Genetic Variation of α-Glycerophosphate – Dehydrogenase Isoenzymes in Clupeoid and Salmonoid Fish

The enzyme α-glycerophosphate-dehydrogenase (α-GPDH; EC 1.1. 1.8) represents a widely distributed dehydrogenase. Its metabolic role has been intensively studied in the insect flight muscle[1–5]. Furthermore the enzyme has been thoroughly explored biochemically[6,7]. α-GPDH is a dimeric molecule, and has a molecular weight of 78,000[8,9]. It catalyzes the reaction: Dihydroxyacetone Phosphate + NADH ⇄ α-Glycerophosphate + NAD+. The occurrence of multiple molecular forms of this enzyme was demonstrated especially in *Drosophila*, indicating the existence of a genetically determined polymorphism[10–14]. Hardly any investigations have been undertaken to study this enzyme with vertebrates[15,16]. To the best of our knowledge genetic variation was described in 2 species of fish only, *Sebastodes alutus*[17] and *Katsuwonas pelamis*[18].

In the course of experiments designed to study more closely the diploid-tetraploid relationship occuring among members of the fish order *Isospondyli*[19–22], α-GPDH was introduced as a genetic marker. It will be shown that the 4 species examined exhibit a rather complex electrophoretic pattern, including a number of variants of this isoenzyme system which, nevertheless, can be interpreted by a simple genetic model.

Materials and methods. The species examined are: *Clupea harengus* (herring), *Osmerus esperlanus* (smelt), both obtained from a fish store in Freiburg; *Salmo trutta* (brown trout), *Salmo irideus* (rainbow trout), obtained from trout hatcheries in the surroundings of Freiburg.

In general, homogenates of heart and kidney were used. From a number of animals of each species the following organs were also examined: brain, eye, gills, intestine, 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 20.000 × *g* twice for 30 min. The clear supernatant was then subjected to horizontal starch gel electrophoresis with 0.01 *M* *Tris*-PO_4-buffer, pH 7.4 in 14% starch gel at 12 V/cm for 5 h; bridge: 0.1 *M* *Tris*-PO_4-buffer, pH 7.4. In the case of the phenotype AA'BC in smelt electrophoresis was performed with pH 8.0 (see Figure 2, e-g). After electrophoresis, the sliced gel surfaces were stained for α-GPDH by incubation in the following staining solution: 50 mg NAD, 30 mg 3,3'-dianisoyl 4,4'-*bis* [2, (4-nitrophenyl) 5-phenyltetrazolium chloride], 10 ml Na-glycerophosphate 0.1 *M*, 1 ml NaCN 0.1 *M*, 5 mg phenazine methosulfate suspended in 150 ml *Tris*-HCl 0.05 *M*, pH 8.0 The formazan reaction is dependent on the presence of NAD and Na-glycerophosphate. The α-GPDH

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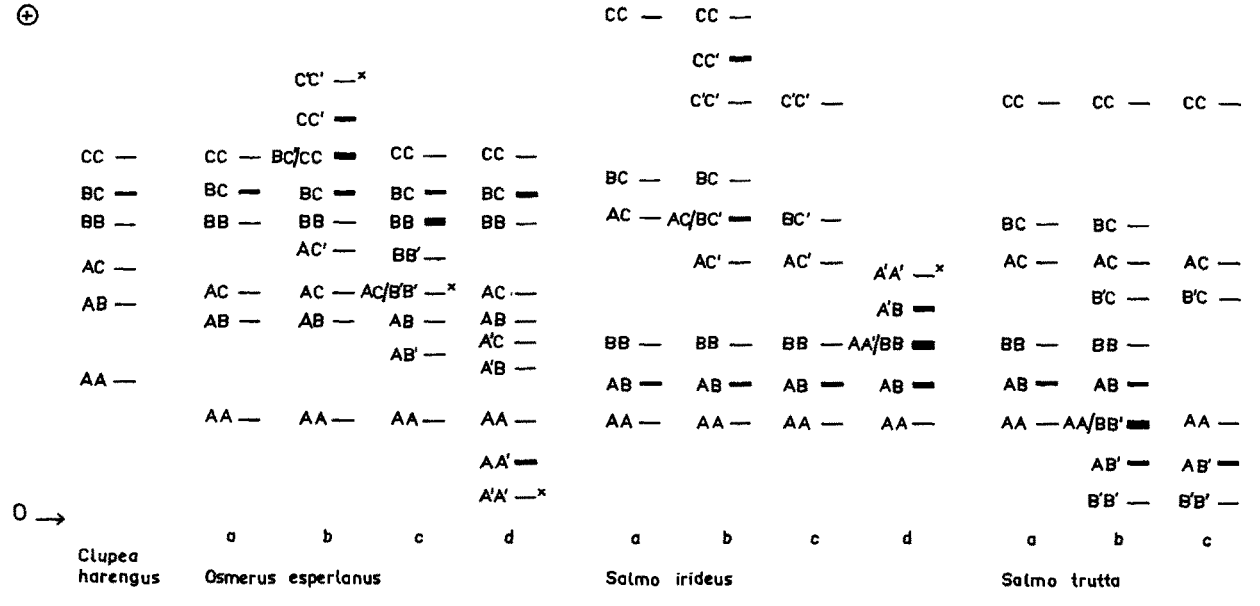

Fig. 1. Schematic presentation of the various α-GPDH isoenzyme phenotypes observed and tentative designation of the subunit composition. Variants occuring only in the heterozygous state are marked by an asterisk.

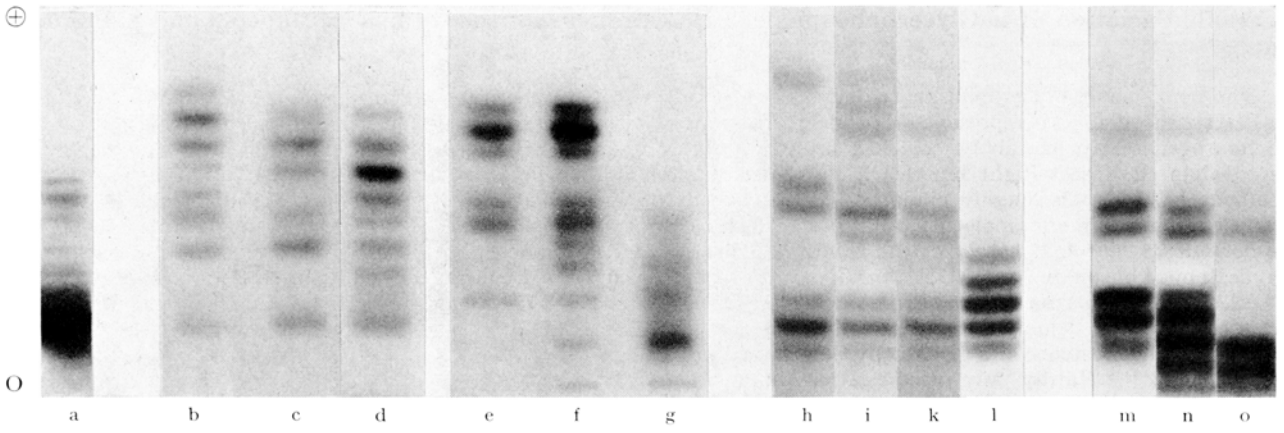

Fig. 2. α-GPDH-isoenzymes (a) in heart tissue of *Clupea harengus*; (b-f) in kidney and (g) in muscle tissue of *Osmerus esperlanus*; (h-l) in heart tissue of *Salmo irideus*; (m-o) tissue in heart in *Salmo trutta*.

Phenotypes: a, ABC; b, ABCC′; c, ABC; d, ABB′C; e, ABC; f, AA′BC; g, the same fish as in f; h, ABC; i, ABCC′; k, ABC′; l, AA′B (muscle tissue was used from which C cannot be demonstrated); m, ABC; n, ABB′C; o, AB′C.

bands coincided neither with bands of lactate-dehydrogenase nor alcohol-dehydrogenase.

From 3 samples each of brown trout, rainbow trout and herring, mitochondria were isolated out of heart and muscle tissue (method according to HENDERSON[23]); they were likewise subjected to electrophoresis.

Results and comments. α-GPDH activity was found in the extracts of all tissues subjected to electrophoresis. In mitochondria, no NAD-dependent α-GPDH activity could be demonstrated. Organ variability extended to both the number and intensity of electrophoretic bands.

The different phenotypes observed in the 4 species are summarized in Figure 1. Under the assumption of a dimeric structure of the molecule and random association of the subunits, the subunit composition can be specified as given in Figure 1.

Accordingly, a total of 3 different gene loci A, B and C coding for α-GPDH is to be assumed in each of the 4 species. Individuals which are homozygous at all 3 loci are referred to as representing the wild type. Among the variants observed, the finding of the respective variant homozygote demonstrates allelic polymorphism at the loci B and C; the occurrence of alleles at locus A can be derived from the heterozygote pattern with high probability. The number of specimens examined and the number of the various phenotypes found are summarized in the Table.

Number of animals examined in each species and electrophoretic findings

Species	No. of individuals	Electrophoretic pattern					
		ABC	AA′BC	ABB′C	AB′C	ABCC′	ABC′
Clupea harengus	40	40	—	—	—	—	—
Osmerus esperlanus	179	176	1	1	—	1	—
Salmo irideus	60	19	1	—	—	31	9
Salmo trutta	43	32	—	10	1	—	—

In the herring (*Clupea harengus*), only the wild type pattern was found among the 40 specimens examined (Figure 2a). In the smelt (*Osmerus esperlanus*), 176 out of 179 animals exhibited the wild type. The electrophoretic position of the presumed homomeric isoenzymes BB and CC is identical to that of the herring; the AA homomer, however, migrates slower to the anode in the smelt than in the herring occupying the same position as in the two trout species (Figure 1).

In the smelt 3 variant phenotypes representing different heterozygotes were designated as A′, B′, and C′ which, however, remain to be confirmed by the identification of the respective variant homozygotes (Figures 1, and 2, b-g).

In the 2 trout species examined, the isoenzymes formed of A and B subunits occupy identical electrophoretic positions. The position of the CC homomer in *Salmo irideus* is further anodical than that of *Salmo trutta*. While *Salmo trutta* invariably showed the wild type CC-band, a variant C′ was found in *Salmo irideus* occupying the same position as C in *Salmo trutta*. A complete polymorphism was observed at the B locus in *Salmo trutta* (Figure 2, m-o), and at the C locus of *Salmo irideus* (Figure 2, h-k). A heterozygous phenotype of *Salmo irideus* was interpreted as an A variant (Figure 2, l).

By examination of several organs it was hoped to obtain information on possible homologies of the isoenzymes between the species. The results obtained, however, were disappointing in this respect: In homogenates of testis almost exclusively the fastest cathodally migrating bands were found in the rainbow trout, brown trout and smelt; in the herring, on the contrary, the position near the origin was occupied. Homogenates of swim-bladder from all species revealed isoenzymes near the origin. Liver of the rainbow trout and smelt showed most intensive positions near the origin, whereas in the herring the farther cathodal positions were occupied. In brain from rainbow trout and herring the slower positions were stained more intensively, smelt, however, shows a greater activity for the faster positions.

Inspite, the comparison of the complete pattern in the 4 species examined leads to the distinction of 2 types of pattern in such a way that the herring and smelt at the

[23] N.S. HENDERSON, J. exp. Zool. *158*, 263 (1965).

one side, the 2 trout species at the other exhibit close similarities: While the herring and smelt show a single homomeric band (A) close to the start position, the 2 other homomers (B and C) migrating farther to the anode, the 2 trout species exhibit the homomeric bands A and B close to the origin, the C homomer migrating farther.

According to the diploid-tetraploid relationship established between members of the order *Isospondyli*, herring and smelt represent species on the diploid level, while the trout species have undergone tetraploidization during their recent evolution[20]. Thus, each of the 2 types of α-GPDH pattern coincides with one of the two ploidy levels. Since the number of gene loci in the diploids is apparently the same as in the tetraploids, the question of homology between the gene loci involved is of particular interest. Possibly the gene locus of the A isoenzyme was maintained in all species during evolution, whereas the loci of the B and C isoenzymes could have originated in different ways in the two groups of species. The 3 genes present could have arisen from a smaller number of ancestral genes through tandem duplication in the dipliods and through polyploidization in the tetraploids.

Addendum. The offspring of rainbow trout type ABC × type ABC' investigated meanwhile revealed the expected heterozygous pattern ABCC'. The confirms the existence of two alleles at the C locus of rainbow trout.

Zusammenfassung. Die Isoenzyme der α-GPDH lassen bei diploiden (Hering, Stint) und tetraploiden (Regenbogen-, Bachforelle) Fischen der Ordnung *Isospondyli* auf jeweils 3 verschiedene Genloci schliessen. Die stammesgeschichtlichen Beziehungen zwischen den verschiedenen Untereinheiten werden kurz diskutiert.

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Karyotypes of the Panamint Kangaroo Rat (*Dipodomys panamintinus* (Merriam))

Chromosomal polymorphism is not uncommon in mammals and has been reported by several authors (BAKER and MASCARELLO[1]; PATTON and DINGMAN[2]; HSU and ARRIGHI[3]). Usually this takes the form of interpopulational rather than intrapopulational variation and points up the fact that describtion of the karyotype of a species should only be made after sampling at least all of the subspecies. This is particularly true if some of the subspecies are well isolated from one another.

In this light, we wish to report on the karyotypes of the Panamint kangaroo rat, *Dipodomys panamintinus* (Merriam). This species is divided into 5 subspecies (HALL and KELSON[4]): *leucogenys* and *mohavensis* which have a continuous distributrion; *argusensis* and *panamintinus* which are in doubtful contact with each other and probably were in recent contact with *leucogenys* and *mohavensis*; and *caudatus* which is isolated from the other 4 subspecies by extremely arid, low desert which is atypical of the habitat of this species (Figure 1).

The only reports of the karyotype of this species have been from *D. p. mohavensis* (CSUTI[5]; JACKSON and HUNSAKER[6]). In this study, specimens were collected at or near the type localities of each subspecies. Karyotypes were prepared by the method of PATTON[7]. The results are shown in the Table and Figures 2 and 3. Figure 2 shows a karyotype of *D. p. leucogenys* which is typical of the species. Figure 3 shows the karyotype of *D. p. caudatus* which is quite distinct from the other 4 subspecies. The diploid number is constant (64) in all subspecies and agrees with the number given by the other investigators. From our sample, the sex chromosomes appear to be the same in all subspecies, i.e., a metacentric *X* and a small metacentric *Y*. CSUTI[5] found a small submetacentric *Y* and JACKSON and HUNSAKER[6] indicated a small metacentric as the *Y*. We differ in ratios of biarmed and uni-

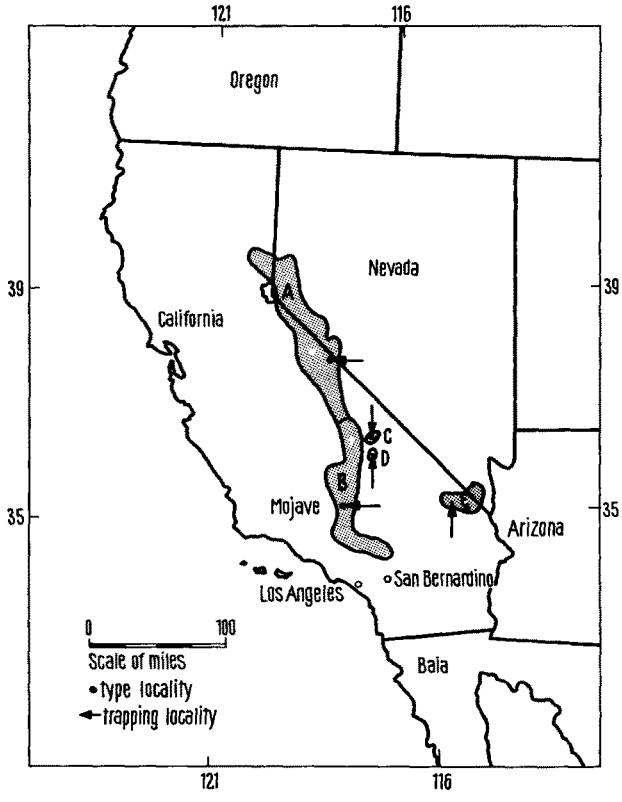

Fig. 1. Distribution of *Dipodomys panamintinus* and subspecies. A) *D. p. leucogenys*. B) *D. p. mohavensis*. C) *D. p. panamintinus*. D) *D. p. argusensis*. E) *D. p. caudatus*.

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