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ORIGINAL PAPER

Genetic diversity and population structure of the Chinese mitten crab *Eriocheir sinensis* in its native range

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Abstract The Chinese mitten crab *Eriocheir sinensis* is an indigenous and economically important species in China, but can also be found as invasive species in Europe and America. Mitten crabs have been exploited extensively as a food resource since the 1990s. Despite its ecological and economic importance, the genetic structure of native mitten crab populations is not well understood. In this paper, we investigated the genetic structure of mitten crab populations in China by screening samples from ten locations covering six river systems at six microsatellite loci. Our results provide further evidence that mitten crabs from the River Nanliujiang in Southern China are a genetically differentiated population within the native range of *Eriocheir*, and should be recognized as a separate taxonomic unit. In contrast, extremely low levels of genetic differentiation and no significant geographic population structure were found among the samples located north of the River Nanliujiang. Based on the reproductive biology of mitten crabs and the geography of their habitat we argue that both natural and human-mediated gene flow are unlikely to fully account for the similar allele frequency distributions at microsatellite loci. Large population sizes of mitten crabs suggest instead that a virtual absence of genetic drift and significant homoplasy of microsatellite alleles have contributed to the observed pattern. Furthermore, a coalescent-based maximum likelihood method indicated a more than two-fold lower effective population size of the Southern population compared to the Northern Group and low but significant levels of gene flow between both areas.

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Introduction

The Chinese mitten crab *Eriocheir sinensis* is a euryhaline brachyuran with a native range extending from the eastern pacific coast of China to the Korean Peninsula (Gu and Zhao 2001). Nevertheless, in the past 100 years, the species has also been introduced inadvertently into continental Europe, the United Kingdom, San Francisco Bay, Chesapeake Bay and the mid-Atlantic coast of North America and has become one of the most notorious aquatic invaders in these areas (Carlton 1985; Cohen and Carlton 1997; Hänfling et al. 2002; Herborg et al. 2003; Rudnick et al. 2003; Ruiz et al. 2006).

In China, exploitation of mitten crab populations has a long tradition and today it is one of the most economically important species for freshwater aquaculture. Traditionally,

megalopa larvae were captured from river estuaries during their upstream migration and restocked in lakes for grow-out (Zhang et al. 2002; Liu et al. 2007). However, with intensifying exploitation and habitat destruction, natural stocks and the availability of larvae for aquaculture have decreased dramatically since the 1960s. For example, the average natural yield of mitten crab megalopa larvae in the Yangtze River estuary was 6,820 kg in the period of 1970–1981 but dropped to 660 kg in the period of 1982–1998. The same trend occurred in the average annual fishery landings of adult crabs, which were about 50,000 and 16,000 kg, respectively, during the two periods (Yu et al. 1999). Fortunately, benefiting from advances in larviculture techniques (Zhao 1980), mitten crab aquaculture based on reproduction in captivity has developed extensively since the early 1990s, with annual aquaculture production of 500,000 mt in 2005, valued at 2.2 billion USD (FAO 2007). Nowadays, instead of wild caught megalopa or juveniles, those produced from hatcheries have been the major source for mitten crab farming. They are usually reared in outdoor earthen ponds or released into net enclosures in lakes for grow-out.

In its native range, the mitten crab is distributed in three main drainages (the rivers Liaohe, Yangtze and Ou) and a number of smaller adjacent river systems in Northern and Central China (Zhang et al. 2000). The stocks originating from the Yangtze River basin are regarded as the most valuable due to a number of attributes including better growth patterns, larger size and good flavor (Li et al. 2000). However, the shortage of broodstock and juveniles from the Yangtze River basin caused by over-exploitation has driven frequent stock transfer among the different geographical populations (mainly transport from the rivers Liaohe and Ou area to the Yangtze river basin) in the 1990s. Thus, anthropogenic dispersal and restocking programs may in the past have caused gene flow between spawning populations from different river systems and potentially compromised their genetic identity (Qiu 1998; Wang and Li 2002). However, such stocking practices became very rare recently due to lacking availability of wild megalopa and juveniles.

The taxonomy of the mitten crab populations from Southern China (e.g., the rivers Zhujiang and Nanliujiang) remains unclear. Based on morphological studies (e.g., carapace, cheliped, ambulatory leg, abdomen, etc.) and their distinct geographical distribution, they were first recognized as a subspecies of the Japanese mitten crab, *E. japonicus hepuensis* by Dai (1988, 1991) and later as a separate species, *E. hepuensis* by Guo et al. (1997). An early genetic study based on allozymes and quantitative variation could not confirm this hypothesis (Li et al. 1993), suggesting instead that the mitten crab populations in Southern China and Northern China might belong to a single species. More recently, an analysis of mitochondrial sequence variation indicated marked sequence divergence among individuals from Northern China, Southern China and Japan (Wang et al. 2008). These data support the hypothesis of three allopatric mitten crab species (Tang et al. 2003): *E. japonica*, *E. sinensis* and *E. hepuensis*.

The genetic characterization of stocks using molecular markers has provided fisheries managers with a powerful tool to design and manage sustainable fishing practices (Valle-Jimenez et al. 2005; Ward 2000). A number of previous studies on native mitten crab populations described low levels of differentiation among the geographically distinct populations using enzymatic proteins and RAPD markers (Wang and Yu 1995; Gao and Zhou 1998). However, a conclusive pattern of geographical population structure has not yet emerged. Methodological problems such as low polymorphism at the analyzed allozyme loci and low repeatability for RAPD markers, may limit their value for accurately detecting low levels of population differentiation. Such problems can potentially be avoided by analyzing hyper-variable microsatellites loci that can be used to detect low levels of population structure. Microsatellite markers have been successfully employed in marine species with high levels of current gene flow such as cod, *Gadhus morhua* (Hutchinson et al. 2001) and Atlantic herring, *Clupea harengus harengus* (Mariani et al. 2005) or past connectivity, such as whelk, *Buccinum undatum* (Weetman et al. 2006a). Microsatellites have further proved to be useful tools to investigate the population divergence and colonization process of *Eriocheir sinensis* in its invasive range (Herborg et al. 2007; Muirhead et al. 2008). Catadromous species are particularly interesting for population genetic studies because they combine characteristics of both freshwater and marine species making predictions about their genetic population structure difficult. For example molecular studies have challenged the hypothesis of a panmictic spawning population for the catadromous European eel (Wirth and Bernatchez 2001) and suggest an unexpectedly low effective population size (Pujolar et al. 2006). The life history of mitten crabs provides certainly opportunities for both geographic isolation due to the existence of geographically separated breeding grounds in river estuaries, but also gene flow during the planktonic larval phase and during active migrations of both juveniles and adults. Furthermore, effective population size which determines the extent of genetic drift is a similarly unknown quantity in *E. sinensis.*

The aim of this study was to use microsatellite markers to identify the key evolutionary processes and associated ecological factors shaping genetic population structure of a catadromous species. Furthermore, a better knowledge of genetic diversity and structure of the Chinese mitten crab could provide vital input in support of sustainable exploitation and conservation of natural populations.

Materials and methods

Mitten crab life history

The Chinese mitten crab has catadromous life cycle, including reproduction in brackish water, a marine larval stage and an adult stage which is spent in freshwater. In late autumn the adults migrate downstream with a daily migration rate of 24–40 km (Zhang and Li 2002) in order to reproduce in a marine environment. After mating in the brackish water of estuaries females move toward higher salinities and carry the eggs over winter for a period of 4 months until hatching. Both male and female crabs die after reproduction. The planktonic larvae pass five zoeal stages over a 1–2 month period before metamorphosing into megalopa. The megalopa settle in brackish water in late spring and move upstream along with the tidal currents at a speed of 8–12 km per day (Du 2004). After 7–10 days, the megalopa develop into benthic juveniles which migrate continuously upstream (1–3 km per day) into freshwater often for considerable distances where they remain for 2–3 years.

Sample collection and geographical information

Between 2001 and 2002, a total number of 352 mitten crabs was collected from nine locations (18–55 specimens each) spanning the entire range of *Eriocheir* in mainland China. Sampling locations were distributed in six river systems, namely River Liaohe (PJ), Haihe (HH), Huanghe (KL), Yangtze (RD, YZ, NJ and YR), Ou (WZ) and Nanliujiang (HP) (Table 1; Fig. 1). The HP sample falls within the range of populations previously described as a separate taxon (Guo et al. 1997; Tang et al. 2003), whereas the remaining samples are assumed to belong to the *E. sinensis* sensu lato and will in the following be referred to as the Northern Group. Data sets of a Chinese population (Liaohe, LH) previously analyzed in Herborg et al. (2007) were also integrated in the analysis.

Microsatellite amplification and screening

The pleopod of the adults or juvenile crabs was taken and preserved in 98% ethanol for subsequent DNA extraction. Total genomic DNA was extracted using a modified cethyltrimethyl ammonium bromide (CTAB) protocol based on Doyle and Doyle (1985) (Table 2). The samples were genotyped at six dinucleotide microsatellite loci *Esin42*, *Esin55*, *Esin67*, *Esin74*, *Esin75* and *Esin87* as described in Hänfling and Weetman (2003). PCR products were run on Pharmacia ALF Express automated sequencers (Amersham, UK) with at least three internal size standards included to permit reliable size-scoring using FRAGMENT MANAGER 1.2 (Amersham, UK). Additionally, two previously genotyped individuals from the data set of Herborg et al. (2007) were included on each gel to improve cross-referencing among gels and data sets. Raw data were checked for scoring errors due to stuttering, larger allele dropout, or null alleles using the program MICRO-CHECKER 2.2.3 (Van Oosterhout et al. 2004).

Data analysis

Genetic diversity of individual samples was described as observed and unbiased expected heterozygosities (H_O and H_E; Nei 1978) using GENEPOP 3.2 (Raymond and Rousset 1995) and as the expected average number of alleles at the size of the smallest sample (allelic richness, A) which was estimated by rarefaction, using FSTAT 2.9 (Goudet 1995). Deviations from Hardy–Weinberg equilibrium were tested via separate one-tailed tests for heterozygote excess and deficiency for each locus in each population, to obtain unbiased estimates of the exact P value in GENEPOP 3.2 with 100 batches of 5,000 iterations. To combine probabilities from across loci and populations we applied the Bernoulli equation: $P = [N!/(N-K)!K!] \times \alpha^K (1-\alpha)^{N-K}$, where N is the number of total tests and K is the number of tests below the designated type I error rate, α (Moran 2003)

Table 1 Sampling locations of mitten crab populations and number of individuals typed at 6 microsatellite loci

Location	Number of individuals	Geographical information		
		Latitude	Longitude	River system
Panjin (PJ)	27	41°00′N	122°07′E	Liaohe, China
Liaohe (LH)	49	41°21′N	122°38′E	Liaohe, China
Haihe (HH)	55	39°13′N	117°20′E	Haihe, China
Kenli (KL)	30	37°59′N	118°54′E	Huanghe, China
Rudong (RD)	45	32°30′N	121°80′E	Yangtze, China
Yizheng (YZ)	30	32°27′N	119°16′E	Yangtze, China
Nanjing (NJ)	30	32°04′N	118°78′E	Yangtze, China
Shanghai (YR)	44	31°10′N	121°28′E	Yangtze, China
Wenzhou (WZ)	18	28°02′N	120°65′E	Ou, China
Hepu (HP)	19	21°33′N	109°20′E	Nanliujiang, China

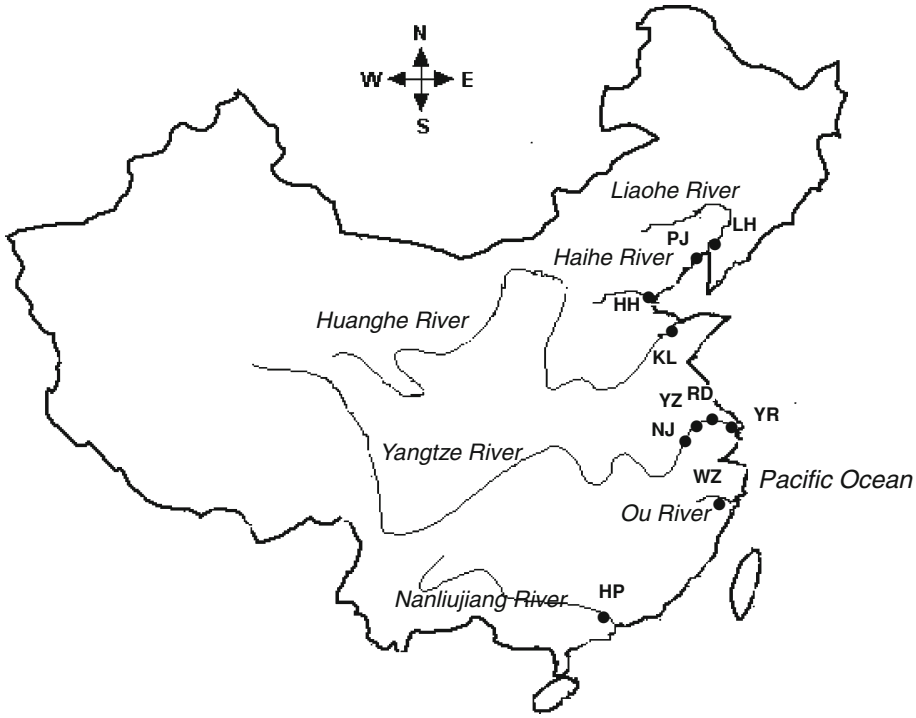

Fig. 1 Map of native mitten crab populations sampled for the current study. See Table 1 for population codes

which was set at 0.05. Following the terminology of Kinnison et al. (2002), we refer to this as the 'binomial likelihood method' (LM). Since the LM always requires repeatability of individually significant results across tests to yield an overall $P < 0.05$, it has the desirable property of not being influenced by single extremely low P values in contrast with the commonly applied Fisher's method. Linkage disequilibrium was examined using the exact test in GENEPOP 3.2 with 100 batches of 5,000 iterations. To determine whether pairs of loci were significantly linked, the LM method was applied across sample sites (within locus pairings) since sites can be viewed as replicates tests of an overarching hypothesis. Within populations lack of independence among locus pairings meant that it was necessary to correct α for inflated type I error by the sequential Bonferroni procedure (Rice 1989).

Global and pairwise genetic differentiation was quantified using Weir and Cockerham's (1984) method to estimate F_{ST} using GENEPOP 3.2. Significance of pairwise differentiation was tested by computing probabilities of homogeneity of allele frequencies among populations for individual loci using the exact χ^2 test in GENEPOP 3.2 (option "genic differentiation"). The LM method was applied to test for significance across loci and the sequential Bonferroni procedure was used to correct type I error for multiple testing across populations. An AMOVA was carried out among the multiple samples collected from the rivers Liaohe and Yangtze using the program Arlequin 3.1 (Excoffier et al. 2005) and using "river" as a hierarchical level.

Principal component analysis (PCA) was used to produce a visual representation of differentiation among the samples using the program PCA-GEN (available from J. Goudet at http://www2.unil.ch/popgen/softwares/pcagen/htm).

Partial Bayesian cluster analysis of individuals was performed using BAPS 5.1 (Corander et al. 2003, 2006). The aim of which is to identify the optimum number of partitions (K) among groups of samples. The analysis was run using maximum K settings from 1 to 10, repeating the analysis 5 times at each value. The overall posterior probability for each value of K was calculated from the log likelihood values across all runs. Furthermore, the option to carry out cluster analysis with a predefined number of K was carried out in order to test the specific hypothesis that the samples consisted of two different taxa ($K = 2$). Admixture analysis was not carried out given the limited power at low levels of population structure (Hauser et al. 2006).

Isolation by distance analysis of native mitten crab populations was carried out by comparing matrices of genetic population divergence measured as $F_{ST}/1 - F_{ST}$ with geographical distance using Mantel tests. The geographical distances were calculated in two ways: (1) the shortest aquatic distance between each pair of sampling sites based on the assumption that geographical locations within rivers might represent different populations; (2) the shortest aquatic distance between estuaries of the rivers in which samples were caught based on the assumption that dispersal occurs mainly during the larval stage. For this analysis the mean across samples of the same river system was used.

Table 2 Genetic diversity of mitten crab populations at 6 microsatellite loci

Name of populations	Genetic diversity							
		Esin42	*Esin55*	*Esin67*	*Esin74*	*Esin75*	*Esin87*	Mean
Panjin (PJ)	H_E	0.90	0.93	0.71	0.93	0.96	0.50	0.82
	H_O	0.81	0.96	0.69	0.96	0.92	0.44	0.80
	A	10.65	16.22	7.63	15.45	18.89	6.48	12.55
Liaohe (LH)	H_E	0.91	0.95	0.77	0.92	0.96	0.42	0.82
	H_O	0.83	0.84	0.76	0.90	0.77	0.45	0.76
	A	11.99	18.18	8.36	15.36	18.05	5.36	12.88
Haihe (HH)	H_E	0.89	0.95	0.81	0.93	0.96	0.46	0.83
	H_O	0.84	0.93	0.82	0.94	0.94	0.45	0.82
	A	12.13	17.11	7.44	15.00	19.19	5.78	12.77
Kenli (KL)	H_E	0.91	0.94	0.71	0.95	0.96	0.55	0.84
	H_O	0.87	0.90	0.77	0.93	0.90	0.57	0.82
	A	12.22	17.10	7.94	17.14	18.29	5.04	12.95
Rudong (RD)	H_E	0.89	0.95	0.68	0.89	0.96	0.37	0.79
	H_O	0.82	0.93	0.66	0.93	0.95	0.38	0.78
	A	11.09	17.87	6.42	14.07	18.29	4.89	12.10
Yizheng (YZ)	H_E	0.91	0.96	0.75	0.91	0.96	0.31	0.80
	H_O	0.83	0.96	0.73	0.87	0.76	0.31	0.74
	A	11.78	19.13	10.05	15.02	17.85	4.54	13.06
Nanjing (NJ)	H_E	0.91	0.94	0.82	0.94	0.96	0.51	0.84
	H_O	0.93	0.93	0.90	0.97	0.87	0.47	0.84
	A	11.88	17.20	8.21	16.00	18.21	7.66	13.19
Shanghai (YR)	H_E	0.90	0.96	0.74	0.96	0.95	0.53	0.84
	H_O	0.86	0.93	0.66	0.93	0.94	0.38	0.78
	A	11.27	18.56	6.36	17.85	17.19	6.84	13.01
Wenzhou (WZ)	H_E	0.90	0.92	0.76	0.93	0.94	0.31	0.79
	H_O	0.89	1.00	0.83	0.83	0.88	0.28	0.78
	A	11.54	16.00	7.75	14.19	15.00	6.33	11.80
Hepu (HP)	H_E	0.87	0.85	0.76	0.92	0.84	0.36	0.77
	H_O	0.95	0.84	0.89	0.95	0.83	0.32	0.80
	A	8.81	9.36	12.38	14.67	7.97	2.98	9.36
Total	No. of alleles	21	47	21	32	43	15	
	Allele range (base pair)	223–273	81–179	137–179	137–199	156–258	123–161	

H_E expected heterozygosity (unbiased estimated), H_O observed heterozygosity, *A* allelic richness

The coalescent-based maximum likelihood method implemented in MIGRATE 2.06 (Beerli and Felsenstein 1999, 2001) was used to estimate ratios of migration rate:mutation rate (M) and θ ($= 4N_e\mu$; N_e is effective population size and μ is mutation rate). MIGRATE uses a likelihood approach using a Markov-chain-Monte-Carlo (MCMC) search algorithm. The analysis was carried out assuming the two population model identified from the cluster analysis. The Brownian motion model was used as an approximation of the stepwise mutation model, and following initial trials, search criteria for the MCMC sampler were set to 20 short chains of 20,000 steps and three long chains of 200,000 steps. Searches were combined across all long chains (Geyer 1994) and a static heating scheme with four temperatures (1; 1.5; 3; 6) was used (Geyer and Thompson 1995). In order to obtain reliable parameter estimates, seven subsequent runs were performed using the parameter estimates from the previous run as start values. Convergence was accepted if confidence intervals for all parameters overlapped between runs. MIGRATE tends to overestimate migration rates in a scenario where real values are small or zero. We therefore re-run the analysis using a restricted model of no migration and used a likelihood ratio to test whether this model is equally likely than a unrestricted model (i.e., a model in which both θ and M are estimated).

Results

MICRO CHECKER found no evidence of scoring errors or null alleles in any sample. None of the combined tests for HWE across loci and populations was significant. No evidence of linkage disequilibrium was observed at any of the six loci investigated, thus allowing allelic variation at all loci to be treated as independent.

The six microsatellite loci showed varying numbers of alleles, ranging from 15 at *Esin87* to 47 at *Esin55* (Table 2). Ranging from 11.80 (WZ) to 13.19 (NJ), the values of mean allelic richness (A) in the populations of the Northern Group (excluding the HP samples) were larger than those in the Southern native population (HP, 9.36) and the invasive population (Elbe, 7.87). The same trend also showed in mean H_E across all the loci but not so pronounced as for A. Estimates of H_E across the Northern Group varied from 0.79 (WZ and RD) to 0.84 (KL, NJ and YR), while the value of HP (0.77) was slightly smaller or similar to the lower margin.

The global F_{ST} value was 0.021 across the entire data set and statistically significantly different from zero ($P < 0.01$), indicating genetic differentiation. Relatively high pairwise F_{ST} values were obtained between the HP population and other Chinese populations (0.024–0.040). Differentiation among the remaining Chinese populations was much lower (global $F_{ST} = 0.007$, pairwise $F_{ST} < 0.015$) and 18 out of 36 tests for genic differentiation were not significant (Table 3). Surprisingly differentiation among samples from different river systems within the Northern Group ($F_{ST} = 0.000 - 0.015$) was not higher than differentiation between samples from the same river system ($F_{ST} = 0.002 - 0.010$). An AMOVA among the samples of the rivers Liaohe and Yangtze showed that there was no significant differentiation on the level "among rivers" ($P > 0.3$). The absence of a geographical association is also reflected in the PCA in which samples from the same river system fail to cluster together. Isolation by distance analyses within the Northern Group showed no significant correlation using either the shortest aquatic distance between samples or between estuaries (breeding grounds).

An individual-based Bayesian cluster analysis among all Chinese samples supported $K = 10$ as the most likely partition. However, eight of these clusters where represented by a small number of individuals which appeared to be randomly distributed among clusters indicating that the real number of clusters might be overestimated due to some genotypes which show by chance a higher divergence (Latch et al. 2006). Nevertheless, a clear geographic pattern became apparent. 80–100% of individuals from the Northern Group but only two individuals of the population HP were assigned to cluster 9 whereas 78% of individuals from HP were assigned to clusters 1, 3 and 4 compared to 10% of individuals from Northern Group samples. When K was fixed to two a clear separation between the Northern Group (99% cluster 1; 1% cluster 2) and the HP population (26% cluster 1; 74% cluster 2, Figs. 2, 3) was apparent.

Based on the results from both cluster analysis and F statistics, a two-population model was used for the estimation of migration rates and effective population sizes. For this analysis, all samples of the Northern Group were pooled and regarded as one population. The second population included only individuals of the sample HP. Estimates of θ for the Northern Group was more than twice as high as for the Southern population with non overlapping credibility intervals. Since θ represents the product of effective population

Table 3 Genetic differentiation using estimation of pairwise F_{ST} values (Weir and Cockerham 1984) among mitten crab populations and significant differentiation based on genic differentiation

Probabilities across loci were adjusted using the Bernoulli equation, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Significant tests are in *bold*. Pairwise comparisons within the river systems (Liaohe and Yangtze) are *boxed*

	PJ	LH	HH	KL	RD	YZ	NJ	YR	WZ	HP
Panjin (PJ)		Liaohe								
LH	**0.0088** *									
Haihe (HH)	0.0020	0.0044								
Kenli (KL)	0.0018	**0.0066** *	0.0053							
Rudong (RD)	0	**0.0136** *	**0.0063** *	**0.0083** **		Yangtze				
Yizheng (YZ)	0.0042	**0.0147** **	**0.0084** **	0.0060	0.0031					
Nanjing (NJ)	**0.0048** *	0.0029	0.0001	0.0010	**0.0098** **	**0.0062** *				
Shanghai (YR)	0.0020	**0.0073** **	**0.0036** *	0.0001	**0.0092** *	0.0053	0.0016			
Wenzhou (WZ)	0.0047	**0.0073** *	**0.0091** **	**0.0085** *	**0.0122** *	0.0047	0.0065	**0.0088** *		
Hepu (HP)	**0.0324** ***	**0.0317** ***	**0.0325** ***	**0.0339** ***	**0.0400** ***	**0.0244** ***	**0.0244** ***	**0.0347** ***	**0.0263** ***	

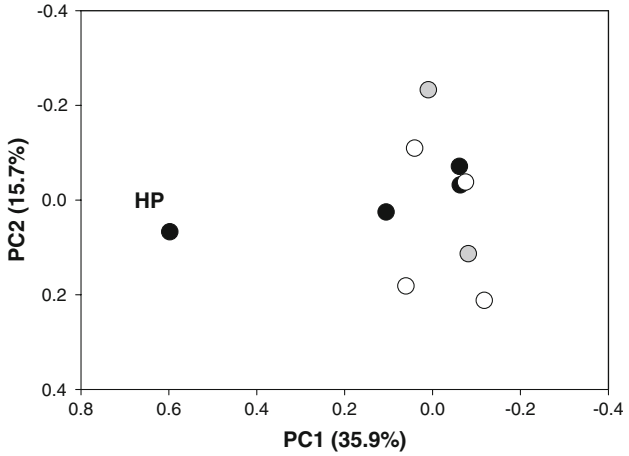

Fig. 2 Principle component analysis based on pairwise F_{ST} values (global $F_{ST} = 0.034$). Wild native populations are represented by *circles*, the hatchery stock by a *square*. *Symbols* representing samples within the river Yangtze catchment are colored *white*, those from the river Liaohe are *gray*

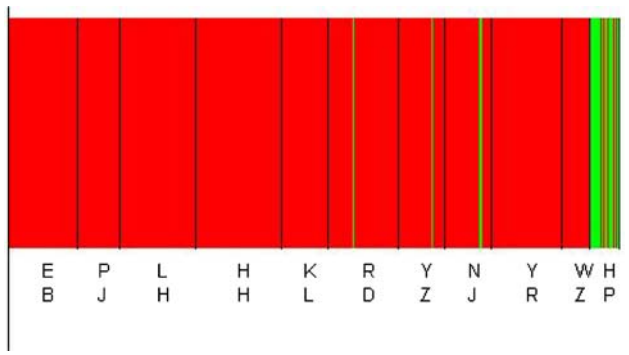

Fig. 3 Individual based cluster analysis using BAPS 5.1 with number of clusters fixed to two

size and mutation rate the former can be calculated under the assumption of the later. Mutation rates can vary considerably among microsatellite loci and species but often range between 10^{-3} and 10^{-4} (Ellegren 2000, 2004). Assuming an average mutation rate of 5×10^{-4} these estimates translate into long-term effective population sizes (N_e) of approximately 6×10^3 and 2×10^3, respectively (Table 4). However, effective population size might be substantially higher if the mutation rate is lower than average as it has been reported for some invertebrates (Ellegren 2000; Weetman et al. 2006b). ML estimates of immigration rates were low ($m < 0.002$, Table 4), but provided a significantly better fit to the data than an alternative model with zero migration ($P < 0.001$).

Table 4 Maximum likelihood estimates (MLE) of effective population size (N_e) and migration rate (m) for the two mitten crab populations identified in the cluster analysis their credibility intervals (lower percentile = 0.05 and upper percentiles = 0.95)

	Northern group			HP		
	0.05	MLE	0.95	0.05	MLE	0.95
N_e	5,148	5,671	6,268	2,246	2,342	2,431
m	0.0011	0.0012	0.0013	0.0014	0.0015	0.0017

Values were calculated from MIGRATE estimates of θ and M, respectively, assuming a mutation rate of 5×10^{-4}

Discussion

The current study revealed that the sample from the river Nanliujiang (HP) in Southern China is the only clearly differentiated population within the native range of *Eriocheir* in China. This is in agreement with earlier morphological studies proposing that this population should be recognized as a separate taxonomic unit (Dai 1988, 1991; Guo et al. 1997). Furthermore, recent studies on mitochondrial DNA variation showed that both groups belong to distinct phylogenetic clades (Tang et al. 2003; Wang et al. 2008). Nevertheless, our analysis also provided evidence that there is small but significant gene flow between both taxa. This is consistent with the results of Wang et al. (2008) who detected secondary contact and mitochondrial introgression between the two groups in two smaller drainages located in-between the Northern Group and the population HP. Further research on nuclear genome variation in these contact zones is needed to establish the nature of this hybrid zone. The long-term effective population size of the Northern Group appears to be approximately 2.5 times larger than the Southern population. The Northern Group also occupies a substantially larger geographic area and is therefore expected to have larger census population size assuming similar densities. Such a concordance between effective and census population size in turn suggests that mitten crab population might indeed be in mutation drift equilibrium, although further tests based on an expanded dataset are required to investigate this hypothesis further (Hänfling and Weetman 2006). In the absence of detailed estimates of census population sizes a rough estimate might be obtained from fishery catch data that are available at least for the Northern Group. It has been estimated that in the period of 1997–2004, the average yearly catch of adult crabs in the fishing season (November and December) in Yangtze River estuary alone was 2,400 kg and around 17,000 individuals (calculated on the base of average individual body weight of 140 g; Liu et al. 2007). This translates into census populations sizes of at least 100,000 individuals assuming that not more than 50% of the adult population was harvested and the Yangtze river constitutes only around 30–50% of the total mitten crab population. Therefore, the census population size of mitten crabs is at least 20 times larger as the long-term effective population size. This is not surprising, given the population crash

experienced by this species in the 1970s and 1980s (Yu et al. 1999).

A number of results indicate that there is little geographically patterned structure among the Northern Group populations. Firstly, the rather low F_{ST} value (0.007) among the Northern populations compared to the value across the whole data set (0.021). Secondly, differentiation among river systems was not significantly larger than differentiation within river systems. Thirdly, population genetic differentiation does not appear to be related to geographical distances since no significant isolation-by-distance relationship was detected among the native populations. Finally, Bayesian cluster analysis did not support a geographic association of clusters other than the distinction between the Northern and Southern Group. Next, we will discuss five potential mechanisms that may have contributed to the low genetic differentiation between populations of the Northern Group: (1) Natural gene flow homogenizing genetic differentiation, (2) deliberate or unintentional translocation through aquaculture, (3) lack of power to detect differentiation when analyzing highly variable loci, (4) low level of genetic drift due to large population size, and (5) microsatellite allele size homoplasy.

Firstly, gene flow is often invoked as the "default" explanation if an absence of genetic differentiation among geographically distinct populations is found. Chinese mitten crabs do have a high dispersal capacity and their life cycle as a catadromous species is characterized by migration events. Adult crabs migrate up to 1,000 km upstream within rivers and have also been observed to walk on land in order to cross migration barriers such as hydroelectric power dams. Furthermore, there is also potential for larval drift, as their planktonic zoeal larvae live in the sea before they become benthic and migrate upstream into freshwater. However, we argue that such dispersal capacity is unlikely to cause large-scale gene flow. First, there are no freshwater connections among the different river systems sampled in this study that could facilitate adult migration across watersheds. Second, the distance among the main river estuaries in which mitten crab reproduction takes place is large (e.g., 300 km between River Haihe and Huanghe, and more than 1,000 km between River Liaohe and Yangtze). Although mitten crab release their larvae few kilometers from the coast and during the first three development stages larvae drift with coastal currents this phase is limited to 10–20 days. Such a relatively short planktonic phase together with the absence of strong currents along the Northern Chinese coast (Cheng YX, Shanghai Fisheries University, personal communication) suggests that larval drift is unlikely to promote large scale gene flow over hundreds of kilometers.

Secondly, deliberate and unintentional translocation through aquaculture may have contributed to a genetic homogenization of wild populations (Qiu 1998; Wang and Li 2002). Driven by the high market price of mitten crab in the Yangtze River area, the transport of broodstock and juveniles from north and south to the hatcheries and farms in this area, and vice versa, have been carried out since the early 1990s. Although a number of anecdotal reports of large-scale accidental releases from mitten crab aquaculture exist (Cheng YX, Shanghai Fisheries University, personal communication) detailed data about their extent are not available. Such translocation and escapees could theoretically have resulted in gene flow among geographically distinct populations. However, population genetic theory predicts that migrants have negligible impact on allele frequencies of large recipient populations (Crow and Kimura 1970). Furthermore, large populations are often resilient to immigration due to what has been termed monopolization (De Meester et al. 2002). Given the large census and effective population sizes of natural mitten crab populations, translocation events would have to be very frequent and involve large numbers of individuals in order to result in significant change of allele frequencies during only the few generations in which this practice has been carried out.

Thirdly, microsatellites are highly variable markers that have limited power to reveal genetic differentiation when using traditional F_{ST}-based statistics. Genetic differentiation is defined as $G_{ST} = 1 - Hs/Ht$, where Hs and Ht are expected heterozygosities in each population and the total of the combined populations, respectively. As Hedrick (1999) demonstrated, G_{ST} is always smaller or equal to $1 - Hs$, the expected homozygosity in populations. Given the high level of heterozygosity ($Hs > 0.8$), G_{ST} remains close to zero, suggesting low level of differentiation. However, a number of non F_{ST}-based approaches such as Bayesian cluster analysis and tests for homogeneity of allele frequencies also suggest an absence of geographic structure among native mitten crab populations of the Northern group.

Fourthly, our results also indicate that long-term effective population size of mitten crab populations is in the order of magnitude of thousands and census population sizes exceed hundreds of thousands within each river system. It is therefore likely that reduced genetic drift has contributed to the observed low level of genetic differentiation. The differentiation between native and the invasive populations observed in a previous study and using the same set of microsatellite markers (Herborg et al. 2007) is also interesting in that context. Herborg et al. (2007) showed that the colonization of non-native habitats in Europe and North America was associated with severe demographic bottlenecks. As a consequence of such reduction in effective population size F_{ST} values between invasive and native populations were more than fivefold higher than the average values among populations of the Northern group reported in the present study. Such comparison demonstrates that genetic differentiation among mitten crab populations

would increase rapidly if population sizes would be low, providing indirect evidence that this is not the case.

Finally, observed genetic differentiation at microsatellite loci might have been further underestimated by allele size-homoplasy (Estoup et al. 2002). Homplasy is expected to be a particular problem when investigating large populations. The probability of a new mutation arising in a large population is higher than in a small population during each generation. Microsatellite mutations in particular might also increase in rate with increasing heterozygosity linking it to demographic history (Amos et al. 2008).

In summary, our results provide further evidence that the mitten crab populations of Southern China belong to a different taxonomic unit. It appears to be unlikely that gene flow alone whether through natural or human-mediated dispersal can explain the absence of genetic structure among mitten crab populations of the Northern group. Large effective population sizes and high gene diversity will have prevented genetic differentiation through drift in general and through homoplasy at microsatellites in particular. The results are relevant for both applied research and evolutionary biology. Our study provides the first clear evidence that neutral processes such as genetic drift and isolation have not resulted in significant genetic differentiation among geographic locations of this catadromous species. Such a mechanism can therefore clearly be ruled out as cause of previously reported differences in adaptive traits such as growth rates and body size (Li et al. 2000). The alternative hypotheses are that such variation has in fact no genetic basis or that strong selection has driven divergence as previously reported for quantitative traits across a wide range of organisms (Reed and Frankham 2001). The absence of a geographic population structure has also implications for the study of mitten crab invasions since it provides clear predictions when evaluating specific hypothesis about colonization history (Hänfling 2007).

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