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POPULATION STRUCTURE IN THE SWORD PRAWN (*PARAPENAEOPSIS HARDWICKII*) FROM THE EAST CHINA SEA AND TAIWAN STRAIT INFERRED FROM INTRON SEQUENCES

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A B S T R A C T

Sequence analyses on the intron from the elongation factor-1α gene were conducted to examine the population structure of sword prawn (*Parapenaeopsis hardwickii*) in the East China Sea and Taiwan Strait. Five samples including 207 individuals were separately collected from the north of East China Sea (NECS) and waters off Tamsui, Taichung, Putai, and Cheding, and 102 alleles were identified. Despite no phylogeographic structure in alleles, pairwise F_{ST} values and analysis of molecular variance (AMOVA) showed significant genetic difference between the NECS and the other four samples. The UPGMA tree of five samples showed two distinct clusters; one included the NECS sample; the other included the rest of samples. The results suggest that two populations exist in the East China Sea and Taiwan Strait. To ensure resource sustainability and maintenance, the sword prawn in the Taiwan Strait and the north of the East China Sea should be treated as two separate populations and then be separately managed in future. Both Tajima's *D* and Fu and Li's *D* statistics and analysis of mismatch distribution for overall alleles suggested that sword prawn in studied area had experienced population expansion.

KEY WORDS: Elongation factor-1α gene, East China Sea, population structure, *Parapenaeopsis hardwickii*, Taiwan Strait

INTRODUCTION

Information on population structure is essential for the management and conservation of genetic resources in exploited marine organisms (Hillis et al., 1996). Recent researches on population genetic structure have utilized new analytical and technical tools that provide high-resolution genetic information. These tools include polymerase chain reaction (PCR) amplification, sequencing of DNA fragments, and the phylogenetic analysis of allelic variants. Many of the data for these studies have come from sequences of mitochondrial genes. However, the analysis of mitochondrial DNA sequence variability is widely recognized as suffering from two main weaknesses. First, the mitochondrial genome is only a single genetic locus. A second problem is that such analyses on mitochondrial DNA sequence allow only the reconstruction of maternal lineages (Wilson et al., 1985). The nuclear markers would differ from mitochondrial markers in their rates of evolution and biparental mode of inheritance. The ideal nuclear marker for intraspecific studies would show relatively high levels of neutral variation. One approach to find such markers has been to target introns in highly conserved nuclear genes (Bradley and Hills, 1997). An additional benefit of such intron is that "universal" primers can be designed that will anneal to regions in the highly conserved exons flanking noncoding introns (Palumbi and Baker, 1994).

Analyses on population genetic structure of marine biota have frequently revealed that organisms with high dispersal capacity would have little genetic distinction over large geographic scales (Hellberg, 1996). These studies suggest that there are high levels of gene flow between marine populations. However, there is growing evidence that widespread marine organisms are more genetically structured than expected given their high dispersal potential and apparent lack of barriers to dispersal in the ocean (Palumbi, 1997; Benzie, 1999; Briggs, 1999). Thus, there may be limits to the actual dispersal of marine organisms with high dispersal potential (Benzie and Williams, 1997). These limits vary widely with species, habitats, local ocean conditions or historical events, and they may produce sufficient chances for genetic distinction (Palumbi, 1994).

Sword prawn, *Parapenaeopsis hardwickii* Miers, 1878, is distributed mainly in the Indo-West Pacific from Pakistan to Japan and lives at 5 to 90 m in the sand bottom areas. This species is one of the most abundant and highly valued species in the East China Sea and Taiwan Strait (Wu, 1985; Song and Ding, 1993). The life history of the sword prawn, with an offshore planktonic larval phase, an estuarine postlarval and juvenile phase, and an offshore adult and spawning phase (Dall et al., 1990), may allow moderate gene flow among populations. Two morphologically distinguishable populations of sword prawn in the East China Sea and

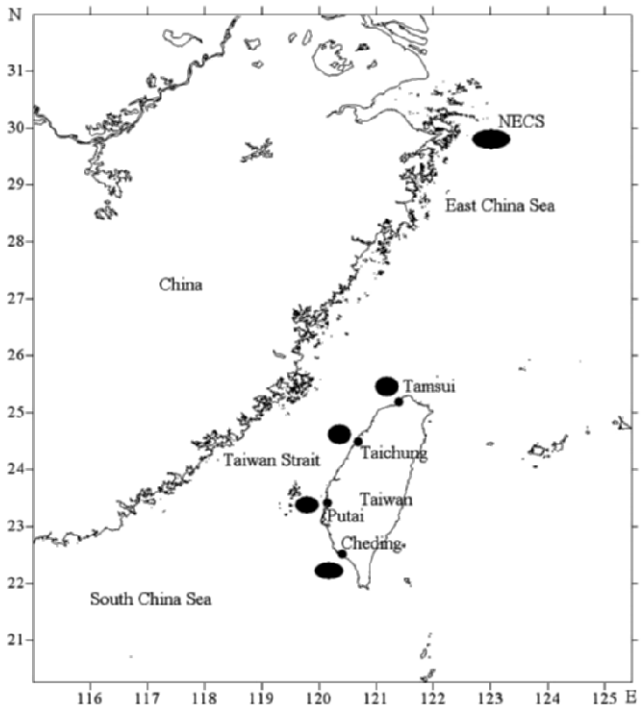

Fig. 1. Shadowed areas showing the sampling areas from the East China Sea and Taiwan Strait.

Taiwan Strait were discriminated (Tzeng, 2004). However, the variation of morphological characters could be affected by genetic and environmental factors, so that discrimination of populations based on morphological variation must be verified by genetic evidence to confirm that the variation reflects the true degree of reproductive isolation rather than environmental isolation (Pepin and Carr, 1992). In this paper, sequence analyses of the intron from the elongation factor-1α gene were conducted to elucidate the population genetic structure of sword prawns in the East China Sea and Taiwan Strait.

Materials and Methods

Sample Collection

Five samples of sword prawn including 207 specimens were collected from commercial shrimp trawlers during October 2002 and February 2003 (Fig. 1 and Table 1). They were separately sampled from the north of East China Sea (NECS) and waters off Tamsui (Tamsui), Taichung (Taichung), Putai (Putai), and Cheding (Cheding). Specimens were iced or frozen immediately after capture and later kept at −75°C before DNA extraction.

DNA Extraction, Amplification and Sequencing

Total genomic DNA was extracted from frozen muscle tissue using QIAamp Tissue Kit (QIAGEN) and was kept at −20°C until analyses. A fragment of the intron sequence in the elongation factor-1α gene was amplified using the primers EF3s (5′-GACAAGGCCCTCCGTCTTCC-3′) and EF4s (5′-GGGCACTGTTCCAATACCTC-3′) (France et al., 1999). PCR was run on a GeneAmp 2400 thermal cycler (Perkin-Elmer Corp.) with an initial denaturation of 60 seconds at 94°C, followed by 30 cycles of 15 seconds at 94°C, 15 seconds at 53°C, 15 seconds at 72°C, and finally a 3-min extension at 72°C. PCR products were visualized on an agarose gel and purified using the QIAgen PCR Purification kit. Purified PCR products were ligated into a pGEMT vector, and subsequently transformed with JM109 competent cells (Promega, Madison, USA), following manufacturer's instructions. White clones were selected from the plate. Their plasmids were prepared and then sequenced.

Table 1. Sample code, sampling locality, sample size, number of allele, and nucleotide diversity (π) with their standard deviation (SD) in five sword prawn samples in the East China Sea and Taiwan Strait.

Sample code	Sampling locality	Sample size	Number of allele	π ± SD
NECS	North of the East China Sea	38	23	1.360 ± 0.163%
Tamsui	Waters off Tamsui	47	26	1.861 ± 0.195%
Taichung	Waters off Taichung	41	26	1.920 ± 0.231%
Putai	Waters off Putai	41	29	1.964 ± 0.228%
Cheding	Waters off Cheding	40	22	1.437 ± 0.125%
Total		207	102	1.835 ± 0.085%

Sequence Analyses

DNA sequences were aligned using the PILEUP program in GCG (Genetics Computer Group, version 7.0; Devereux et al., 1991). The beginning (5′-GT) and end (3′-AG) of the intron sequence were confirmed by comparing with published sequence of *Penaeus vannamei* Boone, 1930 (France et al., 1999). Subsequent analyses were based on the intron sequence obtained from 207 individuals. The number of variable sites was assessed with MEGA3 (Kumar et al., 2004). Each different allele was assigned a number, and the distribution of the alleles in each sampling area was determined. The nucleotide diversity (π) (Nei, 1987) in each sample was calculated using DnaSP Version 4.10 (Rozas et al., 2003). Allele network was constructed using the median-joining method (Bandelt et al., 1999) in Network Version 4.2.0.1.

To examine whether two of the samples are genetically different from each other, the values of F_{ST} statistic (Wright, 1965) between five samples were estimated and tested using the program ProSeq (Filatov, 2002). Gene flow (N_m) was also estimated using the program ProSeq. The statistical significance of the estimate was tested through 1000 permutations. The dendrogram of five samples was constructed using unweighted pair-group method with arithmetic means (UPGMA) based on the F_{ST} values with MEGA3.

Analyses of molecular variance (AMOVA) implemented in ARLEQUIN Version 2.000 (Schneider et al., 2000) were performed to test the geographic divisions among samples. Different groupings of samples were suggested by: 1) UPGMA tree of sampling areas, 2) F_{ST} values between samples, and 3) geographic distribution. The significance of these Φ statistics is evaluated by 1000 random permutations of sequences among samples. The groupings that maximize values of Φ_{CT} and are significantly different from random distributions of individuals are assumed to be the most probable geographic subdivisions.

To check for the deviations from neutrality, Tajima's *D* statistical test (Tajima, 1989) and Fu and Li's *D* statistical test (Fu and Li, 1993) were carried out to assess evidence for population expansion using DnaSP. Population demographic history was examined by calculating mismatch distribution overall alleles with DnaSP.

Results

The intron sequence from the elongation factor-1α gene (206 bp in length) was identified and used for the following analyses. Nucleotide diversity (π) was 1.835 ± 0.085% for all samples, with values from 1.360 ± 0.163% (NECS) to 1.964 ± 0.228% (Putai) (Table 1). Among the 207 individuals sequenced, 102 alleles were identified. In total, 78 variable sites, including 46 singletons and 32 parsimoniously informative sites, were observed. The most common allele was observed in all samples except NECS and shared by 41 individuals; 14 specimens were from the Taichung, 10 from the Tamsui, 8 from the Putai, and 9 from Cheding samples. The second most common allele was observed in all samples and shared by 15 individuals; 1 specimen was from the Taichung, 6 from the Tamsui, 1 from the Putai, 6 from Cheding and 1 from NECS samples. The third most common allele was observed in all samples and share by 9 individuals; 1 specimen was from the Taichung, 3 from

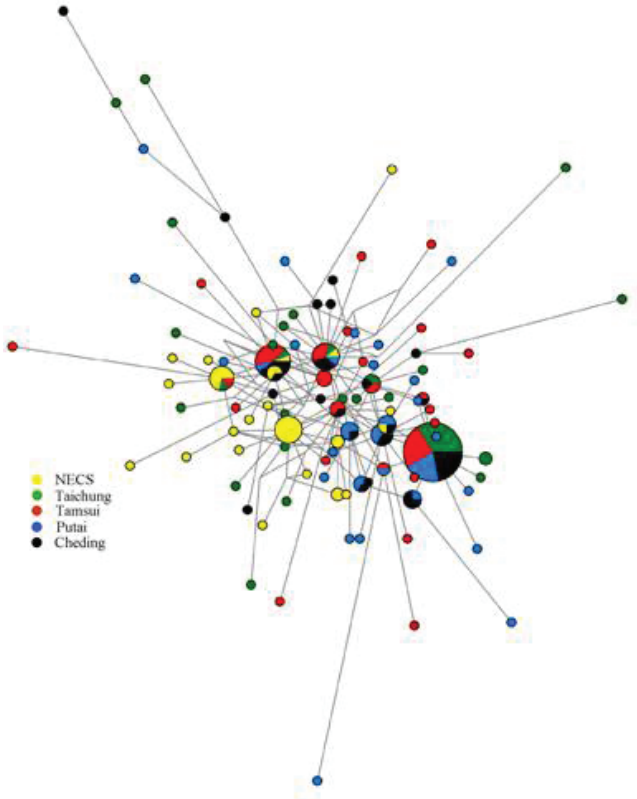

Fig. 2. Allele network of sword prawn in the East China Sea and Taiwan Strait. Different symbols represent different sampling areas. Small symbols indicate 1 individual, while large ones indicate more than 1 individual.

the Tamsui, 1 from the Putai, 3 from Cheding and 1 from NECS samples. Another allele was also shared by 9 individuals, but all these ones were from NECS sample. The median-joining network for the 102 alleles appeared star-like and showed no notable allele clustering (Fig. 2). This suggests that sword prawn probably underwent recent population expansion.

The F_{ST} and N_m values are shown in Table 2. Pairwise F_{ST} values between NECS and the rest of the samples revealed significant genetic differences, but genetic variation among the other four samples were not significant. The N_m values between all pair-wise comparisons ranged from 0.0309 (NECS-Taichung) to ∞(Tamsui-Taichung). The UPGMA tree of the five is shown in Fig. 3. The five samples were clustered into two distinct groups, with NECS constituting the first group and the other four samples making up the second group.

Various groupings of samples were tested using AMOVA, but only two groupings showed significant variation (Table 3). In the first grouping, the AMOVA for five samples yielded a significant Φ_{ST} value of 0.0827, indicating that at least one of pair-wise comparisons revealed significant heterogeneity. In the second grouping, five samples were classified into two groups. One included NECS, the other included Tamsui, Taichung, Putai and Cheding. Significant Φ_{CT} value of 0.1801 was observed, indicating that genetic discontinuity occurred in the NECS population.

Table 2. F_{ST} (below the diagonal) and N_m values (above the diagonal) between five sword prawn samples in the East China Sea and Taiwan Strait. Abbreviations for sampling locations are defined in Table 1.

	NECS	Tamsui	Taichung	Putai	Cheding
NECS	—	1.2408	0.0309	0.0972	0.9789
Tamsui	0.1677**	—	69.2553	15.4259	∞
Taichung	0.1952**	0.0036 ns	—	42.0443	29.9052
Putai	0.2401**	0.0159 ns	0.0059 ns	—	41.2861
Cheding	0.2034**	0.0000 ns	0.0083 ns	0.0060 ns	—

** Significant at $P < 0.001$; ns, not significant.

The model of population expansion could not be rejected because of the distribution of the pairwise number of differences in the intron alleles fitted the population expansion model well (Fig. 4). This outcome was also supported by the low Harpending's Raggedness index ($r = 0.0166$). The significant negative values of Tajima's D ($D = -2.3821$, $P < 0.01$) and Fu and Li's D ($D = -6.6935$, $P < 0.02$) were consistent with population expansion.

To investigate whether there was the presence of more than one locus in the elongation factor-1α intron in *Parapenaeopsis hardwickii*, 8 additional sword prawn specimens were sequenced and compared. We sequenced 8 clones per individual. Four different sequence sizes (205, 206, 223 and 228 bp in length) were identified in 64 obtained sequences (Table 4). The numbers of sequences of 205, 206, 223 and 228 bp in length were 7, 47, 6 and 4, respectively. There is one specific deletion (position 199) when comparing sequences of 205 and 206 bp in length. Five deletions and 25 specific variable sites were found when comparing sequences of 223 and 228 bp in length. Number of times in which identical and different sequence were calculated when comparing two sequences of 206 bp in length from the same individual. Multiple sequences from an individual were classified as different if they differed by at least four characters, or, when they differed by fewer than four characters, if the differences between sequences could not be attributed to polymerase error. Base the expectations under 1- and 2- locus models presented by Duda and Palumbi (1999), only one locus exists in the sequence size of 206 bp, because the number of time for identical sequence (87) is significant larger than the number of time for different sequence (31). We, therefore, concluded that there are at least four elongation factor-1α loci within *Parapenaeopsis hardwickii*.

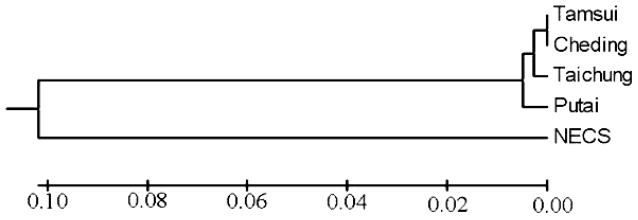

Fig. 3. UPGMA dendrogram illustrating the genetic relationships among sword prawn samples from the East China Sea and Taiwan Strait based on F_{ST} values.

Table 3. The results of AMOVA. Abbreviations for sampling locations are defined in Table 1.

Groupings	Source of variation	Percentage of variation	Φ-statistics	*P*
One group for all samples				
1 Group 1(NECS, Tamsui, Taichung, Putai, Cheding)	Among locations	8.28	$\Phi_{ST} = 0.0827$	< 0.0001
Two groups				
2 Group 1 (NECS) Group 2 (Tamsui, Taichung, Putai, Cheding)	Among groups	18.10	$\Phi_{CT} = 0.1810$	< 0.0001

Discussion

Although the median-joining network for the 102 alleles revealed no genealogical branches or geographic clusters, result of cluster analysis, sequence statistic (F_{ST}), and AMOVA indicated significant genetic division between the five samples. The cluster analysis indicated that the five samples could be clustered into two groups. One included the NECS sample, and the other included the other four samples (Fig. 3). F_{ST} values between the NECS and the other four samples showed significant genetic differences (Table 2), indicating at least two isolated populations exist in studied area. Results of the AMOVA revealed two different populations in the East China Sea and Taiwan Strait (Table 3). Base on the above analyses, the sword prawns in the East China Sea and Taiwan Strait can be discriminated into two distinct populations. The first population is in the Taiwan Strait, and the second one in the north of the East China Sea. The present result is in agreement with the previous outcome that two morphologically distinguishable stocks separately exist in the East China Sea and the Taiwan Strait (Tzeng, 2004). Thus, to ensure resource sustainability and maintenance, the sword prawn in the Taiwan Strait and the north of the East China Sea should be treated as two separate stocks and then be managed separately in the future.

To form and maintain discrete stocks, a moderately high degree of reproduction isolation is essential. Reproduction isolation can be developed through spawning dates, spawning frequency and spawning location. Two spawning areas were found in the Taiwan Strait and the East China Sea. One is located in the middle and north of the Taiwan Strait (Guo, 1993), but the other is located in the north of the East China Sea (Zheng and Li, 2002). In the Taiwan Strait, two peaks of spawning season were found (Guo, 1993); one is between February and April, and the other is between October and November. In the north of the East China Sea, the spawning season lasts from May to September, with the peak usually occurring between June and July (Zheng and Li, 2002). Reproductive separation in spawning date, frequency, and location may develop and keep these two discrete sword prawn stocks in the Taiwan Strait and the north of East China Sea.

Sword prawns migrate from inshore to offshore as they grow to specific size or life stage, but the migratory distance is limited (Dall et al., 1990). Thus, the dispersal of larvae is the primary source of gene flow, and ocean currents play a major role in the dispersal of this species. In the north of the East China Sea, the spawning season lasts from May to September, with the peak usually occurring between June and July (Zheng and Li, 2002). Along the eastern coast of China, the larvae of sword prawns from the north of the East China Sea may be transported to the Taiwan Strait by the China coastal current, but may not be large enough to

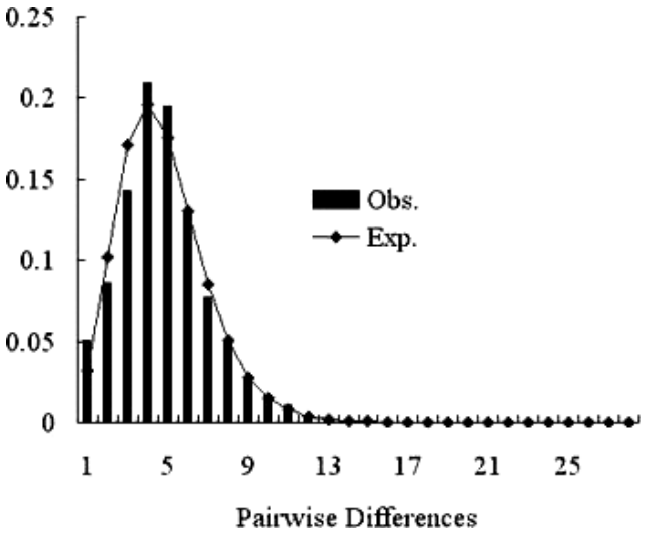

Fig. 4. Mismatch distribution constructed using pairwise differences among intron sequences of the sword prawn in the East China Sea and Taiwan Strait.

Table 4. Number of sequence size for 8 sword prawn specimens. Number of times in which identical or different sequence were obtained when comparing two sequences of 206 bp in length from the same individual.

	Sequence size					
Specimen	205 bp	I[a]	206 bp	D[b]	223 bp	228 bp
1	1	11	7	10	0	0
2	1	9	7	12	0	0
3	1	6	4	0	1	2
4	1	5	5	5	1	1
5	0	15	6	0	1	1
6	1	15	6	0	1	0
7	0	15	6	0	2	0
8	2	11	6	4	0	0
Total	7	87	47	31	6	4

[a] number of time for identical sequence.
[b] number of time for different sequence.

eliminate the genetic difference between the NECS and Taiwan Strait populations (Table 2, 3), and that is supported by lower values of N_m between NECS and the other four samples in Taiwan Strait (from 0.0309 to 1.2408, Table 2). During the spawning season of the NECS population the China coastal current is not strong enough to flow through the Taiwan Strait, and it only spreads to the north and middle of the Taiwan Strait (Wu, 1982) and results in the occurrence of sword prawn larvae mixed in the north and middle of the Taiwan Strait. This may explain why the values of nucleotide diversity in the samples from Tamsui, Taichung, and Putai are higher than ones in NECS and Cheding samples.

In the Taiwan Strait, two peaks of spawning season were found (Guo, 1993); one is between February and April, and the other is between October and November. During the late spring, the South China Sea warm water dominates the Taiwan Strait (Wang and Chern, 1989). In general, if the N_m value is greater than about 1, the gene flow is considered sufficient to maintain a relatively homogeneous gene pool (Slatkin, 1987). The N_m values between all pair of four samples from Taiwan Strait are high (15.4259 to 69.2553) and prevent population differentiation between the four samples (Table 2).

Because of the fluctuation in population size, the neutrality cannot be tested accurately. The Tajima's *D* and Fu and Li's *D* statistics are sensitive to the factors such as bottlenecks or population expansion which tends to drive the values of Tajims's *D* and Fu and Li's *D* towards more negative values (Tajima, 1996; Martel et al., 2004). Indeed, significant negative values of these two statistics indicated that sword prawn in the East China Sea and Taiwan Strait has experienced population expansion. The unimodel mismatch frequency distribution pattern accorded well the predicted distribution under a model of population expansion (Fig. 4, Rogers and Harpending, 1992). This unimodel pattern has also been observed for other shrimp species, *Farfantepenaeus aztecus* Ives, 1891 and *Farfantepenaeus duorarum* Burkenroad, 1939 (McMillen-Jackson and Bert, 2003; 2004). Past geological and climatic events have undoubtedly played a major pole in terrestrial biogeography. During the Pleistocene glaciations, sea level was 130-150 m lower than the present level in the East China Sea and 100-120 m lower in the South China Sea. Consequently, the entire Yellow Sea and Taiwan Strait were exposed, and the East China Sea was reduced into an elongated trough (Wang and Sun, 1994). The disappearance of habitat had restricted marine species to the relatively limited areas and caused the mixing among populations and reduced the genetic variation between populations (Benzie and Williams, 1997). The distribution of sword prawns gradually extended corresponding to the rise of the sea level of the East China Sea and Taiwan Strait since late Pleistocene. The similar magnitude of nucleotide diversity in NECS and Cheding samples provide part of the evidence that sword prawn in the studied waters share common ancestry (Table 1).

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