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Centric Fusion and Trisomy for the LDH-B Locus in Brook Trout, Salvelinus fontinalis

Abstract. *Cytogenetic analyses showed that a trisomic male brook trout of genotype* BB′B″ *for one of the lactate dehydrogenase subunit loci had a karyotype with two extra arms appearing as a metacentric chromosome. The metacentric chromosome probably arose through centric fusion of two acrocentric or telocentric chromosomes—one of which carried the locus for subunit B—followed by nondisjunction.*

In the course of screening a population of brook trout for use in intragenic recombination studies (*1*) at the locus specifying the B subunit of the ubiquitous system of lactate dehydrogenase (LDH) in trout (*2*), a male showed a zymogram pattern that indicated trisomy for three *LDH-B* alleles—genotype *BB′B″*. Breeding and cytogenetic analyses of this male confirmed the trisomy and indicated that it probably arose through a spontaneous centric fusion of two acrocentric or telocentric chromosomes, followed by nondisjunction.

Starch gel electrophoresis of eye tissue (*2*) permits unambiguous detection of all genotypes for the *LDH-B* locus. This is so because gene dosage effects, as well as allelic differences, are reflected in differentially stained homo- and heterotetramers of unique electrophoretic mobility formed from the A and B subunits specified by the *LDH-A* and *LDH-B* loci (Fig. 1).

The breeding results for the parental generation in which the trisomic male was found (family M290), for two families produced by this male (0-37 and 0-38), and for families produced by two heterozygous male sibs (0-39 and 0-40) are presented in Table 1. The trisomic male arose in a family in which the proportions of offspring indicate the expected segregation of *LDH-B* alleles, if the trisomic offspring is excluded.

The trisomic male was testcrossed to two females of *BB* genotype to give the families 0-37 and 0-38. In each family the ratio of six offspring genotypes fit that expected if there had been random assortment of three chromosomes into equal numbers of functional n and $n + 1$ gametes. Moreover, the data from the two families are homogeneous enough to be combined ($\chi^2 = 0.82$, $P > .95$); as expected, the number of progeny with the six genotypes are nearly equal, as are the number of trisomic and disomic offspring (55 and 53, respectively). Typical zymogram patterns used to classify the six genotypes are shown in Fig. 1. The high concentration of the B_4 homotetramer in genotypes *BBB″* (slot 1) and *BBB′* (slots 3 and 5) contrasts to the lower concentration of this tetramer in genotypes *BB″* (slot 6) and *BB′* (slot 4). More bands are present in the zymogram from a *BB′B″* fish (slot 2), and the second band from the origin in this zymogram is a doublet representing A_3B' and A_3B'' heterotetramers.

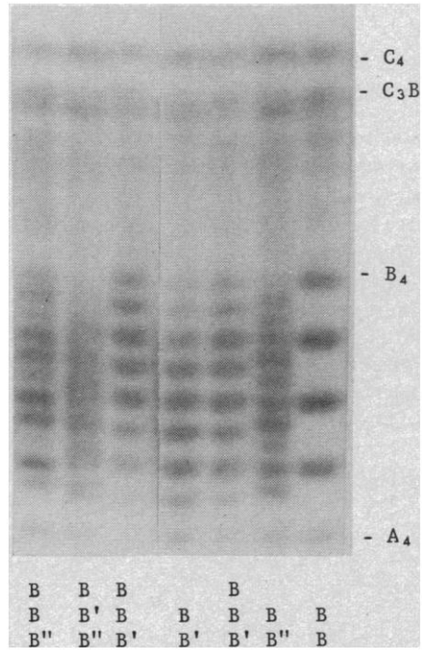

Fig. 1. Starch gel zymograms of eye tissue LDH from progeny of a *BB* disomic female crossed with a *BB′B″* trisomic male brook trout. Genotypes are designated at the bottom of each slot; homotetramers of A and B subunits of the ubiquitous system as well as tetramers of the C subunit of the eye system are shown along the side (origin and cathode at bottom; anode at top).

Two *B′B″* male sibs from family M290 were testcrossed to *BB* females to produce the families 0-39 and 0-40 (Table 1); the ratios of offspring in both families are close to the 1 : 1 expected for offspring of disomic heterozygotes. Also, one female and three male sibs of the *B′B″* genotype from family M290 were used in other crosses (*1*); all four produced results expected of normal disomic segregation. Thus, no unusual genetic results were recorded from six normal siblings of the trisomic male studied.

Cytological studies were made on the trisomic male after the breeding experiments and on some of his progeny from family 0-37. Squash preparations were made from gill and kidney tissues that were finely minced, treated with hypotonic saline, and fixed in Carnoy's fixative; the technique has been described (*3*). Chromosomes were stained with 1 percent aqueous crystal violet and photographed with a Leitz Ortholux photomicroscope. The normal brook trout modal chromosome number is $2n = 84$, with 100 total arms distributed as 16 metacentrics and 68 acrocentrics (*3*, *4*). In contrast, all 25 cells examined from the trisomic male had two extra arms. The modal count (15 cells) was 85 chromosomes with 17 metacentrics and 68 acrocentrics. One cell had 87 chromosomes with 15 metacentrics and 72 acrocentrics; six cells had 86 chromosomes with 16 metacentrics and 70 acrocentrics; and three had 84 chromosomes with 18 metacentrics and 66 acrocentrics. While some of this variation might be due to counts made on disrupted cells from squash preparations, such intraindividual variation due to Robertsonian variation or centric fusion and fission is common in Salmonidae (*3*, *5*). A karyotype of a cell showing the modal chromosome number with the extra metacentric appears in Fig. 2.

Among young fry from family 0-37, limited chromosome counts on known LDH genotypes were informative but not definitive. One metaphase in a *BB′B″* individual showed 102 arms distributed as 85 chromosomes with 17 metacentrics, a distribution identical to

that of the male parent. Three *BBB′* individuals had a modal count of 85 chromosomes with 16 metacentrics, a total of 101 arms. Two cytological classes (with 100 and 101 arms, respectively) appeared among *BB″* disomics. All had modal counts of 84 chromosomes, but one group had 16 metacentrics and the other had 17 metacentrics. All other disomics had 100 arms with a modal count of 84 chromosomes (16 metacentrics and 68 acrocentrics).

Table 1. Breeding results from matings of brook trout of various *LDH-B* locus genotypes.

Family	Parental genotypes		Number of offspring with genotype:							*P* of χ^2
	♀	♂	*BB*	*BB′*	*BB″*	*B′B″*	*BBB′*	*BBB″*	*BB′B″*	
M290	*BB″*	*BB′*	7	9	16	12			1*	>.20
0-37	*BB*	*BB′B″**	6	10	11		13	5	11	<.50
0-38	*BB*	*BB′B″**	7	7	12		12	5	9	<.50
0-39	*BB*	B′B″		41	36					>.50
0-40	*BB*	B′B″		121	113					>.50

* Original trisomic.

The cytological and zymogram analyses show that the extra metacentric possessed by the original trisomic of genotype *BB′B″* was not an isochromosome. An isochromosome would have resulted in only one extra arm, and the ratios of genotypes and of disomics to trisomics among the progeny would have departed significantly from equality. If the extra metacentric were a trisomic isochromosome, the original male with three different alleles would have been tetrasomic for the chromosome bearing the *LDH-B* locus. Rather, the data indicate that the metacentric arose from centric fusion (Robertsonian translocation) of two acrocentric chromosomes, one of which carried the *LDH-B* locus. It is likely that the other acrocentric involved in the centric fusion was the chromosome carrying the *LDH-A* locus, because pseudolinkage of *LDH-A* and *LDH-B* alleles occurs among males of trout species and their hybrids and is correlated with centric fusion and fission (*2, 3*). If this hypothesis is correct, the trisomic chromosome set also possessed three doses of the *LDH-A* gene, because the normal complement of chromosomes was present in addition to the extra metacentric. In some zymograms the A_4 band appears heavier in trisomics than in disomics. However, the lack of allelic differences for the *LDH-A* locus in the *BB′B″* trisomic made definitive analysis impossible.

The extra metacentric probably carried the *B″* allele. The one chromosome count made on a *BB′B″* offspring from family 0-37 showed 102 arms, including an extra metacentric; two *BB″* disomic offspring had an extra metacentric, while the extra chromosome in three *BBB′* progeny was an acrocentric. Also, all *BB′* offspring had 100 arms, whereas *BB″* offspring had cells with 101 and 102 arms as well. If *B″* was carried on the extra metacentric, the *BB″* disomics with the normal 84 chromosomes and no extra chromosome arms must have resulted from fission of the metacentric, followed by segregation of the *B″* acrocentric to one gamete and the *B* and *B′* acrocentrics to the other. The latter would give rise to *BBB′* offspring with one extra arm; three such offspring were found. The failure to find disomics for *LDH-B* with an extra acrocentric (presumably bearing the *LDH-A* locus) may represent some univalent loss during parental gamete formation or may be simply the result of sample size. Unfortunately, we did not obtain data, such as cytological analysis of the *BBB″* progeny, that would have shown whether the metacentric carried the *B″* allele.

The equal numbers of the six genotypes and of disomics and trisomics among the progeny of the male trisomic suggest that meiosis in this male must have been precise. The metacentric bearing the *B″* allele probably formed a bivalent and a univalent randomly. Disjunction of the bivalent members plus random segregation and regular inclusion of the univalent in meiotic products must then have occurred. It is possible that other gametic combinations are produced but are lethal.

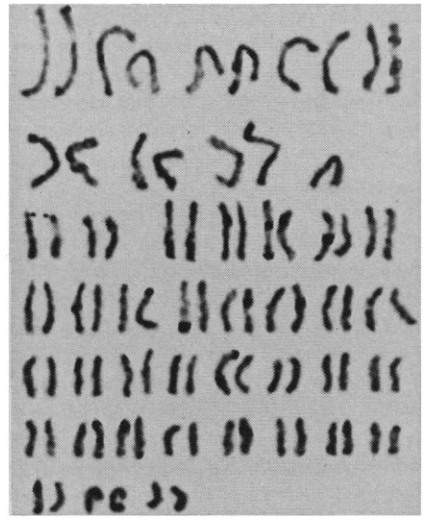

Fig. 2. Karyotype of a centric-fusion trisomic male brook trout. Two extra arms as a metacentric are at the end of the second line ($2n = 85$ with 17 metacentrics; fundamental arm number = 102). A satellite pair of chromosomes is in the third line.

We had hoped to identify a specific chromosome as the bearer of the *LDH-B* locus and its linkage group. This was not possible because of the relative lack of differences among the 68 acrocentric chromosomes in a normal array (Fig. 2). New staining techniques may make such identification possible. We showed that aneuploidy can be superimposed on the Robertsonian variation extant in species of Salmonidae and that it can be recognized when it involves chromosomes carrying alleles for biochemical traits with distinguishable genotypes.

Two additional points should be made. In most other animals, including humans, even partial trisomy is usually deleterious (*6*). In contrast, neither differences in size and viability nor abnormalities in phenotype or meiotic segregation were associated with trisomy in these fish. Second, it has been proposed that salmonids are ancient tetraploids. Recurrent trisomy may have been one mechanism for gradually attaining polyploidy and for creating a duplicated genome on which the selective processes of evolution could act (*7*). Many lines of evidence point to the importance of the flexible genomes of these fishes in evolution and in our understanding of evolutionary processes.

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Selection for Circadian Eclosion Time in *Drosophila melanogaster*

Abstract. *Early and late eclosion strains were developed from* Drosophila melanogaster *cultures. The Oregon-R parent strains (isolated in 1925) showed significantly more selectability than the W² parent strain collected at the beginning of this study (1971). This is consistent with the hypothesis that the selective advantage of circadian behaviors is reduced in laboratory conditions.*

Many characteristics of circadian rhythms have been investigated in detail, for example, phase response (*1*), free-running period (*2*), temperature independence (*3*), and numerous physiological factors (*4*). Maintenance of these characteristics in various taxa [such as plants, protozoa, insects, and mammals (*1, 5*)] implies that they have general adaptive value.

Bunning (*6*) crossed plants with different period lengths and suggested Mendelian segregation of period length, because intermediate periods occurred in F_1 plants and the original periods appeared in following generations. The same was evident in Danilevskii's work with the butterfly *Aeronycta rumicus* (*7*).

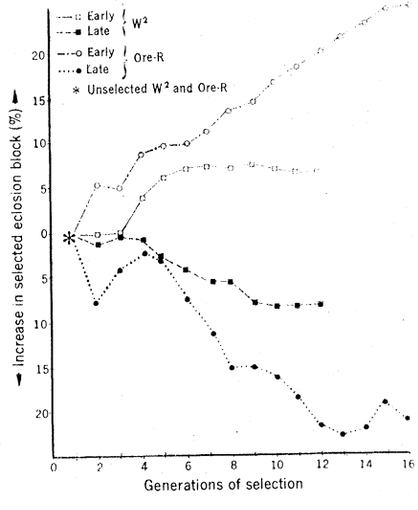

Fig. 1. Selection for early and late eclosion strains of *Drosophila melanogaster* wild-caught (W^2) and laboratory-reared (Ore-R) populations (*13*). The values on the ordinate are the percentages eclosing in block 1 of the early strain and block 3 of the late strain minus the percentages eclosing in these collection blocks of the unselected stocks (Table 1). The mean number per data point is 362 flies; the range is 153 to 652.

Konopka and Benzer (*8*) showed that free-running periods in *Drosophila* exhibit classical Mendelian characteristics and have further localized the mutant gene or genes affecting period length to a very short segment of the X chromosome. These mutant stocks were developed by using the mutagen ethyl methanesulfonate. It is likely that flies with varying period lengths also occur in nature. Maintenance of 24-hour periods in natural populations implies selection against other periods. In this report we discuss the selectability of wild and laboratory-reared populations of *Drosophila* for early and late eclosion.

Selection lines for early and late eclosions from the pupae case were developed by using laboratory and wild populations of *Drosophila melanogaster*. The laboratory population was of the Oregon-R strain (Ore-R) isolated in 1925 by D. E. Lancefield (*9*) and obtained from A. Yanders (Michigan State University) in 1966. The wild population (W^2) was collected at the beginning of this study (September 1971) in Walla Walla, Washington.

The selection schedule consisted of three consecutive 4-hour collection blocks (periods) beginning 6 hours before and ending 6 hours after dawn. Flies eclosing in blocks 1 and 3 were used as breeding stock for successive generations of the early and late lines, respectively. Block 2 flies were counted and discarded. All flies were reared in 31-g shell vials containing 10 cm^3 of media and maintained at 25°C in a cycle of 12 hours of light followed by 12 hours of darkness (LD 12 : 12).

If selection pressures have been altered by laboratory rearing, one might expect differences in (i) the initial eclosion band shape and (ii) the responses to artificial selection when laboratory-reared flies were compared to wild-caught flies. No difference was seen in the initial eclosion band (Table 1, $P > .975$ by χ^2 analysis). The collection blocks were broad (4 hours), and shorter blocks (1 hour) might have shown a subtle difference. On the other hand, selection for early and late eclosion did demonstrate striking differences in variability of the two parent strains ($P < .01$ by sign test). This is true for comparisons of both early and late eclosion. In both cases the laboratory strain showed greater variability, a result suggesting a relaxation of selection pressures (Fig. 1).

These results are, in one respect, counterintuitive. Forty-seven years of laboratory culturing have no doubt subjected the Ore-R strain to considerable inbreeding, which reduces genetic variability (*10*). In addition, selective pressures different from those in which the species has developed tend to truncate gene distribution. One might, then, expect laboratory rearing to reduce genetic variability unless selective pressures in natural conditions are even more limiting than the forces of inbreeding and artificial (laboratory) selection.

Pittendrigh (*11*) suggested that humidity levels in nature are effective in selecting for eclosion times. Eclosion in *D. pseudoobscura* is more limited to early dawn, when humidity is highest,

Table 1. Eclosion of unselected (generation 1) laboratory (Ore-R) and wild (W^2) populations of *Drosophila melanogaster* in three consecutive 4-hour collection blocks. Dawn of the LD 12 : 12 cycle occurred 2 hours into block 2 (*N*, number of flies eclosing).

Collection block	Eclosion in strain			
	Ore-R		W^2	
	N	%	*N*	%
1	73	11.2	43	10.8
2	503	77.3	310	77.5
3	76	11.6	47	11.8