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Analysis of VNTR loci in fish genomes using synthetic oligodeoxyribonucleotide probes

(Multilocus DNA fingerprinting; variable number tandem repeat; genetic distance; microsatellites; populations)

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SUMMARY

A set of synthetic oligodeoxyribonucleotide (oligo) probes, OAT18, OMS1 and OAT24 carrying the $(TGG)_6$, $(GGAT)_4$ and $(GACA)_6$ repeat motifs, respectively, was used to analyze the variable number tandem repeat (VNTR) loci in the genomes of *Oncorhyncus mykiss* (rainbow trout; family Salmonidae), *Oreochromis mossambicus* and *Oreochromis niloticus* (both tilapia belonging to family Cichlidae). Of all the oligos and enzymes (*Alu*I, *Mbo*I, *Hae*III and *Hin*fI) used, the OAT18/*Hae*III combination was found to be most informative for detecting DNA fingerprinting in rainbow trout, while the OMS1/*Mbo*I combination gave the most informative pattern for the *Or. niloticus* genome. In the rainbow trout genome, all three repeat loci were hypervariable, revealing varying degrees of polymorphism as compared to tilapia genomes. Startlingly, the OAT24 probe did not cross-hybridize with *Or. mossambicus* and lamprey salmon (*Lampetra japonica*) although GACA repeats have been reported to be evolutionarily conserved in all eukaryotes studied thus far. Cluster analysis with respect to GGAT repeat loci revealed that *Or. niloticus* diverged from *Or. mossambicus* before the separation of *On. mykiss*, suggesting the relatively recent evolution of these loci in rainbow trout, compared to the tilapia genomes. These highly informative probes will find application in various genetic studies of fishes.

INTRODUCTION

The advent of DNA fingerprinting (Jeffreys et al., 1985a,b) has revealed the existence of genetic loci that are conserved but sufficiently polymorphic to serve as marker for analyzing eukaryotic genomes. The tandem arrays of DNA sequences include mono-, di-, tri- and tetranucleotide repeat motifs that extend over less than 300 bp and behave like variable number tandem repeat (VNTR) loci. Probes based on various tri- and tetranucleotide repeats also detect numerous polymorphic loci that can be resolved on Southern blots (Ali et al., 1986; Schaefer et al., 1988). The technique of DNA fingerprinting allows simultaneous detection of highly polymorphic loci and offers improved prospects for studying migration, genetic drift and overall population structure,

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Abbreviations: Bkm, banded krait minor; bp, base pair(s); kb, kilobase(s) or 1000 bp; *L.*, *Lampetra*; nt, nucleotide(s); oligo, oligodeoxyribonucleotide; *Omo, Or. Mossambicus; Omy, On. mykiss; On, Oncorhyncus*; *Or*, *Oreochromis*; P, probable degree of divergence; PCR, polymerase chain reaction; PIC, polymorphic information content; RAPD, randomly amplified polymorphic DNA; RT, room temperature; SAHN, sequential agglomerative hierarchical and nested; SDS, sodium dodecyl sulfate; SSC, 0.15 M NaCl/0.015 M Na_3·citrate pH 7.6; SSPE, 0.15 M NaCl/10 mM NaH_2PO_4·$2H_2O$/1 mM EDTA pH 7.4; TAE, 0.04 M Tris·acetate/0.001 M EDTA pH 8.3; T_m, melting temperature; UPGMA, unweighted pair group method with arithmetic means; V, volt(s); VNTR, variable number tandem repeat.

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thereby substituting the conventional protein and biochemical markers. This has led to its widespread use in many organisms (Lloyd et al., 1989; Fields et al., 1989; Nanda et al., 1990; Rico et al., 1991; Harris et al., 1991). Unfortunately, for the analysis of different species of fishes, no single marker is available which can identify particular strain and lend support to quantitative genetic analysis useful for breeding program.

Multilocus VNTR probes like $(GACA)_4$ and $(CT)_9$ reveal significant population differences in the allelic frequencies of the outbred, abundant and vagile species of *Poecilia latipinna* (Laughlin and Turner, 1994). Similarly, based on the DNA fingerprinting pattern generated by $(GACA)_4$ repeat sequences, an 'anonymous' fry was correctly assigned to its nest (Gross et al., 1994). Genetic markers based on mini- and microsatellites derived from fish genome were used to discriminate genotypes at single and multiple loci to study evolutionary ecology of three-spine Stickleback *Gasterosteus aculeatus* (L) (Rico et al., 1993). Similarly, evaluation of male reproductive tactics in the three-spine Stickleback (Rico et al., 1992) and genetic variability in sympatric brown trout population (Prodoehl et al., 1992) are some of the areas of fisheries where DNA fingerprinting approach has found its applications. Rainbow trout supports important aquacultural sport and commercial fishing industries whereas tilapia are important in warm climate areas of the world and serve as a quick-breeding logistically convenient model for quantitative genetic studies of fishes.

We studied three different repeat loci of these fishes using synthetic oligo probes having tri- and tetranucleotide repeat motifs. For Southern blot hybridization, all the DNA samples from fishes, barring catfish DNA, were from Japan. In our subsequent dot blot hybridization studies, we also included DNA sample of *Oreochromis mossambicus* of Indian origin which enabled us to ascertain the organizational and evolutionary differences in the repeat loci within an identical but geographically isolated species.

RESULTS AND DISCUSSIONS

(a) Genetic variability of different repeat loci

The different oligo probes used in the present study to detect genetic variability of three repeat loci in *Or. mossambicus* (*Omo*) and *Or. niloticus* (*Oni*) (family: Cichlidae) and *On. mykiss* (*Omy*) (family: Salmonidae) are listed in Table I. The oligo probe OAT24, based on the evolutionarily conserved tetranucleotide repeat 5′-GACA-3′, used for hybridization with *Alu*I, *Hae*III, *Hin*fI and *Mbo*I-digested DNA from *Omo*, *Oni* and *Omy* showed cross hybridization only with *Omy* (rainbow trout) genome. In rainbow trout, this probe detected polymorphisms with *Mbo*I and *Hin*fI-digested DNA samples with variable bands in the range of 10–24 kb (Fig. 1). However, the signals were faint and bands were not discernible with *Hae*III and *Alu*I-digested DNA samples (data not shown). Our results on the rainbow trout were in accordance with earlier data on DNA fingerprinting obtained using the GACA probe (Lloyd et al., 1989). The GACA repeat is a major component of Bkm sequences and has been reported to be conserved and organized in a sex specific manner in several vertebrate species (Epplen, 1988) including some fishes (Nanda et al., 1990). However, we failed to detect any sex specific hybridization pattern in any of the species studied. It was interesting to note that the OAT24 probe did not cross hybridize with the DNA samples from *Omo* and *Oni* digested with any of the four enzymes, however, strong signals were detected in dot blot hybridization studies with *Oni* DNA.

Similarly, rainbow trout DNA digested with *Mbo*I and *Hae*III enzymes showed polymorphic bands with OAT18 probe based on trinucleotide PuPuPy repeat 5′-TGG-3′ sequences (Fig. 2). The probabilities of any two random

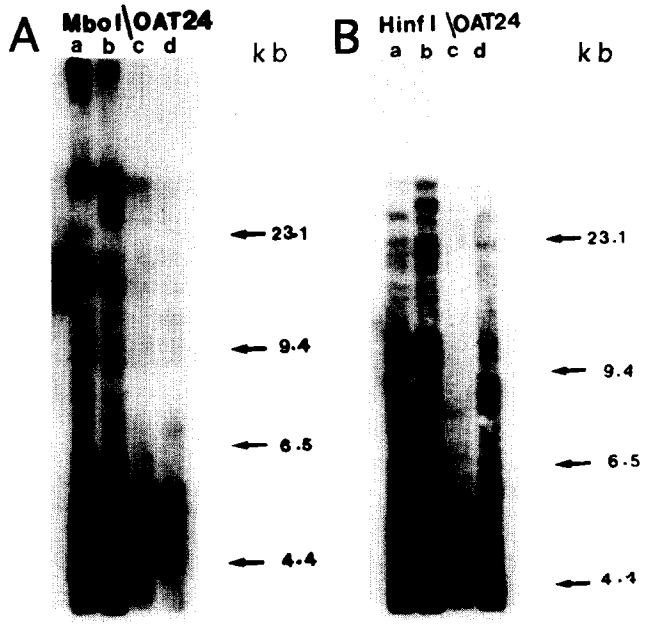

Fig. 1. Hybridization pattern of rainbow trout (*On. mykiss*; *Omy*) genomic DNA digested with *Mbo*I (panel **A**) and *Hin*fI (panel **B**) and probed with OAT24. Lanes a–d in both the panels represent random DNA samples of rainbow trout. DNA was isolated from blood leukocytes (Ali et al., 1986), digested with the respective enzymes and the digests were resolved on 0.85% agarose in 1 × TAE at 1.5 V/cm. After alkali transfer (using 0.4 N NaOH) and 2 h of prehybridization in 5 × SSPE/5 × Denhardt's solution/0.1% SDS/sonicated and denatured *E. coli* DNA (10 μg/ml), the membranes were hybridized in the same buffer containing ^{32}P-labeled probes at the recommended temperatures (Table I). Post hybridization washings were carried out with 6 × SSC for three times at RT followed by a hot wash for 15 min at the hybridization temperature with 6 × SSC for 15 min. Blots were exposed to X-ray film (Konica) for 2 days in −70°C.

TABLE I

Synthetic oligos used as probes for DNA profiling of fish genomes

Sl No.	Probes	Sequences (repeat unit)	Number of repeat unit	Total length of the probe (nt)	T_m (°C)	Hybridization temperature (°C)
1	OAT24	5′-GACA-3′	6	24	72	65
2	OAT18	5′-TGG-3′	6	18	60	55
3	OMS1	5′-GGAT-3′	4	16	48	43

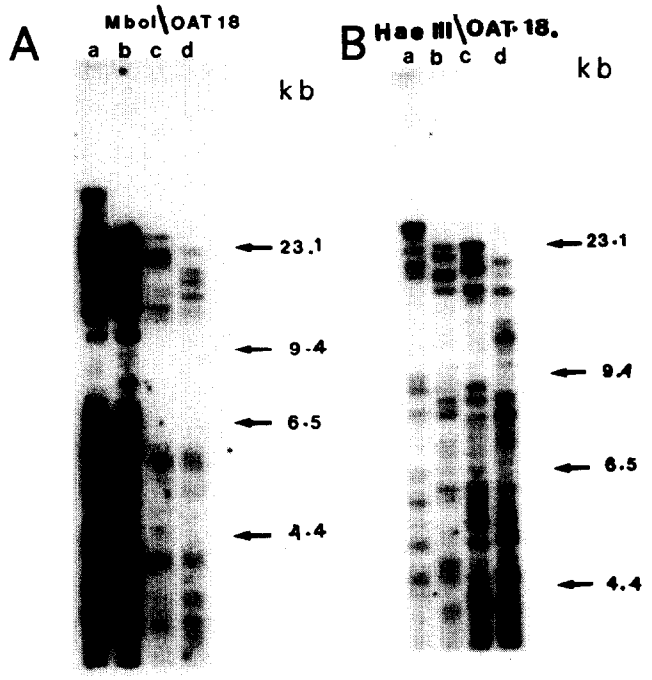

Fig. 2. Hybridization pattern of rainbow trout genomic DNA digested with *Mbo*I (panel **A**) and *Hae*III (panel **B**) enzymes (lanes are same as in Fig. 1) and probed with OAT18. The experimental approach was same as that described in Fig. 1. The highest level of polymorphism is noticed with OAT18/*Hae*III probe/enzyme combination compared to any other ones.

individuals of the species to have identical band profiles with *Mbo*I and *Hae*III enzymes were calculated (Jeffreys et al., 1985) to be 1.806×10^{-5} and 2.14×10^{-7}, respectively, with an approximate heterozygosity of 80%. The OAT18 probe, known to detect sequences polymorphism in the human and several animal species (Ali and Wallace, 1988), failed to cross-hybridize with DNA samples of both the species of tilapia digested with four different enzymes.

(b) Evolutionary conservation of repeat loci

The absence of GACA and TGG repeat sequences in tilapia genome is intriguing. It is possible that the sites complementary to these sequences are destroyed by the enzymes used for digestion. To resolve this, simple dot blot hybridizations were carried out using DNA samples from human, rat, rabbit, caiman, alligator, catfish, two species of tilapia (*Omo* and *Oni*), rainbow trout (*Omy*), Sockey salmon (*Oncorhyncus nerka*), masou salmon (*On. masou*) and lamprey salmon (*L. japonica*). Of the two species of tilapia from Japan, *Oni* showed signals with TGG and GACA repeat sequences whereas *Omo* DNA did not show cross-hybridization (Figs. 3 and 4). Interestingly, DNA from the same species *Omo* from India, showed strong signal with both TGG and GACA repeat sequences. Similarly, dot blot hybridization of Lamprey salmon *L. japonica* DNA did not cross-hybridize with GACA repeat (Fig. 3, spot 22) though the same was found to be positive with the TGG repeat sequence probe (Fig. 4, spot 22). To exclude the possibility of procedural artifact or hybridization failure being

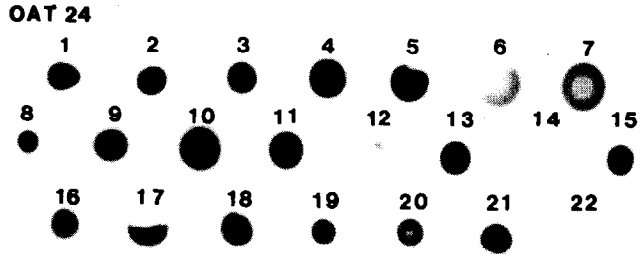

Fig. 3. Dot blot hybridization of genomic DNA using OAT24 probe. The hybridization temperature is as given in Table I and in the legend to Fig. 1. The numbers 1–22 represents DNA samples from: Human male (1) and female (2); Rats, both males (3 and 4); Rabbit male (5); Caiman male (6) and female (7); Alligator male (8) and female (9); Catfish male (10) and female (11); *Omo* both males from Japan (12 and 14); *Omo* male (13) and female (15) both from India; *Oni* both males from Japan (16 and 17); Rainbow trout both males (18 and 19); sockey salmon (20); Masou salmon (21) and Lamprey salmon (22). Note the absence of cross hybridization of GACA repeat with *Omo* from Japan (12 and 14) and Lamprey salmon (22).

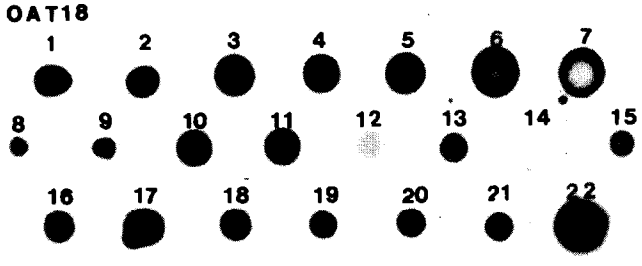

Fig. 4. Dot blot hybridization of genomic DNA from different species using OAT18 probe. The order of the samples represented is the same as that given in Fig. 3. Note the absence of cross hybridization of the repeat TGG with the DNA of *Omo* from Japan (spot 12 and 14) and strong signal in lamprey salmon (spot 22).

responsible for the absence of signal, we reprobed the same blot representing Fig. 3 and 4 with OMS1 probe carrying the GGAT repeats. DNA samples from all the species cross hybridized to generate strong signals (Fig. 5). However, lamprey salmon showed relatively reduced signal (Fig. 5, spot 22). The detailed dot blot profiles of genomic DNA from different species hybridized with three different oligo probes are summarized in Table II. This clearly indicated that the absence of signal with TGG and GACA repeats in dot blot hybridization was not an experimental artifact. This was surprising since TGG in general and GACA sequences in particular

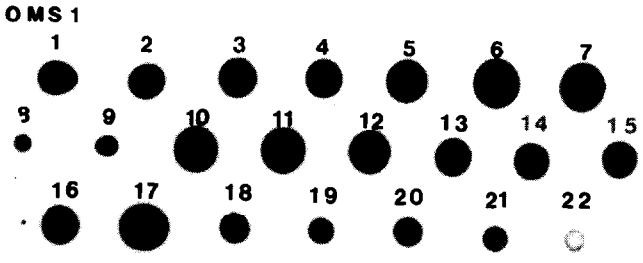

Fig. 5. Dot blot hybridization of genomic DNA from different species using OMS1 probe. The order of the samples represented is the same as that given in Fig. 3. Note the cross hybridization of the repeat GGAT sequences with all the DNA samples with varying signal intensities.

TABLE II

Dot blot hybridization profiles of genomic DNA from different vertebrate species generated with three oligo probes

Sl No.	Source of DNA[a]	Probes[b]		
		OAT24	OAT18	OMS1
1	Human (M)	+ +	+ +	+ + +
2	Human (F)	+ +	+ +	+ + +
3	Rat (M)	+ +	+ + +	+ + +
4	Rat (M)	+ +	+ + +	+ + +
5	Rabbit (M)	+ + +	+ + +	+ + +
6	Caiman (M)	(+)	+ +	+ + +
7	Caiman (F)	(+)	+ +	+ + +
8	Alligator (M)	+	+	+
9	Alligator (F)	+ +	+	+
10	Catfish (M)	+ + +	+ + +	+ + +
11	Catfish (F)	+ + +	+ + +	+ + +
12	*Omo* (J)	(−)	(+)	+ + +
13	*Omo* (I)	+ +	+ +	+ + +
14	*Omo* (J)	(−)	−	+ + +
15	*Omo* (I)	+ +	+	+ + +
16	*Oni* (M)	+ +	+ + +	+ + +
17	*Oni* (M)	+ +	+ + +	+ + + +
18	Rainbow trout (M)	+ +	+ +	+ +
19	Rainbow trout (M)	+ +	+ +	+ +
20	Sockey salmon	+ +	+ +	+ +
21	Masou salmon	+ +	+ +	+ +
22	Lamprey salmon	(−)	+ + +	(+)

[a] M = male, F = female, J = Japan, I = Indian.

[b] Symbols: + denotes cross-hybridization and signal intensity, (+) denotes faint signal, and (−) denotes complete absence of hybridization.

have been reported to be evolutionarily conserved in all eukaryotes (Epplen et al., 1983; Epplen, 1988). The total absence of these repeat sequences in some species and their presence and genetic variability in others might have been a gradual phenomenon during the course of evolution of the fish genomes. This is supported by the fact that we observed a gradation in the signal intensity in the genomes of *Oni* and *Omy* where (GACA) and (TGG) sequences though cross-hybridized showed varying signal intensity with almost same amount of DNA.

Spandidos and Holmes (1987) have proposed that VNTR's are functional and hence they are expected to be conserved throughout evolution. Incidentally, no clear and consistent evolutionary pattern exists for the presence or absence of VNTR loci in the species studied by us. In another independent study on rat, northern blot analyses showed that GACA sequences are transcribed both in testis and several other somatic tissues such as kidney, brain, lung, liver, spleen and muscle. In case of testis, the level of expression varied with the stages of development (manuscript under preparation). This observation is in accordance with earlier report where GACA/GATA repeat sequences have been reported to transcribe (Schafer et al., 1986). While this substantiates the above view that VNTR's play some functional role, its absence in some genomes is still inexplicable.

(c) Organization and polymorphic information content (PIC) of different repeat motifs

Earlier report suggests that the PuPuPy sequences in the salmonid genome are highly dispersed generating high background signals in Southern blots. However, AP-PCR amplification with PuPuPy sequences showed more of common shared bands between individuals of this species (Bentzen et al., 1991). In our study, salmonid DNA probed with TGG and GACA motifs showed discernible polymorphic bands suggesting that these repeat sequences are organized in clustered form.

Another probe OMS1 representing 5-'GGAT-3' sequences hybridized with *Mbo*I, *Hae*III, *Hin*fI and *Alu*I-digested DNA samples from *Omo*, *Oni* and *Omy* revealed marked difference in the hybridization pattern in all the species. With *Hae*III-digested DNA samples of *Oni* and *Omy*, OMS1 probe revealed a highly polymorphic band pattern while with *Omo*, a monomorphic pattern was detected (Fig. 6A). The PIC of OMS1/*Hae*III combination was found to be 8.5×10^{-2} in *Oni* and 1.79×10^{-6} in *Omy*. Interestingly, *Hin*fI-digested DNA of rainbow trout revealed more polymorphic bands with PIC = 3.7×10^{-4}, while the DNA from both the species of tilapia digested with *Hin*fI and *Alu*I enzymes did not show cross hybridization with OMS1 probe (data not shown).

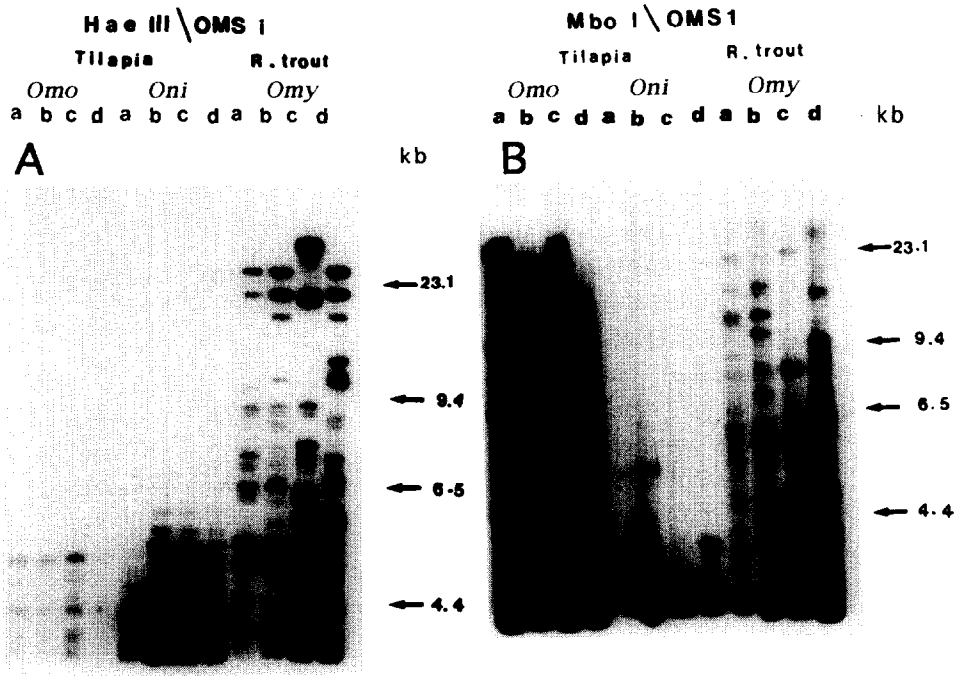

Fig. 6. Hybridization profile of OMS1 probe with the *Omo* (Japan), *Oni* and *Omy* genomic DNA digested with *Hae*III (panel **A**) or *Mbo* (panel **B**). Lanes a–d represent random DNA samples from these species where clearly discernible highly polymorphic bands in the range of 5–24 kb are seen in *Omy*. Note in panel **A** the fewer polymorphic bands in the *Oni* and a few monomorphic bands in the *Omo* genome detected with this probe. Also note in panel **B** the marked differences in the signal intensities and DNA band pattern in the two species (both from Japan) of tilapia, *Omo* and *Oni*. The *Omy* showed several discernible polymorphic bands (5–24 kb).

(d) Phylogenetic relationship of tilapia genomes with rainbow trout

The degree of divergence of these three species became obvious when the OMS1 probe was used against *Mbo*I-digested DNA samples (Fig. 6B). With this probe/enzyme combination, *Oni* genome revealed polymorphic bands in the lower molecular weight region with PIC of 6.5×10^{-4}. The *Omo* DNA showed heavy smears without resolvable bands while rainbow trout DNA showed more number of resolvable polymorphic bands in the range of 2.3 kb to 23 kb. The PIC calculated for OMS1/*Mbo*I probe/enzyme combination for the rainbow trout was 2.37×10^{-4}.

The OMS1 probe showed marked organizational variations within the three species. Dot blot hybridization using OMS1 probe revealed less signal intensity with rainbow trout DNA compared to that in both the species of tilapia (Fig. 5) indicating loss of repeat sequences in this genome during the course of evolution. Southern blot hybridization pattern of the OMS1 probe with *Hae*III-digested genomic DNA from *Omo*, *Oni* and *Omy* (Fig. 6) supports the earlier assumption concerning the GACA and TGG repeat motifs that the rainbow trout (*Omy*) has evolved rather recently as compared to tilapia *Omo* and *Oni* genomes. This suggests that hypervariability at GGAT conserved loci was generated after the divergence of *Oni* and rainbow trout *Omy* from the *Omo* genome (Fig. 7). The monomorphic bands (absence of polymorphism) in the *Omo* genome suggests that, GGAT repeat loci probably do not participate in the recombinatorial activity since this is one of the mechanisms responsible for generating variability (Jeffreys et al, 1985b).

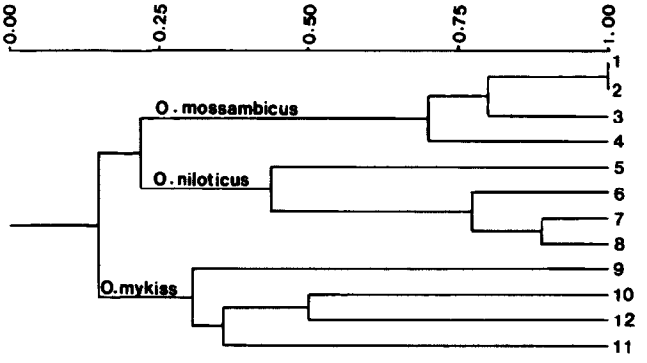

Fig. 7. Phylogenetic relationships of the tilapia genomes *Omo* and *Oni* with the rainbow trout *Omy* using OMS1/*Hae*III probe/enzyme combination. The band profile observed after Southern blot hybridization was scored as binary codes '1' for the presence and '0' for the absence of a band (allele) in an individual sample. From the coefficient of dissimilarity measured between individual genome using the Jaccard's coefficient, the unweighted pair group method, arithmetic averages (UPGMA) were calculated and individuals were clustered using sequential agglomerative hierarchical nested (SAHN) clustering method (NTSYS-pc version 1.7, Rohlf and Slice, 1992). Note the clustering of both the species of tilapia, *Omo* and *Oni* and the degree of genetic variability among members of each species. The salmonid, *Omy* deviated from the two species of tilapia as a separate genetic group.

The organizational variation of the 5′-GGAT-3′

sequence between *Omo* and *Oni* within the family Cichlidae may be due to extensive divergence of the two species of tilapia genomes (Kornfield, 1984). This is evident from the fact that *Mbo*I-digested genomic DNA of *Omo* revealed heavy smear without discernible bands. The *Oni* DNA revealed resolvable polymorphic bands below 4.0 kb (Fig. 6B) indicating short arrays of the repeats organized in clustered manner which probably participates in the recombinatorial activity. The thick smeary signals in the *Omo* detected with OMS1 probe, despite the amount of DNA being equal (the blot in Fig. 6B is a repeat of earlier blot in Fig. 1), may be due to the presence of numerous repeat units of varying lengths in the genome with frequent *Mbo*I sites.

Our results show that the hypervariability detected, with oligo probes at these loci in both the species of tilapia and rainbow trout genomes, is higher than that reported earlier with the myoglobin 33.15 and 33.6 probes (Bentzen et al., 1991). The DNA band patterns of the salmonid *Omy* (rainbow trout) using all the three synthetic oligos were clear, distinct and highly polymorphic even in the high molecular weight regions. The most informative probe/enzyme combination was found to be OAT18/*Hae*III with a PIC of 2.14×10^{-7} followed by OMS1/*Mbo*I combination with a PIC of 6.5×10^{-4} for identical band profile between random individuals in the rainbow trout, *Omy* and tilapia *Oni* species, respectively.

A comparative genome analysis conducted on a single species *Omo* from two different geographically isolated populations showed genomic variation with respect to GACA and TGG motifs (Figs. 3 and 4). The overall hybridization profiles of Southern blots of the three genetic markers for *Omo*, *Oni* and *Omy* genomes are summarized in Table III. The genome organization of Japanese population with respect to OAT24 loci, is therefore distinctly different since GACA sequences are absent in this species compared to that of Indian population. It would be informative if similar information is made available on this species from other parts of the world to substantiate or otherwise the evolutionary conservation of these repeat loci in this species.

TABLE III

The polymorphic information content (PIC) of different probe/enzyme combinations used in the analysis of fish genomes

Species	Probe	Enzyme	Hybridization pattern	Probability of identical DNA profile[a]
Om	OAT18	*Alu*I	Smear	–
	OMS1	*Alu*I	Smear	–
		*Hae*III	Monomorphic	–
		*Mbo*I	Smear	–
Oni	OAT18	*Alu*I	Smear	–
	OMS1	*Alu*I	Smear	–
		*Hae*III	Polymorphic	8.15×10^{-2}
		*Mbo*I	Polymorphic	6.50×10^{-4}
Omy	OAT24	*Hae*III	Polymorphic	NS
		*Hin*fI	Polymorphic	NS
		*Mbo*I	Polymorphic	NS
	OAT18	*Alu*I	Polymorphic	NS
		*Hae*III	Polymorphic	2.14×10^{-7}
		*Mbo*I	Polymorphic	1.80×10^{-5}
	OMS1	*Alu*I	Polymorphic	NS
		*Hae*III	Polymorphic	1.79×10^{-6}
		*Hin*fI	Polymorphic	3.70×10^{-4}
		*Mbo*I	Polymorphic	2.37×10^{-4}

[a] NS, not scorable; –, does not apply.

(e) Conclusions

(*1*) Synthetic oligo probes may provide a reliable tool to discriminate the geographical distribution of the population and genetic make-up of a species between the two populations. When highly polymorphic genetic markers like synthetic oligos are available, this problem may be circumvented by rearing all the genetic groups together, thus nullifying the non-genetic variations.

(*2*) Many commercially available fishes exhibit great deal of variations in their survivability at the early stages depending on their genotype/environment interactions. The genetic variability detectable by polymorphic marker allows identification of the progenies within the families and assignment of parentage of larvae which would then help in assessing the genetic and environmental influences on the survivability. The marker also obliterates the need for preselection of the parental stock for suitable phenotypic markers.

(*3*) The oligo probes will be useful markers in evaluating genetic contamination of wild stocks with the aquaculture production of exotic and domestic strains to: (*a*) identify different strains, (*b*) evaluate the level of inbreeding, and (*c*) monitor the effects of deliberately released stocks in the wild.

(*4*) Unlike conventional protein and biochemical markers, these synthetic DNA based markers allow analysis of population structure, migratory habits and even the extent of mixing of populations detectable at molecular level.

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REFERENCES

Ali, S. and Wallace, R.B.: Intrinsic polymorphism of variable number tandem repeat loci in the human genome. Nucleic Acids Res. 16 (1988) 8487–8496.

Ali, S., Muller, C.R. and Epplen, J.T.: DNA fingerprinting by oligonucleotide probes specific for simple repeats. Hum. Genet. 74 (1986) 239–243.

Bentzen, P., Harris, A.S. and Wright, J.M.: Cloning of hypervariable minisatellite and simple sequence microsatellite repeats for DNA fingerprinting of important aquacultural species of Salmonids and Tilapia. In: Berk, T., Dolf, G., Jeffreys, A.J. and Wolff, R. (Eds.), DNA Fingerprinting: Approaches and Applications. Birkhauser Verlag, Basel, 1991, pp. 243–262.

Epplen, J.T.: On simple repeated GATA/GACA sequences: a critical reappraisal. J. Hered. 79 (1988) 409–417.

Epplen, J.T., Cellini, A., Shorte, M. and Ohno, S.: On evolutionarily conserved simple repetitive DNA sequences: do 'sex-specific' satellite components serve any sequence dependent function? Differentiation 23 (1983) 60–63.

Fields, R.W., Johnson, K.R. and Thorgaard, G.H.: DNA fingerprint in rainbow trout detected by hybridisation with DNA of bacteriophage M13. Trans. Am. Fish. Soc. 118 (1989) 78–81.

Gross, M.L., Kapuscinski, A.R. and Faras, A.J.: Nest specific DNA fingerprints of small mouth bass in lake Opeongo, Ontario. Trans. Am. Fish. Soc. 123 (1994) 449–459.

Harris, A.S., Bieger, S., Doyle, R.W. and Wright, J.M.: DNA fingerprinting of tilapia *Oreochromis niloticus*, and its application to aquaculture genetics. Aquaculture 92 (1991)157–163.

Jeffreys, A.J., Wilson, V. and Thein, S.L.: Hypervariable 'minisatellite' regions in human DNA. Nature 314 (1985a) 67–73.

Jeffreys, A.J., Wilson, V. and Thein, S.L.: Individual specific 'fingerprints' of human DNA. Nature 316 (1985b) 76–79.

Kornfield, I.: Descriptive genetics of Cichlid fishes. In: Turner, B.J. (Ed.), Evolutionary Genetics of Fishes. Olenum, New York, 1984, pp. 591–616.

Laughlin, T.F. and Turner, B.J.: Multilocus DNA fingerprinting detects population differentiation in the outbred and abundant fish species *Poecilia latipinna*. Mol. Ecol. 3 (1994) 263–266.

Lloyd, M.A., Fields, M.J. and Thorgaard, G.H.: Bkm minisatellite sequences are not sex associated but reveal DNA fingerprint polymorphism in rainbow trout. Genome 32 (1989) 865–868.

Nanda, I., Feichtinger, W., Schmid, M., Schroder, J.H., Zischler, H. and Epplen, J.T.: Simple repetitive sequences are associated with differentiation of the sex chromosomes in the guppy fish. J. Mol. Evol. 30 (1990) 456–462.

Prodoehl, P.A., Taggart, J.B. and Ferguson, A.: Genetic variability within and among sympatric browntrout (*Salmo trutta*) population; multilocus DNA fingerprinting analysis. Hereditas 117 (1992) 45–50.

Rico, C., Kuhnlein, U. and Fitzgerald, G.J.: A DNA probe that yields highly informative DNA fingerprints for the three spine Stickle back. Trans. Am Fish Soc 120 (1991) 809–815.

Rico, C., Kuhnlein, U. and Fitzgerald, G. J.: Male reproductive tactics in the threespine Stickle back – an evaluation by DNA fingerprinting. Mol. Ecol. 1 (1992) 79–87.

Rico, C., Zadworny, D., Kuhnlein, U. and Fitzgerald, G.J.: Characterization of hypervariable microsatellite loci in the three spine Stickle back *Gasterosteous aculeatus*. Mol. Ecol. 2 (1993) 271–272.

Rohlf, F.J. and Slice, D.E.: NTSYS-pc. Numerical taxonomy and multivariate analysis system: version 1.70. Applied Biostatistics, New York, 1992.

Schafer, R., Boltz, E., Becker, A., Bartels, F. and Epplen, J.T.: The expression of the evolutionarily conserved GATA/GACA repeats in mouse tissues. Chromosoma 93 (1986) 496–501.

Schaefer, R., Zischler, H. and Epplen, J.T.: $(CAC)_5$, a very informative oligonucleotide probe for DNA fingerprinting. Nucleic Acid Res. 16 (1988) 5196.

Spandidos, D.A. and Holmes, L.: Transcriptional enhancer activity in the variable tandem repeat DNA sequence downstream from the Ha-ras I. FEBS Lett. 218 (1987) 41–46.