International Journal of Biomedical Research

MICROSTELLITES: SIMPLE SEQUENCES WITH DYNAMIC ROLE IN FISH GENETICS

Ankur Singh, Prashant Katiyar*****, Mahavir Yadav and Archana Tiwari

School of Biotechnology, Rajiv Gandhi Proudyogiki Vishwavidalaya, Bhopal-462036, Madhya Pradesh, India

Corresponding author*: prashantkat_27@yahoo.co.in

ABSTRACT

Microsatellite or short tandem repeats (STRs) represent codominant molecular genetic markers and are scattered ubiquitously distributed within genomes of all prokaryotic and eukaryotic organisms. Being highly polymorphic and relatively small, these markers are extensively used in an array of fundamental and applied fields of life and medical sciences. Rapid exploitation of microsatellite loci has provided biologists with a set of molecular tools with unsurpassed versatility. In the field of aquaculture, microsatellites are proven tools for the characterization of genetic stocks, broodstock selection, constructing dense linkage maps, mapping economically important quantitative traits, identifying genes responsible for these traits and application to marker-assisted breeding programmes. In this review, functional and practical applications of microsatellites are considered, with special emphasis on fish genetics and aquaculture.

KEY WORDS: Microsatellites, fisheries, Genetic diversity, Conservation Genetics, genetic mapping

INTRODUCTION

Recent years have witnessed increasingly rapid development of molecular phylogenetics and systematics. This is caused by the development of new varied methods of analysis of molecular DNA markers. These methods allow researchers to evaluate genetic relationships among taxa at a new, more advanced level and obtain new facts pertaining to their phylogeny and biodiversity.

The microsatellites: simple sequences with complex evolution are among most variable types of DNA sequence in the genome[19]. Microsatellites or simple sequence repeats (SSRs) are short tandem (satellite) DNA repeats, and are found in greater or less abundance in the genomes of just about every known organism and organelle; deserve special consideration because high variability makes them a tool of population genetics.

Microsatellites are organized in continuous clusters with the “head-to-tail” orientation of monomer units. The length of monomers in a cluster ranges from several to several thousand base pairs. These short tandem repeats (STRs) demonstrate high levels of allele polymorphism. These properties have led to their extensive use in population genetic structure analyses, gene mapping, and paternity identification[84,47]. The microsatellites are codominant markers of relatively small size, which can be easily amplified with the polymerase chain reaction. These features provide the foundation for their successful application in a wide range of fundamental and applied fields of biology and medicine, including forensics, molecular epidemiology, parasitology, population and conservation genetics, genetic mapping and genetic dissection of complex traits.

In the field of fisheries and aquaculture, microsatellites are useful for the characterization of genetic stocks, broodstock selection, constructing dense linkage maps, mapping economically important quantitative traits and identifying genes responsible for these traits and application in marker assisted breeding programmes[120].

Even though microsatellites are considered selectively neutral markers, they contribute to DNA structure, chromatin organization, regulation of DNA recombination, transcription and translation, gene expression and cell cycle dynamics. To date, microsatellites are far from being completely identified and characterized, providing intriguing perspectives for discovery of new properties and characteristics of microsatellites, which will help the design of new research fields and the practical use of these markers.

There is an increasing interest for genetic improvement to maximize efficiency of aquaculture production. In order to establish a selection program and minimize the effects of inbreeding, fish progeny needs to be traced back to their family groups. In the last decade, several studies have demonstrated the ability to establish parentage in fish reared communally using highly polymorphic markers, particularly microsatellites. These studies established that microsatellites are useful markers for the construction of genetic maps and have wide applications in fish biology and genetics in aquaculture.[24,34,33,45,40,81,9]. Recently, the application of microsatellite has extended to more areas of studies, from population genetics, molecular ecology, and molecular breeding to the management of fishery resources.

Applications of Microsatellites in Fisheries

Molecular markers provide direct assessment of pattern and distribution of genetic variation[28] and thus help in answering, “whether the population is a single unit or composed of subunits”. Several evolutionary forces affect the amount and distribution of genetic variation among populations and thereby population differentiation[27]. Geographic distance and physical barriers enhance reproductive isolation by limiting the migration and increased genetic differentiation between populations. Impact of migration and gene flow on genetic differentiation also depends upon effective size of receiving population and number of migrants received. Increased computational power and mathematical models have enhanced the scope of conclusions that can be drawn out of genotype data generated through molecular markers. Some of the possibilities are assignment of migrants[79], determination of genetic bottlenecks[65], besides genetic variation and differentiation estimations[115,73,85]. These have been extensively employed across various taxonomic groups (mosquito:[30] turtle:[101] amphibians:[49] vertebrates:[72].

In fisheries biology, these distinctive markers are extensively used to evaluate the effective population size of stocks[87], stock identification[102], levels of inbreeding[108], population structure and gene flow[18], parentage[54], and quantitative traits[46].

Genetic diversity

Genetic diversity or variation and its measurement have vital importance in interpretation, understanding and management of populations and individuals. The genetic variation in and between populations is the outcome of several factors, such as mutation rate, breeding size of the population, breeding strategy, migration and, above all, selection[12]. The most common measures of genetic diversity are heterozygosity, allelic diversity, and proportion of polymeric loci[60,74]. Habitats with greater biodiversity are more resilient and recover from various disturbances[3]. The conservation of genetic variation is an essential component of many species management programmes. Ultimately, it is genetic variation that allows species to adapt to changing environmental conditions and respond to selection=breeding programmes. To manage any biological resource effectively, researchers must identify the level of genetic variation within and among populations. Measuring genetic diversity in wild fish populations or aquaculture stocks is essential for interpretation, understanding and effective management of these populations or stocks.

The conservation of various fish species has traditionally been done through restocking and genetic variability is pivotal to maintaining the capability of restocked fish to adapt to a changing environment[2]. One approach that have been successfully used in uncovering cryptic population structure in freshwater is microsatellite markers.

Microsatellites, being highly variable genetic markers, are sensitive and promising in studying genetic variation, especially among closely related populations or populations sampled over a reduced geographical scale[26,119]. These markers have been chosen to study population diversity in most major taxa of organisms and successfully used in revealing population genetic diversity[98, 99] for the reason that they are co-dominant and highly polymorphic.

Microsatellite have been isolated and characterized in a large number of fish species and have been used in a wide range of applications, as in evolutionary biology, population genetics and ecology[23]. By characterizing the geographical distribution of allele frequencies, population sub-structuring can be detected and local populations identified. The levels of genetic diversity between fish populations revealed with microsatellite markers are much higher than those obtained with phenotypic or allozyme markers[68,103,110,14] for the reason that microsatellites exhibit higher levels of polymorphism and abundance in genomic DNA than allozyme markers[93].

In recent years, more and more microsatellite-related reports have been issued in fish, such as Northern pike *Esox lucius*[100], Indian major carp, *Catla catla*[67], common carp, *Cyprinus carpio*[121], Atlantic sturgeon, *Acipenser oxyrinchus oxyrinchus*[42], *Silurus glanis*[110] cichlid fish, *Oreochromis niloticus*[7], *Portuguese Guadiana*[91], *M. nemurus*[112], hucho trout, *Hucho taimen*[62], sea bass, *Dicentrarchus labrax*[10], Arctic charr, *Salvelinus alpinus*[66], and so on.

Microsatellite markers also find application in aquaculture to assess loss of genetic variation in hatcheries, comparison of variation estimates between hatchery stocks and wild counterparts. The information is found useful to monitor farmed stocks against inbreeding loss and to plan genetic upgradation programmes. A major aspect of such studies addressed is concerned with the assessment of escapes into the natural population and introgression of wild genome. For instance, genetic diversity of Atlantic salmon from Ireland and Norway were analyzed using 15 microsatellite markers[77]. To summarize the genetic diversity through microsatellite analyses, there is a trend toward increased genetic variation in the progression from freshwater to anadromous to marine fish.

Population and Conservation Genetics

The molecular structure and genetic variability of microsatellites is comprehensively exploited in evolutionary studies of a wide variety of fish species. Microsatellite loci are always a prime choice for the appraisal of the dynamics of populations, including demographic bottlenecks[106],[37], population size fluctuations and effective population sizes. Common measures of genetic diversity are heterozygosity, allelic diversity, and the proportion of polymorphic loci[83]. Marked decreases in the observed heterozygosity and reduced number of observed alleles of tested microsatellites[11] and severe, temporary reduction in size might be accredited to the action of population genetic bottlenecks.

Because bottlenecks may influence the distribution of genetic variation within and among populations, the genetic effects of reductions in population size have been studied extensively by evolutionary biologist[119]. Loss of genetic diversity may reduce the potential of small populations to respond to selective pressures[1], and increased inbreeding may reduce population viability[75,116]. Changes in heterozygosity are often measured to detect bottlenecks[60]. Microsatellite DNA markers have been used to assess bottlenecks in many fish species[106, 21, 53].

The genetic mapping

Genetic mapping epitomize one of the major research fields in which microsatellite markers have been applied. Microsatellites remain the markers of choice for the assembly of linkage maps, because they are highly polymorphic and informative, and entail a small amount of DNA for each test. The enrichment of linkage maps assembled by microsatellites greatly benefits the mapping and characterization of genes responsible for medically, agriculturally and evolutionarily important complex traits. This also provides a good opportunity for marker-assisted selection (MAS) in commercially significant species[80,114].

A review of literature reveals that microsatellites have been isolated and used for genetic mapping in various economically important fishes such as Atlantic salmon[56], channel catfish[64,114, 50], Nile tilapia[13], rainbow trout[90] and zebrafish[51].

Individual and parentage identification

Microsatellites represent codominant single-locus DNA markers. For each simple sequence repeat of microsatellite, a progeny inherits one allele from the male parent and another allele from the female parent. This simple inheritance pattern can explain the extreme popularity of polymorphic simple sequence repeat loci in paternity testing. In forensic science, microsatellites are widely used for individual DNA identification because of their relatively stability in degraded DNA due to their small size[94].

Microsatellites are significantly exploited for paternity and relatedness analysis of natural populations, hatchery broodstocks and trade control of fish products, including those from aquaculture[63]. These DNA markers have also been report to resolve paternity and reproductive contribution in wild and farmed populations of various economically significant species such as bluegill sunfish *Lepomis macrochirus*[72], red sea bream *Pagrus major*[17], turbot *Scophthalmus maximus*[8], chinook salmon *Oncorhynchus tshawytscha* and rainbow trout[4], Atlantic salmon[55].

In addition, microsatellite loci show a high degree of allelic variability, and thus exclusivity. Moreover, they remain relatively stable in bone remnants and dental tissue, providing the basis for the successful application of ancient DNA for molecular analysis[6]. Furthermore, microsatellites obtained from historical fish scale collections has helped to explicate demographic declines in abundance, which resulted in the complete collapse of populations of lake trout in the upper Laurentian Great Lakes of North America during the past 40 years[37]. Other cases where DNA from old fish scales was used to characterize historic populations involve northern pike *Esox lucius*[68, 59] and brown trout *Salmo trutta*[38]. Also the membrane lining of fish otoliths contains DNA, which can be used to genotype historic populations [Atlantic cod *Gadus morhua*:[44], New Zealand snapper *Pagrus auratus*:[39]].

Propagation assisted rehabilitation programmes

Habitat alterations and overharvesting have contributed to the decline or disappearance of numerous natural populations. In addition, reinforcement programs of wild populations based on releases of hatchery-reared fish of non-native origin compromise the conservation of remnant native trout resources. Effect of these programmes through releases in natural populations has been studied in many fishes through molecular markers. Brown trout population structure in an alpine lake with three major recruitment streams was assessed by analysis for eight DNA microsatellite markers and compared with the non-native population.[41]. Microsatellites have increased application in genome mapping[16], paternity testing[71], and forensics[48]. These markers have increasingly been used in population studies of both contemporary[97, 107, 104] and historical samples[20].

Identification of microsatellite loci through cross-species amplification

Microsatellite markers are developed through microsatellite enriched partial genomic library construction. The procedures involve high skill, cost and time. In view of large number of animal species that require genetic diversity analysis, cross priming between related species can be a useful strategy to save cost and time. The sequences flanking the microsatellites are conserved among related species and provide great potential for using primers developed in one species to characterize loci in other species as[122]. However, to large extent, flanking sequences have been found conserved within the families but conservation among distantly related species is also reported in some vertebrates.

Knowledge of microsatellite loci that are conserved between families and orders will be of evolutionary significance and find application in generating population genetics data for wide range of species. [96] have reviewed cross-species amplification of microsatellite markers in various taxonomic groups.

Among fishes, cross priming has been demonstrated in various groups and used as tool to identify polymorphic microsatellite marker. A survey of literature reveals that cross species amplification has been reported within various families e.g., Percidae[117,61], Blennioidei[36], Pangasidae[76], Cottidae[22], Sebastidae[111]. Cross species amplification has also been reported within and between subfamilies[77,105]. [123] demonstrated the possibility of using primers interspecifically among cyprinids. Primers of microsatellite loci from *Catla catla* were used to cross amplification of particular microsatellites locus in *Labeo rohita*[69]. Microsatellite primers designed for common carp *Cyprinus carpio* were successfully amplified in silver carp *Hypophthalmichthys molitrix* and bighead carp *Aristichthys nobilis*[109]. [5]developed microsatellite primers in fathead minnow *Pimephales promelas*, which were screened in two congeneras, *Pimephales notatus* and *Pimephales vigilax* and other five species, *Notropis stramineus, Cyprinelle lutrensis, Semotilus atromaculatus, Campostoma anomalum* and *Cyprinus carpio*. Several other reports demonstrate successful amplification of heterologous or homologous microsatellite loci among various fish species[88,78,36,95,69,58,70,35,57,86,76,43]. These data indicate that success rate cross species amplification is locus-dependent, and support the finding that cross species amplification decreases with the increase of the divergence time[25].

Future perspectives

In this review, with special prominence on fishes, we considered microsatellites as structural genomic components and as genetic markers, which have specific evolutionary mechanisms, functions and applications. Microsatellites exhibit attributes that make them particularly suitable as genetic markers for numerous applications in aquaculture and fisheries research.

The microsatellite technique opens new perspective for studying the structure of closely related populations, population samples over a reduced geographic scale and less isolated populations. It is also a unique tool for tracking pedigrees in breeding programs and marker-assisted selection. Microsatellite loci have a high efficacy for creating a genetic framework onto which other markers and genes are integrated using various mapping strategies. In aquaculture, microsatellites represent the markers of choice for genetic monitoring of farmed stocks in view of breeding programs through the analysis of genetic variability and pedigree structure to design beneficial crosses, select genetically improved stocks, minimize inbreeding and increase selection response[15,52]. Microsatellite-based techniques can also applied in genome scans in natural populations to search for the genetic basis of adaptive selection and biodiversity in an increasing number of species[89].

Large-scale DNA marker-based approaches derived from population and conservation genetics will greatly assist the management of wild fish stocks. As advanced genomic resources become more and more available in model and economically important organisms. Availability of such data could be very useful for stock management, selective breeding programs and sustainable use of wild resources.

ACKNOWLEDGEMENTS

The authors would like to acknowledge Dr. Madhu Yashpal, Department of Anatomy, Institute of Medical Sciences, Banaras Hindu University for her assistance in editing the manuscript.

REFERENCES

1. Allendorf, F., W., and Leary, R.,F., “Heterozygosity and fitness in natural populations of animals.” In: M.E. Soulé (ed.), Conservation Biology; the Science of Scarcity and Diversity. Sinauer. Sunderland, MA. (1986) pp. 57-76.
2. Avise, J., C., “Molecular Markers, Natural History and Evolution.” Chapman and Hall (1994) New York.
3. Beardmore, A., L., Mair, C.,G., and Lewis, C.,G., “Biodiversity in aquatic systems in relation to Aquaculture,.” Aquac. Res. (1997) Vol .28 pp. 829–839.
4. Bentzen, P., Olsen, J., B., McLean, J.,E., Seamons, T.,R., and Quinn, T.,P., “Kinship analysis of Pacific salmon: insights into mating, homing, and timing of reproduction.” J. Hered. (2001) Vol .92 pp.127–136.
5. Bessert, M.,.L., and Orti, G., “Microsatellite loci for paternity analysis in the fathead minnow, Pimephales promelas (Teleostei: Cyprinidae).” Mol. Ecol. Notes (2003) Vol. no.34 pp. 532-534.
6. Burger, J., Hummel, S., Hermann, B., and Henke, W., “DNA preservation: a microsatellite–DNA study on ancient skeletal remains.” Electrophoresis (1999) Vol .20 pp.1722–1728.
7. Carleton, K., L., Streelman, J.,T., Lee, B.,Y., Garnhart, N., Kidd, M., and Kocher., TD., “Rapid isolation of CA microsatellites from the tilapia genome.” Anim. Gene. (2002) Vol.33 pp. 140–144.
8. Castro, J., Bouza, C., Presa, P., Pino-Querido, A., Riaza, A., Ferreiro, I., et al. “Potential sources of error in parentage assessment of turbot (*Scophthalmus maximus*) using microsatellite loci.” Aquaculture, (2004) Vol.242 pp.119–135.
9. Castro, J., Bouza, C., Presa, P., Pino-Querido, A., Riaza, A., Ferreiro, I., et al. “Microsatellite marker tool for parentage assessment in Senegal sole (*Solea senegalensis*): genotyping errors, null alleles and conformance to theoretical assumptions.”Aquaculture, (2006) Vol .261 pp.1194–1203.
10. Chistiakov, D., A., Hellemans, B., Tsigenopoulos, C.,S., Law, A.,S., Bartley,N., Bertotto, D., et al. “Development and linkage relationships for new microsatellite markers of the sea bass (Dicentrarchus labrax L.).” Anim. Gene. (2004) Vol.35 pp.53–57.
11. Chistiakov, D.,A., Hellemans B., Haley, C.,S., Law, A.,S.,

Tsigenopoulos, C.,S., Kotoulas, G., Bertotto, D., Libertini, A., Volckaert, F.,A : A microsatellite linkage map of the European Seabass (*Dicentrarchus labrax*) L. Genetics (2005) Vol. 170: pp.1821-1826.

12. Claes, R.,"Biodiversity and intraspecific genetic variation" Pure. Appl. Chem. (1998) Vol.70 pp.2079-2084.
13. Cnaani, A., Ron, M., Hulata, G., and Seroussi, E., "Fishing in silico: searching for tilapia genes using sequences of microsatellite DNA markers." Anim. Gene. 2002 Vol. 33 pp. 474–476.
14. Corujo, M., Blanco, G., Vazquez, E., and Sanchez, J., A., "Genetic structure of northwestern Spanish brown trout (*Salmo trutta L.*) populations, differences between microsatellite and allozyme loci." Hereditas (2004) Vol .141 pp.258–271.
15. Davis, G., P., and DeNise, S., K., "The impact of genetic markers for selection." J. Anim. Sci. (1998) Vol. 76 pp 2331–2339.
16. Dietrich, W., F., A., "Genetic map of the mouse with 4,006 simple sequence length polymorphisms." Nat. Genet. (1994) Vol.7 pp.220−225.
17. Doyle, R., W., Perez-Enriquez, R., Takagi, M., and Taniguchi, N., "Selective recovery of founder genetic diversity in aquacultural broodstocks and captive, endangered fish populations" Genetica(2001) Vol .111 pp.291–304.
18. DeLeon, F., J., G., L., Chikhi, and F., Bonhomme. Microsatellite polymorphism and population subdivision in natural populations of European sea bass *Dicentrarchus labrax* (Linnaeus, 1758). Molecular Ecology (1997) Vol.6: pp.51–62.
19. Ellegren, H., "Microsatellites: simple sequences with complex evolution." Na. Rev. Genet. (2004) Vol .5 pp.435-445.
20. Ellergen, H., "DNA typing of museum birds." Nature (1991) Vol .354 pp.113.
21. Elliott, N., G., and Reilly, A., "Likelihood of bottleneck event in the history of the Australian population of Atlantic salmon (*Salmo salar L.*)." Aquaculture, (2003) Vol .215 pp.31-44.
22. Engelbrecht, C., C., Freyhof, J.,and Nolte, A., "Phylogeography of the bullhead Cottus gobio (Pisces: Teleostei: Cottidae) suggests a pre-Pleistocene origin of the major central European populations." Mol. Ecol. (2000) Vol .9 pp.709-722.
23. Estoup, A., and Angers, B., "Microsatellites and minisatellites for molecular ecology: theoretical and empirical considerations." In: Carvalho, G.R. (ed) Advances in Molecular Ecology, IOS 1998; Press: Amsterdam pp.55–86.
24. Estoup, A., Presa, P., Krieg, F., Vaiman, D., and Guyomard, R., "(CT)n and (GT)n microsatellites: a new class of genetic markers for Salmo trutta L.(brown trout)." Heredity (1993) Vol .71 pp.488–496.
25. Estoup, A., Tailliez, C., Cornuet, J.,M., and Solignac, M., "Size homoplasy and mutational processes of interrupted microsatellites in two bee species, Apis-Mellifera and Bombus- Terrestris (Apidae)." Mol. Biol. Evol.(1995) Vol .12 pp.1074-1084.
26. Estoup, A., Rousset, F., Michalakis, Y., Cornuet, J., M., "Adriamanga M, Guyomard R. et.al Comparative analysis of microsatellite and allozyme markers: a case study

investigating microgeographic differentiation in brown trout (*Salmo trutta*).” L. Mol. Ecol. (1998) Vol .7 pp.339–340.

27. Felsenstein, J., “Confidence limits on phylogenies: an approach using the bootstrap.” Evolution (1985) Vol .39 pp.783-791.

28. Ferguson, A., McGinnity, P., Stone, C., Clifford, S., Taggart, J., Cross, T., et al. “The genetic impact of escaped farm Atlantic salmon on natural populations.” Aquaculture, (1995) Vol.137 pp.55– 56.

29. Fitz Simmons, N., N., Moritz, C., and Moore, S.,S., “Conservation and Dynamics of Microsatellite Loci over 300 Million Years of Marine Turtle Evolution Department of Zoology, and Commonwealth Scientific and Industrial Research Organisation.” Mol. Biol. Evol. (1995) Vol .12 (3) pp.432-440.

30. Fonseca, D., M., Lapointe, D., A., and Fleischer, R., C., “Bottlenecks and multiple introductions: population genetics of the vector of avian malaria in Hawaii.” Mol.Ecol. (2000)Vol .9 pp.1803–1814.

31. Galbusera, P., Volckaert, F., A., Hellemans, B., and Ollevier, F., “Isolation and characterization of microsatellite markers in the African catfish Clarias gariepinus (Burchell, 1822).” Mol. Ecol. (1996) Vol .5 pp.703–705.

32. García de León, F.,J., Canonne ,M., Quillet, E., Bonhomme, F., Chatain, B., “The application of microsatellite markers to breeding programmes in the sea bass, Dicentrarchus labrax.” Aquaculture, (1996) Vol .159 pp.303–316.

33. Garcia de Leon, F., J., Chikhi, L., and Bonhomme, F., “Microsatellite polymorphism and population subdivision in natural populations of European sea bass Dicentrarchus labrax.” Mol. Ecol. (1997) Vol. 6 pp.51–62.

34. Goff, D., J., Galvin, K., Katz, H., Westerfield, M., Lander, E.,S., and Tabin, C.,J., “Identification of polymorphic simple sequence repeats in the genome of the Zebrafish.” Genomics (1992) Vol.14 pp.200–202.

35. Gopalakrishnan, A., Musammilu, K., K., Mohammed, M., Lal, K., K., Dhurendra, K.,Ponniah, A.,G.,and Mohindra, V., “Microsatellite DNA markers to assess population structure of red tailed barb Gonoproktopterus curmuca.” Acta. Zoo.l Sin. (2004) Vol .50 pp.686 – 690.

36. Guillemaud, T., “Interspecific utility of microsatellites in fishes: a case study of (CT)n and (GT)n markers in the shanny Lipophrys pholis (Pisces: Blenniidae) and their use in other Blennioidae.” Mar. Biotechnology (2000) Vol . 2, pp 248-253.

37. Guinand, B., Scribner, K., T., Page, K., S., and Burnham-Curtis, M.,K., “Genetic variation over space and time: analyses of extinct and remnant lake trout populations in the Upper Great Lakes.” Proc. R. Soc. Lond. B (2003) Vol .270 pp.425–433.

38. Hansen, M.,M., Ruzzante, D.,E., Nielsen, E.,E., Bekkevold, D., and Mensberg, K.,L., “Long-term effective population sizes, temporal stability of genetic composition and potential for local adaptation in anadromous brown trout (Salmo trutta) populations.” Mol. Ecol. (2002) Vol.11 pp.2523–2535.

39. Hauser, L., Adcock, G.,J., Smith, P.,J., Ramirez, J.,H.,B., and Carvalho, G.,R., “Loss of microsatellite diversity and low effective population size in an overexploited population of New Zealand snapper (Pagrus auratus).”

Proc. Natl. Acad. Sci. USA. (2002) Vol .99 pp.11742–11747.

40. Hayes, C., N., Sonesson, A.,K., Gjerde, B., "Evaluation of three strategies using DNA markers for traceability in Aquaculture, species."Aquaculture, (2005) Vol .250 pp.70–81.

41. Heggenes, J., Roed, K.,H., Hoyheim, B.,and Rosef, L., "Microsatellite diversity assessment of brown trout (*Salmo trutta*) population structure indicate limited genetic impact of stocking in a Norwegian alpine lake Ecol. Freshwat. Fish. (2002) Vol .11 pp. 93–100

42. Henderson, A.,A., and King, T.,L., "Novel microsatellite markers for Atlantic sturgeon (*Acipenser oxyrinchus*) population delineation and broodstock management." Mol. Ecol. Notes (2002) Vol .2 pp. 437–439.

43. Hogan, Z.,S and May, B., "Twenty-seven new microsatellites for the migratory Asian catfish family Pangasiidae." Mol. Ecol. Notes. (2006) Vol .2 pp.38-41.

44. Hutchinson, W.,F., Van Oosterhout, C., Rogers, S.,I., and Carvalho, G.,R., "Temporal analysis of archived samples indicates marked genetic changes in declining North Sea cod (Gadus morhua)." Proc. R. Soc. Lond. (2003) B 270 pp. 2125–2132.

45. Jackson, T.,R., Martin-Robichaud, D.,J and Reith, M.,E., "Application of DNA markers to the management of Atlantic halibut (Hippoglossus hippoglossus) broodstock" Aquaculture,. (2003)Vol.220 pp.245–259.

46. Jackson, T., R., M. M. Ferguson, R., G., Danzmann, A.,G., Fishback, P., E., Ihssen, M. O'Connell, and T., J.,Crease. Identification of two QTL influencing upper temperature tolerance in three rainbow trout(*Oncorhynchus mykiss*) half-sib families. Heredity (1998) Vol.80: pp.143–151.

47. Jarne, P., and Lagoda, P., J.,L., "Microsatellites from molecules to populations and back." Trends. Ecol. Evol. (1996) Vol .11 pp.424-429.

48. Jeffreys, A.,J., Maxine, J.,A., Erika, H., and Andreas, S., "Identification of the Skeletal Remains of Mengele, Josef, by DNA Analysis." Forensic Sci. Int. (1992) Vol .56 pp.65–76.

49. Jehle, R., and Arntzen, J.,W., "Microsatellite markers in amphibian conservation genetics." J. Herpetol. (2002) Vol .12 pp.1-9.

50. Karsi, A., Li,P., Kim, S., Dunham, S., and Liu, Z.,J., "Performance trait-linked DNA markers and marker-assisted selection." Plant and Animal Genome VIII Abstracts (2000) Available on http://www.intl-pag.org/pag/8/abstracts/pag8723.html.

51. Knapik, E.,W., Goodman, A., Ekker, M., Chevrette, M., Delgado, J., Neuhauss, S., et al. "A microsatellite genetic linkage map for Zebrafish (*Danio rerio*)." Nat. Genet. (1998) Vol .18 pp.338–343.

52. Knibb, W., "Genetic improvement of marine fish—which method for industry?." Aquac. Res. (2000) Vol .31 pp.11–23.

53. Kohlmann, K., Kersten, P., and Flajshans, M., "Microsatellite-based genetic variability and differentiation of domesticated, wild and feral common carp (*Cyprinus carpio L.*) populations" Aquaculture,, (2005) Vol .247 pp. 253–266.

54. Knight, M. E., G. F. Turner, C. Rico, M. J. H. van Oppen, and G. M. Hewitt. Microsatellite paternity analysis on captive Lake Malawi cichlids supports reproductive isolation by direct mate choice. Molecular Ecology (1998) Vol.7: pp.1605–1610.

55. King, T. L., S. T. Kalinowski, W. B. Schill, A. P. Spidle, and B. A. Lubinski. Population structure of Atlantic salmon (*Salmo salar* L.): a range-wideperspective from microsatellite DNA variation. Molecular Ecology (2001) Vol.10 (4): pp. 807-821.

56. Koop, B.,F., and Davidson, W.,S., "ESTs, microarrays and genomic research in salmon (GRASP)." Plant and Animal Genome XIII Abstracts (2005) Available on http://www.intl-pag.org/13/abstracts/ PAG13_W022.html.

57. Kreig, F., Estoup, A., Triantafyllidis, and Guoymond, R., "Isolation of microsatellite Loci in European Catfish Silurus glanis." Mol. Ecol. (1999) Vol.8 pp. 1257-1969.

58. Lal, K.,K., Chauhan, T., Mandal, A., Singh, R.,K., Khulbe, L., Ponniah, A.,G., and Mohindra, V., "Identification of microsatellite DNA markers for population structure analysis in Indian major carp, Cirrhinus mrigala (Hamilton-Buchanan, 1882)." J. Ichthyol. (2004) Vol .20 pp. 87–91.

59. Larsen, P., F., Hansen, M.,M., Nielsen, E.,E., Jensen, L.,F., and Loeschcke, V., "Stocking impact and temporal stability of genetic composition in a brackish northern pike population (*Esox lucius L.*), assessed using microsatellite DNA analysis of historical and contemporary samples." Heredity (2005) Vol .95 pp. 136–143.

60. Leberg, P., L., "Effects of population bottlenecks on genetic diversity as measured by allozyme electrophoresis." Evolution, (1992) Vol. 46 pp. 477-494.

61. Leclerc, D., Wirth, T., and Bernatchez, L., "Isolation and characterization of variable microsatellite markers in the yellow perch (*Perca flavescens*), and cross species amplification within the family Percidae." Mol. Ecol. (2000) Vol.9 pp.995-997.

62. Liang, L.,Q., Chang, Y.,M., Dong, C.,Z., and Sun, X.,W., "Genetic analysis for Hucho taimen in Wusuli River with microsatellite." J. Fish China, (2004) Vol. 28 pp. 241–244 (In Chinese with English abstract).

63. Liu, Z.,J and Cordes, J.,F., "DNA marker technologies and their applications in Aquaculture, genetics." Aquaculture,, (2004) Vol. 238 pp. 1–37.

64. Liu, Z.,J., Li, P., Kocabas, A., Ju, Z., Karsi, A., Cao, D., and Patterson, A., "Microsatellite-containing genes from the channel catfish brain: evidence of trinucleotide repeat expansion in the coding region of nucleotide excision repair gene RAD23B." Biochem. Biophys. Res. Commun. (2001) Vol. 289 pp. 317– 324.

65. Luikart, G., Allendorf, F.,W., Cornuet, J.,M., and Sherwin, W.,B., "Distortion of allele frequency distributions provided a test for recent population bottlenecks ." J. Hered. (1998) Vol . 89 pp. 238–247.

66. Lundrigan, T.,A., Reis, J., and Ferguson, M.,M., "Microsatellite genetic variation within and among Arctic charr (Salvelinus alpinus) from Aquaculture, and natural populations in North America." Aquaculture, (2005) Vol . 244 pp. 63–75.

67. McConnell, S.,K.,J., Leamon, J., Skibinski, D.,O.,F., and Mair, G.,C., "Microsatellite markers from the

Indian major carp species, *Catla catla*.” Mol. Ecol. Notes, (2001) Vol .1: pp.115–116.

68. Miller, L.,M., and Kapuscinski, A.,R., “Microsatellite DNA markers reveal new levels of genetic variation in northern pike.” Trans. Am. Fish. Soc. (1996) Vol .125 pp. 971–977.

69. Mohindra, V., Mishra, A., Palanichamy, M., and Ponniah, A.,G., “Cross-species amplification of *Catla catla* microsatellite locus in *Labeo rohita*.” Indian J. Fish. (2001) Vol .48: pp.103–108.

70. Mohindra, V., Lal, K.,K., Chauhan, T., Mandal, A., Singh, R.,K., Khulbe, L., and Ponniah, A.,G., “Microsatellite DNA markers for population-genetic studies of *Labeo dyocheilus* (McClelland, 1839).” J. Appl. Ichthyol. (2004) Vol . 21 pp. 487.

71. Morin, P.,A., Wallis, J., Moore, J.,J., and Woodruff, D.,S., “Paternity exclusion in a community of wild chimpanzees using hypervariable simple sequence repeats.” Mol. Ecol. (1994) Vol .3 pp. 469–478.

72. Neff, B.,D., and Gross, M.,R., “Microsatellite evolution in vertebrates: Inference from AC dinucleotide repeats.” Evolution (2001) Vol .55 pp. 1717–1733.

73. Nei, M., and Chesser, R.,K., “Estimation of fixation indices and gene diversities.” Ann. Hum. Genet. (1983) Vol .47 pp. 253–259.

74. Nei, M., Maruyama, T., and Chakraborty, R., “The bottleneck effect and genetic variability in populations.” Evolution (1975) Vol . 29 pp. 1–10.

75. Newman, D., and Pilson, D., “Increased probability of extinction due to decreased genetic effective population size: Experimental populations of Clarkia pulchella.” Evolution (1997) Vol .51 pp. 354–362.

76. Ngamsiri, T.,N., Sukumasavin, O., Nakajima, M., Na-Nakron, U., and Taniguch, N., “Characterization of microsatellite DNA markers in a critically endangered species, Mekong giant catfish, Pangasianodon gigas, Junbin zhang, Zeping cai and Hui haung. Isolation and characterization of microsatellite loci from mangrove red snapper Lutjanus argentimaculatus.” Mol. Ecol. Notes, (2006) Vol . 6 pp. 313.

77. Norris, A., T., Bradley, D.,G., and Cunningham, E.,P., “Microsatellite genetic variation between and within farmed and wild Atlantic salmon (*Salmo salar*) populations.” Aquaculture, (1999) Vol .180 pp. 247–264.

78. Patton, J.,C., Gallaway, B.,J., Fechhelm, R., and Cronin, M.,A., “Genetic variation of microsatellite and mitochondrial DNA markers in broad whitefish (*Coregonus nasus*) in the Broad whitefish Sagavanirktok and Colville Rivers in northern Alaska.” Can. J. Fish Aquat . Sci. (1997) Vol.54 pp. 1548-1556.

79. Piry, S., Alapetite, A., Cornuet, M.,J, Paetkau, D., Baudouin, L., Estoup, A., et al., “GENECLASS2: a software for genetic assignment and first-generation migrant detection.” J. Hered. (2004) Vol . 95 pp.536–539.

80. Poompuang, S., and Hallerman, E.,M., “Toward selection of quantitative trait loci and marker-assisted selection in fish.” Rev. Fish. Biol. Fish (1997) Vol. 5 pp. 253–277.

81. Porta. J., Porta, J.,M., Martínez-Rodríguez, G., Alvarez, M.,C., “Genetic structure and genetic relatedness of a hatchery stock of Senegal sole (*Solea senegalensis*) inferred by microsatellites." Aquaculture, (2006) Vol . 251 pp. 46–55.

82. Primmer, C.,R., Muller, A.,P., and Ellegren, H., "A wide-range survey of cross-species microsatellite amplification in birds." Mol. Ecol. (1996) Vol .5 pp. 365-378.

83. Pujolar J.,M, Maes G.,E., Vancoillie, C., Volckaert, FAM (2005) Growth rate correlates to individual heterozygosity in European eel, *Anguilla anguilla* L. Evolution Vol.59: pp.189–199.

84. Queller, D.,C., Strassmann, J.,E., and Hughes, C.,R ., "Microsatellites and kinship." Trends Ecol. Evol. (1993) Vol . 8 pp. 285-288.

85. Raymond, M., and Rousset, F., "An exact test for population differentiation." Evolution, (1995) Vol . 48 pp. 1280–128.

86. Revaldaves, E., Luiz, H.,G., Pereira, Fausto, F., and Oliveira, C., "Isolation and characterization of microsatellite loci in Pseudoplatystoma corruscans (*Siluriformes: Pimelodidae*) and cross-species amplification." Mol. Ecol. Notes (2005) Vol . 5 pp. 463.

87. Reilly, A., N., G., Elliott, P., M., Grewe, C., Clabby, R.,Powell, and R., D., Ward. Genetic differentiation between Tasmanian cultured Atlantic salmon(*Salmo salar* L.) and their ancestral Canadian population:comparison of microsatellite DNA and allozyme and mitochondrial DNA variation. Aquaculture (1999) Vol.173: pp.459–469.

88. Rico, C., Rico, I., and Hewitt, G., "470 million years of conservation of microsatellite loci among fish species." Proc. R. Soc. Lond. B (1996) Vol . 263 pp. 549-557.

89. Rogers, S.,M., and Bernatchez ,L., "Integrating QTL mapping and genome scans towards the characterization of candidate loci under parallel selection in the lake whitefish (*Coregonus clupeaformis*)." Mol. Ecol. (2005) Vol . 14 pp. 351–361.

90. Sakamoto, T., Danzmann, R.,G., Gharbi, K., Howard, P., Ozaki, A., Khoo, S.,K., et al., "A microsatellite linkage map of rainbow trout (Oncorhynchus mykiss) characterized by large sex-specific differences in recombination rates." Genetics (2000) Vol .155 pp. 1331–1345.

91. Salgueiro, P., Carvalho, G.,R., Collares-Pereira, M.,J and Coelho, M.,M., "Microsatellite analysis of genetic population structure of the endangered cyprinid Anaecypris hispanica in Portugal: implications for conservation." Biol. Conserv. (2003) Vol .109 pp. 47–56.

92. Schlotterer, C., Amos, B., Tautz, D., Stalling, L., Ford, F., Torney, D., Hildebrand, C., and Moyzis, R., "Conservation of polymorphic simple sequence in cetacean species." Nature (1991) Vol .354 pp. 63–65.

93. Schlötterer, C., "Evolutionary dynamics of microsatellite DNA."Chromosoma (2000) Vol .109 pp. 365–371.

94. Schneider, P.,M., Bender, K., Mayr, W.,R., Parson, W., Hoste, B., and Decorte, R., et al., STR analysis of artificially degraded DNA- results of a collaborative European exercise, Forensic Sci. Int. (2004) Vol .139 pp.123–134.

95. Schrey, A., and Heist, E.,J ., "Microsatellite Analysis of Population Structure in the Shortfin Mako (Isurus oxyrinchus)." Can. J. Fish. Aquat. Sci. (2003) Vol .60 pp. 670-675.

96. Scribner, K.,T., and Pearce, J.,M., "Microsatellites: evolutionary and methodological background and empirical applications at individual, population, and phylogenetic levels." In: Baker, A. (ed.) Molecular Methods in Ecology. Blackwell Science Ltd., London, England (2000) pp. 235–271.

97. Scribner, K.,T., Arntzen, J.,W., and Burke, T., "Comparative analysis of intra and interpopulation genetic diversity in Bufo bufo, using allozyme, single-locus microsatellite, minisatellite and multilocus minisatellite data." Mol .Biol. Evol. (1994) Vol .11 pp. 737-748.

98. Sekino, M., and Hara, M., "Microsatellite DNA loci in Pacific abalone Haliotis discus discus (Mollusca, Gastropoda, Haliotidae)." Mol. Ecol. Notes (2001) Vol . 1 pp. 8–10.

99. Selkoe, K.,A., and Toonen, R.,J., "Microsatellites for ecologists: a practical guide to using and evaluating microsatellite markers." Ecol. Lett. (2006)Vol . 9 pp. 615-629.

100. Senanan, W., and Kapuscinski, A.,R., "Genetic relationships among populations of northern pike (*Esox lucius*)." Can. J. Fish. Aquat. Sci. (2000) Vol .57 pp. 391-404.

101. Shanker, K., Ramadevi, J., Choudhury, C., Singh, L., and Aggarwal, R.,K., " Phylogeography of olive ridley turtles (Lepidochelys olivacea) on the east coast of India: implications for conservation theory." Mol. Ecol. (2004). Vol.13 pp.1899–1909.

102. Shaklee, James, B., Bentzen, Paul. Genetic Identification of Stocks of Marine Fish and Shellfish University of Miami - Rosenstiel School of Marine and Atmospheric Science Bulletin of Marine Science, March (1998) Vol. 62: (2) pp. 589-621(33).

103. Shaw, P.,W., Turan, C., Wright, J.,M., O'Connell, M., and Carvalho, G.,R., "Microsatellite DNA analysis of population structure in Atlantic herring (*Clupea harengus*) with direct comparison to allozyme and mtDNA RFLP analyses." Heredity (1999) Vol .83 pp. 490–499.

104. Slatkin, M., "A measure of population subdivision based on microsatellite allele frequencies." Genetics (1995) Vol .139 pp. 457–462.

105. Smith, C.,T., Koop, B.,F., and Nelson, R.,J., "Isolation and characterization of coho salmon (*Oncorhynchus kisutch*), microsatellites and their use in other salmonids." Mol. Ecol. (1998) Vol. 7 pp.1613–1621.

106. Spencer, C.,C., Neigel, J.,E., and Leberg, P.,L., „Experimental evaluation of the usefulness of microsatellite DNA for detecting demographic bottlenecks." Mol. Ecol. (2000) Vol . 9 pp.1517-1528.

107. Taylor, A.,C., Sherwin, W.,B., and Wayne, R.,K., "Genetic variation of simple sequence loci in a bottlenecked species: the decline of the northern hairy-nosed Wombat (*Lasiorbinus krefftii*) ." Mol. Ecol, (1994) Vol .3 pp. 277-290.

108. Tessier, N., L., Bernatchez, and J., M., Wright. Population structure and impact of supportive breeding inferred from mitochondrial and microsatellite DNA analyses in land-locked Atlantic salmon (*Salmosalar*) L. Molecular Ecology (1997) Vol.6: pp.735–750

109. Tong, J., Wang, Z., Yu, X., Wu, Q., and Chu, K., H., "Cross-species amplification in silver carp and bighead carp with microsatellite primers of common carp." Mol. Ecol. Notes (2002) Vol .2 pp. 245–247.

110. Triantafyllidis, A., Krieg, F.,C., Cottin, T.,J., Abatzopoulos, C.,T., and Guyomard, R., "Genetic structure and phylogeography of European catfish (*Silurus glanis*) populations." Mol. Ecol. (2002) Vol . 11 pp. 6.

111. Uchida, D., G., Hoffman, E., A, Ardren, W., R., and Banks, M., A., "Microsatellite markers for the heavily exploited canary (*Sebastes pinniger*) and other rockfish species." Mol. Ecol. Notes (2003) Vol.3 pp.387-389.

112. Usmani, S., Tan, S., G., Siraj, S.,S., and Yusoff., K ., "Population structure of the Southeast Asian river catfish Mystus nemurus." Anim. Gene. (2003) Vol . 34 pp. 462-464.

113. Waldbieser, G.,C. ,and Wolters, W.,R., "Application of polymorphic microsatellite loci in a channel catfish, Ictalurus punctatus, breeding program." J. World. Aquac. Soc. (1999) Vol .30 pp. 256–262.

114. Waldbieser, G.,C., Bosworth, B.,G., Nonneman, D.,J.,and Wolters ,W.,R., "A microsatellite based genetic linkage map for channel catfish(*Ictalurus punctatus).*" Genetics (2001) Vol .158 pp. 727–734.

115. Weir, B., S., and Cockerham, C., C., "Estimating F-statistics for the analysis of population structure." Evolution (1984) Vol .38 pp. 1358–1370.

116. Westemeier, R.,L., Brawn, J.,D., Simpson, S.,A., Esker, T.,L., Jansen, R.,W., Walk, J.,W., et al. Tracking the long-term decline and recovery of an isolated population. Science (1998) Vol . 282, pp 1695-1698.

117. Wirth, T., Saint-Laurent, R., and Bernatchez., L., "Isolation and characterization of microsatellite loci in the walleye (*Stizostedion vitreum*), and cross-amplification within the family Percidae." Mol. Ecol. (1999) Vol. 8 pp. 1961–1963.

118. Jonathan, M., W., and Bentzen, P., "Microsatellites: genetic markers for the future." Rev. Fish. Biol. Fish (1994) Vol .4 pp. 384-388.

119. Wright, S., "Evolution in Mendelian populations." Genetics (1931) Vol. 16 pp. 97–159.

120. Xiao-Gu, Z., Jin-Gou, T., and Bang-Xi, X., "Applications of microsatellite markers in studies of genetics and breeding of fish." Chin. J. Agric. Biotechnology (2006) Vol.3 pp. 83–87

121. Yue, G.,H., and Orban, L., "Polymorphic microsatellites from silver crucian carp (Carassius auratus gibelio Bloch) and cross-amplification in common carp (Cyprinus carpio L.)." Mol. Ecol. Notes (2002) Vol. 2 pp. 534-536.

122. Zardoya, R., Vollmer, D.,M., Craddock, C., Streelman, J.,T., Karl, S., and Meyer., A., et al. "Evolutionary conservation of microsatellite flanking regions and their use in resolving the phylogeny of cichlid (Pisces: Perciformes)." Proc. R. Soc. Lond. B (1996) Vol .263 pp. 1589–1598.

123. Zheng, W., Stacey, N., E., Coffin, J., and Strobeck, C., "Isolation and characterization of microsatellite loci in the goldfish (Carass*ius au auratus*)." Mol. Ecol., (1995) Vol. 4 pp. 791–792.