

NOTE

mt-DNA sequences and possible invasion pathways of the Chinese mitten crab

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ABSTRACT: The Chinese mitten crab *Eriocheir sinensis* is an invasive species that inflicts considerable economical and ecological damage in European and American freshwaters. Sequence variation at the mitochondrial *COI* gene was investigated from Chinese, European and American populations in order to infer the invasion history. Firstly, we found a decrease in genetic variability from the source populations to those that have recently been established. Secondly, Central European populations that are about 100 yr old seem to have originated from multiple invasion events. Thirdly, the sequence data suggest high levels of gene flow across drainages within continents. Fourthly, the 2 American populations sampled in San Francisco Bay were fixed for a single haplotype. This haplotype was also found in European but not in Chinese populations; this observation provides circumstantial evidence for the unexpected hypothesis that the American populations were founded by a single introduction event from a European source.

KEY WORDS: Brachyura · Crustacea · Estuary · Molecular marker

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Global expansion in travel and trade has resulted in an increasing number of translocations of species (Everett 2000). Some species succeed in establishing founder populations and thereby extending their geographic range into new areas. A small proportion of such invading alien species have caused devastating effects on native biodiversity and ecosystems (Pimentel et al. 2000). Some of the most significant impacts have been recorded in aquatic habitats, including for example, the zebra mussel in North American freshwaters and the Nile perch in Lake Vic-

toria (Brown & Lomolino 1998). In addition to active transfers, the ballast water of ships has been identified as a major vector for aquatic invaders, resulting in complex and sometimes unexpected colonisation routes (Ricciardi & MacIsaac 2000). Identification of source populations and reconstruction of possible pathways of invasion are key issues in our understanding of the invasion process and especially in the design of effective programmes to minimise colonisation by alien species (Myers et al. 2000). Molecular markers, especially sequence variation, provide effective tools to investigate invasion histories (Kreiser et al. 2000, Cristescu et al. 2001, Patti & Gambi 2001).

The Chinese mitten crab *Eriocheir sinensis* H. Milne-Edwards 1854 is a euryhaline species with a catadromous life style and is a successful aquatic invader. *E. sinensis*, whose native range is in the temperate and tropical region along the Chinese Pacific coast, colonised Europe around 100 yr ago and has recently arrived in America. In Europe, this crab has colonised most of the drainages to the North Sea and the Baltic (Panning 1939), where it causes considerable economic and ecological damage due to its burrowing activities as well as predation of commercial fish. During the early 1980s, the European distributional area of *E. sinensis* had enlarged to include the Baltic Gulf of Gascogne (Vigneux et al. 1993). Very recent records have shown that the crab has now reached the Tagus estuary in Portugal (Cabral & Costa 1999). This species was also reported from several sites in North America, but in most cases it was not able to sustain a viable founder population. However, in 1993 the first established population was reported in San Francisco Bay (Cohen & Carlton 1997). Cohen & Carlton (1997) suggested that the most likely origin for these Pacific populations would be from Asia. Here, we apply molecular markers to examine the genetic relationships of mitten crabs

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sampled in Asia, Europe and America in order to infer gene flow and to reconstruct the history and possible pathways of the invasion.

Samples of *Eriocheir sinensis* were collected in 1999 and 2000 from: (1) 3 sites within the native distributional area in China, River Liahoe (122°38'E, 41°21'N), River Yangtze (120°45'E, 31°58'N) and Hangzhou Bay (120°35'E, 30°10'N); (2) 5 sites within Europe, lake Laascher See (11°25'E, 53°02'N) the rivers Elbe (12°02'E, 52°45'N), Weser (8°09'E, 53°06'N), Thames (0°25'E, 51°27'N) and Tagus (9°01'W, 38°53'N); and (3) 2 sites in North America, Sacramento delta (121°41'W, 38°07'N) and South San Francisco Bay (122°22'W, 37°39'N; Fig. 1). Tissue samples of all individuals were stored in 98% ethanol for DNA analysis. Total genomic DNA was extracted using a modified CTAB protocol based on Doyle & Doyle (1985). A fragment of 586 bp, which is part of the mitochondrial *COI* gene, was amplified using the universal primers LCO1490 and HCO2198 from Folmer et al. (1994). PCR was carried out in 25 µl using 10 to 30 ng genomic DNA, 0.2 mM of each dNTP, 3 pmol of each primer, 1 × NH₄ buffer (Bioline) and 0.2 U of *Taq* polymerase (Bioline). Reactions were amplified for 35 cycles with the following parameters: 45 s at 95°C, 45 s at 53°C and 3 min at 72°C. This was followed by a final extension step of 10 min at 72°C. The reaction product was sequenced directly using the Thermo sequenase fluorescent labelled cycle sequencing kit (Thermo RPN 2580, Amersham Pharmacia) and an ALFexpress™ automatic sequencer (Amersham Pharmacia). The sequences were aligned by eye. Gene diversity as a measure of genetic variability within populations was

estimated according to Nei (1987). An exact test using a Markov chain algorithm was applied to test the null hypotheses of no genetic differentiation between samples (Raymond & Rousset 1995) and pairwise F_{ST} values estimated differentiation among samples. Additionally, a hierarchical analysis of molecular variance (AMOVA, Excoffier et al. 1992) was performed with the samples grouped by continent. All analyses were performed using Arlequin ver. 2.0 2000 software (Schneider et al. 2000).

Seven different haplotypes were identified across the 120 individuals analysed (6 to 16 per sample; Tables 1 & 2). All haplotypes were closely related and could be linked in an unambiguous most parsimony network (not shown), where neighbouring haplotypes differ by 1 mutation. The low sequence divergence among haplotypes indicates that the analysed populations of *Eriocheir sinensis* have diverged very recently, which is consistent with the age of the European populations (ca. 100 yr). The number of haplotypes as well as within-sample gene diversity was highest in the Chinese populations (0.75 ± 0.026 ; mean \pm SD calculated across populations), slightly smaller in the European populations (0.61 ± 0.15) and markedly lower in the American samples (0 ± 0 ; Table 2). ANOVA reveals significant differences between continents ($F_{2,7} = 26.8$, $p < 0.001$); however, the difference is based on the very low gene diversity of the American samples with significant differences observed only between Chinese and American as well as European and American populations (Scheffé post-hoc test; $p < 0.05$; Scheffé 1953). We also found some variation in gene diversity within Europe: the recently established populations in Portu-

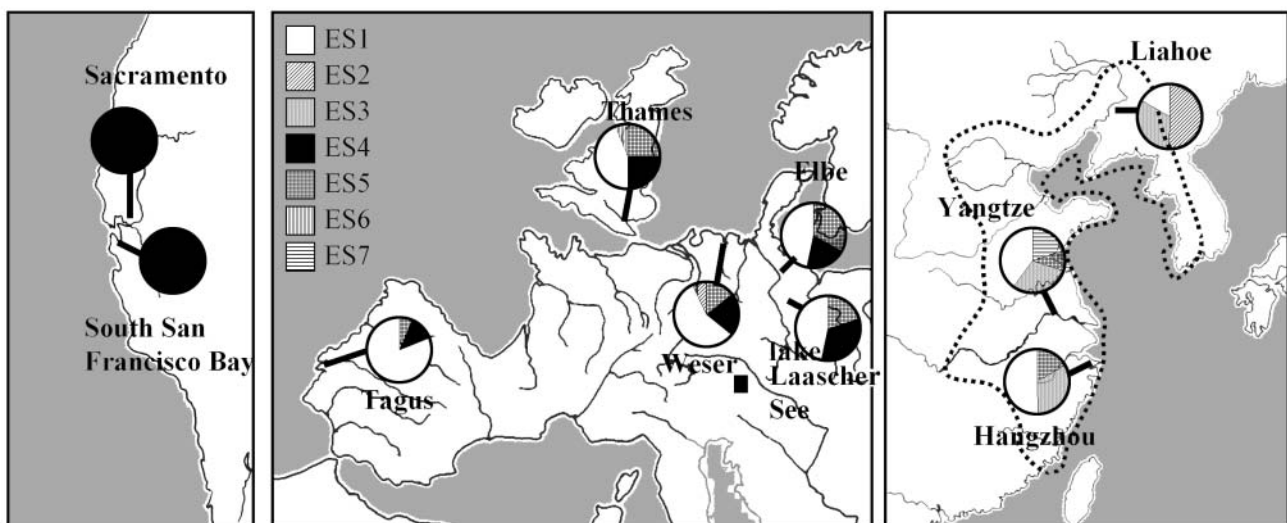


Fig. 1. *Eriocheir sinensis* sample sites in North America (left), Europe (centre) and China (right) and the relative distribution of the 7 identified *COI* haplotypes (ES1–ES7) within each sample. The dotted line indicates the native distribution of *E. sinensis*

gal showed only about half of the gene diversity of central European populations (Table 2). When the gene diversity value from the Portuguese population (0.34) is excluded, all means were significantly different (Scheffé post-hoc test; $p < 0.05$). Overall, our data show a consistent decrease of gene diversity from the native distributional area to regions where the most recent invasions have occurred (Portugal and America); this is a clear indication of the erosion of genetic diversity during successive founder events. However, it was somewhat surprising to find such a high number of haplotypes in the central European population. Two mutually non-exclusive possibilities can be proposed: (1) a large number of individuals contributed to the founder population during a single colonisation; and/or (2) successive invasion events fuelled the genetic diversity. Although somewhat speculative, we think that the second explanation is more likely because it is consistent with the observation of strong differentiation among the European and Chinese populations, indicating a relatively small founding population.

A global F_{ST} -value of 0.39 indicated considerable differentiation among samples. However, pairwise F_{ST} -values and tests for genetic differentiation among samples (Raymond & Rousset 1995) showed that significant differentiation occurred only among samples from different continents, whereas all the pairwise tests within continents were non-significant. Pairwise F_{ST} -values ranged from -0.06 to -0.04 among Central European populations, from 0.02 to 0.12 among Central European and the Tagus populations, from -0.11 to 0.09 among Chinese populations and from 0.06 to 0.81 among populations from different continents. Hierarchical AMOVA corroborated this pattern. We found genetic homogeneity within (1%, $p > 0.3$), but significant differentiation among continents (38%, $p = 0.000$). Such a pattern indicates high levels of gene flow among populations and across drainages within continents, which is in contrast to the strong genetic differ-

Table 1. Variable sites from 589 bp sequences of mitochondrial *COI* genes sampled across 120 individuals from 10 populations of *Eriocheir sinensis*. All sequences for the 7 haplotypes (ES1–ES7) have been submitted to GenBank (Accession numbers: AF 435113 to 9)

| Haplotype | Nucleotide position | | | | | |
|-----------|---------------------|-----|-----|-----|-----|-----|
| | 130 | 167 | 295 | 328 | 365 | 509 |
| ES1 | A | G | G | C | G | T |
| ES2 | . | . | . | T | . | . |
| ES3 | . | . | A | . | . | . |
| ES4 | G | . | A | . | . | . |
| ES5 | . | . | A | . | . | C |
| ES6 | . | . | A | . | G | C |
| ES7 | . | A | A | . | . | C |

entiation across drainages typically seen in other freshwater organisms (Ward et al. 1994, Hänfling & Brandl 1998). Since *Eriocheir sinensis* spends most of its life cycle in freshwater habitats, our results suggest that considerable larval drift occurs during the short period between hatching of larvae in the sea and the active migration of larvae into freshwater. During the first 3 developmental stages, larvae drift passively with the coastal currents and thereby secure a high potential for large-scale gene flow. Only during the last developmental stage (the megalopa) does the larva actively migrate towards the freshwater by remaining in the benthos at low tide and dispersing into the water column at high tide (Panning 1939).

The haplotype distribution of the Portuguese and American populations shows greater similarity to the Central European populations than to the Chinese populations (Table 2). Within our American samples, we found only 1 haplotype (ES4), indicating that the San Francisco population was established by a single event. This is of particular interest and unexpected, since recent findings of a study at the University of California at Berkeley (D. Rudnick unpubl. data) have

Table 2. Distribution of the 7 haplotypes (see Table 1) found across the 10 sampled populations of *Eriocheir sinensis*. We also give the gene diversity and associated standard error within each population and the sample sizes

| Site | Haplotype | | | | | | | Gene diversity | SE | n |
|-------------------|-----------|-----|-----|-----|-----|-----|-----|----------------|-------|----|
| | ES1 | ES2 | ES3 | ES4 | ES5 | ES6 | ES7 | | | |
| Liahoe | 1 | 3 | 2 | | | | | 0.733 | 0.155 | 6 |
| Yangtze | 4 | 3 | | | 1 | | 2 | 0.778 | 0.091 | 10 |
| Hangzhou | 3 | 2 | | | 1 | | | 0.733 | 0.155 | 6 |
| Elbe | 7 | | | 3 | 5 | | | 0.676 | 0.070 | 15 |
| Lake Laascher See | 7 | | | 5 | 3 | | | 0.676 | 0.070 | 15 |
| Weser | 8 | | 1 | 3 | 2 | | | 0.648 | 0.116 | 14 |
| Thames | 7 | | | 4 | 4 | 1 | | 0.725 | 0.069 | 16 |
| Tagus | 13 | | | 2 | 1 | | | 0.342 | 0.140 | 16 |
| Sacramento | | | | 7 | | | | 0.000 | 0.000 | 7 |
| San Francisco | | | | 16 | | | | 0.000 | 0.000 | 16 |

shown clear differences in morphology and feeding behaviour between individuals from the Sacramento delta and South San Francisco Bay. The haplotype found in America is quite common in Europe but absent in our Chinese populations (Table 2). The Chinese populations were not sampled comprehensively and we are not able to exclude the possibility that the haplotype ES4 may also occur at low frequencies within Chinese populations. However, the F_{ST} -values suggest a high level of gene flow among populations within a continent. Thus, we do not expect that other Chinese populations have a considerably different haplotype distribution to that presented here. Furthermore, our sampling covered the waters in proximity to Shanghai which is China's most important port and thus the most likely source for the invasion to Europe and America.

The populations of the mitten crab in San Francisco Bay may originate from larvae in ballast water of large vessels but, since mitten crab is an important food species, it may also have arisen from intentional introductions (Cohen & Carlton 1997). Cohen & Carlton (1997) concluded from shipping data of large commercial vessels and human food consumption patterns that the most likely source of the introduction is Asia. Clearly, our genetic data provide an unexpected alternative hypothesis about the invasion history of the American populations of the mitten crab, i.e. the source may be Europe and not Asia. A possible mechanism for such a translocation could be the transport of mitten crab from Europe to America for food consumption since mitten crabs are caught and marketed by commercial fishermen in the river Elbe, Germany.

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