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Population Genetic Structure of Pacific White Shrimp (*Litopenaeus vannamei*) from Mexico to Panama: Microsatellite DNA Variation

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Abstract: Genetic variation and population structure of wild white shrimp (*Litopenaeus vannamei*) from 4 geographic locations from Mexico to Panama were investigated using 5 microsatellite DNA loci. The genetic diversity between populations was indicated by the mean number of alleles per locus and mean observed heterozygosity, which ranged from 7.4 to 8.6 and from 0.241 to 0.388, respectively. Significant departures from Hardy-Weinberg equilibrium were found at most locations at each locus, with the exception Guatemala at *Pvan0013*, and were caused by high heterozygote deficiencies. Genetic differences between localities were detected by pairwise comparison based on allelic and genotypic frequencies, with the exception of locus *Pvan1003*. Significant pairwise F_{ST} values between locations and total F_{ST} showed that the white shrimp population is structured into subpopulations. However, population differentiation does not follow an isolation-by-distance model. Knowledge of the genetic diversity and structure of *L.vannamei* populations will be of interest for aquaculture and fisheries management to utilize and preserve aquatic biodiversity.

Key words: genetic structure, microsatellites, Pacific white shrimp, *Litopenaeus vannamei*, population genetics.

INTRODUCTION

The white shrimp *Litopenaeus vannamei* is one of the major commercial shrimp species of the Eastern Pacific, with fisheries in Mexico (Martínez-Córdova and Campaña-Torres, 1999), Guatemala, El Salvador (Dore and Frimodt, 1987), and aquaculture production in the United States, Mexico, Panama, Colombia, Ecuador (Martínez-Córdova and Campaña-Torres, 1999), and Brazil. For instance, of a total of 90,287 metric tons of shrimp production in Mexico during 2001, 52% came from cultured shrimp (SAGARPA, 2002), of which the main species is *L. vannamei*. This species has a wide range of distribution from northern Mexico to northern Peru (Perez-Farfante and Kensley, 1997) and inhabits coastal lagoons and open coastal waters to depths of 70 m. Its life cycle consists of an oceanic planktonic larval stage, an estuarine postlarva-to-juvenile stage, and a return to the marine environment as an adult to mature and spawn.

The management and conservation of exploited resources must include aspects of the genetic diversity of the

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species. The genetic definition of stocks has been a major concern in understanding natural resources to ensure sustainability of fisheries resources. Because different broodstocks or seedstocks might differ in growth rate, or disease resistance, among other characteristics (Lester and Pante, 1992; Klinbunga et al., 2001), identification of wild shrimp stocks is also important to provide an available source of wild genetic diversity in domestication and selective breeding programs (Benzie, 2000; Klinbunga et al., 2001). The use of genetic markers has been greatly improved our understanding of population structure in penaeids (Benzie, 2000). Allozyme analysis has been the most commonly used method to determine the levels of variation and the degree of genetic subdivision for several shrimp species (Lester and Pante, 1992; de la Rosa-Velez et al., 2000; Garcia-Machado et al., 2001). Use of molecular markers such as mitochondrial DNA restriction fragment length polymorphisms (RELPs) (Benzie et al., 1993; Klinbunga et al., 1999, 2001; Garcia-Machado et al., 2001), randomly amplified polymorphic DNAs (Aubert and Lightner, 2000; Benzie, 2000), and microsatellites (Wolfus et al., 1997; Brooker et al., 2000; Supungul et al., 2000) has revealed more variability than allozymes. Despite the economic importance of the white shrimp *L. vannamei*, knowledge of the genetic structure and diversity of the natural populations throughout its range of distribution is limited. Sunden and Davis (1991) found very low levels of genetic variation, heterozygosity, and population subdivision for 3 wild populations of *L. vannamei* from Mexico, Panama, and Ecuador using allozymes. In contrast, de la Rosa-Velez et al. (1999) found higher allozyme variation of *L. vannamei* in a sample from northern Mexico. High heterozygosity values in 5 domestic populations and a single wild female broodstock population of *L. vannamei* were found using one microsatellite locus (Wolfus et al., 1997).

Many marine species, including the penaeids, are thought to have a high dispersal capacity throughout their geographic range during their larval planktonic phases, caused by oceanographic current (Féral, 2002). However, since the habitats of coastal species are rarely continuous, heterogeneous genetic diversity could be generated by some environmental factors (e.g., currents and physical barriers) and biological factors (e.g., reproductive behavior) (Féral, 2002). In fact, geographic differentiations have been observed in some penaeids (Aubert and Lightner, 2000; de la Rosa-Velez et al., 2000; Garcia-Machado et al., 2001; Xu et al., 2001).

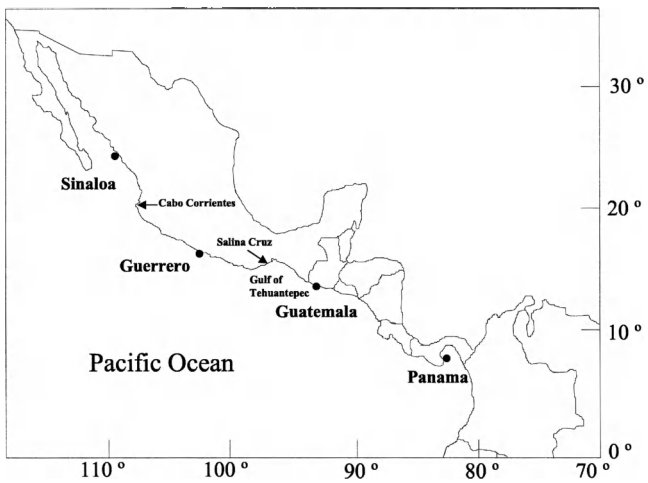

Figure 1. Collection sites of *Litopenaeus vannamei* samples in the Eastern Pacific.

Oceanographic currents and local hydrographic events affect the environmental conditions along the Eastern Pacific (de la Lanza, 1991). Three major currents occur along the Pacific coast from Mexico to Panama: the North Equatorial Current, the Equatorial Counter Current, and the Costa Rica Coastal Current (de la Lanza, 1991). These processes may be responsible for the habitat fragmentation along the Pacific coast, acting as barriers to gene flow and contributing to the genetic differentiation of the species. In fact, genetic structure has been shown for the mollusk *Pinctada mazatlanica* along this region (Arnaud et al., 2000). Our hypothesis is that the white shrimp could be genetically structured along its latitudinal range of distribution owing to geographic heterogeneity. To evaluate the genetic structure of wild *L. vannamei*, 5 microsatellite loci were use to analyze samples from 4 geographically distant locations along its area of distribution in the Eastern Pacific.

Materials and Methods

Sample Collection Sites and Storage

Samples of wild white shrimp (*L. vannamei*) were collected from 4 locations in the Eastern Pacific: Sinaloa and Guerrero in Mexico, Guatemala, and Panama (Figure 1). All samples were collected at coastal lagoons and individually measured (Table 1). For all samples muscle tissue from at least 50 individuals was removed from each specimen and stored in 95% ethanol. The Panama sample consisted of postlarvae and juveniles that were pooled and treated as a

Table 1. Collection Sites of *Litopenaeus vannamei* populations from Eastern Pacific

Location	Sampling date	No. specimens	Mean L.C.[a](mm)	SD
Sinaloa	Mar 2002	57	51.6	4.8
Guerrero	Apr 2002	50	28.8	1.9
Guatemala	Dec 2001	50	NA	
Panama	Jun 2001	20 (juveniles)	NA	
Panama	Jun 2001	30 (postlarvae)	8.0[b]	0.07

[a]Length of cephalothorax (L.C.); NA indicates not available.
[b]Total length.

single sample after statistical analysis indicated no differences in allelic or genotypic frequencies.

DNA Extraction

Total genomic DNA was extracted from each specimen using 700 µl TNES-urea (10 mM Tris-HCl, pH 7.5, 1.5 M NaCl, 10 mM EDTA, 0.5% sodium dodecylsulfhate [SDS], 4 M urea) and 5 µl of proteinase K (50 µg/µl final conc.). The mixture was incubated overnight at 37°C for 16 hours. DNA was organically extracted in 2 successive steps with phenol–chloroform–isoamyl alcohol (25:24:1) and chloroform–isoamyl alcohol (24:1), and precipitated with a double volume of 99% cold ethanol and 50 µl of 3 M sodium acetate trihydrate. The DNA pellet was washed with 70% ethanol, air-dried for 20 minutes, resuspended in 100 µl TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.2), and preserved at 4°C.

Microsatellite Loci Amplification

The microsatellite loci for white shrimp were amplified by the polymerase chain reaction (PCR) with 5 species-specific primers: *Pvan0013, Pvan0040, Pvan1003, Pvan1758*, and *Pvan1815* (Cruz et al., 2002). Primers were ordered from Sigma-Genosys, with the reverse primer 5′-end-labeled with biotin. Amplification was done in a 25-µl reaction volume containing 50 ng of DNA, 10 pmol of each primer, 2.1 mM $MgCl_2$, 0.2 mM each dNTP, and 0.25 U of *Taq* polymerase (Roche). PCR amplifications were done in a Genius thermal cycler (Techne) with the following profile: 4 minutes at 95°C, 35 cycles of 1 minute at 95°C, 1 minute at the annealing temperature, and 1 minute at 72°C, and a final extension at 72°C for 4 minutes (Cruz et al., 2002). PCR products were separated by electrophoresis on 8 M urea 6% denaturing polyacrylamide gels. DNA from gels was transferred to a nylon membrane (Nytran-N) and visualized with the chemiluminescent Phototope-Star Detection Kit (New England Riglabs) (Cruz et al., 2002). The emitted light was detected by exposing the membrane to an x-ray film (Kodak X-Omat). A standard M13 sequencing reaction was used as DNA size marker utilizing the SequiTherm Cycle Sequencing Kit (Epicentre Tech).

Variation in Microsatellite Loci and Departure from Hardy-Weinberg Equilibrium

The observed heterozygosity (H_o) was calculated directly from observed genotypes. The number of the alleles (A) and expected heterozygosity (H_e) were determined for each population at each locus using GENEPOP Version 3.3 (Raymond and Rousset, 1995). The effective number of alleles (a_e) was calculated by $a_e = 1/\Sigma x^2_i$, where x_i is the frequency of the ith allele for each locus. Heterozygous deficiency or excess at each locus was calculated by $D = (H_o - H_e)/(H_e)$. Departures from Hardy-Weinberg equilibrium (HWE) at each population were tested, and a global test of hetererozygote deficit across loci and across populations was conducted by GENEPOP. The Markov chain method was employed to estimate the probability of significant deviation from HWE using GENEPOP with the following parameters: dememorization = 1000, batches = 100, and iterations = 1000. Critically significant levels for the test were adjusted using a sequential Bonferroni approach (Rice, 1989). We tested each pair locus across all populations for linkage disequilibrium, under the null hypothesis of no association between genotypes in GENEPOP, using Fisher's test (dememorization = 1000, batches = 100, iterations = 1000). The Wright's F statistics were estimated by the analogue θ (Weir and Cockerham, 1984) by FSTAT Version 2.9.3 (Goudet, 2001). The significance of F_{IS}, which estimates the reduction in the average proportion of heterozygous genotypes within populations, was estimated by a random permutation procedure (n = 1000; genotypes were permuted not assuming HWE within samples) that tests whether or not the values differ from zero (Goudet, 2001).

Genetic Differentiation Among Localities

F_{ST}, which measures the reduction in the average proportion of heterozygous genotypes among localities, was

estimated by FSTAT to determine population genetic differentiation. Its significance values were based on permuting multilocus genotypes among samples (Goudet, 2001). Pairwise F_{ST} values between all populations, with their significances, were also calculated with GENEPOP. Differences in the number of alleles and genotypes among locations at each locus were tested by Wilcoxon signed rank tests. Critically significant levels for both tests were adjusted using a sequential Bonferroni approach (Rice, 1989).

The genetic divergence among localities was examined by an exact test and a log-likelihood G test by GENEPOP, which estimates the probability of whether or not the allelic and genotypic distribution was identical between all pairs of populations. A regression analysis of F_{ST} versus the logarithm of the geographic distance for all pairs of localities was done to test isolation by distance using GENEPOP. A Mantel test with 1000 permutations was used to test the significance of this correlation.

Results

Variation in Microsatellite Loci and Departure from Hardy-Weinberg Equilibrium

The genetic variability of *L. vannamei* from 4 populations at 5 microsatellite DNA loci is shown in Table 2. The number of alleles per locus (A) across all 4 populations combined ranged from 3 to 13.2, the mean observed heterozygosity (H_o) ranged from 0.164 to 0.535, and the mean expected heterozygosity (H_e) ranged from 0.385 to 0.857. The mean number of alleles per locus and the mean observed heterozygosity were similar among across all populations, and ranged from 7.4 to 8.6 and from 0.241 to 0.388, respectively. Within populations, the number of alleles ranged from 2 to 16, the effective number of alleles (a_e) ranged from 1.5 to 10.5, and the observed heterozygosity (H_o) from 0.045 to 0.614. We found 2 groups of microsatellites, one with lower allele numbers (less than 5) (*Pvan0013, Pvan1003*) and other with higher allele numbers (more than 7) (*Pvan0040, Pvan1758, Pvan1815*). The larger variation in the number of alleles was for the 3 large microsatellite loci (Table 2).

In 19 of 20 possible tests, significant deviations from HWE were observed after the sequential Bonferroni procedure (Table 2). All populations at each locus exhibited heterozygous deficiencies, except Guatemala with *Pvan0013* that was in HWE. Global tests both across loci within each population and across populations within each locus showed departures from HWE ($P < 0.05$). A HWE test for null alleles was performed using the EM algorithm in GENEPOP. First, the frequency of a null allele suspected at a locus was estimated, then the data set was corrected, and HWE was tested again. The results based on the "null allele" data set showed that no heterozygous deficiencies occurred in any of the populations at any loci. Moreover, *Pvan0013* and *Pvan1815* loci showed a significant excess heterozygosity, suggesting that the presence of null alleles could be negligible. The linkage disequilibria analyses were nonsignificant for all pair of loci, indicating no association between genotypes; therefore, we could consider each locus as an independent estimate (data not shown).

The size frequency distribution of microsatellite alleles varied among the 4 populations studied (Figure 2). However, in 3 loci, *Pvan0013, Pvan0040,* and *Pvan1003*, the most common allele was the same in all populations. Loci *Pvan1758* and *Pvan1815* showed the highest allele size variation, and several unique alleles were present in each population (Figure 2).

The F_{IS} positive values indicated a significant reduction in the average proportion of heterozygous genotypes within populations at all loci, owing to heterozygous deficiency in one or more populations (Table 3). The values of F_{IS} were significantly higher than zero, both globally and at individual loci (Table 3).

Genetic Differentiation Among Populations

An overall significant F_{ST} value of 0.055 indicates substantial genetic differentiation of the *L vannamei* populations sampled at all loci except for *Pvan1003* (Table 3). The pairwise F_{ST} values showed significant genetic differences between all pairs of populations (Table 4). According to the Wilcoxon's signed rank test, the number of alleles and genotypes at the 5 microsatellite loci did not differ significant between pairs of populations.

Similar to F_{ST}, most pairwise test comparisons of allelic and genotypic frequencies showed a genetic differentiation among populations at most loci with the exception of *Pvan1003* (Table 5). Additionally, Sinaloa showed no genetic differences in genotype frequencies with Guerrero at *Pvan0040*, or with Guatemala at *Pvan0013* and *Pvan0040*. A pairwise comparison of allelic and genotypic frequencies based on the null allele data set using the EM algorithm was

Table 2. Genetic Variability of *Litopeneaus vannamei* Population at Four Locations and Five Loci

Population[a]	Locus					
	Pvan0013	*Pvan0040*	*Pvan1003*	*Pvan1758*	*Pvan1815*	Mean
Sinaloa						
Sample size	39	44	44	41	40	41.6
A	4	11	3	13	12	8.6
a_e	3.1	3.1	1.5	9.4	8.7	5.2
H_o	0.179	0.250	0.136	0.415	0.475	0.291
H_o	0.678	0.675	0.354	0.894	0.885	0.697
D	–0.735	–0.630	–0.615	–0.536	–0.463	–0.596
P[b]	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	(0.001)	**(0.001)**	**(0.001)**	**(0.001)**	**(0.001)**	
Guerrero						
Sample size	38	31	40	35	44	37.6
A	2	7	3	10	16	7.6
a_e	2.0	2.6	1.8	5.3	5.3	3.4
H_o	0.263	0.161	0.300	0.600	0.614	0.388
H_e	0.499	0.621	0.454	0.811	0.811	0.639
D	–0.472	–0.740	–0.339	–0.260	–0.244	–0.411
P[b]	**0.0037**	**<0.001**	**0.0334**	**<0.001**	**<0.001**	**<0.001**
	(0.001)	**(0.001)**	**(0.001)**	**(0.001)**	**(0.001)**	
Guatemala						
Sample size	26	20	42	39	48	35.0
A	3	5	3	13	16	8.0
a_e	2.2	2.7	1.6	9.5	10.5	5.3
H_o	0.500	0.200	0.238	0.282	0.583	0.361
H_e	0.548	0.631	0.357	0.895	0.905	0.667
D	–0.088	–0.683	–0.333	–0.685	–0.356	–0.429
P[b]	0.4524	**<0.001**	**0.0031**	**<0.001**	**<0.001**	**<0.001**
	(0.1582)	**(0.001)**	**(0.001)**	**(0.001)**	**(0.001)**	
Panama						
Sample size	34	22	44	29	32	32.2
A	5	7	3	13	9	7.4
a_e	3.2	5.5	1.6	5.8	4.7	4.2
H_o	0.324	0.045	0.227	0.138	0.469	0.241
H_e	0.684	0.819	0.373	0.828	0.786	0.698
D	–0.527	–0.945	–0.391	–0.833	–0.404	–0.620
P[b]	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	(0.001)	**(0.001)**	**(0.001)**	**(0.001)**	**(0.001)**	
Total, all populations						
Sample size	137	117	170	144	164	732
Mean no. of alleles	3.5	7.5	3	12.25	13.25	7.9
Mean H_o	0.317	0.164	0.225	0.359	0.535	0.320
Mean H_e	0.602	0.687	0.385	0.857	0.847	0.675
Mean D	–0.456	–0.750	–0.420	–0.579	–0.367	–0.514
P[b]	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**

[a]A indicates number of alleles; a_e, effective number of alleles; H_o, observed heterozygosity; H_e, effective heterozygosity; D, deficit or excess of heterozygous $(H_o - H_e)/(H_e)$; P, probability of significant deviation from HWE (Markov chain procedure, $P < 0.05$).

[b]Number in parentheses indicates the probability (P) of significant heterozygosity deficiency. Significant criteria were adjusted using sequential Bonferroni technique (Rice, 1989), and significant P values are in boldface.

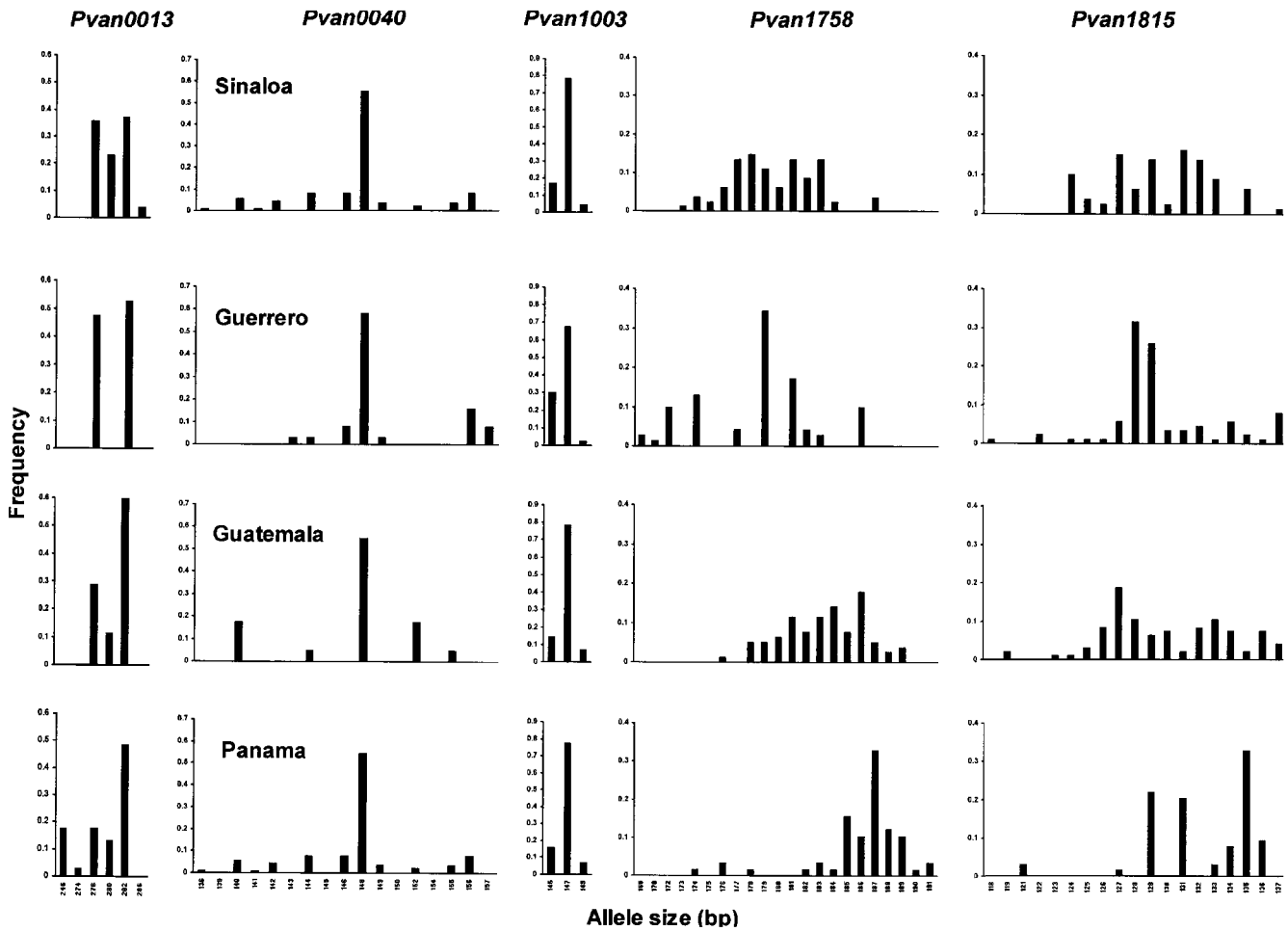

Figure 2. Histograms of allele frequencies at each microsatellite locus of *Litopenaeus vannamei*.

Table 3. *F* Statistics of Four Locations of *Litopenaeus vannamei*[a]

Locus	F_{IS}	F_{ST}	F_{IT}
Pvan0013	0.516**[b]	0.046**[b]	0.539**[b]
Pvan0040	0.744**[b]	0.053**[b]	0.758**[b]
Pvan1003	0.426**[b]	0.004	0.429**[b]
Pvan1758	0.582**[b]	0.072**[b]	0.612**[b]
Pvan1815	0.374**[b]	0.065**[b]	0.415**[b]
All	0.533**[b]	0.055**[b]	0.559**[b]

[a]Data were obtained using FSTAT (Goudet, 2001).
[b]Probability that the estimate is not different from zero based on a permutation procedure given in FSTAT (Goudet, 1995). $^{**}P < 0.01$.

Table 4. Pairwise F_{ST} Estimates Among Populations of *L. vannamei*[a]

Population	Sinaloa	Guerrero	Guatemala	Panama
Sinaloa				
Guerrero	0.0415			
Guatemala	0.0171	0.0511		
Panama	0.0625	0.1043	0.0614	

[a]Data were obtained using GENEPOP (Raymond and Rousset, 1995). For all values, $P < 0.008$, indicating highly significant genetic differentiation tested by Fisher's technique.

done. The analysis of the rescored data set showed genetic differentiation between populations in both allelic and genotypic frequencies, including *Pvan1003*. The isolation by distance analysis showed no significant results with the Mantel test ($P = 0.121$), indicating that the genetic differences were not explained by geographic distance (Figure 3).

Discussion

Genetic Diversity

The number of alleles per locus in this study (2–13) was lower than that reported in other species of penaeids (Benzie, 2000; Xu et al, 2001). In *Penaeus monodon*, mi-

Table 5. *P* Values for Pairwise Comparison of Allelic and Genotypic (in Parenthesis) Frequencies Among Populations[a,b]

Comparison	*Pvan0013*	*Pvan0040*	*Pvan1003*	*Pvan1758*	*Pvan1815*
Sinaloa vs. Guerrero	**0.000 (0.000)**	**0.010** (0.131)	0.119 (0.198)	**0.000 (0.000)**	**0.000 (0.000)**
Sinaloa vs. Guatemala	**0.042** (0.131)	**0.002** (0.071)	0.711 (0.742)	**0.000 (0.000)**	**0.000 (0.002)**
Sinaloa vs. Panama	**0.000 (0.003)**	**0.000 (0.000)**	0.848 (0.055)	**0.000 (0.000)**	**0.000 (0.000)**
Guerrero vs. Guatemala	**0.001 (0.000)**	**0.000 (0.000)**	0.026 (0.877)	**0.000 (0.000)**	**0.000 (0.000)**
Guerrero vs. Panama	**0.000 (0.000)**	**0.000 (0.000)**	0.055 (0.096)	**0.000 (0.000)**	**0.000 (0.000)**
Guatemala vs. Panama	**0.004 (0.022)**	**0.000 (0.000)**	0.961 (0.967)	**0.000 (0.000)**	**0.000 (0.000)**

[a]Data were obtained using GENEPOP (Raymond and Rousset, 1995).
[b]Significant criteria were adjusted using sequential Bonferroni technique, and significant *P* values in boldface.

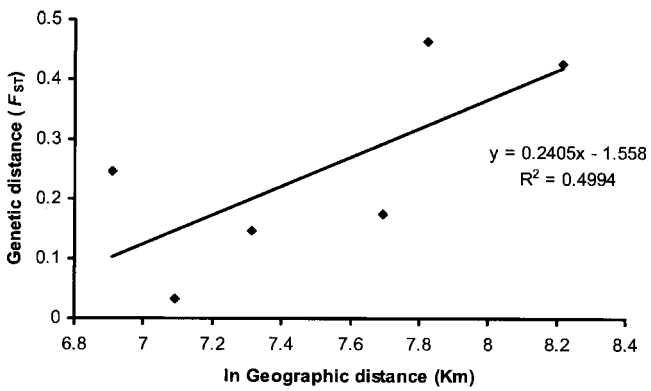

Figure 3. Isolation by distance: regression between paired F_{ST} values and the logarithm of the geographic distance.

crosatellite surveys revealed between 14 and 28 alleles at 2 loci (Supungul et al., 2000), and between 19 and 30 alleles at 5 loci in Thailand (Tassanakajon et al., 1998), 34 and 84 alleles at 3 loci in Australia (Brooker et al., 2000), and 6 and 54 at 6 loci in the Philippines (Xu et al., 2001). The only previous work for *L. vannamei* with microsatellites (Wolfus et al., 1997) reported from 4 to 23 alleles at one locus. Nevertheless, these diverse allelic estimates are higher than those reported for allozymes (Benzie, 2000). As expected the number of alleles was also slightly higher in our study than that of a farm sample using the same 5 microsatellites (Cruz et al., 2002). The moderate allele variation of the microsatellite loci observed in this study showed enough resolution to allow differentiation of populations. This is an advantage over loci with more alleles in which larger sampling sizes might be needed (Hedrick, 1999). The results regarding heterozygosity also indicate that other penaeids have shown more microsatellite variation than *L. vannamei*, with values between 0.425 and 0.964 (Benzie, 2000), compared with the range reported here between 0.164 and 0.535.

Deviation from Hardy-Weinberg Equilibrium

We detected highly significant deviations from HWE for almost all tests at each locus and each population (19 of 20) owing to homozygous excess. This phenomenon has been reported for other shrimp species such as *P. monodon*: while Supungul et al. (2000) found significant deviations from HWE in 19 of 25 possible tests, Xu et al. (2001) reported it in 8 of 24 tests. The high heterozygous deficit has been commonly observed in other aquatic organisms using allozymes (Zouros and Foltz, 1984; Gaffney et al., 1990) and microsatellite loci (Pemberton et al., 1995; Ruzzante et al., 1996). Furthermore, allozyme heterozygous deficit in some shrimp species has been found in some loci of *Farfantepenaeus californiensis* and *Litopenaeus stylirostris* (de la Rosa et al., 2000), and in *Farfantepenaeus notialis* (Garcia-Machado et al., 2001). Xu et al. (2001) found for *P. monodon* 5 cases of heterozygous deficit for 3 microsatellite loci in 4 populations.

Several explanations for heterozygous deficit have been proposed, including mating of close relatives (inbreeding), null alleles, technical artifacts, population mixing (Wahlund effect), mixture of cohorts ("family" Wahlund effect), and others (Gaffney et al., 1990; Castric et al., 2002). When inbreeding is present in a population, we would expect that all loci were correlated (linkage disequilibrium) (Castric et al., 2002); therefore, because we did not observe linkage disequilibrium between each pair loci, inbreeding does not explain the heterozygous deficit.

Another explanation for heterozygous deficit could be the presence of null alleles, i.e., the failure to amplify one of the alleles in an individual or misscoring due to stutter bands (Pemberton et al., 1995). The presence of null alleles has been suggested for *P. monodon* (Xu et al., 2001) and

Litopenaeus setiferus (Ball et al., 1998). In our case Mendelian inheritance tests (Cruz et al., in press) with the 2 most variable loci, *Pvan1758* and *Pvan1815*, have shown that one of them (*Pvan1815*) could show null alleles when PCR conditions were not optimal (unpublished data). It is common for null alleles to be expected at one locus in all populations; however, we found this condition not only for one locus, but also for all loci. Considering that the analysis of a null allele data set (Xu et al., 2001) decreased the number of departures from HWE, we cannot rule out the possibility of null alleles, especially in 3 loci, *Pvan0040, Pvan1003*, and *Pvan1758*. The presence of technical artifacts (shadow or stutter bands), rather than single discrete bands of microsatellite loci, could have caused misclassification of heterozygotes for closely sized alleles as homozygotes. This effect is normally present in dinucleotide-repeat loci as a result of slipped-strand misparing during PCR. The microsatellite loci used in this study were mostly of dinucleotide repeats, and 2 microsatellites included tetranucleotide repeats (Cruz et al., 2002). We found that the number of stutter bands was variable among loci and even among individuals, but the normal pattern was a single strong band in all loci, with weak stutter bands (normally not more than 2). Thus we scored alleles with the strong band following Xu et al. (2001). We believe, therefore, that misscoring due to stutter bands was not the major cause of the homozygous excess in our study.

We could not eliminate the possibility that the deviation from HWE in *L. vannamei* along the Pacific coast could be due to a sweepstakes reproductive success effect among larval cohorts. This process has been proposed for the fish *Clupea pallasi* (O'Connell et al., 1998) and also for the shrimp *P. monodon* (Supungul et al., 2000).

Genetic Structure and Isolation by Distance

Microsatellites have revealed higher population substructure than allozymes in other species of shrimps (Brooker et al., 2000; Supungul et al., 2000; Xu et al., 2001). We also found higher and significant levels of genetic structure (F_{ST}) for *L. vannamei* populations in the Eastern Pacific using microsatellites, compared with extremely low levels of population subdivision using allozymes for the same region reported by Sunden and Davis (1991). This genetic structure is also evident between each region, as indicated by the pairwise F_{ST}, suggesting that each location should be treated as a separate population.

The lack of correlation between F_{ST} and geographic distance indicates that the genetic differentiation of *L. vannamei* populations is not chiefly determined by the effect of distance between populations. Although *L. vannamei* has a 2-week pelagic larval stage before moving into coastal lagoons and thus can potentially disperse over large distances by ocean currents, resulting in a panmictic population, the combination of physical, oceanographic, and biological factors could act to limit gene flow (Launey et al., 2001). In our case, we could divide the study area in 3 regions. One is the lower Gulf of California, where the continental shelf is wide (85 km), with alluvial sediments deposited by river systems, and with ocean-current patterns determined by strong winds in winter flowing from northwest parallel to the coast, producing gyres and coastal upwelling, pushing waters offshore, and from the southeast in summer that would push surface waters inshore (de la Lanza, 1991). Another is the middle part of Mexico, between Cabo Corrientes and Salina Cruz (Figure 1), where the continental shelf is narrow (10–15 km) with fewer estuarine systems, and a "warm water pool" front of Guerrero and Michoacan (Mexico), where surface waters show little movement and maintain temperatures above 28°C throughout the year (Trasviña et al., 1999). In the Third region, south of Salina Cruz (Mexico) to Panama, the continental shelf is wide, and the coastal waters are mainly affected by the wind; during winter, strong winds flow perpendicular to the coast in the Gulf of Tehuantepec in Mexico, the Gulf of Fonseca in Honduras, and in the Gulf of Panama, producing large warm eddies and intense offshore current jets (Trasviña et al., 1999; Brenes et al., 2003; Rodríguez-Rubio and Wolfgang, 2003), which, could be transporting postlarvae offshore and preventing mixture of populations. In relation to reproductive biology, a latitudinal cline in spawning numbers has been observed for *L. vannamei* along the Mexican Pacific coast (Sepúlveda-Medina, 1991), and closely linked to the water temperature (Cabrera-Jimenez, 1997), that over time might act as a biological barrier.

In summary, our findings suggest that populations of *L. vannamei* should be treated as genetically subdivided. This information could be important for fishery and aquaculture management and conservation activities. Further studies on a narrower geographic range and including samples from the open sea are needed for better understanding of the genetic structure of white shrimp populations.

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