LINKAGE RELATIONSHIPS BETWEEN THE HOMOLOGOUS MALATE DEHYDROGENASE LOCI IN TELEOSTS

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GENE duplication is an important evolutionary mechanism and may have led to large increases in genome size and diversity early in vertebrate evolution (OHNO 1970). The role of duplicate genes may be further elucidated by biochemical and genetic analyses of multigenic isozyme system in modern teleosts.

Gene duplications are generally detected by the biochemical multiplicity of gene products. Apparent duplications have been found in vertebrates for a number of enzyme systems (SHAW 1969; MARKERT and WHITT 1968). Extensive gene duplication has occurred in salmonid and cyprinid fish as a result of tetraploid evolution (OHNO 1970). Specific duplications have occurred in most teleosts. Examples of teleost loci arising from duplication are the lactate dehydrogenase *E* and *B* loci (WHITT 1969, 1970; WHITT, CHILDERS and WHEAT 1971) and the supernatant malate dehydrogenase *A* and *B* (*MDH-A* and *MDH-B*) loci (KARIG and WILSON 1971). The inheritance of *MDH-B* has been studied in interspecific bass hybrids (WHEAT and WHITT 1971; WHEAT *et al.* 1971). In addition, BAILEY *et al.* (1969, 1970) have biochemically analyzed both the supernatant MDH-A and MDH-B isozymes of salmon. These MDH isozymes are very similar in kinetic properties, amino acid composition, and immunochemical cross-reactivity (BAILEY *et al.* 1970). The good correlation between immunological similarity and sequence homology established for other enzymes (PRAGER and WILSON 1971) suggests extraordinary homology between MDH-A and -B. Furthermore, the structural similarity of the supernatant MDH-A and -B polypeptides in interspecific centrarchid hybrids is confirmed by the random assembly of these subunits into dimers both *in vivo* and *in vitro* (WHEAT and WHITT 1971; WHEAT *et al.* 1971). The present study is one of the first analyses of linkage relationships between homologous loci specifying such closely related enzymes.

Interspecific hybrids between bluegills (B) (*Lepomis macrochirus* Rafinesque)

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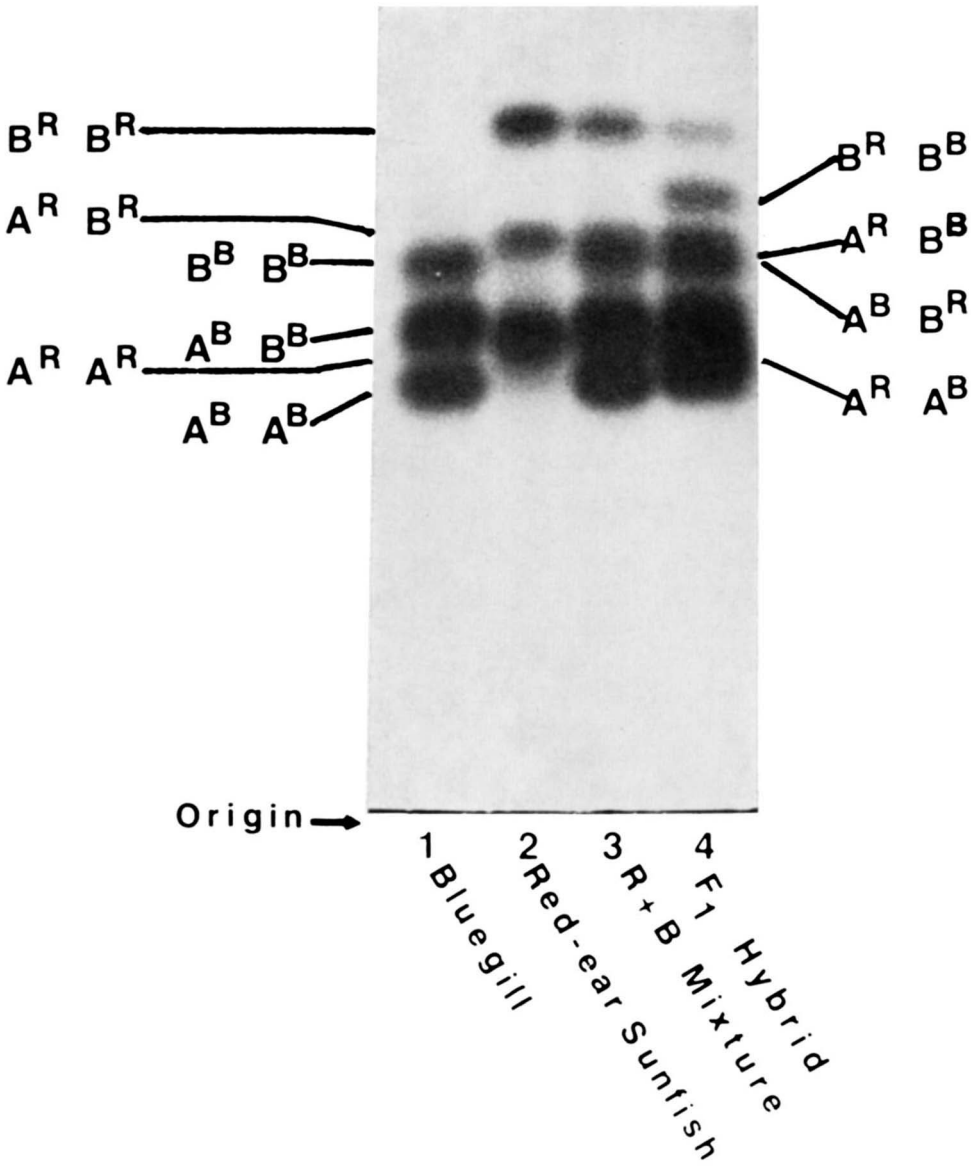

FIGURE 1.—Supernatant malate dehydrogenase isozymes of bluegill, red-ear sunfish, and their F_1 hybrid. 1—Bluegill skeletal muscle; 2—Red-ear skeletal muscle; 3—Mixture of equal volumes of extracts from bluegill and red-ear skeletal muscle; 4—F_1 hybrid skeletal muscle. The isozyme pattern is generated by the random assortment of subunits to form all possible dimers. The F_1 hybrid exhibits 10 isozymes: 3 of each parental type and 4 molecular hybrids. These molecular hybrids are not observed in the simple mixture of parental extracts.

and red-ear sunfish (R) (*L. microlophus* Günther) were selected for the backcross. These hybrids are fertile (CHILDERS 1967), and the chromosomes of the parental species are morphologically and numerically indistinguishable (ROB-

ERTS 1964). F_1 hybrids (♀ B × ♂ R) were produced in the laboratory and grown to maturity in the wild. In the spring of 1971, several ♂ F_1 hybrids and ♀ red-ears were placed in an east–central Illinois pond containing no other fish. Successful backcrossing occurred. At an age of about 2 months, 412 backcross progeny were collected by seining and were immediately frozen. Whole fish were individually homogenized, subjected to electrophoresis, and specifically stained for malate dehydrogenase (MDH) (EC 1.1.1.37, L-malate:NAD oxidoreductase) as described previously (WHEAT *et al.* 1971).

Figure 1 shows the supernatant MDH isozyme pattern observed in skeletal muscle of the two parental species (bluegill-A^BA^B/B^BB^B; red-ear-A^RA^R/B^RB^R) and their F_1 hybrid (A^RA^B/B^RB^B). Subunit composition of the isozymes and the tissue specificity of their synthesis were determined as previously reported (WHEAT *et al.* 1971).

The number of observed testcross progeny is shown in Table 1. The ratio of homozygotes to heterozygotes does not differ significantly from the expected 1:1 for either locus (*MDH-A*: $\chi^2 = 1.90$, $P > 0.15$, 1 d.f.; *MDH-B*: $\chi^2 = 0.350$, $P > 0.60$, 1 d.f.).

The distribution for all four classes does not differ significantly from the 1:1:1:1 ratio expected for unlinked loci ($\chi^2 = 3.22$, $P > 0.30$, 3 d.f.). Calculation of the maximum likelihood limit (MATHER 1951) demonstrates that these loci undergo recombination at a frequency of 47.6% ± 6.35% (99% confidence limit). These data are not significantly different from random assortment of codominant alleles at unlinked loci, although loose linkage cannot be excluded. However, it is clear that the *MDH-A* locus is not closely linked to the *MDH-B* locus in this system.

The absence of close linkage between such similar homologous loci may reflect the mechanism which generated them and their evolutionary history. On the basis of DNA content as well as chromosome morphology and number (OHNO and ATKIN 1966; ROBERTS 1964), the bluegill and red-ear sunfish appear to be typical of fish considered to be diploid as opposed to the tetraploid members of the Salmonidae and Cyprinidae which have about twice the DNA content and chromosome arms compared to other teleosts. Therefore, the supernatant MDH gene duplication in the sunfish could not have arisen during a recent tetraploidization event like that of salmonid and cyprinid fish.

Our data are consistent with tandem duplication of the ancestral supernatant MDH locus and suggest that some selective force favored the separation of the

TABLE 1

Distribution of MDH-A and MDH-B phenotypes among backcross progeny

	B^RB^R	B^RB^B	Total
A^RA^R	118	102	220
A^RA^B	94	98	192
Total	212	200	412

duplicate loci. However, we cannot exclude gene duplication during an extremely ancient polyploidization event. OHNO (1970) has proposed that extensive gene duplication, including polyploidization, was an important factor in the evolution of primitive chordates prior to the adaptive radiation of fish and other vertebrates.

In summary, the closely related duplicate gene loci encoding the supernatant malate dehydrogenase isozymes of fish are not closely linked. The absence of linkage between these closely related loci may be related to the means of gene duplication and/or the specificity of their regulation.

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