Asymmetric Hybridization and Introgression between Pink Salmon and Chinook Salmon in the Laurentian Great Lakes[1]

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Abstract.—Among Pacific salmon collected in the St. Marys River, five natural hybrids of pink salmon *Oncorhynchus gorbuscha* and chinook salmon *O. tshawytscha* and one suspected backcross have been detected using morphologic, meristic, and color evidence. One allozyme (LDH, L-lactate dehydrogenase from muscle) and one nuclear DNA locus (growth hormone) for which species-specific fixed differences exist were analyzed to detect additional hybrids and to determine if introgression had occurred. Restriction fragment length polymorphism of mitochondrial DNA (mtDNA) was used to identify the maternal parent of each hybrid. Evidence of introgression was found among the five previously identified hybrids. All hybrid specimens had chinook salmon mtDNA, indicating that hybridization between chinook salmon and pink salmon in the St. Marys River is asymmetric and perhaps unidirectional. Ecological, physiological, and sexual selection forces may contribute to this asymmetric hybridization. Introgression between these highly differentiated species has implications for management, systematics, and conservation of Pacific salmon.

Morphologic, meristic, and coloration data have been used to detect natural hybrids of pink salmon *Oncorhynchus gorbuscha* and chinook salmon *O. tshawytscha* (Rosenfield 1998). These data also suggest, but cannot confirm, that introgression has occurred between the Great Lakes populations of these fishes. Here, we use molecular genetic techniques to answer two questions that cannot be answered using morphological characteristics alone: Is the recent hybridization between chinook salmon and pink salmon symmetric or asymmetric? And, has genetic introgression occurred between these two species?

Rosenfield (1998) documented the morphologic, meristic, and coloration characters of five hybrids (specimens A, B, C, D, and E). Using meristic measures and size, he was able to confirm the probable hybrid origin of five additional salmon, including three originally classified as pink salmon by Kwain (1987). In addition one salmon from the St. Marys River (specimen X) was classified as a suspected backcrossed hybrid. However, classical systematic data such as meristics, morphometrics, and coloration are of limited use in detecting introgression or the direction of hybridization between pink salmon and chinook salmon.

If their recent hybridization in the Great Lakes leads to introgression, it could produce rapid change in one or both species. For example, Leary et al. (1987) and Smith (1992) presented evidence that introgressive hybridization has played a role in the evolution of genus *Oncorhynchus.* Whereas introgression is common among less derived members of *Oncorhynchus* (e.g., Campton 1987; Allendorf and Leary 1988; Dowling and Childs 1992), among the five, more derived, Pacific salmon species it appears to be uncommon, and some evidence of resistance to introgression exists among these species (e.g., May et al. 1975; Bartley et al. 1990). The fertility of hatchery-reared pink

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salmon × chinook salmon hybrid offspring (Foerster 1935; Chevassus 1979) demonstrates that introgression is possible between these two species.

Fisheries managers and researchers need to know the pattern of introgression, if it occurs, because gene flow between these two species could alter population dynamics of Great Lakes pink salmon and chinook salmon. Introgression could also lead to dramatic changes in the growth rate of these fish in the Great Lakes; hybridization between pink salmon and chinook salmon was first detected when a hybrid caught in Lake Huron was submitted as a record-size pink salmon (Rosenfield 1998).

The direction of hybridization between pink salmon and chinook salmon (i.e., which species provides maternal genetic material) may foreshadow the evolutionary consequences of introgression. For instance, unidirectional hybridization will allow transfer of mitochondrial DNA from the maternal species only. In addition, if the hybridization is asymmetric or unidirectional it may reveal the reproductive isolating mechanisms that operate in these species' native range and why they fail in the Great Lakes.

Molecular techniques are uniquely suited to provide information about introgression and the direction of hybridization (Avise 1994). We employed restriction fragment length polymorphism (RFLP) analyses to uncover fixed differences between pink salmon and chinook salmon nuclear and mitochondrial DNA (mtDNA). Species-specific fixed differences in nuclear DNA, mtDNA, and allozymes were then used to determine (1) whether the recently discovered hybridization between Great Lakes' pink salmon and chinook salmon is symmetric or asymmetric and (2) whether this hybridization has lead to genetic introgression.

Methods

Specimen collection.—During September and October 1992–1994, personnel at the Lake Superior State University Aquatic Research Laboratory (ARL) netted 71 pink and chinook salmon from the St. Marys River, a short (approximately 110 km), wide waterway that connects Lake Superior to Lake Huron. Among this sample was one individual (specimen X) that was later classified as a suspected hybrid based on color and meristic evidence (Rosenfield 1998). Although most of the specimens were collected from the river directly adjacent to the ARL facility, several individuals from both species were captured on the St. Marys

rapids, adjacent to the Sault Ste. Marie Locks, approximately 0.75 km upstream from ARL. The ARL also provided five hybrids (Rosenfield 1998) caught by anglers on the St. Marys River during the 1993 and 1994 spawning seasons. Specimens were frozen at approximately −32°C for 1–13 months before being transported to the University of Michigan and stored at −32°C.

Personnel at ARL performed an artificial cross between female chinook salmon and male pink salmon in the fall of 1993. Five progeny of this cross and five hatchery-reared chinook salmon were provided for molecular comparison to wild-caught specimens. The reciprocal hybridization failed during 1993 and 1994. Additional tissue samples were taken from a chinook salmon produced at the Wolf Lake Hatchery (Michigan Department of Natural Resources, Mattawan) near Lake Michigan and a pink salmon from the French River hatchery (Minnesota Department of Natural Resources, French River) on Lake Superior.

Allozyme electrophoresis.—Skeletal muscle and liver tissue were sampled from 80 salmon and frozen at −72°C until processing. Tissue samples were thawed and ground in a grinding buffer (0.1 M tris, 0.001 M EDTA, 10^{-4} M NAD, and 10^{-4} M NADP adjusted to pH 7.0 with HCl), centrifuged, and then stored at −32°C until used in electrophoresis. Due to repeated freezing and thawing and the delay (up to 30 months) between fish capture and allozyme analysis, tissue samples of nonhatchery fish were of marginal quality before grinding.

Utter et al. (1973) documented the diagnostic electrophoretic mobility patterns of phosphoglucomutase (PGM; Enzyme Commission, EC, number 5.4.2.2), superoxide dismutase (SOD; EC 1.15.1.1), L-lactate dehydrogenase (LDH; EC 1.1.1.27), creatine kinase (CK; EC 2.7.3.2), and two muscle proteins in pink and chinook salmon. Enzyme and locus nomenclature is based on that of Shaklee et al. (1990); EC numbers are those of IUBMBNC (1992).

We assayed for these proteins using the methods of Utter et al. (1973), Shaklee and Varnavskaya (1994), and Adams et al. (1994). Tissue samples from 54 St. Marys River salmon were used, including two hatchery-produced pink salmon × chinook salmon hybrids and two juvenile chinook salmon from ARL. We used 12% starch gels and several buffers described in Shaklee and Keenan (1986) and Aebersold et al. (1987). Buffers used were (1) a Ridgway buffer (Ridgway et al. 1970); (2) a TECB (tris–EDTA–citric acid–boric acid) buffer (pH 8.7); (3) a bis–tris buffer (*N,N*-bis(2-

hydroxyethyl)imino–tris; pH 7.0) and; (4) a tris–glycine buffer (pH 8.5). Gels were run from 5 to 7 h at 4°C to maximize band separation. Enzyme stain recipes were modified from Shaw and Prassad (1970) and Shaklee and Keenan (1986).

Nuclear DNA amplification, restriction, and electrophoresis.—The DNA was extracted from tissues of 27 salmon caught in the St. Marys River and identified previously using meristic, morphometric, and color data (Rosenfield 1998). Salmonids sampled included five natural hybrids (specimens A through E), one suspected backcross individual (specimen X), 10 pink salmon, and 11 chinook salmon. In addition, DNA was extracted from one Lake Superior pink salmon supplied by the French River Hatchery, one Lake Michigan chinook salmon produced at the Wolf Lake Hatchery, and one pink salmon × chinook salmon hybrid produced by ARL.

The DNA was extracted using Chelex-100 resin (catalog number 143-2832, Bio-Rad Laboratories, Richmond, California) as per Walsh et al. (1991). The procedures of Walsh et al. (1991) were modified in that proteinase-K digestions were not performed in nuclear DNA extractions. After DNA had been extracted from the tissue samples, growth hormone genes were amplified by polymerase chain reaction (PCR) using *Taq* polymerase. Twenty microliters (μL) of solution from Chelex extractions was added to 80 μL of a PCR buffer solution containing 2 units of *Taq* polymerase with final reaction concentrations as follows: (1) 20 mM tris–HCl; (2) 50 mM KCl; (3) 1.95 mM MgCl$_2$; (4) 0.2 mM dNTP (deoxynucleotide triphosphate); and (5) 0.25 mM of each primer. The PCR was conducted using a thermocycler with 1 cycle of 94°C for 5 min and 45 cycles of 94°C for 1 min, 52°C for 1 min, and 72°C for 2 min. The primer extension step was extended by 1 s/cycle and the reaction ended with a 7-min polymerization period.

We used primers that bind to regions flanking the first four introns of the two growth hormone genes found in Pacific salmon (*GH1A,* coding strand in exon 1: 5′-AGAAAACCAACGGCT-CTTCAA-3′, and *GH4B,* complementary strand of exon 4: 5′-GGGTACTCCCAGGATTCAATC-3′) (B. A. Shields, Oregon State University, by contract). Growth hormone copy "A" (*GHA*) is roughly 300 base pairs (bp) longer than growth hormone copy "B" (*GHB*). Amplifications were judged to be successful if they produced roughly equal amounts of both growth hormone DNA copies, as determined by fluorescent intensity after electro-

phoresis through 1% agarose gels and staining with ethidium bromide (EtBr).

Amplification products were digested with the restriction enzyme *Msp* I, which cuts the larger copy of the growth hormone gene (*GHA*) in chinook salmon, but does not cut *GHA* from pink salmon. Digests were performed at 37°C for at least 4 h using between 0.49 and 0.54 units of *Msp* I per microliter of total reaction volume. Digested fragments were separated by electrophoresis through gels made with 1% agarose and 1% Synergel (catalog number SYN-100, Diversified Biotech, Boston) and either TBE (tris–borate–EDTA) or TAE (tris acetate–EDTA) buffer. All gels were poststained with EtBr and visualized using ultraviolet light.

The heterozygous *Msp* I digestion pattern of hybrids could be interpreted as an incomplete digest of chinook salmon *GHA* (Figure 1), leading to misclassification. To avoid this potential error and ensure complete digestion of PCR products, we tried to combat the effects of declining restriction enzyme activity as the digestion progressed. Growth hormone DNA from specimens that produced a heterozygous *Msp* I restriction pattern was subjected to a final digestion using two equal aliquots (7 units each) of *Msp* I enzyme, one added at the beginning of the digestion period and the second added after 2.5 h of digestion. Digestions proceeded for a total of at least 4.5 h. Final enzyme concentration (not accounting for enzyme degradation) was 0.77 units per microliter of total reaction volume.

Mitochondrial DNA amplification, restriction, and electrophoresis.—Mitochondrial DNA was extracted from 18 specimens: 5 natural hybrids (specimens A through E); 1 suspected backcross (specimen X); 1 ARL-produced hybrid; 5 wild-caught chinook salmon; 1 ARL-produced chinook salmon; and 5 pink salmon. The ARL-produced hybrid and the ARL-produced chinook salmon had different chinook salmon mothers, so their inclusion in the sample increased to seven the number of independent chinook salmon mtDNA haplotypes analyzed. Chelex-100 extraction of mtDNA was performed using the procedures of Walsh et al. (1991).

Primers with sequences 5′-TTGGGTTTCTCG-TATGACCG-3′ and 5′-AGAGCGTCGGTCTTG-TAAACC-3′ (P. Evans, Brigham Young University, personal communication) were employed to copy and amplify the mtDNA control region (D-loop) using PCR. Twenty microliters of solution from Chelex extractions was added to 80 μL of a
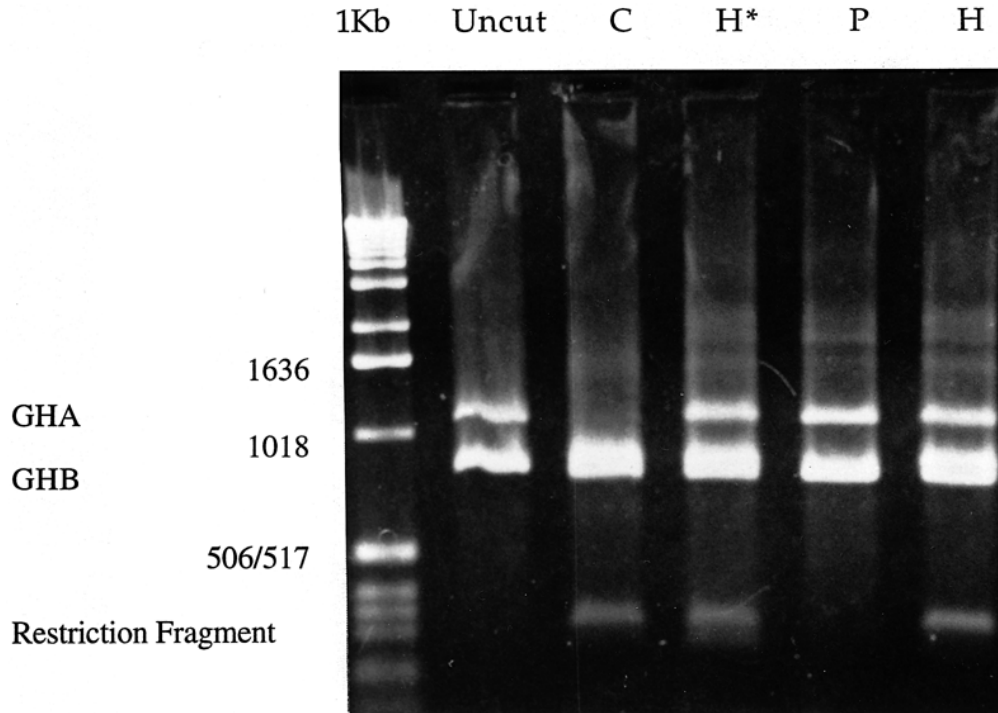
FIGURE 1.—Growth hormone DNA from chinook salmon (C), pink salmon (P), a hatchery-produced hybrid of pink and chinook salmon (H*), and a natural hybrid (H) after PCR amplification and treatment with *Msp* I restriction enzyme. Neither copy of growth hormone DNA (GHA, GHB) is cut among pink salmon. In chinook salmon, *GHA* is cut by the restriction enzyme, producing a small restriction fragment. After treatment with *Msp* I, hybrids retain an uncut copy of *GHA* (as with pink salmon) and yield a restriction fragment (as with chinook salmon). Uncut chinook salmon growth hormone DNA and a 1-kilobase (1Kb) size standard are presented for reference.

PCR stock solution with final reaction concentrations as follows: (1) 20 mM tris–HCl; (2) 50 mM KCl; (3) 1.8 mM MgCl$_2$; (4) 0.2 mM dNTP; and (5) 0.2 mM of each primer. Two units of *Taq* polymerase were added after this solution was heated to 94°C for 5 min. Samples were then exposed to the following sequence for 45 cycles: 94°C for 1 min, 52°C for 1 min, and 72°C for 2 min. The reaction ended with a 7-min polymerization period. Amplification products were electrophoresed on a TAE-based gel made of 1% agarose and 1% Synergel. When exposed to ultraviolet light, successful amplifications produced a single fluorescent band after staining with EtBr.

The PCR amplification products were digested using the five-base restriction enzyme *Bst*n I at 60°C for at least 14 h. Restriction fragments were separated electrophoretically on TAE or TBE-based gels containing 1% agarose and 1% Synergel. Restriction fragment sizes for nuclear DNA and mtDNA were estimated by comparison with the known size profiles of 1-kbp or 100-bp size standards run on each gel. Fragment size profiles were then compared with size profiles of chinook salmon (Cronin et al. 1993) and expected profiles based on the sequence data of Shedlock et al. (1992).

Results

Nuclear DNA

The growth hormone primers amplified both copies of growth hormone DNA. The *GHB* was roughly 800 bp long and *GHA* was approximately 1,100 bp long (Figure 1). Pink salmon *GHA* was not cut by *Msp* I among 10 previously identified pink salmon or one pink salmon from the French River hatchery. Chinook salmon *GHA* was completely cut by *Msp* I and produced a visible fragment approximately 300 bp long; the other cutting product appeared to comigrate with the uncut *GHB* band. This pattern was visible in each of the 11 chinook salmon analyzed; specimen X, the suspected backcross, also displayed this restriction
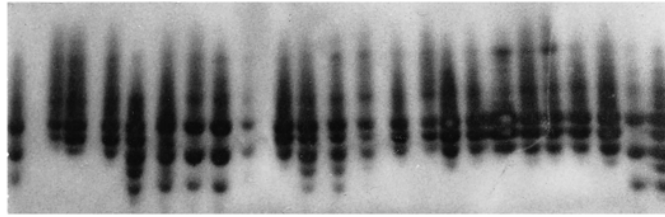
FIGURE 2.—L-Lactose dehydrogenase (LDH) from muscle tissue of chinook salmon (C), pink salmon (P), a hatchery-produced hybrid of pink and chinook salmon (H*), and four natural hybrids (H), previously identified by Rosenfield (1998) as noted below the starch gel patterns. Three LDH-A1 patterns are visible: A/A, B/B, and the heterozygote A/B. Each of these patterns was described previously by Utter et al. (1973). One previously identified hybrid, specimen E, displayed the LDH banding pattern of a chinook salmon (12th column from the right), indicating that it is a hybrid backcross. All previously identified pink salmon displayed the A/A banding pattern typical of that species, all chinook salmon displayed the B/B pattern, and three natural hybrids and a hatchery-produced hybrid displayed the heterozygous A/B pattern. An additional previously identified hybrid (not shown) also revealed the heterozygous LDH-A1 A/B pattern.

fragment profile. The hatchery-produced hybrid retained both the uncut *GHA* band, as in pink salmon, and the 300-bp restriction fragment found in chinook salmon. All five previously identified natural hybrids (specimens A through E), displayed a restriction profile identical to the hatchery-produced hybrid, indicating that each inherited a pink salmon *GHA* and a chinook salmon *GHA*. No other specimen had this heterozygous restriction pattern.

Allozymes

Only one allozyme, LDH from muscle tissue, consistently produced scorable banding patterns. Figure 2 shows the banding pattern of LDH from muscle tissue of 24 salmon from the St. Marys River after electrophoresis for 6 h on a Ridgway-buffered gel (Ridgway et al. 1970). Another Ridgway gel, run simultaneously and containing muscle tissue extract from 24 additional salmon revealed staining patterns analogous to those in Figure 2.

Three different LDH phenotypes were recorded from the starch gels (Figure 2). Sixteen fish displayed well-separated bands at *LDH-A1**–*A2** and were homozygous for a slow-migrating allele scored as LDH-A1 A/A. All fish exhibiting this pattern had been previously classified as pink salmon (Rosenfield 1998). Twenty-four fish had an *LDH-A1** phenotype that migrated more an-odally than the previous phenotype and were scored as homozygotes, LDH-A1 B/B. Twenty-two of the 24 specimens with this pattern were previously classified as chinook salmon (Rosenfield 1998). Specimen X also displayed this chinook salmon pattern, and the additional chinook salmon pattern was produced by hybrid specimen E. Six specimens had an *LDH-A1** phenotype with bands that spanned the entire zone of LDH-A1 A/A and LDH-A1 B/B. Scored as heterozygotes, LDH-A1 A/B, two of these specimens were known pink salmon × chinook salmon hybrids from ARL, and the other four specimens were previously identified hybrids (specimens A, B, C, and D). No specimens that had previously been classified as pink salmon or chinook salmon displayed this heterozygous phenotype.

Mitochondrial DNA

Mitochondrial DNA of chinook salmon revealed a *Bst*n I restriction profile with five bands, approximately 560, 380, 240, 190, and 160 bp in length (Figure 3). The close match between this restriction fragment pattern and that anticipated by the D-loop nucleotide sequence for chinook salmon reported by Shedlock et al. (1992) confirmed that the pattern observed was produced by chinook salmon mtDNA. Using D-loop primers within the region we amplified, Shedlock et al. (1992) reported a sequence with *Bst*n I restriction sites at
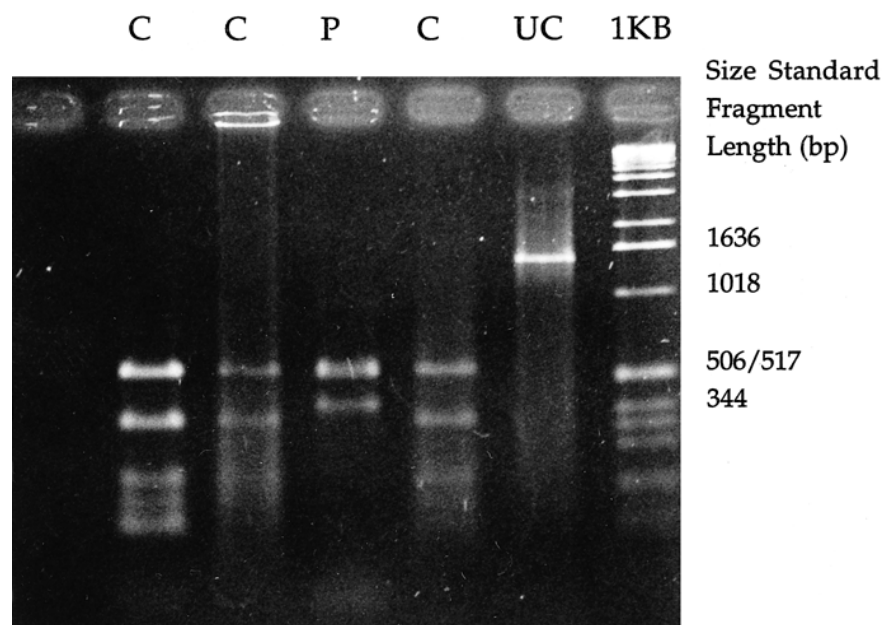
FIGURE 3.—Restriction profiles of mtDNA from three chinook salmon and a pink salmon cut with *Bst*n I restriction enzyme. When cut, the chinook salmon mtDNA (C) produced five visible fragments (two well-separated bands, three weakly separated bands) and the pink salmon mtDNA (P) produced fragments of only two distinguishable sizes. Also, the size difference between the two largest bands in the chinook salmon restriction profile was greater than the difference between the two bands of the pink salmon profile. The chinook salmon mtDNA restriction pattern was visible in each of the five wild hybrids identified previously (Rosenfield 1998). A 1-kilobase size standard (1KB) and uncut mtDNA from a chinook salmon (UC) are displayed for reference; fragment sizes are described in the text.

base pairs 449, 678, and 870 in chinook salmon. This sequence thus predicts that *Bst*n I digestion of chinook salmon D-loop will produce at least four fragments and that one fragment will be roughly 230 bp in length (resulting from cutting the site at Shedlock's bp 449 and the site at bp 678) and another will be approximately 190 bp in length (due to cutting at bp 678 and bp 870). Cronin et al. (1993) reported a qualitatively similar pattern (e.g., two well-separated bands and three smaller, weakly separated bands) for chinook salmon from Oregon, Canada's Yukon Territory, and Alaska. Their fragment length estimates were considerably greater than those presented here.

Restriction of St. Marys River pink salmon mtDNA with *Bst*n I produced fragments roughly 560 and 440 bp long (Figure 3). Again, the sequence described by Shedlock et al. (1992) confirmed that the observed pattern resulted from *Bst*n I digestion of pink salmon mtDNA. Restriction sites described by Shedlock et al. (1992) were expected to produce at least one 430-bp restriction fragment. The two known *Bst*n I restriction sites should have produced at least three restriction fragments; there was some indication from the intensity of the restriction profile that there were actually two fragments in the 560-bp band.

Mitochondrial DNA from each of the five previously identified natural hybrids and specimen X, the suspected backcross, revealed a chinook salmon mtDNA restriction fragment profile indicating that their female parents carried chinook salmon mtDNA.

Discussion

Introgression

Specimen E, the only male hybrid identified, is almost certainly the product of introgression between pink and chinook salmon. The hybrid origin of specimen E is evidenced by a mosaic of unambiguous pink salmon characters (e.g., 177 scales in the lateral series, 17 gill rakers on the lower limb of the first gill arch), unambiguous chinook salmon characters (e.g., 16 branchiostegal rays), and results of three principle component analyses (Rosenfield 1998). The heterozygous *Msp* I restriction fragment pattern for *GHA* confirms

specimen E's hybrid inheritance. In the context of these results, its homozygous chinook salmon banding pattern for *LDH-A1** strongly suggests that specimen E is the product of a hybrid breeding with a chinook salmon. This pattern could also be produced in an F_2 hybrid, but the low numbers of hybrid specimens detected before this specimen was caught (Fall 1994) suggest that a mating between two hybrids was improbable. The four female hybrids (specimens A through D) displayed heterozygous inheritance at both the nuclear DNA and allozyme locus. This finding corroborates their identification as hybrids between pink and chinook salmon, but it does not reveal whether the four individuals are F_1, F_2, or backcross hybrids.

The nuclear DNA, allozyme, and mtDNA loci analyzed here did not identify cryptic hybrids among specimens previously identified as either chinook or pink salmon by Rosenfield (1998) nor did they reveal interspecific parentage for specimen X. Thus, these individuals are not F_1 hybrids, although more distant hybrid inheritance (introgression) cannot be ruled out.

Direction of Cross

The D-loop restriction pattern found in each of the five natural hybrids was identical to that found in chinook salmon mtDNA (Figure 3). Specimen E's mtDNA may have been inherited from a chinook salmon female mating with a male hybrid or from a pink × chinook salmon hybrid female mating with a male chinook salmon. The lack of any hybrid specimens with pink salmon mtDNA suggests that successful, natural hybridization between the two species is asymmetrical and perhaps unidirectional, involving female chinook salmon. Additional hybrid specimens should be analyzed to test this hypothesis.

Mechanisms

Successful hybridization between pink salmon and chinook salmon appears to be occurring regularly, though perhaps at a low rate, in the St. Marys River and nearby tributaries. The pink × chinook salmon hybrids discussed here were caught on or near the St. Marys River spawning grounds in two different years (1993 and 1994). In addition, Rosenfield (1998) presented size and meristic evidence that two specimens caught in the St. Marys River in 1992 and three fish caught in a tributary to Lake Huron's North Channel (near the St. Marys outflow) in 1985 by Kwain (1987) were also hybrids. In every year since 1994, ARL staff members have detected hybrids using char-

acters described by Rosenfield (1998). For example, during the 1998 spawning season, an ARL netting effort targeted at catching pink salmon produced 20 hybrids among 627 pink salmon caught (R. Greil, unpublished data). Also, more hybrids are brought to ARL each year by anglers. From this evidence, it is not clear whether frequency of hybridization, search effort for hybrids, or both are responsible for the increase in the number of hybrids detected.

Unlike previous cases of hybridization between Pacific salmon (e.g., Bartley et al. 1990), there is almost no chance that hybrids between pink and chinook salmon have been accidentally produced in a hatchery. To our knowledge, no hatchery has produced pink salmon for release into the Great Lakes since their accidental introduction in 1956. It would be very difficult for even an untrained hatchery worker to confuse sexually mature pink salmon and chinook salmon.

Hybridization between pink salmon and chinook salmon is probably largely driven by differences between the physical conditions found in the Great Lakes drainage basin and those of the Pacific Coast watersheds these species normally inhabit (Rosenfield 1998). Salmon spawning migrations in the St. Marys River end at the Sault Ste. Marie locks, approximately 110 km from the river's mouth. At the foot of this barrier are rapids that constitute the only suitable mass-spawning grounds on the St. Marys River (other spawning grounds exist on its tributaries). This inability to migrate far upstream, combined with the limited salmonid spawning grounds in the main stem of the St. Marys River, probably forces spawning chinook and pink salmon into close proximity—a situation that rarely occurs in their native Pacific Coast habitats.

Their placement in a novel evolutionary environment may explain why pink salmon and chinook salmon hybridize in the Great Lakes, but the frequency and directionality of that hybridization require additional study and explanation. Physiological mechanisms that prevent or limit production of hybrids from crosses between chinook salmon males and pink salmon females seem unlikely. Smirnov (1972) reported excellent hatching of pink salmon eggs fertilized by chinook salmon sperm, and Chevassus (1979) reported that both crosses produced viable male offspring. However, success in hatchery breeding experiments does not guarantee hybrid viability in the wild (e.g., Hatfield and Schluter 1999).

Other ecological factors may play a role in the asymmetric hybridization between pink salmon

and chinook salmon. For example, Hubbs (1955, 1961) pointed to great disparities in abundance of spawning individuals on the breeding grounds as a force that could lead to hybridization and influence its direction. In the St. Marys River, chinook salmon spawn from June to November with peak spawning occurring from late September to early October. Pink salmon may spawn from August through early October with the peak spawning period in mid-September (Greil, unpublished data). Thus, chinook salmon are spawning during the entire time that pink salmon spawn in the St. Mary's River. If disparities in spawning population size (between species) or sex ratio (within species) play a role in hybridization, the period during which hybridization occurs is probably limited to the tails of the pink salmon spawning season (August or October).

Behavioral mechanisms may also limit production of chinook salmon × pink salmon hybrids in the wild while encouraging the reciprocal cross. Sexual selection is strongly size-dependent among salmon (Gross 1985; van den Berghe and Gross 1989). Male pink salmon may be attracted to female chinook salmon because the latter are so much larger than pink salmon females. Pink salmon males might gain access to mature chinook salmon females by employing a sneaker tactic. The sneaking reproductive tactic is well documented in *Oncorhynchus* (e.g., Gross 1985) and particularly in pink salmon (Keenleyside and Dupuis 1988; Noltie 1990). Size-dependent sexual selection has been proposed as a mechanism for unidirectional hybridization between other fish species (Konkle and Philipp 1992; McGowan and Davidson 1992). If hybridization between pink and chinook salmon is driven by male selection for large females, F_1 hybrids with pink salmon mothers should be rare or nonexistent.

Implications

Hybridization and introgression between these two species presents questions and problems for those who study and manage Pacific salmon. The implications discussed here depend, in part, on the frequency of hybridization and the fitness of hybrid salmon. Hybrids are often expected to be less fit than their parent species; but, this is not always, or even usually, the case (Arnold and Hodges 1995; Arnold 1997).

If survival and fecundity of hybrids are less than those in the parent populations, hybridization would represent a drain on natural population growth of pink salmon and chinook salmon in the Great Lakes. State fishery management agencies and anglers will no doubt be interested in the growth rate of the hybrids and backcrosses because one hybrid has already confused size-record awards (Rosenfield 1998). If the hybrid population grows large enough, hybrid salmon could have significant and unforeseen effects on the Great Lakes ecosystem.

In addition to fast growth (Greil, unpublished data), hybrid salmon may experience higher fitness than members of their parent species if their heterozygous genomes provide added physiological, developmental, or behavioral options (Arnold 1997). Some hybrid organisms successfully colonize habitats beyond the range of their parental species (Arnold 1997; Echelle et al. 1997). If hybrid salmon can accomplish such a range expansion, they may invade neighboring ecosystems or closely related gene pools. The ecological consequences of such a range expansion are unforeseeable.

Natural introgressive hybridization between pink salmon and chinook salmon may create problems for those concerned with salmonid speciation and systematics. It is clearly unacceptable to recognize pink salmon and chinook salmon as two species where they remain distinct (the Pacific Coast) while recognizing only one species where introgression occurs (the Great Lakes). However, under most species criteria in use today, populations that exchange genetic information in the wild must be classified as members of the same species (Arnold 1997). Whether F_2 hybrids or backcross salmon are selected for or against is undetermined, but this is not necessarily important with regard to species delineation since permanent transfer of genetic information is possible even when hybrids and backcrosses are selected against (Arnold 1997). These issues extend beyond this species pair. Introgression within the genus *Oncorhynchus* is well documented, particularly among the Pacific trout (e.g., Loudenslager et al. 1986; Leary et al. 1987; Dowling and Childs 1992). Even though we know of no other modern cases of introgression between Pacific salmon species, Smith (1992) presented evidence for historical introgression between pink salmon and chum salmon *O. keta,* and natural hybridization between Pacific salmon species has occurred in modern times (Hunter 1949; Bartley et al. 1990). Members of this genus are highly differentiated (Stearly and Smith 1993) and very old (Smith 1992; McKay et al. 1996); yet, reproductive barriers between the species appear to be incomplete.

The frequency of hybridization and introgression within this genus presents the opportunity to reconsider dominant species definitions. Intrinsic reproductive barriers seem insufficient to delineate species within *Oncorhynchus.* Equally inadequate are species definitions that rely purely on diagnostic characters, as these characters can be exchanged during introgression. Instead, species delineation within this taxon should focus on lineage irreplaceability (Smith et al. 1995; Templeton 1998) and incorporate a wide variety of factors that create and maintain species integration, continuity, and distinction (Van Valen 1976; Smith et al. 1995).

At this time, the possibility of frequent introgression or hybrid escape to the other Great Lakes seems remote because the number of hybrids found each year remains small. However, Arnold (1997) has observed that production of F_1 hybrids is often the major barrier to extensive introgression. Also, as the spread of pink salmon through the Great Lakes demonstrates, establishment and expansion of populations in a novel environment are not predictable. Future studies of this phenomena should attempt to uncover mechanisms that support or encourage hybridization, document the survival and reproductive success of hybrids and backcrosses, and explore differences (if any) between the ecology and behavior of hybrids and the parental species.

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