Journal Home page : www.jeb.co.in ★ E-mail : editor@jeb.co.in

JEB
Journal of Environmental Biology

ISSN: 0254-8704
CODEN: JEBIDP

Study of population genetic polymorphism and gene flow rate in Indian snow trout, *Schizothorax richardsonii* fish of Himalaya, India

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Publication Info

Paper received:
09 January 2013

Revised received:
23 June 2013

Re-revised received:
15 September 2013

Accepted:
05 October 2013

Abstract

The genetic polymorphism and gene flow rate among the Indian snow trout fish population *S.richadsonii* from three different locations viz., Chirapani stream of Champawat district, Kosi and Gola river of Nainital district, Uttarakhand State, India were assessed by employing twenty numbers of Randomly Amplified Polymorphic DNA (RAPD) markers. The overall percent polymorphisms among these three populations were 14.76 with 6.56, 4.92 and 3.28 in Chirapani, Kosi and Gola river population, respectively. Chirapani population had higher proportion of polymorphic loci as compared to the Kosi and Gola. The higher value of genetic distance (0.1565) was obtained between Chirapani and Gola population and the lower value of genetic distance was observed between Chirapani and Kosi (0.1058) river population. The cluster analysis revealed that in the formation of two clusters, one consisted of Chirapani and Kosi and the other was Gola fish population. Gst estimates among these populations showed some extent of homogeneity with lower genetic differentiation rate between populations and further suggested that higher tolerance to mutation, as expected that RAPD bands, arose from both coding and non-coding DNA regions. The findings revealed that the rate of gene flow in three populations seemed very low *i.e.* highly conserved its genetic diversity in their natural waterbodies and indicative of little migration among populations (geographically isolated and not the possibilities man made interventions/ introduction of similar kind of fish species). It is further concluded that the Chirapani, Kosi and Gola river populations of *S.richardsonii* were being conserved naturally in their habitat and the species actual genetic potential were being maintained (adaptation to local climatic conditions, reproduction, production traits and disease resistance trait etc) in their natural habitat.

Key words

Fish population, Genetic distance, Gene flow, Genetic polymorphism

Introduction

India has significant cold water/ hill fishery resources starting from the north-western to the north-eastern Himalayan region and some parts of Western Ghats. Coldwater fishery is one of the emerging contributors to the national fish basket and is about 1.5%, to the inland fish production. Fisheries provide a promising role in terms of providing nutritional security, good quality proteins and employment opportunities to millions of fisher folk. The mahseers, schizothoracids, minor carps, trouts, and exotic carps are widely distributed and are important for food as well as recreation (Tripathi, 2005). Native fish species are an important component of the region's biodiversity and are a valuable genetic resource for future like adaptability to local climatic conditions, maintenance of unique production and reproduction traits and disease resistance traits, etc and may have some unique traits which may be needful in future. The Indian snow trout constitutes an important part of cold water fishery in the Himalayan region and is an economically important fish of the fast flowing snow fed streams and lakes (Barat *et al*., 2012). Fish is being considered as one of very specific experimental model animal system for indicating global warming and fishes exhibit enormous diversity in size, shape, biology and different geographic habitats (Brander, 2007). The recent years

have noticed a great concern towards the protection and conservation of aquatic resources by implementation of convention on biodiversity (CBD) and Biodiversity Act 2002. Unfortunately, fish population biodiversity is getting reduced drastically due to over fishing and indiscriminate killing (Lakra *et al*., 2007), destructive fishing methods, aquatic pollution and emergence of diseases (Hatanaka and Galette, 2003), introduction of exotic fishes (Nayler *et al.,* 2000, Lakra, 2007), habitat modification and climate change (Morrongiello *et al*., 2011), construction of hydro-electric projects (Zhong and Power, 1996), deforestation, agricultural runoffs, pesticides, fertilizers, sewage and chemical pollutants (Lakra *et al*., 2007) and reduction in surface water flow (Blackburn *et al*., 2010) and is greatly threatened which leads to endangered or extinction of some of the most valuable fish species (Harris et al., 2012). The conservation assessment and management program (CAMP, 1998) has identified 327 threatened freshwater fishes in India (Lakra *et al*., 2007) and many fish species have experienced drastic reduction in number. Ecosystems and native fish species are important in sustaining human life but the pollution of natural water bodies is occurring at faster rate. Many places experienced with overfishing cause reduction in fish population size and lead to inbreeding depression and loss of genetic biodiversity. Recent developments made in the molecular methods will help to assess the actual genetic potential of the fish genetic stock and exploitation of DNA polymorphism has opened many vistas in genetic improvement, wherein the influence of environmental effect can be nullified. DNA- based analysis would help a conservationist to assess the population dynamics and identification of species at population level about genetic classes (Comincini *et al*., 1998), genetic diversity (Gharaei *et al*., 2005) and evolutionary relationship (Posto and Prather, 2003). Knowledge on genetic diversity within and between wild population is extremely useful to gather information on individual's identity (Barmintsev *et al*., 2001), breeding patterns (Apostol *et al*., 1996), degree of relatedness (Faddagh *et al*., 2012) and disturbance of genetic variation among them (Schierwater *et al*., 1994; Matoso *et al*., 2011) and is crucial for the conservation of species (Haig, 1998; Hatanaka and Gallet, 2003). Keeping in view, the present study was envisaged to study the population dynamics, survival fitness in terms of genetic polymorphism, genetic biodiversity/ genetic distance, gene flow rate and breeding structure within and between these *S. richardsonii* populations from three different locations of Himalayan water bodies by employing RAPD marker in spite of several million years of evolution.

Materials and Methods

Indian snow trout *(Scizothorax richardsonii*) fin tissue samples (n= 24 from each population) were collected from the Kumaon Himalayan rivers such as Gola near Kathgodam (515m above mean sea level) and Kosi near Ratighat (1000m above mean sea level) in Nainital district and Chirapani stream (1620m above mean sea level) in Champawat district of Uttarakhnad State, India using cast nets (Fig.1).

Extraction of DNA: DNA was isolated following the method of Sivaraman *et al*. (2010) from fin tissues, without killing the fish. The concentration of DNA was estimated by measuring the absorbance at 260 and 280 nm in a UV-visible spectrophotometer and, the good quality DNA having OD ratio at 1.7 to 1.9, was subjected to PCR amplifications.

PCR was performed with 50 ng of genomic DNA, 100 pM of random primer, 200 µM of each dNTP, 2.5 µl 10 X PCR reaction buffer with $MgCl_2$ and 0.5U of Taq DNA polymerase. Amplification was carried out with an initial denaturation at 95ºC for 5 min followed by 35 cycles consist of denaturation at 94C for 1 min, primer annealing at 36C for 1 min, primer extension at 72C for 1 min and final extension at 72C for 5 min. The amplified products were checked on 1.0 % submarine agarose gel electrophoresis and visualized in the Gel Doc system. 20 numbers of OPY OPY 01 to OPY 20) series primers were employed in this study and 10 primers produced polymorphic loci across three population (Table. 1).

Statistical analysis of RAPD profile: RAPD data were scored manually, based on the presence or absence of band of identical molecular size and were recorded in a binary matrix. If a band was present, it was recorded as '1' and if absent, as '0'. For all statistical tests were chosen at significance level of = 0.05 and a simulation of 1000 permutations. Genetic diversity was calculated as observed number of alleles (na), effective number of alleles (ne) (Kimura and Crow, 1964), number of polymorphic bands, Nei's gene diversity 'h' (Nei, 1973), Shannon's information Index (Lewontin, 1972), total genetic diversity in population (H_t), within sample gene diversity (H_s). G_{ST} was calculated using the Nei method, from the total genetic diversity in the pooled populations (H_t) and mean diversity within each population (H_s) as: $G_{st} = 1-H_s/H_t$. Geneflow {$Nm= 0.5(1- G_{st})/ G_{st}$, for RAPD marker. Cluster analysis was then performed to create a dendrogram, using UPGMA, by SAS Software (version 6.12).

Table 1 : Primer sequences and amplification profiles

Primer name	Primer Sequence (5'- 3')	G+C (%)	Polymorphic
OPY-01	5'-GTGGCATCTC-3'	60	Yes
OPY-02	5'-CATCGCCGCA-3'	70	Yes
OPY-04	5'-GGCTGCAATG-3'	60	Yes
OPY-07	5'-AGAGCCGTCA-3'	60	Yes
OPY-10	5'-CAAACGTGGG-3'	60	Yes
OPY-11	5'-AGACGATGGG-3'	60	Yes
OPY-13	5'-GGGTCTCGGT-3'	70	Yes
OPY-14	5'-GGTCGATCTG-3'	60	Yes
OPY-16	5'-GGGCCAATGT-3'	60	Yes
OPY-20	5'-TGAGGGTCCC-3'	70	Yes

Results and Discussion

RAPD- PCR polymorphisms at 118 presumptive loci were used to examine the breeding structure of *S. richardsonii* fish populations from the Himalayan waterbodies in India. The proportion of polymorphic loci among Chirapani stream, Ratighat and Ranibag river population were 62.3, 70.5 and 60.6% respectively from the 20 number of 10 mer random OPY series primers and significant differences in band frequencies from different locations of fish population (Fig.2). The average number of observed alleles and effective alleles were 1.9508 and 1.4945 among these fish populations with genetic diversity of 0.2962. The overall genetic polymorphisms among *S. richardsonii* from three different locations were 14.76 % with primers (n= 20) employed. Percent polymorphisms within the population were 6.56, 4.92 and 3.28 in Chirapani, Kosi and Gola, respectively (Table.2). Variance in allelic frequencies (na) was observed between and within these three fish population. The Chirapani population had a higher proportion of polymorphisms as compared to other two locations which is in accordance with the present study. Das *et al.* (2005) observed varied range of 42.6, 31.7, 30, 19.2, 16.8 and 14.3% polymorphic loci in six *Labeo* species carp species from Odisha. Similarly, Hatanaka and Galetti (2003) in *Prochilodus marggravii* from three collecting sites of Sao Francisco river (Brasil), Dergam *et al.* (2002) in fresh water fish Hoplias malabaricus (trahira) from Rio Doce lake and Macacu river basin (Brazil); Aranishi and Okimoto (2004) in Pacific Oyster Crassostrea gigas, Thunberg in Hiroshima and Goseong in Japan and Grapputo *et al.* (2006) also employed 3 to 5 random primers in different fish species from different locations and could amplified 31 to 74 total numbers of amplified fragments with a size ranging from 300bp to 1500bp and 10- 20% genetic polymorphism among the population studied. Whereas, Faddagh *et al*. (2012), Liu and Chu-Wu (2007) and Aranishi and Okimoto (2004) observed a high proportion of polymorphisms in among eight cyprinid fish species of Iraqi inland water (84.4%); Hiroshima fish population (86.00 to 92.11%) and 92.29 to 93.32% in Goseong populations, respectively. It is further suggested that the existence of higher proportion of genetic polymorphisms among the fish populations have an important implication for the conservation of the genetic diversity.. However, Yoon and Kim (2001) employed only 5 random primers and observed a maximum of 1344 numbers of amplicon, were Liu *et al*. (1999), observed 462 amplified fragments (200-1500bp) by using 75 primers. Further noticed RAPD- PCR revealed the presence of large number of genetic polymorphisms among *S.richardsonii* population and was a suitable tool to characterize the genetic structure of the population. Thus, RAPD has been used in population studies in fisheries and genetic variation analysis of population with differential degrees of geographical isolation (Shanmughavalli *et al*., 2013).

RAPD can be effectively utilized to differentiate geographically and genetically isolated populations and also 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). The average genetic heterogenicity/ distance was 0.1334, which was more than *S.richardsonii* population (0.122) in Kumaon's river (Sivaraman *et al*., 2010), using RAPD-PCR analysis. The largest genetic distance (0.1565) was obtained between Chirapani and Gola population, whereas the smallest genetic distance was formed between Chirapani and Kosi (0.1058) followed by Kosi and Gola (0.1385) and showed that extensive genetic variation was found between locations. The population genetic differentiation can be driven by ecological, evolutionary and historical factors and the existence genetic differentiation is possibly related to its local adaptation with its distinct environmental conditions and / or inbreeding within the *S.richardsonii* fish population (Hatanak and Galleti, 2003; Hendry *et al*., 2000). The presence of variability among populations as well as individuals within a population is essential for their ability to survive and successfully respond to environmental changes (Ryman *et al*., 1995). Furthermore, the natural water bodies contain metapopulations composed of distinct breeding units (Carvalho, 1993; Hansen and Loeschcke, 1994). Although, further studies are required to obtain repeatable results with high precision by using more number of sample size and primers. The dendrogram showed two clusters formation, one consisting of Chirapani and Kosi and the other was Gola population (Fig. 3). The principal aspect of UPGMA dendrogram was the striking separation of Gola

Table 2 : Within population genetic variability among Indian snow trout fish populations of *S. richardsonii*

Populations	Sample Number	na	ne	h	I	Polymorphic loci
Champawat	20	1.6230	1.4327	0.2466	0.3610	38
(Chirapani Stream)		(0.4887)	(0.3892)	(0.2094)	(0.2983)	(62.3%)
Ratighat	20	1.7049	1.4098	0.2406	0.3619	43
(Uttarbahini River)		(0.4599)	(0.3723)	(0.1952)	(0.2738)	(70.5%)
Ranibag	20	1.6066	1.3585	0.2091	0.3133	37
(Gola river)		(0.4926)	(0.3786)	(0.2022)	(0.2867)	(60.6%)
Mean	20	1.9508	1.4945	0.2962	0.4523	39.3
		(0.2180)	(0.3355)	(0.1596)	(0.2059)	(64.46%)

]SD within parentheses; na= observed no. of alleles; ne= effective no. of alleles; h= Nei's gene diversity'; I = Shannon's Index

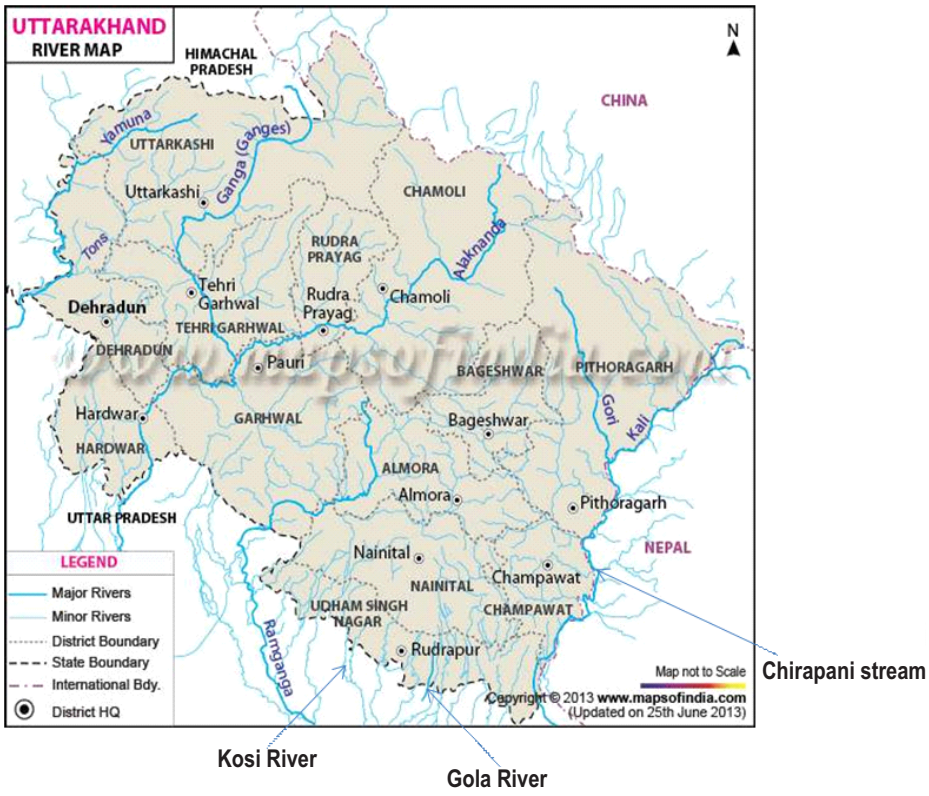
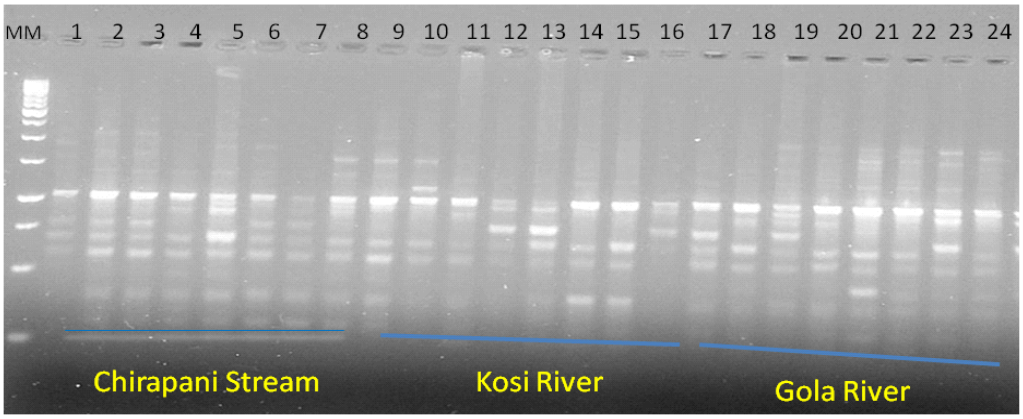


Fig. 1 : Locations of fish samples collection in Himalayan water bodies of Uttarakhand state

RAPD-PCR amplification patterns in the individual samples of *S. richardsonii* populations



Lanes from left to right: MM: double digest λ-Hindi III/Eco RI digest; 1-8: Population 1; 9-16: Population 2; 17-42: Population 3

Fig. 3 : Dendrogram based genetic distance by UPGMA among Indian snow trout fish population

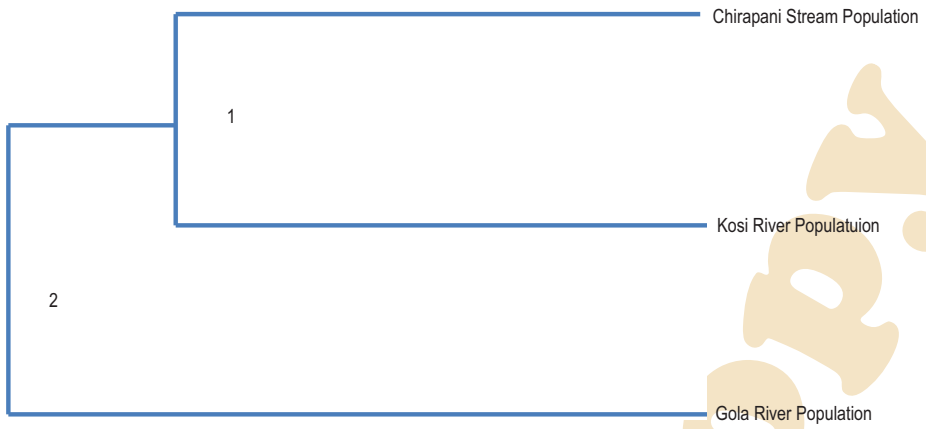


Fig. 2 : RAPD profiles from *S.richardsonii* fish population from three different location using OPY series primers

population from the other two, which were closely grouped. Similar to the present study, Baradakci and Skibinski (1994) studied the phylogenetic variation among three species of tilapia genus *Oreochromis* and four subspecies of *O.niloticus,* and found that this technique would be able to distinguish the tilapia species even at subspecies level and was also more successful than allozyme and mitochondrial DNA analysis. Comincini *et al.* (1998) found that RAPD method could able to distinguish different six species of cervidae at subfamily level and the result is in total agreement with the morphological, paleontological, molecular and cytological studies. Callejas and Ochando (2002) studied eight species of the genus *Barbus* in Iberian Peninsula and exhibits two main branches; one contains Barbus species from North-eastern Spain and Spanish species and second branch contains Atlantic basins and Mediterranean region. Barman *et al.* (2003) in four Indian major carps, *Labeo rohita*, *L.calbasu*, *Catla catla* and *Cirrhinus mrigala* and found that kalbasu is the closest to rohu and farthest from mrigal. Das *et al.* (2005) also studied cluster analysis in six *Labeo* species and observed that two main clusters, one with *L.calbasu*, *L.rohita*, *L.fimbriatus* and *L.gonius* and another with *L.bata* and *L.dyocheilus* and suggested that RAPD could be used for genetic differentiation of closely related species. Aranishi and Okimoto (2004) in cultured populations of Pacific oyster *Crassostrea gigas* from Hiroshima, Japan and Goseong, Korea and dendrogram clearly revealed two separate cluster of Japan and Korea population. Li *et al.* (2007) observed that *L.seabae* and *L.stellatus* were closely related and cluster in one branch whereas, *Lutjanus vita*, *L.fulvus*, *L.fulviflamma* were in other cluster and observed that distinct phylogenetic relationship existed among these species in relation to the evolutionary history or geographic distributions and further suggested that the geological events of freshwater species are important factors for their genetic differentiation and dispersal rate.

The *S.richardsonii* population from 3 different locations was nested spatial design to analyse the pattern of gene flow from the effective migration rates (Nm) among the fish population from the F_{ST} by assuming as island model of migration. This *S.richardsonii* population was split into many geographically isolated subpopulations and each subpopulation was assumed to be sufficient for genetic drift to be negligible. The allelic frequency in migrants among subpopulations was assumed to be equal to average allele frequencies in the overall population. The genetic variation and allelic frequency of migrating populations can vary due to man- made introduction (ranging) and interconnecting natural water bodies. Gst estimate revealed some extent of homogeneity among these three populations from different geographical locations and the lower differentiation rate between populations revealed RAPD data since the priming site at target DNA had higher tolerance to mutation and the RAPD amplicons arose from both coding and non-coding regions (Peng *et al*., 2009). Moreover, in the present study, the lower genetic homogenicity might be attributed to sampling fluctuation (n= 24), local adaptation, inbreeding, genetic drift and subpopulation with distinctive reproductive patterns etc. The estimates of effective number of migrants per generation (Nm) from G_{st} values were 1.81 which further suggested that the gene flow rate in the three populations seemed to be very low and indicative of little migration among these populations i.e. confined to its locations and no man- made interventions had taken place either by the introduction of the same fish species from other locations or releasing the cultured fish into nature. Similar to the present study, Barman *et al.* (2003) observed limited migration rate with low levels of within species genetic variation in four species of Indian major carps (Shanmughavalli *et al*., 2013) in molly fish species, *P.latipinna* and *P.sphenops.* Kim and Sappington (2004) suggested that one genetically effective migrant per generation between populations would be sufficient to prevent fixation of an allele and

significant divergence in allele frequencies between populations can occur up to 10 migrants per generation. Moreover, the presence of large number of polymorphic loci, revealed by RAPD- PCR, allowed the distribution of F_{ST} and linkage disequilibrium to be examined among loci and demonstrated that small samples inflate F_{ST} and linkage disequilibrium. The present study reveals that there was no linkage disequilibrium maintained among the detected 118 loci and might be due to epistasis.

The present finding highlights an important aspect of genetic conservation of indigenous fish population *S.richardsonii in vivo* where in no man-made intervention has taken place.

Acknowledgment

We acknowledge the financial assistance provided by the Indian Council of Agricultural Research, New Delhi, India to carry out this research project.

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