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Using SSR Marker to Trace Chinese Shrimp *Fenneropenaeus chinensis* Released in Natural Sea — A Feasible Strategy for Assessment of Release Effect in Natural Resources Recovery Program

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Abstract Enhancement release has been proven effective in natural resources recovery of Chinese shrimp *Fenneropenaeus chinensis* in the last several decades in China, however, to assess the effectiveness of enhancement release, we still need to develop a high-efficient and easy-operational method to replace those physical-tagging release method with labor intensive, size- and number-limited. In the present study, single (with maternal known) parentage identification using eight simple sequence repeat (SSR) markers genotype fingerprint was used to trace Chinese shrimp released in Bohai Bay in 2013. A total of 884 shrimp spawners were collected from two hatcheries in Tianjin City respectively after enhancement release of shrimp larvae in May 2013. A total of 844 shrimp samples were recaptured around the release location approximately 4 months after the shrimp larvae were released into the natural sea. Genotype data of 8 SSR loci of the 1,726 samples were used for maternal-offspring parentage identification using CEervus 3.0 software. The allele number in each locus ranged from 8 to 68 with an average value of 33.25, which produced the cumulative exclusion probability with one parent known of all these sight loci up to 99%. Among the 844 recaptured shrimp samples, 448 (♂:♀=212:232, gender information was lost for 4 samples) were successfully traced to their 337 maternal parents using a logarithm of odds (LOD) > 3.0 threshold. Among these 337 maternal parents, 253 had a single offspring, 62 had two offspring, 18 had three offspring, 3 had four offspring, and 1 had five offspring. For the first time, a large number of released shrimp were identified from recapture samples, and this study showed that it is possible to trace all released Chinese shrimp without using any type of physical tag in enhancement release activities. This not only means more precise recapture ratio assessment than ever expected, but also this method demonstrates an effective method for large-scale hatchery release as well as for organisms used in hatchery enhancement which are not suitable for physical tagging.

Key words *Fenneropenaeus chinensis*; Parentage identification; SSR; Enhancement release

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Introduction

One of the most important native marine shrimp species in China, with smaller populations also found along the west coast of the Korean Peninsula, the Chinese fleshy shrimp (*Fenneropenaeus chinensis*) has played an indispensable role in Chinese fishery and marine culture (Deng, 1998). However, since the 1980s, the once plentiful captures of Chinese shrimp in the Yellow and Bohai Seas has dramatically declined. This decline has been attributed to an increase in fishing, pollution and natural habitat destruction. Additionally, the traditional spring shrimp fishing season disappeared approximately in 1986 (Deng *et al*, 2001). After several decades of continuous practice, hatchery release enhancement was proven to be an alternative strategy for natural population recovery, with the fishing industry also gradually benefiting from the strategy (Wang *et al*, 2006; Li *et al*, 2012). During this period, different methods were developed to assess the recapture rate. These methods mainly include marker- recapture methods with physical markers such as eye tags, visible implant elastomers (VIE, http://www.nmt. us), code wire tags (CWT, http://www.nmt.us) and radio frequency identification (RFID, http://www.biomark. com) (Klima, 1965; Luo *et al*, 2008; Gao *et al*, 2014). However, they do have deficiencies in common, such as labor-intensive tagging processes, damage to tagged individuals, and impracticality for large-scale application, all of which make it difficult to calculate precise recapture rates for assessing the effect of the release. Therefore, there is an urgency to develop a new marker for precise recapture rate assessments.

Wang *et al* (2014) introduced a new method that was based on individual identification and pedigree tracing using simple sequence repeat (SSR) markers, and it was successfully applied in release effect assessments in Jiaozhou Bay and Bohai Bay in China in 2012. In that study, a large number of shrimp from several full-sib families were replaced and functioned as the previously physically tagged individuals, thereby skipping the labor-intensive tagging process and significantly increasing the tagged individuals' number at the same time, consequently producing a more precise recapture rate. However, during this "molecular marker-recapture" process, several problems emerged, particularly regarding family development, which was tedious and prone to failure, and the number of shrimp carrying the "molecular marker" was still finite compared to the whole released stock (Wang *et al*, 2014). This prompted us to develop a more thorough and precise assessment method, where all released shrimp could be traced through parentage identification using an SSR marker. A previous study has verified the accuracy of SSR markers on single- parent parentage identification in Chinese shrimp (Zhang *et al*, 2014). In the current study, all of the 884 spawners from the two hatcheries responsible for larvae hatchery release in Tianjin City were collected and involved in a four-month-later single-parent parentage identification using 844 recaptured samples and genotype information from 8 SSR loci. For the first time, all offspring from those 884 spawners were identified from recapture samples; further, we could theoretically expect that all released shrimp could also be traced.

Materials and methods

Sample collection

In early March 2013, approximately 2000 shrimp spawners (sexual matured female shrimp carrying spermatophore) were collected along their spawning migration route in the Yellow Sea. The spawners were cultured at the Bohai Fishery Enhancement Station of the Bohai Fisheries Research Institute of Tianjin and the Dashentang Shrimp Seedling Plant in Tianjin. These stations are the two exclusively designated hatcheries for Chinese shrimp larvae production used for enhancement release in Bohai Bay in Tianjin City. After one and half month indoor domestication and nutrient enrichment period, a total of 884 spawners in both hatcheries started a 15-day spawning period. During this time, any dead individuals were collected and stored at –20℃. After the spawning period, all 884 spawners (successful and unsuccessful) were numbered and transported at –20℃ to the laboratory in the Yellow Sea Fisheries Research Institute (YSFRI), Qingdao, P. R. China and stored at –75℃ until use.

In early June, after a 45-day growth period, the fertilized eggs in the two hatcheries had developed into the post larval phase with body lengths of 1.2 cm, and an estimated 1.487 billion post-larvae were released into the Bohai Sea near Tianjin City. By the end of August, just before the natural Chinese shrimp population initiated their wintering migration, 844 shrimp were recaptured from the area around the original release location. The recaptured shrimp were transported alive to the YSFRI laboratory. The body length, weight, and gender were recorded for further analysis. All samples were maintained at –75℃ for DNA isolation.

Methods

The swimming apparatus of each shrimp sample was dissected for genomic DNA isolation using the protocol described by Wang (2008). Pure DNA was dissolved in double-distilled water to obtain a final concentration of 50 ng/μl. A total of 8 unlinked mutually SSR loci were used in genotyping all DNA samples, among which, 6 were the same as those used in previous reports by Zhang *et al* (2014) and Wang *et al* (2014). The first four of these six were EN0032, RS0622, FCKR002, and FCKR013, which formed a high-temperature group (HTG) of the quadruple SSR-PCR system to save PCR

and genotyping processing time. The other two were RS1101 and FCKR005. In the present study, two new SSR loci, FC019 and RS0683, were introduced to replace the previous FCKR005 and FCKR007 loci for more ideal genotyping results. The PCR reaction components and procedures were adopted according to the description of Li *et al* (2012), and all other SSR primer details were displayed in Tab. 1 (Li *et al*, 2012; Dong *et al*, 2014). An ABI 3130 auto-sequencer (Applied Biosystems Inc.) was used for the PCR amplification product separation. Allele size estimation and recording were completed using the DNA marker GeneScan-500 Liz (Applied Biosystems Inc.) and Genemapper software (Applied Biosystems Inc.). Cervus 3.0 software (http://www. fieldgenetics.com), a computer program for the assignment of parents to their offspring using genetic markers, was used for single-parent (maternal only) parentage identification.

Tab.1　Details of 4 SSR markers composing one set of quadruple-fold fluorescent-labeled PCR system and four single SSR-PCR systems

SSR-PCR system	Locus name	GenBank No.	Annealing temperature (℃)	Primer sequences (5′~3′) and labeled fluorescent dye
Quadruple-fold fluorescent-labeled SSR-PCR system	EN0033	AY132813	64	F: 6-FAM-CCTTGACACGGCATTGATTGG R: TACGTTGTGCAAACGCCAAGC
	RS0622	AY132778	66	F: ROX-TCAGTCCGTAGTTCATACTTGG R: CACATGCCTTTGTGTGAAAACG
	FCKR002	JQ650349	60	F: HEX-CTCAACCCTCACCTCAGGAACA R: AATTGTGGAGGCGACTAAGTTC
	FCKR013	JQ650353	61	F: TAMRA-GCACATATAAGCACAAACGCTC R: CTCTCTCGCAATCTCTCCAACT
Single-fold PCR system	RS1101	AY132811	52	F: 6-FAM-CGAGTGGCAGCGAGTCCT R: TATTCCCACGCTCTTGTC
	FC019	/	45	F: ROX-GTTGATGCCAGCAGTTAT R: TTCCAAGGGTCAGAGGTG
	RS0683	AY132823	64	F: HEX-ACACTCACTTATGTCACACTGC R: TACACACCAACACTCAATCTCC
	FCKR009	JQ650352	52	F: TAMRA-GCACGAAAACACATTAGTAGGA R: ATATCTGGAATGGCAAAGAGTC

Note: 6-FAM, ROX, HEX and TAMRA were the labeled fluorescent dyes

Results

The genotyping results of 8 SSR loci indicated that 1728 shrimp samples, including 884 spawners and 844 recapture samples, displayed high genetic variability. The allele number at each locus ranged from 8 to 68 with an average value of 33.25 (Tab.2). The polymorphic information content (PIC) values were 0.776~0.951 with an average value of 0.857. Further more, the cumulative exclusion probability with one parent known [combined non-exclusion probability (NE-1P): 8.913×10^{-5}] of all 8 SSR loci was in excess of 0.99% (Tab. 2), with which high reliable parentage identification results were expected.

Subsequently, 1728 samples were involved in single-parent parentage identification analysis between the 884 spawners and 844 recapture samples using the 8 SSR loci and Cervus 3.0 software. The results indicate that a total of 448 offspring from the 844 recapture samples were traced to 337 of the 884 spawners with a LOD value>3.0. The identified maternal-offspring relationship was comprised of the six following cases: among most of traced spawners (253), only one offspring was detected; in 62 traced spawners, two offspring were detected; in 18 traced spawners, three offspring were detected; in 3 traced spawners, four offspring were detected; and in one traced spawner, one offspring was detected.

Discussion

Parentage identification

With both parents known, only several SSR loci (given there are abundant alleles in each locus) could produce a high enough cumulative exclusion probability to meet authentic parentage identification, even in high-capacity sample analysis, as shown in previous research (Li *et al*, 2012; Dong *et al*, 2014). However, in practice, it is not always the case that both parents are simultaneously available, especially in some crustacean species that mature asynchronously. In the instance of Chinese shrimp, the male first transfers the spermatophore to the female during winter migration and then dies in the following autumn. In the following spring, the females

Tab.2 Summary of genetic variability of 8 SSR loci crossing the whole 1728 samples and exclusion probability they could offer

Locus	*k*	*N*	H_{Obs}	H_{Exp}	PIC	NE-1P	NE-2P	NE-PP
EN0033	68	1680	0.768	0.953	0.951	0.170	0.094	0.015
RS0622	26	1703	0.854	0.912	0.905	0.304	0.179	0.052
FCKR002	40	1423	0.921	0.952	0.950	0.177	0.097	0.017
FCKR013	27	1720	0.885	0.925	0.921	0.259	0.149	0.036
RS1101	23	1697	0.778	0.804	0.776	0.558	0.382	0.200
FC019	43	1725	0.774	0.901	0.894	0.323	0.193	0.057
RS0683	8	1718	0.856	0.615	0.541	0.809	0.667	0.515
FCKR009	31	1517	0.697	0.926	0.921	0.258	0.149	0.036
Mean number of alleles per locus	33.25							
Mean observed heterozygosity			0.817					
Mean expected heterozygosity				0.874				
Mean polymorphic information content (PIC)					0.857			
Combined NE-1P						8.913×10^{-5}		
Combined NE-2P							1.790×10^{-6}	
Combined NE-PP								1.009×10^{-10}

Note: *k*: Number of alleles at the locus; *N*: Number of individuals typed at the locus; H_{Obs}: Observed heterozygosity; H_{Exp}: Expected heterozygosity; NE-1P: Average non-exclusion probability for one candidate parent; NE-2P: Average non-exclusion probability for one parent given the genotype of a known parent of the opposite sex; NE-PP: Average non-exclusion probability for a candidate parent pair

bearing spermatophores simultaneously produce eggs and release the matured sperm from spermatophore at the spawning sites. In this case, single parentage identification using more SSR markers than used when oth parents are known is an alternative for tracing released individuals or other related studies. The accuracy of parentage identification using SSR markers has been confirmed by an indoor sealed pond experiment using physically tagged individuals (VIE) as the reference (Li *et al*, 2012). The accuracy of maternal only parentage identification was indirectly confirmed by two enhancement release studies of Chinese shrimp in Bohai Bay and Jiaozhou Bay in 2012 (Wang *et al*, 2014). In the present study, two SSR loci from the previous study, FCKR005 and FCKR007, were replaced with two newly introduced loci, FC019 and RS0683 for better genotyping resolution (Wang *et al*, 2014; Dong *et al*, 2014). There were 43 and 8 alleles detected at FC019 and RS0683, respectively. The sum of alleles is slightly less than what was detected at loci FCKR005 and FCKR007 in the previous study, indicating that the cumulative exclusion probability produced by the present 8 SSR loci is slightly less than or equal to that produced by the previous 8 SSR loci. Moreover, in the present study, at each assignment where the 8 SSR loci matched between the candidate parent and offspring, only when the locus-by-locus logarithm (base 10) of odds (LOD) score was≥3.0. Could it be declared as a true parentage relationship?

Previous studies have shown that where the LOD score was not considered in the parentage assignment, even when alleles at each locus from offspring matched the parents or a single parent, the chance of a false positive would significantly increase. In regards to the precision of tracing released individuals using SSR markers, the following measures were suggested in the study by Bravington *et al* (2004): (i) Use as few brood-stock as possible. Generally, a wild Chinese shrimp spawner can produce 400,000 to 1,800,000 fertilized eggs, among which, 40%~60% could develop to the post larvae phase in plants and be released into the sea. In practice, plants fishing shrimp spawners along their spawning migration route at each spring for shrimp larvae production. Then, for a better profit, they usually produce as many post larvae as possible using as few spawners as possible, which in fact increases the chance to produce authentic parentage identification results, meanwhile increasing the risk of inbreeding depression. (ii) Obtain paternal as well as maternal genotypes. Chinese shrimp are a type of asynchronous-maturing crustacean, in other words, the male transfers the spermatophore to the female and subsequently dies. The female bearing the spermatophore simultaneously releases the egg and the sperm the next spring after they have finished their over-wintering phase. Removing the spermatophore directly would seriously damage the female. Therefore, in the present study, only the genotype information from the female was involved in the parentage identification analysis. (iii) Stock as many as possible, are similar to premise (i). In fact, the two shrimp seedling plants produced and released approximately 1.2×10^9 post larvae shrimp using only

approximately 1,200 captured spawners in the spring of 2012. (iv) Finally, capture and genotype are as many as prawns as possible. The number of recaptured samples in this study was 844, among which, a total of 448 released individuals were identified, representing two large sample sizes. On account of the above-mentioned measures, the accuracy of the parentage identification results in this study is reliable.

Release effect assessment

Using SSR markers to trace released individuals played an important part in assessing the accuracy of the effect of hatchery enhancement of Chinese shrimp. Due to the tiny size (the released shrimp post larvae were approximately 1.2 cm), and the large scale release number (approximately 0.5×10^9~1.5×10^9 shrimp post larvae were released into Bohai Bay every year), there was no appropriate physical marker that could be used. This problem has persisted over several decades which was one of the most puzzling problems in the accuracy assessment of the effect of hatchery release since the Chinese shrimp hatchery release activity was initiated. Bravington *et al*(2004) evaluated the potential of using SSR markers for estimating the proportion of hatchery-reared offspring in an enhancement program, which prompted us to introduce the use of SSR markers in the assessment of the effect of Chinese shrimp hatchery enhancement. The first attempts were carried out in Jiaozhou Bay and Bohai Bay in 2012. In each bay, six full-sib families were released with a hatchery stock. During the late autumn fishing season, a total of 12 individuals from 7 families were identified from 5739 recaptured samples using SSR markers. This was the first time that molecular marker recapture assessment was applied to the Chinese shrimp hatchery enhancement (Wang *et al*, 2014). However, due to the small number of molecular-marked individuals released from the 12 full-sib families (but far more than previous studies that released physically tagged shrimp), the ratios between the molecular-marked individuals and the hatchery stock in Bohai Bay and Jiaozhou Bay were approximately 1:800 and 1:300, respectively. The reliability of the recapture ratio using molecular marker recapture in that study still needs be considered, although it produced a molecular marker recapture ratio very close to those of traditional methods (Wang *et al*, 2014).

To improve the accuracy of molecular marker recapture assessment in Chinese shrimp, one strategy could be the release of all stock throughout full-family development. In that case, all released shrimp could be traced using SSR genotyping information under the condition that all parents were known. However, it was only theoretical, since large scale artificial fertilization is labor-extensive and easily fails. Additionally, mated female over-wintering cultures and specific family culture installations are only available at small plants. Since hatcheries release spawner shrimp every spring and the spawners that finished producing could be collected, the alternative is parentage identification with only maternal parents known. In the present study, the number of shrimp spawners used in the seedling development was close to 2000. The original plan was to sample all of these spawners after they finished spawning. However, for uncontrolled reasons, only 844 spawners were sampled for subsequent SSR genotyping, and only 337 individuals were identified as the previously released individuals from the 844 recaptured samples, with the remaining 505 individuals originating from both the wild population and the released stock. The genetic variation between the released stock and the wild population could not be further assessed in this study. This problem has been assessed in the Chinese shrimp hatchery enhancement field for the last thirty years.

Before they initiate their over-wintering migration in the late autumn, all shrimp, including the wild population and the released stock concentrate and forage in a relatively fixed area. When all spawner shrimp used in the hatchery enhancement are sampled in an area (for example, Bohai Bay), then there is no difficulty in discriminating the wild population from the released stock in the recaptured samples. Additionally, It could also be clarified whether the released stock "dilute" the genetic diversity of the wild population (Kitada *et al*, 2009). Further, the number and proportion of the released stock in the following year's spawner population could be accurately identified by investigation over consecutive years, which is also critical to evaluate the effect of the hatchery enhancement and has not yet been assessed. Chinese shrimp exhibit migration behavior over their entire life period, and their migration route spans northern latitudes from 33° (over-wintering) to 41° (spawning) (Deng *et al*, 1990). Deng *et al* (1990) investigated the distribution of the migration routes of the species, including spawning migration, foraging migration and over-wintering migration using physical tagging release assessment. Due to the limited number of physically tagged shrimp, however, the different geographic distribution of the population still needs to be evaluated. Using the parentage identification strategy in this study, this puzzle may be clarified in the future.

References

Bravington MV, Ward RD. Microsatellite DNA markers: Evaluating their potential for estimating the proportion of hatchery-reared offspring in a stock enhancement programme. Molecular Ecology, 2004, 13(5): 1287–1297

Deng JY, Ye CC, Liu YC. Penaeid shrimp—Its biology and management in the Yellow-Bohai Seas. Beijing: Ocean Press,

1990, 256–258 [邓景耀, 叶昌臣, 刘永昌. 渤黄海的对虾及其资源管理. 北京: 海洋出版社, 1990, 256–258]

Deng JY, Zhuang ZM. The cause of recruitment variation of *Penaeus chinensis* in the Bohai Sea. Journal of Fishery Sciences of China, 2001, 7(4): 125–128 [邓景耀, 庄志猛. 渤海对虾补充量变动原因的分析及对策研究. 中国水产科学, 2001, 7(4): 125–128]

Deng JY. The research status of shrimp fishery biology. Chinese Bulletin of Life Sciences, 1998, 10(4): 191–194, 1997 [邓景耀. 对虾渔业生物学研究现状. 生命科学, 1998, 10(4): 191–194, 1997]

Dong SR, Luan S, Kong J. Screening of microsatellite DNA markers associated with growth traits in a single *Fenneropeneaeus chinensis* family. Journal of Fishery Sciences of China, 2014, 21(5): 936–943 [董世瑞, 栾生, 孔杰. 中国明对虾单个家系中与生长性状相关微卫星标记的初步筛选. 中国水产科学, 2014, 21(5): 936–943]

Gao H, Yan BL, Lai XF, *et al*. Progress on the identification technology used in stock enhancement for crustacean organisms. Transactions of Oceanology and Limnology. 2014(1): 94–100 [高焕, 阎斌伦, 赖晓芳, 等. 甲壳类生物增殖放流标志技术研究进展. 海洋湖沼通报, 2014(1): 94–100]

Kitada S, Shishidou H, Sugaya T, *et al*. Genetic effects of long-term stock enhancement programs. Aquaculture, 2009, 290(1–2): 69–79

Klima EF. Evaluation of biological stains, inks, and fluorescent pigments as marks for shrimp. Washington: U.S. Department of the Interior, Bureau of Commercial Fisheries, 1965

Li WY, Wang WJ, Kong J, *et al*. Establishment of the microsatellite quadruple PCR technology for *Fenneropenaeus chinensis* and its application in the evaluation on the effect of releasing simulation. Acta Oceanologica Sinica, 2012, 34(5): 213–220 [李伟亚, 王伟继, 孔杰, 等. 中国对虾微卫星四重 PCR 技术的建立及其在模拟放流效果评估方面的应用. 海洋学报, 2012, 34(5): 213–220]

Li ZY, Wang J, Zhao ZL, *et al*. Resources enhancement of *Fenneropenaeus orientalis* in the Bohai Sea. Progress in Fishery Sciences, 2012, 33(3): 1–7 [李忠义, 王俊, 赵振良, 等. 渤海中国对虾资源增殖调查. 渔业科学进展, 2012, 33(3): 1–7]

Luo K, Zhang TS, Kong J, *et al*. Study on techniques of internal tagging by visible implant fluorescent elastomer in *Fenneropenaeus chinensis*. Marine Fisheries Research, 2008, 29(3): 48–52 [罗坤, 张天时, 孔杰, 等. 中国对虾幼虾荧光体内标记技术研究. 海洋水产研究, 2008, 29(3): 48–52]

Wang QY, Zhuang ZM, Deng JY, *et al*. Stock enhancement and translocation of the shrimp *Penaeus chinensis* in China. Fisheries Research, 2006, 80(1): 67–69

Wang WJ, Zhang K, Luo K, *et al*. Assessment of recapture rates after hatchery release of Chinese shrimp *Fenneropenaeus chinensis* in Jiaozhou Bay and Bohai Bay in 2012 using pedigree tracing based on SSR markers. Fisheries Science, 2014, 80(4): 749–755

Wang WJ. Ⅰ Genetic mapping of the Chinese shrimp *Fenneropenaeus chinensis* using AFLP markers and commercial traits QTL mapping. Ⅱ Genetic linkage mapping using AFLP markers and primarily study on sex-determination of bluegill sunfish (*Lepomis macrochirus*). Doctoral Dissertation of Ocean University of China, 2008 [王伟继. Ⅰ中国对虾(*Fenneropenaeus chinensis*)AFLP 分子标记遗传连锁图谱的构建以及相关性状的 QTL 定位分析 Ⅱ蓝鳃太阳鱼(*Lepomis macrochirus*)AFLP 分子标记遗传连锁图谱的构建及性别决定机制初探. 中国海洋大学博士研究生学位论文, 2008]

Zhang K, Wang WJ, Kong J, *et al*. Accuracy of short sequence repeats on single-parent parentage identification in Chinese shrimp *Fenneropenaeus chinensis*. Aquatic Biology, 2014, 20(1): 45–51

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