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Running head: Westslope cutthroat x rainbow trout hybrids

WESTSLOPE CUTTHROAT TROUT X RAINBOW TROUT HYBRIDS: HATCHING SUCCESS, GROWTH, AND SURVIVAL

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Hybrids of female westslope cutthroat trout (Salmo clarki lewisi) x male rainbow trout (Salmo gairdneri) had slight but significantly greater 'eyed' and hatching success than pure westslope cutthroat trout. By 90 days after fertilization, however, the hybrids showed a significantly slower growth rate and lower survival than pure westslope cutthroat trout. The results indicate that there may be more genetic incompatibility between these species than is generally assumed, or that greatly different levels of genetic incompatibility exist between different populations of rainbow trout and westslope cutthroat trout.

Key words: rainbow trout, westslope cutthroat trout, hybrids, introgression

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25 April 1983

Dr. Robert Behnke
Department of Zoology
Colorado State University
Fort Collins, CO 80523

Dear Dr. Behnke:

Enclosed are two manuscripts that we have recently submitted for publication that address the issue of hybridization between native and introduced salmonid fishes in western North America. Because of your interest in these fishes, and this phenomenon, we thought that you would be interested in the results. Any comments or criticism of the manuscripts will be greatly appreciated.

Sincerely,

Robb F. Leary

Robb F. Leary

RFL:mb

Encs.

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TETRAPLOIDY AND THE EVOLUTION OF SALMONID FISHES

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1. INTRODUCTION: POLYPLOIDY IN VERTEBRATE EVOLUTION

Polyploidy has long been recognized to have played an important role in the evolution of plant species (Lewis, 1980). However, polyploidy has only recently been recognized as a potentially important process in the evolution of vertebrates (Ohno, 1974; Fisher et al., 1980; Bogart, 1980; Schultz, 1980). Mammals and birds generally possess more DNA per cell than fish and other chordates. This observation, and the existence of many duplicated gene loci, have led Ohno and coworkers (1967; 1968; 1970a; 1974) to propose that genome doubling has taken place at least once in the evolution of vertebrates. One tetraploid event apparently took place about 500 million years (Myr) ago in a common ancestor of all vertebrates. Fisher et al. (1980) describe isozyme studies that are consistent with this idea. Other tetraploid events may have taken place in major lineages of vertebrate evolution, possibly including a genome doubling in a reptilian ancestor of mammals (Ohno, 1967; Comings, 1972).

Results have also shown that recent polyploid events are much more common than was previously suspected. Polyploid amphibians and reptiles are surprisingly common (Bogart, 1980). It is curious that all of the polyploid frogs and toads that have been described are bisexual, while all of the polyploid salamanders and lizards are parthenogenetic or gynogenetic triploids. In spite of the many examples of polyploidy in amphibians and reptiles, polyploidy has apparently not been an important process in progressive evolution of these groups; all of the polyploid species have closely related diploid species and no higher polyploid taxa have been found (Bogart, 1980).

A number of cases of polyploidy in fish have also been reported (Schultz, 1980). Some species of primitive fish, including the paddlefish, Polyodon spathula (Dingerkus and Howell, 1976), the shovelnose sturgeon, Scaphirhynchus platorhynchus (Ohno et al., 1969a), the lungfish, Protopterus dolloi (Vervoort, 1980), and the spotted gar, Lepisosteus productus (Ohno et al., 1969a), appear to be tetraploid. Closely related diploid and tetraploid forms are found in the spinous loach, Cobitis biwae (Kobayasi, 1976; Sezaki and Kobayasi, 1978). There also appears to be a diploid-tetraploid relationship among species of armoured catfish of the genus Corydoras (Dunham et al., 1980) and among some eels of the genus Misgurnus (Raicu and Taisescu, 1972).

Three species of cyprinids, the barb (Barbus barbus), the carp (Cyprinus carpio), and the goldfish (Carassius auratus) appear to be tetraploid (Ohno et al., 1967; Muramoto et al.,1968; Wolf et al., 1969; and Ohno, 1974). In addition, some unisexual goldfish strains are triploid or tetraploid in relation to the normal bisexual goldfish (Cherfas, 1966; Liu et al., 1978).

Recent polyploids have been found in two genera of livebearing fishes (Poecilia and Poeciliopis) in Mexico (Schultz, 1980). The triploid unisexual forms all reproduce by gynogenesis

and have hybrid origins. These fish, and related diploids, have been intensively studied and have provided useful model systems to examine the origin and adaptive value of polyploidy (Schultz, 1980). In addition, Echelle and Mosier (1981) have recently described a unisexual species of Menidia (Atherinidae) that are apparently triploid.

There are only two known cases of polyploidy in fish giving rise to an entire family. The suckers of the family Catostomidae are a large evolutionary group (12 genera, 58 species; Nelson, 1976) that apparently share a common tetraploid origin. Uyeno and Smith (1972) have suggested that the catostomids "evolved by tetraploidy from a cyprinid-like ancestor" over 50 Myr ago on the basis of an apparent doubling of chromosome numbers and DNA contents. They consider the catostomids to be one of the most evolutionarily successful groups of fishes in North America. The suckers have been an especially valuable group for study of evolutionary changes following a tetraploid event (Ferris, this volume).

The Salmonidae are the second family of fish that apparently share a common tetraploid origin. There are three major salmonid taxa that are classified as subfamilies by most taxonomists: Coregoninae (whitefish and cisco), Salmoninae (trout, salmon, and char), and Thymallinae (grayling) (Norden, 1960; Nelson, 1976). These fish have been further classified into 9 genera and some 68 species, Table I (Nelson, 1976). The scientific and common names of all species discussed in this paper are given in Table II.

These two tetraploid-derived families differ in that the catostomids apparently quickly returned to a diploid state of chromosome pairing, while the salmonids are still going through the "diploidization" process of restoring disomic inheritance. The evolutionary success of these two families is an exception to the common view that polyploidy is not an important factor in progressive evolution beyond the species level (Stebbins, 1977; Dobzhansky et al., 1977; White, 1978). The objective of this paper is to describe the evolution and genetics of salmonids with the principal purpose of increasing the understanding of the significance of polyploidy as an evolutionary process.

2. THE SALMONID TETRAPLOID EVENT

2.1. Evidence for Ancestral Polyploidy

The idea that salmonids have a polyploid origin was first proposed by Svardson (1945). He observed that chromosome numbers in salmonid species seemed to fall into multiples of 10; Atlantic salmon have about 60 chromosomes; the brown trout, Arctic char, brook trout, and the common whitefish have about 80 chromosomes; the grayling has about 100 chromosomes. These observations, plus variations in the numbers of bi-armed chromosomes among species and the observation of multivalents in meiotic preparations from several species, led Svardson to propose that the basic chromosome number in salmonids is n=10, and that the variations in chromosome number were the result of polyploid events. This proposal was shown to be incorrect when Rees (1964) demonstrated that cellular DNA contents and total chromosome lengths were similar in the Atlantic salmon and brown trout. Further evidence against Svardson's proposal accumulated (Booke, 1968). Svardson's proposal that salmonid fish are polyploid has subsequently been presented as a disproved example of polyploidy in animals (White, 1973 and 1978).

Ohno and coworkers later proposed a new model of polyploidy in the ancestry of salmonids (Ohno et al., 1968 and 1969b; Ohno, 1970a, 1970b, and 1974). They proposed that salmonids as a group were tetraploid in comparison to related salmonoid and clupeoid fishes such as the smelt, herring, and anchovy.

There are four major lines of support for an ancestral tetraploid event in salmonid evolution (Ohno, 1970b). (1) Salmonid fish, with about 80% as much DNA per cell as mammals, have approximately twice the amount of DNA per cell as closely related fish. (2) Salmonids typically have about 100 chromosome arms, twice as many as closely related fish. (3) Multivalents have commonly been observed in meiotic preparations from several salmonid species. (4) Salmonids show a high incidence of duplicated enzyme loci. Subsequent studies reviewed in this paper support such a polyploid event in an ancestral salmonid.

2.2. Time of the Event

It is difficult to determine when the tetraploid event occurred. Bailey and his coworkers (Lim et al., 1975; Lim and Bailey, 1977) have attempted to do this by estimating the amount of divergence that has accumulated between duplicate loci using quantitative immunological methods. Using two different pairs of duplicated LDH loci produced by the tetraploid event, they have found amounts of divergence that are similar to that found between two homologous lactate dehydrogenase (LDH) genes in species that have been separated an estimated 100 Myr.

This estimate is fraught with potential problems. First, it is based on the principle of the molecular clock, i.e. homologous proteins tend to evolve at similar rates in different lineages.

This concept has been widely used to estimate the timing of evolutionary events but its validity has been seriously questioned (see review by Selander, 1982).

Even if we accept the validity of the molecular clock in general, there are reasons why it may not be provide an accurate estimate in this instance. This estimation of the time of the tetraploid event assumes that genes in separate species will evolve at the same rate as duplicate genes in a single species. We would expect duplicate genes to diverge more rapidly because deleterious mutations that would normally be removed by natural selection can become established at one of the loci as long as the other locus continues to perform the normal function. This effect would cause an over estimation of the time since the tetraploid event.

A third potential problem is that the divergence of the two loci resulting from the duplication of a single locus cannot begin until disomic inheritance has been established for these loci. This is demonstrated by the many pairs of duplicated loci in salmonids for which there is no evidence of divergence (Bailey et al., 1970; Allendorf and Utter, 1976). The absence of detectable divergence within these pairs indicates that the disomic inheritance of these loci has been established in the comparatively recent past. In fact, there have been recent reports that some pairs of duplicate loci have still not evolved complete disomic inheritance (Wright et al., 1980; May et al., 1982). The length of time that the LDH loci have been diverging is therefore a minimum estimate of the time since the tetraploid event.

It should also be possible to estimate the time of the tetraploid event using the fossil record. The event had to occur after the salmonids diverged from the nearest ancestor and before the three subfamilies diverged. Unfortunately, the fossil record of the salmonids is extremely scanty for the critical time period (Norden, 1961; Cavender, 1970). The divergence of the three subfamilies apparently occurred somewhere between 25 and 100 Myr ago (R. J. Behnke, personal communication; Obruchev, 1967, cited in Schmidtke et al., 1979; Norden, 1961). The estimate from the biochemical evidence is thus at least not contradicted by the fossil record. Thus, we are left with this estimate of 25-100 Myr, although we must be aware of its weaknesses.

Schmidtke et al. (1979) and Schmidtke and Kandt (1981) have recently seriously questioned this view of a single ancient tetraploid event in salmonid evolution on the basis of DNA reassociation kinetics. They propose that polyploidy within the salmonids is the result of at least four separate polyploid events. They suggest that the grayling is an ancient polyploid lineage and that Salmo, Salvelinus, and Coregonus are all the products of much more recent (3 Myr ago) and separate tetraploid events.

– fossils

We believe that their proposed history is untenable in view of the evidence from isozyme studies. Salmo, Salvelinus, and

Thymallus species have nearly identical genetic systems of control for many different enzymes (Massaro, 1973; Section 4). Similar patterns of genetic control and tissue expression are also present in Coregonus and Prosopium species (Clayton and Franzin, 1970; Massaro, 1972; unpublished data). It is extremely unlikely that these same patterns of genetic control and tissue expression would have evolved independently in separate lineages. Thus, we are left with the conclusion that these similarities result from all of these species sharing a common ancestor after the polyploid event.

It is also impossible to reconcile the amount of loss and divergence of duplicate gene expression that has occurred in Salvelinus and Salmo with an ancestral polyploid event of only three Myr ago. Schmidtke et al. (1979) attempt to avoid this difficulty by suggesting that the loci reported to no longer be duplicated have not yet diverged so that the duplication is not detectable. Both studies (Allendorf et al., 1975; May, 1980) reporting an approximate 50% loss of duplicate gene expression in these genera were aware of this difficulty and therefore only included loci that were polymorphic so that this possibility could be excluded. In addition, the amount of structural divergence found between duplicate LDH loci is incompatible with recent tetraploidy (Lim et al., 1975; Lim and Bailey, 1977). May (1980) has presented evidence that approximately 90% of the loss or retention of duplicate gene expression is shared by salmonid genera, including Thymalus.

In addition, it is extremely unlikely that an event as rare as polyploidy would have occurred independently in all salmonid genera. We therefore believe that the available evidence overwhelmingly supports a single ancient tetraploid event in a common ancestor of all salmonid fishes.

2.3. Nature of the Tetraploid Event

There apparently was substantial homology between the contributing genomes at the time of the tetraploid event. This is supported by the presence in current salmonids of multivalents at meiosis (Table III), by the existence of some duplicate loci pairs with no evidence of any divergence, and by apparent examples of tetrasomic inheritance of some of these loci (May et al., 1982; Section 5.4.2). Although the possibility of segmental allopolyploidy cannot be excluded, it is likely that the salmonid genome was doubled through autopolyploidy. This is in contrast to the catostomids that apparently had an allotetraploid origin (Ferris and Whitt, 1980).

Many possible 'barriers' to tetraploidy in animal species have been proposed. One reason why polyploidy may be rare is that it might require the independently-arisen tetraploids of opposite sexes to mate and start the tetraploid strain. One way of avoiding this problem is a possible two-step process as proposed by Schultz (1969) and Astaurov (1969): (1) The origin of a unisexual triploid strain, followed by (2) occasional fertilization of the unisexual triploid by a normal diploid to

produce fertile tetraploids. Astaurov (1969) has successfully carried out these steps in the laboratory to produce a sexually reproducing allotetraploid strain of silkworms. As mentioned earlier, unisexual triploid strains of several species of fish are known.

For a unisexual triploid strain of fish to reproduce, it must produce a high frequency of unreduced eggs and be capable of initiating development parthenogenetically or, if using sperm from a donor male, gynogenetically. Extant salmonids are apparently capable of producing high frequencies of unreduced gametes in some cases (Thorgaard and Gall, 1979). In addition, Melander and Monten (1950) described a possible case of parthenogenetic reproduction in the common whitefish. Hybridization between species sometimes might act to suppress the second meiotic division of the egg (Uyeno, 1972) and induce polyploidy in salmonids (Capanna et al., 1974) and other fishes (Vasilev et al., 1975; Marian and Krasznai, 1978; Beck et al., 1980). Thus, hybridization and unisexuality may both play an important role in polyploidy of fish species (Schultz, 1969 and 1980).

The objection that polyploidy would upset the chromosomal sex-determining mechanism (Muller, 1925) may not apply to salmonids. The rainbow trout shows evidence of having a "dominant Y" sex-determining mechanism (Thorgaard and Gall, 1979). Such a mechanism, if present in an ancestral salmonid, would not have been subject to Muller's objection because XXXY inidividuals would be expected to be males and not sterile intersexes.

Another objection to polyploidy in animals has been that they might not tolerate the changes in cell size because of their tissue complextity (White, 1973). Although polyploid mammals are inviable (Niebuhr, 1974) there are many examples of viable spontaneous and induced polyploids in salmonids and other fishes (Section 5.3). It appears that none of the traditional barriers to polyploids in animals necessarily apply to salmonids.

3. EVOLUTION OF CHROMOSOMES

3.1. Ancestral and Extant Karyotypes

The diploid ancestor of salmonids probably had 48 acrocentric chromosomes. This is the most common karyotype among fish species, and it is found in a variety of distantly related fish taxa (Ohno, 1974), including many relatives of the salmonids (Sola et al., 1981). If the ancestral karyotype was not 48 acrocentric chromosomes it is likely to have been very similar to that; the majority of fish species have chromosome numbers in the range of 2n=44 to 52 (Gold et al., 1980), with predominantly acrocentric or subtelocentric chromosomes.

The ancestral tetraploid salmonid thus probably had 96 acrocentric chromosomes. Karbe (1964, in Sola et al., 1981) reported such a karyotype in three species of the genus Coregonus, but two of these same species were later reported to have 2n=80 by Nygren et al. (1971b). Most salmonids have about 100 major chromosome arms and diploid chromosome numbers between 56 and 84 (Table II). The general trend since the tetraploid event has been a reduction in chromosome number by centric fusion, while conserving the chromosome arm number at about 100 (Fig. 1). Two major exceptions are the Atlantic salmon, with 74 chromosome arms, and the grayling, with 170 chromosome arms. Many pericentric inversions or unequal translocations have apparently become established in these two species.

Salmonids appear to have undergone much more rapid chromosomal divergence than other groups of fish (Fig. 1). For example, the closely related species of the genus Oncorhynchus (Utter et al., 1973) have chromosome numbers ranging between 52 and 74. In contrast to the large variation in chromosome numbers among salmonid species, the vast majority of species of the family Cyprinidae, for example, have 48-52 chromosomes, and all species of the family Centrachidae have 46 or 48 chromosomes (Sola et al., 1981). All species of the family Catostomidae apparently have 96-100 chromosomes (Beamish and Tsuyuki, 1971; Uyeno and Smith, 1972); thus, a tetraploid ancestry alone does not insure that rapid chromosomal divergence will take place. Perhaps the historical isolation of salmonid populations by glaciation and other geological events in combination with their reproductive homing behavior (Behnke, 1972) have promoted rapid chromosomal evolution within the salmonids. The difference in rates of chromosomal evolution in the salmonids and catostomids is probably also due, to some extent, to the difference between an auto- and allo-polyploid ancestry.

3.2. Evolution of Disomy

In a new autotetraploid, or segmental allotetraploid, some multivalent formation and tetrasomic inheritance is expected. Because there is an increased frequency of non-disjunction when chromosomes associate in multivalents, this should lead to aneuploid gametes and reduced survival of zygotes. Selection for

decreased infertility should cause a reduction of multivalent pairing and the restoration of disomy; what were four homologs begin to pair as two pairs of chromosomes (Sybenga, 1972). In addition to the advantage of decreased infertility, disomy might also be favored because it allows the structural and regulatory divergence of the newly duplicated gene loci.

Experiments with induced autotetraploid maize are representative of what might occur after a new tetraploid event; the average frequency of quadrivalents declined from 8.47 to 7.46 after only 10 generations (Gilles and Randolph, 1951). Similar results were found after selecting for fertility in Brassica campestris (Swaminathan and Sulbha, 1959). However, some naturally occurring tetraploid frog species function well with a high frequency of multivalent pairing (Bogart, 1980).

The primary mechanism for the restoration of disomic inheritance after tetraploidy is structural divergence of the four homologs into two pairs (Sybenga, 1972). A few studies have shown the effect of chromosome rearrangements on meiotic pairing in polyploids; Grell (1961), in Drosophila, and Shaver (1963), in maize, have shown that an inversion in a homolog can reduce the likelihood that it will pair with a normal chromosome. The effectiveness with which a rearrangement reduces pairing between homologs depends upon the meiotic mechanism of a species. In tetraploid Rhoeo discolor, for example, there is no preferential pairing between isosequential chromosomes, probably because the initiation of pairing in that species is restricted to small regions (Walters and Gerstel, 1948). In contrast, the well known Ph locus acts to "amplify" the structural differences between the three component genomes in hexaploid wheat and allow disomic inheritance (Sears, 1976). It apparently brings this about by promoting a premeiotic somatic pairing of homologs.

The presence of multivalents at meiosis (Table III) and evidence for tetrasomic inheritance of some loci suggest that the process of return to disomic inheritance ("diploidization") is not yet complete in the salmonids. Some of the multivalents might also reflect translocation heterozygosity. As previously discussed, changes in chromosome number without a change in chromosome arm number ("Robertsonian" changes) are common among salmonid species. A number of examples of intraspecific Robertsonian polymorphisms are also known (Ohno et al., 1965; Roberts, 1968 and 1970; Gold and Gall, 1975; Thorgaard, 1976) that could be contributing to the observed multivalents.

A curious aspect of the meiotic multivalents and tetrasomic inheritance in salmonids is that these are both apparently restricted to males. This is difficult to explain but might be caused by differences in the initiation of pairing at meiosis. For example, by analogy with the situation in Rhoeo discolor (Walters and Gerstel, 1948), males may initiate pairing in small regions and not recognize the differences between homeologs that females do detect. Differences in the genetic control of meiosis between sexes in Drosophila (Baker et al., 1976) and other animals (White, 1973) are well documented.

Many centric fusions appear to have taken place since the ancestral tetraploid event. Ohno and coworkers (1969b) proposed that the fusion of ancestral homologs may have been an important type of chromosomal rearrangement in the diploidization process in salmonids. If this were true, we would expect to observe loose linkage of duplicated gene loci. The present evidence, however, suggests that duplicated loci do not show classical linkage (May et al., 1979b, 1980, and 1982; Wright et al., in press). Thus the linkage data do not support Ohno's model of diploidization by fusion of homeologs.

Other types of centric fusions and structural rearrangements have presumably helped to differentiate homeologous chromosomes. The effects of specific rearrangements on meiotic pairing and the diploidization process in salmonids are not known. Similarly, it is unknown what, if any, changes to promote disomy (analogous to the Ph locus of wheat) have taken place in the meiotic system of salmonids. The Atlantic salmon, with only 74 chromosome arms, shows striking divergence from the typical, and probably ancestral, salmonid chromosome arm number of about 100; nevertheless, this species still shows multivalent pairing at meiosis (Table III). If these reflect homeologous pairing, it means that the many rearrangements in the lineage of the Atlantic salmon still were not sufficient to bring about disomic pairing for all chromosomes.

It will be difficult to assess what sorts of small chromosomal rearrangements have taken place and what ancestral similarities between homeologs are still present in salmonid species until improved chromosme banding techniques are developed. Zenzes and Voiculescu (1975) reported that they could arrange C-banded brown trout chromosmes into groups of four, but the apparent homologies are not striking. One case in which a rearrangement has taken place in a specific chromosome since the tetraploid event, is the chromosome pair bearing satellites. In those cases reporting satellited chromosomes in salmonids, they have been present on only one pair and not on two as expected in a 'fresh' tetraploid (Cuellar and Uyeno, 1972; Gold and Gall, 1975; Thorgaard, 1976 and 1978; Loudenslager and Thorgaard, 1979).

4. EVOLUTION OF PROTEINS

A newly arisen autotetraploid is endowed with four doses of every gene at a single tetrasomically inherited locus. The four homologs are eventually transformed into two independently inherited pairs of chromosomes as discussed in the previous section. In this way, all of the protein loci are duplicated.

4.1. Possible Fate of Protein Loci

The evolution of protein loci following autotetraploidy can be conceived as occurring in three different periods (Li, 1980). The first period occurs between tetraploidization and the reestablishment of disomic segregation. During this period, the chromosome, and not the individual locus, is the unit of importance.

The second period begins with the reestablishment of disomic inheritance. During this period, the original locus is functionally duplicated. That is, there are now two genetically independent loci that are equivalent to the ancestral unduplicated locus. It is during this period that divergence of the two duplicate loci can begin.

The third period begins when structural or regulatory divergence of the duplicate loci has proceeded to a substantial extent. With regard to structural divergence, this period starts when different alleles have become "fixed", or nearly so, at the two duplicates. This period starts with regard to regulatory divergence when different ontogenetic or tissue-specific patterns of expression have been established at the two loci. It is impossible, however, to detect such regulatory divergence without the presence of some structural divergence.

The term 'duplicated locus' has been applied to all three of these periods. This is a potential source of confusion. Strictly speaking, 'duplicated' loci are present only during the second period. During the third period, the loci have diverged so that they may no longer perform duplicate functions. We think it is important to differentiate among these three periods when referring to a pair of loci that share a common ancestral locus.

We therefore recommend the following nomenclature in this paper. During the first period, a locus should be referred to as being 'tetrasomic'. A pair of genetically distinct loci that still share identical alleles, so that variant alleles cannot be unmbiguously assigned to one locus or the other, will be referred to as 'isoloci'. (Gall et al. (1976) have referred to these loci as being "isoqualitic".) After the completion of the second period, we will refer to such pairs of loci as being 'paralogous' (Fitch, 1976). We will use the term 'duplicated' as a general term, without reference to any of these three specific time periods.

These three time periods are not necessarily discrete. Both

the reestablishment of disomy and the divergence of isoloci are continuous processes. Nevertheless, the recognition of these periods and the associated nomenclature should make it easier to understand and discuss the post-autotetraploidy evolution of protein loci.

4.1.1. Loss of Duplicate Gene Expression

Haldane (1933) first suggested that one of the duplicate loci may become non-functional through the accumulation, and eventual fixation, of deleterious (i.e., null) mutations at one locus while the other locus continued to perform the original function. The theoretically expected rates of such loss of duplicate gene expression has been recently explored by a number of authors (reviewed in Li, 1980).

The recent discovery of widespread pseudogenes in the genomes of vertebrates provides evidence that such loss of gene expression may indeed be a common evolutionary event. Pseudogenes are DNA segments that show homology to functional genes but have nucleotide changes so that they are not expressed (Proudfoot, 1980). Pseudogenes are apparently duplications that have become nonfunctional by the accumulation of mutations.

The loss of detectable function has been a common 'fate' for enzyme loci in the salmonids (Allendorf et al., 1975; May et al., 1980; May, 1980; Kijima and Fujio, 1980). Approximately 50% of the additional loci created by tetraploidy are no longer detectable. These estimates are similar to those from other tetraploid-derived fish species that show between a 25% and 75% loss in duplicate gene expression (Li, 1980). The existence of three non-duplicated loci in a single linkage group (Odh-Mpi-Gpi3; Wright et al., in press) suggests that part of the diploidization process may have involved whole sections of DNA.

4.1.2. Retention of Duplicate Gene Expression

Those cases in which both duplicated loci are retained fall into different categories. The first is tetrasomic loci or isoloci in which disomic inheritance has only recently evolved or is still not complete. Loss of duplicate gene expression may eventually occur in these cases. A second category is loci that have diverged structurally but not with regard to their regulation. These systems are also potential candidates for future loss of duplicate gene expression. As long as both loci are always equally expressed, a null allele at one locus may be sheltered from selection by the presence of the normal allele at the other locus.

Another category is loci that have diverged in their developmental or tissue-specific expression. Such regulatory divergence can occur in two different ways: unidirectional and bidirectional (Ferris and Whitt, 1979). In unidirectional divergence, the enzyme products of one locus predominate in all

tissues in which the two loci are not equally expressed. In bidirectional divergence, there is no consistent predominance of one locus or the other in tissues showing unequal expression.

When a locus is uniquely expressed in a particular tissue or developmental stage it is unlikely to become fixed for a null allele because the other locus can no longer shield it from natural selection. Therefore, either loss of duplicate gene expression or evolution of unique expression is the probable eventual fate of all duplicated loci.

Two possible modes of selection could also 'protect' against the loss of duplicate gene expression. A benefit in having large amounts of enzyme product could select against the establishment of null alleles at either locus. This is unlikely to occur for enzyme loci in eukaryotes since the quantity of enzyme present is unlikely to be rate limiting (Kacser and Burns, 1981). Also, benefit in having two different alleles present (overdominance) would cause selection against null alleles (Spofford, 1969; Allendorf, 1978).

4.2. Salmonid Enzymes

The genetic control of a large number of enzymes in salmonids has been described. We do not intend to review all of these results; rather, we will first describe the genetic systems of control of the most studied enzymes: lactate dehydrogenase (LDH) and malate dehydrogenase (MDH). These two enzymes present extreme differences in the amount of divergence between duplicated loci. Finally, we describe the present genic control of 33 ancestral loci in a species that has been intensively studied: the rainbow trout. Table IV lists all of the enzymes and their abbreviations. We have used the system of nomenclature proposed by Allendorf and Utter (1979) with the additions suggested by May et al. (1979b).

4.2.1. Lactate Dehydrogenase

LDH was the first duplicated enzyme system in salmonids to be described (Goldberg, 1965; Markert and Faulhaber, 1965; Morrison and Wright, 1966). The diploid ancestor of the salmonids apparently possessed three LDH loci: a muscle specific locus, LDH-A; an eye specific locus, LDH-C; and a third locus found in all tissues, LDH-B (Markert et al., 1975). As many as eight LDH loci have been suggested to be present in extant salmonids (Massaro and Markert, 1968). This estimate, however, is based on zymogram patterns alone, without supporting inheritance studies, and is apparently inflated.

Wright et al. (1975) summarized their studies of the genetic control of LDH in Salmo and Salvelinus. Five loci code for LDH activity in species of these genera. The two loci (Ldh-1 and -2) descendant from the ancestral LDH-A locus have different common alleles resulting in a common five-banded pattern for muscle specific LDH. These two loci show no evidence of regulatory divergence as determined by patterns of tissue-specific

expression.

The two loci (Ldh-3 and -4) descendant from the ancestral LDH-B locus also have different common alleles. These two loci have also evolved different tissue-specific patterns of expression (Figure 2). Only the products of Ldh-4 are found in liver tissue while Ldh-3 locus products predominate in heart tissue. These loci thus show bidirectional regulatory divergence. There is only one locus coding for the eye specific from of LDH. These results have been confirmed by several other investigations (e.g., Bailey et al., 1976).

A similar system of genetic control and tissue-specific expression of LDH loci is found in the grayling (Massaro, 1973; Figure 2). However, there is only one isozyme of the muscle specific form of LDH. This could be caused by either the comigration of the products of the Ldh-1 and -2 loci or by the loss of expression of one of these loci. A null allele at Ldh-1 has been found to be common in brown trout populations and is even fixed in one Swedish population (Ryman et al., 1979). Null alleles at the Ldh-1 locus have also been found in rainbow trout and sockeye salmon (unpublished results). A null allele at Ldh-2 has also been described in Snake Valley cutthroat trout (Klar and Stalnaker, 1979).

Whitefish LDH is also encoded by five loci. However, the two descendant LDH-B loci do not show the tissue-specific pattern of expression that is found in the trout, char, and grayling (Figure 2). Rather, these two loci are nearly equally expressed in all tissues (Clayton and Franzin, 1970; Massaro, 1972). The similar expression of both loci is the primitive condition while the highly specific pattern of expression seen in the tissues of trout, char, and grayling is a derived characteristic (synapomorphy) that is likely to have evolved only once. Thus, this result indicates that the trout, char, and grayling apparently shared a common ancestor longer after the tetraploid event than did the whitefish. The pattern of expression of other enzyme loci supports these relationships.

4.2.2. Malate Dehydrogenase

The loci encoding the cytosolic form of MDH show a different picture of post-tetraploidy evolution than the LDH loci. The LDH loci have all been "diploidized" for a long time. This is supported by the estimates of 100 Myr divergence time for the LDH-A and LDH-B descendants (Lim et al., 1975; Lim and Bailey, 1977) and the loss of one of the LDH-C loci.

The pre-tetraploidy ancestral salmonid had two MDH loci; one that predominated in skeletal muscle tissue, MDH-B, and another that predominated in liver tissue, MDH-A (Bailey et al., 1970). The present descendants of these loci show very little evidence of diverergence, either structurally or in their tissue expression.

Both MDH-B loci (Mdh-3,4) in all salmonid species that have

been described have the same common electrophoretic allele. Figure 3 shows the five phenotypes resulting from two alleles segregating at these loci in rainbow trout. This lack of detectable divergence indicates that the diploidization of these loci has occurred recently. Inheritance experiments indicate that this process is still apparently not complete in the rainbow trout (May et al., 1982; Section 5.4.2).

A similar pattern is seen for the MDH-A loci. In most species studied, both loci have the same electrophoretic allele. In some species, however, the two MDH-A loci have diverged in structure and in tissue-specific patterns of expression. For example, the closely related brown trout and Atlantic salmon share the same three-banded pattern for MDH-A indicating that the two descendant loci have different common alleles. In the brown trout, however, only one of these two loci is expressed in skeletal muscle while both of these loci are expressed in the skeletal muscle of Atlantic salmon.

4.2.3. Rainbow Trout Isozymes

Table V summarizes the status of 33 ancestral protein loci in the rainbow trout. Other species in the same subfamily differ very little from these results (May et al., 1982; May 1980). We have not included a similar table for species in the other two subfamilies because there is not sufficient information available. Many enzymes and a large number of individuals must be examined to acquire the required information. For example, if a single isozyme is seen for a particular enzyme (e.g., Adh), the number of loci controlling this isozyme (or electromorph) cannot be determined until electrophoretic variation is detected. Many of the enzymes represented by a single electromorph in salmonids are also characterized by a low amount of genetic variation (e.g., Adh, Ck-B, and Gpd). Thus, there is a bias against detecting loci at which duplicate gene expression has not been retained. Many individuals must therefore be examined in order to accuately estimate the prportion of gene duplication lost.

Thirty percent (10 out of 33) of the ancestral loci in rainbow trout do not show any evidence of duplicate gene expression. Twenty-four percent (8 out of 33) of the loci are isoloci showing no evidence of divergence. The remaining 45% of the loci are duplicated and show evidence of structural or regulatory divergence.

4.3. Regulation of Enzyme Loci

The examination of genetic variation at enzyme coding loci using gel electrophoresis in the last 15 years has revealed a large amount of genetic variation in plants and animals. Demonstrating the evolutionary significance of this variation has proven to be a much more difficult problem. Several authors have suggested that changes in the regulation of enzyme producing loci may be of more evolutionary significance than changes in the enzymes themselves (Wallace, 1963; Wilson, 1976). The evidence for this view remains largely indirect. Differences between the

rate of change at structural loci encoding enzymes and the rate of change in phenotypes having adaptive importance suggests that significant evolutionary changes may be due to changes in gene regulation (Wilson, 1976). Further indirect evidence of the evolutionary importance of gene regulation comes from paleontology (Valentine and Campbell, 1975; Gould, 1980) and developmental genetics (Flickinger, 1975; Whitt, 1981).

The eventual acceptance of these views awaits direct evidence of the amount and adaptive significance of variation at regulatory genes. It is necessary to find intraspecific genetic variation for the control of enzyme producing loci to explore the adaptive significance of gene regulation. Paralogous isozyme loci in the salmonids show considerable divergence in tissue-specific expression. This relatively rapid divergence is in contrast to the commonly observed evolutionary conservation of patterns of tissue-specific expression of enzyme loci (Markert et al., 1975; Shaklee and Whitt, 1981). We would therefore expect salmonids to have a greater amount of intraspecific genetic variation for the regulation of enzyme loci because of the differential rates of divergence among pairs of duplicated loci. That is, we expect duplicate loci in the early stages of divergence to be polymorphic for the types of differences in tissue-specific patterns of expression that have been established between paralogous loci.

Salmonids are therefore potentially valuable for studying the evolutionary significance of changes in the regulation of enzyme loci. Wright et al. (1975) have described intraspecific variation for the ontogenetic schedule of expression of an LDH locus in the brook trout. A mutant allele resulting in a greater than 100 fold increase in the liver-specific expression of a PGM locus has been reported in the rainbow trout (Allendorf, 1980; Allendorf et al., in press). Results of inheritance experiments are consistent with a single regulatory gene having additive inheritance being responsible for the differences in the tissue-specific expression of this locus (Allendorf et al., in press). The presence or absence of this isozyme in the liver gives rise to important differences in several phenotypic characteristics of adaptive significance (developmental rate, developmental stability, body size, and age at first reproduction). We have also detected several other putative allelic variants in the tissue-specific expression of enzyme loci in other salmonid species (unpublished data).

5. THE CURRENT SALMONID GENETIC SYSTEM

All aspects of genetics and evolution in salmonids are affected by their tetraploid ancestry. Because of this we feel it is important to consider the general features of the genetic system of present-day salmonids in light of their tetraploid origin.

5.1. Sex Chromosomes and Sex Determination

Little was known about the mechanism of sex determination in salmonids until recently. The most widely studied species has been the rainbow trout; this species has an XY male, XX female sex chromosome system. This conclusion is based on chromosomal studies (Thorgaard, 1977; Thorgaard and Gall, 1979) and breeding studies with sex-reversed fish (Okada et al., 1979; Johnstone et al., 1979). Chromosomal evidence also supports male heterogamety in the sockeye salmon (Thorgaard, 1978). The coho salmon also appears to be male heterogametic based on breeding studies with hormonally sex-reversed fish (Hunter et al., in press) and the production of all-female lots after diploid gynogenesis using irradiated sperm and cold shock (Refstie et al., in press).

The fact that XXY triploid rainbow trout are males (Thorgaard and Gall, 1979) suggests that a "dominant Y" sex determining mechanism is operative. Such a system would avoid one of Muller's (1925) objections to polyploidy in animals.

5.2. Genetic Recombination

Building a linkage map for salmonids is a challenging task because of their many chromosomes and relatively long generation times. Nevertheless, several examples of classical linkage have recently been reported in salmonids (reviewed in May et al., 1982). Comparison of linkage relationships in different species should provide insight into the process of post-tetraploidy chromosomal evolution in salmonids. Unfortunately, all of the available linkage data are restricted to a single subfamily: Salmoninae. Those genera that have been studied (Salmo, Oncorhynchus, and Salvelinus) apparently show conservation of linkage groups (May et al., 1981).

Males show a much lower frequency of recombination than females (Table VI). This difference between sexes is in agreement with the general observation that when there is a difference in recombination rates between the sexes, it is the heterogametic sex that shows reduced frequencies (Swanson et al., 1981). The differences in recombination frequencies and in the occurrence of meiotic multivalents between sexes suggest that there are substantial differences in meiosis between male and female salmonids.

5.3. Aneuploidy and Polyploidy

Polyploid plant species tolerate aneuploidy much better than diploid species. Salmonids might also be expected to be relatively tolerant to aneuploidy because of their tetraploid origin. There are several reports of aneuploids in salmonids, supporting this contention. Davisson et al. (1972) found a male brook trout that was trisomic for a chromosome carrying a Ldh-B enzyme locus. This fish was fertile and produced euploid and trisomic offspring in equal frequencies; the trisomics were similar in size, appearance, and viability to the normal fish. Ohno (1970a) reported finding "a few" trisomic and monosomic rainbow trout during a survey of nearly 500 hatchery fish; these aneuploids also had no obvious differences from normal fish. Aneuploid salmonids thus seem to be common and viable. Nevertheless, more information about the frequency and effects of aneuploidy in salmonids and other fishes is needed before conclusions about the effect of the tetraploid ancestry of salmonids on aneuploids can be made.

Polyploid salmonids also occur naturally and have been induced artificially. Spontaneously occurring triploid rainbow trout have been found (Cuellar and Uyeno, 1972; Grammeltvedt, 1974; Thorgaard and Gall, 1979); these fish are normal in appearance but sterile. Naturally occurring polyploid brook trout have also been found; Allen and Stanley (1978) concluded on the basis of red blood cell nuclear volumes that these fish were mosaics composed mainly of triploid cells but also contained cells of other ploidy levels. Triploidy has also been induced in salmonids using a variety of treatments (Allen and Stanley, 1979; Smith and Lemoine, 1979; Chourrout, 1980; Thorgaard et al., 1981). These findings suggest that triploid salmonids may not have significantly reduced viability.

Although no spontaneously occurring tetraploid salmonids have been reported, Refstie (1981) described tetraploid rainbow trout produced by treating fertilized eggs with cytochalasin B. There was an increased frequency of abnormalities among these fish but some males were able to produce milt.

The fact that triploid, and possibly tetraploid, salmonids are reasonably viable is consistent with results with other fish species (Swarup, 1959; Purdom, 1972; Valenti, 1975; Gervai et al., 1980; Schultz, 1980; Wolters et al., 1981) and with the idea that an ancestral salmonid could tolerate a polyploid step.

5.4. Patterns of Genic Inheritance

The autotetraploid ancestor of present day salmonids had four copies of each chromosome. Because the homologs were initially structurally similar, these chromosomes would pair randomly and the genes on these chromosomes would be inherited

tetrasomically. The chromosomal diploidization process would eventually transform the four homologs into two pairs of two, resulting in disomic inheritance. The status and inheritance of ancestral gene loci (Table V) can be used to determine how far the diploidization process has proceeded. Duplicated loci that show no evidence of divergence are possible candidates for tetrasomic inheritance.

Most of the isozyme loci in rainbow trout have completed the diploidization process; only eight out of 33 ancestral loci remain isoloci (Table V). Thus, approximately three-quarters of the salmonid genome has become diploidized. This assumes that isozyme loci are an accurate reflection of the entire genome and that rainbow trout are typical of other salmonids. We believe that both of these assumptions are good ones. The diploidization process is a chromosomal rather than genic process. Therefore, the 33 ancestral isozyme loci spread throughout the genome should simply act as chromosomal markers. The pattern seen in rainbow trout is similar to that seen in other salmonids; we have chosen to use rainbow trout because it has been intensively studied with regard to patterns of inheritance (see Stoneking et al., 1979; May et al., 1979b and 1980; Wright et al., 1980 for analogous results with Salvelinus species).

5.4.1. Inheritance of Duplicated Loci

The many multivalents observed in meiosis (Table III) suggest that some of the duplicated loci in salmonids may be inherited tetrasomically. Several loci have been reported to be inherited tetrasomically in salmonids without any supporting inheritance results: Idh in rainbow trout (Wolf et al., 1970); Sdh in rainbow trout and common whitefish (Engel et al., 1970); and H6pdh in brook trout (Stegeman and Goldberg, 1972). It has been shown, however, that it is impossible to demonstrate tetrasomic inheritance in the absence of inheritance results (Allendorf et al., 1975). These papers have continued to be cited as evidence of tetrasomic inheritance in salmonids even though they are apparently erroneous.

There are a variety of possible modes of inheritance that must be considered when distinguishing between isoloci and a single tetrasomic locus (Table VII). In a two allele system, the critical genotype for examining inheritance is the duplex phenotype (Burnham, 1962) having two copies of both alleles i.e., AAaa). The expected disomic inheritance ratios will be different depending on whether the parent is a double homozygote (AA;aa) or double heterozygote (Aa;Aa). The segregation ratios produced by the double heterozygote can be affected by linkage. Thus, the double homozygote parental genotype must be observed to establish disomic inheritance.

The segregation ratios produced by tetrasomic inheritance are affected by the frequency of quadrivalent formation and the distance of the locus from the centromere (Burnham, 1962).

Cross-overs between the locus and the centromere in a quadrivalent during the first meiotic division can produce so-called double reduction gametes that carry two copies of identical chromatids from a single chromosome. The maximum frequency of such double reduction divisions is 1/6 (Burnham, 1962). Thus, the proportion of Aa gametes produced by tetrasomic inheritance from the AAaa parental genotype varies between 67% and 55% (Table VII).

The study of isoloci presents special problems. Variant alleles cannot be assigned to a particular locus on the basis of electrophoretic phenotypes. Therefore, these loci cannot be treated individually when estimating allelic frequencies in population samples. First-generation inheritance results do not solve this problem. An enormous number of mutiple generation inheritance experiments are necessary to assign variation observed in a single population sample to one locus or the other. In addition, inheritance studies with duplicated have presented special problems. Beginning with Morrison and Wright (1966), inheritance ratios have been reported that do not agree with any simple genetic model of either disomic or tetrasomic inheritance.

5.4.2. Unusual Inheritance Results at Duplicated Loci

Morrison and Wright (1966) were the first to report inheritance results of duplicated loci in salmonids. They reported linkage of the two loci resulting from the duplication of the ancestral Ldh-B locus in hybrids between brook and lake trout. Additional results demonstrated that these inheritance ratios could not be explained by classical linkage because the nonparental types were found to be in excess, rather than the parental types (Morrison, 1970). This phenomenon was first referred to as "pseudolinkage" by Davisson et al. (1973). A series of papers from the laboratory of Prof. James E. Wright has continued to further the understanding of the mechanisms producing these unusual segregation ratios (Lee and Wright, 1981; May et al., 1979ab, 1980, and 1982; Wright et al., 1975 and 1980). In this paper, we have chosen not to present an historical review of this important work. Rather, we believe the essential findings can be presented much more clearly by beginning with the simplest possible genetic models and then adding further complexity only when it becomes neccessary.

Bailey et al. (1970) were the first to describe the inheritance of isoloci. They concluded that Mdh-B was controlled by two disomically inherited loci in chinook salmon. As pointed out in the previous section, disomic and tetrasomic inheritance can only be distinguished in a two allele system by examining the segregation of individuals with two doses of each allele (AAaa). This was not done by Bailey and his coworkers. Therefore, their data are also compatible with tetrasomic inheritance.

One of us (Allendorf, 1975) performed a series of

experimental matings with this same system in rainbow trout to distinguish between disomic and tetrasomic inheritance (Table VIII). These matings were done using Chambers Creek anadromous rainbow trout (steelhead) from the Washington State Department of Game. These results are incompatible with tetrasomic inheritance; only Bb gametes were produced by three male fish having the BBbb genotype (families C25, C37, and C39). Four additional BBbb types produced gametes compatible with the 1:2:1 segregation of BB:Bb:bb types expected with disomic inheritance. We thus concluded that Mdh-B in rainbow trout is controlled by two disomically inherited loci (Mdh-3,4) that have apparently only recently evolved disomic inheritance.

A more recent series of experiments with a different strain of rainbow trout (Jocko River State Trout Hatchery, Arlee, Montana) has provided some surprising results (Table IX). All four females examined produced gametes in agreement with the previous results of disomic inheritance found in the Chambers Creek fish. Five of the six males, however, produced families that can only be explained by a mixture of disomic and tetrasomic inheritance (G1, H6, H7, H18, and H19).

Families H6 and H18 demonstrate the clearest examples of tetrasomic inheritance. In these families, males having two doses of both the common and variant alleles (BBbb) were crossed with females having only the common allele (BBBB). Two different segregation ratios are possible with disomic inheritance. If the male is a double homozygote (BB;bb), then all progeny should be (BB;Bb); if the males is a double heterozygote (Bb;Bb), then the progeny should segregate 1:2:1 for the BBBB:BBBb:BBbb types. Neither of these two ratios is obtained in these families. It appears that these males were double homozygotes but that some homeologous pairing has produced infrequent BB and bb gametes.

The male producing family G1 shows a pattern of gamete formation that is similar to the male parents of H6 and H18. This male, however, was crossed with a female segregating 1:1 for BB and Bb gametes. The male parents of H7 and H19 were apparently double-heterozygotes (Bb;Bb); homeolog pairing in these males produced gamete frequencies intermediate between the 1:2:1 expected with disomic inheritance and the 1:4:1 expected with tetrasomic inheritance.

In this strain of rainbow trout, segregation ratios in males for Mdh-B are intermediate between disomic and tetrasomic inheritance. May et al. (1982) have reported similar results at this locus for two males (they did not use any BBbb females) in another strain of rainbow trout and for an Aat locus in brook trout (Wright et al., 1980). They have referred to this phenomenon as residual tetrasomic inheritance. We can thus conclude that the 'diploidization' of all chromosomes in salmonids is apparently not complete.

These observations are in agreement with reports of

multivalent formation in males but not females (Table III). We can apparently explain these results by the pairing of homeologs in males only, resulting from differences in meiosis between males and females. There is a problem with this simple explanation however. The union of sperm carrying two homologs with an egg carrying two homeologs will produce progeny having three copies of one homolog and only one copy of the other homolog. Such individuals should show tetrasomic gamete formation because they do not possess the two copies of each homolog necessary for disomic segregation. This should be true regardless of the sex of the individual. Frequent homeologous pairing in one sex should thus quickly restore tetrasomic inheritance in the whole population.

We must explain the stable differences between the sexes in multivalent formation and pairing of homeologs as detected by the inheritance of isozyme variants. This may be explained by a two-stage pattern of pairing in males in which homologous chromosomes pair first followed by homeologous pairing. Disjunction so that paired chromosomes pass to opposite poles would insure that each gamete receives one copy of each homeolog. Exchanges between homeologs would produce segregation ratios approaching tetrasomic expectations (Fig. 4).

This model would produce a mixture of disomic and tetrasomic inheritance, depending upon the map distance between a locus and the centromere. Loci near the centromere would show disomic inheritance and distal loci would show ratios near those expected with tetrasomic inheritance. Exchanges between homeologs would keep the distal part of the homeologous chromosomes from diverging. Homeologs would maintain their integrity because of divergent sequences near the centromeres (see Fig. 4).

A simple extension of this model can also explain the observations of preferential production of non-parental gamete types at the paralogous Ldh-3 and Ldh-4 loci in brook-lake trout hybrids (Morrison and Wright, 1966; Morrison, 1970). These loci show only disomic inheritance; we would therefore assume that they lie near the centromere in the diverged chromosomal segment. The excess of non-parental gametes by males would result if there is preferential secondary pairing between homeologs from the same species followed by disjunction so that paired chromosomes pass to opposite poles (Fig. 5). The preferential pairing of homeologs from the same species is expected because they should have greater pairing affinity because of secondary tetrasomic segregation within each species so that there would be more differences between homeologs from different species.

Many of the results that this model is based on comes from recent work from the papers previously mentioned coming from Wright and his colleagues. They have proposed a model involving diverged and undiverged chromosomal segments to explain unusual inheritance ratios seen at two AAT loci in

brook trout (Wright et al., 1980). Their model, however, is more specific and assumes major chromosomal rearrangement producing homeologous pairing between acrocentric and metacentric chromosomes. Our model is more general in that the diverged and undiverged chromosomal segments result from differential rates of divergence between homeologs because of differences in the frequency of crossovers between a locus and the centromere.

They have referred to both the partial tetrasomic ratios at isoloci and the excess of non-parental types at paralogous loci as 'pseudolinkage' (Wright et al., 1980; May et al. 1982). We believe that the use of the term pseudolinakge should be restricted to the excess of non-parental types at paralogous loci and the term residual tetrasomic inheritance be used to refer to the intermediate disomic-tetrasomic segregation ratios seen at isoloci.

We would like to summarize the evidence for our proposed model of segregation and to suggest possible ways that it could be tested. First, the continued disomic inheritance in females implies that homologs must separate at the first meiotic division in males so that each gamete receives one copy of each homolog. Therefore, pairing of homologs must always occur in males. However, males also produce gamete types expected only with tetrasomic inheritance. Therefore, there must be some secondary pairing of homeologs in males. Preferential secondary pairing of homeologs from the same lineage followed by separation in the first meiotic division of paired chromosomes would produce an excess of non-parental types (i.e. pseudolinkage).

There is additional evidence of this pattern of segregation. Double reduction divisions (the production of gametes containing two copies of an allele carried by sister chromatids) are expected to result if three things occur (Burnham, 1962): (1) formation of multivalents (2) crossovers between the locus and the centromere; and (3) the chromatid products of such crossovers pass to the same pole in anaphase I followed by random separation of the chromatids in anaphase II. We have never seen any of the exceptional progeny expected from double reduction in the examination of thousands of progeny at Mdh-3,4 in rainbow trout (Allendorf et al., in preparation). However, we know that requirements (1) and (2) do occur in males. Therefore, the homeologous chromosomes that crossover in males must pass to opposite poles.

This model predicts that loci near the centromere should become diploidized more quickly than distal loci. Therefore, the same loci in different lineages should show the same relative rates of divergence. This has been found to be the case. For example, the Mdh-B loci are isoloci in all salmonid species that have been studied in all three major salmonid lineages: trout, salmon, and char (as described in this paper); whitefish Imhoff et al., 1980); and grayling

(Massaro, 1972; unpublished data). The muscle AAT loci are also isoloci in most salmonid species and aberrant inheritance ratios have been reported in both char (Wright et al., 1980) and trout (Allendorf and Utter, 1976).

May et al. (1982) discuss this similarity among salmonid species because it is not predicted by their model involving specific chromosome rearrangements since there is no reason to expect the same chromosomes to be involved in such rearrangements in different lineages. Such similarities are expected to result with our model simply as a function of the distance between a locus and its centromere. We do not suggest that our model can or should replace their model. Rather, ours is a simpler (and therefore perhaps more general) model sufficient to explain the available segregation data. Their model, however, is in agreement with recent cytological evidence from studies of meiosis (Wright et al., in press) and we believe that their model (or a similar one) is required to explain meiotic behavior of some salmonid chromosomes.

Our model predicts that loci that are commonly isoloci should be more distal than loci that should have long established diploidization. We are presently testing this prediction by production of diploid gynogenetic genetic offspring produced by fertilization with irradiated sperm and suppression of the second meiotic division by temperature shock (Chourrout, 1980; Thorgaard et al., 1981). The amount of recombination between a locus and its centromere can be estimated by the proportion of heterozygous progeny from heterozygous females.

Segregation ratios intermediate between disomy and tetrasomy have been reported previously in tetraploid plants (Lewis, 1945; Gerstel and Phillips, 1958). These ratios have been explained by occasional homeologous pairing. There is no reason that the model we have described should be limited to salmonids. We would expect any tetraploid species in the process of establishing disomic inheritance to show these same effects, i.e., residual tetrasomic segregation of distal loci and pseudolinkage in hybrids between different lineages descendent from the same polyploid event. We are not aware, however, of any segregation studies with polyploid plants showing similar results. This is at least partially due to the fact that most inheritance studies with polyploid plants have been done with recessive morphological markers in which only two phenotypes can be distinguished in comparison to the five phenotypes that can be distinguished with isozyme markers.

Segregation ratios intermediate between disomic and tetrasomic expectations have also been reported in the autopolyploid frog Hyla versicolor at two isozyme loci (Danzmann and Bogart, 1982; MS). Surprisingly enough, these loci code for the same enzymes (MDH and AAT) for which tetrasomic ratios have been discovered in salmonids. This implies that perhaps the loci encoding these enzymes are distant from the centromere in both of these groups.

5.5. Implications of Non-Disomic Inheritance

Loci in the salmonid genome are inherited in a variety of different patterns, ranging from non-duplicated to at least partially tetrasomic. The possible effects of this mosaic genetic system must be taken into account when considering the inheritance of phenotypic variation in salmonids. In the next sections, we consider the possible effects on the inheritance of monogenic and quantitative traits.

5.5.1. Monogenic Traits

We are aware of only a few descriptions of the genetic control of morphological traits in salmonids. We should consider both the expected frequency and the inheritance of such traits if controlled by a tetrasomic locus or isoloci as compared to a single non-duplicated locus. Most of these traits are rare (e.g., albinism) and are apparently maintained in populations by a balance between mutation and natural selection. The simplest model to consider is one in which the mutant allele is recessive and effectively lethal when homozygous.

At equilibrium, the frequency of such a trait in a population is equal to the mutation rate (u) and is independent of the mode of inheritance. However, the allelic frequencies and the inheritance pattern of the trait will differ depending upon the mode of inheritance.

At a disomic locus, equilibrium will occur when the frequency of the recessive phenotype (q^2, where q is the frequency of the recessive allele) is equal to the mutation rate. Thus, the allelic frequency at equilibrium will be $\sqrt{u}$. The mode of inheritance is usually determined by crossing the variant phenotype (aa) with the common normal phenotype (AA) and then backcrossing the resulting offspring (Aa) with the variant phenotype. The expected ratios from these matings are presented in Table X.

At a tetrasomic locus, the allelic frequency at equilibrium will be equal to the fourth-root of u (assuming no double reduction divisions). Thus, the allelic frequency at a tetrasomic locus of will be much higher than at a disomic locus. For example, if u is 10^{-6} for albinism, then the allelic frequency at a tetrasomic locus will be 0.032, as compared to 0.001 at a disomic locus. The inheritance at a tetrasomic locus will be more complex. The expected frequencies of genotypes in a population at equilibrium are shown below:

Genotype	Frequency	
AAAA	p^4	0.878
AAAa	$4p^3q$	0.116
AAaa	$6p^2q^2$	0.006
Aaaa	$4pq^3$	0.000
aaaa	q^4	0.000

If we cross an albino with a normal fish having the genotype AAAA, then we expect a normal:albino ratio in back-cross progeny of 5:1 and in F_2 progeny of 35:1, as compared to the 1:1 and 3:1 ratios expected at a disomic locus. If, however, we cross an albino with a AAAa fish, then we will get a 1:1 mixture of AAaa and Aaaa fish in the first generation. The first of these genotypes will produce back-cross and F_2 progeny ratios identical to those if we initially used a AAAA normal parent. The second genotype will produce segregation ratios identical to those expected at disomic locus if the normal parent was AA. Thus, tetrasomic inheritance for morphological traits should produce inheritance ratios in which the recessive phenotype is much less frequent than expected with disomic inheritance (Table X).

The situation becomes more complex in the case of isoloci controlling such morphological variation. This system has been considered in some detail by Christiansen and Frydenberg (1977). There is not a single equilibrium solution for allelic frequencies. Rather, at equilibrium, the allelic frequencies will satisfy the equation

$$u = (Q_a^2)\ (Q_b^2)$$

This equation defines a hyperbola in the plane of all possible allelic frequencies. Once allelic frequencies reach equilibrium on this line, they will be free to move along through the effects of genetic drift.

The expected inheritance results again depend on the genotype of the fish used in the original cross. This fish may be of any of the following genotypes: AABB, AaBB, AABb, AaBb, Aabb, or aaBb. The expected segregation ratios are presented in Table X. As with tetrasomic inheritance, isoloci coding for a recessive trait will result in inheritance ratios in which the recessive phenotype is observed less frequently than expected with disomic inheritance (Table X).

All reported inheritance studies of morphological variants have found non-duplicated disomic inheritance. Both albinism and golden coloration in rainbow trout have been found to be controlled by single Mendelian loci (Bridges and Von Limbach, 1972; Wright, 1972). Kincaid reported an iridescent blue color variant in rainbow trout that appeared to be inherited as a single locus recessive with incomplete penetrance (Kincaid, 1975). We would expect only those loci that have lost duplicate gene expression not to show non-disomic inheritance ratios. Assuming that isozyme loci are representative of the entire genome, we would only expect 30% of all morphological loci to show disomic inheritance.

Why have such non-disomic ratios not been reported for morphological loci? There are several possible explanations. The simplest explanation is that simply by chance a morphological locus retaining duplicate gene expression has not been studied.

However, perhaps loci controlling color variation are less likely to retain duplicate gene expression than are isozyme loci. A third possibility is that there may have been some reluctance of investigators to report such unexpected results. There is very little extensive inheritance data available in the literature for any salmonid morphological locus. No conclusions can be drawn until more results are available.

5.5.2. Quantitative Traits

The two principal genetic characteristics of quantitative traits are the amount of genetic variation for a particular trait and the expected response of that trait to either natural or artificial selection. These two characteristics are usually closely related for a particular trait; the presence of more genetic variation is associated with more rapid response to selection. A tetrasomic locus is in some ways a contradiction to this rule. We expect a tetrasomic locus to possess more allelic variation but respond slower to selection than a disomic locus because of the presence of the additional gene copies. This same principle also applies to a trait controlled by isoloci. We examine this effect by considering the relative rates of change expected under natural selection for traits controlled by duplicated and non-duplicated loci.

5.4.2a. Selection for a Recessive Trait.

Assume that selection changes so as to favor a recessive allele that had been previously deleterious. The present fitness of the recessive phenotype is twice that of the other genotypes. Previously, its fitness was zero so that it was maintained in the population by selection-mutation balance.

Figure 6 shows the response to this situation at a tetrasomic and a disomic locus, using the recurrence equation of Li (1975). It will take an expected 10000 generations at a tetrasomic locus for this trait to be incorporated into the population. This is almost exactly ten times longer than expected at a disomic locus. This is true even though the recessive allele was intitially much more common at the tetrasomic locus; 12% of the individuals in the initial tetrasomic population carried the recessive allele, as compared to only 2% in the disomic population. Thus, the tetrasomic possessed more initial variation but responded much more slowly to selection.

This same general situation will also prevail at an isolocus, although the precise dynamics will depend upon the initial frequencies of the recessive allele at the two loci. However, if the initial frequencies are equal, then the response is similar to a tetrasomic locus. Perhaps more importantly, the same mutation must occur at both loci to be expressed at isoloci. Therefore, in general, a duplicated locus will respond much more slowly to selection for a recessive trait than will a non-duplicated locus.

5.4.2b. Selection for a Dominant Trait

Assume that a dominant advantageous mutation having a five percent advantage in fitness occurs in a population containing one hundred breeding adults. Both tetrasomic and disomic loci respond very quickly to selection for a dominant allele under these conditions (Fig. 7). It is interesting to note, however, that the dominant allele is quickly nearly fixed at a disomic locus but at a tetrasomic locus there is a plateau at a frequency of about 0.8. Thus in this case, the tetrasomic locus will respond to selection nearly as rapidly as a disomic locus and will also retain a greater amount of genetic variation. In addition, a dominant advantageous mutation is more likely to occur at a tetrasomic locus because of the extra copies of the gene.

The response at isoloci will be identical to a disomic locus since the mutation is likely to occur only at one locus. Thus, isoloci will phenotypically respond as quickly as a disomic locus but will maintain the original allele at the other locus.

5.4.2c. Selection for an Overdominant Trait

Both a disomic and tetrasomic locus will initially respond similarly to selection for a mutant allele resulting in heterozygous superiority. This will be true because the initial dynamics will be identical to selection for a dominant trait. However, the equilibrium conditions will be very different. If we assume that both homozygotes have equal fitness, then the equilibrium allele frequencies will be 0.5. In the disomic case, the homozygous phenotypes with reduced fitness will be present at a frequency of $(p^2 + q^2) = 0.5$; this is the so-called segregation load. At a tetrasomic locus the homozygous phenotypes will be present at a frequency of $(p^4 + q^4) = 0.125$. Thus, the segregation load is much reduced at a tetrasomic locus.

At isoloci, selection will cause the alternative alleles to be fixed alternative loci so that all individuals in the population will be fixed 'heterozygotes' (Spofford, 1969). The segregation load in the case of overdominant selection at isoloci wil be zero.

5.4.3. Inbreeding Depression

Inbreeding depression is caused by two different effects: the fixation of deleterious alleles and the loss of allelic variation at loci where there is heterozygous superiority (Falconer, 1981). Both of these effects are the result of genetic drift causing random changes in allele frequencies.

Tetrasomic loci are 'buffered' against the effects of genetic drift by virtue of having twice as many gene copies at a locus. The relative rate of loss of genetic variation at disomic and tetrasomic loci depends upon the population size (p. 342, Li, 1975). With selfing it will take a tetrasomic locus 3.80 times as long to reach the same amount of homozygosity as a disomic

locus, in comparison to 2.67 times as long with mating between full-sibs (Wright, 1951). Figure 8 shows the relative rate of loss of heterozygosity at a disomic and tetrasomic locus with continuous full-sib mating.

Isoloci are even more buffered against the effects of inbreeding depression. Deleterious recessive alleles would have to become frequent at two loci before their phenotype would be expressed. In addition, because there is no allelic variation present at equilibrium for overdominant selection, such loci will be completely unaffected by inbreeding.

5.4.4. Additional Considerations

Our consideration of the potential implications of the unusual genetic system of salmonids suggests a variety of experiments that are needed. First, more inheritance data are needed for morphological traits apparently controlled by single loci. Also, quantitative genetic analyses of salmonids must consider the possible effects of partial tetrasomic inheritance of polygenic traits. The assumption of disomic inheritance when estimating certain parameters (e.g., heritability) may lead to erroneous estimates. For example, the dominance covariance between half-sibs at a tetrasomic locus will not be zero as it is for a disomic locus. Experiments could be designed to estimate what proportion of the genotypic variation controlling polygenic traits is acting in a simple disomic manner.

The incomplete disomic inheritance of some loci may also have important practical implications. Results with autotetraploid ferns have shown that chromosomes that are inherited disomically may return to partial tetrasomic inheritance in hybrids between different lines (Hickok, 1978). This will have the effect of 'releasing' genetic variation that was not present in either of the original lines. For example, consider a recessive trait for which both lines are fixed for normal and mutant alleles (A1A1a2a2 and A1A1a2a2); homeologous pairing in the hybrids will produce gametes with two doses of the recessive allele (aa), resulting in recessive phenotypes appearing in the F_2's. Such recessive phenotypes will be at a much higher frequency in the F_2's if the two lines have become fixed for the mutant allele at different loci. The crossing of different lines (or species) of salmonids may therefore produce new strains that have more genetic variation that expected by simply adding the genetic variation of the two original lines.

Perhaps the most surprising thing about the inheritance of phenotypic variation in salmonids is that it appears to be so 'normal'. The chromosomal evidence and observed patterns of inheritance at isozyme loci indicate that the salmonids have an unusual genetic system. It is tempting to speculate that the evolutionary and domestic success of these fish is at least partially due to their tetraploid ancestry; we will consider this possibility in the next section.

6. ADAPTIVE SIGNIFICANCE OF POLYPLOIDY

The evolutionary success of the tetraploid salmonids and catostomids stands in contrast to the general lack of long-term evolutionary success of polyploids among reptiles, amphibians, and plants (Bogart, 1980; Stebbins, 1977). The explanations for this success currently fall into the realm of conjecture. Nevertheless, we believe a consideration of the possible adaptive significance of polyploidy in the salmonids may be valuable. Uyeno and Smith (1972) have suggested that the increase in heterozygosity of catostomids is at least partially responsible for the evolutionary success of these fish.

6.1. Short-term Success

A newly created polyploid is faced with direct competition from its diploid progenitor. To survive, the polyploid must either avoid such competition by being adapted to a different niche or by displacing its diploid ancestral species. This competition should be more of a problem for an autopolyploid than an allopolyploid because of the absence of any genetic distinction between the diploid and polyploid forms. Schultz (1980) has reviewed the evidence of the relative success of polyploid fish and their diploid ancestors.

Perhaps the most important immediate difference between ploidy-types is cell size and associated characteristics. Large increases in nuclear DNA amounts are accompanied by increased cell size, decreased metabolic rates, and slower development (Bachmann et al., 1972). Cavalier-Smith (1978) has argued that differences in cell size and development rates are of fundamental importance and that autopolyploidy in plants and animals "may commonly result from selection for increased cell size".

The characteristics accompanying increased nuclear DNA amounts would be favored in K-selected species. The relatively few large eggs and the slow development rates of salmonids, in comparison to other fish species, certainly are in agreement with the hypothesis of Cavalier-Smith. In addition, salmonids have apparently maintained increased cell sizes, unlike tetraploid cyprinid species (Schmidtke et al., 1975). Thus, the ancestral polyploid salmonid may have displaced its diploid progenitor because of differences associated with increased cell size.

Another possible explanation of the short-term success of the polyploid may be the possible asexual intermediate stage. A parthenogenetic form that produces all female offspring is expected to replace the competing sexual form because of the 'cost' of producing 50% males (Maynard Smith, 1978). Given that all else is equal, the frequency of parthenogenetic females will continue to increase each generation until the sexual form is eliminated.

6.2. Long-term Success

There are two conflicting views of the importance of polyploidy for major evolutionary changes. One view holds that the duplicate genes created will increase 'evolutionary inertia' thereby reducing the chances of evolving new genetic types (Stebbins, 1977; White, 1978). This is correct in that to replace an allele shared by two disomic loci or a single tetrasomic locus is a much longer process than is replacing an allele at a single disomic locus. Thus, advantageous recessive mutations are much less likely to be successfully incorporated at a tetrasomic locus.

In contrast to this view, however, Ohno has argued that gene duplications are absolutely essential for progressive evolution (Ohno, 1970ab). Ohno sees evolutionary changes as being caused by the addition of genes with new functions. This can only occur following gene duplication so that one copy of the gene can be conserved to perform the initial function of that gene.

Metabolic complexity has evolved by increasing the number and specificity of enzymes controlling biochemical reactions. For example, most of the secreted proteins of vertebrates, although currently widely divergent in function, have apparently originated from a few digestive enzymes secreted by an ancestral species (Hartley, 1974). Similarly, many of the dehydrogenases share homologies resulting from their divergence from a single ancestral gene. On a smaller scale, the at least eight loci coding for hemoglobins in humans (Harris, 1980) have all evolved from a single globin gene existing at the time of the origin of vertebrates approximately 500 Myr ago.

How has the additional tetraploid event of 25-100 Myr ago contributed to the evolutionary success of salmonids? The additional gene duplication has resulted in the further specialization of particular enzyme loci. For example, the vertebrate Ldh-B gene (Markert et al., 1975) is now represented in salmonids by two separate loci that are distinct in both structure and function. Other non-enzymatic classes of loci may show analogous specializations in place or time of expression.

6.2.1. Specialization of Duplicate Genes

It is tempting to suggest that the success of many salmonid species in living in different environments (e.g. freshwater and saltwater) is related to tetraploidy. Perhaps the salmonids' unparalleled anadromous success results from their having different genes expressed during the freshwater and marine parts of the life cycle.

Such a suggestion is certainly speculative but it could be tested. One way would be to determine those genes likely to be of importance in surviving in either a freshwater or marine environment. This hypothysis predicts that salmonids would be more likely than other anadromous fishes to have different gene products expressed during the marine and freshwater phases of the life-cycle. This could be tested by examining the patterns of tissue-specific expression of duplicated enzyme loci during

different phases of the life cycle. We have preliminary evidence of differences between individual Atlantic salmon smolts in the expression of enzyme loci in the kidney (Leary and Allendorf, unpublished data) that may fit this pattern. Another potential way of testing this hypothesis would be to examine the mRNA produced during the marine and freshwater phases of salmonids and other anadromous fishes in critical tissues. This hypothesis predicts that salmonids would be more likely to have greater differences between the mRNA's produced during these two phases.

6.2.2. Evolutionary 'Inertia' of a Polyploid Genome

It has been found that the more primitive teleost species tend to have higher cellular DNA content than more the specialized forms (Hinegardner, 1976). The salmonids with their doubled DNA content and primitive morphology are no exception to these observations. One proposed explanation for this relationship is that the presence of multiple copies of genes would have a 'buffering' effect on the phenotypic effects of allelic substitutions and thus have a conservative effect on evolutionary change (Pederson, 1971).

We have already seen by looking at the effects of natural selection that such effects do result from the presence of gene duplication. These effects are also the reason that some authors feel that polyploidy in plants has not played an important role in progressive evolution beyond the species level. However, such inertia does not hold for dominant mutations; we would expect advantageous dominant mutations to be incorporated more often in a tetraploid species than a diploid species. Evolutionary conservatism, however, is not necessarily all bad. More specialized forms may be more successful in the short-term but also tend to have higher exinction rates. Thus, the 'inertia' inherent in the salmonid genome would resist highly specialized morphological adaptations but at the same time may have increased the probability of long-term survival of salmonid lineages.

6.2.3. Population Structure

The genetic population structure of many salmonid species is characterized by many small subpopulations or demes (Behnke, 1972; Ryman et al., 1979). The loss of genetic variation within such isolated demes is a potential problem. The tetraploid genome of the salmonids will have a buffering effect against the deleterious effects of losing genetic variation. Such isolated demes may therefore be more successful in salmonids than in a comparable diploid species.

Lande (1979) has shown that that rate of fixation of chromosomal rearrangements is inversely related to local deme size. We would therefore expect salmonid lineages to show a high rate of chromosomal divergence. This effect would be intensified by the chromosomal instability following tetraploidy. Thus the high rate of chromosomal divergence in salmonids is perhaps related to their polyploid ancestry and population structure.

Such chromosomal rearrangements usually lower the fitness of heterozygotes and are thought to be an important mechanism promoting speciation. We would therefore expect salmonid lineages to have a high potential for speciation. This notion is supported by the complex patterns of relationship among taxa within salmonid genera (Behnke, 1972).

The long-term evolutionary success of a group of organisms is dependent upon avoiding extinction of species and the creation of new species. We believe that the autopolyploid nature of the salmonid genome may have been important in both of these aspects and has resulted in the evolutionary success of the salmonid fishes.

7. SUMMARY

All the fish of the family Salmonidae are apparently descended from a single tetraploid event that occurred 25-100 Myr ago. They differ from the only other known entire family of fish with a tetraploid ancestry, the Catostomidae, in that the salmonids are still in the diploidization process of restoring disomic inheritance. Multivalents have been described at meiosis in males of several salmonid species. The salmonids have undergone rapid chromosomal divergence; chromosome numbers range from 52 in the pink salmon to 102 in the European grayling. This divergence has involved many Robertsonian changes as well as other types of structural rearrangements.

Protein loci in salmonids reflect their polyploid ancestry; only 10 of 33 ancestral loci in the rainbow trout do not show evidence of duplicate gene expression. Fifteen of 33 loci have retained duplicate gene expression but have apparently been completely 'diploidized' in that the two remaining loci show structural or regulatory divergence. Eight of the 33 loci have retained duplicate gene expression and show no evidence of structural or regulatory divergence between the remaining duplicates. These pairs of loci sharing structural alleles have been termed 'isoloci'.

These isoloci have not diverged because they have not yet been fully diploidized. The Mdh-B isoloci in rainbow trout (Mdh-3,4) show normal disomic segregation in females. However, secondary pairing of homeologs in males coupled with homeologous exchanges between the loci and the centromere produce segregation ratios approaching those expected with tetrasomic segregation.

This meiotic model can also explain the aberrant segregation ratios reported at duplicate loci showing excess of non-parental types (pseudolinkage). Preferential secondary pairing of parental homeologs combined with directed disjunction of paired chromosomes will produce an excess of non-parental types.

The autopolyploid ancestry of the salmonids provides the genetic architecture upon which the evolutionary forces of mutation, natural selection, genetic drift, and migration have acted during the history of these fishes. The presence of diploidized duplicate copies for many genes allows specialization of the two loci for different metabolic functions. This is demonstrated by the differences in tissue-specific expression of many duplicate pairs. We have suggested that the salmonids unparalleled anadromous success may partially result from the expression of different duplicates during the freshwater and marine stages.

The unusual patterns of inheritance in salmonids have important implications for the evolutionary potential of these fishes. The duplicate copies of many gene loci will allow the accumulation of more genetic variation than in a diploid because of the greater number of mutations and relaxed selection against deleterious mutations. The duplicate copies will also

act as a buffer against the harmful effects of inbreeding depression. However, the duplicate copies of many genes may also have a buffering effect on the rate of progressive evolutionary change by natural selection.

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Table I. Subfamilies and genera of the family Salmonidae (Nelson, 1976).

Subfamily	Genus	No. species
Coregoninae	Coregonus	25
	Prosopium	6
	Stenodus	1
Salmoninae	Brachymystax	1
	Hucho	3-5
	Oncorhynchus	7
	Salmo	13
	Salvelinus	6
Thymallinae	Thymallus	4

Table II. Chromosome and arm numbers of salmonid fish species (Gold et al., 1980; Sola et al., 1981). Short arms on subtelocentric chromosomes are not counted in calculating arm numbers. Common names are from Robins et al. (1980) for North American species, Maitland (1977) for European species, and Ricker (1962) for Asian species.

Species	Common name	Reported chromosome numbers	Chromosome arm numbers
Coregonus albula	cisco	80	96
Coregonus artedii	lake herring	80	96
Coregonus clupeaformis	lake whitefish	80	100
Coregonus hoyi	bloater	80	90
Coregonus lavaretus	common whitefish	80,96	92-128
Coregonus nasus	broad whitefish	80,96	92,96
Coregonus oxyrhynchus	houting	96	96
Coregonus peled	peled	80	92,98
Coregonus pidschian	Arctic whitefish	80,96	92-98
Coregonus reighardi	shortnose cisco	80	92
Coregonus ussuriensis	Amur whitefish	80,82	100
Coregonus zenithicus	shortjaw cisco	80	98
Prosopium abyssicola	Bear Lake whitefish	72	100
Prosopium coulteri	pygmy whitefish	82	100
Prosopium cylindraceum	round whitefish	78	100
Prosopium gemmiferum	Bonneville cisco	64	100
Prosopium spilonotus	Bonneville whitefish	74	100
Prosopium williamsoni	mountain whitefish	78	100
Stenodus leucicthys	inconnu	74	108
Brachymystax lenok	lenok	90	116
Oncorhynchus gorbuscha	pink salmon	52	100,104
Oncorhynchus keta	chum salmon	74	100
Oncorhynchus kisutch	coho salmon	60	106,112
Oncorhynchus masou	masu salmon	66	100
Oncorhynchus nerka	sockeye salmon	56-58	102,104
Oncorhynchus rhodurus		66	100
Oncorhynchus tschawytscha	chinook salmon	68	100,104
Salmo aquabonita	golden trout	58	104
Salmo apache	Apache trout	56	106
Salmo carpio		80	98
Salmo clarki bouvieri	Yellowstone cutthroat trout	64	104
Salmo clarki clarki	coastal cutthroat trout	68,70	104,106
Salmo clarki henshawi	Lahontan cutthroat trout	64	104
Salmo clarki lewisi	westslope cutthroat trout	64-66	104
Salmo gairdneri	rainbow trout	58-65	104
Salmo gilae	Gila trout	56	105

Salmo obtusirostris	Adriatic salmon	82	94
Salmo salar	Atlantic salmon	54-60	72-74
Salmo trutta	brown trout	77-82	96-102
Salmo sp.	redband trout	58	104
Salvelinus alpinus	Arctic char	78,80	96,100
Salvelinus fontinalis	brook trout	84	100
Salvelinus leucomaenis	Siberian char	84	100
Salvelinus malma	Dolly Varden	80,82	98
Salvelinus namaycush	lake trout	84	100
Thymallus thymallus	European grayling	102	170

Table III. Reports of multivalents in meiosis of salmonid fishes.

Species	Sex	Meiotic Stage	Multivalents Observed	Reference
Pink salmon	M	Diakinesis	No	Simon, 1964
Coho salmon	M	Metaphase I	Yes	Ohno, 1970b
Golden trout	M	Metaphase I	Yes	Gold and Gall, 1975
Rainbow trout	F	Metaphase I	Yes	Ohno et al., 1965
	F	Pachynema	No	Thorgaard and Gall,1979
	M	Diakeinesis	Yes	Simon, 1964
	M	Metaphase I	Yes	Ohno et al., 1965; Ohno et al., 1968
Atlantic salmon	M	Metaphase I	Yes	Svardson, 1945; Nygren et al., 1972
Brown trout	M	Metaphase I	Yes	Svardson, 1945
	M	Metaphase I	No	Nygren et al., 1971
Arctic char	M	Metaphase I	Yes	Svardson, 1945
	M	Metaphase I	No	Nygren et al., 1971
Brook trout	F	Pachynema	No	Davisson et al., 1973; Lee and Wright, 1981
	M	Metaphase I	Yes	Davisson et al., 1973; Lee and Wright, 1981
Lake trout	F	Pachynema	No	Davisson et al., 1973
	M	Metaphase I	Yes	Davisson et al., 1973

Table IV. Enzymes and proteins examined in rainbow trout for loss of duplicate gene expression.

Enzyme	EC number	Abbreviation
Aspartate aminotransferase	2.6.1.1	AAT
Adenosine deaminase	3.5.4.4	ADA
Alcohol dehydrogenase	1.1.1.1	ADH
Creatine kniase	2.7.3.2	CK
Diaphorase	1.6.4.3	DIA
Fructose diphosphatase	3.1.3.11	FDP
Glyceraldehyde-phosphate dehydrogenase	1.2.1.12	GAP
Glucosephosphate isomerase	5.3.1.9	GPI
Isocitrate dehydrogenase	1.1.1.42	IDH
Lactate dehydrogenase	1.1.1.27	LDH
Malate dehydrogenase	1.1.1.37	MDH
Malic enzyme	1.1.1.40	ME
Mannosephosphate isomerase	5.3.1.8	MPI
Para-albumin		PAL
Phosphoglucomutase	2.7.5.1	PGM
Phosphogluconate dehydrogenase	1.1.1.44	PGD
Phosphoglycerate kinase	2.7.2.3	PGK
Sorbitol dehydrogenase	1.1.1.14	SDH
Transferrin		TFN

Table V. Present status of 33 ancestral protein loci in the rainbow trout.

Locus	Duplicated		Single locus
	Isoloci	Diverged	
Aat-A	-	Yes	-
-B	Yes	-	-
Aat-m	-	Yes	-
Adh	-	-	Yes
Ada	-	-	Yes
Ck-A	-	Yes	-
-B	-	-	Yes
-C	-	Yes	-
Dia	-	-	Yes
Fdp	-	-	Yes
Gap-A	-	Yes	-
-B	-	Yes	-
Gpi-A	-	Yes	-
-B	-	-	Yes
Idh-A	Yes	-	-
-m	-	Yes	-
Ldh-A	-	Yes	-
-B	-	Yes	-
-C	-	-	Yes
Mdh-A	Yes	-	
-B	Yes	-	-
Mdh-m	-	Yes	-
Me-A	Yes	-	-
-m	Yes	-	-
Mpi	-	-	Yes
Pal	Yes	-	-
Pgd	-	-	Yes
Pgk	-	-	Yes
Pgm-A	-	Yes	-
Pgm-B	Yes	-	-
Sdh	-	Yes	-
Sod	-	-	Yes
Tfn	-	-	Yes
Totals	8	15	10

Table VI. Comparative recombination rates in male and female brook trout - lake trout hybrids from May et al. (1980. The number in parentheses is the number of individuals examined.

Loci		Mean Recombination Rate	
		Females	Males
Cpk-1	Gus	0.199 (2)	0.088 (1)
Gpi-3	Mpi	0.299 (1)	0.000 (1)
Ada-1	Agp-2	0.154 (1)	0.031 (4)
Idh-3	Me-2	0.086 (4)	0.048 (7)

Table VII. Expected segregation ratios for duplicated loci with disomic and tetrasomic inheritance. A semicolon separates disomic loci. For example, the genotype AA;aa is homozygous at two disomic loci.

Parental Genotypes	Frequency of Gametes*		
	AA	Aa	aa
AA;Aa	0.50	0.50	0
AAAa	0.50 $(1+\alpha/2)$	0.50 $(1-\alpha)$	0.25 (α)
AA;aa	0	1.00	0
Aa;Aa (trans)	0.50 (r)	1-r	0.50 (r)
Aa;Aa (cis)	0.50 (1-r)	r	0.50 (1-r)
AAaa	0.17 $(1+2\alpha)$	0.67 $(1-\alpha)$	0.17 $(1+2\alpha)$

* α is the frequency of double reduction divisons and r is the frequency of recombination.

Table VIII. Observed and expected disomic segregation ratios at Mdh-B rainbow trout from Chambers Creek.

Family	Parental Genotypes		Progeny Phenotypes					X^2
	Female	Male	BBBB	BBBb	BBbb	Bbbb	bbbb	
C21	BB;BB	Bb;Bb	114 (115)	238 (230)	109 (115)	0	0	0.60
C22	BB;BB	Bb;Bb	21 (23)	50 (46)	20 (23)	0	0	1.11
C25	BB;BB	BB;bb	0	40 (40)	0	0	0	--
C31	BB;BB	Bb;Bb	27 (25)	51 (50)	23 (25)	0	0	0.33
C32	BB;Bb	Bb;Bb	12 (12)	34 (37)	38 (37)	15 (15)	0	0.84
C37	BB;Bb	BB;bb	0	41 (40)	39 (40)	0	0	0.05
C39	BB;BB	BB;bb	0	40 (40)	0	0	0	--

Table IX. Observed and expected disomic and tetrasomic segregation ratios at Mdh-B in rainbow trout from the Jocko River Hatchery. The first expected ratios are for disomic inheritance assuming double homozygosity (BB;bb), the second for double heterozygosity (Bb;Bb), and the third for tetrasomic inheritance with random chromosome inheritance.

	Parental Genotypes		Progeny Phenotypes					X^2
Family	Female	Male	BBBB	BBBb	BBbb	Bbbb	bbbb	1 df
G1	BB;Bb	BB;bb	2	50	45	5	0	
			-	(56)	(56)	-	-	-
			(13)	(38)	(38)	(13)	-	17.90
			(8)	(42)	(42)	(8)	-	7.06
G5	Bb;Bb	Bb;bb	0	20	30	30	6	
			-	-	(43)	(43)	-	-
			-	(11)	(32)	(32)	(11)	1.26
			-	(7)	(36)	(36)	(7)	11.40
G6	Bb;Bb	BB;BB	32	57	19	0	0	
			-	(108)	-	-	-	-
			(27)	(54)	(27)	-	-	0.33
			(18)	(72)	(18)	-	-	9.38
H5	BB;BB	Bb;Bb	52	92	58	0	0	
			-	(202)	-	-	-	-
			(50)	(101)	(50)	-	-	1.60
			(34)	(135)	(34)	-	-	40.55
H6	BB;BB	BB;bb	14	266	9	0	0	
			-	(289)	-	-	-	-
			(72)	(144)	(72)	-	-	20.43
			(48)	(193)	(48)	-	-	83.70
H7	BB;BB	Bb;Bb	49	127	46	0	0	
			-	(222)	-	-	-	-
			(55)	(111)	(55)	-	-	4.61
			(37)	(148)	(37)	-	-	8.94
H13	BB;bb	BB;BB	0	44	0	0	0	
H17	BB;bb	BB;BB	0	249	0	0	0	
H18	BB;BB	BB;bb	12	184	5	0	0	
			-	(201)	-	-	-	-
			(50)	(100)	(50)	-	-	138.75
			(34)	(134)	(34)	-	-	55.97
H19	BB;BB	Bb;Bb	38	107	37	0	0	
			-	(182)	-	-	-	-
			(46)	(91)	(46)	-	-	5.63
			(30)	(121)	(30)	-	-	5.08

Table X. Expected inheritance patterns for a recessive trait at a single locus and duplicated loci for matings between affected and normal parents.

Inheritance Pattern	Parental Genotypes		Frequency of Progeny Phenotypes	
	Normal	Affected	Normal	Affected
Non-duplicated	AA	aa	1.00	0
	Aa	aa	0.50	0.50
Isoloci*	AA;AA	aa;aa	1.00	0
	Aa;AA	aa;aa	1.00	0
	Aa;Aa	aa;aa	0.75	0.25
	Aa;aa	aa;aa	0.50	0.50
Tetrasomic**	AAAA	aaaa	1.00	0
	AAAa	aaaa	1.00	0
	AAaa	aaaa	0.83	0.17
	Aaaa	aaaa	0.50	0.50

* Assuming no linkage

** Assuming random chromsome inheritance

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CONSISTENTLY HIGH MERISTIC COUNTS IN NATURAL HYBRIDS BETWEEN BROOK TROUT AND BULL TROUT

ROBB F. LEARY, FRED W. ALLENDORF, AND KATHY L. KNUDSEN

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(369)

Abstract.—We detected hybrids between the bull trout (*Salvelinus confluentus*) and the brook trout (*S. fontinalis*) in three samples from the Upper Columbia River drainage in Montana using 10 isozyme loci that differentiate these species. The occurrence of only first generation, male hybrids indicates that these hybrids are almost certainly sterile.

We counted 10 meristic characters from all of the bull trout, brook trout, and their hybrids in the sample from the South Fork of Lolo Creek. Univariate and multivariate analyses of the meristic data conclusively indicated that the hybrids are not meristically intermediate to the parental species. The hybrids had counts higher than either of the parental species or counts similar to the parental species with the higher count. The consistent tendency for the hybrids to have high meristic counts is suggested to be due to reduced developmental rate caused by genetic incompatibility between the parental genomes. [Bull trout; brook trout; *Salvelinus*; hybrids; electrophoresis; meristics; developmental rate.]

Hybridization between fish species in nature is common (Hubbs, 1955; Schwartz, 1972). Identification of naturally occurring hybrid fishes has usually relied on the assumption that hybrids are morphologically intermediate to the parental species. Although widely accepted, this assumption remains largely untested. The morphology of hybrid fishes has seldom been described when hybrid status was known independent of morphology.

Neff and Smith (1979) used hybrids produced in the laboratory to test the assumption of hybrid intermediacy and the appropriateness of multivariate analysis to identify naturally occurring fish hybrids. Interspecific hybrids of *Notropis* and *Lepomis* were generally, but not uniformly, intermediate in multivariate space as assessed by discriminant function and principal component analyses. A number of other investigators have also provided evidence of deviations from morphological intermediacy among hybrids produced in the laboratory (Hubbs and Strawn, 1957; Smitherman and Hester, 1962; West and Hester, 1964; Simon and Noble, 1968; Berry and Low, 1970; Ross and Cavender, 1981).

There are limitations, however, to the usefulness of laboratory produced hybrids. Such hybrids are generally raised under different and less environmentally variable conditions than naturally produced hybrids. The morphology of fishes is influenced by the environment as well as genetics (Barlow, 1961; Garside, 1966; Ali and Lindsey, 1974; MacGregor and MacCrimmon, 1977). Thus, laboratory hybrids may not accurately represent the morphology of natural hybrids. Furthermore, laboratory hybrids are usually derived from a small number of parents. Samples of natural hybrids, however, will generally contain individuals from many parents. These samples, therefore, will usually be more genetically variable than artificially produced hybrids. Thus, the morphology of naturally produced hybrids probably will be more variable than that of laboratory hybrids because of increased environmental and genetic variation.

Natural hybrids can be unambiguously identified without knowledge of their morphology when the hybridizing populations do not have the same alleles at electrophoretically detectable protein loci (Reinitz, 1977; Solomon and Child, 1978; Beland et al., 1981). Morphological data from known hybrids and both parental species can, therefore, be collected from hybridizing natural populations. The morphology of interspecific hybrid fishes

May 17, 1983

Mr. Robb F. Leary
Department of Zoology
University of Montana
Missoula, MT 59812

Dear Mr. Leary:

Many thanks for the copies of manuscripts. Several weeks ago I reviewed the paper on Westslope cutthroat x Eagle Lake rainbow hybrids for the Canadian Journal of Fisheries and Aquatic Science. I assume that you have received my review comments by now (I initialed the review). Mainly, I suggested alternative explanations for the delayed mortality of the hybrids -- that the "genetic incompatibility" may be only the incompatibility of the Eagle Lake genome to the hatchery environment and diet, not incompatibility between the DNA of the parental species.

I found the bull trout x brook trout hybrid paper quite interesting. This is the first report of lack of hybrid fertility between species of Salvelinus. In my 1980 paper on Salvelinus in the book: "Charrs, salmonid fishes of the genus Salvelinus" (E. Balon, ed.), I discuss bull trout on p. 467. I mention bull trout x brook trout hybrids I found in Long Creek in Upper Klamath Lake basin. Cavender mentioned these hybrids in his 1978 paper.

Enclosed is a copy of a report on bull trout written for Glacier Park. In it I mention that Long Creek hybrids and state that.. "as far as known, all Salvelinus hybrid combinations are fertile."

With such sharp electrophoretic differences between bull trout and brook trout, can Fedd Allendorf perhaps arrange with Fred Utter to obtain material for further electrophoretic comparisons with northern and southern subspecies of Dolly Varden (S. malma) and also Arctic charr (S. alpinus)?

In Table 3, meristic values for paired structures are the sum of the left and right sides. This is an unusual procedure and a footnote explanation should be made that the values are the combined counts from the left and right sides (typically meristic data are based on left side only). Evidently all rudimentary rays in the dorsal and anal fins are included. Typically, only principal rays are counted (see Hubbs and Lagler, Fishes of the Great Lakes, for standard taxonomic procedure).

The vertebrae counts are about four too few. S. fontinalis typically has 58-60 vertebrae and S. confluentus has about 64-65. Evidently, the last three upturned vertebrae were not counted and perhaps the first vertebrae, fused to the basioccipital was not counted.

I might mention that Ted Cavender (unpublished) has examined the karyotypes of Montana bull trout and found 2N=78.

I also found Allendorf and Thorgaard's paper on tetraploidy of great interest. You can ask Fred if he wants my comments or if the paper is already in press. I recall a paper by Rosen and Heingardner (?) several years ago on DNA content in teleost cells. I believe they had evidence of polyploidy in some families of catfishes (Siluriformes).

Sincerely,

Robert Behnke

Mr. Robb F. Leary
Department of Zoology
University of Montana
Missoula, MT 59812

(enclosure)

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