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Zoological Research

Microsatellite Variations Among Four Populations of *Eriocheir sinensis*

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Abstract：Using microsatellite markers，we analysed genetic polymorphism in four populations of *Eriocheir sinensis*，sampled from Jiangsu，Anhui，Liaoning and Tianjin．Twenty-four pairs of primers were used to amplify the target fragments ranging from 80 bp to 445 bp，which included 16 pairs designed in our laboratory and eight pairs published internationally．Two to 10 alleles per locus in four populations were amplified，and there were 155 alleles in all populations．The average number of alleles per locus was 6.458．The average number of effective alleles per locus（*Ne*）was 4.3491 to 4.7234；the average observed heterozygosity（*Ho*）was 0.5690 to 0.6722；and the average expected heterozygosity（*He*）was 0.7238 to 0.7546．Hardy-Weinberg equilibrium analysis（χ^2 test，$P < 0.05$）revealed that seven loci in the four populations were in equilibrium．The genetic distances between the four populations were calculated and revealed that the Anhui，Jiangsu and Tianjin crabs belong to a Yangtse River population，while the Liaohe crabs form another branch．

Key words：*Eriocheir sinensis*；Microsatellite；Population polymorphism

利用微卫星分子标记分析四个中华绒螯蟹群体的遗传多样性

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摘要：利用本实验室克隆的16个和国际上发表的8个微卫星标记，对4个中华绒螯蟹群体（江苏、安徽、辽宁、天津）的遗传多样性进行检测。所检测到的扩增片段长度为80—445 bp，在群体间扩增出2—10个等位基因，共计155个等位基因，平均等位基因6.458个。4个中华绒螯蟹群体的平均有效等位基因数（*Ne*）为4.3491—4.7234，平均观察杂合度（*Ho*）为0.5690—0.6722，平均期望杂合度（*He*）为0.7238—0.7546，并通过基因型的P值，确定了7个座位处于Hardy-Weinberg平衡；同时对4个群体的遗传距离进行了估算，聚类分析结果表明，安徽、江苏、天津聚为一支，属于长江河蟹类型，辽河种群单独聚为一支。

关键词：中华绒螯蟹；微卫星；群体多样性
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The Mitten crab（*Eriocheir sinensis*）is a very important aquatic commercial species in China．It is distributed mainly in the Yangtse，Liaohe and Oujiang Rivers and their neighboring regions．These populations are very difficult to distinguish from their phenotypes but their growth speeds are very different．The Yangtse River crabs are

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the biggest in body form and it is thought to be the most valuable breed (Zhao et al, 1998).

Microsatellites occurs in most eukaryote genomes. They can be very informative in genetic studies and are multi-allelic and reproducible (Powell et al, 1996; Quan et al, 2005; Russell et al, 1997; Wei et al, 2001; Zhang et al, 2006). Many studies have been done to investigate the population genetics of *E. sinensis*, using anatomy (Xu et al, 1997; Li et al, 1999; Dai et al, 1998), biochemistry (Xu et al, 1996; Wang et al, 1995; Zhao et al, 1999) and random amplified polymorphism DNA (RAPD) (Qiu et al, 1997; Gao et al, 1998; Xiang et al, 1998; Zhou et al, 1999; Xie et al, 1999; Li et al, 1999). For example, Qiu (Qiu et al, 1999) used RAPD to study the genetic differences of three populations of *E. sinensis*, and found that the differences between populations were significant; Xie et al (1999) used RAPD to describe the relationship between *E. sinensis*, *E. japonicus* and *E. japonicus hepuensis*; Zhao & Zhao (1999) analysed the biochemical genetic differences of two varieties of mitten crabs (*E. sinensis* and *E. japonicus*) from the Liaohe, Yellow, Yangtse, Oujiang, Pearl and Nanliujiang Rivers, and found that there were signigicant differences in biochemical genetic characters of the *E. sinensis* from the Liaohe and Yangtse Rivers. However there are few reports on microsatellites in *E. sinensis*.

With the development of commercial breeding of *E. sinensis*, crabs from different populations have been interbred and the natural resource exhausted. Because of this it is urgent to decipher the genetic differences of different populations. In this study, we used 24 microsatellite loci to analyse the genetic differences between the Yangtse River population (samples are from Jiangsu, Anhui and Tianjing) and the Liaohe River population (samples are from Liaoning). This work will shed light on the population structure, population history and conservation of *E. sinensis*.

1 Materials and Methods

1.1 Materials

The samples of *Eriocheir sinensis* were collected from Jiangsu (J), Anhui (A), Tianjin (T) and Liaoning (L). Thirty samples (15 male and 15 female) were collected from each of Jiangsu, Anhui and Tianjin. In addition 29 samples (14 male and 15 female) were collected from Liaoning. Samples from Jiangsu, Anhui and Tianjin are all from the Yangtse River population, while samples from Liaoning belong to the Liaohe River population.

1.2 Methods

We used the phenol/chloroform method to extract DNA.

Microsatellies were constructed from the *E. sinensis* genome by combining the biotin magnetic beads capture method with radioactive labeling hybridization. The online program Primer 3 was used to design and filter primers. Finally we selected 16 primer pairs to study the population diversity of *E. sinensis*. The primers are given in Tab 1.

We obtained eight pairs of primers that have been published, which were synthesized by Watson BioTechnologies Inc., Shanghai. These eight pairs are polymorphic in *E. sinensis*. Characterization of these primers is shown in Tab. 2.

The Touch-down PCR program was used with all primers to acquire more pure products. PCR conditions were as follows: an initial denaturation step at 94℃ for 3 min; 2 cycles of 94℃ for 30 s, annealing (Ta + 6℃) for 30 s and extension at 72℃ for 30 s; 2 cycles of 94℃ for 30 s, annealing (Ta + 4℃) for 30 s and extension at 72℃ for 30 s; 2 cycles of 94℃ for 30 s, annealing (Ta + 2℃) for 30 s and extension 72℃ for 30 s; and 23 cycles of 94℃ for 30 s, annealing (Ta + 2℃) for 30 s and extension at 72℃ for 30 s. Final extension was at 72℃ for 10 min in a PCR system (PE9700). There were 29 cycles in all.

Products were detected by electrophoresis using 8% polyacrylamide, 120 V electrophoresis for 4 h and buffer (0.25% Methylene Blue trihydrate, 40% cane sugar). After electrophoresis, products were dyed using 0.1% silver nitrate for 10 min and stained with color-coded solution for 15 min. A scanner was then used to register the results. All the images were analysed using Gel-Pro Analyzer 4.5.

1.3 Statistical analysis

Because microsatellites are codominant, we can easily calculate the allele frequency (P), which is a measure of the relative frequency of an allele on a genetic locus in a population. The following variables were measured: Mean observed heterozygosity (Ho); mean expected heterozygosity (He); effective numbers of alleles (Ne); genetic similarity index (I); and genetic distance (Ds) (Nei, 1972). The following formulas were used:

Expected heterozygosity: $h_i = 1 - \sum_{i=1}^{n} P_i^2$

Tab. 1 Characterization of *Eriocheir sinensis* microsatellites

Locus	Repeat type	Temperature (℃)	Primer sequences	Length of products
ESA42HLJ	$(ca)_{17}$	54	5′ GAC CGCCTCTTAC TCAT 5′ CCCAAACCCCACCCT ACA	315–349
ESA67HLJ	$(ca)_{31}N_2$ $(ca)_4$	52	5′ TCAGACCAGGATGAAGCA 5′ TGTGGGATTATCGCAGAG	213–309
ESB25HLJ	$(ca)_{23}N_2$ $(ca)_{16}$	52	5′ AAGGACAACACGATGACA 5′ AAGAGGAGGAAGAGGCAG	211–280
ESB72HLJ	$(ca)_{52}$ $(ga)_{47}$	60	5′ GGAGGAAAGGCAACCAGG 5′ GCGAAGAGGCGACCGATA	237–422
ESB88HLJ	$(ac)_{18}$	52	5′ TACGGCAAATCCATCCTC 5′ ACGCCAATAAACTGACCAA	213–270
ESC11HLJ	$(aga)_{22}$ $(aca)_7$	52	5′ CATTAGGACCACCACCAA 5′ ATAAGGCAACGAATCACG	257–393
SC20HLJ	$(aca)_{15}N_3$ $(aca)_9$	50	5′ AACCAGGTGTTTCCCAGC 5′ GGGTTCCTGAGAGCG	193–257
ESC29HLJ	$(tgt)_3$ $(tgc)_6$ $(tgttgctgatgt)_3$	50	5′ CCTCTTTTCTCTTAGCCG 5′ AACTGAATGAAGCCAAGC	245–287
ESC34HLJ	$(ca)_{25}$	50	5′ AACAACTACCCAGCACCT 5′ CTCATCACGCTACCACCT	134–189
ESC56HLJ	$(tac)_{12}N_3$ $(tac)_4$	50	5′ CATCTAAAACGGGTCCTA 5′ TGTTCTACAACGCTTCCT	193–236
ESC57HLJ	$(tgt)_3N_9$ $(tgt)_3$ $(tct)_5$	50	5′ TTCGGTGTCGTCAGCGTT 5′ GGAAGTCAAGTCGGAGGC	222–231
ESC65HLJ	$(aga)_5N_3$ $(aga)_7$	50	5′ AAGGAATGTTAGCAGGTC 5′ TGAGGGTTCAGGGAGATA	321–341
ESC86HLJ	$(tgt)_{16}$ $(tct)_9N_9$ $(tct)_{13}$	50	5′ AGGCAGCCTAGTAATGAG 5′ ACTTTGGATACCTGGAGA	328–444
ESD02HLJ	$(tgt)_{16}$ $(tct)_9N_9$ $(tct)_{13}$	60	5′ AGGCAGGTGGGATTACAT 5′ CACAGTCATTAGCGAGGG	125–176
ESD11HLJ	$(ct)_{26}N_{52}$ $(aca)_7N_{23}$ $(ca)_{29}$	53	5′ TGAGGAGGAAAATGGTGC 5′ TTTGGTCCCGTTCTTGTG	186–290
ESD52HLJ	$(ct)_{26}N_{52}$ $(aca)_7N_{23}$ $(ca)_{29}$	53	5′ CGGAGTGTTTTGTTTGTC 5′ ATCATCAGCAGCAACCAC	53–171

Tab. 2 Characterization of *Eriocheir sinensis* microsatellites

Locus	Repeat type	Temperature (℃)	Primer sequences	Length of products
Esin06	$(ca)_{14}$	58	5′ CCCTTCCATTATCTTAACCTG 5′ CTGTGCTTCGTCTGTGTATG	105–180
Esin18	$(gt)_{26}$	58	5′ CACCGTAAGGTTCCGTAA 5′ AAGCACCCATAAGTCAATGTA	170–225
Esin36	$(ca)_{29}$	50	5′ GAGCGAGTATGCAAATGAGTAAT 5′ TTCATTCACGAACAAAACACTAA	227–430
Esin38	$(gt)_{30}$	50	5′ CTCATCAGTGTTTATGCAACA 5′ TGGAAAACTATTCAACTTATCAC	90–185
Esin42	$(ac)_{19}$	53	5′ GCACCGCAGTGATAATGTAGTGG 5′ GATCCTCGTGTGGGCGTGCTTAC	235–275
Esin67	$(gt)_{11}$	53	5′ TTTGGGATTCACCTTGTCAACTT 5′ CGACGCACGACAGAGGAGAGG	105–170
Esin74	$(ac)_{16}$	58	5′ ACAGCAAGTGGCAACAGGTAAAC 5′ CCGCCCAGCCTCCCGTCAAC	105–195
Esin75	$(ac)_{10}$	53	5′ CGGCAGTGAAAGATTACAGGCTG 5′ TTCCAAATAGTTATGACGGATGA	165–260

Mean expected heterozygosity: $He = \sum_{i=1}^{r} h_i / r$

Effective numbers of alleles: $He = 1/\sum_{i=1}^{n} P_i^2$

where n is the number of alleles in a locus; P_i is the frequency of the i allele; h_i is the expected heterozygosity of the i locus; r is the number of loci; He is the mean expected heterozygosity.

Genetic distance: $Ds = -\ln I$

Genetic similarity index: $I = J_{xy}/\sqrt{J_x J_y}$

$$J_x = \sum_{i=1}^{r}\sum_{j=1}^{k} X_{ij}^2/n,\quad J_y = \sum_{i=1}^{r}\sum_{j=1}^{k} Y_{ij}^2/n,\quad J_{xy} = \sum_{i=1}^{r}\sum_{j=1}^{k} X_{ij}Y_{ij}/n$$

where I is the genetic similarity index; X_{ij} and Y_{ij} are frequency of the k allele of the r gene locus in X, Y populations respectively; and n is the number of loci.

We used PHYLIP V3.6 to calculate the genetic distance between the populations and then used MEGA3 (http://www.megasoftware.net/) to draw the phylogenetic tree.

2 Results

2.1 The results of PCR

Special bands can be amplified in all of the 24 pairs of microsatellite primers in the four crab populations. We amplified more than 2000 bands in all. There were 2–10 alleles in each locus and each band was between 80–445 bp. In 24 loci, we acquired a total of 155 alleles and the mean number of alleles was 6.458. Among these loci, es36, ESB72 and ESD11 each had 10 alleles, while ESC57 had only two alleles. The gene frequency of all microsatellites in different populations was very different (Tab. 3). Some alleles of ES36 are special in some samples of the Liaohe population (Fig. 1, Tab. 3). It is believed that *E. sinensis* is diploid (2n = 146, n = 73) and most of the primers amplified only one or two bands (Fig. 2). However four primer pairs amplified three bands in five samples (♀) of the Jiangsu population. Molecular weight showed that the three bands were all normal alleles, as shown by es74 (Fig. 3).

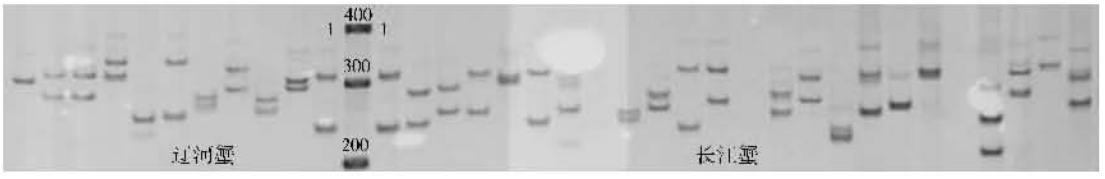

Fig. 1 Genetic diversity in locus ES36 of the Liaohe and Changjiang *Eriocheir sinensis* populations
The two tracks beside the marker are the same product from a Yangtse River crab for convenient comparison.

Tab. 3 Allele frequencies of three microsatellite loci in four *Eriocheir sinensis* populations

Loci	Length of allele (bp)	Anhui (A)	Jiangsu (J)	Tianjin (T)	Liaohe (L)
Es06	165	0.0667	0.1833	0.1667	0.0517
	150	0.1500	0.1333	0.3167	0.3966
	144	0.2833	0.1833	0.2300	0.1379
	132	0.3667	0.2167	0.1167	0.3793
	126	0.1333	0.2833	0.1700	0.0345
Es18	230	0.0167	0.0167	0.0667	0.0172
	220	0.2667	0.1000	0.0667	0.0862
	200	0.2667	0.2833	0.3333	0.2586
	190	0.3167	0.2500	0.3500	0.2241
	180	0.1167	0.2167	0.1333	0.2069
	170	0.0167	0.1333	0.0500	0.2069
Es36	381	0.0000	0.0000	0.0000	0.0172
	377	0.0000	0.0000	0.0000	0.0172
	350	0.0000	0.0000	0.0000	0.0862
	342	0.0000	0.0000	0.0167	0.0862
	340	0.1500	0.0833	0.0500	0.1379
	330	0.2667	0.3000	0.2167	0.1724
	322	0.2667	0.2000	0.1333	0.1207
	310	0.1000	0.2167	0.2333	0.2414
	307	0.1833	0.1333	0.1000	0.0690
	300	0.0333	0.0667	0.2500	0.0517

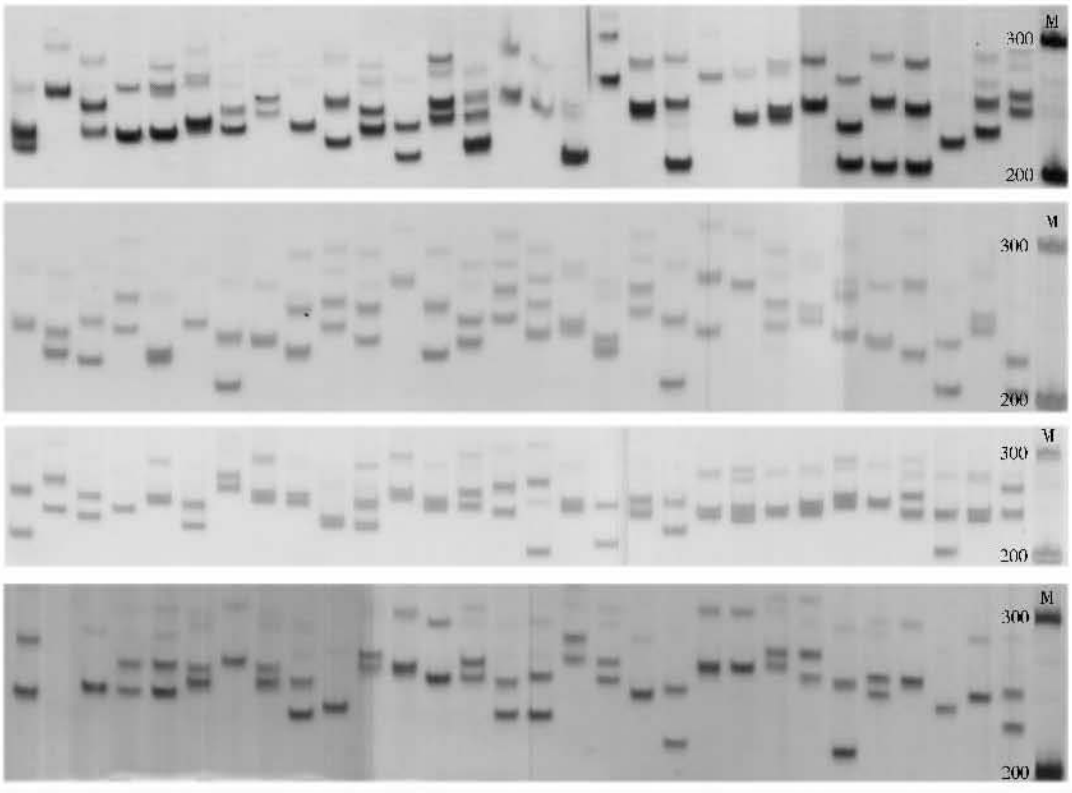

Fig. 2 Genetic diversity in locus ESB25 of the four *Eriocheir sinensis* populations
Figures from upper to lower are Anhui crabs (A), Jiangsu crabs (J), Tianjin crabs (T) and Liaohe crabs (L) respectively.

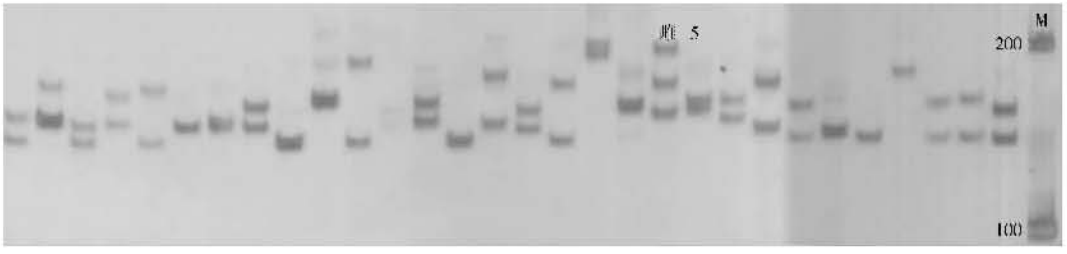

Fig. 3 Amplification result in locus es74 of the Jiangsu *Eriocheir sinensis* population

2.2 Population diversity

The number of alleles, mean observed heterozygosity (Ho), effective number of alleles (He) and the Hardy-Weinberg equilibrium χ^2 test are shown in Tab. 4. Effective number of alleles (Ne), mean observed heterozygosity (Ho) and mean expected heterozygosity (He) are shown in Tab. 5. A multi-group test of all loci showed that seven microsatellite loci (es42, es74, ESB88, ESC20, ESC29, ESC34, ESC57) of the four populations are in Hardy-Weinberg equilibrium, while the other loci are not. Except for the seven loci which are in equilibrium in all four populations, loci es06, es18, es36, es38, es67, es75, ESB72, ESC56, ESC65, ESC86 in the Anhui population, loci es36, es75, ESA67, ESB25, ESC56, ESC65, ESD02, ESD11, ESD52 in the Jiangsu population, loci es36, es38, es67, es75, ESB25, ESB72, ESC11, ESC65, ESD02, ESD in the Tianjin population and loci es38, es67, ESA42, ESA67, ESC56, ESD02 in the Liaohe population are also in Hardy-Weinberg equilibrium. All other loci are not in equilibrium.

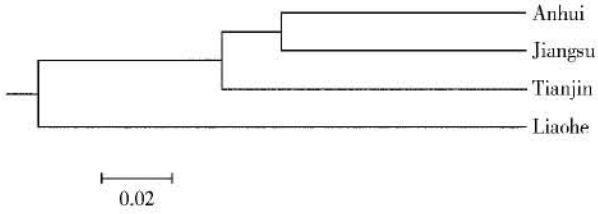

Fig. 4 Cluster analysis tree of the four *Eriocheir sinensis* populations using the UPGMA method

Tab. 4　Mean observed (*Ho*) and expected (*He*) heterozygosity and results of χ^2 tests for 24 microsatellites loci in the four *Eriocheir sinensis* populations

Loci	Numbers of allele	Anhui		Jiangsu		Tianjin		Liaohe	
		Ho	*He*	*Ho*	*He*	*Ho*	*He*	*Ho*	*He*
		χ^2 test (value)		χ^2 test (value)		χ^2 test (value)		χ^2 test (value)	
Es06	5	0.6000	0.7531	0.5333	0.8181	0.3000	0.7102	0.4138	0.6878
		0.056575		0.000006		0.000006		0.000000	
Es18	6	0.5333	0.7559	0.7000	0.7955	0.5333	0.7497	0.4483	0.8034
		0.259131		0.016404		0.010527		0.006569	
Es36	10	0.7333	0.8040	0.9333	0.8073	0.8667	0.8192	0.6552	0.8705
		0.606778		0.202744		0.134485		0.000001	
Es38	8	0.7667	0.8028	0.6333	0.8503	0.7000	0.8198	0.7586	0.8227
		0.196516		0.007541		0.263658		0.113682	
Es42	6	0.7000	0.6232	0.8333	0.7249	0.8333	0.7164	0.7241	0.7586
		0.816605		0.591352		0.182617		0.559707	
Es67	5	0.7000	0.7215	0.5667	0.7158	0.4333	0.4435	0.3793	0.5802
		0.294127		0.008081		0.610114		0.258940	
Es74	6	0.8000	0.7616	0.7000	0.7938	0.7667	0.7593	0.7931	0.7568
		0.101654		0.268924		0.916901		0.512258	
Es75	6	0.8000	0.8045	0.8667	0.7571	0.8667	0.7672	0.7241	0.7768
		0.454535		0.235921		0.477564		0.008824	
ESA42	7	0.8000	0.7718	0.6667	0.7684	0.5667	0.7605	0.5862	0.6836
		0.038601		0.002408		0.000000		0.053696	
ESA67	9	0.5333	0.8548	0.7667	0.8090	0.4333	0.8124	0.5862	0.8379
		0.000007		0.788996		0.000002		0.121854	
ESB25	7	0.5667	0.8503	0.8333	0.8469	0.8000	0.8136	0.5517	0.6933
		0.007377		0.865549		0.821936		0.000000	
ESB72	10	0.7333	0.8232	0.7000	0.8740	0.7000	0.7994	0.5172	0.8421
		0.281200		0.000000		0.907443		0.000000	
ESB88	9	0.8000	0.8390	0.8000	0.8565	0.8333	0.8271	0.7241	0.8167
		0.279852		0.055937		0.692561		0.531609	
ESC11	9	0.6000	0.8452	0.7000	0.8503	0.7000	0.8621	0.7241	0.8711
		0.000015		0.001037		0.158963		0.000012	
ESC20	8	0.8667	0.7169	0.7667	0.8090	0.8000	0.7859	0.8276	0.7368
		0.942787		0.395429		0.836010		0.870949	
ESC29	5	0.4667	0.4678	0.3333	0.3249	0.3000	0.3181	0.2069	0.1887
		0.344977		0.647473		0.751618		0.573572	
ESC34	8	0.9000	0.8045	0.8333	0.8226	0.9333	0.8186	0.8276	0.7768
		0.325269		0.665497		0.834279		0.482589	
ESC56	6	0.6667	0.7136	0.5333	0.6989	0.4333	0.7435	0.4828	0.7205
		0.492071		0.069970		0.007488		0.218028	
ESC57	2	0.1000	0.0966	0.1000	0.0966	0.0667	0.0655	0.0690	0.0678
		0.815391		0.815391		0.894626		0.892738	
ESC65	7	0.7000	0.8367	0.8333	0.8215	0.8000	0.8463	0.6207	0.8004
		0.099058		0.121728		0.415133		0.001226	
ESC86	8	0.7000	0.8252	0.5333	0.8531	0.6000	0.8316	0.5517	0.8276
		0.168661		0.002287		0.000945		0.000026	
ESD02	6	0.7667	0.6751	0.4667	0.7441	0.5333	0.6672	0.3793	0.7048
		0.011709		0.071076		0.450965		0.064467	
ESD11	10	0.6333	0.8763	0.8000	0.8768	0.7667	0.8706	0.6207	0.8742
		0.014359		0.270516		0.624529		0.028292	
ESD52	9	0.5000	0.8701	0.7000	0.7944	0.4333	0.8520	0.4828	0.8700
		0.000000		0.879545		0.000000		0.000000	

2.3　Phylogenetic relationship of the four populations

The genetic similarity between Anhui and Jiangsu crabs is very high (0.8675, Tab. 6). The genetic similarity between Jiangsu and Tianjin crabs is 0.8460 and between Anhui and Tianjin crabs is 0.8311, which are very similar values. However the genetic similarity between the Liaohe crabs and the three other populations is much lower. (Tab. 6). We drew a UPGMA tree using MEGA3 (Fig. 4). The Jiangsu and Anhui crabs cluster together to form the first branch. The Tianjin crabs

Tab. 5 Genetic diversity parameters of the four *Eriocheir sinensis* populations

	Effective numbers of allele (*Ne*)	Observing value of mean heterozygosity (*Ho*)	Expected value of mean heterozygosity (*He*)
Anhui	4.5381	0.6653	0.7467
Jiangsu	4.7234	0.6722	0.7546
Tianjin	4.3491	0.6250	0.7275
Liaohe	4.3730	0.5690	0.7238

Tab. 6 Genetic distance (lower-left triangle) and the genetic similarity index (upper-right triangle) among four populations of *Eriocheir sinensis*

	Anhui	Jiangsu	Tianjin	Liaohe
Anhui		0.8675	0.8311	0.7884
Jiangsu	0.1422		0.8460	0.7264
Tianjin	0.1850	0.1672		0.7472
Liaohe	0.2378	0.3197	0.2915	

form the next branch and the last are Liaohe crabs forming the final branch (Fig. 4). The result is in accordance with the geographical distribution of the samples. In this way, we can deduce that microsatellites are suitable for detecting the genetic distance between different regional populations of the species. We can also see that different regional populations of *E. sinensis* have different levels of diversity.

3 Discussion

3.1 The validity of loci

Microsatellites usually have very high polymorphism. The mutation rate in different species, as well as in different loci in the same species, and even different alleles in the same locus can be very different (Xin et al, 2000; Zhang et al, 2003). Natural selection is just one of the reasons causing high polymorphism in microsatellites. Geographical isolation is another reason. In this study, we employed 24 pairs of microsatellite primers and amplified 144 alleles in four crab populations. The polymorphism gene frequency is between 0.0167 and 0.8966. Microsatellites es36 and ESC57 each have 10 alleles and are the most polymorphic. The allele frequency of ESC57 in the Tianjin and Liaohe populations is over 0.95. According to the criteria of polymorphism (Gu et al, 2004) this locus is monomorphic, while the other 23 loci are all polymorphic.

3.2 The genetic structure

Biodiversity is the basis of life's adaptation to the environment. Maintaining maximum genetic diversity is the precondition and basis of sustainable use of species. The value of genetic heterozygosity reflects the degree of a population's genetic similarity. The lower the genetic heterozygosity, the higher the genetic similarity, which means that the population has very little genetic variance and the genetic diversity is very low. In this study, we found that the genetic heterozygosity and similarity of the four populations are all at a medium level. The mean observed heterozygosity of the three Yangtse River populations (Jiangsu, Tianjin and Anhui) is 0.6250 – 0.6722, which reveals that the genetic diversity of Yangtse River crabs is very high, especially in the Jiangsu population. The mean observed heterozygosity of Liaohe crabs is much lower, at just 0.5690.

Microsatellites are neutral and are not pressured by natural selection. Therefore in an ideal population, the frequency of all alleles must be steady. However using the Hardy-Weinberg equilibrium *P* value to do a multi-group test (Tab. 3), we found that some microsatellite loci have departed from Hardy-Weinberg equilibrium and some of them are notable departures. There are many reasons for this, for example, artificial selection, transfer and mutation. However it is still not clear which reasons cause the departure from Hardy-Weinberg equilibrium in *E. sinensis*.

3.3 The genetic diversity of four populations

Thorp (1982) reported that: the genetic similarity between different genera in the same family is $I = 0.1 - 0.5$ ($Ds = 0.5 - 0.9$); the genetic similarity between different species is $I = 0.2 - 0.8$ (genetic distance $Ds = 0.2 - 0.8$); and between different species in the same population is $I = 0.8 - 0.97$ ($Ds = 0.03 - 0.2$). The genetic difference between the Jiangsu, Tianjin and Anhui populations is $I = 0.8311 - 0.8675$ ($Ds = 0.1422 - 0.1850$), so they must belong to the same crab population. This result is in accordance with the geographical distribution of the samples. Jiangsu, Anhui and Tianjin crabs are all from the Yangtse River. The genetic difference between Liaohe crabs and the other crabs $I = 0.7264 - 0.7884$ ($Ds = 0.2378 - 0.3197$), so they must belong to a different population. This result supports Qiu et al (1997) and Zhao et al (1999).

The clustering reflects these genetic relationships (Xing et al, 2003). Anhui crabs and Jiangsu crabs cluster together first, showing that these two populations are the most similar. They then cluster with the Tianjin crabs and lastly the Liaohe crabs. The genetic distance between Jiangsu crabs and Anhui crabs is just

0.1422, and between these and the Tianjin crabs is 0.1672. However between Jiangsu and Liaohe crabs the distance is 0.3197, which is a much bigger value. This shows that the Yangtse River crabs and Liaohe crabs are two different populations.

3.4 The reasons for amplification of three bands

Three bands were amplified in some samples. The potential reasons are: (1) there may have been some mistakes in our experiments. However we repeated the experiments and contrasted the bands with the products of three other pairs of primers, and found that the samples still had three very clear bands. Therefore we can exclude experimental mistakes; and (2) Some crabs may be autopolyploid. There are only a few studies on the chromosomal karyotype of Decapods. It is hard to count the number of the chromosomes, because Decapods have a large number of chromosomes which are very short and the centromeres are hard to identify. Roberts (1969), Farmer et al (1974) and Hughes (1982) reported the number of chromosomes of the Decapoda, but the results were all very different. They believed that there might be some supernumerary chromosomes. If this is the reason for the three bands in some crabs, we can identify autopolyploid crabs in nature using molecular markers.

3.5 The prospect of using microsatellites

In this study, we used only a few microsatellite loci, but we failed to find a locus that can clearly identify Liaohe and Yangtse River populations. Therefore we must isolate more microsatellite loci to find some special markers that can identify different populations, and establish the genetic criteria of *E. sinensis*. In this way, we can scientifically protect and utilise *E. sinensis*.

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