Three Runs Creek has never received heated effluent, whereas both Pen Branch and Four Mile Creek have received thermal effluent for 18 to 19 years. Water temperatures are above lethal limits for vertebrates near the reactors and decrease to ambient after the creeks flow into the Savannah River swamp.

TABLE 1

ALLELE FREQUENCIES FOR THE POLYMORPHIC LOCI* IN MOSQUITO FISH, LARGEMOUTH BASS, AND BLUEGILL

Sample locality; water condition†	*Gambusia affinis*			*Micropterus salmoides*			*Lepomis macrochirus*					
	Pgi-2^a	Pgi-2^b	N‡	Mdh-1^a	Mdh-1^b	N‡	Es-3^a	Es-3^b	Es-3^c	Got-2^a	Got-2^b	N‡
Par P. (1); S, A				0.47	0.53	34						
Par P. (2); S, A	0.81	0.19	37									
Par P. (3); S, A							0.87	0.11	0.02	0.28	0.72	30
Par P. (4); S, A	0.85	0.15	30				0.82	0.15	0.03	0.18	0.82	30
Par P. (5); S, A				0.44	0.56	25						
Par P. (6); S, A	0.79	0.21	33	0.38	0.62	28	0.78	0.18	0.04	0.22	0.78	60
Pond B (7); S, P	0.82	0.18	25	0.58	0.42	26						
Pond B (8); S, P							0.94	0.06	0	0.11	0.89	40
Pond C (9); S, T	0.90	0.10	25	0.66	0.34	28	0.78	0.18	0.04	0.14	0.86	40
Par P. (10); S, T	0.84	0.16	25	0.43	0.57	58	0.88	0.07	0.05	0.21	0.79	21
Par P. (11); S, A				0.46	0.54	24						
Par P. (12); S, A	0.84	0.16	25				0.96	0.04	0	0.27	0.73	24
Steel Cr. (13); R, P	0.90	0.10	20									
Steel Cr. (14); R, P	0.92	0.08	25									
Pen Br. (15); R, T	0.94	0.06	16									
Pen Br. (16); R, T	0.96	0.04	40									
Four Mile Cr. (17); R, T	0.96	0.04	25	0.33	0.67	49						
U. Three Runs (18); R, A	0.98	0.02	25	0.35	0.65	30	0.26	0.74	0	0.83	0.17	19

*PGI, phosphoglucose isomerase; MDH, malate dehydrogenase; ES, esterase; and GOT, glutamate oxalate transaminase.

†See Fig. 1 for identification of sample location. Water condition: S, standing: R, running; A, ambient temperature; T, temperature above ambient owing to thermal effluent; and P, ambient temperature in a post-thermal recovery area.

‡Sample size.

MATERIALS AND METHODS

Specimens were obtained by hook and line, seines, or rotenone. Fish were placed on ice and transported to the laboratory for processing. Procedures described by Avise and Smith (1974) were used for the preparation of tissue extracts for electrophoresis.

Recipes for stains and buffer systems and techniques for starch gel electrophoresis are given in Selander et al. (1971). The buffer systems used were similar to those reported by Avise and Smith (1974) with the following exceptions: for largemouth bass, glutamate oxalate transaminases (GOT) with potassium phosphate (pH 7.0); for mosquito fish, GOT with tris EDTA borate; and for mosquito fish, isocitrate dehydrogenases (IDH) with discontinuous tris citrate (pH 6.3). Genotypic frequencies in all populations closely approximated Hardy–Weinberg expectations, and banding patterns of the various enzymes were consistent with the presumed structure of the enzymes as observed in other organisms. Zymograms of the fish obtained by rotenone demonstrated no differences from those caught by seining and angling.

Samples collected from localities near one another (e.g., sites 15 and 16 in Fig. 1) were pooled when their allele frequencies were homogeneous as tested by chi-square analysis (Sokal and Rohlf, 1969). Heterozygosities were obtained by direct count, with polymorphic loci defined as those in which the frequency of the common allele was $\leqslant 0.95$.

RESULTS

The zymogram patterns of enzymes in largemouth bass, mosquito fish, and bluegill were generally similar to those previously described for *Astyanax mexicanus* (Avise and Selander, 1971) and several species of *Lepomis* (Avise and Smith, 1974). Although a large number of proteins were assayed for variability, only two loci in bluegill (ES-3 and GOT-2) and one each in bass and mosquito fish (MDH-1 and PGI-2, respectively) were polymorphic. Allele frequencies at these loci for the various populations are listed in Table 1. Genotype frequencies at these loci were subjected to heterogeneity chi-square tests for evidence of significant differences between populations (Table 2). In general, populations within each pond or stream were homogeneous, but populations living in different ponds and streams were often heterogeneous. Most of the interlocality variation was due to comparisons between populations in lentic vs. those in lotic environments. There was no significant heterogeneity due to varying thermal conditions (Table 2).

DISCUSSION

Despite the large size of Par Pond and the considerable distance between collecting sites (up to 15 km; Fig. 1), the polymorphic loci in each species are

TABLE 2

GENOTYPIC HOMOGENEITY CHI-SQUARE (χ^2) VALUES FOR POLYMORPHIC LOCI OBTAINED FROM POPULATION COMPARISONS*

Comparisons	Degrees of freedom	Homogeneity† by χ^2 test	Probability of greater homogeneity by χ^2 test
Mosquito fish, *Gambusia affinis* (PGI-2)			
Among populations	20	42.4	<0.01
Among ponds	4	3.3	0.50
Within Par Pond	8	5.3	0.73
Among creeks	6	12.9	0.04
Among cold creeks	1	2.7	0.10
Among hot creeks	2	4.0	0.14
Hot vs. cold creeks	2	3.8	0.15
Creeks vs. ponds	2	24.0	<0.01
Largemouth bass, *Micropterus salmoides* (MDH-1)			
Among populations	18	34.6	0.01
Among ponds	4	12.2	0.02
Within Par Pond	8	4.7	0.79
Pond B vs. Pond C	2	0.9	<0.64
Among creeks	4	1.9	0.76
Creeks vs. ponds	2	15.8	<0.01
Bluegill, *Lepomis macrochirus* (ES-3)			
Among populations	28	130.6	<0.01
Among ponds	8	34.3	<0.01
Within Par Pond	16	14.1	0.59
Pond B vs. Pond C	4	24.9	<0.01
Bluegill, *Lepomis macrochirus* (GOT-2)			
Among populations	14	147.3	<0.01
Among ponds	4	8.9	0.06
Within Par Pond	8	6.2	0.62
Pond B vs. Pond C	2	0.3	0.88

*Separate collections within Par Pond were pooled before calculating the among-ponds comparisons.

†Expected frequencies, calculated using correction for small samples (Canning and Edwards, 1969).

homogeneous in allele frequency. A similar situation was found by Avise and Smith (1974) for other bluegill populations in which only 5% of the variance in allele frequency within a drainage system could be attributed to populations within reservoirs. Thus we cannot eliminate the possibility that a fish species within a large reservoir is one extensive panmictic unit. Alternatively, relevant selection pressures for the loci concerned could be uniform throughout the lake. However, since water temperature is clearly not uniform within Par Pond (maximum surface temperatures in winter at the input of the heated reactor effluent range from 30 to 35°C, and those in cooler parts of the lake range from 10 to 15°C), water temperature cannot be the most important factor determining allele or genotype frequencies for these polymorphic loci. This argument is enforced by the general lack of correspondence between water temperature and allele frequency for populations in other streams and ponds on the SRP area.

The observed homogeneity of allele frequency in Par Pond may indicate extensive gene flow over considerable areas. Bluegill and largemouth bass are strong swimmers, and long-distance movements are known to be common (Gibbons and Bennett, 1971). Mosquito fish are much smaller fish and spend their entire lives very close to shore (Krumholz, 1948). But even for these fish very small amounts of gene flow between populations may be sufficient to maintain homogeneity if the alleles have similar selective values [under certain conditions, with neutral alleles, the exchange of one individual per generation between local populations is sufficient to maintain homogeneity (Kimura and Ohta, 1971)]. Thus with the present data we cannot distinguish between possible effects of gene flow and uniform selective pressures in the maintenance of similar allele frequencies throughout Par Pond. We can, however, conclude that water temperature is not the primary determinant of allele frequencies at these loci.

In each locus there is significant genotypic heterogeneity when comparisons among all populations are made (Table 2). In most cases this heterogeneity is not correlated in any consistent fashion with water temperatures. However, there are several significant comparisons between localities (Table 2), suggesting that among the populations heterogeneity can be partially attributed to locality effects that appear independent of thermal conditions.

Significant differences in allele frequencies are seen in comparisons involving creek and pond populations of mosquito fish and largemouth bass (Table 2). Similar comparisons were not made for bluegill, because this species does not commonly occur in the creeks on the SRP area. The shift in allele frequency from ponds or lakes to creeks is illustrated by PGI-2 in mosquito fish (Table 1). In lotic waters the allele frequency ($Pgi\text{-}2^a$) ranged from 0.90 to 0.98, but in lentic situations it ranged from 0.79 to 0.90 (Table 1). There is no overlap in frequencies of MDH alleles in bass from running vs. standing waters. Future work is needed to substantiate whether or not these allele-frequency differences can

be attributed to different selective regimes associated with standing or running waters.

One shift in allele frequency could possibly be attributed to thermal selection. Mdh-1^a in largemouth bass varied in frequency from 0.44 in Par Pond (parts of which are near ambient temperature) to 0.58 in Pond B (a post-thermal recovery area) to 0.66 in Pond C (currently receiving thermal effluents). One possibility is that Mdh-1^a has a selective advantage over Mdh-1^b in the hot water in Pond C and that the population in Pond B is returning to the frequency characteristic of populations nearer ambient temperature. Certain other community and population measures do show the intermediate type of response hypothesized for populations in the post-thermal environment (Parker, Hirshfield, and Gibbons, 1973). The population at the hot end of Par Pond does not show an elevated frequency of Mdh-1^a (Table 1), but this could be due to the effects of extensive gene flow with other populations in Par Pond not similarly selected. The population in Four Mile Creek (which receives thermal effluents) has a low frequency of Mdh-1^a as does the population in the normal temperature of Upper Three Runs Creek. Thus, if temperature is an agent in determining these allele frequencies, it is certainly complicated by interactions with lentic–lotic factors.

In this study no clear relationship has been established between allele frequency and thermal conditions. Perhaps few loci in the genome are involved in the adaptation to thermal stress. Another possibility, that no changes in allele frequency occur as a result of selection due to the thermal effluents, does not seem justified, considering the indication of relatively short-term evolutionary changes in fish populations subjected to different temperature regimes (Johnson, 1971, and Holland et al., 1974). On the basis of the limited number of loci examined thus far, definitive statements on the extent of genetic alteration necessary for thermal adaptation to reactor effluents would be premature.

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