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A Preliminary Note on the Chromosomes and Enzymatic Patterns of Three Forms of Sticklebacks¹⁾

By

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(With 2 Text-figures)

In recent years a good deal of information has been accumulated to show that cytogenetic data involving the morphology and number of the chromosomes serve as significant criteria for the classification of plant and animal species, since the chromosomes provide the basic material for hereditary changes. To date, it is generally accepted that species specificity has based on chromosomal aspects from lower to higher animals. Indeed, cytogenetic analyses gave taxonomy a new meaning and impetus.

Current knowledge of genetics has shown that data from the molecular level are useful for classification in taxonomy. Skeletal muscle proteins of fishes have provided important information in classification at the generic, familial, and higher taxonomic levels, and in most cases at the species level as well (Tsuyuki and Roberts 1966, Tsuyuki *et al.* 1967, Tsuyuki *et al.* 1968). Hori and Kamada (1967) reported that species specificity was defined in electrophoretically distinct forms of glucose 6-phosphate dehydrogenase in some mammals.

The present paper deals with some cytotaxonomic aspects of three forms of sticklebacks, *Gasterosteus aculeatus aculeatus* (Linnaeus), *G. a. microcephalus* (Linnaeus) and *Pungitius sinensis* (Guichenot).

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Materials and Methods: For chromosome studies, each fish from three forms of sticklebacks as mentioned above received an intramuscular injection of 0.1 to 0.2 cc of 50 µg colchicine/1 ml dist. water 1.5 to 2 hours prior to sacrifice. Upon sacrifice, small pieces of the gill, spleen, and/or testis were cut with a pair of scissors and put into 0.075 M KCl for 20 minutes. They were then fixed in Carnoy's solution. Cells on slides were flamed after 1 hour of fixation. The slides were stained with Giemsa solution (for technical details, see Itoh 1968).

For enzymatic studies, skeletal muscle was frozen on the dry ice immediately after

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sacrifice, homogenized with 3 volumes of homogenizing solution and centrifuged at 15,000 g for 20 minutes. After electrophoresis for about 2 hours at room temperature, the gels were cut halves longitudinally, the half was stained with a solution containing glucose 6-phosphate and the other half stained with a solution containing galactose 6-phosphate. For processes in detail, refer to Hori *et al.* (1966).

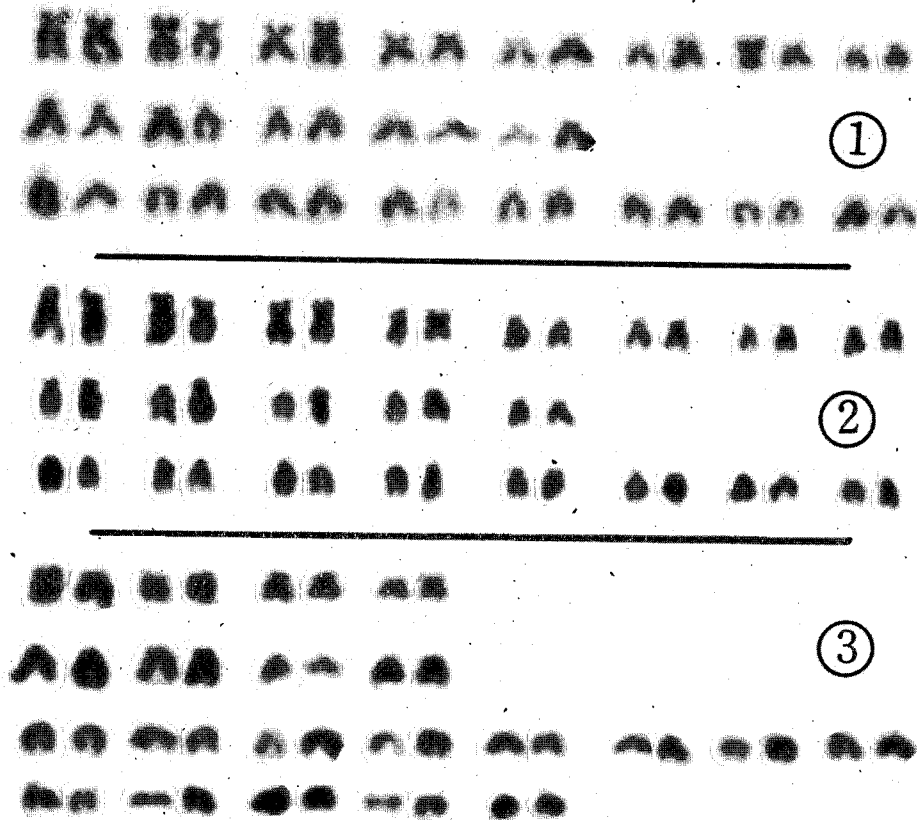


Fig. 1. Karyotype of *Gasterosteus aculeatus aculeatus* (Linnaeus) prepared from a metaphase from the gill. 8 pairs of meta- and submetacentrics constitute the top row. 5 pairs of subterminals occupy the middle row and 8 pairs of acrocentrics constitute the bottom row. Fig. 2. Karyotype of *Gasterosteus aculeatus microcephalus* (Linnaeus) prepared from a splenic cell is identical with that of *Gasterosteus aculeatus aculeatus* (Linnaeus). Fig. 3. Karyotype from the gill of *Pungitius sinensis* (Guichenot). 4 pairs of meta- and submetacentrics are aligned on the first row. 4 pairs of subterminals occupy the second row and 13 pairs of acrocentrics constitute the third and the fourth rows.

Results

Cytological Findings

Gasterosteus aculeatus aculeatus (Linnaeus): This is a member of the family Gasterosteidae. The specimens for study were obtained in the stream in Ôno City, Fukui Prefecture. Only a small number of analyzable mitotic figures was available for study in gill-tissue cells derived from five specimens. In karyotype analysis, the chromosomes were classified into three groups as follows: 1) definitely two armed chromosomes including metacentrics and submetacentrics which can not be confused with acrocentrics, 2) subterminals which were sometimes confused with acrocentrics, and 3) acrocentrics. This practice was done in the study of the other two species as well, since subterminals were often difficult to distinguish from acrocentrics on account of their small size.

The diploid chromosome number of this species was determined as 42 which consist of 8 pairs of meta- and submetacentrics, 5 pairs of subterminals and 8 pairs of acrocentrics. A karyotype made from a gill specimen is shown in Figure 1.

Gasterosteus aculeatus microcephalus (Linnaeus): This species belongs also to the family Gasterosteidae. The specimens were captured in the stream in Ôgaki City, Gifu Prefecture. Four fishes were subjected for the chromosomal study. The number of cells available for the chromosomal counts was only 12 in total. It was shown that this species had the karyotype morphologically identical with that of the former species as given above, showing 42 chromosomes (Figure 2).

Pungitius sinensis (Guichenot): This species is also a member of the family Gasterosteidae. The specimens were caught in the stream in Takefu City, Fukui Prefecture. As seen in Figure 3, the karyotype of this species was different from those of the two above forms, though the number of chromosomes was the same being 42. Analysis revealed that the diploid complement was made of 4 pairs of meta- and submetacentrics, 4 pairs of subterminals and 13 pairs of acrocentrics.

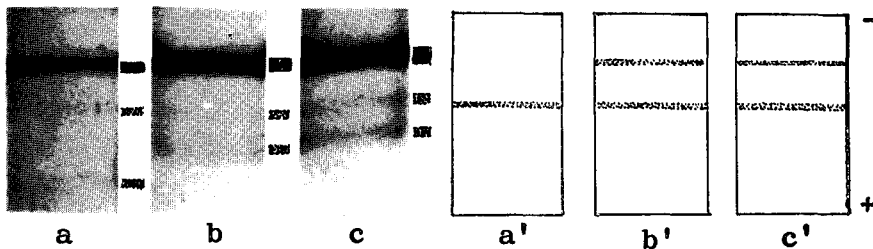


Fig. 4. Electrophoretic patterns of glucose-6-phosphate dehydrogenase and lactose-6-phosphate dehydrogenase in extracts from skeletal muscle of three forms of sticklebacks. The left half was developed in glucose-6-phosphate at photograph and right half in galactose-6-phosphate at diagram. a and a' in *Pungitius sinensis*, b and b' in *Gasterosteus aculeatus aculeatus*, and c and c' in *G. a. microcephalus*.

Electrophoretical Findings

Two subspecies, *Gasterosteus aculeatus aculeatus* (Linnaeus) and *G. a. microcephalus* (Linnaeus) demonstrated electrophoretically an identical type for glucose-6-phosphate dehydrogenase. Electrophoretical patterns of those two subspecies were shown as three bands. The slowest band showed the strongest G-6-PD activity. The middle and fastest bands represented almost similar weak activity for G-6-PD. The type of G-6-PD of *Pungitius sinensis* (Guichenot) was similar to that of the above two subspecies for the slowest and middle bands, while the fastest band showed faster moving to anodal direction than the fastest band of the above two subspecies.

On the other hand, the electrophoretical patterns of galactose-6-phosphate dehydrogenase for two subspecies (*G. a. a.* and *G. a. m.*) corresponded to the slowest and middle bands of G-6-PD. The only one band of *P. s.* for Gal-6-PD was homologous to the middle band of that of the two subspecies. The bands of Gal-6-PD were too weak to demonstrate in photograph although they were visible in the gels. Electrophoretical patterns are as shown in Figure 4.

Discussion

It is very interesting whether the changes of chromosomes and the variations at enzymatic level are present, or not, between species or subspecies, in relation to taxonomical classification.

A comparison of the electrophenograms and the chromosomes of the three forms of sticklebacks here considered demonstrated a remarkable parallelism between electrophoretic patterns and chromosome features among those closely related species. No differences of the two subspecies, *G. a. a.* and *G. a. m.*, were obtained at the chromosomal level and within the limitation of electrophoretic patterns of two kinds of enzymes, G-6-PD and Gal-6-PD. The electrophoretic study indicated that the two subspecies *G. a. a.* and *G. a. m.* and one species *P. s.* shared the same alleles except for one allele at those gene loci. Ohno (1968) reported that the wolf and the coyote belonging to the genus *Canis* were indistinguishable from the domestic dog, not only in the chromosome constitution, but in the electrophoretic types of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase.

It has been shown that the glucose-6-phosphate dehydrogenase is produced by a sex-linked gene in man and some other mammals (Childs *et al.* 1958, Young *et al.* 1964, Ohno *et al.* 1965, Trujillo *et al.* 1965). In the present cases, it has remained undecided whether, or not, the glucose-6-phosphate dehydrogenase was under the control of sex-linked gene, because the present study was done with male specimens for the most part. Two forms of glucose-6-phosphate dehydrogenase, A and B, have been reported in deer mouse tissues (Shaw and Barto 1965). Moreover, Shaw (1966) described that the B enzyme was equally active toward glucose-6-phosphate dehydrogenase and galactose-6-phosphate dehydrogenase in deer mouse, human and horse livers, and Ohno *et al.* (1966) also found the same relationship as

that of Shaw. A corresponding feature was obtained in our enzymatic study with skeletal muscle.

The study is in preparation on the chromosomes and the enzymatic patterns of the anadromous form of *Gasterosteus aculeatus aculeatus* (Linnaeus), in comparison with those of the land-locked type of *G. a. a.* here studied.

Summary

Three forms of sticklebacks were studied on the bases of chromosomes and electrophoretic patterns of two enzymes, G-6-PD and Gal-6-PD. Two subspecies, *Gasterosteus aculeatus aculeatus* (Linnaeus) and *G. a. microcephalus* (Linnaeus), gave no visible difference in the chromosomal constitution and electrophoretic patterns. A slight difference was noted in *Pungitius sinensis* (Guichenot) with respect of the chromosomes and electrophoretic patterns from the above two subspecies.

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