Asian Fisheries Science 6(1993):149-160.
Asian Fisheries Society, Manila, Philippines
https://doi.org/10.33997/j.afs.1993.6.2.003

DNA Fingerprinting as a Tool in Fish Biology

YING-PEI ZHANG and YU-PING TANG

Department of Aquaculture
Shanghai Fisheries University
Shanghai 200090, China

Abstract

DNA fingerprinting is a relatively new technique by which a set of polymorphic markers can be simultaneously detected, resulting in a pattern unique to a species, strain or individual (DNA fingerprint). The DNA fingerprinting probes applicable to fish include human minisatellite DNAs, bacteriophage M13, mouse sequence related to *Drosophila Per* gene and some oligonucleotide sequences such as (GATA)4 and (GACA)4. The fingerprints can also be obtained by combination of a number of hypervariable single locus markers. The methods employed to reveal DNA fingerprints include hybridization and the polymerase chain reaction (PCR). With arbitrarily-primed PCR, DNA fingerprints can be obtained from a wide range of species. The technique has proven to be a powerful tool in various fields of fish biology, such as individual and stock identification and breeding.

Introduction

DNA-level polymorphic markers are useful tools in fisheries science, especially in population biology and breeding (Hallerman and Beckmann 1988). One of the detection methods is RFLP (restriction fragment length polymorphism) analysis. Small-scale changes in DNA will sometimes create or destroy specific restriction endonuclease cleavage sites (Jeffreys 1979). Such changes in mitochondrial genomes can be detected simply by restriction analysis (Lansman et al. 1981), but those in nuclear genomes cannot be identified unless appropriate DNA probes are used (Beckmann and Soller 1986). RFLPs are dimorphic (presence or absence of a restriction site) and thus heterozygosity can never exceed 50%. Moreover, this method requires specific probes which are not available for most fish. Fortunately, high polymorphic markers can be produced

from most fish with a new technique, DNA fingerprinting (Jeffreys et al. 1985a), which reveals DNA banding patterns unique to a species, strain and individual (DNA fingerprint).

Probes and Methods Applicable to Fishes

Human Minisatellites

In the first intron of the human myoglobin gene, there is a region comprised of tandem repeats of a 33-base pair (bp) sequence (minisatellite). Using the polymer of the 33-bp sequence as a probe, Jeffreys et al. (1985a) identified several minisatellite clones (e.g., 33.5, 33.6 and 33.15) in the human genomic library. Each of these 33-related minisatellites consists of tandem repeats of short sequences. When the restriction digestion of human genomic DNA was hybridized with these minisatellites (for example, 33.15) under low stringency conditions, a set of hypervariable loci consisting of similar sequences could be detected simultaneously. The loci revealed by these minisatellites usually have a high level of heterozygosity (90% or so). Therefore, the resulting pattern is unique to an individual. The DNA fingerprints are somatically stable and the bands detected are inherited in a Mendelian fashion (Jeffreys et al. 1985b).

Comparison of the repeated sequences of various 33-related minisatellites revealed a 10 bp core sequence, GGGCAGGAXG. The sequence is similar to the signal of generalized recombination in *Escherichia coli*. Computer simulation suggests that the minisatellite regions are hotpoints of recombination. The minisatellite loci are hypervariable in length, probably as a result of unequal recombination which alter the number of the repeat units (Jeffreys et al. 1985a). These minisatellites can also detect hypervariable regions in other animals including mice (Jeffreys et al. 1987) and birds (Burke and Bruford 1987; Wetton et al. 1987).

Oligonucleotide Probes

Synthetic oligonucleotides corresponding to ubiquitous simple sequences can also reveal DNA fingerprints when used as probes in hybridization. The oligonucleotide sequences which are highly informative in fish are $(CAC)_5$, $(GACA)_4$ and $(GATA)_4$ (Nanda et al.

1990; Turner et al. 1990). A minisatellite consisting primarily of tandem repeats of GATAs or GACAs can cross-hybridize with W chromosomes of birds (Jones and Singh 1985), X-chromosomes of *Drosophila melanogaster* (Singh et al. 1981) and Y chromosomes of mice (Epplen et al. 1982; Jones and Singh 1982). The minisatellites, termed BKm sequences, were first isolated from a female banded krait snake (Singh et al. 1980). BKm-related sequences are useful in the evolutionary study of sex-chromosome differentiation.

Mouse Sequence Related to Drosophila Per *Gene*

The *Per* locus codes for a clock gene in *Drosophila*. Mutations at this locus change some rhythmic activities (Young and Judd 1978). The homologous sequences have also been detected in mouse, chicken and human DNA (Shin et al. 1985). Comparison of the *Per* sequences from *Drosophila* and mice shows that each contains long tandem repetitions of the sequence ACNGGN, which are predicted to code for poly (Thr-Gly) tracts (Shin et al. 1985). The mouse sequence has been used as a probe in DNA fingerprinting of various species including fish.

M13 Phage

Another minisatellite probe is derived from a widely used cloning vector, M13 phage (Vassart et al. 1987). This was discovered by chance in a study of RFLPs associated with the human thyroglobulin gene. In the absence of salmon sperm DNA, polymorphic patterns, which are distinct from those obtained with the 33-related probes, can be detected in the human genome with the M13 probe. If excess salmon sperm DNA is added to the hybridization mixture, as under classical hybridization conditions, the polymorphism will be obscured.

The effective sequence in M13 is located within the Protein III gene, which contains a 15-bp motif repeated in tandem at two places. The consensus sequence of the 15-bp motif, GAGGGTGGXGGXTCT, encodes a stretch of peptide GluGlyGlyGlySer. Because the M13 phage is available from many commercial suppliers, DNA fingerprinting can now be used in more laboratories.

Fish Minisatellites

Besides these non-fish originated probes, four hypervariable minisatellite single locus probes have been isolated from Atlantic salmon (*Salmo salar*) by screening the genomic library with probes 33.5 and 33.15 (Taggart and Ferguson 1990). Preliminary results showed that heterozygosities for these loci are very high (59-72%). These probes should be useful in fish identification (individual and family) especially when jointly used.

Simple Sequences Consisting of Repeated Short Motifs

Simple sequences consisting of stretches of monotonously repeated short nucleotide motifs are generally considered hypervariable in length. This hypervariability does not seem to be limited to particular types of simple sequences or particular places of a genome (Tautz 1989). Extensive simple sequence length polymorphisms (SSLPs) have been observed in (CAG)n (Tautz 1989), (CT)n (Tautz 1989), (GT)n (Tautz 1989), (CA)n (Webber and May 1989) and (TG)n (Litt and Luty 1989) blocks, even some of which are in coding regions (Tautz 1989). The length variation is probably caused by DNA slippage during replication and repair. The heterozygosity for these loci is very high. Litt and Luty (1989) reported that 12 different allelic fragments in (TG)n blocks were observed in 37 unrelated people, 32 of whom were heterozygous. For a given locus, different alleles vary exactly in the number of the repeat unit. For the CAG stretch in the *Notch* gene of *Drosophila*, the change is always the times of the triplets and for the CT stretch in pilot whales, the times of the dinucleotides (Tautz 1989).

The variations among a multiple allelic series are relatively small, sometimes only two bases (Tautz 1989). This, however, can be detected by polymerase chain reaction (PCR) plus electrophoresis through a sequencing gel. Tautz (1989) showed that several independent loci could be analyzed simultaneously. This implies that individual-specific patterns can be obtained by combined analysis of a set of such loci. Since the simple sequences may comprise up to 5% of a eukaryotic genome, an almost unlimited number of highly polymorphic loci are potentially available from fish.

Arbitrarily-Primed PCR Amplification

PCR amplification has proven to be of great value in many fields (Sambrook et al. 1989). A novel application of PCR has been

described by Williams et al. (1990). According to their report, genomic DNA was amplified using single short nucleotides (10 bp or more) of arbitrary sequence as primers; DNA polymorphisms were observed in the amplification products resolved on an agarose gel. The RAPD (random amplified polymorphic DNA) markers are interpretable on the basis of simple Mendelian inheritance. The possible sources of the detected polymorphisms include the mismatch between the primer and the DNA template, deletion of the priming site and insertion or deletion of DNA fragments between the priming sites.

This method is independent of sequence information, therefore, applicable to a wide range of species including human, soybean, corn, *Neurospora crassa* and *E. coli.*

Although DNA fingerprints cannot be produced with single primer in some cases, a set of primers should be capable of providing sufficient information.

A similar method independently developed by Welsh and McClelland (1990; 1991) which they termed AP-PCR (arbitrary primed PCR), is characterized by two cycles of low stringency PCR followed by many cycles of high stringency PCR. At a sufficiently low temperature, a variety of mismatches will occur between primers and template DNAs. If two priming sites are only a few hundred base pairs apart, the DNA fragment between these sites can be amplified. In the following high stringency cycles, the fragments will be further amplified to a sufficiently high concentration. Reproducible and species-specific (sometimes strain-specific) banding patterns can be revealed with this method (Welsh and McClelland 1990; Welsh et al. 1991).

Although there are only a limited number of application reports at present, this method should be applicable to any species (Welsh and McClelland 1990).

The procedures employed by most researchers to reveal DNA fingerprints involve digestion of genomic DNA with restriction endonuclease(s), separation of the resulting fragments by electrophoresis, transfer and immobilization of the DNA fragments on nitrocellulose or nylon membranes and hybridization with radiolabeled DNA or RNA probes under low stringency conditions (Jeffreys et al. 1985a, 1985b; Carter et al. 1989). Compared to these procedures, PCR-based methods are simple and rapid. Because only

a small amount of template DNA is needed for amplification, this method is particularly valuable in the analysis of scarce specimens.

Application of the DNA Fingerprints in Fish Biology

Jeffreys et al. (1985a, 1985b) reported that the fingerprints detected by the 33-related minisatellites were almost totally individual-specific in humans, therefore, the fingerprints can be used as a powerful marker system in human individual identification. For the same reason, DNA fingerprints can be used to verify the identity of cultured cell lines (Devor et al. 1988) and various lines of clonal fishes, including those obtained by gynogenesis and androgenesis.

Because the bands are inherited strictly in a Mendelian fashion, all bands of the offspring should be from either father or mother. A putative parent of an individual can be identified by analysis of the fingerprints of the individual, the putative parent and the known parent. Therefore, DNA fingerprints are useful tools in demographic analysis of fish populations.

Mapping of a genome requires a lot of polymorphic markers. In most fishes, only a limited number of such loci have been well characterized. In these cases, the arbitrarily-primed PCR should be of great use, as it requires little knowledge of sequence information.

As RFLP markers, the fragments detected by DNA fingerprinting can also be used in gene linkage analysis. If a commercially important gene is tightly linked to a fingerprinting marker, the transmission of the gene can be determined by inspection of the marker. This will be of great value in genetic improvement of fish.

Welsh and McClelland (1990) reported that the DNA fingerprints unique to some species of *Staphylococcus* and to some strains of the human pathogen *Streptococcus pygenes* could be produced with the AP-PCR. It is expected that this method can also be used to identify fish pathogens.

So far, the value of DNA fingerprints has not been fully exploited. A brief review of some applications of DNA fingerprinting to fish biology follows.

Individual and Population Identification

Jeffreys and Morton (1987) first demonstrated that human DNA probes can detect multiple DNA fragments in fish. Individual specific patterns have been observed in rainbow trout

(Oncorhynchus mykiss), Atlantic salmon, chum salmon *(O. keta)*, coho salmon *(O. kisutch)* (Fields et al. 1989) and barbels *Barbus barbus* (Georges et al. 1988) with M13 phage or other probes. As in mammals, the bands revealed by M13 probe are stably inherited in a Mendelian fashion in rainbow trout (Fields et al. 1989) and striped bass *Morone saxatilis* (Wirgin et al. 1991), and can therefore be used in family analysis. However, use of the M13 probe produced a weak signal and uninformative banding patterns in tilapia *Oreochromis niloticus* (Harris et al. 1991). Rico et al. 1991 reported the use of DNA fingerprinting to determine paternity of wild collected broods of three-spined stickleback *Gasterosteus aculeatus* demonstrating its use in determining reproductive behavior from familial relationships.

Striped bass is quite monomorphic in allozymic loci and mitochondrial DNA, but population-specific banding patterns were detected with the DNA fingerprinting M13 probe (Wirgin et al. 1991). Wirgin and Maceda (1991) also demonstrated that nuclear RFLP analysis can be used to distinguish populations of striped bass. DNA fingerprinting with oligonucleotides $(CAC)_5$ and $(GACA)_4$ revealed a high degree of heterogenity in natural populations of two clonal fishes, *Poecilia formosa* and *Rivulus marmoratus*. For example, three laboratory-maintained clonal lines of *R. marmoratus* were previously separable only by histocompatibility tests. According to the DNA fingerprints, they can be easily discriminated (Turner et al. 1990). Turner et al. (1991) presented data from three studies of repetitive and minisatellite sequences which revealed considerable intra-and inter-population genetic variation not detected by other methods.

Study of Sex Chromosome Differentiation

Sex chromosomes are highly differentiated in mammalian species. The evolution of sex chromosomes often correlates with a significant accumulation of simple repetitive sequences and heterochromatins, but the mechanisms have not been fully understood. Comparative studies of the sex chromosomes at early evolutionary stages can provide valuable clues to the evolutionary process. Fish are well suited for this purpose (Llyod and Fields 1989). The GACA-GATA sequences, due to their relationship with heterochromatins of sex chromosomes in many species, should be useful probes in these studies.

In guppy fish (*Poecilia reticulata*), the (GACA)4 detected a male-specific simple tandem locus in the Y-chromosome in outbred populations, but the typical bands were observed only in two of eight laboratory strains. The probe (GATA)4 could detect a specific band in all red male fish, but not in others (Nanda et al. 1990). The polymorphisms of Y chromosomes were also detected with other methods. The C-banding studies of guppy chromosomes revealed apparent difference between the extent of heterochromatization within the Y chromosome. In some specimens, the process of heterochromatization had reduced the amount of euchromatin in the Y chromosome. In others, the euchromatin of the Y chromosome remained unchanged, but the heterochromatic region was duplicated. Based on these observations, Nanda et al. (1990) suggested that the evolution of heterogamety in conjunction with the establishment of heteromorphic sex chromosomes might not be the result of a single structural change, but develops in several subsequent steps.

In another study, Lloyd and Fields (1989) reported that the GATA-GACA minisatellite sequences isolated from *Drosophila* could hybridize extensively to genomic DNA and detect DNA fingerprinting polymorphisms in rainbow trout. The sequences, however, were not sex-associated in this fish. These observations suggested that the accumulation of these sequences might not be an important step in early sex-chromosome differentiation or the mechanisms for sex-chromosome evolution in rainbow trout might not be the same as in many other species.

Nanda et al. (1990) recently reported that long (CA)n sequences are accumulated specifically in the W chromosome of female black molly *(Poecilia sphenops)*. This probe will allow us to study the evolution of sex-chromosome differentiation in ZZ/ZW fish in the future. Devlin et al. (1991) isolated a Y-chromosome specific DNA probe from Chinook salmon (*O. tshawytsha*) and demonstrated its value in examining male contamination in all-female broodstocks.

Genetic Breeding

Artificial gynogenesis can be induced by fertilization of fish eggs with irradiation-inactivated sperms followed by diploidization (Purdom 1983). Suppression of the second division of meiosis of the fertilized eggs will result in meiotic gynogens, which are partly

heterozygotic due to recombinations between the loci distal to centromeres. The suppression of first mitosis should produce fully homozygous gynogens, which can be used for various purposes such as rapid establishment of inbred lines. Because there is an overlap in the induction window for meiotic and mitotic gynogenesis, appropriate genetic markers are needed to distinguish the two types of gynogens.

Carter et al. (1991) reported that human minisatellite probes could detect the heterozygotic loci in the tilapia *(Oreochromis aureus)* which had been regarded as mitogynes. The probes, however, cannot distinguish mitogynes and meiogynes unambiguously due to the low heterozygosity at minisatellite loci and lack of knowledge of allelism in the fish. PCR-based methods should be useful for this purpose because pair-wise combination of probes can detect sufficient polymorphism (Welsh and McClelland 1991) and the allelism can be easily analyzed in some cases. Harris et al. (1991) reported the potential use of DNA fingerprints with human minisatellite probes for estimating inbreeding ratio and marking hatching stocks.

DNA fingerprinting is useful in assessing paternal genomic contribution in gynogenetic fish. Carter et al. (1991) detected the transmission of paternal DNA with human minisatellite probes in the progeny of a cross between two putative mitogynes of *O. aureau*. Several years ago, Jiang et al. (1983) reported that the gynogenetic offspring of crucian carp *(Carassius auratus gibelio)* displayed significant heterosis, especially when fertilized with sperm from a red variety of carp *(Cyprinus carpio)*. The paternal influence has been speculated as the possible mechanism, but the explanation is disputable for lack of DNA-level evidence (Zhang et al. 1990). We plan to use DNA fingerprints as markers to study the mechanism.

Conclusion

Although there are currently only few reports on the application of DNA fingerprinting to fish biology, its advantage has been clearly demonstrated. Compared with other commonly used methods such as allozymic and mitochondrial DNA RFLP analysis, this method reveals more genetic variations. Moreover, the revealed loci are usually highly heterozygous, therefore more informative than

allozymic and mitochondrial RFLP loci. More applications can be expected in the near future, especially in the following fields: a) population genetics; b) identity labelling of individuals, pedigrees, somatic cell lines, inbred lines and gynogenetic lines; c) paternity identification in the study of reproductive behavior; d) assessment of paternal contribution to the evolution of natural gynogenetic or clonal fish; e) linkage analysis, which will provide clues to inspection of the transmission of some important traits in breeding; f) sex control and evolutionary study of the sex differentiation; and g) diagnosis of fish pathogens. With the development of the technique, novel applications can be envisaged.

References

Beckmann, J.S. and M. Soller. 1986. Restriction fragment length polymorphisms and genetic improvement of agricultural species. Euphytica 35:111-124.

Burke, T. and M.W. Bruford. 1987. DNA fingerprinting in birds. Nature (Lond.) 327:149-152.

Carter, R.E., G.C. Mair, D.O.F. Skibinski, D.T. Parkin and J.A. Beardmore. 1991. The application of DNA fingerprinting in the analysis of gynogenesis in tilapia. Aquaculture 95:41-52.

Carter, R.E., J.H. Wetton and D.T. Parkin. 1989. Improved genetic fingerprinting using RNA probes. Nucleic Acids Res. 17:5867.

Devlin, R.H., B.K. McNeil, T.D.D. Groves and E.M. Donaldson. 1991. Isolation of salmonid Y-chromosomal DNA probes and their application to monosex culture, p. 273. *In* A.P. Scott, J.P. Sampler, D.E. Kime and M.S. Rolge (eds.) Reproductive physiology of fish. Proceedings of the Fourth International Symposium on the Reproductive Physiology of Fish, 7-12 July 1991, University of East Anglia, Norwich, UK.

Devor, E.J., A.K. Ivanovich, J.M. Hickok and R.D. Todd. 1988. A rapid method for confirming cell line identity: DNA fingerprinting with a minisatellite probe from M13 bacteriophage. Biotechniques 6:200-202.

Epplen, J.T., J.R. McCarrey, S. Sutou and S. Ohno. 1982. Base sequence of a cloned snake W-chromosome DNA fragment and identification of a male-specific putative mRNA in the mouse. Proc. Natl. Acad. Sci. USA. 79:3798-3802.

Fields, R., K.R. Johnson and G.H. Thorgaard. 1989. DNA fingerprinting in rainbow trout detected by hybridization with DNA of bacteriophage M13. Trans. Am. Fish. Soc. 118:78-81.

Georges, M., A.-S. Lequarre, M. Castelli, R. Hanset and G. Vassart. 1988. DNA fingerprinting in domestic animals using four different minisatellite probes. Cytogenet. Cell Genet. 47:127-131.

Hallerman, E.M. and J.S. Beckmann. 1988. DNA-level polymorphism as a tool in fisheries science. Can. J. Fish. Aquat. Sci. 45:1075-1087.

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

Jeffreys, A.J. 1979. DNA sequence variants in the G-gamma, A-gamma, delta and beta globin genes of man. Cell 18:1-10.

Jeffreys, A.J. and D.B. Morton. 1987. DNA fingerprints of dogs and cats. Animal Genet. 18:1-5.

Jeffreys, A.J., V. Wilson, R. Kelly, B.A. Taylor and G. Bulfield. 1987. Mouse DNA "fingerprints" analysis of chromosome localization and germ-line stability of hypervariable loci in recombinant inbred strains. Nucleic Acids Res. 15:2823-2836.

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

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

Jiang, Y., S. Liang, B. Chen, H. Yu, S. Shan, D. Yang, S. Lin and G. Shen. 1983. Biological effect of heterologous sperm on gynogenetic offspring in *Carassius auratus gibelio*. Acta Hydrobiol. Sin. 18:1-3. (In Chinese).

Jones, K.W. and L. Singh. 1982. Conserved sex-associated repeated DNA sequences in vertebrates, p.135-154. *In* G.A. Dover and R.B. Flavell (eds.) Genome evolution. Academic Press, New York.

Jones, K.W. and L. Singh. 1985. Snakes and the evolution of sex chromosomes. Trends Genet. 1:55-61.

Lansman, R.A., R.O. Shade, J.F. Shapiro and J.C. Avise. 1981. The use of restriction endonucleases to measure mitochondrial DNA sequence relatedness in natural populations. III. Techniques and potential applications. J. Mol. Evol. 17:214-236.

Litt, M. and J.A. Luty. 1989. A hypervariable microsatellite revealed by *in vitro* amplification of a dinucleotide repeat with the cardiac muscle actin gene. Am. J. Human Genet. 44:397-401.

Lloyd, M.A. and M. Fields. 1989. BKm minisatellite sequences are not sex associated but reveal DNA fingerprint polymorphisms in rainbow trout. Genome 32:865-868.

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

Purdom, C.E. 1983. Genetic engineering by manipulation of chromosomes. Aquaculture 33:287-300.

Rico, C., U. Kuhnlein and G.J. Fitzgerald. 1991. Spawning patterns in the three-spined stickleback (*Gasterosteus aculeatus* L.): An evaluation by DNA fingerprinting. J. Fish. Biol. 39 (Suppl. A): 151-158.

Sambrook, J., E.F. Fritsch and T. Maniatis. 1989. Molecular cloning: A laboratory manual. 2nd edition. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Shin, H.S., T.A. Bargiello, B.T. Clark, F.R. Jackson and M.W. Young. 1985. An unusual coding sequence from a *Drosophila* clock gene is conserved in vertebrates. Nature (Lond.) 317:445-448.

Singh, L., I.F. Purdom and K.W. Jones. 1980. Sex chromosome associated satellite DNA: Evolution and conservation. Chromosoma 79:137-157.

Singh, L., I.F. Purdom and K.W. Jones. 1981. Conserved sex-chromosome-associated nucleotide sequences in eukaryotes. Cold Spring Harbor Symp. Quant. Biol. 45:805-814.

Taggart, J.B. and A. Ferguson. 1990. Hypervariable minisatellite DNA single locus probes for the Atlantic salmon, *Salmo salar* L. J. Fish Biol. 37:991-993.

Tautz, D. 1989. Hypervariability of simple sequence as general source for polymorphic DNA markers. Nucleic Acids Res. 17:6463-6471.

Turner, B.J., J.F. Elder, Jr., T.F. Laughlin and W.P. Davis. 1990. Genetic variation in clonal vertebrates detected by simple-sequence DNA fingerprinting. Proc. Natl. Acad. Sci. USA. 87:5653-5657.

Turner, B.J., J.F. Elder, Jr. and T.F. Laughlin. 1991. Repetitive DNA sequences and the divergence of fish populations: some helpful beginnings. J. Fish. Biol. 39 (Suppl. A): 131-142.

Vassart, G., M. Georges, R. Monsieur, H. Brocas, A.S. Lequarre and D. Cristophe. 1987. A sequence in M13 phage detects hypervariable minisatellites in human and animal DNA. Science (Wash.) 235:683-683.

Webber, J.L. and P.E. May. 1989. Abundant class of human DNA polymorphisms which can be typed using polymerase chain reaction. Am. J. Hum. Genet. 44:388-396.

Welsh, J. and M. McClelland. 1990. Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res. 18:7213-7218.

Welsh, J. and M. McClelland. 1991. Genomic fingerprinting using arbitrarily primed PCR and a matrix of pairwise combination of primers. Nucleic Acids Res. 19:5275-5279.

Welsh, J., C. Peterson and M. McClelland. 1991. Polymorphisms generated by arbitrarily primed PCR in the mouse: Application to strain identification and genetic mapping. Nucleic Acids Res. 19:303-307.

Wetton, J., R.E. Carter, D.T. Parkin and D. Walters. 1987. Demographic study of a wild house sparrow population by DNA fingerprinting. Nature (London) 327:147-149.

Williams, J.G.K., A.R. Kubelik, K.J. Livak, K.A. Rafalski and S.V. Tingey. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 18:6531-6535.

Wirgin, I.I., C. Grunwald and S.J. Garte. 1991. Use of DNA fingerprinting in identification and management of a striped bass population in the southeastern United States. Trans. Am. Fish. Soc. 120:273-282.

Wirgin, I.I. and L. Maceda. 1991. Development and use of striped bass - specific RFLP probes. J. Fish. Biol. 39 (Suppl. A): 159-167.

Young, M.W. and B.H. Judd. 1978. Nonessential sequences, genes and the polytene chromosome bands of *Drosophila melanogaster*. Genetics 88:723-742.

Zhang, Y.P., H. Liu and Y.D. Lou. 1990. Comparative studies on the isozymes of allogynogenetic crucian carp (Qi River) and its parents. Acta Genetica Sin. 17:34-37. (In Chinese.)

Manuscript received 13 May 1992, accepted 11 May 1993.