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Monitoring the genetic diversity of three Asian arowana (*Scleropages formosus*) captive stocks using AFLP and microsatellites

Gen Hua Yue[a], Yang Li[a], Lian Chuan Lim[b], Laszlo Orban[a,c,*]

[a] *Reproductive Genomics Group, Temasek Life Sciences Laboratory, Singapore, Singapore*
[b] *Ornamental Fish Centre, Agri-Food and Veterinary Authority, Singapore, Singapore*
[c] *Department of Biological Sciences, National University of Singapore, Singapore, Singapore*

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Abstract

Asian arowana (*Scleropages formosus*) is listed by CITES as a highly endangered fish species. The genetic diversity and population structure of three Asian arowana captive stocks was analyzed using microsatellites and AFLP markers on 32 randomly collected individuals from each stock. Six AFLP primer pairs amplified a total of 324 bands across the three sample sets. The green stock showed the highest percentage of polymorphic bands (15.6%) and expected heterozygosity (0.26), followed by the red (13.0% and 0.24), then the golden one (12.7% and 0.22). Microsatellite analysis showed high allelic and gene diversity in all three varieties. The green stock showed higher allele number (100) and higher gene diversity (0.75) than the red (98 and 0.74) and golden ones (85 and 0.71), respectively. The estimates of long-term effective population size by two different methods ranged from 4741 to 7288 and from 25,056 to 64,641, respectively. Tests of heterozygosity and allele frequency distribution indicate that a recent bottleneck in any of the three captive populations is highly unlikely. Altogether, the data suggest that the captive breeding program for conservation and sustainable use of the endangered Asian arowana will be successful. In addition, we found that the differentiation among populations was intermediate ($F_{ST}=0.047$, $R_{ST}=0.103$) and the genetic distance was the smallest between the green and red, whereas the largest between the red and golden varieties.

Keywords: Dragonfish; DNA markers; Genetic diversity; Effective population size; Conservation

* Corresponding author. Reproductive Genomics, Temasek Life Sciences Laboratory, 1 Research Link, The NUS, Singapore 117604, Singapore. Tel.: +65-6872-7413; fax: +65-6872-7007.
E-mail address: laszlo@tll.org.sg (L. Orban).

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1. Introduction

The Asian arowana (dragonfish; *Scleropages formosus*) is an ancient fish species from the *Osteoglossidae* family and one of the most primitive teleostean forms (Greenwood et al., 1996). The natural distribution of the Asian arowana covers vast areas of southeast Asia, including Cambodia, Indonesia, Laos, Malaysia, Philippines, Vietnam and possibly Thailand (Dawes et al., 1999). The typical habitat of the species is swamps and flooded forests, but they also occur in lakes, rivers, reservoirs and waterways. The diet of Asian arowana is wide-ranging, including insects, arachnids, nonwoody roots and tubers (Scott and Fuller, 1976). A fully grown individual may weigh over 7 kg and its length could exceed a meter (Alfred, 1964). There are three main color varieties of the species: green, golden and red, with additional subtypes within the main varieties. The natural origin of different color varieties is thought to be different according to the available information (Dawes et al., 1999). From the three main varieties, the green variety was widely distributed throughout the region, including Malaysia, Indonesia, Thailand, Cambodia, Laos, Philippines and Vietnam, the golden originated from Indonesia's Sumatra and Malaysia, whereas the red one was only located in Indonesia's Kalimantan province (Goh and Chua, 1999).

The reproductive biology of Asian arowana is unusual for a fish: individuals mature quite late (at the age of 3–4 years), they produce few (30–100) eggs of giant size and they show an advanced degree of parental care. The fertilized eggs and larvae are protected in the mouth of males. The two sexes of Asian arowana are quite difficult to distinguish visually because there are no obvious phenotypic signs of sexual dimorphism, especially in young adults (Dawes et al., 1999; Scott and Fuller, 1976). However, a sex-associated DNA marker has been identified recently from the green variety of Asian arowana (Yue et al., 2003).

The demand for Asian arowana in the ornamental fish industry has increased substantially since 1960s, especially for the red and golden varieties. An adult red or golden Asian arowana individual might cost over US$20,000 at the ornamental fish market. Population sizes of Asian arowana in their natural habitat keep decreasing due to overfishing, low fecundity and long generation interval. The species was classified by the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) as highly endangered and listed under Appendix 1 in 1975 (Greenwood et al., 1996).

It is crucial for endangered species to retain as much genetic variation as possible to enhance the chance for their recovery (Hedrick et al., 2000). The Asian arowana was first bred in captivity in the Sembawang Field Experimental Station (PPD, Singapore) in 1981. In the recent years, several other captive broodstocks were established in Singapore, Malaysia and Indonesia (Dawes et al., 1999). However, no information is available on the genetic diversity of these captive populations, although such information could facilitate the captive breeding programs for conservation and production of the species for the ornamental fish industry.

Molecular markers are useful tools in the assessment of genetic diversity (Powell et al., 1996). Polymorphic microsatellites were isolated from the genome of Asian arowana by Yue et al. (2000), and analysis of AFLPs as well as microsatellites has been shown to have

higher power than RAPD for the detection of genetic diversity in populations of this species (Yue et al., 2002b). This paper describes the genetic diversity and population structure in three captive Asian arowana stocks obtained from the same Singaporean farm as evaluated by microsatellite and AFLP markers.

2. Materials and methods

2.1. Fish samples and DNA isolation

Samples were collected from the stock of Rainbow Fish Farm (Singapore), which was established in the early 1980s. The founder stocks were constructed from wild-caught Asian arowana individuals. Individuals from the three different color varieties were kept, raised and bred in separate ponds. Individuals from different generations were raised in different ponds; every individual was tagged with electronic PIT tags.

For our study, fin clips were collected from 32 adult unrelated F_2 individuals of green, Indonesian golden and blood red stocks of the Rainbow farm (Singapore), respectively. Tissue samples were kept in absolute ethanol at 4 °C until use. DNA was isolated as described by Miller et al. (1988).

2.2. Genotyping of microsatellites

From 21 microsatellites isolated by Yue et al. (2000), nine markers (D11, D31, D33, D38, D42, D72, D85, D92 and D94) were selected for genotyping based on polymorphism in other sample sets. One primer from each pair was labeled with either 6FAM, Hex or Tet (Genset, Singapore), and genotyping of the microsatellites was performed following Yue et al. (2000). Each 25 μl PCR reaction contained 1 × PCR buffer (Finnzymes, Espoo, Finland) with 1.5 mM $MgCl_2$, 200 μM of each dNTP, 0.2 μM of each primer, 30 ng genomic DNA and 1.0 U DyNAzyme II DNA-polymerase (Finnzymes). The cycling conditions were an initial denaturation of 94 °C for 2 min, followed by 34 cycles of 94 °C for 30 s, annealing temperature (50–60 °C) for 30 s, 72 °C for 30 s, with a final extension at 72 °C for 5 min. The PCR products were separated on an ABI377 DNA sequencer (PE/ABI, CA, USA) according to the manufacturer's instructions, and the results were analyzed using Genescan 3.1 and Genotyper 2.0 softwares (PE/ABI, CA, USA).

2.3. AFLP analysis

AFLP analysis was performed using an AFLP™ plant mapping kit according to the manufacturer's protocol (ABI/PE, CA, USA). *Eco*RI and *Mse*I enzymes were used for digestion of 500 ng genomic DNA from each sample. Ligation of *Eco*RI and *Mse*I adaptors and preselective and selective amplifications were carried out according to the manufacturer's protocols. Selective amplification was performed using six fluorescently labeled *Eco*RI–*Mse*I primer combinations with six selective bases. The six primer combinations were *Eco*RI-AAG/*Mse*I-CTC, *Eco*RI-ACC/*Mse*I-CTC, *Eco*RI-AAC/*Mse*I-

CAG, *Eco*RI-AGG/*Mse*I-CAA, *Eco*RI-ACT/*Mse*I-CAA and *Eco*RI-ACA/*Mse*I-CTA. PCR amplification on the PTC-100 thermocycler (MJ Research, CA, USA) and separation of the PCR products on ABI 377 sequencers (ABI/PE) were carried out as described previously (Yue et al., 2002b). The gel was analyzed by using Genescan 3.1 (ABI/PE) software, and the fragment length was calculated against the internal size standard (GS-500-TAMRA) using the Genotyper 2.0 software (ABI/PE). Only the fragments between 50 and 500 bp were scored.

2.4. Data analysis

For the AFLP markers, bands were scored as 1 if present or 0 if absent. Due to the mostly dominant nature of AFLP markers, allele frequencies could not be directly estimated. The estimates for descriptive genetic parameters such as expected heterozygosity (H_e) and percentage of polymorphic loci (P) were generated with the methods described by Lynch and Milligan (1994) and the TFPGA software (Miller, 1997).

Since the microsatellites are co-dominant markers, allele frequencies were estimated by direct count. The inbreeding coefficient index (Fis), pairwise genetic differentiation (F_{ST}), observed heterozygosity (H_o), expected heterozygosity (H_e) and percentage of polymorphic loci (P) were calculated using the software GDA (Lewis and Zaykin, 2000). The same software was used to analyze the private alleles and their frequencies in each population. Deviations from Hardy–Weinberg equilibrium and gametic phase disequilibrium were examined using the chi-square test.

2.5. Effective population size and genetic drift

The effective population size for natural populations was calculated from the estimates of the unbiased expected heterozygosity under both the infinite-alleles model (IAM) and the stepwise mutation model (SMM) according to Nei (1987) and Lehmann et al. (1998) as shown below:

$$\text{IAM}: \quad N_e = H_e/(1-H_e)/4\mu$$

$$\text{SMM}: \quad N_e = \{[1/(1-H_e)]^2 - 1\}/8\mu$$

where N_e is the effective population size, H_e is the average expected heterozygosity and μ is the average mutation rate of the microsatellites used.

Since no information was available on microsatellite mutation rate in the Asian arowana, we used a mutation rate of 2.5×10^{-4}, which was the average from six vertebrate species, including mouse (Dietrich et al., 1992), pig (Ellegren, 1995; Yue et al., 2002a), sheep (Crawford and Cuthbertson, 1996), human (Weber and Wong, 1993), salmon (Steinberg et al., 2002) and zebrafish (Shimoda et al., 1999). Finally, the bottleneck hypothesis was tested using the Bottleneck 1.2.02 software (Cornuet and Luikart, 1996). These methods test for the departure from mutation drift equilibrium based on heterozygosity excess or deficiency. Allele frequency distribution of the microsatellite

loci was also examined by using program Bottleneck 1.2.02, for model shift (Luikart et al., 1998), which may indicate if a recent genetic bottleneck has occurred.

The population structure was analyzed by using both IAM and SMM. Under the IAM model, the weighted analysis of variance of Weir and Cockerham (1984) was applied to estimate the Fis value, the correlation of alleles within individuals and the correlation of alleles within populations for each locus (θ or F_{ST}). Single locus estimates of Fis and θ were weighted as described by Weir and Cockerham (1984) to create a combined estimated overall loci. Under the SMM evolutionary model, the population structure was analyzed for each locus using R_{ST} (Slatkin, 1995), an analog of θ that estimates the correlation of allele sizes within populations. The calculation of all estimates of population structure and their significance were carried out using the software GDA (Lewis and Zaykin, 2000) and Microsat 1.5 (Minch, 1996).

For the AFLP data, genetic distance between populations was calculated according to Nei (1978) and Reynolds et al. (1983) using the program TFPGA (Miller, 1997). For microsatellite data, R_{ST} distance (Slatkin, 1995) was estimated using the Microsat 1.5 software (Minch, 1996) since the majority of mutations at microsatellite loci are stepwise in nature, changing allelic sizes by one or a very few number of repeats. Phylogenetic trees were constructed using the unweighted pair group methods with arithmetic (UPGMA) averages using the TreeView software (Page, 1998).

3. Results

3.1. Genetic variation based on the AFLP analysis

A total of 324 bands—in the size range of 50–500 bp—were amplified across the three sets of samples by six AFLP primer combinations. Fifty-eight (17.9%) bands were polymorphic across the three populations; 49 (15.6%) of them showed polymorphism in the green, 41 (12.7%) in the golden and 42 (13.0%) in the red variety, respectively. Based on the polymorphic bands, the expected average heterozygosity was estimated as 0.26 in the green, 0.22 in the golden and 0.24 in the red variety, respectively.

3.2. Genetic variation based on microsatellite analysis

Among the nine microsatellites studied, eight were polymorphic in all three sample sets, while the remaining one (D33) was polymorphic in the green variety only. At the nine loci, a total of 124 alleles were detected across the three sample sets. Out of these, 100 were found in the green, 98 in the red and 85 in the golden variety. Loci D38 and D92 showed the highest level of polymorphism in all three stocks (for the summary of allelic and genetic diversity, see Table 1). A total of 27 unique (private) alleles were identified at eight loci; 12 such alleles appeared in the red, 11 in the green, but only 4 in the golden variety (Table 2). For example, at locus D94, three alleles (191, 201 and 229 bp) appeared exclusively in the green stock, whereas the 213-bp allele was found in the golden stock only. In general, the frequency of the private alleles was low, ranging from 0.016 to 0.156. The difference of allele size at individual polymorphic microsatellite loci varied from 2 to 60 bp.

Table 1
Genetic diversity in the three Asian arowana stocks tested

Loci	Green					Golden					Red				
	Range (bp)	Allele number	H_e	H_o	Fis	Range (bp)	Allele number	H_e	H_o	Fis	Range (bp)	Allele number	H_e	H_o	Fis
D11	158–172	6	0.79	0.69**	0.13	158–172	6	0.78	0.56*	0.28	158–172	6	0.79	0.69*	0.14
D38	181–231	23	0.94	0.81*	0.14	181–225	20	0.93	0.94	−0.01	179–225	22	0.95	0.94	0.01
D85	143–155	7	0.69	0.44**	0.37	143–157	7	0.80	0.84	−0.07	145–161	7	0.82	0.84	−0.03
D31	223–279	11	0.82	0.81	0.00	223–271	9	0.72	0.66	0.08	223–271	11	0.81	0.81	−0.01
D42	149–199	18	0.88	0.72*	0.19	151–187	15	0.92	0.96*	−0.05	149–209	24	0.96	0.81*	0.14
D94	191–229	10	0.77	0.81*	−0.05	197–227	9	0.78	0.66	0.16	197–207	6	0.59	0.63*	−0.06
D72	101–121	10	0.83	0.84	−0.02	105–111	4	0.64	0.66	−0.07	107–121	8	0.85	0.84	0.01
D33	132–140	3	0.09	0.03*	0.66	136	1	0.00	0.00	–	136–138	2	0.06	0.00*	0.00
D92	148–178	12	0.88	0.69*	0.22	148–186	14	0.82	0.59*	0.28	154–176	12	0.89	0.90	−0.02
Mean	–	11.1	0.75	0.65	0.13	–	9.4	0.71	0.65	0.08	–	10.9	0.74	0.72	0.03

H_e: expected heterozygosity; H_o: observed heterozygosity; Fis: inbreeding coefficient index.

*Loci showing significant ($P<0.05$) deviation from Hardy–Weinberg equilibrium.

**Loci showing highly significant ($P<0.01$) deviation from Hardy–Weinberg equilibrium.

Table 2
Private alleles and their frequencies

Locus	Allele	Frequency	Stock
D38	179	0.0156	red
	221	0.0156	green
	231	0.0156	green
D85	161	0.0312	red
D31	251	0.0312	red
	255	0.0156	red
	279	0.0156	green
D42	153	0.0156	red
	185	0.0468	red
	189	0.0156	red
	191	0.0156	red
	201	0.0156	red
	209	0.0312	red
D94	191	0.0468	green
	201	0.0156	green
	213	0.0156	golden
	229	0.0156	green
D72	101	0.0156	green
	103	0.0156	green
	113	0.1406	red
D33	132	0.0156	green
	138	0.0312	red
	140	0.0312	green
D92	150	0.0312	golden
	152	0.1563	golden
	178	0.0156	green
	186	0.0156	golden

Nearly all the microsatellites showed different allele frequency distributions among the three sample sets. A majority (over 70%) of alleles showed a frequency lower than 0.1 (Fig. 1), and the allele frequency distribution displayed an L shape in all three stocks.

The average expected heterozygosity (gene diversity) at the nine loci was highest (0.75) in the green, followed by the red (0.74) and the golden (0.71) variety. The red population displayed the highest average observed heterozygosity (0.72) followed by the golden (0.65) and green (0.65). The highest Fis value was seen in the green, whereas the lowest was seen in the red population (Table 1).

Hardy–Weinberg disequilibrium was observed at several loci (Table 1). The green stock showed significant ($P<0.05$) deviation from Hardy–Weinberg equilibrium at seven loci, followed by the red (four loci) and the golden (three loci). Gametic phase disequilibrium was significant ($P<0.05$) in 10 out of 36 pairwise comparisons in the green, 6/36 in the golden and 10/36 in the red variety.

Both the IAM and the SMM were applied in the Bottleneck software to test if the microsatellites displayed a departure from the mutation-drift equilibrium. Under the IAM, no population displayed significant heterozygosity excess ($P>0.05$); however, under the

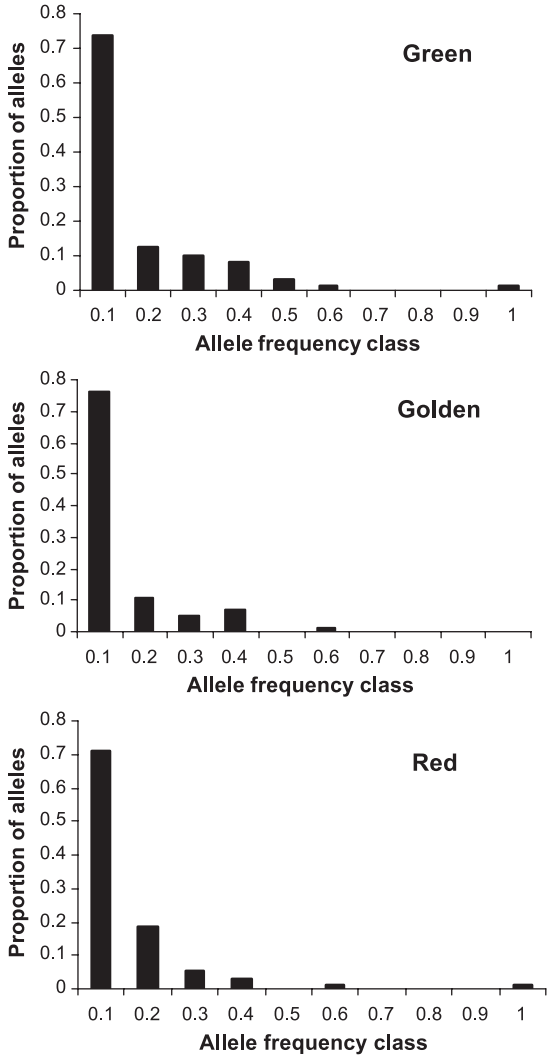

Fig. 1. Allele frequency distribution for all polymorphic microsatellite loci examined in three captive stocks of Asian arowana. An L-shaped distribution is obtained, suggesting that no recent bottleneck affecting the genetic variability occurred.

SMM, the green population exhibited significant ($P<0.01$) heterozygosity deficiency (Table 3).

The effective population size under the IAM was estimated as 4985 for natural populations of the green, 4741 for the golden and 7288 for the red variety, whereas the corresponding values under the SMM were 26,270, 25,056 and 64,641, respectively (Table 4).

Table 3
Sign tests for heterozygosity excess at nine microsatellite loci in the three Asian arowana stocks tested

Variety	IAM		SMM	
	H_e/H_d	P	H_e/H_d	P
Green	7/2	0.21	1/8	0.004
Golden	6/2	0.31	4/4	0.442
Red	7/2	0.20	5/4	0.562

IAM: infinite-alleles model; SMM: stepwise mutation model; H_e: number of loci showing heterozygosity excess. H_d: number of loci showing heterozygosity deficiency. P: statistical possibility under the sign test that the population exhibits overall heterozygosity excess over all loci given the model.

3.3. Genetic differentiation

The F_{ST} estimates within populations ranged from -0.016 to 0.118 with an average of 0.047 (Table 5). The values of the R_{ST} were more variable than the F_{ST} estimates, ranging from -0.002 to 0.361 with an average of 0.103 (Table 5).

3.4. Phylogenetic relationship between the three varieties

The R_{ST} genetic distance calculated based on microsatellite analysis was smallest between the red and green, whereas the largest distance was between the golden and red varieties (Fig. 2). A similar phylogenetic tree was obtained based on the AFLP analysis (data not shown).

4. Discussion

Captive breeding provides an "insurance policy" for natural populations and may be the only hope of survival for some species. It requires consideration of small population vulnerabilities to preserve high levels of genetic diversity (Ryder et al., 2000). The long-

Table 4
Long-term effective population sizes for the three varieties estimated under two different models

Locus	Green		Golden		Red	
	IAM	SMM	IAM	SMM	IAM	SMM
D11	3762	10,838	3545	9831	3762	10,838
D38	15,667	138,389	13,286	101,541	19,000	199,500
D85	2226	4703	4000	12,000	4556	14,932
D31	4556	14,932	2571	5878	4263	13,350
D42	7333	34222	11,500	77,625	24,000	312,000
D94	3348	8952	3545	9831	1439	2474
D72	4882	16,801	1778	3358	5667	21,722
D33	99	104	0	0	64	66
D92	3000	7500	2448	5445	2846	6896
Mean	4985	26,270	4741	25,056	7288	64,641

IAM: the infinite-alleles model; SMM: stepwise mutation model.

Table 5
Overall and per locus Fis, F_{ST} and R_{ST} values for the three stocks of Asian arowana

Locus	Fis	F_{ST}	R_{ST}
D11	0.181	− 0.003	0.018
D38	0.048	0.004	− 0.002
D85	0.077	0.063	0.099
D31	0.024	0.118	0.036
D42	0.093	0.039	0.122
D94	0.021	0.036	0.038
D72	− 0.010	0.114	0.361
D33	0.799	− 0.016	− 0.008
D92	0.158	0.069	0.262
Mean	0.081	0.047	0.103

Fis: inbreeding coefficient index; F_{ST}: pairwise genetic differentiation; R_{ST}: an analog of F_{ST}.

term persistence of an endangered fish species can be influenced by a number of factors, including allelic diversity, gene diversity, effective population size and population structure.

When our data were compared with those obtained from other fish species (for review, see DeWoody and Avise, 2000), the microsatellite allelic and gene diversity of the three Asian arowana stocks were higher than those of freshwater fish, somewhat lower than those of marine fish and similar to those of anadromous fish. The allelic and gene diversity values determined in this study were only slightly lower than those obtained from three other sources including some wild-caught Asian arowana individuals (Yue et al., 2002b), suggesting that the sampled stocks contain high-enough level of allelic and gene diversity. It would be possible to raise the allelic and gene diversity of each population further through introduction of new, unrelated individuals from other fish farms or from the wild populations. Genetic diversities could also be increased theoretically by crossbreeding given that each population contained some private alleles. However, crossbreeding

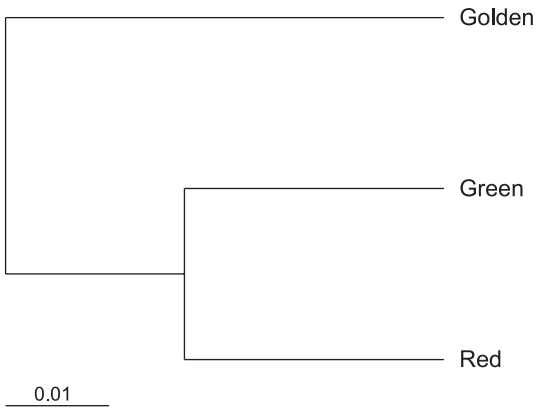

Fig. 2. Phylogenetic relationship between the three Asian arowana varieties based on the R_{ST} distance from the microsatellite analysis. A scale bar is shown under the tree.

between different varieties might not be desirable commercially, as the current market value of different varieties is quite different, unless the new hybrid colors were valued by the customers.

The estimate of effective population size depends on the mutation pattern of microsatellites. Since it has not been clear to date which model would more closely reflect the evolutionary patterns in Asian arowana, both the IAM and SMM models were applied in this study. The estimate also depends on the average mutation rate at microsatellite loci for which we had to use the average mutation rate (2.5×10^{-4}) derived from other species. As these values were derived from pedigrees, they might be underestimated, and their application to Asian arowana could lead to overestimation of the effective population size. On the other hand, the use of genotypes of F_2 individuals from a limited number of captive Asian arowana founders might result in underestimated population size. The fact that Asian arowanas seem to pair up for breeding (or life?) further complicates matters, as their mating cannot be considered random. Therefore, our estimates on effective population size of natural populations, which ranged from 4741 to 64,641 and were greatest in the red variety followed by the green and the golden, should be taken with caution. The estimates seem to suggest that a relatively large number of individuals were likely to be available in the wild for all three varieties at the time, when the founders were captured. However, due to the factors listed above and to the fact that captive broodstocks were established in the early 1980s, the estimates might not hold true for the present natural populations.

At present, the green variety is still thought to be widespread and relatively common in nature, but the red populations have declined considerably due to collecting pressure since the red is one of the most valuable varieties in the Asian arowana trade (Luxmoore, 1990). The species is also thought to be under pressure as a result of habitat loss, for example, by swamp clearance in Malaysia (Scott and Fuller, 1976) and dredging in Thailand (Bain and Humphrey, 1982). Its very low fecundity and relatively late sexual maturity make it particularly vulnerable to such threats. Therefore, it would be desirable to carry out a survey of wild populations in the near future to establish baseline population data and to delineate the distribution of the different color varieties. However, this will not be an easy task since the Asian arowana inhabits swampy and heavily wooded areas. If needed, some captive red individuals—with the highest polymorphism at microsatellite loci—could be returned to their natural habitat to improve the genetic diversity of the wild population, thus also improving the fitness of the fish. Therefore, it is necessary to keep these captive populations for the purpose of conservation and sustainable use of these precious genetic materials. Caughley (1994) pointed out that when measures are taken to avoid inbreeding as part of breeding program, genetic variation of stocks is not necessarily reduced despite the small effective population size. This may be achieved by maintaining pedigree information on all individuals or by genotyping all individuals for several microsatellite markers in breeding programs and using this information to arrange mating pairs. However, for this system, molecular tools for sexing the fish need to be developed and the mating behavior needs to be studied. We have identified a sex-related DNA marker from the green variety of Asian arowana (Yue et al., 2003), which could be useful for such purpose.

Low level of gene diversity has been taken as evidence of bottlenecks in populations known to have undergone severe demographic decline (e.g. Houlden et al., 1997). However, comparison of gene diversities per se is not ideal for studying bottlenecks because a high level of gene diversity may be maintained after a bottleneck (Nei et al., 1975). The number of alleles for a given locus is more sensitive to demographic fluctuations (Nei et al., 1975; Maruyama and Furest, 1985) and is therefore more appropriate for testing whether a bottleneck has occurred in a given population (Cornuet and Luikart, 1996). No recent bottleneck was found in any of the three stocks sampled, when tested under the IAM model. On the other hand, under the SMM model, significant heterozygosity deficiency was detected in the green stock, suggesting either the occurrence of a historical population expansion (Cornuet and Luikart, 1996), founder event, nonrandom mating or appearance of null alleles at some loci. The analysis of allele frequency distribution did not reveal any evidence of a recent genetic bottleneck either (Fig. 1), as a mode shift from L-shaped distribution was not detected in any of the three stocks (Luikart et al., 1998).

In all three sample sets, the microsatellite data showed a slight deficiency of heterozygosity at several loci, and deviation from the Hardy–Weinberg equilibrium was also detected. Similar results have been reported from a wide range of fish species, e.g. European sea bass (DeLeon et al., 1997), Atlantic cod (Bentzen et al., 1996) and vermilion snapper (Bagley et al., 1999). This deviation might either represent real biological phenomenon or could be an artifact resulting from the screening process. However, the possibility of the artifacts being caused by the screening processes should be very low since the microsatellites were genotyped with a highly sensitive automated DNA sequencers and heterozygotes could be easily differentiated from the homozygotes even if the difference between the two alleles was only a few base pairs.

Phylogenetic analysis by AFLP and microsatellite markers showed a clear division between the three stocks derived from different captive populations. The smallest genetic distance was detected between red and green varieties, while the largest was between the red and golden ones. This difference is likely to be a result of geographical separation of the ancestral populations in the wild.

In conclusion, this study showed that both the allelic and gene diversity of the three Asian arowana stocks sampled were high, similar to the estimated effective population size. This suggests that the captive breeding program of Asian arowana for conservation and sustainable use of these genetic materials is successful in avoiding loss of genetic diversity. The markers used in our study can be utilized in future studies to analyze genetic relationships and to monitor allele frequency changes in other captive and wild populations of Asian arowana.

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