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Isolation and characterization of microsatellite loci in hybrid giant tiger grouper

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ABSTRACT. The hybrid giant tiger grouper is a fish that has considerable commercial value and has become increasingly important for aquaculture in South East Asia since 2008. In order to prevent any reduction in genetic diversity in hybrid grouper as a result of aquaculture, we have identified 21 microsatellite markers that can be used to estimate genetic variation in the fish population. The number of alleles at polymorphic microsatellite loci ranged from 2 to 7, and observed and expected heterozygosities varied from 0.0323 to 0.9643 and 0.0921 to 0.7174, respectively. Polymorphism information content values ranged from 0.088 to 0.737. Nineteen of the 21 loci were in Hardy-Weinberg equilibrium ($P > 0.005$) after application of the Bonferroni correction ($k = 10$), the exceptions being ZZLD35 ($P < 0.005$) and ZZLD36 ($P < 0.001$). No linkage disequilibrium was

detected. These 21 microsatellite markers are potentially of great value for analyzing genetic diversity to provide essential information for sustainable management of these fish.

Key words: Conservation; Genetic Diversity; Microsatellite; Hybrid giant Tiger grouper

INTRODUCTION

The hybrid giant tiger grouper is derived from the cross of male *Epinephelus lanceolatus* (considered vulnerable by the IUCN) and female *Epinephelus fuscoguttatus* (considered near threatened by the IUCN) (RBFHG, 2007). At present, Taiwan (China) and Malaysia are the main areas of production of this hybrid grouper. As the fish shows both disease resistance and rapid growth rates, it is of considerable commercial value and has become important in fish culture in South East Asia in recent years. However, the use of a fish such as the hybrid giant tiger grouper may be problematical if only economic aspects receive attention and protection is neglected. It is necessary to maintain its genetic diversity so that the fish can be produced commercially using sustainable development. Additionally, any program to monitor the genetic diversity must also consider the situation of the parental species to ensure successful breeding. In the present study, we sought to evaluate the genetic variation of the germplasm of hybrid giant tiger grouper through analysis of the genetic structure using microsatellite markers. The microsatellite technique has proven to be a valuable means for analyzing genetic diversity in breeding programs (Wolfus et al., 1997). To the best of our knowledge, this approach has not previously been applied to the hybrid giant tiger grouper. Here, we identified 21 microsatellite markers for this hybrid that can be used to examine genetic diversity. Through use of these markers, it should be possible to prevent the loss of rare germplasms and to promote genetic diversity within the farmed populations of hybrid grouper.

MATERIAL AND METHODS

The microsatellite loci were developed using the protocol for fast isolation of amplified fragment length polymorphisms in sequences containing repeats (Zane et al., 2002). Genomic DNA was extracted using a Genomic DNA Extraction kit (Tiangen, Beijing, China) and was digested with *Mse*I (Thermo Scientific, Waltham, MA, USA) at 65°C for 10 min. The digested fragments were ligated to *Mse*I adapter A (5'-ACGATGAGTCCT GAG-3')/*Mse*I adapter B (5'-TACTCAGGACTCAT-3') by T4 DNA ligase at 37°C for 3.5 h. The digestion-ligation mixture was then hybridized to the biotinylated probes $(CT)_{15}$ and $(GT)_{15}$ (Chen et al., 2012). DNA fragments containing microsatellite repeats were collected using Streptavidin MagneSphere® Paramagnetic Particles (Promega, Madison, WI, USA). The recovered DNA fragments were then amplified with the *Mse*I A primer. PCR products were purified using GenCleanPCR (Generay Biotech, Shanghai, China) and ligated to PMD19-T (Takara, Shiga, Japan) at 16°C for 3.5 h. The plasmids were

transformed into *Escherichia coli* and transformants were selected on lysogeny broth agar plates that included ampicillin. Positive clones were confirmed by PCR amplification using the universal M13 primer. The PCR products were separated on 1% agarose gels, and 215 positive clones ranging in size from 450 to 1000 bp were selected and then sequenced by Invitrogen Company (Guangzhou, China). We then designed 49 pairs of primers using Primer Premier 5.0 based on the sequencing results.

All primer pairs were tested using gradient PCR at an annealing temperature range of 50° to 60°C to identify the optimum annealing temperature. DNAs were obtained from a population of 31 hybrid giant tiger grouper collected from Xiamen, China, and used for the subsequent analyses. PCR was performed using 100 ng template DNA, 1X EasyTaq Buffer for PAGE and 2.5 U EasyTaq DNA Polymerase for PAGE (TransGen Biotech, Beijing, China), 0.4 mM dNTPs (Generay Biotech), and 0.4 µM of each primer in a 10-µL final volume with the amplification protocol: initial denaturation at 94°C for 5 min; 35 cycles at 94°C for 30 s, Ta (primer optimal annealing temperature; Table 1) for 30 s, 72°C for 1 min; and a final extension at 72°C for 10 min. Polymorphic amplification products were separated on 6% denaturing polyacrylamide gels with Sequi-Gen Sequencing Cell (Bio-Rad, Hercules, CA, USA). The number of alleles per locus (N_A), observed (H_O) and expected (H_E) heterozygosities, and polymorphic information content (PIC) values were calculated using POPGEN32 (version 1.32) (Yeh et al., 2000) and CERVUS 3.0 (version 2.2.3) softwares. Deviation from the Hardy-Weinberg equilibrium and genotypic linkage disequilibrium were also tested utilizing POPGEN32 version 1.32.

RESULTS AND DISCUSSION

Among the 49 loci tested, 10 were polymorphic with N_A ranging from 2 to 7, H_O from 0.0323 to 0.9643, H_E from 0.0921 to 0.7174, and PIC values from 0.088 to 0.737 (Table 1). Eleven loci were monomorphic. The remaining loci were eliminated as they gave a vague outcome with abundant bands or else failed to provide amplification products. In this study, no linkage disequilibrium was detected after applying the Bonferroni correction (k = 10). Eight of the 10 polymorphic loci were in Hardy-Weinberg equilibrium ($P > 0.005$); the exceptions were ZZLD35 ($P < 0.005$) and ZZLD36 ($P < 0.001$). The deviation from Hardy-Weinberg equilibrium is likely attributable to the small size of the sample as well as to the occurrence of null alleles (Pemberton et al., 1995; Li et al., 2006). The data on genetic diversity obtained here showed that hybrid giant tiger grouper has a medium PIC level, indicating that this population is not in a dangerous condition. Considering that these data provide a snapshot of genetic diversity at the beginning of the breeding of these fish, this finding is reassuring and acceptable.

In summary, 10 microsatellites markers from hybrid giant tiger grouper were developed, showed successful amplification and presented high levels of heterozygosis. These markers provide the basic materials for further study to analyze genetic diversity in these fish and will be of value for conservation of the germplasm, development of breeding strategies, and for sustainable management. We will monitor future progress in grouper aquaculture to gather more extensive data on the genetic structure of hybrid giant tiger grouper to ensure the protection of its genetic diversity and of the parental species.

Table 1. Characteristics of 21 microsatellite loci inhybrid giant tiger grouper.

GenBank accession No.	Locus ID	Primer sequences (5'-3')	Repeat motif	Ta (°C)	N_A	Allele size (bp)	PIC	H_O	H_E
KP064527	ZZLD3	CTCCTGCTGCTTCATCCAAT GGCTCTTAACCCATTCTTCC	$(GT)_{56}$	50	7	270-340	0.501	0.3929	0.4235
KP064534	ZZLD15	TCCAGGACGGACAGACG TAAAGGCCAGGAGGTGC	$(AG)23(GA)_{15}$	56	3	138-156	0.348	0.3793	0.3074
KP064535	ZZLD16	TACACTGGGTTTTGGAGG ATTTTGCTAGTATGCTCACG	$(CT)_{24}$	49	7	285-310	0.699	0.9643	0.6754
KP064537	ZZLD27	AGACAGGGACCATTAGCAGG AAGGCTTTGTTTCTCACATCAC	$(AC)_{16}$	59.8	3	88-92	0.345	0.5806	0.4209
KP064538	ZZLD31	GTCAGGACGCCTCTTTGTG CTCGCCGTCGTCAAGGTAA	$(GT)_{17}$	49	3	149-153	0.410	0.2963	0.3018
KP064539	ZZLD33	CACAAACAGAGCAGAAGT ACAGTAATCCGTATGACAC	$(CT)_8CC(CT)_{15}$	45	2	235-250	0.342	0.6452	0.4370
KP064540	ZZLD35	GAGACAGTGGAGCACAAAGG GCGGCTGAGGTAAATGATAG	$(GA)_{56}$	64	7	158-200	0.737*	0.4074	0.7174
KP064541	ZZLD36	AAAACGAGGTCATAAAATAC TTAGCAAAGCAAACCAAAA	$(CT)_{32}...(CA)_{11}$	49	2	314-318	0.088**	0.0323	0.0921
KP064543	ZZLD41	TCTCGATGGCGCTGTAC AAATGGGCTGCGACTGG	$(CA)_{22}$	64	3	212-238	0.325	0.4194	0.3533
KP064546	ZZLD44	ACAGCCAACAAATGAG TTACGCAAAGCAGGGA	$(GT)_{18}$	55	6	222-253	0.498	0.6667	0.5072
KP064528	ZZLD4	TTATGCGTGGCTCACATTTTAC TGTTGAGTAGGCTGGCTGGAGA	$(AC)_{11}$	64	NA	180	NA	NA	NA
KP064529	ZZLD10	AAGTGGCTCAGATTTCATT TCGCTCTATCCTGTGGGT	$(GT)_{36}(GA)_{30}GG(GA)_{18}$	49	NA	228-240	NA	NA	NA
KP064530	ZZLD11	TATGTTTGGGTCACAATGATGCT AAACCGTTACATTACCTGGACAA	$(TGCC)_3$	65	NA	340	NA	NA	NA
KP064531	ZZLD12	GAGCACTTCAAAGCATCAGG CGGTTTTCAAAACAGCAACA	$(CA)_{16}$	47	NA	315	NA	NA	NA
KP064532	ZZLD13	AGTGATGCCTGGGAGGTTGA AGGGTGATAAAGGGCTGGTG	$(AG)_{24}$	60	NA	148	NA	NA	NA
KP064533	ZZLD14	TACACTGGGTTTTGGAGG ATTTTGCTAGTATGCTCACG	$(TG)_{36}C(GT)_{63}$	52	NA	230	NA	NA	NA
KP064536	ZZLD17	GTCCTAATAGAAGTAAATCTGCTC AGGGTGATAAAGGGCTGGTG	$(AC)_{20}...(GT)_{22}$	61.7	NA	232	NA	NA	NA
KP064542	ZZLD40	TTTCACCTCCACAGTCGC CTCCCTGTTCTTTTCCT	$(CA)_{21}$	49	NA	230	NA	NA	NA
KP064544	ZZLD42	AATCACGGTCACCTTGGC TTGCACTTGTTGTCTCCC	$(AC)_{21}$	53	NA	188	NA	NA	NA
KP064545	ZZLD43	TCGGCTGCTTTCTGGTTC TCCCGTGACTTGATGAGGTT	$(TG)_{18}$	52	NA	188-190	NA	NA	NA
KP064547	ZZLD46	AGGACGCAACCTGAAA GGACGGAAAGTAGAGC	$(CT)_{30}$	49	NA	169	NA	NA	NA

Ta = optimal annealing temperature; N_A = number of polymorphic alleles per locus; PIC = polymorphism information content; *significant deviation ($P < 0.005$), **highly significant deviation ($P < 0.001$) of locus from Hardy-Weinberg equilibrium after Bonferroni's correction (k = 10); H_O = observed heterozygosity; H_E = expected heterozygosity.

Conflicts of interest

The authors declare no conflict of interest.

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REFERENCES

Chen L, Li ZB, Chen XJ, Cao YY, et al. (2012). Isolation and characterization of 15 microsatellite loci in *Acrossocheilus labiatus* Regan (Cyprinidae). *Conserv. Genet. Res.* 4: 167-169.

Li ZB, Appleyard SA and Elliott NG (2006). Population structure of *Haliotis rubra* from South Australia inferred from nuclear and mtDNA analyses. *Acta Oceanol. Sin*. 25: 99-112.

Pemberton JM, Slate J, Bancroft DR and Barrett JA (1995). Nonamplifying alleles at microsatellite loci: a caution for parentage and population studies. *Mol. Ecol.* 4: 249-252.

RBFHG. Researchers Breed First Hybrid Grouper (2007). TheFishSite News Desk. Available at [http://www.thefishsite.com/fishnews/3629/researchers-breed-first-hybrid-grouper]. Accessed December 22, 2014.

Wolfus GM, Garcia DK and Alcivar-Warren A (1997). Application of the microsatellite technique for analyzing genetic diversity in shrimp breeding programs. *Aquaculture* 152: 35-47.

Yeh FC, Yang R, Boyle TJ, Ye Z, et al. (2000). PopGene32, Microsoft Windows-based freeware for population genetic analysis. Version 1.32, Molecular Biology and Biotechnology Centre. University of Alberta, Alberta.

Zane L, Bargelloni L and Patarnello T (2002). Strategies for microsatellite isolation: a review. *Mol. Ecol.* 11: 1-16.

