Li et al. BMC Res Notes (2016) 9:105
DOI 10.1186/s13104-016-1909-6

BMC Research Notes

SHORT REPORT

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Identification and characterization of thirty novel microsatellite DNA markers from the Chinese mitten crab *Eriocheir sinensis* expressed sequence tags

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Abstract

Background: The Chinese mitten crab *Eriocheir sinensis* is an economically important decapod crustacean in China. Despite a widespread distribution and production in China, the resources of *E. sinensis* have experienced a dramatic decline in the past decades. Here we describe a new set of novel polymorphic microsatellite loci to facilitate the investigation of genetic structure and artificial breeding.

Results: In this study, a set of 30 novel polymorphic microsatellite markers for *E. sinensis* was developed from EST databases. The number of alleles per locus ranged from three to twenty. The observed and expected heterozygosities ranged from 0.047 to 0.932 and from 0.047 to 0.935, respectively.

Conclusions: These informative microsatellite markers will be useful in studies of genetics, genomics and marker-assisted selection breeding in *E. sinensis*.

Keywords: Microsatellites, EST, Chinese mitten crab, *Eriocheir sinensis*

Findings

Background

The Chinese mitten crab *Eriocheir sinensis* is one of the most economically important aquaculture species in China [1] due to its taste and nutritious value, with a native range extending from the coastal estuaries of Korea in the north to the Fujian province of China in the south. However, the wild populations of *E. sinensis* have experienced a dramatic decline in the past decades due to overfishing and water pollution [2]. In China, the basic production technology of mitten crab populations has had a long history, with the conventional selective breeding programs based on phenotypic assessment. At present, the yield of *E. sinensis* is almost completely from artificial breeding. Unfortunately, like many other cultured species, the aquaculture performance of *E. sinensis* has declined significantly. In order to protect genetic diversity and prevent population degradation, understanding population genetic structure and genetic connectivity among populations and making a genetic linkage map are necessary.

Microsatellite markers provide a powerful tool in genome researches due to their wide distribution, codominant inheritance and high polymorphism. To date, approximately 83 microsatellite markers have been developed and applied for *E. sinensis* [3–8]. Although the number of described loci is relatively high, much more works is still needed because of the large diploid chromosome number of *E. sinensis* ($2n = 146$) [9]. In this study, we describe a new set of 30 EST-derived microsatellite markers which would aid in characterizing population structure, genetic diversity and constructing linkage map in *E. sinensis*.

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Experimental section

A total of 17067 *E. sinensis* ESTs obtained from the GenBank database (2013) were screened using SSRIT program [10] that was designed to find regions containing microsatellites. The parameters were set for detection of di-, tri- and tetranuclotide motifs with a minimum of six repeats, respectively. Eighty-five microsatellite loci were selected for microsatellite marker optimization. Primers flanking microsatellite were designed using the PRIMER PREMIER 5.0 program.

Sixty cultured *E. sinensis* individuals were randomly captured from Xieyuan Fishing Company in Qilihai region in Tianjin City, China. Genomic DNA was extracted from the leg muscles using a modified phenol-chloroform protocol [11]. Polymerase chain reaction (PCR) amplifications were performed in 10-μL volumes containing 0.25 U *Taq* DNA polymerase (Takara), 1× PCR buffer, 0.2 mM dNTP mix, 1 μM of each primer set, 1.5 mM $MgCl_2$ and about 100 ng template DNA. The PCR profiles for all loci were an initial denaturing at 94 °C for 3 min, followed by 35 cycles of 1 min at 94 °C, 1 min at the annealing temperatures listed in Table 1, and 1 min at 72 °C, with a final extension step of 5 min at 72 °C on a MJ Research PTC-200 DNA Engine (Peltier Thermal Cycler). Amplification products were resolved via 6 % denaturing polyacrylamide gel, and visualized by silver-staining. A 10-bp DNA ladder (Invitrogen) was used as a reference marker for allele size determination. The calculations of observed and expected heterozygosities were estimated with the program MICROSATELLITE ANALYSER software [12]. Tests for linkage disequilibrium and deviations from Hardy–Weinberg equilibrium (HWE) were performed using GENEPOP 4.2 [13, 14].

Results and discussion

Of the 85 potential microsatellite markers, forty-four loci were successfully amplified with the expected products. Thirty of them revealed polymorphism among the tested 60 individuals of *E. sinensis*. The number of alleles at each locus ranged from three to twenty with an average of 8.767 alleles per locus (Table 1). Observed heterozygosities ranged from 0.047 to 0.932 with an average of 0.527, while expected heterozygosities ranged from 0.047 to 0.935 with an average of 0.693. The mean number of alleles per locus, H_O and H_E demonstrated a relatively high genetic diversity within crab individuals. This was similar to reports from studies in other locations [3, 4, 6, 8]. Fourteen of the 30 loci significantly deviated from the Hardy–Weinberg equilibrium after Bonferroni correction. This might be due to the limited sample size, and/or the presence of null alleles at these loci. The high polymorphism of the loci suggests that they would be useful tool in studies of population structure, genetic diversity and the construction of genetic map for *E. sinensis*.

Conclusions

A set of 30 novel hypervariable microsatellite loci in *E. sinensis* was reported in this study. All the characterized microsatellite markers are suited for assessing the genetic diversity and the population structure, and also facilitate marker-assisted selection breeding of *E. sinensis*.

Ethics statement

Every effort was made to minimize animal pain, suffering and distress and to reduce the number of animal used. Sampling of the crabs was approved by Tianjin Diseases Prevention and Control Center of Aquatic Animals.

Availability of the supporting data

The microsatellite sequences are available through the National Centre for Biotechnology Information (http://www.ncbi.nlm.nih.gov); GenBank accession numbers see Table 1.

Table 1 Characterization of 30 EST-SSRs in the Chinese mitten crab *Eriocheir sinensis*

Locus	GenBank accession no.	Repeat motif	Primer sequence (5′–3′)	T_a (°C)	No. of individuals	No. of alleles	Size range (bp)	H_O	H_E	*P* value
ES27	FL571941	$(GT)_{11}$	F: TGTGATGAGAAGAAACCAAAGA	52	59	8	107–123	0.712	0.797	0.0005*
			R: AATACCTGCTGGCGATGA							
ES39	FG359711	$(GT)_{17}$	F: AGGACGAAAGTTGGAGGG	55	56	8	114–128	0.482	0.864	0.0000*
			R: AAATACAAATCTACGGGAGACAC							
ES104	FL569100	$(TG)_{21}$	F: TCACAACTACGAAAACCT	48	53	3	190–200	0.047	0.047	1.0000
			R: GAGTGTCAGTGTATGGAAT							
ES108	FG984086	$(GT)_{19}$	F: GTAAACCCTACGAAACCATA	54	59	15	91–119	0.932	0.932	0.0000*
			R: ACTCCCTAAACTACCTAACTACCA							
ES130	FL570152	$(GGC)_7$	F: CGTTCGTTGTGAGCGTCTGC	57	60	4	145–154	0.167	0.159	1.0000
			R: CGCCTGGTCCATCTCATCG							
ES212	FG984116	$(CCA)_6(CAA)_{10}$	F: GTGACACTGATGCCTGACGA	55	56	4	181–190	0.482	0.583	0.2114
			R: TTATGCCTTTATTGACCGAGAC							
ES271	FG359821	$(TG)_{12}$	F: GCTTCTCACCCGTGATGT	54	49	6	176–186	0.615	0.754	0.0065
			R: CTCCTCCTTTGCTTTCTTTA							
ES352	FL572494	$(GT)_{10}$	F: CACTCGGTACAAACATCAC	53	56	17	91–127	0.768	0.929	0.0000*
			R: AATGGGTATGGATTTAGTGT							
ES582	FL570362	$(GA)_{26}$	F: ACCTCCAAGCCCCTTACC	53	58	5	241–261	0.293	0.296	0.6958
			R: GAACAAACACGAGGGACAAC							
ES584	FL574606	$(CA)_{22}$	F: AGGGAAGTTGTAAAGGTAAGGA	49	56	9	222–240	0.429	0.863	0.0000*
			R: ATGGGAATGAGATGAGGATAGA							
ES645	FL571837	$(AC)_{13}$	F: GACGCACGACAACAACCTC	60	56	11	122–158	0.522	0.912	0.0000*
			R: CCACTCCTAGTCAACGGAAAGA							
ES709	FL574505	$(GCA)_7$	F: GCAGCCACAACCAGCAGAAG	60	60	6	197–212	0.233	0.219	1.0000
			R: CTCGCCATGCAGGATCACC							
ES776	FG357327	$(CA)_{34}$	F: GTTGGTGTTGAAGGAGCCA	53	59	4	204–220	0.410	0.557	0.1180
			R: CTTAATCCGTTGCGTCAGC							
ES789	GE340666	$(GT)_{25}$	F: TCGGGTGAGTTAGGTGTAGG	52	53	18	178–218	0.170	0.929	0.0000*
			R: AGCAAGGCACTTTGAAGC							
ES851	GE340258	$(CAC)_7$	F: TCCAACCAGGCGGCAAAG	54	54	7	216–240	0.778	0.814	0.0580
			R: AGCAAGTCCACCGAACACCAT							
ES911	FL569216	$(TTCA)_7$	F: CGGCGAGACTCACGAACT	56	53	5	242–258	0.453	0.634	0.0012*
			R: CGAGGGTGAAGAGGCATT							

Table 1 continued

Locus	GenBank accession no.	Repeat motif	Primer sequence (5′–3′)	T_a (°C)	No. of individuals	No. of alleles	Size range (bp)	H_O	H_E	*P* value
ES998	FL572952	$(GT)_5N(TG)_5N_2(TG)_{12}$	F: CGACGGTGTCAGATTAGTG R: ACCAACGGGCTCAAGAAG	56	51	8	217–231	0.392	0.860	0.0000*
ES1045	FL575077	$(GT)_{28}$	F: GGAGCACCACCGTAAAGATA R: TCAACACGAAACCGCCAC	55	55	17	112–148	0.582	0.935	0.2415
ES1053	FL572054	$(CA)_{10}$	F: CTACACCAAGACCTCCTCGT R: GGCTGGTTTGTTGGGTAAG	57	58	6	145–155	0.741	0.703	0.3476
ES1126	FG359126	$(AAGG)_{12}$	F: TGTCCAGTCTCCCATCAA R: TGGTATGGTCGCTAATCTC	54	60	14	180–240	0.883	0.907	0.7140
ES1139	FL569992	$(GA)_{11}$	F: ACAGACGCACCTCCAAGC R: TTAGAACAAACACGAGGGACA	55	59	20	110–150	0.644	0.925	0.0000*
ES1171	FG357452	$(AC)_8N_5(TC)_6$	F:CAATCTGCCCTAATCTGTCTGTAA R:GGGAAAGGTAGGAGGATAAGTGA	57	55	8	156–172	0.900	0.871	0.0000*
ES1178	GE341515	$(ACC)_7$	F: TCCCATCGCCGTAGAAAC R: ACGCCAGACTGGACAAGC	55	60	3	138–146	0.150	0.144	1.0000
ES1240	FG982578	$(TACA)_{11}$	F: ATTGTAGCCATACCAGCAT R: ACAAATCTTACAACTACGGC	52	60	13	152–200	0.883	0.890	0.3087
ES1289	FL574500	$(TTA)_{13}$	F: ACCTTGTGGATACCAGCAT R: TTCCCTTCAACCATACATAA	50	55	12	124–169	0.782	0.872	0.0000*
ES1293	FL569028	$(GTA)_7N_8(GTA)_5$	F: GCCTCAATATCGGGCTTAT R: CCTCCCTGCGACTTCTACT	55	59	7	129–176	0.763	0.769	0.2826
ES1300	FL575122	$(TG)_{10}$	F: CCCTTGTTGATTGCCCTA R: GTCACGAAGAAGCACCTC	55	52	5	186–194	0.519	0.608	0.0846
ES1482	FL576108	$(TG)_7$	F: ACTATCCCTGCCTCACTACCG R: GAACAAACATTACCGTCACTCG	57	60	7	115–129	0.250	0.522	0.0000*
ES1507	FL572842	$(AGT)_5N_2(TAG)_9$	F: TGGAGTAGGTCGGTTCGGT R: TAGCAACATCCCTCGTCCTC	57	58	6	197–215	0.603	0.743	0.0299
ES1513	FL574610	$(AG)_9$	F: AACAGTGGCAGGAAACAGAAAG R: AGGGAAGGATGAGTGTGAGCA	57	56	7	100–114	0.217	0.739	0.0000*

T_a annealing temperature, H_O observed heterozygosity, H_E expected heterozygosity

* Significant departure ($P < 0.05$) from expected Hardy–Weinberg equilibrium conditions after correction for multiple tests ($k = 30$)

Authors' contributions
JS was responsible for the design of this study, supervision of the work and contributed to the interpretation of results. JL performed field sampling, data analysis and marker validation, and drafted the manuscript. XG coordinated field sampling and was responsible for the implementation of the study. LC contributed to analysis of sequences. All authors read and approved the final manuscript.

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Acknowledgements
This work was supported by Grants of the National High-Tech Research and Development Program of China (863 programs, 2012AA10A401), National Key Technology R&D Program (2012BAD26B04-05), Tianjin Technical Supporting Program of Tianjin (12ZCDZNC05500) and Research and Extension Projects of Tianjin Fishery Bureau (J2013-21) (J2013-7).

Competing interests
The authors declare that they have no competing interests.

Received: 17 September 2015 Accepted: 3 February 2016
Published online: 17 February 2016

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