SINE sequences detect DNA fingerprints in salmonid fishes

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DNA probes homologous to two previously described salmonid short interspersed nuclear elements (SINEs) detected DNA fingerprint patterns in 14 species of salmonid fishes. The probes showed more homology to some species than to others and little homology to three nonsalmonid fishes. The DNA fingerprint patterns derived from the SINE probes are individual-specific and inherited in a Mendelian manner. Probes derived from different regions of the same SINE detect only partially overlapping banding patterns, reflecting a more complex SINE structure than has been previously reported. Like the human Alu sequence, the SINEs found in salmonids could provide useful genetic markers and primer sites for PCR-based techniques. These elements may be more desirable for some applications than traditional DNA fingerprinting probes that detect tandemly repeated arrays.

Keywords: DNA fingerprints, salmonids, SINEs.

Introduction

Since the advent of DNA fingerprinting (Jeffreys *et al.*, 1985) numerous probes have been identified that detect individual-specific banding patterns based on the variability in the number of tandem repeats (VNTR) of some DNA sequences (e.g., Shin *et al.*, 1985; Vassart *et al.*, 1987; Schafer *et al.*, 1988). Frequently, probes homologous to these tandemly repeated sequences not only detect fingerprint patterns in the organism from which they were isolated but also in distantly related taxa. VNTR probes are useful as markers at the individual and population level (Wetton *et al.*, 1987; Gilbert *et al.*, 1990; Burke *et al.*, 1991; Wirgin *et al.*, 1991; Spruell *et al.*, 1994). However, the presence of a given tandemly repeated element does not reflect the phylogenetic relationships of those species in which it is found.

In addition to tandemly repeated elements, most eukaryotic genomes contain interspersed repetitive elements. Short interspersed nuclear elements (SINEs) comprise one class of such elements. SINEs are typically less than 500 base pairs in length, are found in high copy number, and contain a RNA polymerase III recognition site. SINEs are thought to originate by reverse transcription and are

frequently homologous to tRNAs (for reviews see Deininger & Daniels, 1986; Deininger, 1989; Okada, 1991). The human Alu I repeat is the best-studied example of such a sequence and may provide a model for other systems (Kariya *et al.*, 1987).

Kido *et al.* (1991) identified three SINEs in the fish family Salmonidae and used the polymerase chain reaction to investigate the presence or absence of these elements in 14 species of salmonids. These elements were termed Hpa I, which they reported to be found in all salmonids; Fok I, which was reported to be specific to the genus *Salvelinus*; and Sma I, reported in two species in the genus *Oncorhynchus*. The presence of specific elements has also been used to infer the phylogenetic relationships among salmonids (Murata 1993). We investigated the use of these elements as DNA probes because their reported phylogenetic distribution implied that certain interspecific and intergeneric hybrids and mosaics could be characterized by the presence of a given SINE.

Two results might be predicted when hybridizing a SINE-homologous DNA probe to size-fractionated total genomic DNA. A smear might be detected, reflecting high copy number and a ubiquitous distribution of SINE elements throughout the genome. Alternatively, if discrete bands were detected, limited variation might be expected as SINEs are thought to be stable once integrated into

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the genome (Sawada *et al.*, 1985; Deininger 1989). Thus, the patterns detected should be consistent within a population except for occasional point mutations in restriction enzyme recognition sites.

In salmonids, SINE-homologous probes detected complex, individual-specific banding patterns. We quantified the band pattern detected within a population of steelhead trout (*Oncorhynchus mykiss*) with one of these probes and determined the distribution of various regions of these sequences among salmonids by Southern blotting. While our data are not in disagreement with the findings of Kido *et al.* (1991), they do suggest a more complex system than they reported.

Materials and methods

Samples of either blood, fin or sperm were obtained from the following fishes: steelhead trout (*Oncorhynchus mykiss*), cutthroat trout (*O. clarki*), coho salmon (*O. kisutch*), chinook salmon (*O. tshawytscha*), sockeye salmon (*O. nerka*), pink salmon *(O. gorbuscha)*, Atlantic salmon (*Salmo salar*), brown trout (*S. trutta*), brook trout (*Salvelinus fontinalis*), lake trout (*S. namaycush*), Arctic char (*S. alpinus*), bull trout (*S. confluentus*), dolly varden (*S. malma*), Arctic grayling (*Thymallus arcticus*), mountain whitefish (*Prosopium williamsoni*), northern pike (*Esox lucius*), bridgelip sucker (*Catostomus columbianus*) and striped bass (*Morone saxatilis*). Total genomic DNA was extracted following proteinase K/SDS, digestion modified from Sambrook *et al.* (1989). Tissues (150 μL blood 1:1 in Alsever's solution, 5 μL of undiluted semen or 0.25–0.50 cm^2 of fin clip) were placed in 1.5 mL microfuge tubes and digested in 500 μL of digestion solution (50.0 mM Tris, pH 8; 200 mM NaCl; 50.0 mM EDTA, pH 8; 1.0 per cent SDS; 0.2 per cent DTT; 1 mg mL^{-1} proteinase K) overnight at 60°C with gentle agitation. Undigested material was pelleted by centrifugation for 5 min at maximum speed in a microcentrifuge and the supernant was transferred to a new tube. Two chloroform:isoamyl alcohol (24:1) extractions of two volumes each were then performed to further denature and precipitate proteins. One-fourth volume of 8 M ammonium acetate was then added to each sample and the samples were cooled on ice for 10 min. The samples were centrifuged at maximum speed for 10 min to pellet precipitated proteins and the supernant was transferred to a new tube. DNA was then precipitated with two volumes of 95 per cent ethanol and the samples were cooled on ice for at least 15 min. The DNA was then pelleted by a 20 min centrifugation at maximum speed and washed

with 500 μL of 70 per cent ethanol. After a final centrifugation and removal of the 70 per cent ethanol, the samples were allowed to dry at room temperature and were then resuspended in TE, pH 7.5. We have found that our DNA samples have OD$_{260/280}$ ratios of approximately 1.8 and are digestible with all enzymes we have attempted, even when the standard phenol extractions are omitted.

DNA samples were quantified using a Hoefer fluorometer and 5 μg of each sample were digested to completion with either *Taq*I or *Dpn*II (New England Biolabs) following the manufacturer's directions. The recognition sites for these enzymes are not found in the consensus sequence of either the Hpa I or Fok I SINEs reported by Kido *et al.* (1991) and thus they should cut outside the SINEs, leaving intact elements in the digested DNA. Two μg of each sample were then loaded into either 0.8 or 1.0 per cent agarose gels and electrophoresed in 1 × TAE buffer at 50 volts for 24 h at 4°C with buffer recirculation. DNA was denatured in 1.5 M NaCl; 0.5 N NaOH for at least 30 min and neutralized in 1 M Tris (pH 7.4); 1.5 M NaCl for at least 25 min. DNA was transferred to a nylon membrane (Magnagraph, MSI) by capillary transfer in 10 × SSC using 10 sheets of Quick Draw blotting paper (Sigma). Transfer was allowed to continue until the uppermost blotting pad was saturated.

Oligonucleotides homologous to salmonid SINEs (Table 1) were selected based on the consensus sequences reported by Kido *et al.* (1991). All oligonucleotides were purchased HPLC-purified from Appligene and were directly linked to alkaline phosphatase using either the Lightsmith I TM or Lightsmith II TM kit (Promega).

Prior to hybridization, membranes were prehybridized for 30 min at the temperature of hybridization in 10 mL of 5 × SSC, 2 per cent SDS, 1 per cent casein. Hybridization followed for 30 min in 5 mL of 5 × SSC, 2 per cent SDS, 10 per cent polyethylene glycol plus the appropriate probe at 250 fmol mL^{-1} as recommended in the Lightsmith manual. Two washes of SSC, 1 per cent SDS were followed by two washes of SSC, 1 per cent Triton X-100. Each 250 mL wash was conducted at the concentration and temperature given in Table 1. Membranes were then washed twice in 250 mL of room temperature 1 × SSC. All washes were 5 min in duration.

DNA fingerprint patterns were visualized by equilibrating the membrane in 100 mM Tris-HCl, 100 mM NaCl, 50 mM MgCl$_2$, pH 9.5 for 5 min at room temperature followed by the application of 4 μL of AMPPD (Tropix) diluted in 1.5 mL of the above Tris-MgCl$_2$ buffer. We then exposed the membrane

Table 1 Oligonucleotides and wash conditions used in analysis of distribution and homology of salmonid SINEs. Distribution among genera is also given for each probe

Probe and sequence	Washes	Distribution among genera		
		Oncorhynchus	*Salmo*	*Salvelinus*
Hpa I 5′ GGCAGGGTAGCCTAGTGGTT	0.5 × /59°C	B + + +	B + + +	B +
Hpa I 3′ ACAGGCAGTTAACCCACTGTTCC	1.0 × /56°C	B + + +	B + + +	B +
Fok I 5′ CGTGTGGCTCAGTTGGTAG	1.0 × /48°C	B + +	B + +	B + +
Fok I 3′ ACAAATTGGAAAGTTCATAC	1.0 × /42°C	0	0	S +

Sequences are from Kido *et al.* (1991). Washes represent the concentration of SSC followed by the temperature of washes. Hybridization to taxa is represented by the following: B, banding pattern; S, smear; 0, no hybridization. Pluses indicate the degree to which each probe hybridized to that genus.

to Fuji X-ray film overnight for the oligonucleotide probes or for 4 h for the digoxygenin-labelled probe.

We initially hybridized membranes with two common VNTR-homologous oligonucleotides, one based on Jeffreys 33.6 core fragment (Edman *et al.*, 1988) and the other homologous to bacteriophage M13 (Vassart *et al.*, 1987), which detect DNA finger-prints in salmonids (Spruell *et al.*, 1994). Although fewer bands were detected in the other species than are observed in salmonids, some sharp bands were observed in each species. All DNA samples were thus confirmed to be of adequate quality for DNA fingerprint analysis. Membranes were stripped in two 250 mL washes of 94°C 1 × SSC, 1 per cent SDS and then rinsed in 1 × SSC prior to subsequent hybridizations.

The distribution of Hpa as detected by the Hpa I 5′ probe was consistent with that reported by Kido *et al.* (1991). However, initial results with the oligo-nucleotide homologous to the 5′ end of Fok I were not consistent with the distribution reported by Kido *et al.* (1991). This led us to hybridize membranes with probes derived from the 3′ end of Fok I and subsequently Hpa I to determine which regions of the SINEs were responsible for the distributions reported by Kido *et al.* (1991). In addition to the oligonucleotide probes, a 172 base pair segment of Hpa I was amplified and used as a probe. To accom-plish this, the Hpa I 5′ and 3′ homologous oligonu-cleotides (Table 1) were used as primers to amplify 172 base pairs of the Hpa I element from steelhead trout and label this element with digoxygenin (Lanzillo, 1990). Reactions of 10 µL final volume were performed in an Idaho Technology 1605 AIR Thermocycler with the following profile: an initial

denaturation step at 94°C of 3 min; 5 cycles of 5 s denaturation at 94°C, 5 s annealing at 45°C, 30 s extension at 72°C; 30 cycles of 1 s denaturation at 94°C, 1 s annealing at 45°C, 30 s extension at 72°C; followed by a 10 min extension at 74°C. A sample of each reaction was then electrophoresed and visual-ized with ethidium bromide to ensure that a single product was amplified. The resulting probe was used in hybridizations without further purification.

Mendelian inheritance of the SINE-positive frag-ments was confirmed by examining the patterns detected in two generations of steelhead trout. Banding patterns in five offspring were compared with both parents to determine if any bands were present in the offspring that were not found in one of the two parents.

The degree of similarity of individual banding patterns within a population was measured in 15 steelhead trout from a large hatchery population. Fish were randomly selected from the Dworshak National Fish Hatchery and fingerprinted using the 172 base pair Hpa I probe. Band sharing values were calculated as previously described (Spruell *et al.*, 1994) and compared to values obtained using mini- and microsatellite probes on this same population.

To examine the generality of SINE-generated DNA fingerprints, an oligonucleotide homologous to a highly variable region of the human Alu sequence (CAGGTGATCCACCCACCTCG) was synthesized using the consensus sequence reported by Kariya *et al.* (1987). We hoped that by choosing a highly vari-able region of the Alu I sequence for our probe, only a subset of the Alu sequences present would have sufficient homology to the probe to permit

hybridization. In this manner, we might estimate the degree to which sequence variation was involved in the patterns detected by the salmonid SINE probes. The Alu I-homologous probe was hybridized to restriction enzyme digested human DNA from four individuals at increasing stringency until no signal was detected.

Results

We detected DNA fingerprints in salmonid fishes using probes homologous to regions of salmonid SINEs. The species to which each probe hybridized and the hybridization intensities are summarized in Table 1. Although fingerprints were detected in all salmonids using oligonucleotides homologous to Hpa I, the probe bound strongly to species in the genera *Oncorhynchus* and *Salmo* but weakly to

species in the genus *Salvelinus* and more distantly related salmonids (Fig. 1). The sequence on which these probes were based was obtained from the cherry salmon (*Oncorhynchus masou*) (Kido *et al.*, 1991), thus this observation may reflect divergence in the sequence of the SINE among genera. There also might be fewer copies of the element in *Salvelinus* than in the other genera. There are currently no proposed mechanisms by which SINEs are excised from a lineage once amplified. Thus, if the amplification of Hpa I occurred prior to the divergence of *Salvelinus*, *Salmo* and *Oncorhynchus*, as suggested by Kido *et al.* (1991), it appears that significant divergence has taken place between the *Salvelinus* Hpa I and the Hpa I found in *Oncorhynchus* and *Salmo*.

Probes homologous to both the 5' and 3' regions of the Hpa I sequence and the entire 172 base pair sequence were tested. We expected all of these
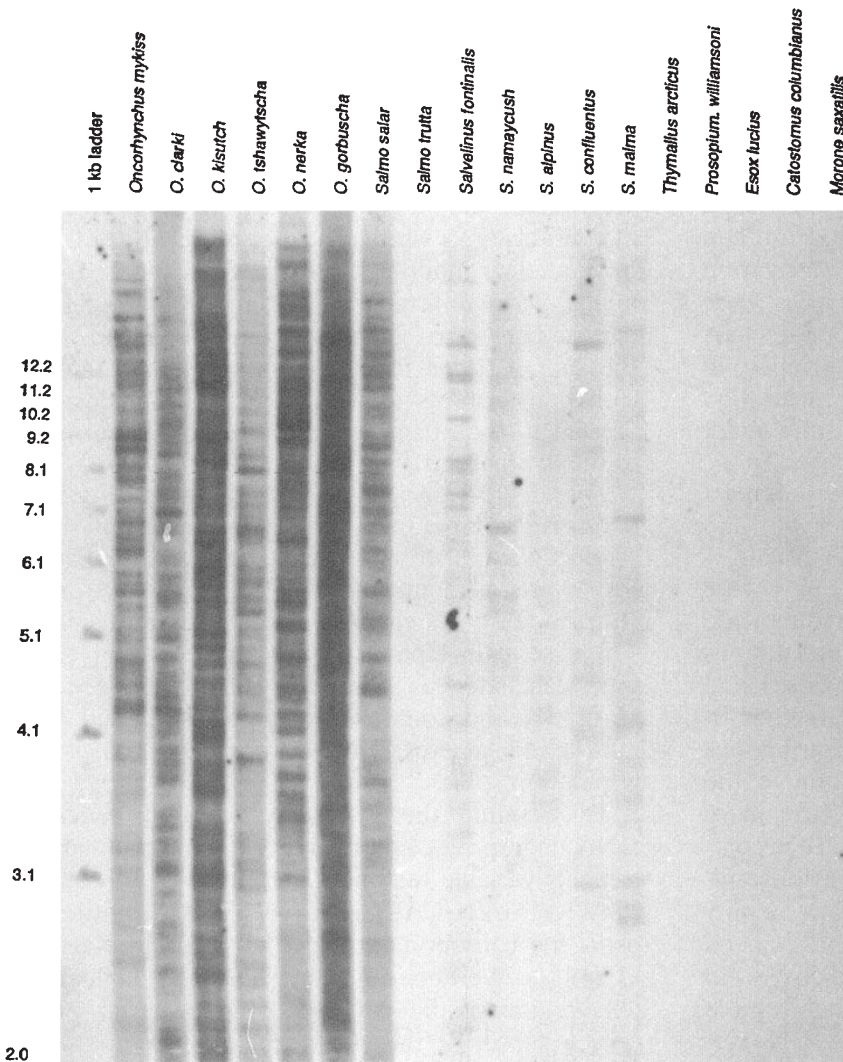
Fig. 1 DNA fingerprints detected in 18 species of fishes by an oligonucleotide probe homologous to the 5' end of the salmonid SINE Hpa I. Genomic DNA was digested with *Taq*I and size fractionated on a 1 per cent agarose gel.
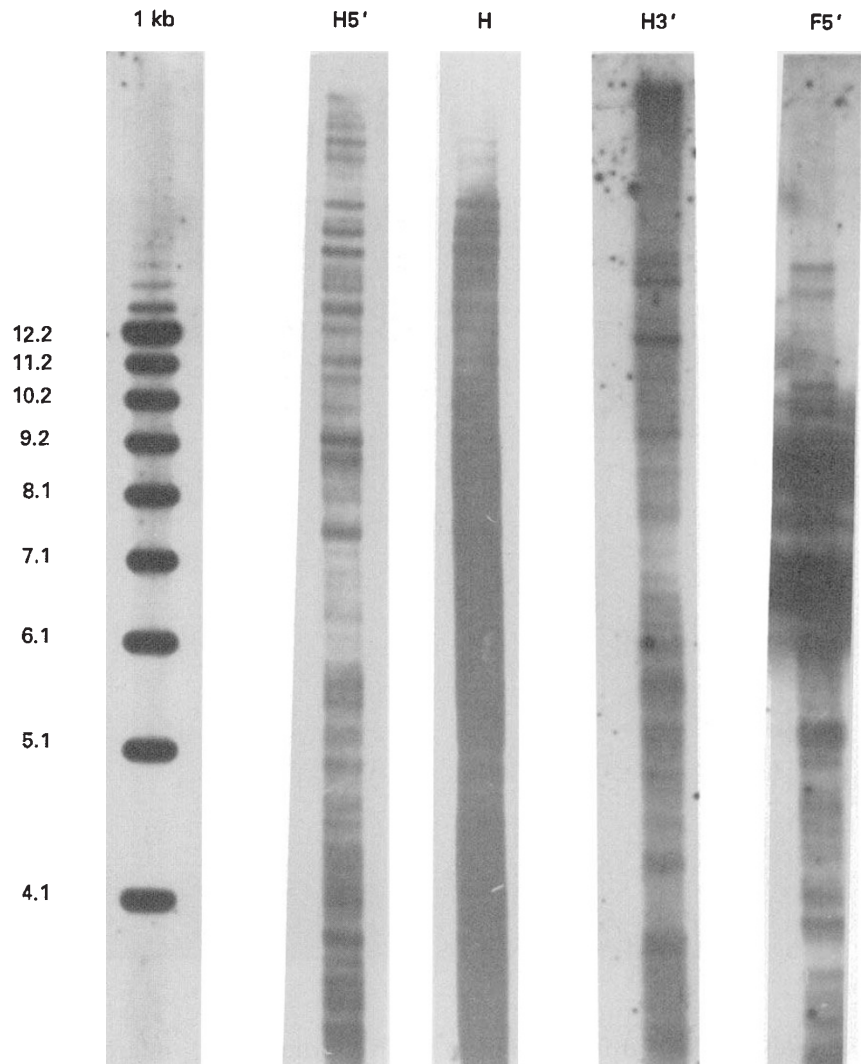
Fig. 2 Comparison of DNA finger-prints detected by oligonucleotide probes homologous to Hpa I 5', Hpa I 3', Fok I 5' and a 172 base pair segment of the Hpa I element. Genomic DNA from steelhead trout was digested with *Taq*I and size fractionated on a 0.8 per cent agarose gel. The same lane of DNA was stripped, sequentially reprobed, and has been aligned for comparison.

probes to reveal the same pattern unless the SINE was cleaved internally by the restriction enzyme. However, even for restriction enzymes which should not cut inside the SINE according to the sequence reported by Kido *et al.* (1991), approximately 50 per cent of the bands detected by the Hpa I 5' and Hpa I 3' homologous probes are shared (Fig. 2). The patterns detected by the 5'-homologous oligonucleotide and the entire element, on the other hand, are nearly identical despite the increased intensity observed with the 172 bp fragment.

A probe homologous to the 5' end of the putative *Salvelinus*-specific Fok I SINE also detects finger-prints in all salmonids and isolated bands in the other species (Fig. 3). A probe homologous to the 3' end of Fok I, however, detects a smear in *Salvelinus* and does not hybridize to other fishes even at low stringency (data not shown). The bands detected by the 5' Fok I probe do not correspond to any of the fragments detected by the Hpa I-homologous probes (Fig. 2) and therefore our observations are not simply the result of cross-hybridization between SINE elements.

Five F_1 steelhead trout and their parents were examined to determine the inheritance patterns of these bands. No bands that could not be attributed to one or both parents were observed in any of the five offspring.

A band sharing index (Wetton *et al.*, 1987) of 0.395 (± 0.098) was calculated for all pairwise comparisons for 15 individual steelhead trout from the Dworshak National Fish Hatchery. This same population has been evaluated using three minis-atellite probes. Band sharing indices for the three
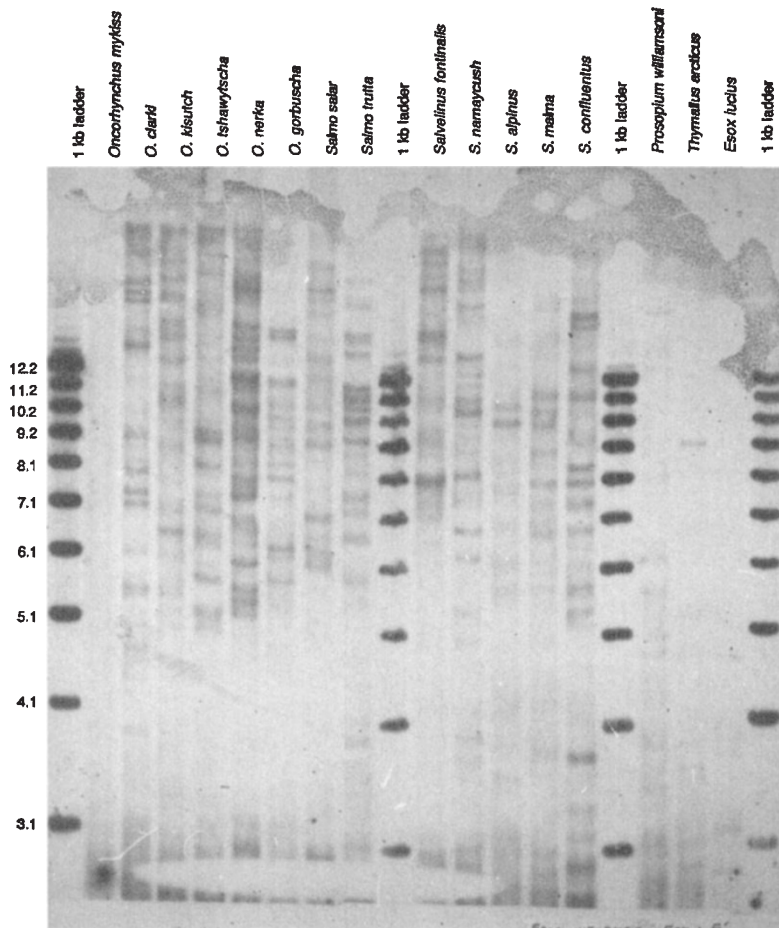
Fig. 3 DNA fingerprints detected in 16 species of fishes by an oligonucleotide probe homologous to the 5' end of the salmonid SINE Fok I. Genomic DNA was digested with *Dpn*II and size fractionated on a 0.8 per cent agarose gel.

minisatellites ranged from 0.387 to 0.556 (Spruell *et al.*, 1994).

We synthesized an oligonucleotide probe homologous to a highly variable region of the human Alu sequence in the hope of detecting DNA fingerprints in humans. Hybridization of this Alu I-homologous oligonucleotide to digested human DNA results in a smear even at the highest stringency at which signal is still detected

Discussion

Probes homologous to salmonid SINE sequences detected DNA fingerprints in members of the family Salmonidae but did not hybridize to the nonsalmonid fishes we examined. It thus seems unlikely that we are detecting a repetitive element other than the SINEs we have targeted. However, if 10 000 copies of each of these elements are randomly distributed throughout the salmonid genome (Kido *et al.*, 1991), the detection of only 35–40 bands per individual is surprising. This observation has several possible

interesting explanations which are currently difficult to evaluate.

The presence of fewer SINEs than has been reported could produce the observed patterns. Kido *et al.* (1991) estimated that 10 000 copies of Hpa I were found in the cherry salmon genome. We did not attempt to fingerprint this species, therefore our results cannot be directly compared to theirs.

It is possible that we may be detecting only a subset of all SINEs under the stringency conditions we are using. Kido *et al.* (1991) sequenced five Hpa I elements from *O. masou* and reported 96.9 per cent homology among these elements. If a perfect match with a 20 base oligonucleotide is demanded for hybridization and if the target elements have 96.9 per cent homology, then 0.969^{20} or approximately 50 per cent of the 10 000 SINEs thought to be present should be detected. Given the resolving power of Southern analysis, a smear, rather than discrete bands would be expected in such circumstances. Thus, this explanation seems unlikely.

Substructuring of the salmonid SINEs could also

lead to the detection of only a fraction of the total SINEs found in the genome. Other taxa contain SINEs that are subdivided into related families (Daniels & Deininger, 1985, 1991). Consistent deviations from the consensus sequence by a subset of the SINEs would result in only a fraction of SINEs being potential targets for our probe. We attempted to evaluate this explanation using the human Alu I sequence. However, either that system is not sufficiently structured to reveal bands, or more likely, too many copies of the element are present to resolve distinct bands.

We may only be detecting DNA fragments that contain several SINE elements. Nonrandom distribution of SINE elements has been reported and could produce DNA fragments that contain multiple SINEs. Korenberg & Rykowski (1988) report the correlation between the distribution of Alu and metaphase chromosome bands. A tendency for SINEs to integrate preferentially near other repetitive elements has also been reported (for review see Rogers, 1985). Other SINE elements occur in tandemly repeated arrays (Nagahashi et al., 1991). All of these observations would lead to clustering of SINEs within the genome and a reduction in the number of bands detected in digested genomic DNA.

In addition to the surprising observation of discrete bands detected with the SINE probes, the source of the hypervariability in the detected patterns also is of interest. As previously discussed, it is possible that some of the SINEs exist as tandem arrays within the genome. Varying the number of repeats at a locus or variation in which SINE loci are tandemly repeated could be responsible for the observed hypervariable patterns. SINEs are mobile elements and episodic amplification events could result in individual-specific patterns. However, these events must not occur every generation or we would not expect the fragments to be inherited in a Mendelian manner. In addition to our confirmation of Mendelian inheritance, Young et al. (1996) examined 75 offspring of two homozygous rainbow trout using the HpaI 5′ probe and found no novel bands in any of these individuals. Other possible sources of hypervariability could include high rates of mutation in restriction enzyme recognition sites near SINEs or adjacent VNTR sequences that are not homologous to the SINEs themselves. Considerable study would be required to determine the relative contributions of these sources to the observed variability.

Probes homologous to different regions of both the Hpa I and Fok I SINEs detected different patterns. A high degree of polymorphism in one region of the SINE could account for bands detected by one probe being absent when the alternative probe is used. However, we would not expect novel bands that are not detected by a longer probe sequence to be detected by either oligonucleotide. It is possible that the complete SINE is not necessary for amplification. Sequences that represent amplified parts of both the human Alu (Quentin, 1992) and the galago SINE have been reported (Daniels & Deininger, 1991). Alternatively, undescribed SINEs might contain elements of the previously reported SINEs coupled to other DNA sequences allowing amplification. This could increase the fluidity that SINEs might confer to the genome; SINEs could alter the genome not only by transposition and homologous recombination between identical SINEs, but related SINEs containing common components could also be sites for recombination.

Although we cannot currently explain all of our observations derived using salmonid SINE-homologous probes, we can draw parallels from the human Alu SINE literature and suggest uses for these sequences beyond those normally associated with VNTR-homologous probes. The human Alu sequence has been used in various PCR applications in which the SINE sequence serves as either one or both PCR primer sites (Ledbetter et al., 1990); salmonid SINEs could be used in an analogous manner. The phlyogenetic distributions of SINE sequences allow their use to identify chromosomes of one species in the genomic background of another genus (Walter & Goodfellow 1993, Peek, 1994).

Our data suggest a more complex distribution of SINEs in salmonids than previously reported by Kido et al. (1991). The SINEs found in salmonids apparently evolved much more recently than the Alu family and may provide insights into the evolution of eukaryotic genomes (Kido et al., 1991; Okada, 1991). In addition to their value in evolutionary studies, these sequences also have utility as genetic markers in applications similar to and beyond traditional VNTR probes.

Acknowledgements

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