

Full Length Research Paper

Population structure and expansion of kuruma shrimp (*Penaeus japonicus*) in the adjacent waters of Taiwan inferred from intron sequences

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Sequence analyses on the specific intron from the elongation factor-1 α gene were conducted to examine the population genetic structure and expansion of kuruma shrimp (*Penaeus japonicus*) off Taiwan. Five populations including 119 individuals were separately sampled from the north of East China Sea (ECS), west of Taiwan Strait (WTS), east of Taiwan Strait (ETS), South China Sea (SCS) and adjacent waters of Kagoshima (JAN). The gene diversity (h) was high for all populations (99.9%), with values from 99.7% (ETS) to 1. Nucleotide diversity (π) for all populations was 0.0421, with values from 0.0368 (JAN) to 0.0437 (ETS). Neighbor-joining tree and network of all alleles showed no significant genealogical structure. Analyses of molecular variance and F_{ST} also revealed no significant genetic structure among five populations, but the F_{ST} value between ECS and ETS was significant. Fu's F statistics and analysis of mismatch distribution for overall alleles suggested that this species in the studied waters had experienced population expansion.

Key words: Intron of elongation factor-1 α gene, *Penaeus japonicus*, population genetic structure, population expansion.

INTRODUCTION

Understanding the population genetic structure is an important component of successful and sustainable long-term management of fishery resources (Hillis et al., 1996), and recent advances in population genetic (Excoffier et al., 1992) and historical demographic analyses (Fu, 1997; Rogers and Harpending, 1992) can be very helpful in providing valuable and complementary

information on catch and age composition data (Pauly et al., 2002).

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 sequences allow only the reconstruction of maternal lineages (Wilson et al., 1985).

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Abbreviations: WTS, West of Taiwan Strait; ECS, East China Sea; JAN, adjacent waters of Kagoshima; ETS, east of Taiwan Strait; SCS, South China Sea.

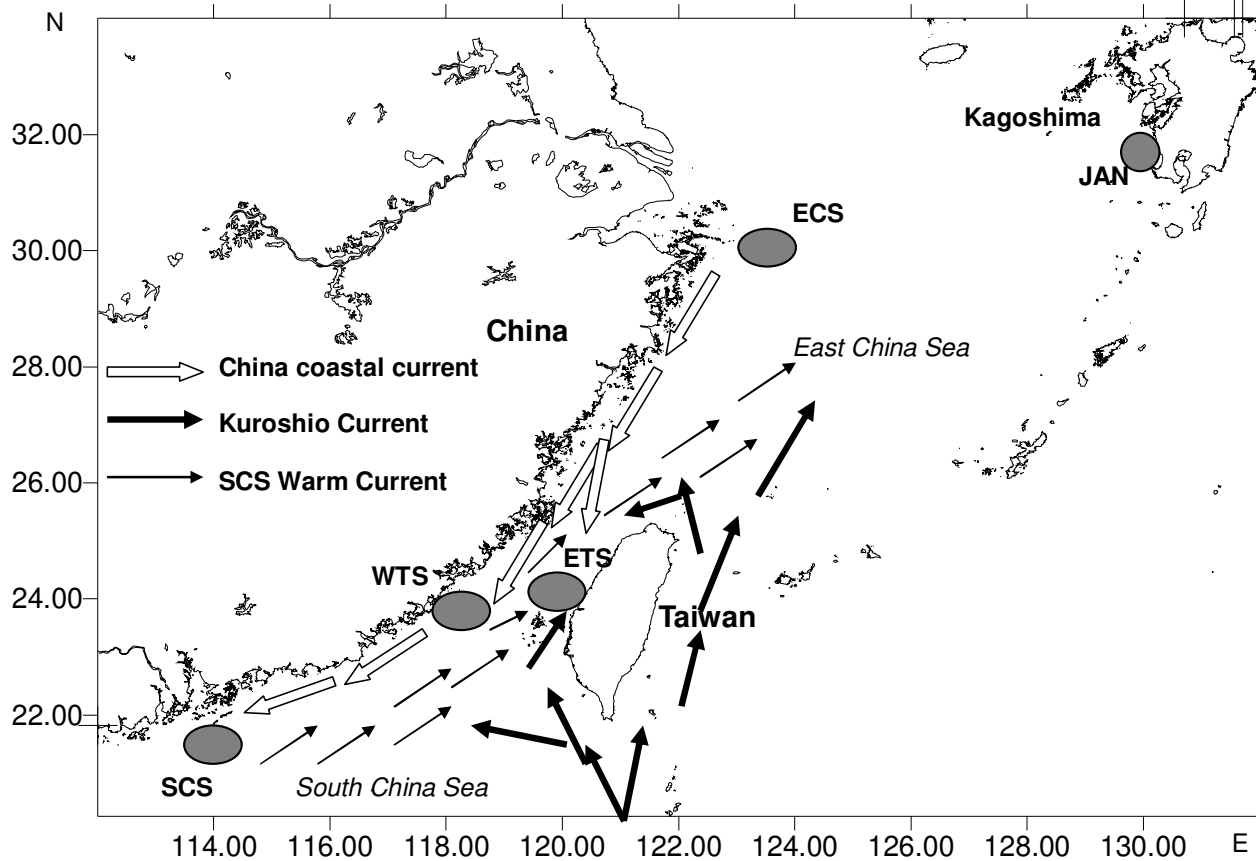


Figure 1. Sampling localities of kuruma shrimp (*Penaeus japonicus*) in the adjacent waters of Taiwan.

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 intron 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). Moreover, the intron sequence could be regarded as haplotype to be analyzed (France et al., 1999). We successfully used the intron sequences from the elongation factor-1 α gene to elucidate the population structure of the sword prawn *Parapenaeopsis hardwickii* from the East China Sea and Taiwan Strait (Tzeng et al., 2008).

The kuruma shrimp (*Penaeus japonicus*) is one of the most important fishery resources of Taiwan. Two morphologically similar but genetically distinct varieties of this species were found (named varieties I and II) and occurred simultaneously in the northern coast of the South China Sea (SCS), Taiwan Strait (TS) and East China Sea (ECS) (Tsoi et al., 2005, 2007; Shih et al., 2011). These two varieties were characterized by diagnostic color banding patterns on the carapace.

Variety I exhibits two characteristic bands extending from the dorsal to the ventral carapace, whereas these two bands do not extend to the lower half of the carapace in variety II. However, the variant I of kuruma shrimp was dominant in the adjacent waters of Taiwan.

In previous studies on the population genetic structure for variety I of kuruma shrimp in the East Asia showed genetically homogeneous (Tsoi et al., 2007; Shih et al., 2011). However, the main molecular markers used in these aforementioned papers were mitochondrial DNA sequences. In the present study, sequence analyses on the intron from the elongation factor-1 α gene were conducted to elucidate the population genetic structure and expansion for variety I of kuruma shrimp in the adjacent waters of Taiwan.

MATERIALS AND METHODS

Sample collection

Five populations including 119 individuals (variety I of kuruma shrimp) were collected from commercial shrimp trawlers (Figure 1, Table 1). They were separately sampled from the north of East China Sea (ECS), west of Taiwan Strait (WTS), east of Taiwan Strait (ETS), South China Sea (SCS) and adjacent waters of Kagoshima

Table 1. Code of sampling site, sample size, number of allele, gene diversity and nucleotide diversity of the kuruma shrimp (*Penaeus japonicus*) in the adjacent waters of Taiwan.

Code	Sampling site	Sampling size	Number of allele	Gene diversity	Nucleotide diversity	Fu's F_s
ECS	East China Sea	32	31	0.998	0.0436	-22.8605**
JAN	Waters off Kagoshima	13	13	1	0.0368	-6.3040**
WTS	West of Taiwan Strait	13	13	1	0.0403	-5.8948**
ETS	East of Taiwan Strait	38	36	0.997	0.0437	-15.5962**
SCS	South China Sea	23	23	1	0.0427	- 8.6135**
Total		119	113	0.999	0.0413	-24.3869**

* $P < 0.05$, ** $P < 0.01$, ^{ns} = Not significant ($P > 0.05$).

(JAN). Specimens were iced or frozen immediately after capture and later kept at -75°C until extracted.

DNA extraction, amplification and sequencing

Total 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-1 α 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 s at 94°C , followed by 30 cycles of 15 s at 94°C , 15 s at 53°C , 15 s 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.

Data analyses

DNA sequences were aligned by ClustalX, version 1.83 (Thompson et al., 1997), then subsequently optimized by eye in BioEdit, version 7.0.5.3 (Hall, 1999). The beginning and end of the intron sequence were confirmed by comparing with published sequence of *Penaeus vannamei* Boone, 1930 (France et al., 1999). All the sequences are shown in Appendix I. The gene diversity (h) and nucleotide diversity (π) (Nei, 1987) in each population were calculated using DnaSP Version 4.10 (Rozas et al., 2003). Nucleotide composition and numbers of variable sites were assessed with ARLEQUIN Version 3.01 (Excoffier et al., 2005). The optimal substitution model for the intron sequences were determined by MEGA 5 (Tamura et al., 2011). The parameter values estimated by MEGA 5 were adopted for the phylogeographic relationship of alleles and the analysis of molecular variances (AMOVA). Phylogeographic analysis was carried out on nucleotide distances by the neighbor-joining (NJ) method implemented in MEGA 5. We used bootstrap analyses with 1,000 replicates to evaluate support for phylogenetic relationships. A network of alleles was also constructed using the median-joining method (Bandelt et al., 1999) in Network Version 4.2.0.1.

To examine whether any two of the populations genetically differed from each other, pair-wise F_{ST} statistics among the 5 populations were estimated and tested using the program, ProSeq (Filatov, 2002). Gene flow (N_m), was estimated using the relationship $N_m = ((1 / F_{ST}) - 1) / 2$. The AMOVA implemented in ARLEQUIN was performed to test the geographic divisions. The significant of these Φ statistics was evaluated by 10000 random permutations of

sequences among populations.

Furthermore, to check for deviations from neutrality, Fu's F_s statistical tests (Fu, 1997) was carried out to assess evidence for population expansion using ARLEQUIN. Meanwhile, the concordance of data with the distribution underlying the expansion model was assessed. Population expansion was investigated by examining the frequency distributions of pair-wise differences between sequences (mismatched distribution) with DnaSP.

RESULTS

The sequence of specific intron allele (246 bp in length) from the elongation factor-1 α gene for each specimen was identified and used for the following analyses. The gene diversity (h) was high for all populations (99.9%), with values from 99.7% (ETS) to 1. Nucleotide diversity (π) was low for all populations (0.0421), with values from 0.0368 (JAN) to 0.0437 (ETS) (Table 1). Among the 119 individuals sequenced, 113 alleles were identified. In total, 88 variable sites, including 37 singletons and 51 parsimoniously informative sites, were observed. The most common allele was shared by 3 individuals from ECS, SCS and WTS populations. Four alleles were separately shared by 2 individuals. All others occurred in only 1 individual. Moreover, the best fit model selected by MEGA 5 was the T92+G+I model with shape parameter of gamma distribution (G)=0.37 and the proportion of invariable sites (I)=0.39; it was used for NJ reconstructions and AMOVA test. Neighbor-joining tree of 113 alleles showed shallow and no significant genealogical branches (Figure 2). The median-joining network for the 113 alleles appeared star-like and showed no notable allele clustering (Figure 3).

The F_{ST} value among all populations showed a non-significant amount of genetic variation ($F_{ST} = 0.0101$, $p = 0.1200$). All F_{ST} values between five populations were not significant except the pair of ECS-ETS (Table 2). Using F_{ST} to estimate gene flow, we obtain $N_m = 24.3954$, a relatively high level of gene flow. The N_m values between all pair-wise comparisons ranged from 8.8229 (ETS-JAN) to ∞ (Table 2). AMOVA of five populations yielded a Φ_{ST} value of 0.00886 ($p = 0.0966$), indicating no significant genealogical structure (Table 3). In addition, Fu's F_s statistical test was performed to determine departure from

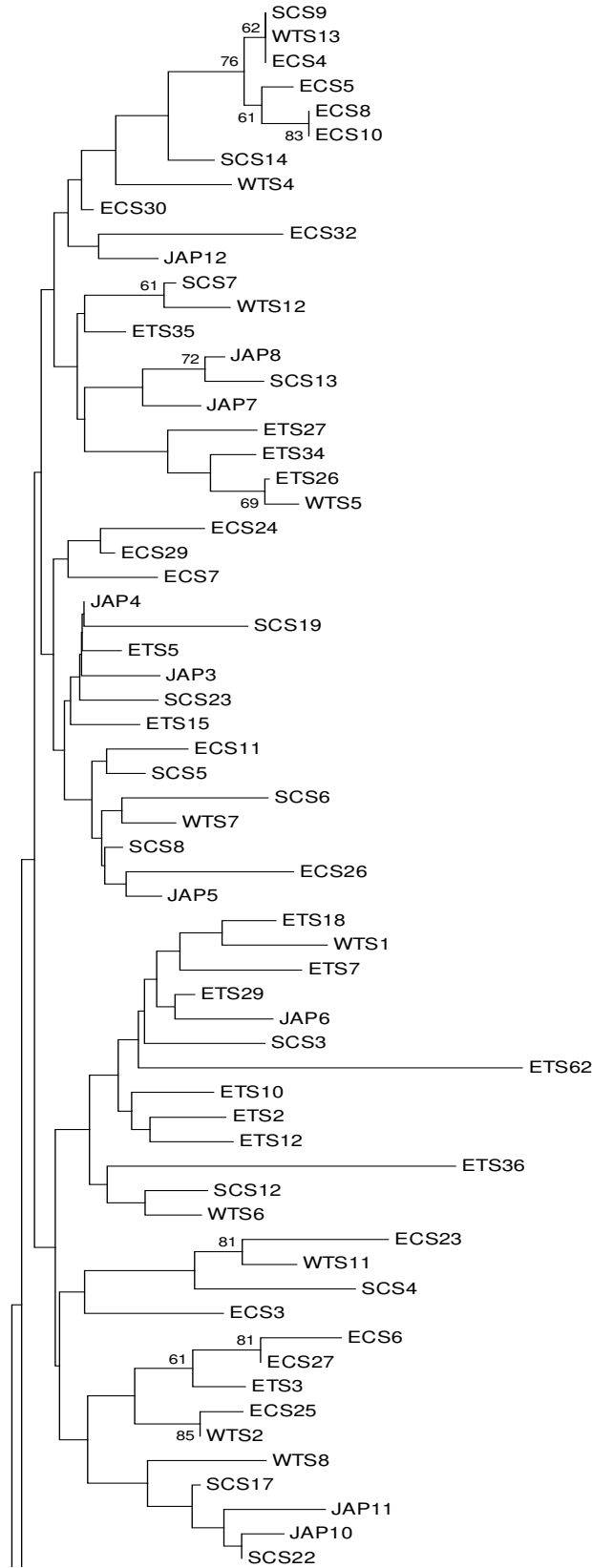


Figure 2. Neighbor-joining tree of alleles of kuruma shrimp (*Penaeus japonicus*) in the adjacent waters of Taiwan. The bootstrap values lower than 60% were not shown.

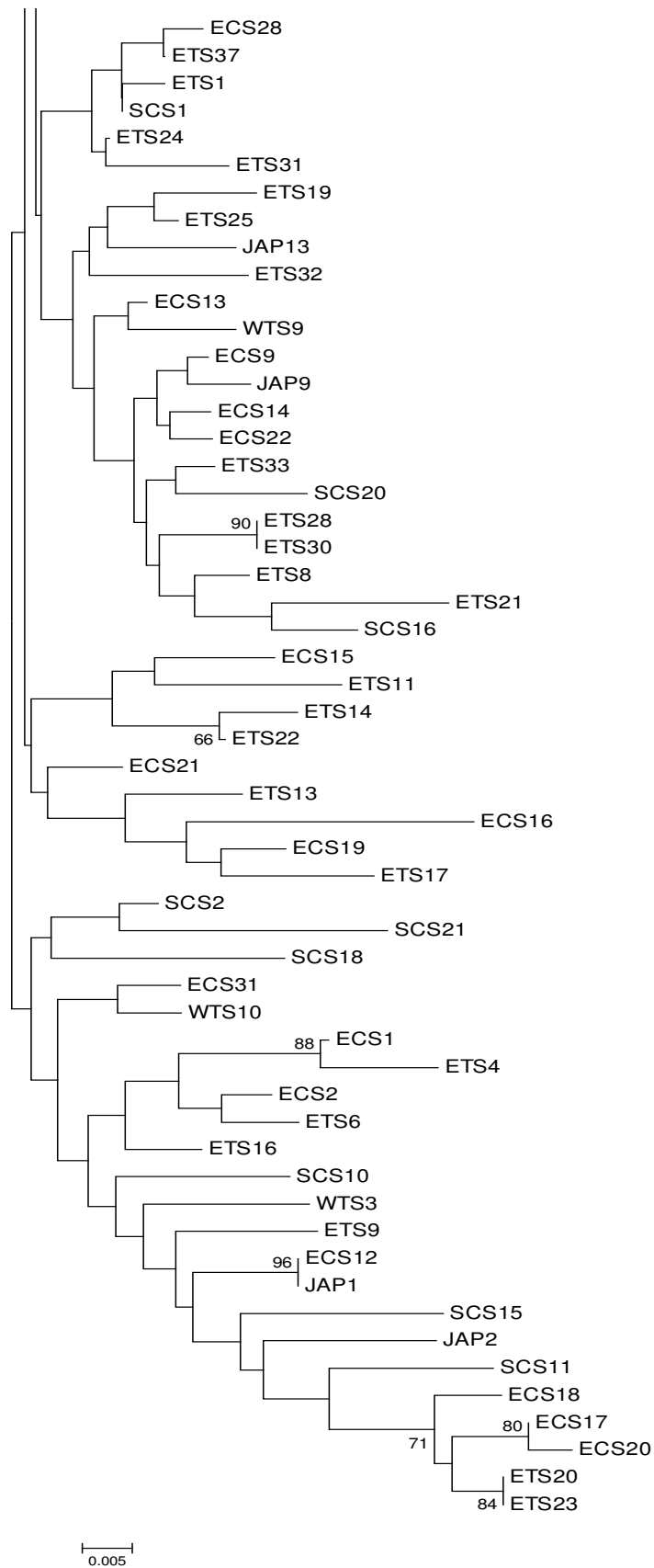


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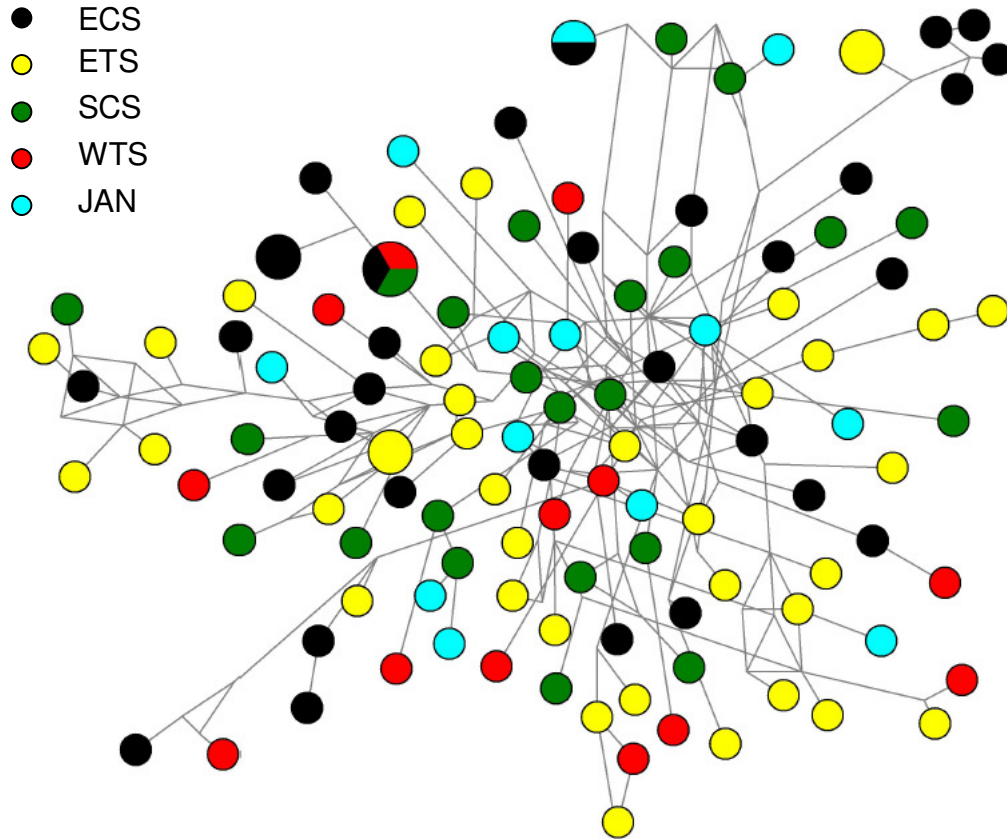


Figure 3. Alleles network of kuruma shrimp (*Penaeus japonicus*) in the adjacent waters of Taiwan. Different symbols represent different sampling areas. The sizes of circles are proportional to frequency. The most common allele was only shared by 3 individuals.

Table 2. F_{ST} (below) and N_m values (above) among populations of *Penaeus japonicus* in the adjacent waters of Taiwan.

Code	ECS	JAN	WTS	ETS	SCS
ECS	-	8.8229	10.4831	10.0305	211.1458
JAN	0.0276 ^{ns}	-	13.8553	10.9031	∞
WTS	0.0233 ^{ns}	0.0177 ^{ns}	-	19.1119	∞
ETS	0.0243 [*]	0.0224 ^{ns}	0.0129 ^{ns}	-	∞
SCS	0.0012 ^{ns}	0 ^{ns}	0 ^{ns}	0 ^{ns}	-

* $P < 0.05$, ^{ns} = Not significant ($P > 0.05$).

Table 3. Result of AMOVA for kuruma shrimp (*Penaeus japonicus*) populations in the adjacent waters of Taiwan.

Source of variation	df	Variance component	Φ -statistics	p
Among populations	4	0.04669	$\Phi_{ST} = 0.00886$	0.0966
Within populations	114	5.22268	-	-
Total	118	5.26937	-	-

neutrality. All F_u 's F_s values were significant for all sampling locations (Table 1). The model of population

expansion could not also be rejected when all populations were combined for F_u 's F_s statistical test. The mismatch

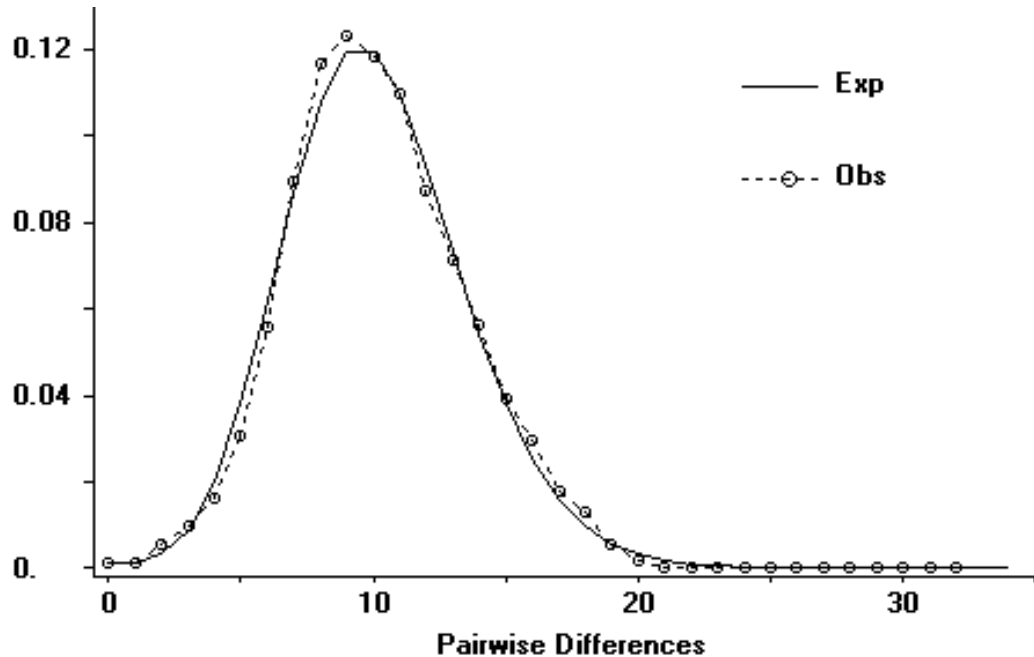


Figure 4. The observed pair-wise differences and the expected mismatch distributions under sudden expansion model of alleles for Kuruma shrimp (*Penaeus japonicus*) in the adjacent waters of Taiwan.

distribution yielded a unimodal distribution, not significantly different (as measure by the sum of squared deviation; $p > 0.05$) from that predicted by the growth expansion model (Figure 4).

DISCUSSION

Neighbor-joining tree and network of all alleles showed no significant genealogical structure, AMOVA did not detect significant differences at all hierarchical levels (Table 3), and most of conventional population F_{ST} statistics were not significant (Table 2), indicating that no significant population structure exists for kuruma shrimp in the adjacent waters of Taiwan. This result is consistent with findings of Tsoi et al. (2007) and shih et al. (2011). Using F_{ST} to estimate gene flow, we obtained $N_m = 24.3954$, a relatively high level of gene flow. In general, if the N_m value is greater than about 5, the gene flow is considered sufficient to maintain a relatively homogeneous gene pool (Slatkin, 1987). The N_m values between all pair-wise comparisons ranged from 8.8229 (ETS-JAN) to ∞ (Table 2), indicating high gene flow among populations reduced genetic heterogeneity.

The kuruma prawns migrate from inshore to offshore as they grow, but the migratory distance is limited. 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. The China Coastal Current brings water of low salinity southward along the Chinese coast. When the northeast winter monsoon winds prevail, the cold China

Coastal Current is driven to flow southward into the South China Sea through the Taiwan Strait. When the southwest summer monsoon winds prevail, the southern part of China Coastal Current is reversed and flows northward into the East China Sea. The spawning season of this species lasts from April until October (Yamada et al., 1986). Along the eastern coast of China, kuruma prawn larvae from the north of the East China Sea may be transported to the Taiwan Strait and the north of South China Sea by the China coastal current. This occurrence of kuruma prawn larvae mixed results in homogeneity among the populations from ECS, Taiwan Strait and SCS. The South China Sea warm water dominates the Taiwan Strait (Wang and Chern, 1989) and provides a northbound gene flow from the north of South China Sea to the Taiwan Strait and East China Sea.

Moreover, since the larvae of kuruma shrimps travel on these currents, the connectivity was therefore high among populations within the studied waters. This is also supported by the seasonal variations in the distribution and abundance of copepod (*Calanus sinicus*). The copepod is transported from population centers in the Yellow Sea and the East China Sea into the coastal waters of Taiwan and the northern part of the South China Sea by the China Coastal Current during the northeast monsoon period in winter (Hwang and Wong, 2005). Census of zooplankton communities showed that zooplankton could intrude into the Taiwan Strait from along the China coast by the China coastal current during winter and from the South China Sea by the South China Sea Surface Current during summer (Hsieh et al., 2005).

These results indicate that planktonic larvae in this region may travel great distances, yielding high connectivity between distant regions.

Furthermore, the significant genetic variation between ECS and ETS populations was found (Table 2). Multiple oceanic currents meeting in the east of Taiwan Strait may partly explain this significant genetic difference. During summer, the SCS warm current (see Figure 1) moves northwards from the SCS to ECS (Niino and Emery, 1961) and the Southwest Monsoon drives water masses from Singapore and Vietnam into the Taiwan Strait (Morton and Blackmore, 2001). The main branch of the warm Kuroshio Current flows northward along the east coasts of the Philippines and Taiwan (Chu, 1972). In winter, a small branch could be deflected by Northeast Monsoon to flow through the east of Taiwan Strait (Farris and Wimbush, 1996) but this up-flowing branch is stopped in the Penghu Island in the Taiwan Strait as the deeper waters beyond Penghu prevent this branch from flowing further north (Jan et al., 2002).

The intron sequences revealed high level of gene diversity (99.9%) and the low level of nucleotide diversity (0.0421) (Table 1). This h was higher than one found in Tsoi et al. (2007), but similar to the one found in Shih et al. (2011). Also, the π obtained herein was lower than 0.094 found in Tsoi et al. (2007), but higher than 0.0355 as reported by Shih et al. (2011). The high gene diversity ($h = 0.999$) and the lower nucleotide diversity ($\pi = 0.0421$) suggests the kuruma shrimp had undergone population expansion (Grant and Bowen, 1998), and this result is the same as the one obtained in Shih et al. (2011). In addition, the neutrality of intron sequence was rejected on the basis of Fu's F_s ($F_s = -24.3869$, $P < 0.01$). The statistic is most sensitive to factors such as bottlenecks or population expansion (Fu, 1997). Indeed, significant negative value of the statistic in this study indicated that the kuruma shrimp had experienced population expansion. The unimodal mismatch frequency distribution pattern accorded well the predicted distribution under a model of population expansion (Figure 4) (Rogers and Harpending, 1992). The neighbor-joining tree showed no significant genealogical branch (Figure 2), and this shallow phylogeny is consistent with a population expansion (Slatkin and Hudson, 1991). The median-joining network showed star-like (Figure 3), and this also suggested that the kuruma shrimp from the studied waters underwent recent population expansion.

Conclusion

Although, two additional populations from WTS and JAN were collected and intron sequences were analyzed, our results indicated that all variant I populations of kuruma shrimp in the ECS, Taiwan Strait and the SCS shared the same gene pool, which was consistent with the report of Shih et al. (2011). However, the gene diversity ($h = 0.999$) and nucleotide diversity ($\pi = 0.0421$) obtained herein from

intron sequence of 246 bp were higher than those obtained from mtDNA sequences (Shih et al., 2011), and this indicated that high variations of this intron sequence were also proper to elucidate the population structure and historical demography.

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