

Invasion of the Red Swamp Crayfish (*Procambarus clarkii*) in China: Genetic Analysis of the Invasion and the Impacts Evaluation

Mitigating Negative Environmental Impacts/Study/09MNE01UM

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ABSTRACT

This research was conducted to study the population genetic structure of red swamp crayfish invasion in China, to identify their invasion centers, to explore if populations derived from single or multiple introduction events, and to investigate the impacts of red swamp crayfish invasion in China.

A total of 1776 crayfish sampled from 37 sites (35 sites from China, one site from America and one site from Japan) were obtained to study population genetic structure and dispersal mechanisms of red swamp crayfish. Twelve microsatellites were used in this research. The allele number ranged from three to twenty seven. The overall observed heterozygosity (H_o) was 0.6723, the overall gene diversity (H_e) was 0.7913, and the overall polymorphism information content (PI_C) was 0.7551. The genetic distance (0.145) between population Xuyi-wild (XYw) and population Xuyi-culture (XYc) was lowest, while the genetic distance (0.999) between population Zhongxian (ZX) and population Japan (Jap) was highest. The Nei's genetic identity (0.865) between XYw and XYc was highest while the Nei's genetic identity (0.368) between ZX and Jap was lowest. The NJ tree consisted of two major branches, one branch included the red swamp crayfish populations in America and Japan, the other branch included all populations collected in China. AMOVA revealed that 91.26% of genetic variation could be explained by the variation within populations.

Six haplotypes were found in the partial COI sequence of *P. clarkii* (145 individuals) with a length of 637bp. The haplotype diversity of the partial COI sequence was 0.419, the variance was 0.00188, and the standard deviation was 0.043. The nucleotide composition of all the haplotypes was 40.6% T, 12.7% C, 26.7% A, and 20.0% G. The nucleotide diversity was 0.267%. Twelve parsimony informative sites and three singleton variable sites were detected in the COI sequences. Three haplotypes were found in the 16SrRNA sequence of *P. clarkii* (142 individuals) with a length of 293bp. The haplotype diversity of the 16SrRNA sequence was 0.431, the variance of haplotype diversity was 0.00158, and the standard deviation of haplotype diversity was 0.040. The nucleotide composition of all the haplotypes was 36.5% T, 9.9% C, 34.4% A, and 19.2% G. The nucleotide diversity was 0.172%. Two parsimony informative sites and one base deletion were detected in the 16SrRNA sequences. The positive F_s values were obtained from analysis of 16SrRNA (0.953 and $P=0.269$) and COI (1.859 and $P=0.124$), which indicated that all red swamp crayfish populations did not experience significant population expansion.

Based on microsatellites and partial mitochondrial DNA data analysis of 37 populations, we speculate that the suburbs of Nanjing, Jiangsu province was the invasion center in China, and crayfish dispersed into Jiangsu province along the Changjiang river. Meanwhile, human-mediated dispersal might have played a role in the expansion and genetic differentiation of this species. Red swamp populations in China probably derived from a single introduction of a large number of individuals from different populations in Japan.

INTRODUCTION

Because of the increased transfer of non-indigenous species (NIS) to new ecosystems and a growing awareness of the potential impacts on recipient ecosystems, studies on biological invasions have increased dramatically over the past 20 years. Recently, much attention has been paid on some aquatic species (LeBlanc et al., 2007; Valentine et al., 2007). The red swamp crayfish (*Procambarus clarkii*), which is native to South central USA and Northeastern Mexico, is one of the most famous invasive species in the world (Huner, 1988; Zhu and Yue, 2008).

Successful invasion requires that a NIS pass through a series of filtering stages that include transport, release, establishment, and, in many cases, dispersal. The red swamp crayfish lacks efficient dispersal capacities such as easily transported resting eggs or highly mobile larval stages. Compared to plants or invertebrate species such as insects or mollusks, the ability of natural dispersal of this species is relatively weak (Geiger et al., 2005). However, anthropogenic activities have played a crucial role in translocation of the red swamp crayfish, and high reproductive output, short development time and flexible feeding habits provide this species a very strong adaptability to various ecosystems.

The red swamp crayfish has become a successful worldwide invader, and in the Mediterranean region it provides a well documented example of the quick expansion of an alien species (Adao and Marques, 1993; Correia and Costa, 1994; Geiger et al., 2005). In 1973, it was introduced to two aquaculture installations located in southwestern Spain (Habsburgo-Lorena, 1983). It has become a widespread species throughout the Mediterranean region and Europe after only for three decades (Adao and Marques, 1993; Correia and Costa, 1994; Stucki and Staub, 1999; Arrignon et al., 1999).

As in Mediterranean region, the invasion status of red swamp crayfish in China is also serious. This crayfish was introduced to Nanjing, China from Japan in 1930s. It is now found in almost all waters including lakes, rivers and even paddyfields in most provinces of China: from Liaoning (northern China) to Guangdong (southern China) and from Taiwan (eastern China) to Sichuan (western China) (Li et al., 2005; Liu et al., 2008).

While less attention is paid to the invasion of this crayfish in China, much effort focuses on technologies of reproduction and breeding (Gong et al., 2008). Crayfish aquaculture has developed rapidly and this species become one of the most important aquatic products in China (Bi et al., 2008). The invasion of red swamp crayfish is a big threat to native crayfish and macrophytes, due to its predatory and grazing activity (Geiger et al., 2005). Red swamp crayfish is an important pest of wet-seeded rice fields (*Oryza sativa*) (Anastacio et al., 2005). As a vector of many diseases, it has a severe impact on preservation and reintroduction of native crayfish (Diéguez-Uribeondo et al., 1995). In addition, it accumulates heavy metals and other pollutants in its organs and body tissues and transmits them to higher trophic levels (Geiger et al., 2005).

The purpose of this study is to evaluate change in population structure of the red swamp crayfish after successful invasion and fast dispersal in China, and to determine impacts of the invasion.

MATERIALS AND METHODS

This study mainly consisted of two components. The first was to study population genetic structure of red swamp crayfish invasion in China, to identify invasion centers and dispersal patterns and to explore whether populations were derived from single or multiple introduction events. The second was to survey impacts of the red swamp crayfish invasion.

Population genetic analysis of red swamp crayfish invasion in China and its ways of dispersal
Our sampling sites covered the major distribution range of red swamp crayfish in China, including one to three sites from each province. Totally, 35 sampling sites were selected from China and one site each from America and Japan (Table 1).

Muscle cuts of 48 individuals were sampled at each site. Samples were stored in the 100% ethanol for DNA extraction. DNA was isolated using ammonium acetate which is now routinely used in our laboratory for preparing DNA. DNA was diluted to a final concentration of 100ng/μL and arrayed on 96-well PCR plates for genotyping of microsatellites.

Twelve microsatellites were used to amplify the DNA solutions. One microsatellite (PCL24) cloned from a partial genomic DNA library enriched for CA- and GA-microsatellites was used; details of genomic library construction and microsatellites cloning were described in Zhu and Yue (2008). An additional eleven microsatellites (PcLG-03, PCLG-04, PcLG-07, PcLG-09, PCLG-10, PCLG-13, PCLG-15, PCLG-17, PcLG-29, PcLG-32, PcLG-48) were selected from Belfiore and May (2000). We performed PCR on an Eppendorf Mastercycler gradient machine in 10 μ L reaction volumes containing 50 ng DNA, one PCR buffer (TaKaRa) with 1.5 mM MgCl₂, 250 nM of each primer, 50 μ M of each dNTP and one unit of DNA polymerase (TaKaRa).

Following amplification, each PCR product was mixed with 1 μ L of sequencing dye. Four microliters of each PCR product were electrophoresed on polyacrylamide gel 8% acrylamide, 650 μ L of 10% ammonium persulfate, and 65 μ L of N,N,N₀,N₀-tetramethylethylenediamine (TEMED). Gel fixation and silver staining were performed following the method described by Sambrook et al. (1989). Sizes of alleles were determined according to a marker of puc18 DNA/*MspI* (TIANGEN).

The number of alleles (*A*), the observed heterozygosity (*H_o*) and the expected heterozygosity (*H_e*) were determined using the software GENEPOP 4.05.2. The Hardy-Weinberg departure value (*D*) was obtained using the equation, $D = (H_o - H_e) / H_e$. The polymorphism information content (*PIC*) was estimated according to the following formula (Botstein et al., 1980):

$$PIC = 1 - \sum_{i=1}^m p_i^2 - \sum_{i=1}^{m-1} \sum_{j=i+1}^m 2 p_i^2 p_j^2$$

where p_i is the gene frequency of the *i*th allele, p_j is the gene frequency of the *j*th allele, and *m* is the allele number.

ARLEQUIN 3.5 was applied for assignments of individuals to populations using a log-likelihood method (Paetkau et al., 1997) and analysis of molecular variance (AMOVA). Ratios of the variance components could then be used to define population structure. Significance was tested by comparing observed values to a null distribution generated by permutation using 10,000 replicates. The bottleneck hypothesis was tested using software BOTTLENECK 1.2.02 (Cornuet and Luikart, 1996) under the infinite allele model (IAM), stepwise mutation model (SMM) and two-phased model of mutation (TPM). Nei's standard genetic distances D_A between all pairs of individuals were estimated to show the phylogenetic relationship among individuals (Nei, 1978). Based on the distance matrix, the software MEGA 5.05 was employed to construct NJ dendrogram.

The genomic DNA of 15 sampling sites (Table 2 and 3) was isolated using ammonium acetate method. The COI gene fragments were amplified using primers LCO 1490 and HCO 2198 from Folmer et al. (1994) under thermocycling conditions. The 16SrRNA gene fragments were amplified with the oligonucleotide primers 1471 and 1472 (Crandall et al., 1995), again under thermocycling conditions. PCR products were directly sent to Invitrogen Biotech (Shanghai) Co., Ltd and Sangon Biotech (Shanghai) Co., Ltd. to sequence. The length of the amplified COI and 16SrRNA fragments were 637 bp and 293bp respectively and could thus be unambiguously aligned by hand.

The variation sites, parsimony informative sites, number of haplotypes, and nucleotide diversity were determined by using the software DnaSP 5.10. The Neutral testing (Fu's *F_s* test) was also considered by DnaSP 5.10. ARLEQUIN v3.5.1.2 was applied for analysis of molecular variance (AMOVA). The genetic distances between populations were calculated by MEGA 5.05 and a NJ dendrogram was constructed.

Survey of impacts of the red swamp crayfish invasion in China

The survey on crayfish impacts was conducted in Sichuan, Jiangsu, Zhejiang, Anhui, Hubei, Hunan and Jiangxi provinces. A questionnaire was used to estimate the effects on native crayfish and macrophytes, diseases, accumulation of heavy metals, areas of destroyed rice fields and destruction of

some water conservation projects caused by the red swamp crayfish. In addition, the internet was another channel to get information about the negative impacts of red swamp crayfish.

RESULTS

Population genetic analysis

Alleles were detected for twelve microsatellite loci over 37 populations (1776 individuals), ranging from a low of three alleles to a high of 27 (Appendix Table 1). The mean frequency of private alleles was 0.03096. The overall observed heterozygosity (H_o) was 0.6723, the overall gene diversity (H_e) was 0.7913, and the overall polymorphism information content (PIC) was 0.7551. The American and Japanese populations displayed similar and highest genetic diversity ($N_a = 14.5833$, $N_e = 8.8149$, $H_e = 0.8799$ and $PIC = 0.8579$ in American; $N_a = 14.3333$, $N_e = 9.3691$, $H_e = 0.8873$ and $PIC = 0.8667$ in Japanese) among the 37 populations, while ZX population showed the lowest relatively ($N_a = 6.4167$, $N_e = 3.6813$, $H_e = 0.7002$ and $PIC = 0.6553$). Overall, all the red swamp crayfish populations evaluated in China had high genetic diversity ($N_a = 6.4167$ -11.7500, $N_e = 3.6813$ -6.4795, $H_e = 0.7002$ -0.8214 and $PIC = 0.6553$ -0.7903) (Table 3). Most loci showed significant deficiency of heterozygosity in almost all populations (Appendix Table 1).

Genetic distance (0.145) between populations XYw and XYc was the lowest, while genetic distance (0.999) between the populations ZX and Japan was the highest (Table 4). The Nei's genetic identity (0.865) between the populations XYw and XYc was highest while the Nei's genetic identity (0.368) between the populations ZX and Jap was lowest (Table 4). The gene flow among populations ranged from a low of 1.284 between DY and PYL population to a high of 10.595 between between XYw and XYc population (Table 5). It can be seen that the gene interchange between XYw and XYc population occurred very frequently and far greater than the gene flow among other populations (range from 1.284 to 6.968). The genetic diversity (F_{st}) in 37 populations of *P. clarkii* ranged from a low of 0.023 between XYw and XYc population to a high of 0.157 between DY and YJ, NX population (Table 5).

The Nei's standard genetic distances for each pair of red swamp crayfish populations (Table 4) was used to construct a neighbor-joining tree based on genetic distances was (Figure 1). The NJ tree consisted of two major branches, which were congruent with regional groupings; one branch included populations collected in America and Japan, the other branch included all red swamp crayfish populations collected in China. The sub-branch, including populations from Baguazhou township, Xiaguan District, Guangfengwei section of Changjiang river, Nantong, and Ningbo, was nearest to the foreign branch. The other sub-branches in the China were not congruent with regional groupings.

AMOVA revealed that 91.26% of genetic variation could be explained by the variation within populations, while the remaining 8.74% came from variation among populations (Table 6).

IAM, TPM, and SMM were applied to test if microsatellites displayed a departure from the mutation-drift equilibrium. Under IAM, the Sign tests revealed that 32 sites may have experienced a bottleneck ($P < 0.05$), while Wilcoxon's signed rank tests detected that all 37 populations may have experienced a recent bottleneck ($P < 0.05$) (Table 9). Under the TPM, the Sign tests revealed that the JX, WXb, XT, DTL, DTLs, and Jap may have experienced a recent bottleneck whereas Wilcoxon's signed rank tests showed 22 populations experienced a bottleneck. Under the SMM, the Sign tests revealed that the population SH, JX, HHL, and CHL may have experienced a recent bottleneck, while Wilcoxon's signed rank tests detected that only the population JX may have experienced a recent bottleneck.

Six haplotypes of mitochondrial COI were found in the partial COI sequence of *P. clarkii*, and the length was 637bp. The haplotype diversity of the partial COI sequence (H_d) was 0.419, variance of haplotype diversity was 0.00188, and standard deviation of haplotype diversity was 0.043. The nucleotide composition of all haplotypes was 40.6% T, 12.7% C, 26.7% A, and 20.0% G. The nucleotide diversity was 0.267%. There were some base substitutions but no base insertions or deletions found. Twelve parsimony informative sites and three singleton variable sites were detected in the COI sequences (Table 8). Under the Kimura two-parameter model, the overall mean pairwise genetic distance of the six haplotypes was 0.011.

Six haplotypes were found in 145 individuals. The frequency of haplotype 1 was highest (107 individuals), mainly distributed among populations collected in China and Japan. The haplotypes 3, 4 and 5 were only detected in populations collected in America (Table 8). The genetic diversity parameters of the COI gene in different populations of *P. clarkii* (Table 9) demonstrated that the highest haplotype diversity was in the SH population ($Hd= 0.571$), the highest nucleotide diversity was the Ame population ($Pi= 0.00614$), and the lowest genetic diversity in the DTL, DTLs, LZL, PYL, CJr, XG, XYw and QJ populations ($Pi= 0.0000$, $Hd= 0.000$).

AMOVA revealed that 52.54% of genetic variation could be explained by variation within populations, whereas 47.46% came from variation among populations (Table 12). Genetic distance (0.005–0.006) between the American population and populations in China was high, and genetic distance varied from 0.000 to 0.006 among all populations. Genetic diversity (Fst) in 15 populations of *P. clarkii* ranged from a low of -0.166 between SH and DY to a high of 0.721 between Ame and five populations in China (DTL, LZL, PYL, XG, XYw and QJ) (Table 13). Based on the genetic distances matrix, the Neighbor-joining tree was constructed among 145 individuals, and showed that the genetic distance between populations collected in America and China was highest (Figure 2).

The NJ dendrogram of six haplotypes detected in 15 populations of *P. clarkii* was constructed which showed that one branch included Hap 1, 2, 3 and 6, and the other one included Hap 4 and 5 (Figure 4).

The detection of population expansion was performed using Fu and Li 's Test. The positive Fs values were 1.859 ($P=0.124$) which indicated that the red swamp crayfish populations did not experience significant population expansion. Moreover, the wave curve was obtained by population size changes analysis showed that the red swamp crayfish populations did not experience significant population expansion as well (Figure 4).

Three haplotypes were found in the 16SrRNA sequence of *P. clarkii*, which had a length of 293bp. The haplotype diversity of the 16SrRNA sequence (Hd) was 0.431, the variance of haplotype diversity was 0.00158, and the standard deviation of haplotype diversity was 0.040. The nucleotide composition of all the haplotypes was 36.5% T, 9.9% C, 34.4% A, and 19.2% G. The nucleotide diversity was 0.172%. Two parsimony informative sites and one base deletion were detected in the 16SrRNA sequences (Table 12). Under the Kimura two-parameter model, the overall mean pairwise genetic distance of the six haplotypes was 0.005. The frequency of haplotype 1 was highest (102 individuals), which mainly distributed among red swamp crayfish populations collected in China and Japan. Haplotype 3 was only detected in populations collected in America (Table 12). The genetic diversity parameters of 16SrRNA gene in different populations of *P. clarkii* showed the highest genetic diversity in the SH population and the lowest diversity in DTL, DTLs, LZL, PYL, CJr, XG, XYw and QJ populations (Table 15).

AMOVA revealed that 53.47% of genetic variation could be explained by variation within populations, whereas 46.53% came from variation among populations (Table 16). The genetic distance (0.004–0.006) between the population in America and populations in China was highest, while genetic distance varied from 0.000 to 0.006 among all populations. The genetic diversity (Fst) in 15 populations of *P. clarkii* ranged from a low of -0.131 between LZL and DTLs population to a high of 0.836 between Ame and three populations in China (DTL, XG, and QJ) (Table 15). Based on the genetic distances matrix, a Neighbor-joining tree was constructed among 142 individuals, and showed that the genetic distance of populations collected in America and China was highest (Figure 5).

The NJ dendrogram of three haplotypes detected in the 15 populations of *P. clarkii* was constructed which showed that one branch only included Hap 1, and the other one included Hap 2 and 3 (Figure 6).

The detection of population expansion was performed using Fu and Li 's Test. The positive Fs values were 0.953 ($P=0.269$) which indicated that the red swamp crayfish populations did not experience significant population expansion. Moreover, the curve was obtained by population size changes analysis showed that the red swamp crayfish populations did not experience significant population expansion as well (Figure 7).

Survey of impacts of the red swamp crayfish invasion in China

The introduction of red swamp crayfish damaged native macrophytes, and often made lakes change from clear state to turbid. The eutrophication of lakes due to dominant phytoplankton, structure of the food web, and trophic state have changed profoundly. In red swamp crayfish stomachs, macrophyte fragments, aquatic adherent organisms, plankton, crops, and aquatic invertebrates (especially insect larvae) were found.

Red swamp crayfish was a medium for infection by fungus (for example: *Saprolegnia parasitica* and *Aphanomyces astaci*). Although it was a carrier for *Aphanomyces*, red swamp crayfish was not affected by it. Red swamp crayfish could carry other pathogenic microbes as well. This could make animals infected in the new aquatic ecosystem which was invaded by red swamp crayfish.

Red swamp crayfish was widely distributed in all kinds of water, especially in static water channels, shallow lakes, ponds and rice fields. Red swamp crayfish has the habit of digging tunnels. Some tunnels were located in the middle of rice fields, and some in the dykes, which could cause bank erosion or collapse. Generally, crayfish holes are more than 1 m deep in mid field, but only about 0.5 m deep in dykes. So they can be destructive to a rice paddy. Tunnels produced by crayfish may also threaten dam security for lakes, reservoirs and rivers.

Red swamp crayfish has an effect on the native biological diversity through competition and predation. Introduction of red swamp crayfish can make the quality of a wetland's habitat decline, directly or indirectly influence the animal and plant species, and lead to species diversity decline. Red swamp crayfish is an omnivorous animal, which mainly feeds on plankton, benthic organisms, algae, small fish, and shrimp.

After nearly 10 years, the market chain of red swamp crayfish has been developed, and it has become a pillar industry in many regions. In this chain, the first link is red swamp crayfish farming, the second processing, and the third tourism services developing by red swamp crayfish as a medium. A successful example is Xuyi Crayfish. At present, the Xuyi Crayfish already has been designated as "Chinese famous brand," "Chinese famous agricultural products," "Chinese famous dish," and "Chinese geographic indication products." The value of crayfish in 2009 was RMB 4.13 billion yuan. For the crayfish industry in Xuyi, Jiangsu Province, more than 20 million mu are in culture for crayfish. Annual trading volume has attained 100,000 tons, the annual businesses have exceeded RMB 1.5 billion yuan, and employees include more than 100,000 people. A similar example is the crayfish industry in Hubei province. The culture model, "Continuous Crayfish Rice," promotes the industry in Hubei province. Presently, the yield of crayfish in Hubei province ranks first in China, and the value of crayfish was 3 billion yuan.

DISCUSSION

Yue et al. (2010) analyzed six populations of *P. clarkii* only collected in China using nine polymorphic microsatellites. In this study, 35 populations of *P. clarkii* were selected from China and one population was selected from America and Japan, which were analyzed using 12 polymorphic microsatellites for the first time. Baguazhou township, Nanjing, Jiangsu province, showed the highest allelic and gene diversity among the populations investigated in China. The microsatellite data obtained by Yue et al. (2010) also showed that the population located in Nanjing displayed the highest allelic and gene diversity among the populations studied. So, Nanjing was probably the place of introduction for *P. clarkii* (Li et al., 2007; Yue et al., 2010).

The overall observed heterozygosity of the 37 populations studied here was 0.6723, the overall gene diversity was 0.7913, and the overall polymorphism information content (*PIC*) was 0.7551. All of the red swamp crayfish populations evaluated by us showed high genetic diversity. High genetic diversity of introduced populations could be caused by multiple introductions (Berg et al., 2002; Kolbe et al., 2004; Barbaresi et al., 2003; Barbaresi et al., 2007; Chu et al., 2007), or single introductions of large numbers of individuals from different populations (Stepien et al., 2002; Barbaresi et al., 2007; Chu et al., 2007; Herborg et al., 2007). Moreover, high genetic diversity of an invasive species may be caused by hybridization and variation after invading the new environment (Xu and Ye, 2003; Chen

and Yan, 2005; Shi and Ma, 2006; Chu et al., 2007). Thus, the high genetic diversity of red swamp crayfish in China might derive from multiple introduction events or a single introduction of large numbers of individuals from different populations.

The genetic diversity of invasive populations would be lower than the origin population after experiencing bottleneck and founder effects if invaders were introduced only once (Amsellem et al., 2000; Dlugosch and Parker, 2008). Lower genetic diversity may be due to the invasive populations experiencing bottlenecks and genetic drift (Friar et al., 2000; Tsutsui et al., 2001). However, if the invasive species originated from multiple introductions, their population genetic diversity would not necessarily be lower than the origin place (Maron et al., 2004; Keller and Taylor, 2008). For example, the genetic diversity and heritable phenotypic variation of *Phalaris arundinacea* in the invasion locations in North America were higher than the origin in Europe. In this study, we found that Baguazhou township, Nanjing, Jiangsu province, showed the highest allelic and gene diversity among the populations investigated in China, however, the diversity was less than in America and Japan. Moreover, most of the red swamp crayfish populations may have experienced a recent bottleneck. Thus, we could speculate that red swamp crayfish populations in China derived from a single introduction of large numbers of individuals from different populations, rather than from multiple introductions. *P. clarkii* may have been introduced only once from Japan to Nanjing in 1929 (Li et al., 2007; Xia et al., 2009). However, Chinese researchers lacked the knowledge of source and dispersal, and cannot exclude the possibility of multiple introductions.

Wang et al. (2009) considered that the genetic distance of Nanjing and Nanchang populations was less than that of Nanjing and Xuyi populations due to the higher rate of gene flow among the Nanjing and Nanchang populations collected along the Changjiang River. The level of genetic differentiation among Xinanjiang and other populations was high, also indicated that exchange among the water system was the main dispersal method of red swamp crayfish. Here, the rate of gene flow among population BGt and other populations along the Changjiang River, such as WX, MAS, CJr, etc, was also higher than that of Nanjing and two Xuyi populations. All of these trends indicated that movement among the water system was the one of main dispersal means for red swamp crayfish.

Dispersal of exotic species is often influenced by human factors (Parker et al., 1999; Suarez et al., 2001), especially in fresh water ecological systems (Maria and Rebelo, 2007). Human activities can result in large and disjointed dispersal of invasive species (Suarez et al., 2001; Tiunov et al., 2006). In our study, the gene flow was high among populations which are separated by a great distance. The AMOVA of microsatellites, mitochondrial COI and 16SrRNA sequences revealed that most genetic variation (>50%) could be explained by the variation within populations. Taken together, human activities, such as movement of aquaculture crops, impelled the exchange among populations of red swamp crayfish, and had an influence on their population genetic structure.

Our results showed that red swamp crayfish may endanger native species by eating native animals and plants. They could sometimes carry and spread diseases, which may also harm native species. Meanwhile, they could destroy rice fields and water conservation projects due to their tunneling habits. Moreover, there are few natural enemies of red swamp crayfish in China (Li et al., 2005), so that red swamp crayfish could establish populations very fast and spread easily. They would endanger local fish, shellfish, and aquatic plants, as well as threaten the local food chain and damage crops and natural vegetation (Gherardi et al., 2001; Renai and Gherardi, 2004). While red swamp crayfish have been included in the list of harmful invasive species, it has also become an important freshwater resource in China. Presently, under the large demands of red swamp crayfish in the domestic and international market, cultivation of red swamp crayfish is increasing in Hubei, Jiangsu and Anhui provinces.

ANTICIPATED BENEFITS

The red swamp crayfish has become a successful worldwide invader. This study used the red swamp crayfish as a model to study changes of population structure due to anthropogenic activities during invasion and fast dispersal after invasion, to identify source area(s) or invasion center(s) as well as the dispersal patterns, to explore populations derived from single or multiple introduction events, and to evaluate the impacts of the red swamp crayfish invasion in China.

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Table 1. Sampling sites for red swamp crayfish, *P. clarkii*.

No.	pop.ID	abbr. of pop.	province
1	Shanghai	SH	Shanghai municipality
2	Ningbo	NB	Zhejiang
3	Jiaxing	JX	Zhejiang
4	Xuyi-culture	XYc	Jiangsu
5	Xuyi-wild	XYw	Jiangsu
6	Wuxi binhu	WXb	Jiangsu
7	Nantong	NT	Jiangsu
8	Xiaguan District	XG	Jiangsu
9	Xiaba village	XBv	Jiangsu
10	Baguazhou township	BGt	Jiangsu
11	Wuxi	WX	Jiangsu
12	Wangjiang	WJ	Anhui
13	Maanshan	MAS	Anhui
14	Guangfengwei section of Changjiang river	CJr	Anhui
15	Chaohu lake	CHL	Anhui
16	Hefei	HF	Anhui
17	Dingyuan	DY	Anhui
18	Sanli township	SLt	Jiangxi
19	Nanbei Port	NBp	Jiangxi
20	Poyang lake	PYL	Jiangxi
21	Nanchang youlan	NCyl	Jiangxi
22	Nanhu lake	NHL	Hubei
23	Yuni lake	YNL	Hubei
24	Xiantao	XT	Hubei
25	Qianjiang	QJ	Hubei
26	Liangzi lake	LZL	Hubei
27	Honghu lake	HHL	Hubei
28	Changhu lake	CHL	Hubei
29	Yuanjiang	YJ	Hunan
30	Ningxiang	NX	Hunan
31	Dongting lake	DTL	Hunan
32	Dongting lakeside	DTLs	Hunan
33	Chongqing suburb	CQs	Chongqing municipality
34	Zhongxian	ZX	Sichuan
35	Jianyang	JY	Sichuan
36	Japan	Jap	Japan
37	America	Ame	America

Table 2. Sampling sites number of samples for mitochondrial COI and 16SrRNA analysis.

No.	pop.ID	abbr. of pop.	Number of samples for COI	Number of samples for 16SrRNA
1	Dongting lake	DTL	10	10
2	Dongting lakeside	DTLs	9	8
3	Liangzi lake	LZL	10	9
4	Poyang lake	PYL	10	8
5	Guangfengwei section of Changjiang River	CJr	8	10
6	Dingyuan	DY	10	10
7	Maanshan	MAS	10	10
8	Xiaguan District	XG	10	10
9	Shanghai	SH	8	10
10	Xuyi-wild	XYw	10	8
11	Sichuan Jianyang	JY	10	9
12	Jiaxing	JX	10	10
13	Qianjiang	QJ	10	10
14	Saitama Prefecture, Japan	Jap	10	10
15	Louisiana, America	Ame	10	10

Table 3. The mean number of alleles (N_a), mean number of effective alleles (N_e), mean polymorphism information content (PIC), mean observed heterozygosity (H_o) and mean expected heterozygosity (H_e) of 12 microsatellite loci in the *P. clarkii* populations.

Population ID	N_a	N_e	H_o	H_e	PIC
SH	11.7500	6.4795	0.7887	0.8214	0.7903
NB	10.4167	5.3597	0.5880	0.8068	0.7738
JX	8.0833	5.4123	0.6632	0.7860	0.7441
XYc	9.5000	5.3707	0.7425	0.7998	0.7623
XYw	9.6667	5.4454	0.7342	0.7933	0.7542
WXb	9.2500	5.7829	0.6416	0.7978	0.7578
NT	10.0833	5.5798	0.6181	0.8211	0.7931
XG	10.0833	6.2292	0.5602	0.8151	0.7792
XBv	10.1667	5.7982	0.7836	0.7877	0.7557
BGt	11.0833	6.7329	0.6285	0.8413	0.8124
WX	10.0000	5.5924	0.7786	0.7889	0.7536
WJ	10.4167	6.1777	0.8129	0.8025	0.7672
MAS	9.5000	5.6025	0.6413	0.7771	0.7359
CJr	10.0000	5.6517	0.5777	0.7984	0.7621
CHL	10.4167	5.7354	0.7413	0.7786	0.7439
HF	8.7500	4.8770	0.6656	0.7623	0.7222
DY	8.7500	4.8386	0.6285	0.7428	0.7018
SLt	10.3333	5.7651	0.7007	0.7847	0.7497
NBp	10.6667	5.4745	0.6991	0.7969	0.7628
PYL	8.9167	4.8893	0.5890	0.7448	0.7086
NCyl	9.7500	5.8021	0.6742	0.7845	0.7433
NHL	10.7500	5.9593	0.7774	0.8194	0.7883
YNL	10.5833	5.8366	0.7361	0.8224	0.7905
XT	9.1667	5.2843	0.7096	0.7940	0.7559
QJ	9.9167	5.5028	0.6764	0.7892	0.7535
LZL	9.6667	5.6394	0.6715	0.8031	0.7658
HHL	10.6667	5.8500	0.7466	0.7924	0.7559
CHL	9.5833	4.8054	0.5875	0.7482	0.7089
YJ	7.9167	4.5335	0.5942	0.7584	0.7162
NX	8.0000	4.4062	0.5569	0.7400	0.6944
DTL	8.6667	5.0804	0.6782	0.7860	0.7459
DTLs	10.2500	5.8721	0.7038	0.8170	0.7850
CQs	9.0000	4.8829	0.7182	0.7593	0.7123
ZX	6.4167	3.6813	0.5748	0.7002	0.6553
JY	7.8333	4.3407	0.6109	0.7512	0.7113
Jap	14.3333	9.3691	0.6283	0.8873	0.8667
Ame	14.5833	8.8149	0.6465	0.8799	0.8579

Table 6. Analysis of molecular variance (AMOVA) within and among *P. clarkii* populations.

Source of variance	degree of freedom	Sum of squares	Variance components	Percentage of variation(%)
Among populations	36	1702.299	0.44426	8.74
Within populations	3515	16300.219	4.63733	91.26
Total	3551	18002.517	5.08159	

Table 7. Sign tests and Wilcoxon tests for heterozygosity excess at twelve microsatellite loci in six *P. clarkii* populations.

Pop	IAM			TPM			SMM		
	HeE	He/Hd	Ps/Pw	HeE	He/Hd	Ps/Pw	HeE	He/Hd	Ps/Pw
SH	7.21	12/0	0.002/0.000	7.1	8/4	0.415/ 0.039	7.16	2/10	0.003/0.983
NB	7.23	9/3	0.230/ 0.032	7.21	8/4	0.440/0.545	7.06	6/6	0.366/0.788
JX	6.98	12/0	0.002/0.000	7.07	12/0	0.002/0.000	7.08	11/1	0.017/0.017
XYc	7.20	11/1	0.020/0.000	7.1	10/2	0.075/ 0.021	7.05	5/7	0.181/0.285
XYw	7.24	11/1	0.021/0.000	7.12	8/4	0.419/ 0.046	7.07	4/8	0.067/0.924
WXb	6.96	12/0	0.001/0.000	6.91	11/1	0.013/0.000	6.88	7/5	0.592/0.424
NT	7.22	11/1	0.020/0.000	7.16	10/2	0.080/0.065	7.02	4/8	0.071/0.954
XG	7.18	12/0	0.002/0.000	7.13	10/2	0.077/ 0.001	7.12	5/7	0.171/0.689
XBv	7.25	11/1	0.021/0.000	7.13	9/3	0.213/0.117	7.01	4/8	0.072/0.954
BGt	7.31	12/0	0.003/0.000	7.14	10/2	0.078/ 0.001	7.09	6/6	0.360/0.515
WX	7.13	11/1	0.018/0.017	7.07	9/3	0.203/ 0.046	7.09	6/6	0.360/0.380
WJ	7.16	12/0	0.002/0.000	7.17	10/2	0.081/ 0.007	7.14	6/6	0.347/0.765
MAS	7.12	12/0	0.002/0.000	7.11	9/3	0.210/ 0.004	7.12	5/7	0.170/0.849
CJr	7.17	10/2	0.080/ 0.000	7.17	9/3	0.221/0.102	7.08	6/6	0.362/0.830
CHL	7.11	11/1	0.017/0.000	7.12	9/3	0.211/0.088	7.09	3/9	0.018/0.961
HF	7.17	10/2	0.080/ 0.000	7.04	7/5	0.600/0.235	7.02	6/6	0.376/0.380
DY	7.08	11/1	0.016/0.000	7.15	8/4	0.426/0.170	7.11	5/7	0.172/0.898
SLt	7.10	12/0	0.002/0.000	7.09	9/3	0.206/ 0.021	7.11	2/10	0.003/0.993
NBp	7.21	11/1	0.020/0.000	7.10	9/3	0.208/0.259	7.07	3/9	0.018/0.998
PYL	6.97	10/2	0.065/ 0.002	7.03	8/4	0.398/0.190	7.12	6/6	0.351/0.867
NCyl	7.17	12/0	0.002/0.000	7.14	7/5	0.576/0.055	7.08	6/6	0.362/0.898
NHL	7.21	12/0	0.002/0.000	7.10	8/4	0.415/ 0.046	7.06	6/6	0.365/0.715
YNL	7.24	11/1	0.021/0.000	7.14	8/4	0.424/0.076	7.08	6/6	0.362/0.830
XT	7.10	12/0	0.002/0.000	7.13	11/1	0.0178/0.002	7.10	4/8	0.064/0.830
QJ	7.18	11/1	0.019/0.000	7.06	8/4	0.405/0.170	7.00	6/6	0.379/0.810
LZL	7.19	12/0	0.002/0.000	7.13	9/3	0.213/ 0.004	7.12	5/7	0.169/0.912
HHL	7.16	11/1	0.019/0.000	7.13	10/2	0.078/ 0.032	7.07	2/10	0.004/0.993

Pop	IAM			TPM			SMM		
	HeE	He/Hd	Ps/Pw	HeE	He/Hd	Ps/Pw	HeE	He/Hd	Ps/Pw
CHL	7.18	10/2	0.081/ 0.026	7.05	7/5	0.597/0.455	7.13	1/11	0.000 /0.999
YJ	7.15	12/0	0.002/0.000	7.15	8/4	0.427/ 0.021	7.10	5/7	0.173/0.898
NX	7.08	12/0	0.002/0.000	7.08	6/6	0.362/0.088	7.14	6/6	0.348/0.924
DTL	7.15	12/0	0.002/0.000	7.14	11/1	0.018/0.000	7.09	4/8	0.065/0.849
DTLs	7.24	11/1	0.021/0.000	7.07	11/1	0.016/0.017	7.06	4/8	0.067/0.924
CQs	7.08	12/0	0.001/0.000	7.18	9/3	0.222/ 0.046	7.11	4/8	0.064/0.961
ZX	6.89	11/1	0.012/0.000	6.99	9/3	0.189/ 0.002	6.98	5/7	0.192/0.830
JY	7.12	11/1	0.018/0.005	7.15	10/2	0.079/ 0.026	7.04	5/7	0.183/0.830
Jap	7.28	12/0	0.003/0.000	7.20	11/1	0.020/0.000	7.04	9/3	0.197/0.259
Ame	7.24	12/0	0.002/0.000	7.10	9/3	0.208/ 0.005	7.09	5/7	0.175/0.677

Table 8. Distribution of haplotypes in COI sequences of *P. clarkia*.

	2	2	2	3	4	4	4	4	4	5	5	5	5	6	DT	DTL	LZ	PY	CJ	D	MA	X	S	XY	J	J	Q	Ja	Am	Total		
	4	4	3	8	9	4	0	4	6	8	2	3	6	9	L	s	L	L	r	Y	S	G	H	w	Y	X	J	p	e			
	2	5	4	8	7	2	8	4	5	6	8	4	5	4	3																	
Hap_1	G	A	A	T	G	A	A	A	G	G	G	A	C	G	T	10	9	10	10	8	5	2	10	4	10	7	8	10	4		107	
Hap_2	A	.	.	.	A	A	.					5	8		4			3	2		6		28	
Hap_3	.	.	.	C	A	.	G	.	A	.	A	.	.	A	.															7	7	
Hap_4	A	.	G	A	A	G	.	G	A	A	.	G	.	A	C															1	1	
Hap_5	A	G	G	A	A	G	.	G	A	.	.	G	.	A	C															1	1	
Hap_6	.	.	.	C	A	.	.	.	A	.	A	.	T	A	.															1	1	
	Total														10	9	10	10	8	10	10	10	8	10	10	10	10	10	10	10	10	145

Table 9. Genetic diversity parameters of COI gene in different populations of *P. clarkia*.

Population	Number of samples(<i>N</i>)	Number of segregating sites(<i>S</i>)	Number of haplotypes(<i>H</i>)	Haplotype diversity(<i>H_d</i>)	Average number of differences(<i>K</i>)	Nucleotide diversity(<i>P_i</i>)
DTL	10	0	1	0.000	0.000	0.00000
DTLs	9	0	1	0.000	0.000	0.00000
LZL	10	0	1	0.000	0.000	0.00000
PYL	10	0	1	0.000	0.000	0.00000
CJr	8	0	1	0.000	0.000	0.00000
DY	10	3	2	0.556	1.667	0.00262
MAS	10	3	2	0.356	1.067	0.00168
XG	10	0	1	0.000	0.000	0.00000
SH	8	3	2	0.571	1.714	0.00270
XYw	10	0	1	0.000	0.000	0.00000
JY	10	3	2	0.467	1.400	0.00220
JX	10	3	2	0.356	1.067	0.00167
QJ	10	0	1	0.000	0.000	0.00000
Jap	10	3	2	0.533	1.600	0.00251
Ame	10	12	4	0.533	3.911	0.00614
Total	145	15	6	0.419	1.702	0.00267

Table 10. Analysis of molecular variance (AMOVA) within and among *P. clarkii* population by mtDNA COI analysis.

Source of variance	degree of freedom	Sum of squares	Variance components	Percentage of variation(%)
Among populations	14	69.83	0.46321Va	47.46
Within populations	130	66.653	0.51271Vb	52.54
Total	144	136.483	0.97583	

Table 11. Genetic distances (below diagonal) and genetic fixations index (above diagonal) of COI gene among *P. clarkii* populations.

	DTL	DTLs	LZL	PYL	CJr	DY	MAS	XG	SH	XYw	JY	JX	QJ	Jap	Ame
DTL		0.012	0.000	0.000	0.000	0.444	0.718	0.000	0.438	0.000	0.222	0.111	0.000	0.556	0.721
DTLs	0.000		0.012	0.012	-0.014	0.248	0.493	0.012	0.224	0.012	0.098	0.042	0.012	0.335	0.610
LZL	0.000	0.000		0.000	0.000	0.444	0.718	0.000	0.438	0.000	0.222	0.111	0.000	0.556	0.721
PYL	0.000	0.000	0.000		0.000	0.444	0.718	0.000	0.438	0.000	0.222	0.111	0.000	0.556	0.721
CJr	0.000	0.000	0.000	0.000		0.407	0.691	0.000	0.396	0.000	0.187	0.080	0.000	0.521	0.694
DY	0.001	0.001	0.001	0.001	0.001		0.078	0.444	-0.116	0.444	-0.022	0.089	0.444	-0.089	0.493
MAS	0.002	0.002	0.002	0.002	0.002	0.001		0.718	0.065	0.718	0.304	0.435	0.718	-0.009	0.440
XG	0.000	0.000	0.000	0.000	0.000	0.001	0.002		0.438	0.000	0.222	0.111	0.000	0.556	0.721
SH	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001		0.438	-0.031	0.077	0.438	-0.095	0.465
XYw	0.000	0.000	0.000	0.000	0.000	0.001	0.002	0.000	0.001		0.222	0.111	0.000	0.556	0.721
JY	0.001	0.001	0.001	0.001	0.001	0.001	0.002	0.001	0.001	0.001		-0.082	0.222	0.074	0.565
JX	0.001	0.001	0.001	0.001	0.001	0.001	0.002	0.001	0.001	0.001	0.001		0.111	0.206	0.611
QJ	0.000	0.000	0.000	0.000	0.000	0.001	0.002	0.000	0.001	0.000	0.001	0.001		0.556	0.721
Jap	0.002	0.002	0.002	0.002	0.002	0.001	0.001	0.002	0.001	0.002	0.001	0.001	0.002		0.470
Ame	0.006	0.006	0.006	0.006	0.006	0.005	0.004	0.006	0.005	0.006	0.005	0.006	0.006	0.005	

Table 12. Distribution of haplotypes in 16SrRNA sequences of *P. clarkia*.

	9	2 2 5	2 9 3	DTL	DTLs	LZL	PYL	CJr	DY	MAS	XG	SH	XYw	JY	JX	QJ	Jap	Ame	Total
Hap_1	A	A	T	10	8	9	8	10	4	2	10	5	8	6	8	10	4		102
Hap_2	G	.	-						6	8		5		3	2		6	2	33
Hap_3	G	G	-															7	7
	Total			10	8	9	8	10	10	10	10	10	8	9	10	10	10	9	142

Table 13. Genetic diversity parameters of 16SrRNA gene in different populations of *P. clarkii*.

Population	Number of samples(N)	Number of segregating sites(S)	Number of haplotypes(H)	Haplotype diversity(Hd)	Average number of differences(K)	Nucleotide diversity(Pi)
DTL	10	0	1	0.000	0.000	0.00000
DTLs	8	0	1	0.000	0.000	0.00000
LZL	9	0	1	0.000	0.000	0.00000
PYL	8	0	1	0.000	0.000	0.00000
CJr	10	0	1	0.000	0.000	0.00000
DY	10	1	2	0.533	0.533	0.00183
MAS	10	1	2	0.356	0.356	0.00122
XG	10	0	1	0.000	0.000	0.00000
SH	10	1	2	0.556	0.556	0.00190
XYw	8	0	1	0.000	0.000	0.00000
JY	9	1	2	0.500	0.500	0.00171
JX	10	1	2	0.356	0.356	0.00122
QJ	10	0	1	0.000	0.000	0.00000
Jap	10	1	2	0.533	0.533	0.00183
Ame	10	1	2	0.467	0.467	0.00160
Total	142	2	3	0.431	0.502	0.00172

Table 14. Analysis of molecular variance (AMOVA) within and among *Procambarus clarkii* population by mtDNA 16SrRNA analysis.

Source of variance	degree of freedom	Sum of squares	Variance components	Percentage of variation(%)
Among populations	14	31.888	0.21465 Va	46.53
Within populations	127	31.331	0.24670 Vb	53.47
Total	141	63.218	0.46135	

Table 15. Genetic distances (below diagonal) and genetic fixations index (above diagonal) of 16SrRNA sequences among *P. clarkii* populations.

	DTL	DTLs	LZL	PYL	CJr	DY	MAS	XG	SH	XYw	JY	JX	QJ	Jap	Ame
DTL		0.181	0.141	0.000	0.000	0.556	0.778	0.000	0.444	0.029	0.268	0.074	0.000	0.476	0.836
DTLs	0.000		-0.131	0.143	-0.039	0.333	0.599	0.181	0.214	-0.086	0.041	0.012	0.181	0.344	0.726
LZL	0.000	0.000		0.107	-0.057	0.362	0.621	0.141	0.242	-0.098	0.063	0.010	0.141	0.355	0.740
PYL	0.000	0.000	0.000		-0.024	0.521	0.755	0.000	0.407	0.000	0.230	0.045	0.000	0.440	0.818
CJr	0.000	0.000	0.000	0.000		0.463	0.704	0.000	0.344	-0.123	0.154	0.006	0.000	0.402	0.791
DY	0.002	0.002	0.002	0.002	0.002		-0.010	0.556	-0.089	0.416	0.031	0.289	0.556	0.151	0.387
MAS	0.003	0.003	0.003	0.003	0.003	0.002		0.778	0.089	0.671	0.290	0.554	0.778	0.388	0.372
XG	0.000	0.000	0.000	0.000	0.000	0.002	0.003		0.444	0.029	0.268	0.074	0.000	0.476	0.836
SH	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002		0.295	-0.056	0.167	0.444	0.078	0.431
XYw	0.000	0.000	0.000	0.000	0.000	0.002	0.003	0.000	0.002		0.107	-0.016	0.029	0.366	0.767
JY	0.001	0.001	0.001	0.001	0.001	0.002	0.002	0.001	0.002	0.001		-0.011	0.268	0.039	0.538
JX	0.001	0.001	0.001	0.001	0.001	0.002	0.002	0.001	0.002	0.001	0.001		0.074	0.129	0.692
QJ	0.000	0.000	0.000	0.000	0.000	0.002	0.003	0.000	0.002	0.000	0.001	0.001		0.476	0.836
Jap	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002		0.577
Ame	0.006	0.006	0.006	0.006	0.006	0.004	0.003	0.006	0.004	0.006	0.005	0.005	0.006	0.004	

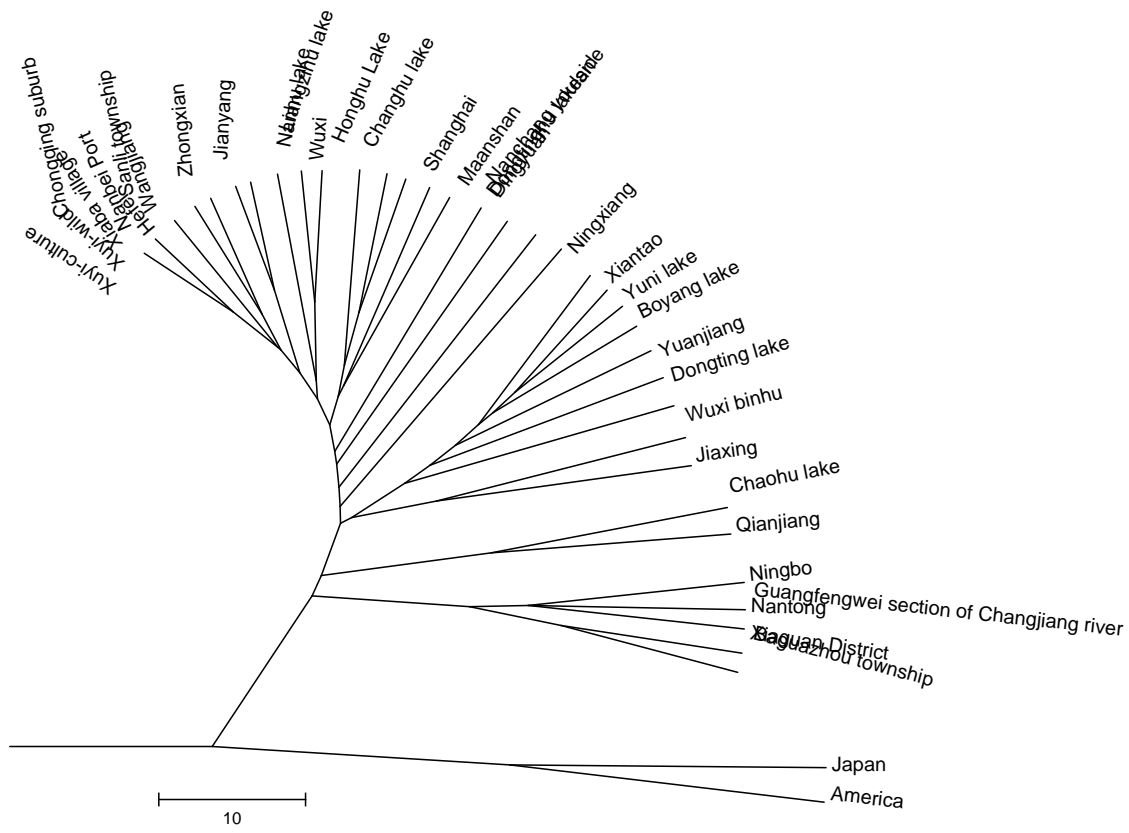


Figure 1. Cluster analysis of 37 red swamp crayfish populations.

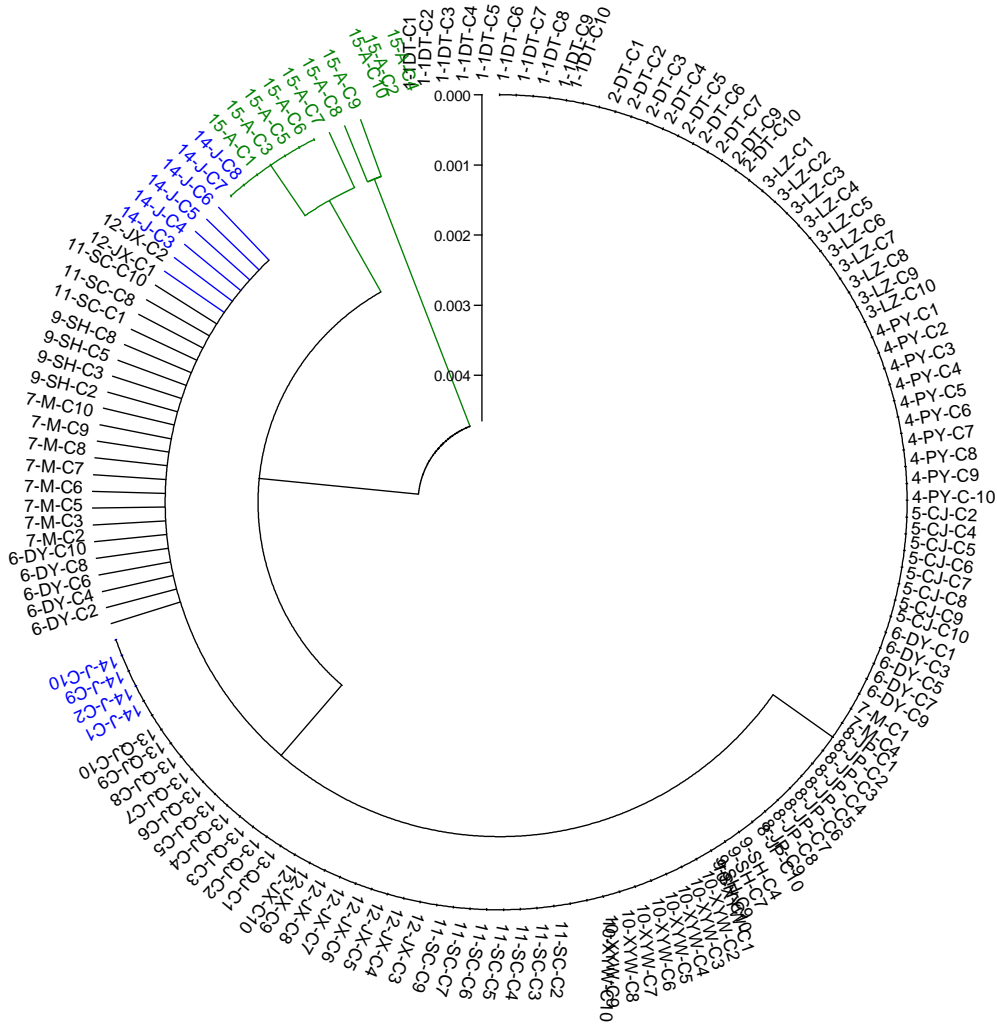


Figure 2. Neighbor-joining tree of COI sequences among 145 red swamp crayfish individuals.

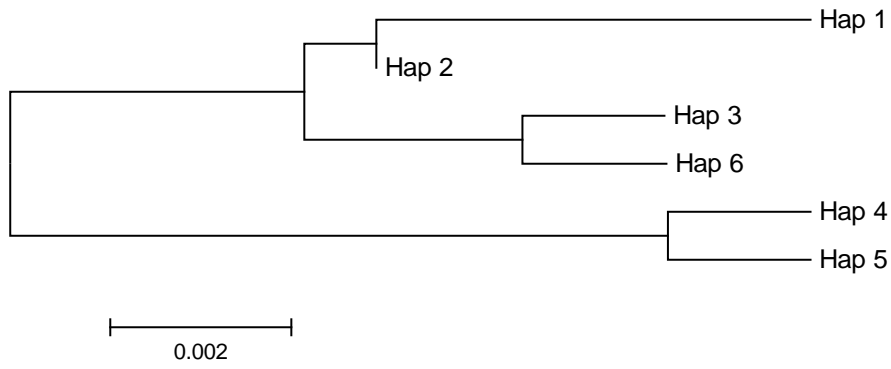


Figure 3. NJ dendrogram of six haplotypes in the 15 populations of *P. clarkii*.

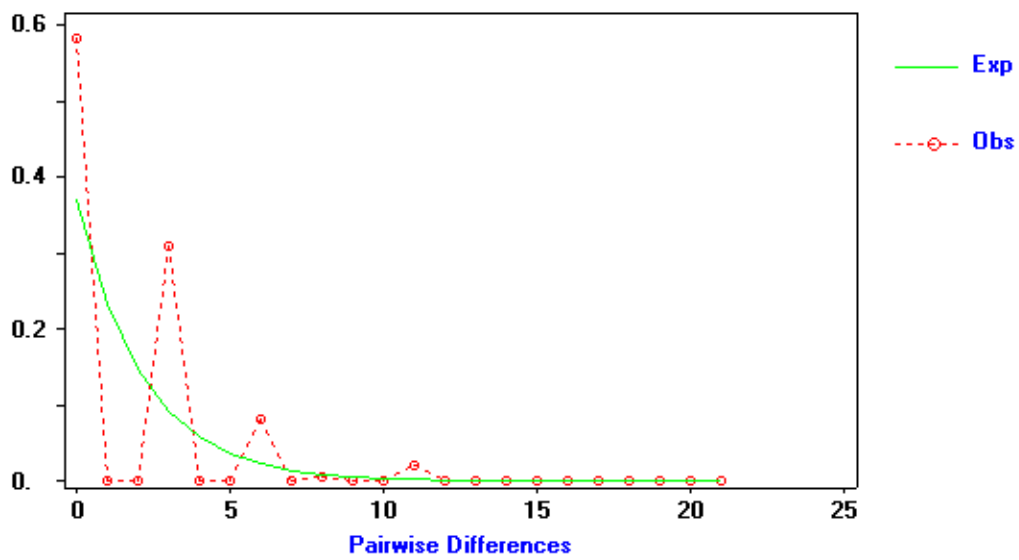


Figure 4. Population size changes analysis of mtDNA COI sequences.

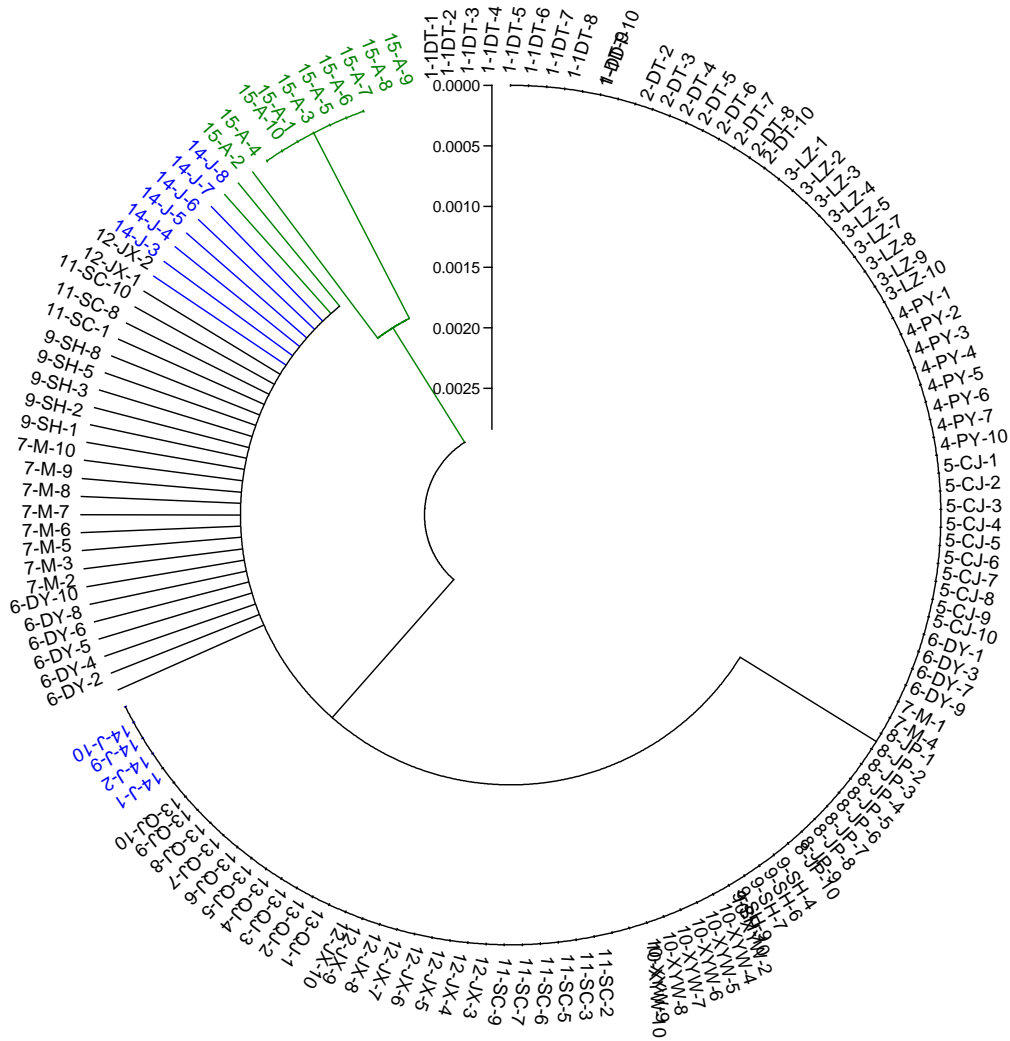


Figure 5. Neighbor-joining tree of 16SrRNA sequences among 142 red swamp crayfish individuals.

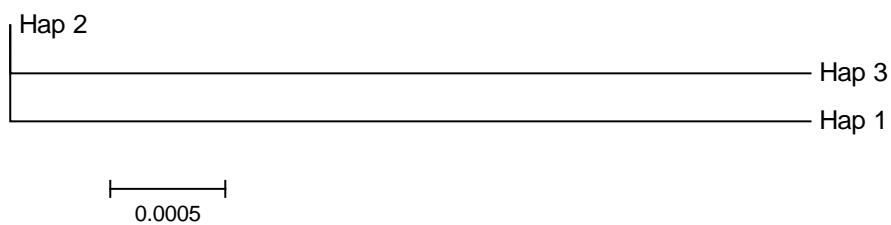


Figure 6. NJ dendrogram of three haplotypes in the 15 populations of *P. clarkii*.

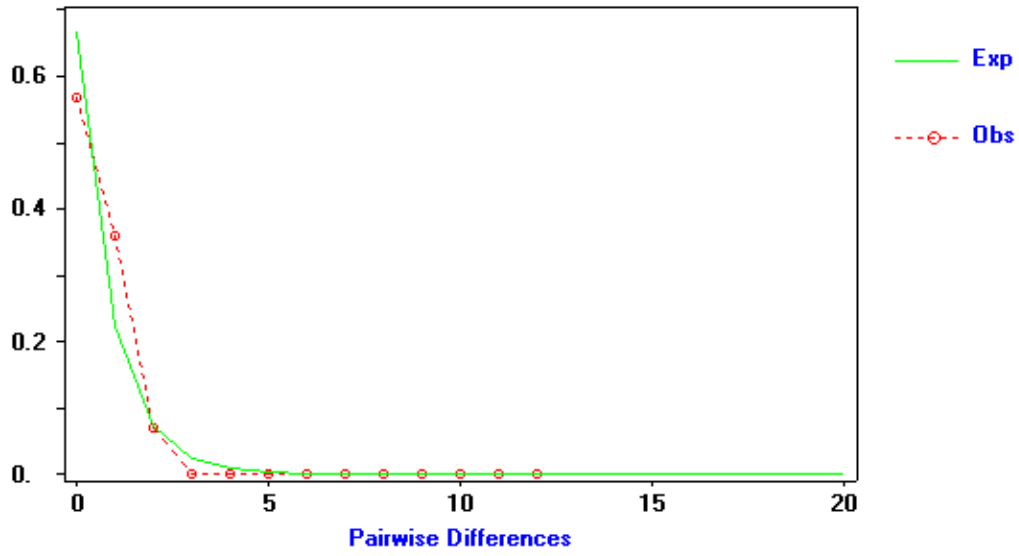


Figure 7. Population size changes analysis of mtDNA 16SrRNA sequences.

Appendix Table 1. Number of alleles (na^*), mean number of effective alleles (ne^*), observed heterozygosity (Ho), expected heterozygosity (He), Hardy-Weinberg departure value (D) and polymorphism information content (PIC), of 12 microsatellite loci in the *P. clarkii* population.

Pop.	Loci	na^*	ne^*	Ho	He	D	PIC
SH	PCL24	4	3.1736	1.0000	0.6921	0.4449	0.6304
	PCLG-03	25	14.2516	0.7660	0.9398	-0.1849	0.9257
	PCLG-04	12	8.9302	0.8542	0.8974	-0.0481	0.8780
	PCLG-07	10	6.0792	0.7917	0.8443	-0.0623	0.8179
	PCLG-09	15	7.3376	0.7917	0.8728	-0.0929	0.8491
	PCLG-10	8	4.5801	0.2609	0.7903	-0.6699	0.7478
	PCLG-13	6	2.5402	0.4792	0.6127	-0.2179	0.5616
	PCLG-15	14	7.3728	0.7917	0.8735	-0.0936	0.8504
	PCLG-17	13	6.5455	1.0000	0.8561	0.1681	0.8318
	PCLG-29	10	4.4869	0.8125	0.7853	0.0346	0.7493
	PCLG-32	14	6.8776	0.9792	0.8636	0.1339	0.8393
	PCLG-48	10	5.5787	0.9375	0.8294	0.1303	0.8019
NB	Loci	na^*	ne^*	Ho	He	D	PIC
	PCL24	7	4.8505	1.0000	0.8022	0.2466	0.7655
	PCLG-03	16	5.1854	0.4681	0.8158	-0.4262	0.7899
	PCLG-04	11	6.1935	0.2292	0.8474	-0.7295	0.8194
	PCLG-07	11	6.8065	0.5625	0.8621	-0.3475	0.8360
	PCLG-09	9	3.2961	0.3125	0.7039	-0.5560	0.6634
	PCLG-10	11	3.9097	0.4255	0.7522	-0.4343	0.7107
	PCLG-13	6	4.0421	0.3750	0.7605	-0.5069	0.7143
	PCLG-15	13	3.3488	0.5833	0.7088	-0.1771	0.6645
	PCLG-17	12	7.6800	0.6458	0.8789	-0.2652	0.8569
	PCLG-29	12	7.0127	0.8085	0.8666	-0.0670	0.8423
	PCLG-32	8	5.6264	0.9375	0.8309	0.1283	0.7976
PCLG-48	9	6.3646	0.7083	0.8518	-0.1685	0.8250	
	Loci	na^*	ne^*	Ho	He	D	PIC
	PCL24	5	3.7504	1.0000	0.7412	0.3492	0.6848
	PCLG-03	18	12.3754	0.7660	0.9291	-0.1755	0.9135
	PCLG-04	9	5.5990	0.4375	0.8300	-0.4729	0.7988
	PCLG-07	9	6.3123	0.7917	0.8504	-0.0690	0.8240
	PCLG-09	10	6.6237	0.5532	0.8582	-0.3554	0.8314
PCLG-10	7	4.4824	0.3488	0.7860	-0.5562	0.7415	

Pop.	Loci	<i>na</i> *	<i>ne</i> *	Ho	He	D	PIC
JX	PCLG-13	8	4.8549	0.3617	0.8026	-0.5493	0.7686
	PCLG-15	3	2.2058	0.3542	0.5524	-0.3588	0.4629
	PCLG-17	7	5.7889	0.9167	0.8360	0.0965	0.8049
	PCLG-29	10	4.9021	0.6875	0.8044	-0.1453	0.7771
	PCLG-32	8	5.4988	0.9583	0.8268	0.1590	0.7948
	PCLG-48	3	2.5540	0.7826	0.6151	0.2723	0.5267
	Loci	<i>na</i> *	<i>ne</i> *	Ho	He	D	PIC
	PCL24	5	3.2797	0.9792	0.7024	0.3941	0.6389
	PCLG-03	16	9.7627	0.6458	0.9070	-0.2880	0.8888
	PCLG-04	17	6.0632	0.8750	0.8439	0.0369	0.8238
	PCLG-07	8	3.5176	0.4375	0.7232	-0.3950	0.6816
	PCLG-09	11	6.1460	0.6364	0.8469	-0.2486	0.8186
	PCLG-10	6	3.4815	0.7872	0.7204	0.0927	0.6629
XYc	PCLG-13	6	4.0457	0.4583	0.7607	-0.3975	0.7139
	PCLG-15	8	4.2353	0.5625	0.7719	-0.2713	0.7273
	PCLG-17	7	4.4223	0.9375	0.7820	0.1988	0.7423
	PCLG-29	10	6.7657	0.8298	0.8614	-0.0367	0.8349
	PCLG-32	12	8.1385	0.8261	0.8868	-0.0684	0.8648
	PCLG-48	8	4.5900	0.9348	0.7907	0.1822	0.7496
	Loci	<i>na</i> *	<i>ne</i> *	Ho	He	D	PIC
	PCL24	5	3.7494	0.8750	0.7410	0.1808	0.6855
	PCLG-03	16	9.4000	0.7447	0.9032	-0.1755	0.8850
	PCLG-04	16	8.1967	0.9362	0.8874	0.0550	0.8667
	PCLG-07	9	3.2823	0.5319	0.7028	-0.2432	0.6443
	PCLG-09	11	7.3027	0.8958	0.8721	0.0272	0.8497
	PCLG-10	6	2.8989	0.5476	0.6629	-0.1739	0.5910
XYw	PCLG-13	8	3.7586	0.4792	0.7417	-0.3539	0.7082
	PCLG-15	5	2.8253	0.3542	0.6529	-0.4575	0.5783
	PCLG-17	8	4.7407	0.9375	0.7974	0.1757	0.7622
	PCLG-29	10	6.2604	0.7609	0.8495	-0.1043	0.8215
	PCLG-32	10	6.4215	0.8936	0.8534	0.0471	0.8273
	PCLG-48	12	6.5085	0.8542	0.8553	-0.0013	0.8302
	Loci	<i>na</i> *	<i>ne</i> *	Ho	He	D	PIC
	PCL24	2	1.9566	0.8511	0.4942	0.7222	0.3694
	PCLG-03	15	7.4128	0.5957	0.8744	-0.3187	0.8524

Pop.	Loci	<i>na</i> *	<i>ne</i> *	Ho	He	D	PIC
WXb	PCLG-04	12	7.1221	0.3542	0.8686	-0.5922	0.8443
	PCLG-07	9	5.7814	0.4783	0.8361	-0.4279	0.8048
	PCLG-09	10	6.7106	0.6341	0.8615	-0.2640	0.8346
	PCLG-10	5	4.0459	0.8478	0.7611	0.1139	0.7105
	PCLG-13	9	6.0393	0.2708	0.8432	-0.6788	0.8173
	PCLG-15	10	4.9073	0.6250	0.8046	-0.2232	0.7737
	PCLG-17	15	9.2903	0.9375	0.9018	0.0396	0.8829
	PCLG-29	11	6.5177	0.8542	0.8555	-0.0015	0.8296
	PCLG-32	10	7.1221	1.0000	0.8686	0.1513	0.8449
	PCLG-48	3	2.4881	0.2500	0.6044	-0.5864	0.5292
NT	Loci	<i>na</i> *	<i>ne</i> *	Ho	He	D	PIC
	PCL24	9	5.1086	0.9792	0.8127	0.2049	0.7802
	PCLG-03	14	5.2803	0.6222	0.8197	-0.2409	0.7937
	PCLG-04	11	6.0711	0.4375	0.8441	-0.4817	0.8164
	PCLG-07	10	6.5177	0.8125	0.8555	-0.0503	0.8288
	PCLG-09	10	5.2186	0.3333	0.8169	-0.5920	0.8346
	PCLG-10	6	3.2582	0.0667	0.7009	-0.9048	0.6407
	PCLG-13	6	4.0209	0.4167	0.7592	-0.4511	0.7121
	PCLG-15	13	5.4409	0.6383	0.8250	-0.2263	0.7988
	PCLG-17	11	7.1002	0.7917	0.8682	-0.0881	0.8439
	PCLG-29	12	6.6590	0.7292	0.8588	-0.1509	0.8325
	PCLG-32	11	6.3471	0.9167	0.8513	0.0768	0.8249
	PCLG-48	8	5.9355	0.6739	0.8407	-0.1984	0.8111
	XG	Loci	<i>na</i> *	<i>ne</i> *	Ho	He	D
PCL24		8	4.5036	0.9362	0.7863	0.1906	0.7458
PCLG-03		17	12.2145	0.6136	0.9287	-0.3393	0.9124
PCLG-04		8	4.5201	0.4667	0.7875	-0.4074	0.7442
PCLG-07		9	3.8933	0.3696	0.7513	-0.5081	0.7052
PCLG-09		7	4.6829	0.2500	0.7947	-0.6854	0.7545
PCLG-10		9	4.7471	0.2093	0.7986	-0.7379	0.7620
PCLG-13		7	4.9529	0.3830	0.8067	-0.5252	0.7690
PCLG-15		6	2.6197	0.1667	0.6248	-0.7332	0.5508
PCLG-17		15	9.4000	0.8085	0.9032	-0.1048	0.8847
PCLG-29		8	5.2068	0.6250	0.8164	-0.2344	0.7811
PCLG-32		12	6.7969	1.0000	0.8620	0.1601	0.8362
PCLG-48		15	11.2132	0.8936	0.9206	-0.0293	0.9040

Pop.	Loci	<i>na</i> *	<i>ne</i> *	Ho	He	D	PIC
	Loci	<i>na</i> *	<i>ne</i> *	Ho	He	D	PIC
	PCL24	8	5.8108	0.9375	0.8366	0.1206	0.8059
	PCLG-03	22	15.8351	0.7292	0.9467	-0.2297	0.9332
	PCLG-04	14	7.1002	0.9167	0.8682	0.0559	0.8442
	PCLG-07	8	3.2428	0.6667	0.6989	-0.0461	0.6603
	PCLG-09	12	6.7665	0.9792	0.8612	0.1370	0.8355
	PCLG-10	7	3.6332	0.9362	0.7326	0.2779	0.6942
XBv	PCLG-13	6	4.1213	0.6596	0.7655	-0.1383	0.7195
	PCLG-15	5	2.2250	0.3542	0.5564	-0.3634	0.5125
	PCLG-17	10	7.0351	0.8750	0.8669	0.0093	0.8421
	PCLG-29	10	5.2118	0.8696	0.8170	0.0644	0.7829
	PCLG-32	10	5.6058	0.9583	0.8303	0.1542	0.7977
	PCLG-48	10	2.9903	0.5208	0.6726	-0.2257	0.6404
	Loci	<i>na</i> *	<i>ne</i> *	Ho	He	D	PIC
	PCL24	8	5.3769	0.9792	0.8226	0.1904	0.7892
	PCLG-03	21	12.2880	0.6250	0.9283	-0.3267	0.9131
	PCLG-04	12	6.8879	0.5417	0.8638	-0.3729	0.8409
	PCLG-07	7	4.0280	0.3125	0.7596	-0.5886	0.7151
	PCLG-09	6	4.3187	0.2500	0.7765	-0.6780	0.7304
	PCLG-10	13	7.5417	0.6875	0.8765	-0.2156	0.8539
BGt	PCLG-13	10	4.1106	0.4375	0.7647	-0.4279	0.7350
	PCLG-15	11	7.3493	0.7292	0.8730	-0.1647	0.8500
	PCLG-17	12	8.7078	0.6304	0.8949	-0.2956	0.8741
	PCLG-29	7	4.3761	0.4583	0.7796	-0.4121	0.7371
	PCLG-32	13	6.3475	0.9318	0.8521	0.0935	0.8257
	PCLG-48	13	9.4620	0.9583	0.9037	0.0604	0.8850
	Loci	<i>na</i> *	<i>ne</i> *	Ho	He	D	PIC
	PCL24	3	2.8533	1.0000	0.6564	0.5235	0.5744
	PCLG-03	22	11.1304	0.8958	0.9197	-0.0260	0.9035
	PCLG-04	14	7.6928	0.8542	0.8792	-0.0284	0.8575
	PCLG-07	8	5.1086	0.7500	0.8127	-0.0772	0.7762
	PCLG-09	7	4.8659	0.7292	0.8029	-0.0918	0.7648
	PCLG-10	10	5.6127	0.6875	0.8305	-0.1722	0.8005
WX	PCLG-13	6	2.9009	0.4681	0.6623	-0.2932	0.6017
	PCLG-15	10	2.2588	0.2500	0.5632	-0.5561	0.5356

Pop.	Loci	<i>na</i> *	<i>ne</i> *	Ho	He	D	PIC
	PCLG-17	9	4.0563	1.0000	0.7614	0.3134	0.7302
	PCLG-29	13	8.4706	1.0000	0.8912	0.1221	0.8704
	PCLG-32	9	5.6959	0.8542	0.8331	0.0253	0.8026
	PCLG-48	9	6.4628	0.8542	0.8542	0.0000	0.8261
	Loci	<i>na</i> *	<i>ne</i> *	Ho	He	D	PIC
	PCL24	6	3.4830	1.0000	0.7204	0.3881	0.6676
	PCLG-03	27	17.4569	0.5778	0.9533	-0.3939	0.9398
	PCLG-04	14	8.0984	0.7917	0.8857	-0.1061	0.8652
	PCLG-07	9	3.4870	0.5745	0.7209	-0.2031	0.6810
	PCLG-09	7	4.5359	0.7826	0.7881	-0.0070	0.7480
	PCLG-10	9	4.4702	0.9333	0.7850	0.1889	0.7474
WJ	PCLG-13	5	3.7403	1.0000	0.7404	0.3506	0.6885
	PCLG-15	9	4.2706	0.6458	0.7739	-0.1655	0.7319
	PCLG-17	15	10.1098	0.9574	0.9108	0.0512	0.8931
	PCLG-29	6	4.5942	0.9167	0.7906	0.1595	0.7481
	PCLG-32	10	6.6039	1.0000	0.8577	0.1659	0.8301
	PCLG-48	8	3.2823	0.5745	0.7028	-0.1826	0.6659
	Loci	<i>na</i> *	<i>ne</i> *	Ho	He	D	PIC
	PCL24	8	6.5085	1.0000	0.8553	0.1692	0.8274
	PCLG-03	21	13.5132	0.6250	0.9357	-0.3321	0.9214
	PCLG-04	14	7.9460	1.0000	0.8836	0.1317	0.8623
	PCLG-07	10	4.5988	0.4375	0.7908	-0.4468	0.7587
	PCLG-09	6	4.5877	0.3617	0.7904	-0.5424	0.7474
	PCLG-10	5	2.5430	0.7708	0.6132	0.2570	0.5513
MAS	PCLG-13	8	5.1601	0.2917	0.8147	-0.6420	0.7801
	PCLG-15	3	1.9567	0.0417	0.4941	-0.9156	0.3880
	PCLG-17	12	7.3143	1.0000	0.8724	0.1463	0.8496
	PCLG-29	7	2.9018	0.6250	0.6623	-0.0563	0.5973
	PCLG-32	11	5.9922	0.9167	0.8419	0.0888	0.8159
	PCLG-48	9	4.2082	0.6250	0.7704	-0.1887	0.7309
	Loci	<i>na</i> *	<i>ne</i> *	Ho	He	D	PIC
	PCL24	7	4.8814	1.0000	0.8035	0.2446	0.7635
	PCLG-03	22	10.7413	0.2500	0.9164	-0.7272	0.9005
	PCLG-04	11	6.7965	0.4792	0.8618	-0.4440	0.8362
	PCLG-07	9	3.3635	0.3958	0.7101	-0.4426	0.6591

Pop.	Loci	<i>na</i> *	<i>ne</i> *	Ho	He	D	PIC
CJr	PCLG-09	5	3.2022	0.5625	0.6950	-0.1906	0.6390
	PCLG-10	7	2.6407	0.0833	0.6279	-0.8673	0.5742
	PCLG-13	6	3.3056	0.4167	0.7048	-0.4088	0.6450
	PCLG-15	10	7.3610	0.6042	0.8732	-0.3081	0.8495
	PCLG-17	10	6.1704	0.7447	0.8469	-0.1207	0.8192
	PCLG-29	10	5.3581	0.3958	0.8219	-0.5184	0.7918
	PCLG-32	13	8.4810	1.0000	0.8918	0.1213	0.8708
	PCLG-48	10	8.4810	1.0000	0.8274	0.2086	0.7962
CHL	Loci	<i>na</i> *	<i>ne</i> *	Ho	He	D	PIC
	PCL24	5	3.4083	0.9583	0.7140	0.3422	0.6532
	PCLG-03	17	9.8273	0.6667	0.9091	-0.2666	0.8898
	PCLG-04	17	10.3318	1.0000	0.9127	0.0957	0.8964
	PCLG-07	6	2.3679	0.4167	0.5838	-0.2862	0.5428
	PCLG-09	7	5.1030	0.8750	0.8125	0.0769	0.7761
	PCLG-10	3	1.7163	0.0000	0.4222	-1.0000	0.3603
	PCLG-13	9	4.3308	0.6875	0.7772	-0.1154	0.7368
	PCLG-15	11	4.9655	0.5833	0.8070	-0.2772	0.7750
	PCLG-17	12	4.3761	0.9583	0.7796	0.2292	0.7393
	PCLG-29	12	7.7315	0.9167	0.8798	0.0419	0.8577
	PCLG-32	14	7.6418	1.0000	0.8783	0.1386	0.8568
	PCLG-48	12	7.0244	0.8333	0.8667	-0.0385	0.8429
	HF	Loci	<i>na</i> *	<i>ne</i> *	Ho	He	D
PCL24		5	3.2224	1.0000	0.6969	0.4349	0.6265
PCLG-03		14	9.1610	0.5417	0.9002	-0.3982	0.8813
PCLG-04		12	8.1413	0.8333	0.8864	-0.0599	0.8648
PCLG-07		7	4.5851	0.3958	0.7901	-0.4991	0.7498
PCLG-09		9	4.0671	0.6458	0.7621	-0.1526	0.7141
PCLG-10		8	3.4621	1.0000	0.7186	0.3916	0.6650
PCLG-13		6	1.9955	0.1277	0.5042	-0.7467	0.4765
PCLG-15		9	4.2548	0.4583	0.7730	-0.4071	0.7381
PCLG-17		9	2.9711	0.8936	0.6706	0.3325	0.6055
PCLG-29		11	7.4638	0.8913	0.8755	0.0180	0.8540
PCLG-32		6	3.8417	0.5957	0.7477	-0.2033	0.7039
PCLG-48		9	5.3581	0.6042	0.8219	-0.2649	0.7866
		Loci	<i>na</i> *	<i>ne</i> *	Ho	He	D

Pop.	Loci	<i>na</i> *	<i>ne</i> *	Ho	He	D	PIC
DY	PCL24	5	3.8241	0.9583	0.7463	0.2841	0.6907
	PCLG-03	23	13.6331	0.5833	0.9364	-0.3771	0.9220
	PCLG-04	8	3.2821	0.9375	0.7026	0.3343	0.6487
	PCLG-07	10	3.9184	0.4583	0.7526	-0.3910	0.7129
	PCLG-09	7	4.4138	0.3125	0.7816	-0.6002	0.7399
	PCLG-10	6	3.1009	0.8125	0.6846	0.1868	0.6166
	PCLG-13	10	4.2548	0.3750	0.7730	-0.5149	0.7372
	PCLG-15	4	1.5479	0.3750	0.3577	0.0484	0.3296
	PCLG-17	8	5.0917	0.8125	0.8121	0.0005	0.7788
	PCLG-29	11	6.5829	0.8750	0.8570	0.0210	0.8315
	PCLG-32	8	5.2483	0.8750	0.8180	0.0697	0.7834
	PCLG-48	5	3.1648	0.1667	0.6912	-0.7588	0.6305
	SLt	Loci	<i>na</i> *	<i>ne</i> *	Ho	He	D
PCL24		6	3.4414	0.8333	0.7169	0.1624	0.6570
PCLG-03		23	14.0917	0.8542	0.9388	-0.0901	0.9249
PCLG-04		13	5.8701	0.9375	0.8384	0.1182	0.8088
PCLG-07		9	5.3457	0.6042	0.8215	-0.2645	0.7883
PCLG-09		8	3.8919	0.4375	0.7509	-0.4174	0.7123
PCLG-10		6	3.7131	0.4792	0.7384	-0.3510	0.6946
PCLG-13		6	3.1114	0.4375	0.6857	-0.3620	0.6265
PCLG-15		4	1.8633	0.3333	0.4682	-0.2881	0.4275
PCLG-17		13	6.9349	0.9111	0.8654	0.0528	0.8398
PCLG-29		8	6.0792	0.7917	0.8443	-0.0623	0.8143
PCLG-32		15	7.0016	0.8723	0.8664	0.0068	0.8424
PCLG-48		13	7.8367	0.9167	0.8816	0.0398	0.8594
NBp	Loci	<i>na</i> *	<i>ne</i> *	Ho	He	D	PIC
	PCL24	9	4.4223	0.9792	0.7820	0.2522	0.7396
	PCLG-03	20	11.2992	0.8723	0.9213	-0.0532	0.9052
	PCLG-04	18	7.1111	0.7708	0.8684	-0.1124	0.8470
	PCLG-07	8	3.8241	0.3958	0.7463	-0.4697	0.7030
	PCLG-09	6	3.7740	0.4375	0.7428	-0.4110	0.6904
	PCLG-10	10	4.9336	0.6250	0.8057	-0.2243	0.7715
	PCLG-13	9	2.7494	0.4167	0.6430	-0.3519	0.6050
	PCLG-15	8	3.1692	0.4792	0.6917	-0.3072	0.6493
	PCLG-17	10	6.3297	1.0000	0.8509	0.1752	0.8247
PCLG-29	8	5.3088	0.7292	0.8202	-0.1109	0.7869	

Pop.	Loci	<i>na</i> *	<i>ne</i> *	Ho	He	D	PIC
	PCLG-32	13	7.7367	0.9130	0.8803	0.0371	0.8572
	PCLG-48	9	5.0361	0.7708	0.8099	-0.0483	0.7739
	Loci	<i>na</i> *	<i>ne</i> *	Ho	He	D	PIC
	PCL24	5	3.1118	1.0000	0.6861	0.4575	0.6155
	PCLG-03	20	7.6041	0.6596	0.8778	-0.2486	0.8564
	PCLG-04	18	9.1850	0.8298	0.9007	-0.0787	0.8821
	PCLG-07	7	3.6302	0.5319	0.7323	-0.2737	0.6765
	PCLG-09	10	4.1033	0.2292	0.7643	-0.7001	0.7314
	PCLG-10	7	3.5832	0.2500	0.7285	-0.6568	0.6856
PYL	PCLG-13	6	4.2314	0.4167	0.7717	-0.4600	0.7284
	PCLG-15	3	1.2072	0.1875	0.1735	0.0807	0.1601
	PCLG-17	7	5.4148	0.9167	0.8239	0.1126	0.7894
	PCLG-29	7	5.1717	0.7500	0.8151	-0.0799	0.7779
	PCLG-32	9	6.2976	0.6087	0.8505	-0.2843	0.8218
	PCLG-48	8	5.1314	0.6875	0.8136	-0.1550	0.7780
	Loci	<i>na</i> *	<i>ne</i> *	Ho	He	D	PIC
	PCL24	7	4.4393	1.0000	0.7829	0.2773	0.7408
	PCLG-03	16	10.3953	0.5106	0.9135	-0.4411	0.8962
	PCLG-04	14	10.6914	1.0000	0.9160	0.0917	0.8988
	PCLG-07	5	2.4792	0.2826	0.6032	-0.5315	0.5207
	PCLG-09	11	6.8496	0.6170	0.8632	-0.2852	0.8375
	PCLG-10	6	2.7378	0.9268	0.6426	0.4423	0.5661
NCyl	PCLG-13	8	3.4491	0.4375	0.7175	-0.3902	0.6800
	PCLG-15	7	3.2565	0.2708	0.7002	-0.6133	0.6449
	PCLG-17	14	10.2269	0.9787	0.9119	0.0733	0.8939
	PCLG-29	7	3.1030	0.6667	0.6849	-0.0266	0.6220
	PCLG-32	8	5.1396	0.4000	0.8145	-0.5089	0.7797
	PCLG-48	14	6.8571	1.0000	0.8632	0.1585	0.8393
	Loci	<i>na</i> *	<i>ne</i> *	Ho	He	D	PIC
	PCL24	7	4.0104	1.0000	0.7586	0.3182	0.7144
	PCLG-03	20	8.9825	0.6042	0.8980	-0.3272	0.8796
	PCLG-04	16	9.7834	0.9167	0.9072	0.0105	0.8897
	PCLG-07	6	3.7433	0.6875	0.7406	-0.0717	0.6950
	PCLG-09	11	7.8635	0.9792	0.8820	0.1102	0.8594
	PCLG-10	9	3.2844	0.5208	0.7029	-0.2591	0.6585

Pop.	Loci	<i>na</i> *	<i>ne</i> *	Ho	He	D	PIC
NHL	PCLG-13	7	4.9389	0.7917	0.8059	-0.0176	0.7711
	PCLG-15	11	4.2905	0.5833	0.7750	-0.2474	0.7466
	PCLG-17	12	7.8904	0.9167	0.8825	0.0388	0.8605
	PCLG-29	8	5.0205	0.7872	0.8094	-0.0274	0.7730
	PCLG-32	8	5.1486	0.8958	0.8143	0.1001	0.7790
	PCLG-48	14	6.5548	0.6458	0.8564	-0.2459	0.8326
	Loci	<i>na</i> *	<i>ne</i> *	Ho	He	D	PIC
	PCL24	6	4.5489	0.8958	0.7884	0.1362	0.7482
	PCLG-03	21	7.7707	0.5417	0.8805	-0.3848	0.8623
	PCLG-04	13	6.8166	0.9375	0.8623	0.0872	0.8398
	PCLG-07	8	4.8403	0.6667	0.8018	-0.1685	0.7627
	PCLG-09	10	6.6398	0.5417	0.8583	-0.3689	0.8319
	PCLG-10	9	3.9150	0.9375	0.7524	0.2460	0.7095
YNL	PCLG-13	10	7.1664	0.4792	0.8695	-0.4489	0.8443
	PCLG-15	11	4.4436	0.7500	0.7831	-0.0423	0.7423
	PCLG-17	13	9.0176	0.9583	0.8985	0.0666	0.8788
	PCLG-29	9	5.5186	0.8542	0.8274	0.0324	0.7984
	PCLG-32	10	6.0792	0.9792	0.8443	0.1598	0.8152
	PCLG-48	7	3.2821	0.2917	0.7026	-0.5848	0.6523
	Loci	<i>na</i> *	<i>ne</i> *	Ho	He	D	PIC
	PCL24	3	2.6995	0.9583	0.6362	0.5063	0.5585
	PCLG-03	20	9.6842	0.5217	0.9066	-0.4246	0.8890
	PCLG-04	13	7.9448	0.5833	0.8833	-0.3396	0.8621
	PCLG-07	8	4.1033	0.5000	0.7643	-0.3458	0.7219
	PCLG-09	9	6.2667	0.9149	0.8495	0.0770	0.8213
	PCLG-10	9	4.6126	0.8958	0.7914	0.1319	0.7616
XT	PCLG-13	7	5.3895	0.4792	0.8230	-0.4177	0.7889
	PCLG-15	8	4.0457	0.5000	0.7607	-0.3427	0.7235
	PCLG-17	6	3.4883	0.9375	0.7208	0.3006	0.6653
	PCLG-29	12	6.0393	0.8542	0.8432	0.0130	0.8150
	PCLG-32	8	5.7171	0.9792	0.8338	0.1744	0.8024
	PCLG-48	7	3.4212	0.3913	0.7155	-0.4531	0.6612
	Loci	<i>na</i> *	<i>ne</i> *	Ho	He	D	PIC
	PCL24	9	3.4936	0.7083	0.7213	-0.0180	0.6786
	PCLG-03	16	6.2693	0.2889	0.8499	-0.6601	0.8244

Pop.	Loci	<i>na</i> *	<i>ne</i> *	Ho	He	D	PIC
QJ	PCLG-04	18	10.4253	0.8958	0.9136	-0.0195	0.8970
	PCLG-07	5	2.1235	0.4167	0.5346	-0.2205	0.4937
	PCLG-09	11	7.2466	0.6739	0.8715	-0.2267	0.8470
	PCLG-10	11	7.1029	0.3830	0.8685	-0.5590	0.8439
	PCLG-13	4	3.4465	0.3750	0.7173	-0.4772	0.6568
	PCLG-15	7	5.2068	0.9583	0.8164	0.1738	0.7793
	PCLG-17	10	6.0711	1.0000	0.8441	0.1847	0.8152
	PCLG-29	10	3.3758	0.8125	0.7112	0.1424	0.6608
	PCLG-32	12	7.4323	0.7083	0.8746	-0.1901	0.8513
	PCLG-48	6	3.8400	0.8958	0.7474	0.1986	0.6943
LZL	Loci	<i>na</i> *	<i>ne</i> *	Ho	He	D	PIC
	PCL24	5	3.3391	1.0000	0.7079	0.4126	0.6420
	PCLG-03	19	9.6463	0.7021	0.9060	-0.2251	0.8880
	PCLG-04	11	5.8551	0.2708	0.8379	-0.6768	0.8076
	PCLG-07	6	2.9811	0.4894	0.6717	-0.2714	0.6117
	PCLG-09	9	4.7554	0.6458	0.7980	-0.1907	0.7634
	PCLG-10	10	4.6762	0.8043	0.7948	0.0120	0.7650
	PCLG-13	7	4.8150	0.4792	0.8007	-0.4015	0.7628
	PCLG-15	9	4.2627	0.6042	0.7735	-0.2189	0.7339
	PCLG-17	10	4.9126	0.9792	0.8048	0.2167	0.7668
	PCLG-29	13	9.1793	0.8750	0.9004	-0.0282	0.8811
	PCLG-32	12	9.5602	0.7083	0.9048	-0.2172	0.8859
	PCLG-48	5	3.6894	0.5000	0.7366	-0.3212	0.6817
	HHL	Loci	<i>na</i> *	<i>ne</i> *	Ho	He	D
PCL24		4	2.8462	1.0000	0.6555	0.5256	0.5793
PCLG-03		15	7.5571	0.1522	0.8772	-0.8265	0.8544
PCLG-04		20	11.6263	0.8723	0.9238	-0.0557	0.9079
PCLG-07		14	7.4323	0.5625	0.8746	-0.3568	0.8530
PCLG-09		12	6.0157	0.9583	0.8425	0.1374	0.8156
PCLG-10		13	8.0217	0.7674	0.8856	-0.1335	0.8629
PCLG-13		5	2.4602	0.8750	0.5998	0.4588	0.5160
PCLG-15		8	2.5671	0.3125	0.6169	-0.4934	0.5834
PCLG-17		12	6.1358	0.9167	0.8458	0.0838	0.8187
PCLG-29		10	6.1114	0.9167	0.8452	0.0846	0.8181
PCLG-32		8	6.2355	0.9375	0.8485	0.1049	0.8196
PCLG-48	7	3.1911	0.6875	0.6939	-0.0092	0.6423	

Pop.	Loci	<i>na</i> *	<i>ne</i> *	Ho	He	D	PIC
CHL	Loci	<i>na</i> *	<i>ne</i> *	Ho	He	D	PIC
	PCL24	5	2.9307	0.9348	0.6660	0.4036	0.5890
	PCLG-03	15	7.6928	0.6042	0.8792	-0.3128	0.8571
	PCLG-04	14	5.1938	0.3953	0.8170	-0.5162	0.7924
	PCLG-07	5	2.3370	0.0889	0.5785	-0.8463	0.4800
	PCLG-09	8	5.2182	0.7174	0.8172	-0.1221	0.7836
	PCLG-10	12	7.0145	0.5909	0.8673	-0.3187	0.8430
	PCLG-13	8	4.0457	0.4792	0.7607	-0.3701	0.7131
	PCLG-15	5	1.4495	0.2708	0.3134	-0.1359	0.2929
	PCLG-17	11	4.2686	0.8511	0.7740	0.0996	0.7342
	PCLG-29	11	5.0805	0.4583	0.8116	-0.4353	0.7829
	PCLG-32	10	5.6569	0.7660	0.8321	-0.0794	0.8022
	PCLG-48	11	6.7761	0.8936	0.8616	0.0371	0.8367
YJ	Loci	<i>na</i> *	<i>ne</i> *	Ho	He	D	PIC
	PCL24	5	3.0396	0.9583	0.6781	0.4132	0.6089
	PCLG-03	9	4.0887	0.1667	0.7634	-0.7816	0.7152
	PCLG-04	10	7.5417	0.8333	0.8765	-0.0493	0.8533
	PCLG-07	7	5.2247	0.6957	0.8175	-0.1490	0.7816
	PCLG-09	8	4.2076	0.2766	0.7705	-0.6410	0.7366
	PCLG-10	10	4.6498	0.5417	0.7932	-0.3171	0.7570
	PCLG-13	7	3.6600	0.2917	0.7344	-0.6028	0.6846
	PCLG-15	5	2.0719	0.4583	0.5228	-0.1234	0.4528
	PCLG-17	8	4.9585	0.6596	0.8069	-0.1826	0.7692
	PCLG-29	12	6.5641	0.8958	0.8566	0.0458	0.8321
	PCLG-32	8	5.5783	0.9362	0.8296	0.1285	0.7970
	PCLG-48	6	2.8166	0.4167	0.6518	-0.3607	0.6065
NX	Loci	<i>na</i> *	<i>ne</i> *	Ho	He	D	PIC
	PCL24	4	2.9501	0.8750	0.6680	0.3099	0.5983
	PCLG-03	12	4.8126	0.1489	0.8007	-0.8140	0.7671
	PCLG-04	19	9.3849	0.8750	0.9029	-0.0309	0.8851
	PCLG-07	7	4.6702	0.8085	0.7943	0.0179	0.7551
	PCLG-09	9	3.5558	0.4222	0.7268	-0.4191	0.6886
	PCLG-10	3	2.3815	0.3043	0.5865	-0.4812	0.5120
	PCLG-13	7	5.5186	0.3958	0.8274	-0.5216	0.7939
PCLG-15	5	2.2143	0.3542	0.5542	-0.3609	0.4917	

Pop.	Loci	<i>na</i> *	<i>ne</i> *	Ho	He	D	PIC
	PCLG-17	8	5.3832	0.8750	0.8228	0.0634	0.7886
	PCLG-29	8	3.4543	0.6458	0.7180	-0.1006	0.6628
	PCLG-32	8	5.8132	0.5870	0.8371	-0.2988	0.8051
	PCLG-48	6	2.7356	0.3913	0.6414	-0.3899	0.5847
	Loci	<i>na</i> *	<i>ne</i> *	Ho	He	D	PIC
	PCL24	6	3.7418	1.0000	0.7408	0.3499	0.6840
	PCLG-03	15	8.0742	0.5814	0.8865	-0.3442	0.8657
	PCLG-04	16	7.8472	0.7447	0.8819	-0.1556	0.8614
	PCLG-07	5	2.8872	0.7083	0.6605	0.0724	0.5967
	PCLG-09	5	3.9685	0.3333	0.7570	-0.5597	0.7041
	PCLG-10	7	3.5611	0.4375	0.7268	-0.3980	0.6778
DTL	PCLG-13	8	6.4538	0.4167	0.8539	-0.5120	0.8267
	PCLG-15	12	6.8674	0.7917	0.8634	-0.0830	0.8387
	PCLG-17	7	4.0907	0.9787	0.7637	0.2815	0.7164
	PCLG-29	9	5.0582	0.8750	0.8107	0.0793	0.7771
	PCLG-32	9	5.5385	0.8958	0.8281	0.0818	0.7980
	PCLG-48	5	2.8764	0.3750	0.6592	-0.4311	0.6037
	Loci	<i>na</i> *	<i>ne</i> *	Ho	He	D	PIC
	PCL24	7	3.9827	1.0000	0.7568	0.3214	0.7074
	PCLG-03	18	9.9525	0.4375	0.9090	-0.5187	0.8915
	PCLG-04	10	3.0157	0.4375	0.6754	-0.3522	0.6344
	PCLG-07	9	4.2627	0.7292	0.7735	-0.0573	0.7323
	PCLG-09	11	7.0244	0.9792	0.8667	0.1298	0.8417
	PCLG-10	8	4.8403	0.8958	0.8018	0.1172	0.7668
DTLs	PCLG-13	8	5.5252	0.3958	0.8276	-0.5217	0.7995
	PCLG-15	9	5.0526	0.6458	0.8105	-0.2032	0.7780
	PCLG-17	11	6.6494	1.0000	0.8586	0.1647	0.8349
	PCLG-29	12	8.8276	0.8958	0.8961	-0.0003	0.8758
	PCLG-32	12	7.4040	0.8000	0.8747	-0.0854	0.8506
	PCLG-48	8	3.9284	0.2292	0.7533	-0.6957	0.7073
	Loci	<i>na</i> *	<i>ne</i> *	Ho	He	D	PIC
	PCL24	5	2.4615	1.0000	0.6000	0.6667	0.5093
	PCLG-03	18	8.9825	0.8750	0.8980	-0.0256	0.8793
	PCLG-04	15	7.9723	0.9167	0.8838	0.0372	0.8627
	PCLG-07	12	5.5054	0.3958	0.8270	-0.5214	0.7974

Pop.	Loci	<i>na</i> *	<i>ne</i> *	Ho	He	D	PIC
CQs	PCLG-09	8	4.6651	0.4318	0.7947	-0.4567	0.7541
	PCLG-10	8	5.7672	0.3750	0.8353	-0.5511	0.8047
	PCLG-13	6	3.6953	0.8750	0.7371	0.1871	0.6809
	PCLG-15	3	1.8893	0.0208	0.4757	-0.9563	0.3693
	PCLG-17	12	5.9305	0.9375	0.8401	0.1159	0.8119
	PCLG-29	6	4.9921	1.0000	0.8083	0.2372	0.7709
	PCLG-32	7	3.7858	0.9362	0.7438	0.2587	0.6952
	PCLG-48	8	2.9482	0.8542	0.6678	0.2791	0.6115
ZX	Loci	<i>na</i>	<i>ne</i>	Ho	He	D	PIC
	PCL24	4	3.2337	0.9792	0.6980	0.4029	0.6356
	PCLG-03	9	3.8793	0.4222	0.7506	-0.4375	0.7159
	PCLG-04	8	4.1626	0.4583	0.7678	-0.4031	0.7296
	PCLG-07	6	3.3907	0.4167	0.7125	-0.4152	0.6576
	PCLG-09	6	4.2769	0.0213	0.7744	-0.9725	0.7281
	PCLG-10	7	3.7864	0.9792	0.7436	0.3168	0.6920
	PCLG-13	7	4.5760	0.3750	0.7897	-0.5251	0.7502
	PCLG-15	2	1.2143	0.1522	0.1785	-0.1473	0.1609
	PCLG-17	5	3.1700	0.9565	0.6921	0.3820	0.6310
	PCLG-29	8	4.7982	0.8043	0.8003	0.0050	0.7599
	PCLG-32	7	3.9294	0.9783	0.7537	0.2980	0.7050
	PCLG-48	8	3.7586	0.3542	0.7417	-0.5224	0.6981
	JY	Loci	<i>na</i> *	<i>ne</i> *	Ho	He	D
PCL24		4	3.5149	1.0000	0.7230	0.3831	0.6632
PCLG-03		15	6.9189	0.4792	0.8645	-0.4457	0.8425
PCLG-04		9	4.8505	0.4792	0.8022	-0.4026	0.7690
PCLG-07		6	3.2867	0.4375	0.7031	-0.3778	0.6458
PCLG-09		7	4.6452	0.0417	0.7930	-0.9474	0.7529
PCLG-10		5	4.1021	0.9574	0.7644	0.2525	0.7163
PCLG-13		7	3.8336	0.3542	0.7469	-0.5258	0.7001
PCLG-15		7	1.7588	0.3191	0.4361	-0.2683	0.4157
PCLG-17		11	5.5855	1.0000	0.8296	0.2054	0.7999
PCLG-29		8	4.8865	0.6875	0.8037	-0.1446	0.7666
PCLG-32		8	4.9389	0.9792	0.8059	0.2150	0.7676
PCLG-48		7	3.7664	0.5957	0.7424	-0.1976	0.6961
	Loci	<i>na</i> *	<i>ne</i> *	Ho	He	D	PIC

Pop.	Loci	<i>na</i> *	<i>ne</i> *	Ho	He	D	PIC	
Jap	PCL24	18	11.8154	1.0000	0.9250	0.0811	0.9092	
	PCLG-03	29	14.7759	0.7660	0.9423	-0.1871	0.9288	
	PCLG-04	13	9.2530	0.4375	0.9013	-0.5146	0.8821	
	PCLG-07	10	6.3911	0.5208	0.8524	-0.3890	0.8246	
	PCLG-09	10	7.4805	0.5000	0.8754	-0.4288	0.8521	
	PCLG-10	15	10.5442	0.5106	0.9149	-0.4419	0.8976	
	PCLG-13	7	3.3295	0.3750	0.7070	-0.4696	0.6680	
	PC							
	LG-15	17	8.7605	0.5625	0.8952	-0.3716	0.8767	
	PCLG-17	11	9.3091	0.4792	0.9020	-0.4687	0.8827	
	PCLG-29	12	9.2427	0.6170	0.9014	-0.3155	0.8819	
	PCLG-32	16	11.9070	0.7917	0.9257	-0.1448	0.9098	
	PCLG-48	14	9.6200	0.9792	0.9055	0.0814	0.8867	
	Ame	Loci	<i>na</i> *	<i>ne</i> *	Ho	He	D	PIC
PCL24		10	7.3880	0.6170	0.8739	-0.2940	0.8502	
PCLG-03		26	17.1301	0.7500	0.9515	-0.2118	0.9387	
PCLG-04		26	14.4000	0.6250	0.9404	-0.3354	0.9265	
PCLG-07		14	8.6292	0.7292	0.8934	-0.1838	0.8731	
PCLG-09		12	9.8837	0.7872	0.9085	-0.1335	0.8900	
PCLG-10		13	5.3644	0.4167	0.8221	-0.4931	0.8011	
PCLG-13		6	4.5669	0.5417	0.7893	-0.3137	0.7482	
PCLG-15		14	8.9130	0.6042	0.8971	-0.3265	0.8780	
PCLG-17		17	8.5810	0.5625	0.8928	-0.3700	0.8724	
PCLG-29		10	5.9305	0.5833	0.8401	-0.3057	0.8103	
PCLG-32		14	8.0419	0.6250	0.8849	-0.2937	0.8640	
PCLG-48		13	6.9502	0.9167	0.8651	0.0596	0.8419	

na: Observed number of alleles; ne: Effective number of alleles.