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Diversity of Fish Genetic Resources below the Species Level, Characterization and Applications in Resource Management

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Intraspecific variation includes the genetic and phenotypic diversity found within and among populations that are often threatened by a reduction in population, local extinctions, climate change, and anthropogenic activities. Documentation of these below-species level variations constitutes the primary step towards conservation. High-resolution genetic markers can potentially be deployed to understand the pattern of genetic diversity at intra and inter-specific levels. The inferences drawn from genetic diversity analysis holds many applications, mainly taxonomy/systematics of species, understanding their genetic connectivity, identifying the breeding populations/parentage assignment, fisheries management, and conservation measures including ranching programs for the purpose of genetic rescue and stock enhancement of wild relatives. These variations form strong foundation for creating base populations in a selective breeding program.

With the adoption of novel high-resolution molecular tools and genome-wide approach, a comprehensive set of applications relevant to fisheries management, aquaculture development, biosecurity, and traceability in the supply chain system, can successfully provide momentum to the sectorial growth. This article highlights the significance and need for intraspecific genetic variabilities; and presents lead research undertaken for registering the genetic stocks and elite germplasm in the country, along with significant breakthroughs globally.

Introduction

Intraspecific variation includes diversity within the species, including from its geographic distribution and encompasses temporal persistence, which results in stability of the populations. As the selection pressure is working on it, in addition to changes on random population sizes and genetic drift, its maintenance is important for the ecosystem's ability to adapt and resilient to climate change, there are consequences of human interventions also, on intraspecific variability (Mimura *et al*., 2016). Roches *et al.* (2021) suggested the rate of loss of intraspecific variation is greater than the species loss.

Biodiversity is among the most important resources, globally. Several biotic and abiotic factors contribute to the sustenance of biodiversity. The FAO's Commission on Genetic Resources for Food and Agriculture (CGRFA) envisions the conservation of biodiversity for Food and Agriculture and promotes its sustainable usage for food security, human well-being, and overall development. India has set national targets within the framework of the Aichi targets. National Biodiversity Target 17 encourages the maintenance of genetic diversity of cultivated plants, farm livestock, and their wild relatives, including other socioeconomically as well as culturally valuable species. It also suggests to develop and implement strategies for minimizing genetic erosion and safeguarding their genetic diversity. Similarly, SDG goals 14 highlights to conserve and sustainably use the oceans, seas, and marine resources, and SDG target 14.4 aims to end overfishing, illegal, unregulated, unreported fishing and destructive fishing practices. Genetic stock identification can be a supporting tool in resolving traceability issues, as done in the 'Fish Pop-Trace' Project by the European Union.

The loss of genetic diversity has been recognized as a serious concern among the scientific communities, which is evident from CBD post-2020 draft that includes genetic diversity among the five main goals. The significance of intraspecies diversity was highlighted in a thematic document of FAO (2021), which has given thrust to collating the genetic information, especially below the species level, e.g. stocks and strains. Intraspecific studies advocate the characterization at both levels to form the baseline information. At present, this baseline information is available for the commercially important fish species, while it is still needed for several other potential species.

Documentation of intraspecific variation, at genotype and phenotype levels, forms the primary step towards its conservation. The distribution and pattern of genetic

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variability at intra/inter-specific level are deciphered, through the use of high-resolution genetic markers. Advances in sequencing technologies can potentially provide molecular resources for generating genotypes/haplotypes. The conclusions from genetic diversity analysis are important for multiple applications including conservation and management, understanding genetic connectivity of fish wild stocks/populations, breeding populations, selection programs and also in taxonomy/systematics. The present paper highlights the significance and role of intraspecies genetic variability, in these areas.

Significance of Intraspecific Variation Studies

1. *Genetic Stock Identification*

In India, fish diversity below the species level has been characterized for several species which are important from a conservation or aquaculture point of view. Genetic variability in natural populations of aquaculture species is documented so as to plan its use in domestication, genetic improvement and conservation of the natural gene pool. Earlier, the Indian Council of Agricultural Research (ICAR) initiated a program of registering the genetic stocks and elite germplasm of potentially cultivable fish species both from natural populations and domesticated sources. This was done to formulate the guidelines related to biodiversity and IPR protection. A total of 71 fish/shellfish species found in Indian waters have been genetically characterised, of which 34 are cultivable species. With an aim to integrate genotype information with biological descriptors, the fish/shellfish species characterised by ICAR-NBFGR include *Catla catla, Cirrhinus mrigala, Labeo rohita, L. calbasu, L. dero, L. dyocheilus, L. dussumieri, Clarias magur, Pangasius pangasius, Horabagrus brachysoma, Gonoproktopterus curmuca, Channa marulius, Etroplus suratensis, Harpadon nehereus Pampus candidus, Rachycentron canadum, Sillago malabarica, Scomberomorus commerson, Rastrelliger kanagurta and invertebrates like Hippocampus kuda, H. trimaculatus, Fenneropenaeus indicus, Penaeus monodon, Panulirus homarus, Thenus unimaculatus, Parapenaeopsis stylifera* and *Perna viridis*.

2. *Resource Management*

Validated molecular markers can be suitably applied for tracing the population and this can be a useful approach to curtail illegal, unreported and unregulated (IUU) fishing in the marine environment. 'Fish Pop trace' is an international programme by European Union to address the problems of IUU fishing in their region. In order to satisfy the increasing number of consumers demanding for edible products of certified origin, the European Commission documented three protection labels including the Protected Geographical Indication (PGI) that indicates a connection with the area, where at least one of the production stages possesses a characteristic feature which links to its geographical origin. In the case of *Sardina pilchardus* (*European pilchard*), the region of the fish origin was identified using mitochondrial markers (Gonzalez *et al*., 2007). Similarly, genetic tools for geographic traceability of cods and soles are worked out from European countries (Bylemans *et al*., 2016) and yellowfin tuna (Pecoraro *et al*., 2018) across its range of natural distribution. The ability to trace the fine scale population structuring depends on marker diversity and species population history. Among the marine species, the genetic divergence varies between species, based on its dispersal capacity, larval distribution, breeding season, behavioural characteristics, etc. In addition, oceanographic discontinuities reduce gene flow and environmental characteristics that determine local adaptation, are sometimes undetectable drivers of population differentiation. Such in-depth genetic studies help to define the conservation and management units and give an indication to the policymakers if they are to be managed as single or multiple populations. However, genetic information has not always been incorporated into fishery management and policy decisions in several countries. At this juncture, a consensus among scientists, governmental organizations, and policymakers are crucial in developing and implementing policies for the sustainable management.

3. *Ranching Programs for Genetic Rescue and Stock Enhancement of Wild Relatives*

Interspecific variations can deplete in many fish species, due to over exploitation and habitat destruction of the wild fish populations, "Freshwater fish are under ever increasing pressure with one in three threatened with extinction, according to the new report published by 16 global conservation organizations." (WWF, 2021). In this direction, captive breeding of threatened populations and reintroduction can be a means of recovery efforts or genetic rescue. By ensuring that the stocked population is imbibed with the genetic diversity from wild population, reintegration of the stocked fish will likely be more successful and portfolio effects will be possibly preserved.

ICAR-NBFGR with Uttar Pradesh State fisheries Department has carried out ranching five carp species including Indian major and minor in the protected areas of river Ganga and its tributaries. For ranching advanced fingerling are produced from wild originated individual tagged broodstock maintained at Live Fish Germplasm Resource Centre of NBFGR at Lucknow. A total of 375000 advanced fingerlings have been stocked during 2017 to 2020 in the areas of river falling under protected or religious sanctuaries, which have low human interference. Similarly, stock-specific, breeding-assisted river ranching of two food fishes (*Horabagrus brachysoma* and *Labeo dussumieri*) has also been successful in Kerala and the landings of *H. brachysoma* after two years increased from 1.8% to 11 % and that of *L. dussumieri* showed an increase from 0.68% to 3.9% of the total landings from the Vembanad Lake and adjacent rivers in the state (Padmakumar *et al*., 2011). Recently, further the stocking program is in place by Kerala state department with seed produced from Peninsular and Marine Fish Genetic Resources Center of ICAR-NBFGR at Kochi. Over 250000 fingerlings of endemic species, *H. brachysoma* and *L. dussumieri* were ranched by the department for enhancing their natural populations. Nilgiri catfish, *Hemibagrus puctatus* a species endemic to Western Ghats from river Cauveri, considered extinct for over twenty years, could be an example for genetic rescue in future. The broodstock raised through wild collection has been bred in captivity and with propagation technology developed, it will be a candidate for ranching and stocking in the native distribution range.

4. *Selective Breeding*

Selective breeding has great potential for genetic improvement of economic traits in aquatic species. Compared to livestock, where the bulk of production comes from less than a dozen species, aquaculture circumvents not less than five dozen species to support nutritional security. The selective breeding program has a prerequisite of the creation of a base population, for which the estimates of genetic diversity and genetic parameters are required. The base populations are mostly selected based on the phenotypic and genotypic performance and evaluation. Genotypic performance evaluation is achieved based on the intra-specific variability studies. Selective breeding has been used largely in European aquaculture and several breeding programs majorly, on growth performance, are ongoing. Of total aquaculture produce in Europe, about 80-83% was obtained from selective breeding (Janssen *et al*., 2017). This includes six major cultured species, salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*), gilthead seabream (*Sparus aurata)*, European seabass (*Dicentrarchus labrax*), common carp (*Cyprinus carpio*) and turbot (*Scophthalmus maximus)*. In rohu carp (*Labeo rohita*), a collaborative program of ICAR-CIFA with the Institute of Aquaculture Research (AKVAFORSK), Norway resulted into a genetic gain of about 17% per generation for growth performance (Jayanti rohu), which is still demonstrating consistent results even after nine generations. The program used base population from five riverine sources for providing broader genetic base to founder individuals. Similarly, in order to develop base population for *Labeo catla,* another major carp, phenotypic and microsatellite marker were used for optimizing the proportions of individuals from each geographic location. The estimates of genetic differentiation and partitioning of genetic variance displayed adequate genetic heterogeneity for selection of populations.

Currently, the black tiger shrimp, *Penaeus monodon,* is being considered for re-introduction into aquaculture, as an alternate species for Indian shrimp farming industry. The Govt. of India has also recognized the need for species diversification for nutritional security and has entrusted RGCA, MPEDA to take lead through PMMSY. In this context, the domestication of *P. monodon* is being carried out at RGCA. The founder populations for developing the SPF domesticated stock were selected on the basis of their diversity profiles, investigated through the molecular markers (Mandal *et al*., 2012). The population for Andaman and Nicobar Islands was identified as founder populations based on the heterozygosity information.

5. *Parentage Assignment*

Success of a breeding program largely depends on ability to minimise inbreeding levels, which requires prior knowledge genetic diversity estimates, below the species level. The molecular markers, particularly microsatellites and SNPs, are used to trace inherited alleles in offspring from a pool of brooder parents and thereby, make an effective mode of pedigree management. Recently, molecular panels were deployed to ensure that white-leg shrimp (*Penaeus vannamei*) lines belonged to multiple genetic sources. Similarly, microsatellite panel was developed for meagre (*Argyrosomus regius*) for parental assignment (Vallecillos *et al*., 2022). In marine species, parentage assignment was explored, Atlantic cod *Gadus morhua* and sole, *Solea solea* (Bylemans *et al*., 2016). For a large variety of commercially reared marine species, escapees and deliberate releases have been reported. Such studies are focused mainly on salmonids and several other important species. In such scenarios, developing baseline genetic information becomes pertinent for aquaculture species.

Way Forward

The characterization of diversity below-the-species level of cultivable fish species is required for a variety of applications, as mentioned above. However, characterization of biodiversity should be extended to many commercially/evolutionary important species through integrative novel technologies of phenomics and genomics. It would help in large scale data collection and analysis at both the levels, for better understanding of the complex association between genotypes and phenotypes, as well as hidden/cryptic variability. Furthermore, scientific evidences indicate the need for adopting the holistic approaches incorporating ecosystem services and functions.

Fishes usually adapt to the changing environmental conditions in response to the climate change scenario and these adaptations are reflected at the genomic level also. Identifying evolutionarily significant units (used to delineate fish stocks), determining connectivity among locations (useful for defining marine protected areas), and generating genetic traceability tools (which allow for assigning individuals back to their birth areas in the case of mixed stock fisheries) needs attention in the changing scenario of fisheries environment. Validated molecular markers can be suitably applied for tracing the population and this can be a useful approach to curtail illegal, unreported and unregulated (IUU) fishing in the marine environment. One example is ‘Fish Pop trace’, an international programme by European Union, to address the problems of IUU fishing in their region,

The advent of newer tools such as environmental DNA (DNA) monitoring the distribution and relative abundance of aquatic organisms, from microbes to sharks and whales, from water samples has made it more useful. This may be proved to be a fast and cost-efficient method and a prerequisite for ecosystem-based management of fishery resources, for monitoring at all trophic levels.

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