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Relationships between genetic variability and life-history features of fishes based on S7 ribosomal protein gene

Tangjie Zhang*

College of Veterinary medicine, Yangzhou University, Jiangsu Yangzhou 225009, (CHINA)

E-mail: slx@yzu.edu.cn

ABSTRACT

To research relationship between genetic variation and life-history variables of *Actinopterygii*, as indicated by common length, maximum length, maximum weight and longevity, and environmental variation, as indicated by three different fishes' living environments, we applied analysis of independent regression and phylogenetically-independent contrasts methods to evaluate life-history variables correlations with rps7 neutral genetic diversity. Polymorphism datasets of rps7 gene, belonging to 48 genera, 25 families and 9 orders, of *Actinopterygii*, was obtained from Polymorphix and Popset of GenBank. Life-history variables were obtained from the AnAge database and fishbase. The results showed that neutral genetic diversity of fishes is significantly negatively associated with common length. No strong level of correlation was found between fish's neutral genetic diversity and maximum size, maximum weigh or maximum longevity. No correlation was found among neutral genetic diversity and fishes' habits (marine, freshwater and marine-freshwater) either.

KEYWORDS

Genetic diversity;
Actinopterygii;
Rps7;
Life-history.

INTRODUCTION

There are two principal types of genetic diversity: adaptive and neutral. Interpreting the genetic diversity in organisms with diverse life and population histories can be difficult since the mechanisms and processes that regulate this diversity are complex and still poorly. The causes of variation could be sample error, variance in gene flow, natural selection, genetic drift and so on. The relative importance of these variation factors in shaping the genetic structure of a population could depend on the life history of the species. For example, to explain mtDNA substitution rate variations, there are some hypotheses-related to life-history- have been proposed: the generation time, the metabolic rate, and the longevity have been advanced to explain variation in DNA substitution rate[28]. In *Actinopterygii*, some studies showed that in marine fishes, the level of genetic diversity is higher and the level of population divergence is lower than in freshwater species, most likely due to the better dispersal capabilities and larger effective population sizes of marine species[14, 37, 11].

Appropriate DNA markers must be selected for the phylogenetic and genetic diversity analyses of

fishes. Mitochondrial DNA (mtDNA) is one of the most widely studied genetic markers in the fields of population genetics and phylogeography. However, a more serious criticism is that mtDNA, which is more prone to hitch-hiking because of the lack of recombination, may not conform to neutral expectations[4, 13]. The RPS7 gene, which belongs to a family of genes called RPS (ribosomal proteins), has been the most popular marker[29, 26, 20] of nuclear neutral molecular diversity in fishes for its high similarity in taxa[6]. In this study, the polymorphism of fish RPS7 gene was therefore used to be analyzed for fish's molecular diversity.

The DNA substitution rate is widely used for the studies of genetic diversity, but accurate estimation of mutation rate is obviously required for accurate molecular dating. Another most fundamental in population genetics is the mutation parameter or genetic diversity $\theta=4Ne\mu$ under the assumption that mutations are effectively neutral. Here μ denotes the expected number of mutations for an individual DNA sequence per generation and Ne denotes the effective population size. The estimate of θ is based on coalescent theory. Watterson's estimator (θ_w,) is commonly used for its simplicity. Under the standard neutral model, mutation rates will definitely positively lead to polymorphism levels. Definitely, the analysis of nuclear genes' polymorphism, which are based on increasing molecular genetic data, are useful to understand the mechanism of molecular diversity because they offer broad and comprehensive coverage.

Molecular genetic data have greatly improved our ability to test hypotheses about organisms' evolution. A large amount of nuclear data has been collected from diverse organism's populations dramatically during past decade or so. In this study, we analyzed genetic diversity by surveying polymorphism of fish's rps7 gene. Our goal of this work was to ascertain if there is a relationship between single nuclear gene (rps7) neutral diversity and life-history variables, such as common length, maximum length, maximum weight and longevity, presented above, and a relationship between fish's living environments and neutral genetic diversity.

MATERIALS AND METHODS

Data collection

Polymorphism datasets of rps7 gene was obtained from Polymorphix[3] and Popset of GenBank. Two sequences were taken not to be clustered if there was a mismatch of >50nt with <80% similarity flanking the CLUSTALW program[35]. Such a mismatch is interpreted as evidence that the sequences represent duplicate genes in distinct genomic contexts. Sequences were inspected by eye and corrected when required.

Data: 146 species of fish belonging to 48 genera and 25 families and 9 orders. Each group was aligned by eye using CLUSTALW. Details of the species sampled are listed in Appendix S.

Polymorphism sequence data analyses

Genetic diversity measures -Watterson's estimator (θ_w) were calculated separately from the intron and synonymous sites of the analyzed fragments of same species, expressed in per-site level of diversity, and the no. of mutation and sites both synonymous and nonsynonymous sites were counted simultaneously.

Life history data

Many researches have been done on life history traits in fishes. Kawasaki (1980) suggested that the grouping of life history traits of fishes differed from the traditional methods developed for terrestrial animals[19]. In this study, common length, maximum length, maximum weight and longevity, data were obtained from the AnAge database[10] and fishbase (http://www.fishbase.org/home.htm).

Body length or size was usually used as a proxy for abundance, given the expected negative relationship between body size and abundance. Indeed, a significant negative relationship between body size and genetic variation was found in mammals based on allozymes[36]. We got two types of body length, common length and maximum length. Using common length as a proxy for abundance allowed us to increase the number of species evaluated, as catch data are only available for a limited number of species.

Statistical tests

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θ_w were arc-sine transformed[34]. Quantitative life-history variables were log transformed. We first calculated θ_s for synonymous sites and θ_i for intron sites and weighted average of θ_s or /and θ_i from rps7 for same species. Analysis of independent regression methods was applied to evaluate one or more life-history correlations with neutral genetic diversity (θ) (synonymous and intron sites).

Phylogenetic reconstructions

We created a phylogenetic hypothesis for the species included in this study by grafting them onto a higher-level phylogenetic supertree of *Actinopterygii* using PhyloWidget[16]. The topology was a composite of information drawn from TreeBASE (http://www.treebase.org/treebase-web/home.html). Phylogenetically-independent contrasts (PIC) were conducted using Phylogenetic Comparative Methods of COMPARE, version 4.6b[23].

RESULTS

Neutral genetic diversity of fishes based on rps7

A smaller amount of life-history data is available especially for max weight, which documented only 17 species in 146 fish species. We thus focused on the effects on maximum size and common length. The common length, however, could been taken as a proxy of population size[21, 29].

To correlate life-variables to all neutral sites' diversity of sampled S7 ribosomal protein gene, we weighted average θ by combining intron with synonymous and performed nonphylogenetic regression (logistic regressions) analyses and phylogenetically regression of independent contrasts. Both the application of phylogenetically independent contrasts and logistic regression analyses showed that θ_w of weighted neutral sites was significantly negatively associated with common length(n=67, p=0.031, n=67, p=0.011). No strong level of correlation was found between θ_w of weighted neutral sites and maximum size, maximum weigh or maximum longevity (TABLE 1).

To investigate the relationship between fish's living environments and neutral genetic diversity, we correlated environments to all neutral sites' diversity of sampled S7 ribosomal protein gene on the basis of three entirely different environments: marine, freshwater and marine-freshwater (TABLE 1). The result showed that no correlation was found between neutral genetic diversity and fish's habits (n=143, p=0.120).

DISCUSSION

Early studies have revealed marked differences in the level of genetic differentiation and genetic diversity between populations of marine and freshwater species, with marine species generally exhibiting lower levels of inter-population differentiation and higher genetic diversity[14, 37, 11]. This general observation has generally been hypothesized to be a result of higher effective population sizes and/or higher inter-population migration rates in marine, compared to freshwater environments and has implications for the conservation of genetic diversity. Lower effective population sizes and/or lower in-

TABLE 1 : Single variable regressions of weighted neutral (introns and synonymous) sites

			Non-phylogenetic			Phylogenetic regression of independent contrasts		
	Trait	**N**	**Slope**	**R^2**	***p*-value**	**Slope**	**R^2**	***p*-value**
	Max size	130	-0.001	0.024	0.078	-0.002	0.036	0.05
	Common length	67	-0.001	0.069	0.031*	-0.001	0.087	0.011*
θ_w	Max weight	17	0.000	0.023	0.545	-0.001	0.053	0.865
	Max Longevity	36	0.001	0.020	0.408	0.002	0.013	0.565
	Environment	*143*	*-0.001*	*0.007*	*0.310*	*-0.002*	*0.008*	*0.120*

ter-population migration rates in the freshwater environment predict that populations of freshwater species are expected to be more prone to extinction than marine species and thus should be of particular conservation concern.

Gene diversities of neutral molecular markers are most influenced by effective population size (Ne)[9, 24]. Diversities are expected to be high in large, stable populations because the magnitude of random drift is less, leading to the retention of a larger proportion of existing diversity and a greater number of new mutations. Although early studies have also indicated that marine fish species have low levels of population genetic differentiation when compared to freshwater species[37, 11], more studies have shown deep divergences on marine species populations. In some cases, the degree of differentiation is so high that speciation events are proposed[8, 27, 31, 7, 5]. Marine organisms disperse much more due to high connectivity between their habitats. Therefore, the degree of genetic differentiation in this environment is attenuated. However, there are some other limits (spatial, directional or temporal) to dispersion that may promote the genetic differentiation in marine organisms. Amongst the different types of limits are physical barriers such as patterns of oceanic circulation, water temperature, gradients of salinity, restrict dispersion of eggs, larval or adults, phylopatry, selection and historic events such as glaciations[33, 30].

The assumptions on the relationship between body weight/length and genetic polymorphism for certain loci, which were confirmed by correlation analysis in small samples based on isozymes, were made[17, 18, 12]. Under natural conditions, one indeed could expect a strong negative relationship between the body length and the proportion of polymorphism[1, 2].

In this study, examination of neutral genetic diversity was based on S7 gene DNA polymorphisms. It was shown that neutral genetic diversity of fishes, measured by θ_w, Watterson's estimator, is significantly negatively associated with common length. Compared to longer length fishes, shorter fishes display higher rates of generation change, resulting in accelerated growth of population size and faster accumulation of genetic variability, which was also found in Vertebrates[12, 25]. Based on common length, used as a proxy for effective population size, our result was with consistent the concept that genetic diversity showed higher in large populations, although this concept has been challenged[28, 4]

Metabolic rate hypotheses proposed that the production of mutagenic free radicals, reactive oxygen species (ROS), increases with increasing rates of respiration; therefore, so does the rate of mutation[25, 22]. Taking body weight as a proxy of metabolic rate, the results of this study show that there was no significant correlation between θ_w of fish weighted neutral sites and metabolic rate. Neither did we find a significant correlation between neutral fish diversity and longevity after multiple regression analyses, which indicated that common length was the most crucial factor of all variables included in affecting nuclear neutral diversity.

Anadromy is a life-history trait of fishes that refers to migration from a freshwater breeding habitat to a marine feeding habitat and back to freshwater for spawning. Several summaries of genetic diversity in fishes have been published[14, 37, 11]. These have shown that there is some evidence the level of genetic diversity and population divergence of anadromous species is intermediate to that of marine and freshwater species. This seems logical given that, for example, the level of between populations migrations may be expected to be higher in rivers connected to the ocean compared to completely isolated water bodies. There, however, has been little statistical support for some of these findings, most probably due to the small number of anadromous populations assessed and to the fact that earlier studies have compared genetic diversity indices based on completely different sets of loci. In this study, we also found there is no correlations between neutral genetic diversity and habits (marine, freshwater and marine-freshwater), even though the anadromous fishes (10 species) sampled were limited.

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Appendix S Species sampled for analyses of rps7 gene polymorphism

Order	Families	Genera	Species
Aulopiformes	Haemulidae	Haemulon	Haemulon parra
			Haemulon scudderi
Cypriniformes	Balitoridae	Oxynoemacheilus	Oxynoemacheilus bureschi
	Cobitidae	Cobitis	Cobitis bilineata
			Cobitis elongata
			Cobitis ohridana
			Cobitis taenia
			Cobitis tanaitica
	Cyprinidae	Codoma	Codoma ornata
		Dionda	Dionda episcopa
			Dionda ipni
			Dionda melanops
			Dionda nigrotaeniata
		Gobio	Gobio carpathicus
			Gobio caucasicus
			Gobio gobio
			Gobio insuyanus
		Lepidomeda	Lepidomeda aliciae
			Lepidomeda copei
		Notropis	Notropis calientis
		Pimephales	Pimephales notatus
			Pimephales promelas
			Pimephales tenellus
		Richardsonius	Richardsonius balteatus
			Richardsonius egregius
		Rutilus	Rutilus frisii
		Tinca	Tinca tinca
		Tribolodon	Tribolodon brandtii
			Tribolodon hakonensis
		Yuriria	Yuriria alta
		Chondrostoma	Chondrostoma duriense
			Chondrostoma nasus
			Chondrostoma polylepis
			Chondrostoma toxostoma
			Chondrostoma willkommii
Cyprinodontiformes	Cyprinodontidae	Poecilia	Poecilia dauli
			Poecilia gillii
Gasterosteiformes	Macroramphosidae	Macroramphosus	Macroramphosus scolopax
	Syngnathidae	Hippocampus	Hippocampus mohnikei
			Hippocampus reidi

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Order	Families	Genera	Species
Osmeriformes	Osmeridae	Hypomesus	Hypomesus japonicus
			Hypomesus nipponensis
			Hypomesus olidus
			Hypomesus pretiosus
			Hypomesus transpacificus
		Osmerus	Osmerus eperlanus
			Osmerus mordax
		Spirinchus	Spirinchus lanceolatus
			Spirinchus starksi
		Thaleichthys	Thaleichthys pacificus
osteoglossiformes	Mormyridae	Campylomormyrus	Campylomormyrus compressirostris
			Campylomormyrus numenius
			Campylomormyrus rhynchophorus
			Campylomormyrus tamandua
Perciformes	Blenniidae	Salaria	Salaria economisidi
			Salaria fluviatilis
			Salaria pavo
	Tripterygiidae	Tripterygion	Tripterygion delaisi
			Tripterygion melanurus
			Tripterygion tripteronotus
	Blenniidae	Coryphoblennius	Coryphoblennius galerita
	Centrarchidae	Pomoxis	Pomoxis nigromaculatus
	Chaenopsidae	Acanthemblemaria	Acanthemblemaria macrospilus
	Cichlidae	Lepidiolamprologus	Lepidiolamprologus kendalli
			Lepidiolamprologus nkambae
	Clinidae	Clinus	Clinus cottoides
			Clinus superciliosus
		Muraenoclinus	Muraenoclinus dorsalis
	Gobiidae	Gillichthys	Gillichthys mirabilis
			Gillichthys seta
		Mesogobius	Mesogobius batrachocephalus
		Neogobius	Apollonia fluviatilis
			Apollonia melanostoma
		Proterorhinus	Proterorhinus semipellucidus
			Proterorhinus marmoratus
			Proterorhinus semilunaris
		Neogobius	Neogobius eurycephalus
			Neogobius gorlap
			Neogobius gymnotrachelus
			Neogobius Kessler
	Haemulidae	Anisotremus	Anisotremus caesius
			Anisotremus davidsoni
			Anisotremus davidsonii
			Anisotremus dovii
			Anisotremus interruptus
			Anisotremus moricandi
			Anisotremus pacifici
			Anisotremus scapularis
			Anisotremus surinamensis
			Anisotremus taeniatus
			Anisotremus virginicus

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Order	Families	Genera	Species
	Labridae	Halichoeres	Halichoeres bivittatus
		Thalassoma	Thalassoma hardwicke
			Thalassoma jansenii
			Thalassoma quinquevittatum
	Nototheniidae	Pagothenia	Pagothenia borchgrevinki
		Trematomus	Trematomus bernacchii
			Trematomus eulepidotus
			Trematomus hansoni
			Trematomus loennbergii
			Trematomus newnesi
			Trematomus nicolai
			Trematomus pennellii.fasta
			Trematomus scotti
	Percidae	Crystallaria	Crystallaria asprella
			Crystallaria cincotta
		Etheostoma	Etheostoma basilare
			Etheostoma blennioides
			Etheostoma burri
			Etheostoma caeruleum
			Etheostoma camurum
			Etheostoma chlorobranchium
			Etheostoma derivativum
			Etheostoma fragi
			Etheostoma longimanum
			Etheostoma maculatum
			Etheostoma mariae
			Etheostoma microlepidum
			Etheostoma nigrum
			Etheostoma obeyense
			Etheostoma olmstedi
			Etheostoma perlongum
			Etheostoma planasaxatile
			Etheostoma podostemone
			Etheostoma punctulatum
			Etheostoma rufilineatum
			Etheostoma sanguifluum
			Etheostoma simoterum
			Etheostoma smithi
			Etheostoma spectabile
			Etheostoma susanae
			Etheostoma uniporum
			Etheostoma virgatum
			Etheostoma vitreum
		Percina	Percina burtoni
			Percina caprodes
			Percina fulvitaenia
	Scaridae	Scarus	Scarus ghobban
	Gobiidae	Zosterisessor	Zosterisessor ophiocephalus
Pleuronectiformes	Pleuronectidae	Hypsopsetta	Hypsopsetta guttulata
Siluriformes	Pimelodidae	Pseudoplatystoma	Pseudoplatystoma corruscans
			Pseudoplatystoma fasciatum
			Pseudoplatystoma metaense
			Pseudoplatystoma orinocoense
			Pseudoplatystoma reticulatum
			Pseudoplatystoma tigrinum

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