and eukaryotes. An example similar to the present one is that reported by SIROTNAK[8] in which mutations in the dihydrofolate synthetase structural gene of *Diplococcus pneumoniae* increase the rate of production of the altered enzyme product.

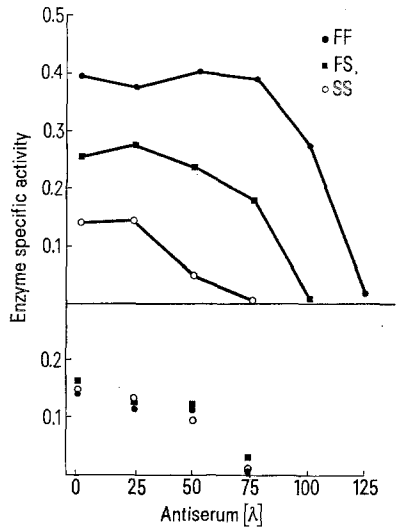

Fig. 2. Alcohol dehydrogenase activity in the supernatant after incubation with antiserum. In the upper figure the crude extracts of the 3 genotypes were undiluted whilst in the lower figure the 3 genotypes were diluted to give similar initial activities.

There is considerable variation in enzyme activity within both fast and slow Adh alleles extracted from natural populations and the variation is susceptible to artificial divergent directional selection[9]. It would be interesting to find out to what extent the variation in enzyme activity within an electrophoretic form is due to changes in the structural gene or background modification which results in changes in the rate of production of the enzyme molecule rather than to changes solely in the affinity of the enzyme molecule for its substrates. The results presented here suggest that some modifiers at least may prove to affect alcohol dehydrogenase activity by changing the rate of enzyme production.

Résumé. Les techniques immunologiques ont démontré que chez *Drosophila melanogaster* une forme électrophorétique «Fast» d'alcool déshydrogénase produit plus de molécules d'enzyme qu'une forme électrophorétique «Slow». On expose les résultats en les comparant à d'autres suggérant des mécanismes autorégulatoire.

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Duplication of the Gene Loci Coding for the Supernatant Aspartate Aminotransferase by Polyploidization in the Fish Family *Cyprinidae*[1]

Aspartate aminotransferase (AAT; E.C.: 2.6.1.1.) is a widely distributed enzyme in plant, animal, and human tissues. It catalyses the reversible reaction L-aspartate + α-ketoglutarate ⇌ oxaloacetate + L-glutamate using pyridoxal phosphate as the coenzyme. Two distinctly different forms of AAT have been described[2,3]. This finding has subsequently been confirmed by several other authors[4–10]. It has been shown that 1 of these 2 forms of AAT is found in the mitochondrial fraction (M-form), while the other one occurs in the supernatant of cell homogenates (S-form). Electrophoretic separation at neutral pH revealed that the M-form migrates towards the cathode and the S-form in anodal direction.

Among the vertebrates studied so far, genetically determined polymorphisms have been found for the S-form in herring[11], for the M-form in mouse (*Mus musculus*)[12], and for the M- and S-form in man[13,14]. A trans-specific variability for both forms has recently been described in primates[15]. The findings in mouse, man, and primates can be interpreted under the assumption of one gene locus existing in different alleles. The data on genetic polymorphism confirm investigations[16,17], suggesting a dimeric structure of either form of AAT.

The supernatant form of this enzyme was introduced as a genetic marker in the course of our studies designed to closer elucidate the diploid–tetraploid relationship established among members of the fish family *Cyprinidae*[18–24], order *Ostariophysi*. The present paper reports our findings in 3 diploid and 3 tetraploid species of cyprinid fish.

Materials and methods. The following species were examined: *Barbus tetrazona* (obtained from a local pet shop), *Rutilus rutilus* (from the Rhine river), *Tinca tinca* (Rhine river and a local fish store), *Barbus barbus*, *Cyprinus carpio*, and the hybrid *Carassius carassius* × *Carassius auratus* (all 3 species from the Rhine river). A number of different tissues were analysed (heart, liver, kidney, muscle, brain, gills, eye, and gonads). In the majority, however, the investigations were limited to heart and liver. In the case of *Barbus tetrazona*, because of its small size, the entire fish was used.

The tissues were homogenized 1:1 in 0.01 *M* PO_4-buffer, pH 7.4, frozen and thawed twice, and centrifuged at 20,000 × *g* until the supernatant was clear, which was subjected to electrophoresis. Gels were made of 0.01 *M* *Tris*–0.0028 *M* citric acid buffer, pH 5.5 in a 14% starch gel. The bridge buffer consisted of a 0.155 *M* *Tris*–0.043 *M* citric acid solution, pH 5.5. Electrophoresis was performed at 12 V/cm for 5 h, and afterwards the gels were sliced and stained in the following solution: 460 mg L-aspartic acid, 200 mg α-ketoglutaric acid, 10 mg pyridoxal phosphate, and 400 mg fast blue BB salt, suspended in 150 ml 0.05 *M* *Tris*-HCl, pH 7.6.

In order to identify the 2 respective forms of AAT, mitochondria were isolated (method according to HEN-

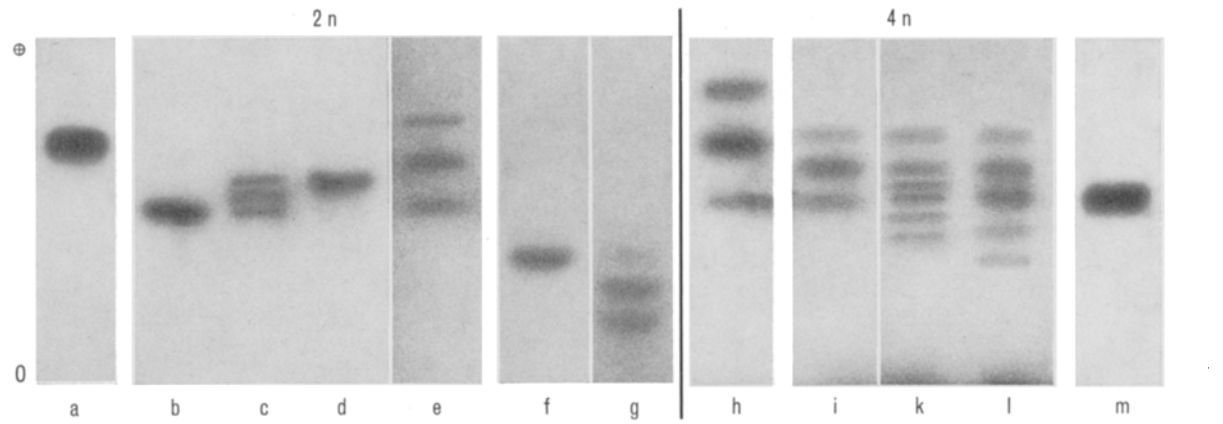

Fig. 1. S-AAT isoenzymes in *Barbus tetrazona*[a], whole fish, in liver-tissue of *Rutilus rutilus*[b–e], heart tissue of *Tinca tinca*[f, g], *Barbus barbus*[h], *Cyprinus carpio*[i–l], and *Carassius carassius* × *Carassius auratus*[m]. Phenotypes: a, AA; b, AA; c, AA′; d, A′A′; e, AA″; f, AA; g, AA′; h, AB; i, AB; k, AA′B; l, AA″B; m, AB.

DERSON[25]) from one representative of each ploidy level (*Rutilus rutilus*, and *Cyprinus carpio*) and subjected to electrophoresis.

Results and discussion. The supernatant form of AAT, as identified by comparison with the mitochondrial form through isolation of mitochondria, migrated anodally in all species examined. The different phenotypes found are shown in Figure 1. The designation of the different electrophoretic bands is given in Figure 2, assuming a dimeric structure of the enzyme and a random association of the subunits.

1. Representatives of the diploid level. a) *Barbus tetrazona*. No variant was found among 10 fishes, all of them exhibiting one single band. b) *Rutilus rutilus*. Among 62 individuals a majority of 40 showed one single band and was therefore referred to as the wild type AA. 19 fishes produced a heterozygote pattern consisting of three bands (AA′), the corresponding variant homozygote type was found twice (A′A′). Another heterozygote variant AA″ occurred in one individual. c) *Tinca tinca*. Out of 33 specimens investigated, 17 exhibited one band designated as the wild type AA, 16 animals were heterozygous (AA′).

2. Representatives of the teraploid level. a) *Barbus barbus*. 71 individuals of this species were examined, a variant, however, could not be detected. The triple band pattern found throughout was assumed to represent the homozygous wild type and might allow us to postulate the existence of 2 loci, A and B. b) *Cyprinus carpio*. In 72 carps 49 times the homozygous wild type pattern AB exhibiting 3 bands was found. 20 individuals showed a slower variant and 3 animals a still slower variant, so that the pattern comprises 6 bands each indicating the existence of 2 different heterozygotes. Whether this polymorphism is due to a variant occurring at the A or B locus cannot be determined because no corresponding variant homozygote was identified. c) *Carassius carassius* × *Carassius auratus*. From this natural hybrid 25 animals were investigated, all of them showing one single band, instead of 3 bands to be expected on the basis of this tetraploid extraction. This failure may be explained by assuming loss or deterioration of one locus, or by postulating identical electrophoretic mobilities of the gene products of 2 loci. Detailed investigations (e.g. quantitative measurements) to clarify this problem are in progress.

A comparison of the electrophoretic mobilities of all species examined yields identical positions of the isoenzymes formed of A subunits in *Rutilus rutilus*, *Barbus barbus*, *Cyprinus carpio* and *Carassius carassius* × *Carassius auratus*; the AA homomer of *Tinca tinca* occupies a position closer to the start, about the same as A″ A″ of *Cyprinus carpio*; the single band of *Barbus tetrazona* takes about the same position as the BB-band of *Cyprinus carpio*. These similarities may indicate possible homologies between the gene loci involved.

Although in *Barbus tetrazona*, *Barbus barbus* and the hybrid *Carassius carassius* × *Carassius auratus*, in which no genetic variants were found, the existence of one locus

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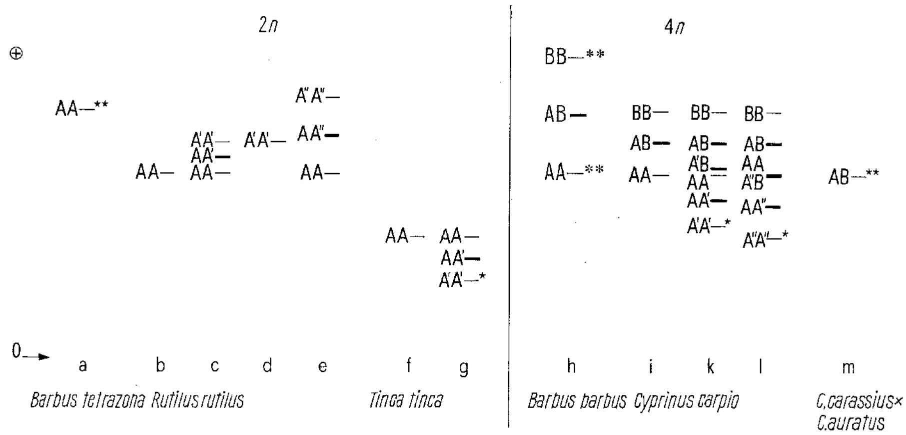

Fig. 2. Schematic presentation of the various S-AAT isoenzyme phenotypes observed and tentative designation of the subunit coposition. Species in which no variantr were observed are marked by 2 asterisks, those in which variants occurred only in the heterozygous state are marked by 1 asterisk.

in the diploids and of 2 loci in the tetraploids seems to be established.

The existence of a diploid-tetraploid relationship has also been established in the order *Isospondyli* (clupeoid and salmonoid fish)[26], and a number of genes coding for different isoenzyme systems (e.g. LDH[26], M-NADP-IDH[27], and SDH[28]) have been shown to be duplicated in the tetraploids. It may be mentioned that this is also valid for S-AAT. The findings of ODENSE[11] in the herring as a diploid species can be interpreted under the assumption that one gene locus with different alleles for S-AAT exists in this species. These data are confirmed by our own results: of 69 herrings investigated, 65 showed a single electrophoretic band (homozygous wild type), and 4 individuals the assumed heterozygous pattern, consisting of 3 bands. *Salmo trutta* and *Coregonus lavaretus*, as representatives of the tetraploid group, are apparently endowed with 2 gene loci for S-AAT: in 60 specimens of *Salmo trutta* and 41 specimens of *Coregonus lavaretus*, 3 electrophoretic bands were consistently found. One individual of *Coregonus lavaretus* showed an assumed heterozygous pattern with 5 bands.

Thus, the S-form of AAT proves to be another useful genetic marker for the demonstration of gene duplication as the consequence of polyploidization. In contrast to some other genes, which have apparently disappeared during evolution of the tetraploids (genes coding for 6-PGD in *Isospondyli* and for SDH in *Ostariophysi*[24]), duplicated S-AAT genes were apparently conserved or modified in such a way that their products fulfill a useful function. From this, it may be concluded that some selective pressure may be responsible for the maintenance of certain duplicated genes in the tetrapolids[29].

Zusammenfassung. Die S-AAT wurde bei Fischen der Familie *Cyprinidae* elektrophoretisch dargestellt. Die Isoenzymmuster lassen bei den diploiden Vertretern dieser Fischfamilie auf einen Genlocus, bei den tetraploiden auf 2 Loci schliessen. Analoge Verhältnisse bestehen offensichtlich auch bei Fischen der Ordnung *Isospondyli*.

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Antennal Proteins Involved in the Neural Mechanism of Quinone Inhibition of Insect Feeding

Findings about several aspects of the energy-transduction mechanism involved when certain quinones inhibit feeding in 2 species of insects were previously reported[1–10]. These chemical messengers react with sulfhydryl groups of macromolecules in nerve membranes in chemosensitive sensilla especially on the antennae of the insects. Such reactions, as monitored by ultraviolet difference spectroscopy[3,5], change the conformation of macromolecules in isolated nerve-membrane fragments. This change in vivo presumably allows altered ion flow through the receptor membrane[9], and this may bring about the generation of an action potential in the neuron[11]. Because our previous studies indicated that proteins (i.e. especially sulfhydryl-containing moieties) were selectively involved in the receptor macromolecules, the binding affinity of the feeding inhibitor, 2-methyl-1, 4-naphthoquinone, for antennal proteins was further investigated. We now present evidence of the resolution, by disc gel electrophoresis, of protein-containing bands from homogenized antennae of *Periplaneta americana* which appear to possess the properties of receptor chemical(s) for this feeding-inhibitory naphthoquinone. Results from in situ exposure of protein-containing materials in the antennae to 2-(^{14}C) methyl-1, 4-naphthoquinone indicated that 2 closely associated bands in the electrophoretically resolved Triton X-100-soluble proteins contained relatively high amounts of label (Figure A) in contrast to a low degree of count associated with saline-soluble proteins (Figure B).

Materials and methods. Antennae of live cockroaches were held in a $2.4 \times 10^{-6} M$ aqueous solution of the radiolabelled naphthoquinone (9.7 mC/m*M*) for 0.5 h. The antennae then were rinsed extensively with tap water which degrades naphthoquinone on the surface of the antennae. The antennal proteins were extracted according