Chapter 3: Phylogeography and the genetic structure of the land-locked freshwater prawn *Macrobrachium asperulum* (Decapoda: Palaemonidae) on the continental island of Taiwan

INTRODUCTION

The geographical distribution and phylogenetic relationships of animal mtDNA variants reflect both contemporary gene flow and biogeographical events in the history of a species (Avise, 1994). A complex history of geological events and climate has shaped current phylogeographical patterns (Hewitt 1996, Avise 2000). The spatially disconnected nature of river systems confers unique phylogeographical constraints on freshwater fauna (Avise 2000). The dispersal of freshwater species between river drainages is normally extremely limited and is dependent on direct connections between drainage basins, such as drainage re-arrangements, short-term connections between drainages, and sea-level changes (Bilton et al. 2001). For this reason, the distribution of freshwater fauna is more likely to reflect historical events than will that of terrestrial species (Kotlik and Berrebi 2001). Historical phylogeographical analyses of freshwater species permit strong inferences regarding the biotic and geological evolution of a region (Lundberg 1993, Berminham and Martin 1998).

Taiwan is a subtropical island, located between the Eurasian continent and the Philippine Sea basin. This island is geologically young, estimated as 5–6 million years old (Sibuet and Hsu 1997 2004) since emerging from the Pacific Ocean resulted from the collision of the Phillippine Sea basin and continental Asia tectonic plates (Page and Suppe 1981). This continental island gradually acquired their floras and faunas from the Eurasian mainland (Shen 1987, Ota 1998). Most island species/populations thus have close phylogenetic links to their mainland relatives; indicate a pattern of colonization from the Asian continent eastward to Taiwan. The gradual

uplifting of the north-south extension of the Central Mountain Rang (CMR) as well as lesser mountain ranges and plateaus (i.e., the Miaoli Plateau of north Taiwan) have produced prominent watersheds and act as dispersal barriers for the freshwater fauna in Taiwan (Tzeng 1986). The cyclic separation and rejoining of Taiwan and the Asian mainland caused by the sea level changes due to glaciations provided opportunities for dispersal of taxa between the two areas (Shen 1987, Ota 1998). The distribution patterns of freshwater fauna on this island certainly have been influenced by geological events and climatic oscillations. Therefore, Taiwan provides an excellent natural laboratory to study biogeographical hypotheses of contemporary phylogeographical patterns.

Studies of phylogeographical patterns of freshwater fauna throughout Taiwan have mostly focused on fishes (Wang et al. 1999, Wang et al. 2000 2004, Lin et al. 2006), and their findings suggest the importance of geographical events and glaciations in shaping their distributions and population structures. Although work on fishes have now sufficiently comprehensive to provide a good sense of their phylogeographical patterns and provide an ideal framework for other freshwater life, it is unlikely that these results are representative of all freshwater organisms. A full elucidation of the phylogeographical patterns of Taiwan will require testing the hypothesis that inferences of historical phylogeography emerge from demonstrating that multiple, independent taxa with overlapping distributions share a common history (Berminham and Avise 1986, Berminham and Martin 1998). However, few studies have examined the patterns of variation in freshwater organisms with differing dispersal abilities and life histories.

The freshwater prawn, *Macrobrachium asperulum* (von Martens 1868), is a land-locked species distributed from S.E. Siberia to S. China and Taiwan inhabiting a wide range of freshwater bodies including middle to upstream reaches of rivers, hill streams, and impounded fresh waters (Holthuis 1950). The prawn is an abundant freshwater macroinvertebrate species, and has a pan-island distribution in Taiwan (Hwang and Yu 1983). *M. asperulum* completes its

life cycle only in freshwater without a pelagic larval stage (Sokita 1977), gene flow between different river systems is limited, and stronger genetic subdivisions among populations are expected (Shokita 1985). Therefore, relationships among lineages also reflect relationships between different areas, minimizing one of the main difficulties (i.e., dispersal) in reconstructing the past biogeographical development of an area (Bohlen and Råb 2001). Due to its wide distribution and limited capacity for dispersal, *M. asperulum* can be considered an interesting subject for use in evaluating historical events on population structures of freshwater species.

In this study, we used mtDNA fragment sequences of both the large subunit ribosomal RNA (16S rRNA) gene and the cytochrome oxidase subunit I (COI) gene to reveal the influence of the geological history and glaciations on the phylogeographical patterns and genetic structure of *M. asperulum* throughout the species' range in Taiwan.

MATERIALS AND METHODS

Sample collection

In total, 195 specimens from 20 populations were collected, belonging to 18 different river systems covering the species' range in Taiwan. In order to obtain a precise view of genetic diversity, samples from two geographically distant sites taken from the same drainages in the Tanshui River system (Peishi River and Tahan River) and Tachia River (Tachia River and Degi Reservoir) were collected to examine dispersal patterns within the rivers (Table 3, Fig. 6). The prawns were immediately preserved in 95% ethanol after been caught in fish traps and were then brought to the laboratory. Two more populations of *M. asperulum* were obtained from the Mulan River and Ming River of Fujian Province, southeastern China. *Macrobrachium sokitai* Fujino & Baba 1973, the closest relatives to *M. asperulum* (Shokita 1977 1996), from the southern Ryukyus and another land-locked species, *M. edentatum* Liang & Yan 1986, from China were used as outgroup for the analysis.

DNA extraction and amplification

Total genomic DNA was extracted from abdominal muscle preserved in 95% ethanol using a standard proteinase K, phenol-chloroform extraction and ethanol precipitation as described in Sambrook and Maniatis (1989). Two fragments of mitochondrial genes, the large subunit ribosomal RNA (16S rRNA) gene and the cytochrome oxidase subunit I (COI) gene, were amplified and sequenced. The 16S rRNA gene was amplified using the primers, 1471 (5'–CCTGTTTANCAAAAACAT–3'), and 1472 (5'–AGATAGAAACCAACCTGG–3') (Crandall and Fitzpatrick 1996). Amplification of the COI sequence was performed using primers HCO2198 (5'–TAAACTTCAGGGTGACCAAAAAATCA–3') and LCO1490 (5'–GGTCAACA AATCATAAAGATATTGG–3') (Folmer et al. 1994). Amplifications were performed using 100 ng μl^{-1} gDNA , 5 µl 10× PCR buffer, 2 µl of a 10 mM dNTP mix, 2 µM of each primer, 2 U of *Taq* DNA polymerase (Promega, USA), and water to a final volume of 50 µl. Amplification conditions involved an initial cycle of denaturation at 94 °C for 5 min, and then 35 cycles of denaturation at 94 °C for 1 min, annealing at 50 °C for 1 min, and extension at 72 °C for 1 min, followed by a final extension at 72 °C for 5 min. Negative controls were included in all PCR runs. PCR products were checked on 1.5% agarose gels, stained with ethidium bromide and purified using the Gel-M Gel Extraction System (Viogene, Taiwan) prior to the sequencing reaction. Double-stranded PCR products were sequenced in both directions using a standard cycle-sequencing protocol of the ABI Big-Dye Ready Reaction Kit (Perkin Elmer, USA), using the same primer pairs. Products of the cycle sequencing reactions were run on an Applied Biosystems automatic sequencer (model 377, Applied Biosystems Inc., Foster City, CA, USA).

Sequence alignment and phylogenetic analyses

All sequences were aligned using MegAlign (DNASTAR, LaserGene). The number of

variable and parsimony informative sites, base composition and transition/transversion (ti/tv) ratios, and inter- and intrapopulation genetic diversities quantified by indices of haplotype diversity and nucleotide diversity (Nei 1987) were calculated using DnaSP version 4.00 (Rozas et al. 2003). For the phylogenetic analysis, for both 16S rRNA and COI genes are in effect linked were combined and that is an appropriate way of dealing with random topological differences that are attributable to sampling error (Hipp et al. 2004). Phylogenetic reconstructions were performed using three different analytical approaches (Neighbour-joining, maximum parsimony, and maximum likelihood) implemented in PAUP* version 4.0b10 (Swofford 2002). Maximum parsimony (MP) (Camin and Sokal 1965) analysis was conducted assuming equal weightings for all characters. Phylogenetic relationships based on Neighbour-joining (NJ) (Saitou and Nei 1987) and maximum likelihood (ML) (Felsenstein 1981) analyses were estimated using an appropriate DNA substitution model calculated with Modeltest version 3.5 (Posada and Crandall 1998). Branch support for both the NJ and MP methods was assessed using bootstrap resampling (Felsenstein 1985) with 1000 replicates to evaluate the reliability of the inferred topologies. For the ML search was performed using the heuristic search option with random sequence additions and the tree bisection-reconnection (TBR) branch-swapping algorithm, bootstrap resampling was run with 100 replicates.

A minimum-spanning network was constructed with the aid of MINSPNET (Excoffier and Smouse 1994) and the ARLEQUIN (version 2.0) package (Schneider et al. 2000). Haplotype networks are generally better suited than phylogenetic trees for depicting relationships within species, as gene flow may lead to reticulate rather than hierarchical or treelike structures between populations. Also, in networks, internal nodes have a clear biological meaning as persistent ancestral haplotypes (Posada and Crandall 2001).

To determine whether the mitochondrial region was evolving according to neutral expectations, neutrality tests (Tajima 1989, Fu and Li 1993) were employed in DnaSP. The

population structure was assessed by analysis of molecular variance (AMOVA; Excoffier et al. 1992) with statistical significance determined by 1000 random permutation analyses in ARLEQUIN. To test for isolation by distance, a test was also carried out by plotting pairwise F_{ST} values against geographical distance (Slatkin 1993). To test the changes in historical population size if the population has undergone a sudden demographic expansion, we employed mismatch distribution analyses (Rogers and Harpending 1992) in DnaSP and ARLEQUIN. A clock-like evolution of the mtDNA sequences was tested with a log-likelihood ratio test (Felsenstein 1988), in PAUP*, using the best-fit model with and without the molecular clock enforced. Although there can be wide errors in estimating divergence times from sequence data (Avise 2000, Porter et al. 2005), we used the sequence divergence to give a crude estimation of a temporal framework of population divergences. The divergence rate of the combined 16S/COI data, calibrated from Jamaican crabs, is 1.17%~1.66% per Myr (Schubart et al. 1998) and 1.4% per Myr for snapping shrimp (Morrison et al. 2004). Assuming a molecular clock and applying this divergence rate (1.17%~1.66% per Myr) for molecular dating.

RESULTS

Genetic characteristics and variations

In total, 195 *M. asperulum* individuals from 20 populations in Taiwan were sequenced (Table 3). The size of the 16S rRNA fragment was 536 bp, with 35 variable and 34 parsimony informative sites. No gaps were detected, and the numbers of transitions outnumbered transversions in all comparisons by approximately 3.3, resulting in 50 unique haplotypes. The size of the COI fragment was 658 bp; there were 127 variable sites and 121 parsimony informative sites, resulting in 77 unique haplotypes. Numbers of transitions outnumbered transversions in all comparisons by 3.6 on average. Base frequencies in both mtDNA genes showed an AT bias (the G+C contents were 35.5% and 39.7% for 16S and COI, respectively).

No evidence was found for saturation of transitions or transversions in either 16S or COI (data not shown). No adjustment was needed when both 16S and COI sequences including the outgroup were aligned, but when the aligned COI sequences were confirmed by translating the DNA sequences into amino acids, no stop codon was revealed, make sure that not pseudogene were amplified (Williams and Knowlton 2001). The combined dataset was 1194 bp long, resulting in 100 unique haplotypes being identified. Populations from different drainage systems were characterized by unique haplotypes; no haplotype was shared by two rivers or by even two sampling sites in the Tachia River (Locality Code TG and DG, Table 3 and Fig. 6). The ranges of haplotype (h) and nucleotide (π) diversities within populations were remarkably wide, at 0%~0.95% and 0%~2.42%, respectively (Table 3). All unique sequences have been deposited in DDBJ database under accession numbers (accession nos.: AB250416~AB250552).

Phylogenetic analyses of mtDNA sequences

The phylogenetic relationships of *M. asperulum* populations was determined from the combined 16S and COI sequences. The best fit model selected by Modeltest was the TrN (Tamura and Nei 1993) +I+G model with base frequencies of A= 0.2965, C= 0.1896, G= 0.1926, and T= 0.3213), shape parameter of gamma distribution (G) = 0.6744, and the proportion of invariable sites (I) = 0.7628; it was used for both NJ and ML reconstructions. The phylogenetic analyses of the NJ tree (Fig. 7) resulted in a well-resolved phylogeny, but the MP and ML analyses were weaker.

A paraphyletic tree revealed two major groups included four distinct lineages belonging to three geographical regions (Fig. 6, 7). One group contains only lineage I, consists of populations collected from northern and northeastern Taiwan. The other group contains lineage I~IV; populations collected from west-central Taiwan were subdivided into two clades as lineages II and III, in which only specimens collected from the Tadu River were distributed in both lineages,

but no haplotypes were shared by the two lineages. The populations collected from southern Taiwan together comprised lineage IV, paraphyletic to lineage III. Two unexpected results were found. First, the haplotypes from the Ming River (at Fujian Province, southeastern China) was at the basal position as being the outgroup, but the haplotypes from the Mulan River, (also at Fujian Province, south of the Ming River, Fig. 6), were nested in lineage I paraphyletic with the Touchien and Chungkang Rivers, separately. Second, the four populations (MK, HKL, TP, and PN) from eastern Taiwan did not comprise a mono-lineage or mono-population cluster; most of the haplotypes nested in lineage III, two haplotypes (TP1 and TP4) from Tapu Pond nested in lineage II, and only one haplotype (PN5) from the Peinan River nested in lineage IV.

Relationships among 100 haplotypes in the minimum-spanning network (Fig. 8) supported the presence of the four discrete lineages identified by the phylogenetic reconstruction (Fig. 7). In lineage I, populations of the Shuang, Touchien, Chungkang, and Houlung Rivers formed a mono-cluster. Haplotypes of the Tahan River were separated into three different clusters. Only the Tashi and Peishi Rivers and two haplotypes from the Tahan River formed a mixed cluster. Haplotypes of the Mulan River of southeastern China were at the tip positions separately linked to the Touchien and Chungkang Rivers, as shown in the phylogenetic tree (Fig. 7). The steps, separating populations in lineage I, were no less than inter-lineage differences. Lineages II~IV, each form a mono-cluster separated to each other with 14~20 steps. Only the haplotypes from the Tadu River were found in lineages II and III also showed in minimum-spanning network belonged to two different clades. In lineage IV, haplotypes of the Tsengwen and Fengkang Rivers each formed a mono-cluster at the tip positions. Haplotypes of four populations from eastern Taiwan did not form a mono-cluster, as shown in the phylogenetic trees, but all were distributed at the tip positions in lineages II, III, and IV.

Genetic structuring among and within lineages

The hierarchical analysis of molecular variance (AMOVA), revealed a significant genetic structure across all hierarchical levels. 58.07% of the variation resulted from differences among regions, 29.65% of the variation occurred among populations within regions, and only 12.68% of the variation was within populations (Table 4). Historical population demography was tested by the pairwise mismatch distribution. Lineages III and IV, excluding the eastern haplotypes, showed a unimodal distribution pattern but without significant *p* values for the "test of goodness-of-fit". The mismatch profile of lineages I and II were shown as multimodal (Fig. 10).

Population differentiation value (F_{ST}) among populations were high and with significant *p* values (Table 5), except for the populations of the Kaoping and Linpien Rivers (lineage IV) which were genetically most similar with a low F_{ST} (F_{ST} = 0.0998, *p* value not significant). No correlation (R^2= 0.030) existed between F_{ST} values and geographical distance (km) for all populations in Taiwan (Fig. 9), but correlations of R^2= 0.467, 0.471, and 0.380 were shown, with significant *p* values in F-test, for the northern, west-central, and southern populations, respectively.

Statistical tests of neutrality

Statistical tests of neutrality were performed to determine departures from neutrality of the mtDNA data. No significant deviations from those expected under neutrality were identified when all haplotypes were analyzed together (Tajima; D = 0.97, $p > 0.10$; Fu and Li's D* =1.31, $p > 0.10$; $F^* = 1.37$, $p > 0.10$), or when four phylogenetic lineages (Fig. 7) were analyzed separately. Most of the populations, except for two, were consistent with neutral evolution. The exceptions were the Tadu River (Tajima's D = 2.09, $p < 0.05$; Fu and Li's D* = 1.28, $p > 0.10$; $F^* = 1.70$, $p < 0.05$) and Tapu Pond (Tajima D = –0.51942, $p < 0.001$; Fu and Li's D* = –1.55, $p < 0.02$; $F^* = –1.70$, $p < 0.02$) of west-central and eastern Taiwan, respectively.

Population divergence

A log-likelihood ratio test detected the evolutionary rate variations were evolving according to a clock-like model (χ^2 = 97.51, d.f. = 98, p >0.1). The population history can be represented by a gene tree with each node indicating a coalescent event (Slatkin and Hudson 1991). Sequence divergences between the four lineages ranged from 2.14% ± 0.35% to 3.15% ± 0.46%. Assuming a molecular clock and applying the divergence rate (1.17%~1.66% per Myr), coalescent events between populations of China (Ming River) and Taiwan, dated back to between the late Pliocene and early Pleistocene (2.63~1.78 Myr). Lineages II and III, distributed in west-central Taiwan, had the smallest divergence of 0.98~0.69 Myr. In addition, the divergence range between population of northern Taiwan and the Mulan River was 0.42~0.29 Myr, indicating more-recent isolation of populations between Taiwan and China.

DISCUSSION

Population genetic structure and differentiation

The hierarchical analysis of molecular variance (AMOVA) (Table 4) revealed a significant genetic structure across all hierarchical levels existed among the 20 populations examined (Table 3), indicating that a high degree of phylogeographical related genetic structure within *Macrobrachium asperulum* in Taiwan was evident. This may be associated with its abundance in each locality and its widespread in Taiwan (Hwang and Yu 1983) as revealed in freshwater fishes (Wang et al. 1999, Wang et al. 2000 2004).

The high diversity and significant genetic differentiation (F_{ST} values) which showed populations are characterized by unique haplotypes, indicating that rare contemporary gene flow occurs among populations even in the same river system (the Peishi and Tahan Rivers belonging to the Tanshui River system) or within river (two sites, DG and TG, in the Tachia River) (Table

3 and Fig. 6). This pattern of population structuring and genetic differentiation is incongruous to the three phylogenetically distant freshwater fishes (Wang et al. 1999, Wang et al. 2000 2004), which showed lower genetic differentiation among population within regions. Some rarely occurs floods at lower elevations, river capture between drainages by some parts of head waters or by river confluence of downstream courses after sea level lowering, may create opportunities for freshwater fishes could disperse across drainage systems, that resulted in the lower genetic differentiation among population within regions (Wang et al. 1999, Wang et al. 2000 2004). The high genetic structure of within-catchment or within-stream populations infers that the freshwater prawn with land-locked life cycle has limited dispersal ability than freshwater fishes have studied. Consequently, the phylogeographic pattern of *M. asperulum* is more reliable in reconstructing the past biogeographical development of Taiwan (Bohlen and Råb 2001).

Populations from different regions are characterized by unique haplotypes, which reflect the effects of geographical isolation (Avise, 1994). Crandall and Templeton (1993) assumed that the most common and widespread haplotype is ancestral. The absence of mtDNA haplotypes shared among rivers may indicate that the observed genetic variation did not necessarily result only from the retention of ancestral polymorphism. It could have arisen through mutations and complete lineage sorting once the populations had become isolated from one another, which was also observed in the introduced populations of eastern Taiwan. However, genealogical mixing of mtDNA from different populations (Fig. 8) was expected to occur even without migration if the ancestral population was polymorphic (Nei 1987). The degree of differentiation among regions is obviously strongly associated with geographical barriers. Within regions, population differentiation is correlated with geographical distances (Fig. 9) and is consistent with the model of “isolation by distance” (Wright 1943, Kimura and Weiss 1964) as suggested in freshwater fishes (Wang et al. 2000 2004). In contrast to the high nucleotide diversity in most populations, some populations, such as those in the Shuang, Chungkang, Houlung, and Fengkang Rivers,

having highly divergent genetic structures (F_{ST} values) among localities and low levels of genetic variability within populations suggests that populations have experienced bottleneck event (Falconer and Mackay 1996). The bottleneck event caused by glaciation is less revealed in the freshwater fishes (Wang et al. 2000), but indicated more happen by the founder effect (Wang et al. 1999, Wang et al. 2000 2004). This result indicated that at glacial period, most population of freshwater prawn was abundant in each locality as it was widespread in Taiwan. The freshwater fishes have retracted to the refugia (0.055 Myr for *Zacco pachycephalus*, Wang et al. 1999), or incursive colonization more recently (0.1–1 Myr for *Acrossocheilus paradoxus*, Wang et al. 2000, 0.085–0.285 for *Varicorhinus barbatulus*, Wang et al. 2004).

Phylogeography and demographic history

The phylogenetic reconstructions present a paraphyletic tree by two major groups (Fig. 7), containing four lineages with significant genetic breaks belonging to three geographical regions (Fig. 6). These two major groups are separated by the Miaoli Plateau (located between the Houlung and Taan Rivers). The phylogeographical patterns of freshwater fishes usually, according to previous studies, reveal a close relatively between populations of the west-central and northern regions (Tzeng 1986, Wang et al. 1999). However, in this study, the phylogenetic reconstruction (Fig. 7) indicates that freshwater prawn populations of the west-central and southern regions are closely related as indicated by two freshwater fishes (Wang et al. 2000 2004). Presumably, it was due to different dispersal time and routs, which different species migrated from mainland China to Taiwan.

The paraphyletic phylogeny of *M. asperulum* populations suggests that it resulted from two incursive colonization scenarios from China (Emerson 2002). One route dispersed to northern Taiwan and formed lineage I, while the other dispersal route was through the west-central region, as the lineage II, then northward and southward dispersal, followed by isolation, lineage sorting,

and allopatric fragmentation into lineages III, and IV, respectively (Avise 2000, Juan et al. 2000). Historical population demography (Fig. 9) indicated that lineages I and II may be presented the ancestral lineages in Taiwan. The allopatric fragmentation was also indicated by the high level of genetic differentiation among lineages and showed in the minimum-spanning network (Fig. 8), four lineages being separated by a long branch length with missing intermediates, is often interpreted as evidence of a past fragmentation event (Avise 1994, Templeton 2004). Fragmentation is predicted to impose strict geographical limits on how widespread the clades can become and to result in the accumulation of mutational differences among clades found in different isolates. Many of the predictions used to detect fragmentation simply follow from the definition of allopatric fragmentation as a nonrecurrent historical event involving subpopulations that are completely or nearly completely non-overlapping geographically with unique haplotype(s) (Templeton et al. 1995).

The paraphyly of the phylogenetic analyses of populations with continental stocks has been highlighted by allozyme studies of small mammals (Yu 1995) and the rice frog (*Rana limnocharis,* Toda et al. 1998). The population of the Mulan River nested in lineage I of north populations and separately linked to haplotypes of the Touchien and Chungkang Rivers, suggests a more-recent back dispersal from Taiwan to China during the Pleistocene (Emerson 2002). The coalescent theory predicts that haplotypes on the tips of the tree are highly likely to be younger than the interior clades to which the tips are connected within a population or set of populations well connected by gene flow (Castelloe and Templeton 1994). The back dispersal from Taiwan to China also indicated by the *Pinus luchuensis* complex (Chiang et al. 2006).

The two incursive colonization scenarios correlated well with current concepts regarding the tectonic evolution of Taiwan and subsequent palaeoclimatic oscillations of glaciations. The present rise in elevation of Taiwan began approximately 5~6 Myr ago (Sibuet and Hsu 1997 2004) and is a result of the collision of the Philippine Sea basin and continental Asian tectonic

plates (Page and Suppe 1981). This was accompanied by a sea level rise and the subsequent isolation of Taiwan from the Asian mainland (Liu and Ding 1984). The connection with the mainland is believed to have been restored periodically throughout the glaciations in the Pliocene and Pleistocene (Huang, 1984). According to the geographical history, the two incursive colonizations from the Asian mainland may have occurred from the late Pliocene to the early Pleistocene (between 2.63~1.78 Myr) after the main orogenic events of Central Mountain Range (CMR) (Sibuet and Hsu 2004). CMR represented a geographic barrier to the west-east dispersal of *M. asperulum* and restricted to the western part of Taiwan, that revealed in freshwater fishes (Wang et al. 1999, Wang et al. 2000 2004, Lin et al. 2006), freshwater crab (Shih et al. 2004 2006) and plants (Shen 1997). The Miaoli Plateau formed a potential geographical barrier, preventing gene flow between the two colonization events (lineages I and II) (Tzeng, 1986).

Historical population demographic analyses by neutrality tests in most populations examined, consistent with neutral evolution and a lack of either recent population bottlenecks or rapid growth. Lineage I and II showed a multimodal mismatch profile (Fig. 10) indicated the strong geographical structure and populations at equilibrium (Rogers and Harpending 1992) maybe present the ancestral lineages in Taiwan. A bottleneck is only likely to be detected if it is very recent and if the sample size is large (Simonsen et al. 1995). Only two populations are indicated to be demographically unstable. The population of the Tadu River of west-central Taiwan, with significantly positive statistical values, reflects population subdivision (Simonsen et al. 1995), while haplotypes of the Tadu River are positioned into two separate lineages (Fig. 7, 8). The Tadu River may be a hybrid zone for populations of two lineages which survived the ice ages in these isolated places but do not exchange genes, and may well be subject to different selection; so they are diverging genetically. When two such diverging genomes expand from different refugia, they may form hybrid zones where they make contact (Hewitt 1999). The population of Tapu Pond of eastern Taiwan, with significantly negative statistical values, reflects

a scenario of recent population expansion (Tajima 1989), will be discussed below.

The introduced populations of east Taiwan

The CMR, composed of more than 100 peaks above 3000 m in elevation, is an obvious geographical barrier for dispersal of populations from west to east and vice versa for freshwater species In Taiwan (Tzeng 1986, Wang et al. 1999, Wang et al. 2000 2004, Shih 2004 2006, Lin et al. 2006). In present study, the haplotypes of the four eastern populations did not form a monophyletic group but instead nested in west-central and southern populations (Fig. 7, 8). These tip haplotypes may represent recently occurring polymorphisms (Golding 1987). The incongruous of the phylogeographic analysis and genetic divergences suggest that the eastern populations are not considered as naturally distributed populations, that likely to have low contemporary gene flow across the CMR and that has permitted lineage sorting, random drift, and generating geographic genetic divergence among populations. It would be rather then by human introduced (Golding 1987, Facon et al. 2003, Kolbe et al. 2004, Mamuris et al. 2005).

The human translocations of freshwater organisms also could be found in freshwater fishes (Wang et al. 1999, Wang et al. 2004), freshwater crab (Shih et al. 2004) and bamboo viper (Creer et al. 2001) in Taiwan. The introduction of *M. asperulum* could be by the fishing activities or commercial trades which *M. asperulum* as food. The genetic variation of eastern populations are not reduced by genetic drift and founder effects, because genetic analyses indicate that multiple introductions have occurred in eastern Taiwan primarily from west-central Taiwan (Fig. 8). The transformation of among-population variations in native ranges to within-population variations in eastern populations by blending genetic variations from different source populations has produced populations that contain substantially more, not less, genetic variation than native populations (Facon et al. 2003, Kolbe et al. 2004, Mamuris et al. 2005). The high genetic diversity in the Peinan River suggest a rapid expansion following multiple introductions, and

genetic variations arising through mutations and complete lineage sorting over a relatively small number of generations. Artificial introduction thus provides a quintessential model for rapid evolution. The impacts of the introduced prawns on the freshwater ecology of eastern Taiwan need to be further studied.