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Genetic Structure and Biogeography of Asian Arowana (*Scleropages formosus*) Determined by Microsatellite and Mitochondrial DNA analysis

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Abstract

The Asian arowana (*Scleropages formosus*) is distributed in Southeast Asia and highly endemic to many river systems. Genetic structure of five strains of arowana was assesed. Twenty-nine microsatellite loci were screened to assess the short-term genetic differentiation. Sequences of *ATPase* subunit 6 and 8 were obtained to estimate the time of divergence. Microsatellite data yielded high value of F_{ST} between each strain. The gene tree constructed based on microsatellite data shows that the Asian arowana is a monophyletic group with two lineages. The green arowana is the outgroup and has a closer relationship with Indonesian gold arowana. All the haplotypes had unique *ATPase* sequences. Sequences of the *ATPase* gene of the arowana were not as variable compared with microsatellites. The mtDNA data yielded a gene tree of different topology as compared to that obtained from microsatellites. The arowana consists of a monophyletic group of mtDNA with three different lineages which represent three different colors red, green and gold. The red arowana is the outgroup but phylogeny was not fully resolved for the gold strains. The phylogenetic structure derived from mtDNA is however not associated with geographical regions. The divergence of the different color strains of arowana was backdated to between the late Pliocene to late Pleistocene era. It is believed that the arowana dispersed in Southeast Asia when Sundaland was formed. The fluctuation of sea level during Pleistocene separated the Indonesian islands with the Southeast Asian mainland and caused the arowana to diverge into distinct strains.

Introduction

Molecular genetic markers have become a well-established and valuable tool for many applications in population genetics, conservation biology and evolutionary studies as well as for mapping projects (Queller et al. 1993: Jarne and Lagoda 1996). These markers have been used to estimate effective population size (Kuhner et al. 1998), past bottlenecks (Luikart and Cornuet 1998), sex-specific gene flow (Latta and Mitton 1997), founder contributions (Carvajal-Carmona et al. 2000) and historical and geographical relationships between groups (Cruzan and Templeton 2000). Different markers are better suited to approach different questions. Selecting appropriate genetic markers is important for a population genetics survey (Sunnucks 2000).

Mitochondrial DNA analysis has become a widely used technique for many applications in population or evolutionary studies (Avise 1986; 1989). Since mtDNA substitution rates are homogenous across lineages, the date of divergence can be estimated based on genetic distance data (Bermingham and Avise 1986).

Due to their exceptional variability and relative ease of scoring, microsatellites are now generally considered the most powerful genetic markers. It is typical to observe loci with more than 10 alleles and heterozygosities above 0.60, even in relatively small samples. Microsatellites are now rapidly replacing RFLPs and RAPDs in most applications in population biology, from identifying relatives to inferring demographic parameters (Bowcock et al. 1994).

The Asian arowana (*Scleropages formosus*) is also known as *dragonfish, Asia Bonytongue, kelisa* or *baju rantai*. It is a primitive fish from the Jurassic era (Bonde 1979). This species is distributed in Peninsular Malaysia, Sumatra, Thailand, Cambodia and Kalimantan. There are four commercial varieties of Asian arowana. Green arowana is found in Malaysia, Thailand, Vietnam and Myanmar (Kottelat et al. 1993). In Malaysia this variety is distributed in Terengganu, Pahang and Johor (Ng and Tan 1999; Suleiman 1999). Malaysian gold arowana is native to Bukit Merah Lake, Perak (Suleiman 1999). The scales may have different shades of colors such as gold, silver or blue. Indonesian gold arowana is found in Sumatra of Indonesia (Dawes et al. 1999). The scales are copper-gold in color. Scales above the lateral line, dorsal fin and upper half of its tail are dark green. The lower half of its tail fin, dorsal fin and anal fin have purplish-red to brownish-red color. Red arowana, the most well known variety is found in Kalimantan, Indonesia (Dawes et al. 1999).

The Asian arowana consists of geographically isolated strains distributed in Southeast Asia. More work is needed to understand patterns of genetic structure and process of diversification in arowana. In this study, the highly variable microsatellites provide a perspective on the diploid nuclear structure of each arowana strains and the less variable mitochondrial DNA sequences provided a phylogenetic perspective.

Material and Methods

Samples

Five strains of arowana were used in this study. The samples comprised of green (N=20), Malaysian red-tail gold (N=22), Malaysian yellow-tail gold (N=23), Indonesian gold (N=11) and Indonesian red arowana (N=15).

DNA extraction, PCR and sequencing

Approximately 30 mg of each scale was used per extraction. Samples were lysed with TNES-Urea buffer (Asahida et al. 1996) and Proteinase K (0.8 mg). The mixture was incubated for 15 h at 50°C followed by conventional phenolchloroform extraction.

All strains were examined for genetic variations at 29 microsatellite loci. Twenty-one loci were described by Yue et al. (1999). Eight loci were isolated from a microsatellite-enriched library using the method of Fisher et al. (1996) and primers were designed for these loci (Sivananthan 2003). Primers for loci D01 to D95, except D35 were suggested by Yue et al. (1999). Primers for locus D35 were redesigned to avoid nonspecific products. Details of all microsatellite loci and PCR condition are given in table 1. PCR amplification for microsatellites was performed on a Hybaid thermal cycler in a total volume of 25 ml. Reactions contained 1x PCR buffer (Promega), 1.5 µM $MgCl_2$ 200 µM of each dNTP, 0.2 µM of each primer (Table 1), 1U *Taq* polymerase (Promega) and 20 ng of genomic DNA. Amplification was carried out using 4 min of initial denaturation followed by 33 cycles of 30s of denaturation at 94°C, 25s annealing at the temperature detailed in table 1 and 25s extension at 72°C with a final extension period of 5 min at 72°C. PCR products were run on a 3.5% (w/v) 1 x TBE horizontal metaphor agarose gel containing 0.5 µg/mg ethidium bromide, at 90 V for 3 to 4 hours.

Phylogenetic study on arowana was based on the nucleotide sequences from the protein coding *ATPase6* and *ATPase8* genes. Two specimens from each strain were used in this study. Two primers ATP8.2L (5'-AAAGCRTYRGCCTTTTAGC-3') and COIII.2H (5'-GTTAGTGGTCAK GGGCTTGGRTC-3') (Sivasundar et al. 2001) were used to amplify an approximately 950 bp fragment flanking these two genes. PCR amplification was carried out in a 25 ml reaction mixture containing 2.5 ml of 10x PCR buffer (Roche), 1.5 µM $MgCl_2$, 1U of *Taq* DNA polymerase (Roche), 10 pmol of each primer, 0.4 µM of dNTPs and 1 to 1.5 ml of template DNA. Amplification was performed on an Eppendorf Mastercycler with the following cycles conditions: 2 min at 94°C, 30 cycles of 1 min at 94°C, 30s at 50°C and 45s at 72°C and the final extension for 8 min at 72°C. The amplified products were sequenced directly using the BigDye Terminator cycle sequencing kit (Applied Biosystem Inc.) with an ABI DNA Sequencer 373A (Perkin-Elmer). Sequencing reactions were carried out as recommended by the manufacturer.

Analysis

Genetic distance between strains was measured by calculating F_{ST} (Weir and Cockerham 1984), using ARLEQUIN version 2.000 software (Schneider

Table 1. Primer sequences of 29 microsatellite loci.

Locus	Repeat motif	Primer sequences (5'-3')	Genebank Accession no.	Annealing Temperature
D01	$(CA)_{10}$	F GAATGCTTAAAGTGGCAGTGAA R CTGGCCTTACGCCCTGTGTTAC	AF219951	57°C
D04	$(GT)_{41}$	F GCTTAAACCCATTACAGACAGG R TTTCTTCATGCAAAACCACTTT	AF219952	55°C
D11	$(GT)_{16}$	F TGGTTTCCACCTACAGTCCAAAGA R GTTACGAGTATCTGGCCCAATGG	AF219953	55°C
D13	$(GT)_{12}(CT)_{23}$	F AGCTGCTGTGTCTGTGGTGGTCTA R CATGCCCATGGAGAGGGAGAG	AF219954	55°C
D14	$(CA)_{12}$	F AAGGGAGCAGCAGTTAGGTAGACG R CCGTGGTGAATTAACATTTCCTCT	AF219955	55°C
D15	$(GT)_{16}$	F GACTGGCGTCCCGTCCTG R AAGGCCTTTTCTGCTGGTAA	AF219956	50°C
D16	$(GT)_{20}$	F CTTGCGCCCTGTGTTGC R AAGGCCTTTTCTGCTGGTAA	AF219957	55°C
D27	$(CA)_{17}$	F GTGTCAGTATAGTGAATCTGTAG R TGACAATGGCAGCATAATGAGAT	AF219958	55°C
D31	$(GATA)_{15}$	F GTTTGTCCCTCCATGCACTGAGAG R GTGATTGCCACATGGTTTTGTTGG	AF219959	55°C
D32	$(CA)_{13}$	F AGCACCCTGTTACTGGAAGAGA R AGTGTGATGCTTTTGCTTTGAGAA	AF219960	55°C
D33	$(CA)_{12}AAC(CA)_4$	F TATTACCATGCGCCCAGCACAC R TGGGTGAGCCAGAAGCAGGACT	AF219961	60°C
D35	$(GT)_{17}$	F CTGGTTTCCTCCCACACAGT R GCCCACACACCTTATTCACC	AF219962	55°C
D37	$(GT)_{51}$	F GCCTTACGCCCTGTGTTGC R TGGATATCTGTGAGTGGTGGTGAA	AF219963	57°C
D38	$(GT)_{24}$	F TTGGGGTCATGCCACTGG R CAATAAATACCAAACAGGGAACC	AF219964	55°C
D42	$(CA)_{19}$	F AGGAACATCACTGACAACACT R TGGACTAACTAGGAGCACAT	AF219965	50°C
D72	$(CA)_{14}$	F AGCAGGTTAATTTGGAGACT R CGACCCTGTATGGGACAAG	AF219966	50°C
D85	$(CA)_{10}$	F GTTCCACAGGGGCTGAGAAAAT R GAGGACGGAACAAAAGCATTGG	AF219967	55°C
D88	$(GT)_{11}$	F TTTCTTTCTGAGACTGAGG R CAACTCTTATCCACCATTT	AF219968	50°C
D92	$(GT)_{13}$	F AGTCGCACACCACCACCTCGA R TCAGCGATAACCCCACACCT	AF219969	55°C
D94	$(CA)_{16}$	F CAGCAGCAGTGACACGGGTTCG R TCGCAGGCTGATTAAAGGTGTG	AF219970	62°C
D95	$(CA)_9$	F CCTGCGGAAGAAGAAAAGACT R CATGGTGTTGGCTGTGAGGAG	AF219971	55°C
K10	$(CA)_{20}$	F GCACCTAACTGAAGAGCATT R AAAATTACCTGCTTGTGTGC	AY173130	57°C
K13	$(CA)_5CG(CA)_3$	F GCACTGTTAAGTTCTGGTGTC R GATACGCATGACATTCTGTG	AY173131	51°C
K16	$(TG)_5$	F CAGTGGTTGCACACTTACAG R AAAGTCGGCATGATGAAATA	AY173136	50°C
K17	$(CA)_6$	F ATATTTCATCATGCCGACTT R TGGTATTTTCTCGTGCATTA	AY173137	50°C
K20	$(CA)_8$	F AGCTGACACTTTGAAGCACT R GTGCTAATTCAGCGACTCTT	AY173134	53°C
K27	$(CA)_{16}$	F CCATTAACCCCTTGTCCTCA R AAGGATGCAGGAGAGCAAAA	AY173135	50°C
K29	$(TG)_5$	F CCCAGTGGTTGCACACTTAC R TGAAAGGAATTTTCAAGGGTTT	AY173133	51°C
K37	$(CA)_4$	F CCATTAGCAAACCCATGCTT R TGGAAATGTGTCATCCTTCAG	AY173132	51°C

et al. 2001). Probability associated with F_{ST} values was evaluated through random permutation procedure (minimum 1000 permutations). A dendrogram of relationships among strains was constructed from the F_{ST} matrix using neighbor joining algorithm (Saitou and Nei 1967) in the MEGA2 program (Kumar et al. 2001).

Sequence alignment of *ATPase6* and *ATPase8* sequence data was performed using Clustal W with gap penalty (5 to 10) and gap length penalty (1 to 5) (Gibson et al. 1996). Percentage of each nucleotide substitution were calculated. The genetic variance among the five color varieties was examined using an analysis of molecular variance (AMOVA; Excoffier et al. 1992) in ARLEQUIN version 2.000. Significance of the estimated f_{ST} value was tested by 1000 times permutation.

Pairwise divergence among arowana was estimated based on individual mtDNA sequence data using the MEGA2 program. Sequence divergence was estimated using the Tamura and Nei (1993) model because of the existence of nucleotide bias in the sequences. Since substitution rates varied at different sites, the gamma correction was applied to the distance estimation with 0.5. A phylogenetic tree topology based on the distance measure was constructed according to a neighbor-joining method (Saitou and Nei 1987) in the MEGA2 program. Tamura and Nei (1993) distance method with gamma correction 0.5 was used to calculate the topology of the phylogenetic tree, 500 bootstrap replicates were used to assess the confidence in the phylogenetic estimate.

Results

From this study, F_{ST} values in table 2 ranged from of 0.2462 between Malaysian yellow-tail gold and Malaysian red-tail gold arowana to 0.4728 between green arowana and red arowana. All pairwise F_{ST} values ($P < 0.05$) were significantly different from zero. This revealed relatively strong genetic differentiation among all strains. The green arowana was the most highly differentiated strain (average F_{ST} = 0.4038), followed by red arowana (average F_{ST} = 0.3830).

The neighbor-joining (NJ) tree in figure 1 obtained by microsatellite data displays the relationship among the five strains of arowana. The tree topology indicates that these five strains of arowana were monophyletic and there were two distinct lineages. The green arowana was an outgroup and formed a sister clade with the Indonesian gold. The red and tow strains of Malaysian gold arowana formed another lineage.

A 842 bp length of DNA containing the *ATPase6* and *ATPase8* subunits were sequenced and 10 haplotypes were identified. The sequences of these haplotypes were submitted to GenBank. The accession numbers for each haplotypes are AY183909 – AY183913, AY184929, AY184930, AY185208, AY185209 and AY186255. There were 35 (4.16%) variable sites (24 transitions and 11 transversions). Most transversions were between guanine and cytosine (45.5%), whereas adenine-cytosine, adenine-thymine and

guaninethymine transversions accounted for 27.3%, 18,2% and 9.09% respectively. Over half (58.3%) of the transitions were between cytosine and thymine (Table 3).

Analysis of molecular variance (AMOVA) using molecular diversity among haplotypes shows significant differentiation between the color strains (f_{ST} = 0.429, P<0.01). This suggests that 42.9% of the total genetic variance observed was due to differences between strains (Table 4).

Pairwise sequence divergence values in table 5 ranged from intrastrain average of 0.2% to interstrain average of 2.6%. Intrastrain divergence of the three strains of gold arowana was low. It ranged from 0.2% (Malaysian yellow-tail gold and Indonesian gold) to 0.5% (Malaysian red-tail gold). Higher divergence was observed in red and green arowana, showing 2.0% and 1.4% respectively. Sequence divergence in the pairwise comparisons among the gold arowana ranged from 0.4% (between Malaysian red-tail gold and Malaysian yellow-tail gold arowana) to 0.6% (between Malaysian red-tail gold and Indonesian gold arowana). Divergence between gold and red arowana ranged from 2.2% (between Malaysian red-tail gold and red arowana) to 2.45% (between Indonesian gold and red arowana). Divergence between gold and green arowana ranged from 1.18% (between Indonesian gold and green arowana) to 1.3% (between Malaysian yellow-tail gold and green arowana).

The neighbor-joining tree topology was supported with 39% to 98% bootstrap values. The NJ tree in figure 2 reveals the monophyly of the arowana, with two different lineages in it. The red arowana was the most distant

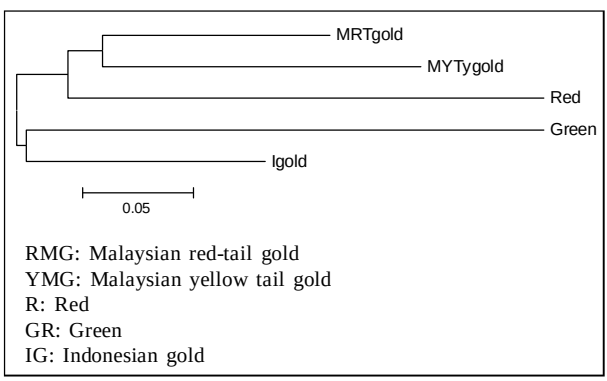

Fig. 1. Neighbor-joining (NJ) tree of arowana based on pairwise microsatellite F_{ST} values

Table 2. Pairwise comparisons of microsatellite F_{ST} among 5 strains of arowana

Strains	GR	RMG	R	YMG	IG
GR					
RMG	0.3512*				
R	0.4728*	0.3434*			
YMG	0.4503*	0.2426*	0.3632*		
I G	0.3407*	0.2603*	0.3524*	0.2848*	

An asterisk indicates a significant genetic distance (P<0.05).
GR: Green
RMG: Malaysian red-tail gold
R: Red
YMG: Malaysian yellow-tail gold
IG: Indonesian gold

Table 3. Variable sites for *ATPase8* and *ATPase6*. Dots indicate identity to the sequence of GR15

	4 2	8 4	1 0 2	1 1 1	1 2 3	1 2 1	2 4 0	2 0 0	2 3 9	2 3 0	3 4 0	3 0 5	3 1 0	3 2 7	3 2 9	3 6 6	3 6 8	3 7 4	4 5 4	5 0 1	5 1 3	5 1 4	5 3 2	5 3 4	6 6 8	6 7 8	7 1 1	7 3 2	7 4 7	7 5 0	7 6 7	8 0 0	8 2 4	8 2 5	8 2 7
GR15	A	T	T	A	G	T	A	C	C	G	T	A	T	C	A	A	A	C	T	C	C	G	C	C	A	C	T	T	G	C	A	C	T	C	A
GR19	G	C	.	G	A	C	.	T	.	A	.	.	.	.	.	G	.	.	.	.	.	.	.	.	G	.	A	.	C	.	.	.	.	.	.	.
MRTG7	.	.	.	.	.	.	.	.	.	.	.	C	.	.	G	G	.	T	.	.	.	.	.	.	.	.	A	.	C	A	.	.	.	.	.	.
MRTG18	.	.	.	.	.	.	.	.	.	.	.	.	T	G	.	.	T	.	.	.	.	.	.	.	.	.	A	.	C	A	.	.	.	.	.	.
R2	.	.	C	.	.	.	C	.	T	.	C	.	.	.	G	.	T	.	.	T	G	A	T	G	.	G	A	G	.	A	G	.	.	.	G	.
R10	G	.	.	.	.	.	.	.	T	.	.	.	.	.	G	.	T	.	.	T	G	.	.	.	.	.	A	.	C	A	G	G	C	.	.	.
MYTG4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	G	.	T	.	.	.	.	.	.	.	.	A	.	C	A	.	.	.	T	.	.
MYTG6	.	.	.	.	.	.	.	.	.	.	.	C	.	.	G	G	.	T	.	.	.	.	.	.	.	.	A	.	C	A	.	.	.	T	.	.
IG1	.	.	.	.	.	.	.	.	.	.	.	.	C	.	G	G	.	T	C	.	.	.	.	.	.	.	A	.	C	.	.	.	.	.	.	.
IG6	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	G	.	T	C	.	.	.	.	.	.	.	A	.	C	.	.	.	.	.	.	.

RMG: Malaysian red-tail gold
YMG: Malaysian yellow tail gold
R: Red
GR: Green
IG: Indonesian gold

Table 4. Results of analysis of molecular variance (AMOVA)

Source of variation	d.f.	Sum of square	Variance components	% of total variance
Among strains	4	28.00	2.10	42.86
Within strains	5	14.00	2.80	57.14
Total	9	42.00	4.90	
Fixation Index	f_{st} = 0.429	P < 0.01		

Table 5. Pairwise mtDNA sequence divergence

Haplotypes	GR15	GR 19	RMG7	RMG18	R2	R10	YMG4	YMG6	IG1	IG6
GR15										
GR 19	0.013									
RMG7	0.008	0.014								
RMG18	0.008	0.017	0.005							
R2	0.020	0.031	0.021	0.021						
R10	0.021	0.027	0.020	0.020	0.019					
YMG4	0.008	0.014	0.002	0.005	0.021	0.020				
YMG6	0.011	0.017	0.002	0.007	0.024	0.023	0.002			
IG1	0.010	0.015	0.006	0.008	0.025	0.024	0.006	0.006		
IG6	0.007	0.013	0.004	0.006	0.021	0.021	0.004	0.006	0.002	

RMG: Malaysian red-tail gold
YMG: Malaysian yellow tail gold
R: Red
GR: Green
IG: Indonesian gold

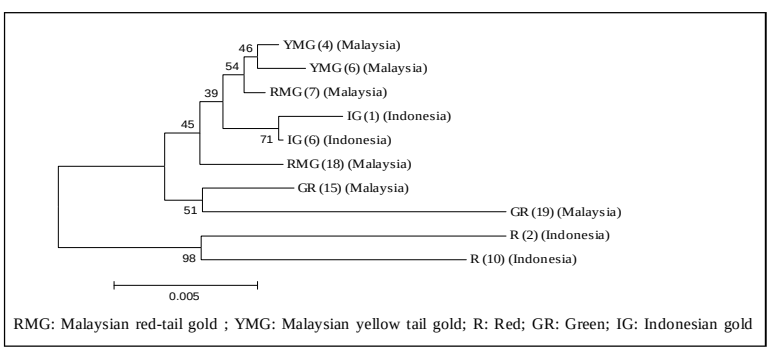

Fig. 2. Neighbor-joining (NJ) tree of mtDNA *ATPase* haplotypes of the arowana

group and had a basal position within the five color strains. The gold arowana formed a sister clade with the green arowana within the lineage. However, the relationship among the three strains of gold arowana was not resolved confidently.

Discussion

Genetic markers such as RAPD and RFLP have been used to differentiate various strains of arowana but the results were not promising (Fernando et al. 1997). Genetic distance using microsatellites yielded a clear division between different color strains of arowana, suggests that microsatellites are more effective. It can be safely assumed that there was no hybridization between different color strains as each strain is endemic to certain river systems. Furthermore, hatcheries only produce pure breeds (Ng H.Y. and Ng K.H. pers. comm.).

The dendrogram derived from microsatellite and mtDNA data showed that the correlation between molecular marker diversity and geographic region is weak. Although green and two strains of Malaysian gold arowana are found in Peninsular Malaysia, the genetic relationship of Malaysian red-tail and yellow-tail gold arowana are closer to Indonesian gold arowana from Sumatra. Sun et al. (1999) suggested that geographically close habitats can be different and conversely, geographical distant habitats can be similar in their environmental conditions. Geological evidence shows that continental Southeast Asian terranes can be classified into two categories, based on their Late Palaezoic tectonic history (Hutchison 1993). The Shan Thai block that includes West Peninsular Malaysia and East Sumatra is characterized with Gondwanan affinities. This terrane is dominated by terrigenous, tilloid-bearing sediments in lower part and carbonate formation in the upper part (Shi and Archbold 1998). On the other hand, East Peninsular Malaysia shows Cathaysian affinities and characterized by predominantly tuffaceous, sandstones and siltstones (Shi and Archbold 1998).

Microsatellite as well as mtDNA data supported the view that red arowana is closely related with Malaysian red-tail gold arowana. However, there were some conflicts between the microsatellite and mtDNA data. Microsatellite data support that the green arowana is the outgroup among the arowana strains. On the other hand the mtDNA data indicated that the red arowana is the outgroup. The relationship between the gold strains was not confidently resolved compared with microsatellite analysis.

Sometimes nuclear DNA such as microsatellite genealogy may not be perfectly concordant with mtDNA because mtDNA data reflects female ancestry only since mtDNA is maternally inherited (Avise 1986). The difference in estimates of genetic differentiation between microsatellites and mtDNA can be influenced by differences in effective population size since the effective population size for mtDNA is one quarter that of microsatellites. Usually differences between the significance of nuclear and mtDNA data suggested male-biased gene flow (Johnson 1989). In this case, all the arowana

strains are highly endemic to certain river systems and no hybridization could have occurred in hatcheries. Hence, male-biased is not proven and both male and female dispersals are limited. Differences in mutation rates between microsatellites and mtDNA may also influence the results. In this study, microsatellites allow a better differentiation among arowana strains. Results showed that microsatellite loci were highly differentiated between strains but differentiation among the gold strains was not fully resolved using mtDNA. Only limited variation in the sequences of mtDNA haplotypes has been observed. Results of the present study indicated that low variability in mtDNA did not reflect low level of genetic variation in the nuclear genome. Most of the microsatellite loci were more variable than mtDNA, indicating that microsatellites evolve faster than mtDNA. Since different regions of mtDNA evolve at different rates, the fast evolving D-loop may be needed to confirm the intraspecific genetic structure.

It is believed that different strains of arowana inhabit separate regions of Southeast Asia and were connected through freshwater habitats during the Pleistocene era (Goh and Chua 1999). Assuming that the sequence divergence rate for *ATPase* determined for fish is 1.3% per million years (Bermingham et al. 1997) the estimated divergence time between the green and red arowana (1.5 to 2.6 MYA) is close to the probable time of the fluctuation of sea level during the late Pliocene to early Pleistocene era. The divergence between the three strains of gold arowana occurred in the early late of Pleistocene. Haplotype sequences of these three strains only differed less than 1%, indicating that dispersal events occurred during Pleistocene.

In Southeast Asia, geological evidence show that there were important sea level fluctuations, especially during the Middle and Late Pleistocene ages. Possibly in the Pleistocene period several episodes of low sea level between 50 and 150 m have occurred (Prentice and Denton 1988). A huge continental shelf called Sundaland was exposed. Indonesian islands such as Sumatra, Java and Borneo were connected with mainland Asia and Peninsular Malaysia with land bridges (Heaney 1985) traversed by several river systems. Malacca Straits River system flowed between Peninsular Malayasia and Sumatra. The Siam River system joined Thailand's Chao Phraya River, with Endau River, Pahang River, Terengganu River, and Kelantan River of Peninsular Malaysia. The North Sunda River ran north from the north-east coast of Sumatra to join Kapuas River from Borneo. The East Sunda River system included rivers of the north coast of Java, the south coast of Borneo and the northern portion of the east coast of Sumatra (Voris 2000). Each time this happened, there were fauna exchange among Peninsular Malaysia, Sumatra, Borneo and Java. During the glacial minima, these islands were separated due to rising of sea level. As a result, several of arowana strains diverged from late Pliocene to middle Pleistocene. The isolated populations evolved in allopatry at intraspecific level. Dodson et al. (1995) reported that west Peninsular Malaysia has greater faunal affinities with Sumatra while east Peninsular Malaysia has greater affinities with the Indochinese fauna. This hypothesis is clearly demonstrated by the distribution of Malaysian and Indonesian gold arowana at west Peninsular Malaysia and eastern Sumatra

respectively. Their close relationships derived from mtDNA data also reflect historical patterns of connections between river systems. On the other hand, green arowana are distributed in east Peninsular Malaysia, Thailand and Indochina. The Malaysian gold and green arowana are only found in west and east Peninsular Malaysian respectively. Most likely east and west Malaysia are separated by a central range of mountains but differences may only be manifested at the intraspecific level as in the case of river catfish, *Hemibagrus nemurus* (Dodson et al. 1995).

Each color strain exhibits high endemism to certain river systems. Since certain morphological traits may represent phenotypic adaptations of specific habitats (Lansman et al. 1983), this leads to the question of what factors are responsible for the evolution of various colors. However, there is lack of proven study that environmental factors, such as water quality and soil composition or different diet would influence the color of arowana after many generations. Further studies are essential to clarify the evolution of different color strains.

Conclusion

There were disagreements between microsatellite and mtDNA results. MtDNA data showed correlation with color trait but not with microsatellites. The phylogenetic structure derived from microsatellite and mtDNA data is not associated with geographical distance. Microsatellite analysis is efficient in estimating short term genetic distance within population level, but mtDNA analysis is more suitable for resolving long term divergence.

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References

Asahida, T., T. Kobayashi, K. Saitoh and I. Nakayama. 1996. Tissue preservation and total DNA extraction from fish at ambient temperature using buffers containing high concentration of urea. Fisheries Science 62: 727-730.

Avise, J.C. 1986. Mitochodrial DNA and the evolutionary genetics of higher animals. Philosophical Transactions of Royal Society of London B 312: 325-342.

Avise, J.C. 1989. Gene trees and organismal histories: a phylogenetic approach to population biology. Evolution 43: 1192-1208.

Bermingham, E., S. McCafferty and A. Martin. 1997. Fish biogeography and molecular clocks: perspectives from the Panamian Isthmus. In: Molecular Systematics of Fishes (ed. T. Kocher and C. Stepien), pp. 112-126. Academic Press, New York.

Bermingham, E. and J.C. Avise. 1986. Molecular zoogeography of freshwater fishes in the Southeastern United States. Genetics 113: 939-965.

Bonde, N. 1979. Paleoenvironment in the “North Sea” as indicated by the fish bearing Mo-clay deposit (Paleocene/Eocene)of Denmark. Mededelingen Werkgroep voor Tertiaire Kwartaire Geologie 16: 3-16.

Bowcock, A.M., A. Ruiz-Linares, J. Tomfohrde, E. Minch, J.R. Kidd, and L.L. Cavalli-Sforza. 1994. High resolution of human evolutionary trees with polymorphic microsatellites. Nature 368: 455-457.

Carvajal-Carmona, L.G., I.D. Soto, N. Pineda, D. Ortiz-Barrientos, C. Duque, J. Ospina-Duque, M. McCarthy, P. Montoya, V.M. Alrarez, G. Bedoya and A. Ruiz-Linares. 2000. Strong Amerind/ white gender bias and a possible Sephardic contribution among the founders of a population in Northwest Colombia. American Journal of Human Genetics 67: 1287-1295.

Cruzan, M.B. and A.R. Templeton. 2000. Paleoecology and coalescence: phylogeographic analysis of hypotheses from the fossil record. Trends in Ecology and Evolution 14: 491-496.

Dawes J, L.C. Lim and L. Cheong. 1999. The Dragon Fish. Kingdom Books, England. 96 pp.

Dodson, J.J, F. Colombani F. and P.K. Ng. 1995. Phylogeographic structure in mitochondrial DNA of a Southeast Asian freshwater fish, *Hemibagrus nemurus* (Siluroidei; Bagridae) and Pleistocene sea-level change on the Sunda shelf. Molecular Ecology 4: 331-346.

Excoffier, L., P.E. Smouse and J.M. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131: 479-491.

Fisher, P.J., R.C. Gardner and T.E. Richardson. 1996. Single locus microsatellites isolated using 5’ anchored PCR. Nucleic Acids Research 24: 4369-4371.

Fernando, A.A., L.C. Lim, K. Jeyaseelan, S.W. Teng, M.C. Liang and C.K. Yeo. 1997. DNA fingerprinting: application to conservation of the CITES-listed dragon fish, *Scleropages formosus* (Osteoglossidae). Aquarium Science and Conservation 1: 91-104.

Gibson, T., D. Higgins and J. Thompson. 1996. Clustal W, EMBL, Heidelberg, Germany and EMBL/EBI, Hixton, UK.

Goh, W. and J. Chua. 1999. The Asian arowana. Dragon Fish Industry, Singapore. 90 pp.

Heaney, L.R. 1985. Zoogeographic evidence for middle and late Pleistocene land bridges to the Philippine Islands. In Modern quaternary Research in Southeast Asia, 9 (ed. G. Bartstra and W.A. Casparie), pp. 127-143. A. A. Balkema, Rotterdam.

Hutchison, C.S. 1993. Gondwana and Cathaysian blocks, Palaeotethys sutures and Cenozoic tectonics in Southeast Asia. Geologische Rundschau 82: 388-405.

Jarne, P. and J.L. Lagoda. 1996. Microsatellites, from molecules to populations and back. Trends in Ecology and Evolution 11: 424-429.

Johnson, C.N. 1989. Dispersal and philopatry in the macropods. In Kangaroo, Wallabies and Rat-kangaroos, Vol. 2 (ed. G. Grigg, I.D. Hume and P.J. Jarman), pp. 593-501. Surrey Beatty and Sons Pty Ltd, Sydney.

Kottelat, M., A.J. Whitten, S. N. Kartikasari and S. Wirjoatmodjo. 1993. Freshwater fishes of Western Indonesia and Sulawesi. Periplus Editions, Hong Kong. 221 pp.

Kuhner, M.K., J. Yamato, and J. Felsenstein. 1998. Maximum likelihood estimation of population growth rates based on the coalescent. Genetics 149: 429-434.

Kumar, S.,K. Tamura and M. Nei. 2001. MEGA. Molecular Evolutionary Genetics Analysis Version 2.1. The Pennsylvanian State University, University Park, PA.

Lansman, R.A., J.C. Avise, C.F. Aquadro, J.F. Shapira and S.W. Daniel. 1983. Extensive genetic variation in mitochondrial DNAs among geographic populations of the deer mouse, *Peromyscus maniculatus*. Evolution 37: 1-16.

Latta, R.G. and J.B. Mitton. 1997. A comparison of population differentiation across four classes of gene markers in limber pine (*Pinus flexilis* James). Genetics 146: 1153-1163.

Luikart, G. and J.M. Cornuet. 1998. Empirical evaluation of a test for identifying recently bottlenecked populations from allele frequency data. Conservation Biology 12: 228-237.

Ng, H.H. and Tan H. H. 1999. The fishes of the Endau Drainage, Peninsular Malaysia with description of two new species of catfishes (Teleostei: Akysidae, Bagridae). Zoological Studies 38: 350-366.

Prentice, M.L. and G.H. Denton. 1988. Deep-sea oxygen isotope record, the global ice sheet system, and homonid evolution. In The evolutionary history of the robust Australopithecines. (ed. F. Grine), pp. 383-403. Aldine de Gruyter, New York.

Queller, D.C., J.E. Strassmann and C.R. Hughes. 1993. Microsatellites and kinship. Trends in Ecology and Evolution 8: 285.

Saitou, N. and M. Nei. 1987. The neighbor-joining method: a new method of reconstructing phylogenetic trees. Molecular Biology and Evolution 4: 406-425.

Schneider, S., J.M. Kueffer, D. Roessli and L. Excoffier. 2001. ARLEQUIN (version 2.000) - A Software for Population Genetic Data Analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland.

Shi, G.R. and N.W. Archbold. 1998. Permain marine biogeography of SE Asia. In: Biogeography and geological evolution of SE Asia (eds. R. Hall and J.D. Holloway), pp. 57-72. Backhuys Publisher, Leiden.

Sivananthan, J. 2003. Isolation and characterization of microsatellites in the Asian arowanas (*Scleropages formosus*). Master thesis, University of Malaya. 75 pp.

Sivasundar, A., E. Bermingham and G. Orti. 2001. Population structure and biogeography of migratory freshwater fishes (Prochilodus: Characiformes) in major South America rivers. Molecular Ecology 10: 407-418.

Suleiman, M.Z. 1999. Pembiakan Ikan Arowana, *Scleropages formosus* (Muller and Schlegel). Risalah Perikanan bil 67. Jabatan Perikanan Malaysia. 17 pp.

Sun, G.L., O. Diaz, B. Salomon and R. von Bother. 1999. Genetic diversity in *Elymus caninus* as revealed by isozyme, RAPD and microsatellite markers. Genome 42: 420-341.

Sunnucks, P. 2000. Efficient genetic markers for population biology. Trends in Ecology and Evolution 15: 199-203.

Tamura, T. and M. Nei. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA. Molecular Biology and Evolution 10: 447-459.

Voris, H.K. 2000. Maps of Pleistocene sea levels in Southeast Asia: shorelines, river systems and time durations. Journal of Biogeography 27: 1153-1167.

Weir, B.S. and C.C. Cockerham. 1984. Estimating *F*-statistics for the analysis of population structure. Evolution 38: 1358-1370.

Yue, G.H., F. Chen and L. Orban. 1999. Rapid isolation and characterization of microsatellites from genome of Asian Arowana (*Scleropages formosus Osteoglossidae, Pisces*). Molecular Ecology 9: 1007-1009.

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