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# Chromosome Cytology in the Cutthroat Trout Series *Salmo clarki* (Salmonidae)

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Chromosome cytology often has been useful in elucidating evolutionary relationships among various taxa (Mayr 1973, White 1973), and has proved especially useful in distinguishing among different lineages in the western North American trouts. The chromosome studies of Wright (1955), Simon and Dollar (1963), Miller (1972) and Gold and Gall (1975) have shown that all the western trout "species" examined differ to some extent in karyotype.

The distribution of karyotypes among the western trouts suggests that most species differ only in the number of Robertsonian fusions (or dissociations) accumulated within each lineage, i. e., they form a Robertsonian series. The rainbow trouts, *Salmo gairdneri* (2n=60), golden trouts, *S. aguabonita* (2n=58), and the unnamed redband trouts (2n=58), each have a fundamental number (number of chromosome arms) of 104 (Wilmot 1974, Gold and Gall 1975). The cutthroat trouts, *S. clarki* (2n=64) and the Apache trouts, *S. apache* (2n=56), however, are reported to have a fundamental number of 106 (Simon and Dollar 1963, Miller 1972). Presumably, the difference between the 104 and 106 chromosome arm groups stemmed from a single centromere shift (unequal pericentric inversion) as pictured in Simon and Dollar (1963).

Simon and Dollar (op. cit.) felt that since the cutthroat trouts have the most primitive distributional pattern (see also Behnke 1972), the simplest phylogenetic interpretation is that a single centromere shift producing 104 chromosome arms from 106 must have occurred subsequent to the divergence of the cutthroat-like trouts from the main western *Salmo* stem. Such reasoning implies a close evolutionary affinity between the cutthroat and Apache trouts. Miller (1972) felt that the zoogeographic and phenetic evidence supported an inland cutthroat trout origin for *S. apache*.

In the present study, the karyotypes of two subspecies of cutthroat trout, (*Salmo clarki*) were found to differ markedly in chromosome number. In both subspecies, the diploid complements contained 104 chromosome arms.

[1] This work was carried out while the senior author was a post-doctoral fellow at the Department of Animal Science, University of California, Davis; and the second author was a pre-doctoral fellow at the Department of Genetics, University of California, Davis, U.S.A.

## Materials and methods

Specimens of the subspecies *S. c. clarki* (the "coastal cutthroat") were sampled by electrofishing from Mill Creek near Trinidad, California; and from Prairie Creek near Orick, California. Both Mill and Prairie Creeks are tributary to the Pacific Ocean. Specimens of the subspecies *S. c. henshawi* (the "inland cutthroat") were sampled by electrofishing from Macklin Creek near Truckee, California. A second sample of inland cutthroat was obtained from Independence Lake near Truckee, California.

The method of chromosome preparation was as outlined in Gold (1974). Anterior kidney tissue was removed from colchicinized fish, gently homogenized, treated with hypotonic KCl, and fixed in 3:1 methanol: acetic acid. Slides were prepared by dropping fixed cells onto microscope slides, air-drying, and staining with undiluted Giemsa. Well-spread metaphases were selected for study.

## Results

The distribution of chromosome counts from individuals of the four *S. clarki* populations examined is presented in Table 1. Consistent modes of 2n=64 chromosomes were found in all individuals from both inland populations—66% of counts in Independence Lake fish and 65% of counts in Macklin Creek fish. This is in agreement with the other report of diploid chromosome number in inland cutthroat trout (Simon and Dollar 1963). In the two coastal populations, consistent modes of 2n=68 chromosomes were found in all individuals—80% of counts from the Mill Creek fish and 66% of counts from the Prairie Creek fish. Simon (in Schreck and Behnke 1971) reported the diploid chromosome number of the coastal cutthroat trout to be 70. However, in a subsequent personal communication to R. J. Behnke, Simon reported observing 68 chromosomes, in agreement with our findings.

Table 1. Distribution of chromosome counts in kidney cells from individuals of four populations of *S. clarki*

| Populations | No. of cells with 2n of | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 61 | 62 | 63 | 64 | 65 | 66 | 67 | 68 | 69 |
| Independence Lake (3)* (inland) | 1 | 3 | 12 | 33 | 1 | — | — | — | — |
| Macklin Creek (4) (inland) | 3 | 7 | 12 | 46 | 3 | — | — | — | — |
| Mill Creek (3) (coastal) | — | — | — | — | — | 1 | 4 | 24 | 1 |
| Prairie Creek (4) (coastal) | — | — | — | — | — | 2 | 17 | 43 | 3 |

* Parentheses refer to the number of individuals examined.

No evidence of aneuploidy or intraindividual chromosomal polymorphism (as found in other salmonids—see Gold 1977) was indicated by the distributions of chromosome counts. From 83–94% of all non-modal cells over all individuals examined were hypomodal, suggesting chromosome loss during preparation or counting errors. The low frequency of hypermodal counts most likely stemmed from counting errors or premature separation of some chromatids.

Karyograms were prepared from photographs of the best spread metaphases, and representatives are shown in Figs. 1 and 2. The karyotypes of the Independence Lake and Macklin Creek trout were identical (Fig. 1), and contained 40 chromosomes

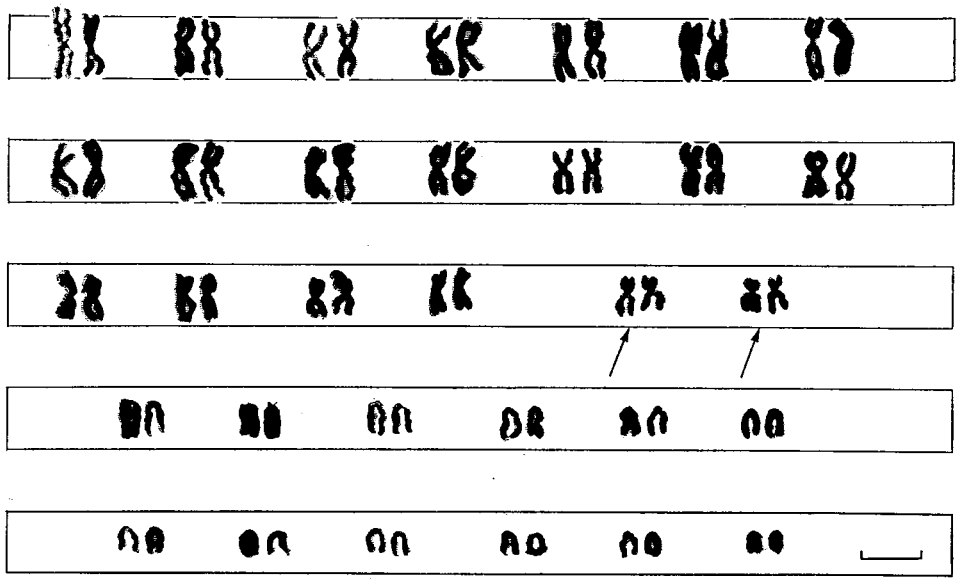

Fig. 1. Somatic metaphase chromosomes (from kidney) of the inland cutthroat trout, *S. c. henshawi* (2n=64). Arrows refer to the two chromosome pairs with submedian centromeres which are not apparent in the coastal cutthroat karyotype (cf. text). Bar.=*ca.* 10 $\mu$.

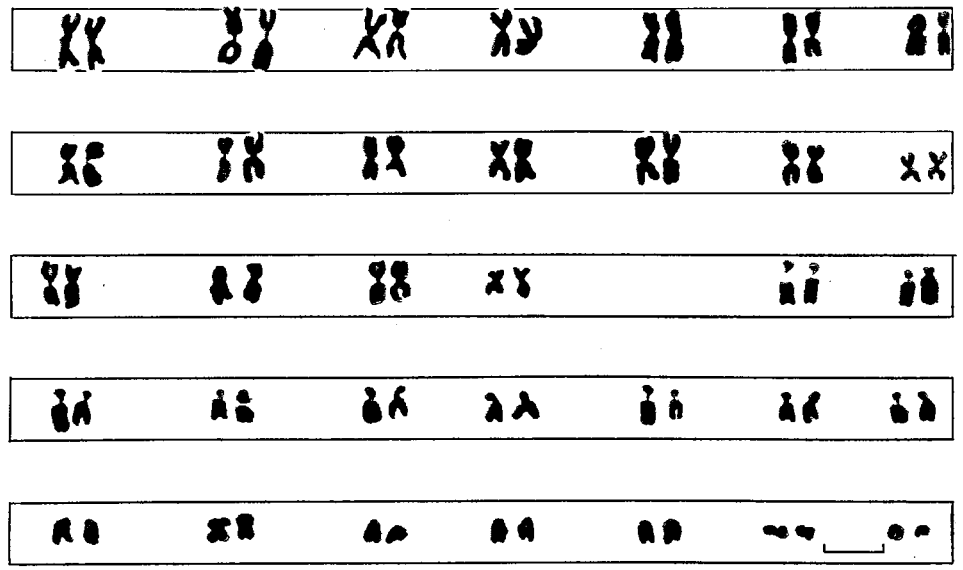

Fig. 2. Somatic metaphase chromosomes (from kidney) of the coastal cutthroat trout, *S. c. clarki*, (2n=68). Bar=*ca.* 10 $\mu$.

with median or submedian centromeres, and 24 chromosomes with subterminal or terminal centromeres. The karyotypes of the Mill and Prairie Creek fish also were identical to each other (Fig. 2), but contained 36 chromosomes with median or submedian centromeres, and 32 chromosomes with subterminal or terminal centromeres. By scoring the chromosomes with median or submedian centromeres as biarmed, and the chromosomes with subterminal or terminal centromeres as uniarmed (as in Gold and Gall 1975), the arm number in both inland and coastal cutthroat trout was estimated to be 104. This estimate is contradictory to that of Simon and Dollar (1963) for the inland form, and to that of Simon (in Schreck and Behnke 1971) for the coastal form.

The simplest explanation for the four chromosome difference in the diploid number between the coastal and inland cutthroat trouts is the past occurrence of two independent centric fusions (or dissociations) involving different pairs of non-homologous acrocentric (or metacentric) chromosomes; with the fusions (dissociations) subsequently becoming homozygous. This would account for the differencen in chromosome number without changing the fundamental arm number. In the inland cutthroat karyotype (Fig. 1), two pairs of chromosomes with submedian centromeres are not readily apparent in the coastal cutthroat karyotype (Fig. 2). These two pair could represent the products of two fusion (dissociation) events which reduced (increased) the chromosome number by four. However, without more discriminating staining techniques, e. g. banding, identification of specific chromosomes involved in chromosomal rearrangements is speculative.

## Discussion

Our finding of 104 chromosome arms in both the inland and coastal cutthroat trout, instead of 106 as previously reported, raises the question as to whether the discrepancy might be due to intraspecific polymorphism, or to inconsistencies among laboratories. During the past two years we have karyotyped some 250 individuals from over six recognized species or subspecies in this taxon, and in no instance have we observed polymorphism of this type. Nor, to our knowledge, are there any conclusive reports of arm number polymorphism in western *Salmo*. Further, Simon and Dollar (1963), in the only report of cutthroat chromosome cytology, employed a much different chromosome preparation technique than the one we used. Judging from their hand-drawn karyotypes (pp. 44–45, op. cit), chromatid separation was far from complete, and centromere placement must have been difficult to observe. On a size and shape basis alone, the chromosome pair No. 21 in their karyotype could easily have been uniarmed instead of biarmed as scored. In lieu of evidence to the contrary, we consider the arm number of the cutthroat trout to be 104.

Therefore, all of the western North American trouts karyotyped to date, with the possible exception of *S. apache* (106 arms), appear to comprise a single Robertsonian series; the difference between species pairs stems from the accumulation of fixed centric fusions or dissociations. No *prima facie* evidence is available to indicate the direction of chromosomal evolution, although fusion would seem the most prob-

able since the lineage with the most primitive distributional pattern (the cutthroats —Behnke 1972) has the highest 2n chromosome number.

The arm number difference between *S. apache* (106 arms—Miller 1972) and the remaining western trouts (104 arms) may be the result of a single centromere shift which became fixed in the line leading to *S. apache*. Although such a shift is, perhaps, of considerable antiquity, a clear understanding will not be possible until the the close geographic relative of *S. apache*, the gila trout (*S. gila*), is examined cytologically.

The situation between the inland and coastal cutthroat trouts is not at all clear. The two forms most definitely have different ecological niches. The single coastal subspecies (*S. c. clarki*) is narrowly distributed along the North Pacific coast, is usually found in the headwater stream portions under densely foliated overhanging banks (although lacustrine populations exist), and is partially anadromous (Behnke 1972). The inland forms consist of many different subspecies, cover a broad geographical swath from British Columbia in the Northwest to Colorado in the Southeast, are found in both lakes and streams, and have no access to salt water.

The difference in chromosome number between *S. c. clarki* and *S. c. henshawi* might provide a cytological mechanism for reduced fertility or fitness in hybrids between the two forms. If this is true, we would expect that if *clarki* and *henshawi* came into contact and interbred, natural selection would favor homotypic matings and the development of complete reproductive isolation. Hybridization studies between the two, which would prove pertinent to the question, have not been carried out.

On the other hand, instances among animals are known in which hybrids between parents differing in chromosome number exhibit only slightly or moderately reduced fertility (e. g. see Baker, Bleier, and Atchley 1975). We do not know what would be the outcome of hybridization between *henshawi* and *clarki*. The ease of hybridization between either form of cutthroat and the rainbow trout, *S. gairdneri* (2n=60; 44 metacentrics and 16 acrocentrics) would suggest that inland×coastal hybrids should be to some extent viable and fertile (Behnke 1965, 1970, 1972). Rigorous attempts to quantify fertility in hybrids of different chromosomal forms are required before we can claim that we fully understand the significance of chromosomal divergence to the process of speciation in the western North American trouts.

## Summary

The karyotypes of two subspecies of the cutthroat trout series, *Salmo clarki*, were determined from anterior kidney cells. One subspecies, *S. c. clarki*, the "coastal" cutthroat, has 2n=68 chromosomes (36 meta- or submetacentrics and 32 acrocentrics). The other subspecies, *S. c. henshawi*, one of the many forms of "inland" cutthroat, has 2n=64 chromosomes (40 meta- or submetacentrics and 24 acrocentrics). The fundamental arm number in both subspecies was estimated at 104. Evidently, chromosomal fusions or dissociations have played a major role in the chromosomal evolution of *S. clarki*.

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