High Incidence of a Male-Specific Genetic Marker in Phenotypic Female Chinook Salmon from the Columbia River

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Numerous populations of anadromous salmonids in the northwestern United States have been declining for many years, resulting in Endangered Species Act listings and in some cases extinction. The degradation of river ecosystems has been proposed as one of the major reasons for the inability of salmon to maintain their populations. However, the specific factors interfering with the reproduction and survival of salmon during the freshwater phase of their life cycle have not been fully described. This study was initiated to determine the incidence of phenotypic sex reversal in wild, fall chinook salmon (*Oncorhynchus tshawytcha*) that returned to spawn in the Columbia River. Fish were sampled at different locations within this watershed to determine whether they were faithfully expressing their genotype. We report a high incidence (84%) of a genetic marker for the Y chromosome in phenotypic females sampled from the wild, which was not observed in female fish raised in hatcheries. It appears likely that female salmon with a male genotype have been sex reversed, creating the potential for an abnormal YY genotype in the wild that would produce all-male offspring and alter sex ratios significantly. *Key words*: chinook salmon, genetic marker, genotype, phenotype, sex linkage. *Environ Health Perspect* 109:67–69(2001). [Online 15 December 2000]
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Numerous populations of anadromous salmonids in the northwestern United States have been declining for many years, resulting in Endangered Species Act listings and in some cases extinction (*1,2*). The degradation of river ecosystems has been proposed as one of the major reasons for the inability of salmon to maintain their populations (*3,4*). However, the specific factors interfering with reproduction and survival of salmon during the freshwater phase of their life cycle have not been described fully. Historically, the Columbia River and its tributaries in Washington, Idaho, and Oregon produced more chinook salmon (*Oncorhynchus tshawytcha*) than any other river system in the world (*5*). Today within the Columbia River watershed only two self-sustaining populations of wild, fall chinook salmon remain (*6*). One of these populations spawns on a downstream tributary (Lewis River) below the first dam, while the other spawns further upstream on the Hanford Reach (Figure 1). The Hanford Reach is the only significant free-flowing part of the mainstem Columbia River; the rest of the river consists of impounded stretches of water contained by hydroelectric dams. Migrating adults of the Hanford Reach population pass over four dams to reach their spawning grounds. Therefore, the wild chinook salmon from the Hanford Reach provide an opportunity to study what may have happened to other salmonid populations that have undergone significant, and in some instances irreversible, declines within this river system.

We examined whether wild male and female chinook salmon spawning on the Hanford Reach of the Columbia River were faithfully expressing their genetic sex. Pacific salmon have a genotypic sex-determining system with male heterogamety; the male is XY and the female is XX (*7*). A molecular test is available for chinook salmon that is based on a DNA marker specific for the Y chromosome (*8*). This DNA sequence is found only in genetically male chinook salmon (i.e., Y chromosome specific) and is not present in genetic females (i.e., XX genotype) (*9*). We tested whether wild chinook salmon with a male phenotype possessed this marker, and conversely whether phenotypic females did not.

Methods

Sample collection. Tissue samples, in the form of a fin clip from the left pectoral fin, were removed from 50 phenotypic female and 50 phenotypic male salmon at each of three sampling locations. Wild chinook salmon were collected after spawning as part of the Washington Department of Fish and Wildlife salmon carcass surveys (November 1999) on the Hanford Reach (Figure 1). Chinook salmon tissue samples were also collected from postspawned adults at two other sites, serving as reference populations: the Priest Rapids Hatchery (November 1999) and Dworshak National Fish Hatchery (August 1999) on the Clearwater River, Idaho (Figure 1). Fish sampled at the Priest Rapids Hatchery are derived from the same genetic stock of fall-run chinook salmon that spawn naturally in the Hanford Reach (*10,11*). The chinook salmon sampled at Dworshak National Fish Hatchery are a spring-run population that begins migration to the spawning grounds earlier in the season. The phenotype of every fish was confirmed by observations of external secondary sexual characters (sexually mature chinook salmon exhibit external sexual dimorphism) and an internal examination of the gonads (either ovaries or testes).

DNA extraction and polymerase chain reaction. Genomic DNA was isolated from 5–10 mg of fin tissue that had been fixed in 95% ethanol using the Puregene DNA Isolation Kit (Gentra Systems, Minneapolis, MN). A protocol for fixed solid tissues supplied by the vendor was followed. Polymerase chain reactions (PCRs) were performed with genomic DNA using reagents supplied in the Taq DNA Polymerase kit (#18038-018; GibcoBRL, Rockville, MD) and primers (*8*) that amplify a male-specific DNA sequence (*9*). This technique permitted the screening of each fish to determine the genetic sex, which was then compared to the expressed phenotype of each individual.

Results

The results of this study show that a high proportion (84%) of phenotypic female chinook salmon from the Hanford Reach of the Columbia River tested positive for the male-specific DNA marker (Table 1). These female chinook salmon had a 209 base pair DNA fragment identical to that of phenotypic males (Figure 2), indicating a Y chromosome within their genome. A minority of the wild females lacked the male-specific DNA. In contrast, we found no evidence for the male-specific DNA marker in female chinook

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salmon sampled from two hatchery populations within the Columbia River watershed (Table 1). All phenotypic male salmon (wild and hatchery) tested positive for the male-specific DNA marker.

Discussion

This is the first report in any wild vertebrate population of a significant proportion of phenotypic females bearing a genetic marker characteristic of the male sex. There are several possible explanations for the high incidence of a male-specific DNA marker, indicating a Y chromosome, in phenotypic female chinook salmon sampled from the Hanford Reach. Perhaps an unusual chromosome translocation event has occurred in the wild chinook salmon population spawning on the Hanford Reach, not previously detected in other chinook salmon populations on the west coast of North America (*9*). If a portion of the Y chromosome containing the male-specific DNA marker amplified in this study had been transferred to another chromosome within the genome, this might explain why the male-specific DNA is present in the genome of some phenotypic females sampled from the Hanford Reach. However, this explanation is difficult to accept given that fish returning to the Priest Rapids Hatchery located at the upper end of the Hanford Reach and wild fish from the Hanford Reach are genetically indistinguishable (*10*) and the male-specific DNA marker was not found in any of the females from Priest Rapids Hatchery.

The low levels of radioactivity entering the Hanford Reach from the adjacent Hanford Nuclear Reservation (*12*) also are unlikely to have caused the results documented. High levels of radioactive exposure in salmonids cause sterility (*13*), and there is no evidence that the exceedingly low levels present in the Columbia River could cause the type of phenotypic changes noted. The most likely explanation is phenotypic sex reversal of fish that develop within the wild on the Hanford Reach.

A large body of evidence shows that factors such as environmental temperature or exogenous sex-steroid treatment can alter sex determination during embryological development in fish (*14*). The results of this study could be evidence that genetic males have been sex reversed and have the appearance of phenotypic females. In this case, it is possible that fluctuations in water temperature during some period of early embryonic development affected sex determination in a proportion of wild male chinook salmon. A report examining the closely related sockeye salmon (*O. nerka*) (*15*) shows that an experimental temperature shift during embryo incubation caused a significant increase in the number of females within the population. This is similar to the well-documented cases in which some reptiles have temperature-dependent sex determination (*16*). Sex determination is controlled environmentally by incubation temperature during embryonic development in these animals. In some lizards and alligators, incubation of embryos at cool temperatures produces a sex ratio skewed significantly toward the female, while the opposite is true for many turtles. Evidence shows that for several fish species, sex determination can also be affected by temperature changes during embryonic development (*17–19*). Water flows and water temperatures within the Hanford Reach are affected daily by upstream activities at dams, which generate hydroelectric power (*11,20*). These fluctuations in water temperature (~2–6°C) could influence sex determination in wild chinook salmon embryos incubating in their redds.

Similarly, it is well known that the phenotype of male salmonids can be changed to that of the female by exposure to estrogenic steroids during the embryonic period of early ontogeny (*21*). Estrogens are implicated as the principal endocrine regulators in the normal development of ovaries in genetic female fish (*22*). An estrogen-sensitive "window" in salmonids occurs around the time of hatching and extends to beyond the time when these fish begin to feed exogenously (*23*); during this window male chinook salmon have been shown to be very susceptible to sex reversal (*24*). Early during this estrogen-sensitive period (at or shortly after hatching) male chinook salmon can be sex reversed by exposure to high concentrations of estrogen for periods as short as one hour (*25*). Later, after exogenous feeding has begun, sex reversal can be induced only by chronic exposure, typically accomplished by feeding food containing estrogens. By two months after hatching, the male gonads are completely differentiated into testes, and beyond this developmental point there is no evidence in salmonids that phenotypic sex can be altered further by exposure to estrogen.

It is possible that wild chinook salmon in the Hanford Reach were exposed to estrogens or compounds that mimic the biological activity of estrogens—the so-called environmental estrogens (*26*) that have caused sex reversal in some genotypic males. Environmental estrogens are chemicals in the form of detergents, plasticizers, and pesticides that derive from a wide range of human activities, such as industry, agriculture, and domestic sewage processing (*27–30*). Some of these compounds (e.g., atrazine, carbofuran,

Figure 1. Map of the northwestern United States showing the Columbia River watershed, dam placements, and the sites where chinook salmon were sampled in this study. Abbreviations: DH, Dworshak National Fish Hatchery; PRH, Priest Rapids Hatchery.

Table 1. The relationship between phenotype and presence/absence of a male-specific DNA marker in chinook salmon populations of wild or hatchery origin from the Columbia River watershed.

	Hatchery Clearwater River		Wild Columbia River		Hatchery Columbia River	
Phenotype	Female	Male	Female	Male	Female	Male
Present	0	50	42	50	0	50
Absent	50	0	8	0	50	0

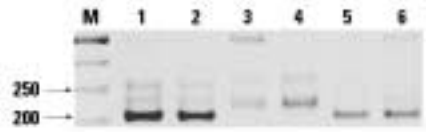

Figure 2. Male-specific DNA (209 base pairs) in phenotypic male (two individuals; lanes 1,2) chinook salmon from the Hanford Reach on the Columbia River, which was absent from some phenotypic females (two individuals; lanes 3,4) but present in other phenotypic females (two individuals; lanes 5,6). DNA products of higher molecular weight are nonspecifically amplified in both sexes and serve as positive controls for the PCRs. Molecular weight markers are indicated in lane M.

lindane, methyl parathion, and dieldrin), known to be estrogenic in rainbow trout (*O. mykiss*) bioassays (*31*), are present in the Columbia River (*32*). The compounds identified have been detected throughout the year in waters of the Hanford Reach (*32*) at annually stable but low levels (> 1–6 ng/L). Unfortunately, no information exists to show that the measured concentrations of these compounds can effectively cause sex reversal in any fish species. The Priest Rapids Hatchery fish were not exposed to such compounds, because their water source comes from wells during the estrogen-sensitive period, and they showed no incidence of sex reversal. Therefore, environmental estrogens remain valid candidates for causing the effects reported.

A number of female fish (16%) sampled from the Hanford Reach did not carry the male-specific DNA marker. Although these females may be wild they could originate from the Priest Rapids Hatchery, which did not show any evidence of the male-specific DNA marker. The Priest Rapids Hatchery is operated to supplement the wild chinook salmon population of the Hanford Reach and mitigate the effect of the Priest Rapids Dam (*11*). Juvenile chinook salmon raised at the Priest Rapids Hatchery are released into the Columbia River and return as adults to the Hanford Reach to spawn. Only ~3% of fish raised at Priest Rapids Hatchery are marked (i.e., adipose fin clip), distinguishing them from wild fish. Some adult salmon of Priest Rapids Hatchery origin home back to the hatchery outflow channel connected to the Columbia River, where they are captured in a trap to obtain gametes for artificial propagation. The remaining adults from Priest Rapids Hatchery spawn on the Hanford Reach. Therefore, we cannot exclude the possibility that some of the "wild" fish sampled from the Hanford Reach were of hatchery origin, and this may account for a proportion of the females sampled that did not test positive for the male-specific DNA marker.

In conclusion, there is a high proportion of phenotypic female chinook salmon from the Hanford Reach of the Columbia River that carry male-specific DNA within their genome. Although this could be explained by a unique chromosomal translocation event, the most likely possibility is that these fish are genetically male (i.e., XY) and have been sex reversed. This characteristic appears to be widespread in fish that develop in the wild on the Hanford Reach, but not found in closely related fish that were raised under hatchery conditions. The most significant implication of these observations is that sex-reversed male salmonids are known to be reproductively functional, producing eggs that carry either an X or a Y chromosome. Normally, chinook salmon females produce all X-chromosome–bearing eggs. Sex-reversed males would contribute to the population Y-chromosome–bearing eggs, which when fertilized with Y-chromosome–bearing sperm will generate an abnormal, genotypic YY individual. It has been shown experimentally that YY coho salmon (*O. kisutch*) and rainbow trout develop properly and are sexually viable (*33–35*). Therefore, we expect that all the offspring of a chinook salmon with a YY genotype would be male, reducing the number of genotypic females in the population with each successive generation. Indeed, this may be occurring in the present population, where 92% of the wild fish sampled carry a DNA marker found on the Y chromosome.

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