Wallago attu populations: distribution and genetic analysis

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Estimation of geographical distribution and genetic characteristics of populations is the need of the hour for conservation genetics program. Study of genetic similarity and genetic distance within and between fish populations and species of fishes is an important application of the DNA based genetic markers. Genetic variation is vital in maintaining the developmental stability and biological potential of an organism. To develop microsatellite markers for population structure analysis was one of the objectives of the present work which revealed genetic variation and relatedness among the *Wallago attu* populations. Average levels of genetic variation and normal levels of genetic relatedness were found in each population whereas a little higher levels of genetic variation were observed between certain pair of population. Reduced genetic variation at minimum number of loci among both the species populations indicates deprived type of genetic resources in the *W. attu* . Information on inter and intra species genetic variation from the present study might be useful not only for breeding and conservation purposes but also in making decision for improvement of the species through selective breeding programs. Besides this, breeders could make a strategy for conservation of this fish species having more or less similar gene pools. As literature on genetic analysis of *W. attu* is very limited, present study could help the researchers in this regard in future. The information generated here is of immense importance for characterization and conservation studies of *Wallago attu* as they are essential for efficient sampling and utilization of germplasm resources and for making decisions regarding choice of brooders. Microsatellite can be an effective tool to differentiate geographically and genetically isolated populations, and has been used to verify the existence of locally adapted

populations within a species that may have arisen either through genetic selection under different environmental conditions or as a result of genetic drift (Fuchs *et al.* , 1998).

Genetic markers that can be used to address questions of relevance to the management and conservation of fauna and flora. Particularly, in fisheries science, these genetic markers have been applied to three areas, stock structure analysis, aquaculture and taxonomy/systematics (Ward and Grewe, 1994) with varying degrees of success (Carvalho and Hauser, 1994). The detection of genetic variation among individuals is a requirement in all applications of genetic markers. Some applications will also require the partitioning of variation among groups of individuals (i. e., groups having different alleles or haplotype frequencies). Although some applications will place greater emphasis on genetic differences among groups (stock structure) (Carvalho and Hauser, 1994) and some will focus on differences between individuals within population (pedigree analysis), the finding of polymorphism remains the crucial. The most common use of genetic markers in fishery biology is to determine if samples from any culture facilities or natural populations are genetically differentiated from each other (Ferguson and Danzmann, 1998). The detection of stock differentiation would suggest that the source groups contain different stocks (Carvalho and Hauser, 1994) and that should be treated as distinct management units (MUs) (Moritz, 1994).

Many characteristics of microsatellites are invaluable to examining population genetic structure of fishes. Microsatellites, being co-dominant in nature and inherited in Mendelian fashion, reveal polymorphic amplified

products and help in characterization of individuals in a population. The existence and uniformity in distribution of microsatellites within most eukaryotic genomes and very high variation rate have fostered its increasing application in genome mapping, forensics and paternity (Gopalakrishnan and Mohindra, 2001). Due to their high levels of polymorphism, they are extensively used in stock structure analysis in a several species (Zardoya *et al.* , 1996; O'Connell and Wright, 1997). In microsatellites the mutation rates are very high. The fast rates of microsatellite evolution are believed to be caused by replication slippage events (Zardoya *et al.* , 1996). Two models for mutation have been proposed for variation at microsatellite loci which are infinite allele mutation model (IAM) and the stepwise mutation model (SMM) (Scribner *et al.* , 1996). The SMM predicts mutation occurs through the gain or loss of a single repeat unit, e. g., GT. This means that some mutations will generate alleles already present in the population. In contrast, the IAM predicts that mutation can only lead to new allelic states and may involve any number of repeat units (Estoup *et al.* , 1995 and O'Connell *et al.* , 1997).

The present study established successful development of microsatellite loci. These microsatellite primers will be useful for population genetic analysis of other Silurids because certain sequences flanking the tandem repeats could be conserved between the different families of order Siluriformes as reported in other fishes by Scribner *et al.* (1996), Zardoya *et al.* (1996). Similarly, studies were also done in a variety of canid species (Gotelli *et al.* , 1994; Roy *et al.* , 1994). Microsatellites are conserved across species as diverse as primates, artiodactyls and rodents (Moore *et al.* , 1991). The above results specify the extremely conserved nature of some microsatellite flanking

regions across orders in different taxa and they can persist for long evolutionary time spans much unchanged. The use of heterologous PCR primers would significantly reduce the cost of developing similar set of markers for other Siluriformes species found in India.

Relative frequencies of microsatellite observed and their types

Fourteen polymorphic single locus microsatellite loci were developed in this study. The tandem repeats of the microsatellite loci observed in the present study are the AG, TG, CA, AC, ATTT, CATC, GA, TGC, ATAG, TAGA, AATA and TGGAG repeats (WAM5, WAM8, WAM16, WAM17, WAM21, WAM23, WAM24, WAM27, WAM28, WAM29, WAM27, WAM30 and WAM32 primers). The study found TG and CA rich microsatellites abundant in *W. attu* which is in conformity with the published reports (Na-Nakorn *et al.* , 1999; Krieg *et al.* , 1999; Usmani *et al.* , 2001; Neff and Gross, 2001; Watanabe *et al.* , 2001;). The types of microsatellite repeats observed in *W. attu* are similar to the ones from salmonids (Estoup *et al.* , 1993; Sakamoto *et al.* , 1994; McConnell *et al.* , 1995 and O'Connell *et al.* , 1997). Normally, most of dinucleotide alleles are always visible as a ladder of bands rather than a single discrete product due to slipped-strand mispairing during PCR (Weber, 1990). This did not happened with the primers used in the present study, which always gave clear and discrete bands.

Genetic variability and Hardy-Weinberg Equilibrium

The number of alleles at different microsatellite loci in *W. attu* varied from 6 to 22 with an average value of 10. Primers WAM8, WAM16, WAM17 and WAM21 exhibited maximum allele number 22, 13, 11 and 11 respectively

compared to other primers (nine to six alleles). High microsatellite allelic variation was found in various marine and freshwater fishes such as whiting (14-23 alleles/locus) (Rico *et al.* , 1997); red sea bream (16-32 alleles/locus) (Takagi *et al.* , 1999) and Atlantic cod (8-46 alleles/locus) (Bentzen *et al.* , 1996) as well as in Thai silver barb (*Puntius gonionotus*) in four microsatellite loci with average of 13. 8 alleles per locus (Kamonrat, 1996). Comparatively low genetic variation was detected among microsatellite loci of brown trout (5-6 alleles /locus) (Estoup *et al.* , 1993), northern pike (3-5 alleles/locus (Miller and Kapuscinski, 1996) and sea bass (4-11 alleles/locus) (Garcia De Leon *et al.* , 1995). Neff and Gross (2001) reported mean number of alleles at different microsatellite loci of 27 species of marine and freshwater fin fishes as 13. 7 + 9. 1 for an average allele length of 23. 0 + 6. 0. A positive relationship between microsatellite length and number of alleles has also been reported by them. In African catfish and various other fish species, low values of mean number of alleles were documented (7. 7; Galbusera *et al.* , 1996); Atlantic salmon (6. 0; McConnell *et al.* , 1995); Chinook salmon (3. 4; Angers *et al.* , 1995) and northern pike (2. 2; Miller and Kapuscinski, 1996). DeWoody and Avise (2000) and Neff and Gross (2001) found that marine species have larger microsatellite allelic variation as compared to freshwater. They also documented that more variation in polymorphism at microsatellite loci that exist between species and classes can be credited to dissimilarities in population biology and life history and to a lesser amount to differences in natural selection relating to the function of the microsatellite loci.

In *W. attu* , the mean observed heterozygosity (Ho) per locus per population was 0. 462 and the mean expected heterozygosity (He) per locus per population was 0. 778. Usmani *et al.* (2003) in *Mystus nemurus* reported a value of mean observed heterozygosity (Ho = 0. 4986), the mean expected heterozygosity was nearly similar to that of present study. In *W. attu* , a significant overall deficiency of heterozygotes was revealed in all the populations. In *Clarias macrocephalus* , Na-Nakorn *et al.* (1999) reported the deficiency of heterozygotes (Ho = 0. 67 and He = 0. 76). But, Watanabe *et al.* (2001) and Usmani *et al.* (2003) reported the significant excess of heterozygotes in bagrid catfishes, *Pseudobagrus ichikawai* (Ho = 0. 54 and He = 0. 56) and *Mystus nemurus* (Ho = 0. 4986 and He = 0. 4817) respectively and in silurid catfish, *Silurus glanis* (Ho = 0. 677 and He = 0. 608) Krieg *et al.* (1999). The one reason for inability to identify all the alleles in population and heterozygote deficiency could be small sample size (Na-Nakorn *et al.* , 1999). However the sample size of each population of *W. attu* for microsatellite study is not small according to Ruzzante (1998), henceforth, this hypothesis is inconclusive. Non-random mating and inbreeding would also effect the heterozygote deficiency (Donnelly *et al.* , 1999). The positive value of F_{IS} at nearly all the loci indicated inbreeding in populations of *W. attu* . Seven of the eight microsatellite loci showed significant deviations ($P <0.05$) from Hardy-Weinberg Equilibrium (HWE). Deviations from HWE is usually results to null alleles (Garcia de Leon *et al.* , 1995), inbreeding or non-random mating (Beaumont and Hoare, 2003) or grouping of gene pools (Wahlund effect) (Gibbs *et al.* , 1997) or selection (Garcia de Leon *et al.* , 1995) could be reasons for deviations from HWE .

Over-exploitation, that leads to decline of this catfish has been recorded in

rivers of India and the species now categorized as endangered as per latest IUCN norms. Due to this, inbreeding can happen, which might result in deficiency of heterozygotes and deviation from HWE (Beaumont and Hoare, 2003). Similar conditions were also reported in other fishes that showed decline in catches due to over-exploitation (Rico *et al.* , 1997; O'Connell *et al.* , 1998; Scribner *et al.* , 1996; Yue *et al.* , 2000).

Significant associations between any pair wise combination of microsatellite alleles were not found indicative of linkage disequilibrium (after Bonferroni correction) in *Wallago attu* . Hence it is assumed that the allelic variation recorded at all microsatellite loci could be independent as observed in many fishes (Usmani *et al.* , 2003; Na-Nakorn *et al.* , 1999 and Scribner *et al.* , 1996;).

Null alleles

When mutations occur at primer sites, certain alleles may not be amplified (null alleles) resulting in false homozygotes (Shaw et. al., 1999). Null alleles are alleles, that do not amplify during PCR due to the mutations at primer binding site changing the DNA sequence in one of the primer sites (mainly at 3′ end), which causes the primer to no longer anneal with the template DNA during the PCR (Van Oosterhout *et al.* , 2004, 2006).

Presence of null alleles possibly be one of the reasons, responsible for the observed heterozygote deficiency (Abdul Muneer, 2012). This could prevent certain alleles from being amplified efficiently by PCR (Paetkau and Strobeck, 1995). In individuals with false homozygote or if null allele is homozygote, this will leads to no PCR amplification. This will show apparent significant

deviations from Hardy- Weinberg equilibrium and non-Mendelian inheritance of alleles (Donnelly *et al.* , 1999). Homozygote individuals found in excess in different populations of *W. attu* in the present study could be due to null alleles or by a real biological phenomenon. But, data analysis using MICRO-CHECKER showed, occurrence of null alleles in all the populations is very unlikely for the seven primer pairs. This was supported by the absence of general excess of homozygotes over most of the allele size classes in MICRO-CHECKER analysis. The overall homozygosity can be due to deviations from panmixia, inbreeding, short allele dominance and stuttering or large allele drop-outs (Abdul Muneer, 2012). If excess of homozygotes are biased towards either extreme of the allele size class – distribution and if there is a general excess of homozygotes and the allele range exceeds more than150 base pairs then dominance of short allele occurs (Van Oosterhout *et al.* , 2004)

Stock-specific markers (Private alleles)

The detection of significant private alleles sixty four in all the population (nine private alleles in Gomti river population, six in Ken river population, ten in Chalakudy River population, nine in Vembanad population, thirteen in Ganga river population, five in Hooghly river population and twelve in Brahmaputra river population) are the clear-cut evidence for no mixing of the gene pools between these populations *Wallago attu* . Na-Nakorn *et al.* (1999) reported twenty stock-specific markers in three loci in four populations of *Clarias macrocephalus* in Thailand. The 22 stock specific alleles in three populations of Chinook salmon from Canada was reported by Scribner *et al.* (1996). In the tuna species populations of the genus *Thunnus,* Takagi *et al.*

(1999) reported the stock specific markers. Coughlan *et al.* (1998) also reported the 5 stock specific alleles in the populations of turbot (*Scophthalmus maximus*) from Ireland and Norway. Private alleles or stock specific microsatellite markers can be used as genetic tags for selection programs and to distinguish the stocks for selective/supportive breeding programmes (Appleyard and Mather, 2000).

Genetic differentiation

Fine scale analysis of samples of *W. attu* with microsatellite markers from different collection sites revealed existence of population subdivision. The combined F_{ST} value (0. 0615) of microsatellite loci in *W. attu* was significantly different from zero ($P < 0.001$), indicates a significant level of genetic differentiation present between the populations. The genetic variations are the outcome of several interactive evolutionary forces that act on the natural population (Ryman, 2002). Most important amongst them are migration, random genetic drift and mutation. The higher rates of mutation (and therefore polymorphism) of DNA markers result in greater power for population differentiation (Goudet *et al* ., 1996; Raymond and Rousset, 1995). The genetic differentiation levels observed in the present study (overall F_{ST} = 0. 0615) are comparable to significant values found in Pacific herring (F_{ST} = 0. 023), Atlantic herring (F_{ST} = 0. 035) and widespread anadromous fish like Atlantic salmon (F_{ST} = 0. 054) (McConnell *et al* ., 1995). The genetic relatedness of *W. attu* populations derived from microsatellite loci, using pair-wise F_{ST} between populations also differed

significantly (P <0. 001) from zero for all the pairs of riverine locations indicating significant heterogeneity between populations.

Genetic relatedness between populations

The genetic relatedness between populations could be explained basically through the geographic distance or isolation by distance between sampling locations. The populations, Gomti, Ganga and Ken River clustered more closely than the other population. The Brahmaputra and Hooghly populations was more close to these populations rather than populations from Kerala. The Vembanad and Chalakudy River populations were also found in one cluster and their genetic distances calculated from microsatellite data agreed with geographic distance.

To conclude, the study using novel microsatellite loci in *W. attu* have shown significant results. The usefulness of these developed markers for population genetic analyses was established. Altogether the eight amplified microsatellite loci were found polymorphic and indicated heterogeneity in allele frequency in *W. attu* populations between different river systems. The study found that the seven natural populations of this species i. e. Gomti, Ken, Chalakudy, Vembanad, Ganga, Hooghly and Brahmaputra Rivers that are divergent in their genetic characteristics and can be identified through microsatellite loci. The baseline data generated will support future studies in genetic conservation and management of the fisheries including designing policies for restoration of declining stocks of *Wallago attu* . In addition, the results of the population screening using microsatellites suggest their wide utility for addressing a variety of basic and applied research questions.