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On the Problem of Regional Gene Duplication in Diploid Fish of the Orders *Ostariophysi* and *Isospondyli**

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Summary. DNA measurements, the number of chromosomes and the analysis of individual gene loci revealed a diploid-tetraploid relationship among members of the fish family *Cyprinidae*. Intermediate DNA values in some diploid species of this family are interpreted as the result of regional duplications. However, the analysis of 8 different isoenzyme systems in these species always confirmed the presence of only the diploid number of genes; this includes the gene for the enzyme 6-PGD which was erraneously reported to be duplicated.

In the order *Isospondyli*, on the other hand, where a diploid-tetraploid relationship has likewise been established, the smelt, *Osmerus esperlanus* provides a clear example for the duplication of single genes. In this representative of the diploid group the genes for S-AAT and PGI are duplicated.

Zusammenfassung. DNS-Messungen, Chromosomenanalysen und die Untersuchung individueller Genloci haben eine Diploid-tetraploid-Beziehung in der Fischfamilie *Cyprinidae* aufgedeckt. Intermediäre DNS-Werte bei einigen diploiden Species dieser Fischfamilie werden als Folge regionaler Duplikationen interpretiert. Die Analyse von 8 verschiedenen Isoenzymsystemen ergab jedoch für diese Species jeweils nur die diploide Anzahl von Genen; das schließt auch den 6-PGD-Locus mit ein, der irrtümlicherweise als dupliziert angesehen worden war. In der Ordnung *Isospondyli*, in der ebenfalls eine Diploid-tetraploid-Beziehung etabliert ist, gibt jedoch der Stint, *Osmerus esperlanus*, ein klares Beispiel für die Duplikation einzelner Genloci. Bei diesem Repräsentanten der diploiden Gruppe sind die für die S-AAT und die PGI codierenden Gene dupliziert.

Introduction

In the fish family *Cyprinidae* the existence of a diploid-tetraploid relationship has been well established (Wolf *et al.*, 1969). On the basis of the electrophoretic pattern of an enzyme, 6-phosphogluconate dehydrogenase (6-PGD; E.C.: 1.1.1.44), Klose *et al.* (1969) assumed that a regionally confined duplication of the 6-PGD gene locus had occurred in some of the diploid species; these diploids resembled electrophoretically the phylogenetically tetraploid Cyprinid species.

In the course of our studies on the consequences of polyploidisation we were not able to confirm these findings of Klose *et al.* (1969). Our electrophoretic results in Cyprinid fish indicate that all the diploids examined are endowed with only one single gene locus for 6-PGD. However, in the order *Isospondyli* in which a diploid-tetraploid relationship has likewise been established (Klose *et al.*, 1968), we were able to demonstrate regional duplications of single gene loci. In the smelt *(Osmerus*

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esperlanus), representing a diploid fish of this order the enzymes phosphoglucose isomerase (PGI; E.C.: 5.3.1.9) and the supernatant form of aspartate aminotransferase (S-AAT; E.C.: 2.6.1.1) exhibit electrophoretic patterns similar to those of the tetraploids.

Material and Methods

The following species were examined: *Barbus tetrazona* (from a local pet shop), *Rutilus rutilus, Tinca tinca, Abramis brama, Leuciscus cephalus* (all 4 species from the Rhine river), *Carassius auratus* (from different local pet shops), *Osmerus esperlanus* and *Clupea harengus*

Table 1. Electrophoretic conditions used for the demonstration of 6-PGD-, PGI-, and S-AAT-isoenzymes

Enzyme	Buffer	Gel			Bridge		Run	
		buffer concentration (*M*)	starch concentration (%)	pH	buffer concentration (*M*)	pH	voltage (v/cm)	hours
6-PGD	K_2HPO_4—KH_2PO_4	0.006	16	7.4	0.25	7.4	12	5
PGI	Tris-citrate	0.008—0.003	14	6.7[a]	0.223—0.068	6.3[a]	12	5
S-AAT	Tris-KH_2PO_4	0.005—0.005	14	7.4	0.05—0.05	7.4	12	5

[a] Adjusted with 3M KOH.

Table 2. Staining solutions for the demonstration of 6-PGD-, PGI- and S-AAT-isoenzymes after starch gel electrophoresis

a) *6-PGD*
30 mg 6-phosphogluconate
24 mg NADP
20 mg NBT
5 mg PMS
50 ml 0.05 M Tris-HCl, pH 8.0 ad 150 ml a. dest.

b) *PGI*
400 mg Agar in 40 ml 0.05 M Tris-HCl, pH 8.0
40 mg Fructose-6-phosphate
15 mg NADP
20 mg NBT
40 mg $MgCl_2$
5 mg PMS
0.07 mg Glucose-6-phosphate dehydrogenase

c) *S-AAT*
460 mg L-aspartic acid
200 mg α-ketoglutaric acid
10 mg Pyridoxal phosphate
400 mg Fast blue BB salt
150 ml 0.05 M Tris-HCl, pH 7.6

(from a local fish store), *Salmo irideus*, *Salmo trutta forma fario* and *Salmo salar* (all 3 species from fish hatcheries near Freiburg) and *Coregonus lavaretus* (from the Lake of Constance). All the fish were worked up shortly after killing, except *Osmerus esperlanus* and *Clupea harengus* which were ice-bound when they arrived in our laboratory.

The following organs were analysed: brain, eye, gills, heart, intestine, kidney, liver, muscle, ovary, spleen, stomach, swim-bladder and testis. The tissues were homogenized 1:1 in 0.01 M PO_4 buffer, pH 7.4, frozen and thawed twice, and centrifuged at 20000 × g twice for 30 min. The clear supernatant was then subjected to horizontal starch gel electrophoresis. Gel conditions and staining solutions are listed in Tables 1 and 2.

The procedure for discriminating between the supernatant and mitochondrial forms of AAT has been described in a previous paper (Schmidtke and Engel, 1972).

Results

A dimeric structure of each of the three isoenzyme systems, 6-PGD (see Kazazian, 1966), S-AAT and PGI (see Darnall and Klotz, 1972) was assumed for our interpretations.

a) 6-PGD Isoenzymes in Cyprinid Fish

1. *Barbus tetrazona* and *Rutilus rutilus*

Our findings in these two species agree with those of Klose *et al.* (1969). In both species a minimum number of one band was found, corresponding to the homozygote, and three bands in heterozygotes (Fig. 1c).

2. *Tinca tinca*

Out of 26 animals 21 showed the assumed single wild type band AA (Fig. 1b, d, e); 3 animals exhibited a pattern of 3 bands (Fig. 1a), representing a heterozygous type AA′, while the respective variant homozygous type A′A′ (Fig. 1f) was found twice.

3. *Abramis brama*

20 specimens of this fish were analysed. 12 individuals showed one single band (Fig. 1i) representing the wild type pattern AA. Among 8 animals three different types of a three banded pattern were found. These types can be interpreted as the heterozygous phenotypes AA′ (2 individuals, Fig. 1h), AA″ (5 individuals, Fig. 1j), and AA‴ (1 individual, Fig. 1k).

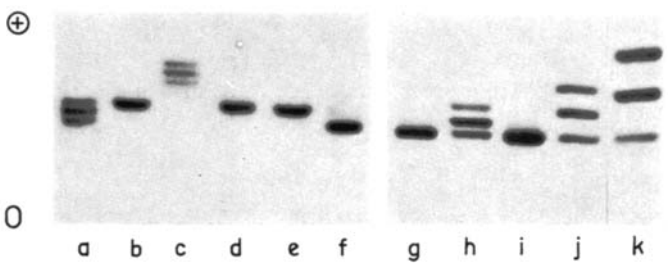

Fig. 1a—k. 6-PGD isoenzymes in diploid species of Cyprinid fish from liver tissue. *Tinca tinca*, phenotypes AA′ (a), AA (b, d, e), A′A′ (f); *Rutilus rutilus*, phenotype AA′ (c); *Leuciscus cephalus*, phenotype AA (g) and *Abramis brama*, phenotypes AA (i), AA′ (h), AA″ (j), AA‴ (k)

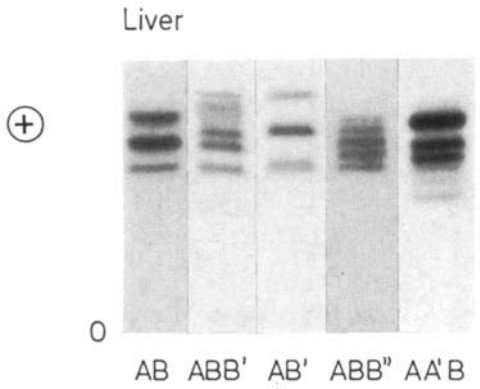

Fig. 2

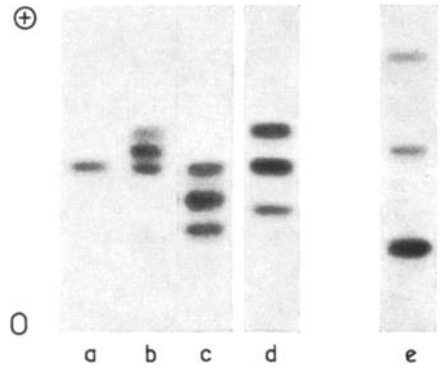

Fig 4a—e

Fig. 2. 6-PGD isoenzymes in different phenotypes of *Carassius auratus*, liver tissue. This picture was placed to our disposal by Dr. S. Kadir, which is gratefully acknowledged

Fig. 4a—e. PGI isoenzymes in fish of the order *Isospondyli: Clupea harengus*, phenotypes AA (a), AA′ (b), AA″ (c) from heart muscle tissue; *Osmerus esperlanus*, phenotype AB (d) from heart muscle tissue; *Salmo salar*, phenotype AB (e) from muscle tissue

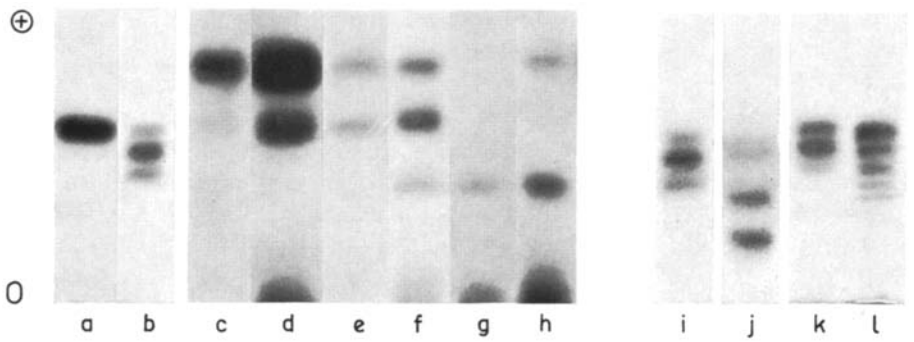

Fig. 3a—l. S-AAT isoenzymes in fish of the order *Isospondyli: Clupea harengus*, phenotypes AA (a), AA′ (b) from heart muscle tissue; *Osmerus esperlanus*, phenotype AB: eye (c), liver (d), stomach (e), kidney (f), muscle (g), heart (h); *Salmo trutta*, phenotype AB: brain (i); *Salmo irideus*, phenotype AB: liver (j); *Coregonus lavaretus*, phenotypes AB (k), AA′B (l) from kidney. Electrophoretic bands close to the start are part of the M form of AAT

4. *Leuciscus cephalus*

An electrophoretic variant was not observed. All the 23 animals examined showed one single band (Fig. 1g).

5. *Carassius auratus*

The 6-PGD isoenzymes of the goldfish have already been studied in some detail by Bender and Ohno (1968) and Klose *et al.* (1969). The investigations of these authors, revealing the existence of two separate gene loci coding for 6-PGD, were confirmed by a population survey of 120 specimens, carried out by Kadir (1969) in our laboratory. He found 103 animals exhibiting the AB wild type pattern,

1 animal with the variant homozygous phenotype AB′ and the following heterozygotes: 9 ABB′, 5 ABB″ and 1 AA′B (Fig. 2).

b) S-AAT and PGI Isoenzymes in Species of the Order Isospondyli

1. S-AAT

Clupea harengus, a diploid fish of this order, exhibits one S-AAT band in the homozygous and three bands in the heterozygous state (Fig. 3a and b), while the tetraploids *Salmo irideus*, *Salmo trutta forma fario* and *Coregonus lavaretus* show a 3 banded pattern in the homozygous state and a 5 banded pattern in the heterozygous state (Fig. 3i—l). This duplication of the S-AAT gene locus in the tetraploids is due to polyploidisation (Schmidtke and Engel, 1972).

In a population survey, 125 specimens of *Osmerus esperlanus*, another diploid species of the order *Isospondyli*, revealed consistently an electrophoretic pattern of three bands for S-AAT (Fig. 3c—h), thus resembling the tetraploids. Although a variant could not be observed, the assumption of two separate gene loci coding for this enzyme is also supported by the discovery of an extensive tissue variability of the S-AAT isoenzymes. While in the eye, swim bladder, liver, stomach, intestine, kidney and brain the faster anodally migrating bands prevail to different degrees (Fig. 3c—f), the situation is reverse in scelet muscle and testis (Fig. 3g). In heart muscle tissue and gills the homomers dominate, while the heteromer shows hardly any staining activity (Fig. 3h), possibly reflecting cell heterogeneity.

2. PGI

Very similar to the findings for S-AAT, *Clupea harengus* shows a single PGI-band in the homozygous state (Fig. 4a) and a set of three bands in two different heterozygotes (Fig. 4b and c). *Salmo salar*, as a representative of the tetraploids exhibits three bands in the wild type (Fig. 4e). Similar as for S-AAT, also for PGI the diploid smelt *(Osmerus esperlanus)* behaves like the tetraploids, exhibiting a pattern of three bands in the 17 specimens investigated (Fig. 4d). A detailed communication on PGI isoenzymes in fish of the orders *Ostariophysi* and *Isospondyli* will be presented elsewhere.

Discussion

As pointed out by Ohno (1970) in some detail, an increase of genetic material at the level of fish and amphibians was a prerequisite for the evolution of higher vertebrates. Gene duplication can be achieved by polyploidisation or by regionally confined duplications of parts of the genome. An example for the latter mechanism was claimed by Klose *et al.* (1969) for the 6-PGD locus in the diploid Cyprinid fish species *Tinca tinca*, *Abramis brama*, and *Leuciscus cephalus*. These species show a DNA content per nucleus (30, 36, 38% of human leucocytes) ranging between the assumed original diploid level (e.g. *Barbus tetrazona:* 20% of human leucocytes) and the tetraploid level (e.g. *Carassius auratus:* 53% of human leucocytes).

Our findings indicate that an unequivocal diploid-tetraploid relationship exists for the 6-PGD gene loci in Cyprinid fish: the diploids are endowed with one locus each, the tetraploids with 2 loci for this enzyme. It might have occurred that the samples taken by Klose *et al.* (1969) happened to include only heterozygotes. The

Table 3. The number of gene loci for various isoenzymes in fish of the order *Ostariophysi*: comparison between species on the diploid and tetraploid level

Gene loci for	*Ostariophysi*	
Genome:	2n	4n
LDH[a]	2^{1}	$2,3,^{1,2}$
6-PGD	$1^{1,3,7}$	$2^{1,3,7}$
S-form NADP-IDH	2^{4}	2^{4}
M-form NADP-IDH	1^{4}	2^{4}
α-GPD	1^{b}	2^{b}
S-form AAT	1^{6}	$1,2^{6}$
PGI	2^{b}	4^{b}
SDH	1^{4}	1^{5}

[a] Tissue specific gene loci observed in several species.
[b] Own unpublished results.

(1) Klose *et al.* (1969); (2) Engel *et al.* (1973); (3) Bender and Ohno (1968); (4) Engel *et al.* (1971); (5) Lin *et al.* (1969); (6) Schmidtke and Engel (1972); (7) This paper.

five banded pattern in liver tissues might then be due to an alteration of the material by prolonged storage, a phenomenon we were likewise confronted with during our own experiments.

Despite of the intermediate DNA values of *Tinca tinca*, *Abramis brama* and *Leuciscus cephalus*, which presumably originated by random duplications of any part of the genome, no example of a gene duplication was detected in these species among all the 8 isoenzyme systems (more than 11 gene loci) studied so far (see Table 3). In contrast to this situation in Cyprinid fish in the order *Isospondyli*, the smelt offers even two examples for the duplication of a single gene though this species possesses the lowest DNA content of all the species of this order examined so far. In both of these duplications, i.e. that for S-AAT and that for PGI, the assumption of 2 separate gene loci remains to be confirmed by the detection of electrophoretic variants. In the case of S-AAT extensive tissue variability is to be considered as rather strong evidence for the existence of two independent gene loci.

Our findings indicate that the higher amount of DNA may not necessarily be reflected in the present-day existence of duplicated structural genes. Although duplications occurred to a large extent, the acquired genetic material may not serve a useful function. A similar mechanism has been supposed for the regulation of genic expression in *Amphiuma*, an amphibian with an excessive amount of DNA (Comings and Berger, 1969). Furthermore, the possibility can not be excluded that the duplicated genes have not undergone allelic mutations and thus cannot be visualized.

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References

Bender, K., Ohno, S.: Duplication of the autosomally inherited 6-phosphogluconate dehydrogenase gene locus in tetraploid species of cyprinid fish. Biochem. Genet. **2**, 101—107 (1968)

Comings, D. E., Berger, R. O.: Gene products of *Amphiuma:* an amphibien with an excessive amount of DNA. Biochem. Genet. **2**, 319—333 (1969)

Darnall, D. W., Klotz, I. M.: Protein subunits: A table (revised edition). Arch. Biochem. Biophys. **149**, 1—14 (1972)

Engel, W., Faust, J., Wolf, U.: Isoenzyme polymorphism of the sorbitol dehydrogenase and the NADP-dependent isocitrate dehydrogenase in the fish family *Cyprinidae*. Anim. Blood Grps. biochem. Genet. **2**, 127—133 (1971)

Engel, W., Schmidtke, J., Vogel, W., Wolf, U.: Genetic polymorphism of lactate dehydrogenase isoenzymes in the carp *(Cyprinus carpio)* apparently due to a "null allele". Biochem. Genet. **8**, 281—289 (1973)

Kadir, S.: Untersuchungen zur Gewebsvariabilität der Isoenzyme, dargestellt am Beispiel der 6-Phosphogluconat-Dehydrogenase vom Goldfisch *(Carassius auratus)*. Dissertation, Freiburg i. Br. 1969

Kazazian, H. H.: Molecular size studies on 6-phosphogluconate dehydrogenase. Nature (Lond.) **212**, 197—198 (1966)

Klose, J., Wolf, U., Hitzeroth, H., Ritter, H., Atkin, N. B., Ohno, S.: Duplication of the LDH gene loci by polyploidisation in the fish order *Clupeiformes*. Humangenetik **5**, 190—196 (1968)

Klose, J., Wolf, U., Hitzeroth, H., Ritter, H., Ohno, S.: Polyploidisation in the fish family *Cyprinidae*, order *Cypriniformes*, II. Duplication of the gene loci coding for lactate dehydrogenase (E.C.: 1.1.1.27) and 6-phosphogluconate dehydrogenase (E.C.: 1.1.1.44) in various species of *Cyprinidae*. Humangenetik **7**, 245—250 (1969)

Lin, C.-C., Schipman, G., Kittrell, W. A., Ohno, S.: The predominance of heterozygotes found in wild goldfish of Lake Erie at the gene locus for sorbitol dehydrogenase. Biochem. Genet. **3**, 603—607 (1969)

Ohno, S.: Evolution by gene duplication. Berlin-Heidelberg-New York: Springer 1970

Schmidtke, J., Engel, W.: Duplication of the gene loci coding for the supernatant aspartate aminotransferase by polyploidisation in the fish family *Cyprinidae*. Experientia (Basel) **28**, 976—978 (1972)

Wolf, U., Ritter, H., Atkin, N. B., Ohno, S.: Polyploidisation in the fish family *Cyprinidae*, order *Cypriniformes*. I. DNA-content and chromosome sets in various species of *Cyprinidae*. Humangenetik **7**, 240—244 (1969)

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