ONLINE RESOURCES

Development of forty-two single-nucleotide polymorphism markers in large yellow croaker (*Larimichthys crocea*)

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[Jiang L., Zhu A., Zhang J. and Wu C. 2015 Development of forty-two single-nucleotide polymorphism markers in large yellow croaker (*Larimichthys crocea*). *J. Genet.* **94**, e31–e34. Online only: http://www.ias.ac.in/jgenet/OnlineResources/94/e31.pdf]

Introduction

The large yellow croaker, *Larimichthys crocea*, is one of the most economically important marine fish species endemic to China. The wild stocks have severely affected from overfishing and they are already at the brink of extinction (Li *et al.* 2007). However, successful techniques for artificial breeding have been established in 1990s in southeast of China (Hong and Zhang 2003). With increasing ponds for artificial farming, several issues such as genetic diversity declining are being taken into consideration in the large yellow croaker farming industry (Guo *et al.* 2005; Wang *et al.* 2012).

Single-nucleotide polymorphisms (SNPs) are nucleotide variations in the DNA sequence of individuals. As the most abundant molecular markers in the genome, they are sequence-tagged markers with codominant inheritance, and thus are ideal for population genetic studies (Morin *et al.* 2004). Therefore, in recent years, SNP have been applied in many aquaculture species such as *Gadus morhua* (Hubert *et al.* 2009) *Salmo salar* (Hayes *et al.* 2007) and *Pinctada fucata* (Huang *et al.* 2014). However, to our knowledge, although the draft genome of *L. crocea* has been established (unpublished data), which could provide a platform for the identification of selection markers. There are no SNP loci which have been identified for large yellow croaker. At present, there are many SNP genotyping systems (Kim and Misra 2007). Our research focussing on developing a batch of SNP markers for population studies, MassArray system, is more flexible with a reasonable efficiency and expense, and has never been applied the identification of SNP markers in aquatic animals but widely used in genotyping, such as populations genetics of human and mammals (Wu *et al.* 2011). In this study, 42 SNP markers were developed in the large yellow croaker. They are the first SNP markers identified for analysing population genetic structure in this species.

Materials and methods

Candidate SNPs were detected from assembled contigs using the Genome Analysis Toolkit (GATK) (Tang *et al.* 2006) with the default parameters. SNP genotyping was performed using the MassArray System (Sequenom iPLEXassay, San Diego, USA). Primers and probes were designed using Primer3 (Rozen and Skaletsky 2000) and OligoCalc (Kibbe 2007), respectively.

Hundred and twenty individuals were sampled randomly from three wild populations in Zhoushan, Zhangpu, Haikou and a farm stock in Ningde, China (table 1). Genomic DNA was extracted from the fins using the standard phenol–chloroform method, and TagSNPs selection was done using the software Haploview (ver. 4.0) with a pairwise tagging approach. The genotypes of all the SNPs were determined by the MassArray system (Sequenom iPLEXassay, San Diego, USA). Approximately 15 ng of genomic DNA were isolated from the fin clips of the croakers. Locus-specific PCR and detection primers were designed using the MassARRAY Assay Design software (ver. 3.0) (Sequenom). The sample DNA was amplified via the multiplex polymerase chain reaction (Multiplex PCR), and the amplification was programmed as follows: an initial denaturation at 94°C for 15 min followed by 94°C for 20 s, 45 cycles of 56°C for 30 s, 72°C for 1 min and 72°C for 3 min. The resulting products were desalted with resin treatment and transferred to a 384-element Spectro CHIP array. The alleles were discriminated with mass spectrometry analysis (Sequenom). Meanwhile, genotyping was performed without knowledge of the case or control status of the subjects. Hundred and twenty random samples were tested in duplicate with different fishes, with a reproducibility of around 89.50%.

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Keywords. large yellow croaker; SNPs; markers; *Larimichthys crocea*.

Table 1. Sampling and stock details of the large yellow croaker (*L. crocea*).

Locality	Stock	Size	Weight (g)	Sampling time
Zhoushan, Zhejiang province	Daiqu population (wild)	30	100–200	2012-10
Haikou, Hainan province	Naozhou population (wild)	30	100–200	2012-05
Ningde, Fujian province	Minyuedong population (cultured)	30	100–200	2012-04
Zhangpu, Fujian province	Minyuedong population (wild)	30	100–200	2012-09

Table 2. Summary of 42 SNP markers in large yellow croaker (*L. crocea*).

SNP ID	Primers (5′–3′)	Probe	H_o	H_e	PIC	SNP		Accession no.
scf386	F: ACGTTGGATGACCAAGTGGATGGAGATCCC R: ACGTTGGATGTTTCTCTCTCTGCCTCTACC	CGTTTCCTCCTGCAA	0.040	0.040	0.038	G	A	KP006746
scf50	F: ACGTTGGATGATGCAGATGCTTGGAGTAAG R: ACGTTGGATGTTAGCCAGGAGGTGGAACAG	GCAGGACTGTTGACAT	0.080	0.078	0.074	A	G	KP006711
scf891	F: ACGTTGGATGTACTTCCTGCTGGACCTATG R: ACGTTGGATGATAGAAACCAGCACAAAGGG	GGAAGCAGATGGCTGA	0.080	0.078	0.074	C	T	KP006714
scf169	F: ACGTTGGATGCATAGATAGTTAAAGAGTGAC R: ACGTTGGATGTCAAATCTGACCTGTGGCCC	tTCCCACCCTCAATCCCTG	0.020	0.040	0.038	G	A	KP006717
scf333	F: ACGTTGGATGGTTTGAAACAGATGAACTTC R: ACGTTGGATGCTCAACAGGTTCATTTTCAGG	ccTTTTCAGGAATCACCCT	0.320	0.372	0.298	G	A	KP006749
scf109	F: ACGTTGGATGCACTAAAGCGTGAAACTCCC R: ACGTTGGATGGGAAACAAGAACACAACAGC	AACACAACAGCTGACTTCT	0.166	0.284	0.239	T	C	KP006733
scf100	F: ACGTTGGATGTGCTTTTGCATTGTGCCTCC R: ACGTTGGATGCCCCATTCAAGTTAGCAGAG	aAAGTTAGCAGAGAGGTAAA	0.520	0.393	0.311	C	A	KP006741
scf305	F: ACGTTGGATGCTCAGCTCACTGAAACCATC R: ACGTTGGATGTGAGGTGTGTCTGAGCAATC	cctccGAGCAATCCTCCAGAG	0.280	0.301	0.252	C	T	KP006712
scf225	F: ACGTTGGATGAGGACTACTTGAGTTACAGG R: ACGTTGGATGGATGTTACCAGTCAGCTTTG	CAGGGTATTTTGTCTTATCTT	0.080	0.078	0.074	G	A	KP006740
scf1080	F: ACGTTGGATGCATGCGTGCGTGTTGACAGT R: ACGTTGGATGTCACGAATGATCAGTGTCGG	ttggGATGCAGAGAGACTGCGA	0.200	0.184	0.164	T	C	KP006736
scf1963	F: ACGTTGGATGCTAGGTTGTGGTCTGAACTG R: ACGTTGGATGGCTGCACGCAAATGTTAAGG	cccccGATGCATACAGCTCCACAG	0.360	0.350	0.284	C	T	KP006721
scf25	F: ACGTTGGATGTTTCCTTTCACACTTGAGGG R: ACGTTGGATGCTGTGTTGTAACAGCTCAGG	GCTCTAAAGTTAGAATACTGATAC	0.280	0.246	0.212	T	C	KP006739
scf719	F: ACGTTGGATGGGTGGTTGAAACACAAAGCC R: ACGTTGGATGTGTTGCTGGTGACTTTACGG	aaacTCTACTCATCGTTTCTTTCAT	0	0.490	0.365	C	A	KP006728
scf708	F: ACGTTGGATGGACTACGGGATTAGAAAGGC R: ACGTTGGATGAACGTTCCCTGGTTTCCAAC	tataAGTTCACTACCTTAAAGAACC	0.792	0.511	0.375	T	C	KP006709
scf233	F: ACGTTGGATGCCCTCTTACCATGTGTATAG R: ACGTTGGATGTAGTAATTGTTCTCTGCGGG	cccACAACAACAGAACTTTCACATTT	0.400	0.470	0.355	T	C	KP006742
scf247	F: ACGTTGGATGCCCAAAGTCTGCCAGTAAAG R: ACGTTGGATGGGTTGTCTATCACGATGCAC	acgcTGCACTACACGAGATAAAGCGA	0.160	0.216	0.189	T	A	KP006750
scf664	F: ACGTTGGATGGCCTCTTGTTCACAATGCTG R: ACGTTGGATGGAAGCCCTTGTGGTTAAAGC	agTCTGTAGTAATCTCACATTGTGTAAT	0.095	0.418	0.325	T	G	KP006792
scf929	F: ACGTTGGATGTCCTGCGTACCAGAATGCTC R: ACGTTGGATGCCACTACTGACAAAGATCAAG	TCAAATTGCGCCAGG	0.042	0.042	0.040	C	T	KP006722
scf818	F: ACGTTGGATGATTTCCTGTTTGGAGCCACG R: ACGTTGGATGATACAGGGCATCCATGTGAC	GGTGAGTGGAGGTCA	0	0.169	0.152	A	C	KP006731
scf434	F: ACGTTGGATGTCTTGCACACAAGATTGCTC R: ACGTTGGATGATCGTGTATGTCATCGTCCG	cCATCGTCCGCCTCAA	0.125	0.191	0.169	T	A	KP006716
scf511	F: ACGTTGGATGTTCTGCTTCATCAGGATCGG R: ACGTTGGATGAGCCAGCGACTCAAACGGA	gaCAAACGGAGCCAGAC	0.522	0.487	0.363	G	T	KP006729
scf182	F: ACGTTGGATGGTATTCACACTCCTAACCTC R: ACGTTGGATGTGAAAAAATGCCGGCACACC	CCGACGTCTCCAGTTTTA	0	0.284	0.239	A	C	KP006715
scf602	F: ACGTTGGATGGCCTGACAGTTATAGAAAAC R: ACGTTGGATGGTGTCCCATTCTGTTGCAAG	cAAGGAGGAATTTCGCAG	0.208	0.503	0.371	C	T	KP006747
scf595	F: ACGTTGGATGTTGCCCAAGGACACTTCAAC R: ACGTTGGATGTGGATGCAATCGAGTGAAAG	cAAAGTTTTCTGTCCTTGC	0.125	0.439	0.337	A	T	KP006748
scf903	F: ACGTTGGATGCGCTTAGCAGAAACTGAAGG R: ACGTTGGATGGCCCTGAGGGTTAGGTAAAC	GGTTAGGTAAACTGGGTGA	0.348	0.464	0.351	A	G	KP006710
scf93	F: ACGTTGGATGCTGTGCTCAGTGTGTCTAAG R: ACGTTGGATGTCTGCTGTCGGATTCTCAAC	cgCAGTGCCAGTTAATACAG	0.333	0.497	0.368	C	T	KP006725
scf688	F: ACGTTGGATGCCGGATGCTAAAGGTTTGAG R: ACGTTGGATGGTGGTTTGTGTTAGCAGCAG	TAGCAGCAGAAAGCTTAACG	0.083	0.284	0.239	C	T	KP006719
scf795	F: ACGTTGGATGTGGGACAGAAGTTAAGCTGG R: ACGTTGGATGATCTGAGTGTCTGATTGGCG	gggtTCTGATTGGCGGACGTG	0.143	0.345	0.280	C	G	KP006737
scf496	F: ACGTTGGATGAGGAGACGTAGTGGTTCTTC R: ACGTTGGATGTGCATCAGGAGAGGCTGGTT	accgCCCGTACACTCCCGTAGA	0.391	0.415	0.323	T	G	KP006723

Table 2 (*contd*)

SNP ID	Primers (5′–3′)	Probes	H_o	H_e	PIC	SNP		Accession no.
scf95	F: ACGTTGGATGTGCTCTGTATGAGGTATGTG R: ACGTTGGATGGACCATAGATAGAGTGAAGG	TCTTAAAAACATACACTGGAGG	0	0.082	0.077	C	T	KP006727
scf175	F: ACGTTGGATGTTCTTAACTTCAACTATCC R: ACGTTGGATGATAGGCCTGCACTACTTCTG	ccCAAATTTAAACCACCCCTTTA	0.042	0.467	0.353	T	C	KP006744
scf540	F: ACGTTGGATGCAGTGGAAGCTAATTCAGTC R: ACGTTGGATGTGCTGACTTTGTTTCCAGCG	tttcaAGCGTTACTCATATTTGTC	0.083	0.156	0.141	C	T	KP006734
scf349	F: ACGTTGGATGAGATGTCTCGAAGGTATCCC R: ACGTTGGATGTTCTTGTGCTGGCTGATGTG	gcttGCTGATGTGTGTTTTTATGT	0.050	0.050	0.045	T	C	KP006713
scf1325	F: ACGTTGGATGGGAACAATGATAACATTTGG R: ACGTTGGATGGTTCAACTAAAAGTCATCCC	tccACTAAAAGTCATCCCTTTTTTT	0.182	0.507	0.373	A	T	KP006745
scf364	F: ACGTTGGATGCTCTGTGAACTACTTGGAGC R: ACGTTGGATGATTACAACTGGCTCCACTGC	ATGGAGTTTATTTGGAGTTTATAAT	0.046	0.046	0.043	A	G	KP006720
scf187	F: ACGTTGGATGCTTCTTGGGGCAGCTACATT R: ACGTTGGATGCTTGGAGCAACAAGTGTTTC	AGTGTTTCAAATTAACTTTCTCTGAA	0.125	0.191	0.169	A	T	KP006726
scf646	F: ACGTTGGATGCCCTTTGCAACATTAGCGAC R: ACGTTGGATGTCAGTAGACCAGGAAAGCTC	ccaatTCCATTTACCATTCAATGAGTC	0.292	0.254	0.218	G	A	KP006724
scf288	F: ACGTTGGATGGTATTTGCGTGTGGACTGTG R: ACGTTGGATGGGAAATCATCTTAGTGGAGG	ATTTATTGCTAAATTATGGATTTAGTT	0	0.089	0.083	G	T	KP006743
scf214	F: ACGTTGGATGGTATTGCTTCTTCTCACTCG R: ACGTTGGATGCGCTGTCACATTGATATTCC	tttgaAGGGTGTGAATGAAAACACAGG	0	0.156	0.141	C	G	KP006718
scf18	F: ACGTTGGATGTTGCCAACACATTTGGCAGG R: ACGTTGGATGTGCGATCATCATCTTTTTGG	ccTTTTTTTTCTTTGTGCCACTTGTGGG	0.353	0.513	0.374	C	G	KP006738
scf462	F: ACGTTGGATGCAGGAGACCTAATATAATGG R: ACGTTGGATGGTGAACAGCAGCAAACTTTG	gggcACAGCAGCAAACTTTGATATCTTG	0.522	0.433	0.338	C	T	KP006735
scf429	F: ACGTTGGATGCTACAAAGATCGCTGTGAAG R: ACGTTGGATGGCTGCAAACAATGTTTATAGG	ggcATGTTTATAGGGATTTTTTTCTTTA	0.450	0.358	0.288	C	T	KP006730

H_o, observed heterozygosity; H_e, expected heterozygosity; PIC, polymorphism information content.

Results and discussion

These 42 SNPs could be classified into four motifs by base substitution type: 25 (59.52%) were A/G (C/T), eight (19.04%) were A/C (G/T), six (14.28%) were A/T and only three (7.14%) were C/G. Besides, the classification result showed that C/T transition was the most frequent motif accounting for 45.24% (19), and C/G transversion was the least common variation, which is same as *S. salar, P. fucata* and *M. galloprovincialis* (Hayes *et al.* 2007; Li *et al.* 2007; Huang *et al.* 2014). Sixty primer groups were designed to identify SNP markers. PCR results showed that 42 primer groups were successfully amplified. A total of 10 genotypes (AA, CA, CC, CT, TT, GG, GA, GT, AT and GC) were found and the minor allele frequency ranged from 0.0561 to 0.491. The expected heterozygosity ranged from 0.273 to 0.320 and observed heterozygosity ranged from 0.190 to 0.235. The polymorphism information constant (PIC) value ranged from 0.215 to 0.247 (table 2). Compared with species of relatively stable population sizes, the dwindling fish species had low genetic diversity, potentially rendering them vulnerable to pathogens and environmental pressures.

In conclusion, this study successfully developed 42 SNP markers for the large yellow croaker. To our knowledge, this is the first report of development of SNP markers in large yellow croaker, and these markers should be useful for evaluation of genetic diversity in this species.

Acknowledgements

This study was supported by the National High-Technology Research and Development Programme of China (2012AA10A403-3) and the Open Foundation from Marine Sciences in the Most Important Subjects of Zhejiang (20130203).

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Received 11 August 2014, in revised form 24 October 2014; accepted 23 January 2015
Unedited version published online: 28 January 2015
Final version published online: 9 June 2015