The evolution of duplicate gene expression in the carp (*Cyprinus carpio*)[1]

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Summary. The carp, Cyprinus carpio (Teleostomi), is a tetraploid species which currently expresses 52% of its duplicate enzyme loci. This level of duplicate gene expression in this cyprinid species is comparable to those observed in tetraploid Catostomidae, but higher than those in tetraploid Cobitidae.

The carp (Cyprinus carpio) is a phylogenetically tetraploid fish[4]. This species has been the object of a number of studies on the effect of polyploidy on gene expression[5–8]. Subsequent to the original tetraploidization event one of the 2 duplicate genes have been silenced in their expression. We wish to determine what fraction of duplicate genes encoding isozymes have been silenced over a given period of time. Such analyses may help us to understand the rates at which initially identical loci can diverge in structure and regulation. We report the result of electrophoretic analyses of isozymes encoded in 21 loci. The extent of functional diploidization is also discussed in relation to the time of origin of the carp, and these results are compared with those for other tetraploid cypriniform fishes.

Materials and methods. 22 carp were captured from streams in central Illinois and stored at −20 °C. Muscle, heart, eye, brain, stomach, gill, liver, spleen, gonad, and kidney were dissected and homogenized 1:2 vol:vol in 0.01 M Tris-HCl pH 7 buffer at 4 °C. Extracts were centrifuged at 27,000 × g for 20 min and the resulting supernatants subjected to vertical starch gel electrophoresis (Buchler instruments). The enzymes, their nomenclature, tissue source and electrophoretic conditions are presented in the table (see also Ferris and Whitt[9] for details of the electrophoresis). Enzyme staining was modified after Shaw and Prasad[10].

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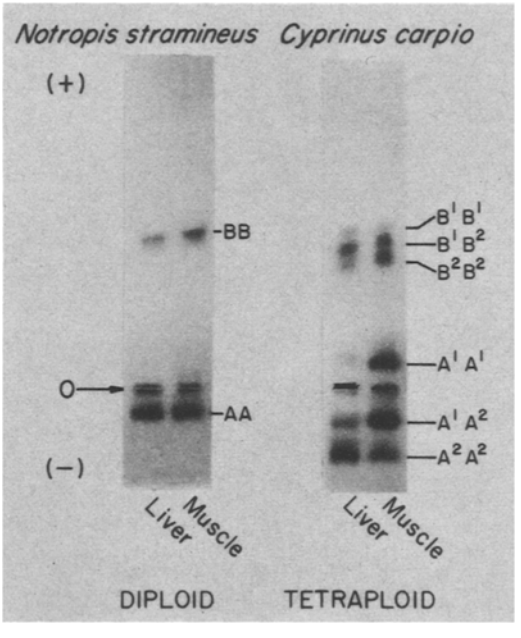
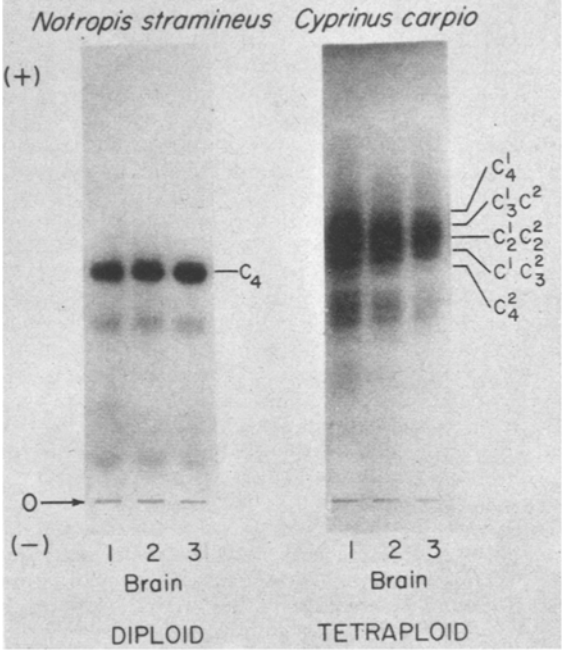


a Supernatant (S-AAT) and mitochondrial (M-AAT) aspartate aminotransferase isozymes of a diploid (cyprinid Notropis stramineus) and a tetraploid cyprinid (Cyprinus carpio). The diploid possesses one S-AAT and one M-AAT locus, designated A and B, respectively. The tetraploid expresses 2 S-AAT loci and 2 M-AAT loci, designated A^1, A^2, B^1 and B^2, respectively.



b Aldolase-C isozymes from brain extracts of 3 individuals of Notropis stramineus (2n) and Cyprinus carpio (4n). The diploid expresses 1 locus. The tetraploid expresses 2 loci. The 5 isozyme phenotype in the tetraploid is consistent with the random assembly of the 2 different subunits into all possible tetramers.

Enzyme	EC number	Locus Abbreviation	Tissues	Electrophoretic conditions	Number loci expressed	References*
Lactate dehydrogenase-B	1.1.1.27	Ldh-B	Muscle, brain	A, B	2	(PS,14,16)
Lactate dehydrogenase-C	1.1.1.27	Ldh-C	Liver	A,B	2	(PS,16)
Mitochondrial malate dehydrogenase	1.1.1.37	M-mdh	Muscle, brain	C	2	(PS,13)
Mitochondrial isocitrate dehydrogenase	1.1.1.42	M-idh	Heart	E	2	(12)
6-Phosphogluconate dehydrogenase	1.1.1.44	6Pgd	Liver, brain	B	2	(PS,15,16)
Glycerol-3-phosphate dehydrogenase	1.1.1.8	G3pdh	Muscle	C	2	(PS,13)
Superoxide dismutase	1.15.1.1	Sod	Liver	A,B	2	(PS)
Soluble aspartate aminotransferase	2.6.1.1	S-aat	Liver, heart	C	2	(PS,19)
Mitochondrial aspartate aminotransferase	2.6.1.1	M-aat	Muscle	C	2	(PS)
Adenylate kinase-A	2.7.4.3	Ak-A	Muscle	C	2	(PS)
Aldolase-C	4.1.2.13	Ald-C	Brain	B	2	(PS)
Glucose phosphate isomerase-A	5.3.1.9	Gpi-A	Brain	A	2	(PS,18)
Alcohol dehydrogenase	1.1.1.1	Adh	Liver	D	1	(PS)
Sorbitol dehydrogenase	1.1.1.14	Sdh	Liver	F	1	(12)
Lactate dehydrogenase-A	1.1.1.27	Ldh-A	Muscle	A	1	(PS,16)
Malate dehydrogenase-A	1.1.1.37	Mdh-A	Liver, brain	C	1	(PS,17)
Malate dehydrogenase-B	1.1.1.37	Mdh-B	Muscle	C	1	(PS,17)
Xanthine dehydrogenase	1.2.1.37	Xdh	Liver	D	1	(PS)
Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.9	Gapd	Muscle	C	1	(PS)
Creatine kinase-A	2.7.3.2.	Ck-A	Muscle	A	1	(PS,11)
Creatine kinase-B	2.7.3.2	Ck-B	Brain	A	1	(PS)
Phosphoglucomutase	2.7.5.11	Pgm	Muscle	C	1	(PS)
Glucose phosphate isomerase-B	5.3.1.9	Gpi-B	Muscle, heart	A	1	(PS,18)
					52% duplication	

A Gel-Tris-EDTA-Borate pH 8.6, 8 V/cm, 18 h[27]. B Tris-citrate pH 7, 7 V/cm, 18 h[28]. C Histidine-NaOH pH 8, 6 V/cm, 7 h[29]. D Boric acid NaOH pH 8.6, 8 V/cm, 7 h[30]. E Phosphate pH 6.5, 12 V/cm, 6 h[31]. F Tris-phosphate pH 6.5, 12 V/cm, 6 h[32]. *Numbers refer to references at end of paper; PS indicates locus was examined in the present study.

Results and discussion. The table summarizes the results of this study as well as results obtained from earlier studies by other laboratories[11–19]. A substantial fraction (52%) of the duplicate loci are still expressed. Isozyme patterns for two such duplicate gene sets are illustrated in the figure. The isozyme phenotypes for mitochondrial aspartate aminotransferase and aldolase-C are shown for both the tetraploid carp and a related diploid species for purposes of comparison.

The data in the table suggest that approximately half the duplicate gene sets in the carp express both loci and half express only one of the loci. Based upon our isozymic analyses 2 main postulates, which are not mutually exclusive, may be generated to account for the detection of only single locus expression at half the duplicate loci.

It is necessary to consider the possibility that the single locus expression of some duplicate locus sets is more apparent than actual. One postulate that might be brought forward is that not enough time has transpired since the polyploidization for duplicate genes to substantially diverge in their structure. If no, or only a few amino acid substitution differences have accumulated between the homologous isozymes, the probability of detecting them electrophoretically is low. However, several lines of evidence suggest that such 'cryptic' duplicate locus expressions are probably not extensive in this species. First, the ratio of staining intensities between allelic isozymes in heterozygous individuals is consistant with disomic inheritance at a single locus for many enzymes (e.g. Ck-A[11] and Pgm, our own unpublished data). Secondly, the carp are believed to have existed in the tetraploid state for a relatively long period of time[12]. They have been diverging from the Catostomidae, for example, for 50–70 million years, inferred from a comparison of hemoglobin α sequences[20,21]. Based on Nei and Chakraborty's formulation[22] for the probability of electrophoretic identity between 2 proteins, it has been shown that for initially identical proteins, which have been diverging for as long as 50 million years, less than 5% of these duplicate enzymes would be expected to possess coincident electrophoretic mobilities (see Ferris and Whitt[9]).

The second, and more likely explanation for the detection of the expression of only one of 2 duplicate loci is functional diploidization. Functional diploidization has been documented in various tetraploid fishes and recently postulated for the carp[13]. Based upon the DNA contents of this species it is unlikely that the functional diploidization is being accompanied by substantial physical loss of DNA[13]. One indication that functional diploidization is continuing to occur in the carp is the presence of null alleles for Ldh-B in European populations[14]. We have also observed these null alleles at the Ldh-B locus in the American populations. The fact that null alleles were not detected at other loci suggests that diploidization, though still occurring, is proceeding at a slow rate.

How does this level of diploidization compare with levels for other tetraploid Cypriniformes? The expression of 52% of the duplicate genes is comparable to the percentages observed in the tetraploid Catostomidae[9]. The average catostomid species expresses 45% (range 35–65%) of its genes in duplicate after 50 million years. Tetraploid cobitids of the genus Botia, however, exhibit relatively lower levels of duplicate gene expression, 15–30% (Ferris and Whitt, in preparation). The relatively low levels of duplicate gene expressions of the cobitids may be attributable to their more recent origin[23].

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The rates at which duplicate genes are silenced may be changing over the course of evolution. In the early evolutionary stages, soon after a disomic pattern of transmission has been established, the 2 loci should be sufficiently similar in structure and regulation, that the silencing of one of these loci would not have serious consequences. In the later evolutionary stages, the 2 loci may have diverged sufficiently in their structure and regulation that a loss of expression at either locus would tend to be selected against. This tendency for duplicate gene expression to be retained would result from both the structural and regulatory divergence of the duplicate genes. One consequence of the divergence in gene structure is that the multiple locus isozymes may eventually acquire different kinetic and physical properties and then come to occupy different metabolic niches of adaptive significance to the organism[24]. Another consequence of this gene divergence is the differential regulation of these duplicate genes during embryogenesis and in the differentiated adult tissues. Such structural and regulatory divergence has been reported for some duplicate loci in all tetraploid fishes examined to date[9, 15, 25, 26]. The extent of this divergence of duplicate locus expression should be able to serve as a very useful gauge of the relative rates of divergence of gene regulation at different periods after a gene duplication event. Furthermore, an estimate of the extent of such regulatory divergence would be helpful in determining how tightly coupled are the rates of evolution of structural and regulatory genes.

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Allozyme constitution of two standard strains of *Drosophila subobscura*[1]

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Summary. 2 reference strains of *Drosophila subobscura* ('Küsnacht' and 'ch-cu-Athens'), widely used for the study of inversion polymorphism in this species, were investigated with respect to their allozyme composition by starch gel electrophoresis in 18 different enzyme systems coded by 18 different gene loci. Both strains are monomorphic for all 18 loci with only 1 exception. A standardized method of designation is proposed, to allow a direct comparison of all enzyme data in the *Drosophila obscura* species group.

Strains. Natural populations of Drosophila subobscura, a European species of the Drosophila obscura group, have been intensively studied for the last 2 decades by several authors with respect to chromosomal inversion polymorphism. For these investigations, mainly 2 different strains have been used as standard strains. Both are homozygous for known chromosomal gene arrangements. The strain 'Küsnacht' originates from a small number of flies caught wild at Küsnacht (Switzerland) and is homokaryotypic for the basic 'Standard' arrangements in all 6 chromosomes[3]. It was used by Mainx[4] and Kunze-Mühl[5] for drawing the chromosomal map of the giant chromosomes of Drosophila subobscura. The other strain is called 'ch-cu-Athens'. It is chromosomally identical with 'Küsnacht' with the exception of being homozygous for the gene arrangement O_{3+4} of chromosome O. (For the designation of gene arrangements see Kunze-Mühl and Sperlich[6].) It is also homozygous for the recessive visible mutant alleles 'cherry' and 'curled', thus any possible contamination with other strains can easily be recognized. The strain is a descendant of the 'β-ch-cu-stock' described by Koske and Maynard-Smith[7]. It was mainly used as a standard strain by Krimbas and coworkers for their studies on inversion polymorphism in Greece.

Symbols for allozymes. Whereas, for inversions of Drosophila subobscura, a common system of designation proposed by Kunze-Mühl and Sperlich[6] has been accepted by practically all investigators, this is not the case for allozymes and the alleles coding for them. Since different authors used different symbols and different strains as controls, some confusion exists. It is the intention of this paper to help to make the situation for D. subobscura more clear.

The terminology we are going to propose here is the one which was used by Lakovaara and Saura[8, 9], Saura et al.[10], Saura[11] and Lakovaara et al.[12–14]. These authors studied the geographical variation of allozyme polymorphism in natural populations of D. subobscura and D. obscura and

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