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# Genetic Susceptibility of Cultured Shrimp (*Penaeus vannamei*) to Infectious Hypodermal and Hematopoietic Necrosis Virus and *Baculovirus penaei*: Possible Relationship with Growth Status and Metabolic Gene Expression

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**Abstract**

Offspring of four crosses (I, II, III, and IV) of *Penaeus vannamei* from known high- and low-growth families were challenged with infectious hypodermal and hematopoietic necrosis virus (IHHNV) and *Baculovirus penaei* (BP) to compare their susceptibility to these viral agents and examine the genetic component involved in disease resistance or susceptibility. Family crosses were made using broodstock from five families developed by the U.S. Marine Shrimp Farming Program. The prevalence of IHHNV infection was highest in cross I and lowest in cross III. Cross I was developed using male and female broodstock from the low-growth family 1.6, and cross III was developed using a female from the high-growth family 1.3 and a male from the low-growth family 1.6. The prevalence of BP infection at Day 4 was highest (100%) in cross IV, which was developed using a female from the low-growth family 1.4 and a male from the high-growth family 1.5. The reciprocal cross, cross III, had the lowest (68%) prevalence at Day 4 postexposure. Both crosses I and II had 88% prevalence

of infection at Day 4. Despite 100% prevalence of BP infection in cross IV at 4 days, animals from this cross and cross II exhibited high survival by Day 18 (85 and 77%). On the other hand, crosses I and III (with 88 and 68% prevalence at Day 4, respectively) showed low survival at Day 18 (19 and 24%). On the basis of prevalence of infection and mortality rates, it was concluded that the susceptibility to BP in penaeid shrimp is governed by the genetic background of the parental crosses. The random amplified polymorphic DNA polymorphisms for crosses I, II, III, and IV, were 43, 45, 53, and 51%, respectively, showing no clear relationship between IHHNV and BP prevalence of infection and levels of nuclear genetic diversity. Though the mtDNA haplotypes in offspring from the different crosses were the same, major differences were observed in both steady-state levels and patterns of expression of the mitochondrial 12s rRNA in offspring obtained at various early developmental stages from each of the four crosses. The possible relationship among disease susceptibility, growth status, and expression of mitochondrial 12s rRNA is discussed in the context of a complex nuclear-cytoplasmic genetic system involved in the regulation of gene expression.

**Keywords:** IHHNV, *Baculovirus penaeid*, *Penaeus vannamei*, genetic diversity, susceptibility, 12s RNA, mtDNA

## Introduction

Viral diseases cause severe economic losses to the shrimp aquaculture industry worldwide (Chamberlain, 1994). So far, at least 18 penaeid viruses have been reported to infect cultured and wild shrimp (Lightner et al., 1994; Overstreet, 1994). Among these viruses, the infectious hypodermal and hematopoietic necrosis virus (IHHNV) and *Baculovirus penaei* (BP) are of considerable economic importance to the industry (Lightner, 1993; Overstreet, 1994).

IHHNV is a parvovirus containing single-stranded DNA that infects cultured Pacific white *Penaeus vannamei* and blue *P. stylirostris* shrimp as well as some wild populations of Pacific American penaeids (Lightner, 1993). Epizootiological data have linked IHHNV infection to "runt deformity syndrome" in cultured *P. vannamei.* The growth rate of infected animals is greatly reduced and a variety of cuticular deformities are observed (Browdy et al., 1993). Bioassay, histopathological techniques, and nonradioactive genomic probes have been developed for detection of IHHNV (Lightner et al., 1994).

*B. penaei*, a double-stranded DNA baculovirus (occluded), was the first shrimp virus reported 20 years ago in penaeid shrimp from the Gulf of Mexico (Overstreet, 1994). BP causes enteric infections resulting in high mortality by infecting the epithelial cells of the hepatopancreas and midgut of all the developmental stages of the host (LeBlanc and Overstreet, 1990; Lightner et al., 1994). Prevalence and severity of BP infection vary depending on the viral strain, inoculum level, resistance and age of host, host species, nutrition, and an undefined "general health state" (Overstreet, 1994).

IHHNV and BP are considered high priority in importance for regulation by international policy for sustainability of shrimp aquaculture and healthy natural resources (Johnson, 1994). A long-term management strategy for viral diseases of shrimp has been implemented by the U.S. Marine Shrimp Farming Program Consortium (MSFP) and accordingly, specific pathogen-free (SPF) *P. vannamei* seedstocks are being supplied to shrimp farmers (Wyban et al., 1993; Lotz et al., 1995). So far, three SPF (Nos. 1, 2, and 3) populations, one

"candidate" SPF (No. 4) population, and 12 families from population 1 have been developed by the MSFP (Wyban et al., 1993; Carr et al., 1994). Differences in growth rate have been reported between these populations and families (Carr et al., 1994). Molecular genetic analysis also revealed considerable variability at the nuclear and mitochondrial DNA (mtDNA) levels between and within these populations (Alcivar-Warren et al., 1994; Garcia et al., 1994). Significant differences in the expression levels of mitochondrial 12S rRNA, cytochrome oxidase subunit I (COI) mRNA, and the nuclear actin mRNA were also observed among individuals of populations 1 and 2 (Alcivar-Warren et al., 1994, 1995, 1996).

Studies with other aquatic animals have revealed a relationship between genetic variability and disease resistance. For instance, a relationship between the bacterial gill disease resistance and genetic (isozyme) heterozygosity of survivors has been presented for rainbow trout (Ferguson and Drahushchak, 1986). These researchers also noticed that resistance to bacterial gill disease was related to size of the fish. This implies that expression of growth or metabolically important genes (like those encoded by the mitochondrial genome) could be involved in susceptibility to disease infection. Examples of genetic variation for disease or pollutant resistance between and within populations of marine species have also been reported by Nevo et al. (1986) and Chevassus and Dorson (1990). However, no information is available regarding the possibility that resistance to viral diseases in shrimp is genetically (inheritable) determined.

We hypothesized that susceptibility of cultured *P. vannamei* to IHHNV and BP infection is genetically determined. This relationship is due in part to the differences in growth performance and the expression of metabolic genes among the families used to perform the crosses. In this study, crosses were performed using individuals from five families of high health *P. vannamei* with the following objectives: (1) to determine if infection of high health shrimp with IHHNV and BP is influenced by the genetic background of the host, (2) to assess the relationship between nuclear and mitochondrial DNA polymorphisms and the prevalence of IHHNV and BP, and (3) to study if the expression of the mitochondrial 12s rRNA gene during development is associated with susceptibility to viral infections and growth status.

## Methods

### *Sample Collection*

The high health shrimp used to perform the crosses for this study were obtained from the Oceanic Institute (OI) in Hawaii and were produced following specifications for stock certification (Lotz et al., 1995). These populations have been regularly screened and found negative to both IHHNV and BP pathogens. Third-generation offspring were obtained from four crosses (I, II, III, and IV) using broodstock from five high health families (Nos. 1.3, 1.4, 1.5, 1.6, and 1.8) of population 1 (Table 1). These broodstock were chosen from families of known high (H) or low (L) growth rates (Carr et al., 1994). Families 1.3, 1.5, and 1.8 had significantly higher growth than families 1.4 and 1.6 (Table 1).

**Table 1.** Details of Family Crosses and Growth Data for the Animals Used in This Study

| Family crosses: | I | II | III | IV |
|---|---|---|---|---|
| (Female × male; population no., family no.) | (1.6 × 1.6) | (1.3 × 1.8) | (1.3 × 1.6) | (1.4 × 1.5) |
| Family growth status (Female × male)[b] | L × L[a] (W379 × W373) | H × H (B490 × G324) | H × L (B492 × W382) | L × H (R391 × W388) |
| Mean weight (g) for families,[c] ±SD | 1.6 = 9.4 ± 2.9 | 1.3 = 17.8 ± 2.0<br>1.8 = 17.3 ± 2.2 | 1.3 = 17.8 ± 2.0<br>1.6 = 9.4 ± 2.9 | 1.4 = 9.4 ± 1.9<br>1.5 = 15.3 ± 2.9 |

a. L, low growth; H, high growth.
b. Letters and numbers in parentheses indicate the tag numbers of male and female broodstock.
c. Taken from the 1992 U.S. Marine Shrimp Farming Program Implementation Plan, unpublished data.

Pooled samples from various developmental stages were obtained from each of the four crosses during an 18-day sample collection period to extract total nucleic acids. The following samples were collected and placed in guanidine isothiocyanate (GT) buffer after confirmation of the developmental stages by microscopic examination: ~2000 nauplii stages 3 and 4 (N3/4), ~2000 zoea stage 1 (Z1), ~2000 zoea stage 3 (Z3), ~1000 mysis 1 (M1), ~1000 mysis 3 (M3), and ~500 each from postlarvae 1 (PL1), PL2, PL4, PL6, PL8, and PL10. Total nucleic acids were also extracted from tail muscle obtained from the broodstock and from 12–15 third-generation juveniles from each of the five families (1.3, 1.4, 1.5, 1.6, and 1.8) used to perform the crosses. Total RNA extracted from the brine shrimp *Artemia*, used to feed the animals at various stages of development, served as controls in the mRNA expression analysis. Postlarvae stage 10 (from the same crosses from which nucleic acids were extracted) were shipped from OI to the Gulf Coast Research Laboratory (Ocean Springs, Mississippi), where these animals were challenged with IHHNV and BP under controlled conditions.

### *Challenge of High Health Shrimp with IHHNV and BP*

Fifteen animals weighing 1–2 g (~3 months old) were stocked into eight 40-L aquaria and challenged with IHHNV. Animals in each tank were fed IHHNV-positive tissue (10% of their body weight) and it is assumed that the amount of virus exposure should have been the same for each tank. Survival at Day 30 was 100% in each of the eight tanks. IHHNV prevalence at Day 30 was determined for the four families after pooling the 30 individuals from the duplicate tanks. Detection of IHHNV was done using nonradioactive probe and dot blot technique on Day 30 (Lightner et al., 1994).

Experiments to test the susceptibility of postlarvae to BP were conducted using stage PL15 of the same spawns used for the genetic analyses. They were performed in eight 38-L aquaria set within a 25–26°C water bath, maintained at 32 ppt salinity and constant light, aerated with air stones and dual sponge filters, and conditioned with No. 9 Fritz-Zyme (nitrifying bacteria). A pair of aquaria was initiated for each of four crosses with about 350 free PL15 per aquarium plus exactly 100 additional specimens in an inserted "egg-cup" for documenting survival at 18 days. The egg-cup was made with a 28-cm-high cylinder of 475-μm mesh nylon screening material fit into a 15-cm glass petri dish base. Shrimp in one aquarium of each pair were exposed to 0.4 g of heavily BP-infected PL material that had

been frozen at –70°C and then thawed. At each of Days 0, 4, 13, and 26 postexposure (PE), the fresh hepatopancreas of 25 shrimp per aquaria were used for light microscopic examination of BP polyhedra (Overstreet et al., 1988). Differences in infections were compared using $\chi^2$ analysis.

***Nucleic Acid Extraction and Randomly Amplified Polymorphic DNA (RAPD)***

Total nucleic acids were extracted using a GT-based protocol (Alcivar et al., 1989) with some modifications as reported earlier (Garcia et al., 1994). The RAPD analysis was performed using six 10-mer oligonucleotide primers (OPA-20, OPB-13, OPB-19, OPG-07, OPM-09, and OPZ-09; Operon Technologies, Alameda, California) using pooled DNA samples from eight different developmental stages (M1, M3, PL1, PL2, PL4, PL6, PL8, and PL10). These six primers were selected after initial screening of 41 primers (Astrofsky et al., unpublished results). The reaction mixture and the thermal cycles for the polymerase chain reaction (PCR) were the same as described by Garcia et al. (1994). The amplified products were run in a 2% agarose gel containing 0.3 μg/ml ethidium bromide in TAE buffer (40 mM Tris, 20 mM acetic acid, 1 mM sodium EDTA) at 1.8 V/cm of gel for 20 hr. The gel was photographed and individual DNA bands were scored as present or absent in each profile.

***Restriction Fragment Length Polymorphisms (RFLPs)***

Seven restriction endonucleases (*Bam*HI, *Bcl*I, *Bst*EII, *Cfo*I, *Eco*RV, *Hha*I, and *Rsa*I from Gibco BRL) were selected for RFLP analysis. All enzyme digestions were carried out as per the manufacturer's instructions. Southern blot hybridizations to detect RFLPs in the mitochondrial COI gene were carried out according to the methods described by Alcivar et al. (1989). Digested DNA was electrophoresed in 0.8% agarose gels, blotted onto nitrocellulose membranes (MSI, Westboro, Massachusetts), and hybridized with a COI DNA probe (Garcia et al., 1994) labeled using the random priming DNA system (Gibco BRL) and [$\alpha$-$^{32}$P]dCTP (Amersham) at specific activities of 1 to 5 × $10^7$ cpm/ml of hybridization solution.

***Northern Blot Hybridizations***

The hybridizations for Northern blots were performed either at 65°C as previously described (Alcivar et al., 1989; Alcivar-Warren et al., 1994) or at 42°C using custom-made hybridization solutions (5 → 3 Inc.) following the procedure suggested by the supplier. A 152-bp DNA of 12s rRNA amplified by PCR was used for probe preparation in Northern blot hybridizations. The primers for PCR amplification of 12s rRNA gene were taken from a published sequence for penaeid shrimp (Palumbi and Benzie, 1991). The PCR-amplified DNA fragment was labeled using the random priming protocol and [$\alpha$-$^{32}$P]dCTP (Amersham) with specific activities ranging from 1 to 3 × $10^7$ cpm/ml of hybridization solution.

## Results

***Susceptibility to IHHNV and BP Infection Varies According to the Genetic Background of the Parental Broodstock***

The prevalence of infection in high health shrimp obtained after challenge with IHHNV is presented in Table 2. There were significant differences in the prevalence of infection to

IHHNV among the four crosses of high health shrimp (Table 2). The prevalence of infection was 48, 36, 6, and 20% for crosses I, II, III, and IV, respectively. The highest ($P < 0.05$) difference in susceptibility to IHHNV was between crosses I and III. Offspring of the most susceptible cross I originated from a homologous cross of broodstock from the low-growth family 1.6, while the most resistant offspring from cross III were derived from a heterologous cross of a female broodstock from the high-growth family 1.3 and a male broodstock from the low-growth family 1.6.

**Table 2.** Genetic Variability and Prevalence of IHHNV and BP in Four Crosses of *Penaeus vannamei*

| Family crosses: | I | II | III | IV |
|---|---|---|---|---|
| IHHNV prevalence | | | | |
| Infected/total (%) at 30 days PE | 15/31 (48)[a] | 5/14 (36)[a] | 2/31 (6)[b] | 3/15 (20)[a,b] |
| BP, % prevalence at | | | | |
| 4 days | 88 | 88 | 68 | 100 |
| 13 days | 88 | 76 | 84 | 100 |
| 26 days | 36 | 52 | 52 | 84 |
| Survival (%) at 18 days PE | 19 | 77 | 24 | 85 |
| % RAPD polymorphisms | 43% | 45% | 53% | 51% |
| COI mtDNA type[1] | | | | |
| *Cfo*I | B × B | B × B | B × B | B × A |
| *Eco*RV | [BD] × [BD] | B × B | B × [BD] | B × [ABC] |
| *Hha*I | B × B | B × B | B × B | B × A |
| *Rsa*I | A × A | A × A | A × A | A × B |
| Expected COI mtDNA types in families of broodstocks | B[BD]BA × B[BD]BA | BBBA × BBBA | BBBA × B[BD]BA | BBBA × B[ABC]AB |
| Observed COI mtDNA types in PL10s of crosses used | BBBA | BBBA | BBBA | BBBA |

1. The mtDNA types of the maternal and paternal (M × P) families from which broodstock were taken; based on analysis of third-generation offspring. Letters in parentheses indicate different mtDNA types detected within the family.

a, b. Different superscripts indicate significantly different at $P < 0.05$.

The prevalence of BP infection at 0, 4, 13, and 26 days PE is shown in Tables 2 and 3. High levels of infection occurred in all exposed groups. There was 100% infection in cross IV at 4 and 13 days PE, which decreased to 84% by 26 days PE. The prevalence of infection was lower (68%) in cross III at 4 days PE. However, prevalence of infection in this cross was increased by Day 13 (84%) followed by a decrease at 26 days (52%). None of the shrimp in any of the control groups exhibited signs of infection.

When the BP data were partitioned at each of the three different periods of PE (4, 13, and 26 days), significant differences ($P < 0.05$) were found among the crosses at Days 4 and 26 but not at Day 13 (Table 3). For cross I, prevalence remained the same at 4 and 13 days and reduced to 36% at 26 days. For cross II, although the prevalence of infection at Day 4 was the same as cross I (88%), it started to decline earlier and more dramatically to a 52% prevalence at 26 days PE. Each cross showed different features with regard to survivability after BP infection. This was in contrast to the prevalence of infection in these crosses. Crosses I and III had very low survivability by Day 18, whereas survivability in crosses II

and IV was about the same as in the controls (Table 3). In cross IV, most of the survivors (84%) remained infected until at least Day 26, whereas in cross II, only 52% of the survivors remained infected, indicating a greater resistance in the later cross.

**Table 3.** Percentage of Postlarvae of Genetic Crosses Infected and Surviving after Exposure to BP

| | | Prevalence of BP infection (%) | | | |
|---|---|---|---|---|---|
| Cross | Test group | 4 days PE[a] | 13 days PE | 26 days PE | Survival in % at 18 days |
| I | Control | 0 | 0 | 0 | 79 |
| L × L | Exposed | 88 | 88 | 36 | 19 |
| II | Control | 0 | 0 | 0 | 94 |
| H × H | Exposed | 88 | 76 | 52 | 77 |
| III | Control | 0 | 0 | 0 | 86 |
| H × L | Exposed | 68 | 84 | 52 | 24 |
| IV | Control | 0 | 0 | 0 | 100 |
| L × H | Exposed | 100 | 100 | 84 | 85 |

a. PE, number of days postexposure to BP

***RAPD Polymorphisms and Susceptibility to IHHNV and BP***

The levels of genetic diversity calculated from the RAPD polymorphism data were 43, 45, 53, and 51% in crosses I, II, III, and IV, respectively (Table 2). A trend between the levels of genetic diversity and the prevalence of IHHNV was noticed. For example, the lower genetic diversity levels observed for crosses I and II may be related to the higher IHHNV prevalence of infection seen in these crosses (48 and 36% for crosses I and II). The crosses III and IV, which showed the lowest prevalence of IHHNV infection, had 53 and 51% genetic polymorphisms.

The BP survival rates, however, did not exhibit a trend with RAPD polymorphisms. For examples, cross IV which showed highest survival had 51% RAPD polymorphisms, whereas cross III with 24% survivability had 53% RAPD polymorphisms. In addition, although the survivability varied significantly between crosses I and II (19 and 77%, respectively) the RAPD polymorphisms were almost equivalent.

Analysis of the RAPD data revealed that two (OPB-13 and OPM-09) of six primers amplified some DNA bands that showed differences in prevalence of IHHNV infection among the four crosses (Table 4). For instance, two OPB-13 bands (~675 and ~725 bp) were found associated 100% with crosses I and II and only 25% of samples in crosses III and IV. The primer OPM-09 amplified a band (~840 bp) predominantly present in crosses III and IV, whereas a ~800-bp band amplified by the same primer was mostly present in crosses I and II (Table 4). Two additional primers (OPB-19 and OPZ-09) amplified bands that could be considered cross-specific. For example, the primer OPB-19 amplified a ~475-bp band which was present in 100% of samples from cross I (from the low-growth family 1.6) and 13% in cross II and absent in crosses III and IV. The primer OPZ-09 amplified a ~375-bp band which was present 100% in crosses I and IV and absent in crosses II and III. Cross I was produced with broodstock from low-growth family 1.6 and the maternal broodstock used to produce cross IV originated from another low-growth family (No. 1.4).

**Table 4.** Potential RAPD Markers Associated with IHHNV Susceptibility under Laboratory Conditions

| RAPD primer | Sequence (5 → 3) | Potential marker size (bp)[a] | Cross I | Cross II | Cross III | Cross IV |
|---|---|---|---|---|---|---|
| OPB-13 | TTCCCCCGCT | 675 | 8/8 | 8/8 | 2/8 | 2/8 |
| | | 725 | 8/8 | 8/8 | 2/8 | 2/8 |
| OPM-09 | GTCTTGCGGA | 840 | 2/8 | 1/8 | 7/8 | 7/8 |
| | | 800 | 6/8 | 7/8 | 1/8 | 1/8 |

a. All base pair sizes are approximations. Only strong DNA bands were considered.

### *Mitochondrial DNA RFLPs*

In addition to nuclear DNA markers, attempts were also made to see if different mitochondrial haplotypes (indicative of cytoplasmic or maternal effects) could be associated with growth or susceptibility to viral diseases. Differences in COI mtDNA haplotypes were examined using samples from (a) offspring and broodstock of families used to perform the crosses ("expected") and (b) siblings at PL10 of animals used for IHHNV and BP challenge experiments ("observed"). The expected results from the families used to perform the crosses are shown in Table 2. Four (*Cfo*I, *Eco*RV, *Hha*I, and *Rsa*I) of the seven restriction enzymes tested showed mtDNA polymorphisms among the families, but no differences were seen using the other three restriction enzymes (*Bam*HI, *Bcl*I, and *Bst*EII). The expected mtDNA haplotypes for *Cfo*I, *Eco*RV, *Hha*I, and *Rsa*I enzymes were most different in the paternal broodstock of cross IV (the most BP susceptible of all the crosses at Day 26 PE, but with the highest survivability). Mitochondrial DNA haplotype D was absent in families used to perform crosses II and IV, both of which had higher survivability in response to BP infection. The observed COI mtDNA haplotypes in the PL10s, siblings of the offspring challenged, were not different among the four crosses with all enzymes tested (Table 2).

### *Mitochondrial 12s rRNA Expression*

The steady-state levels of 12s rRNA expression varied throughout development, from nauplii to PL10 stages, in each of the four crosses examined (Fig. 1). The 12s rRNA levels in cross I (L × L) were generally higher in the early part of development than later. The expression levels were low in N3/4 shrimp and increased to a maximum in Z1 and Z3. The levels remained high in M1 and M3 but gradually decreased when shrimp reached PL6 and PL8 before levels began to increase again in PL10. In cross II (H × H), 12s rRNA levels remained somewhat constant at low levels, only slightly higher from N3 to M3, and decreasing after PL1. A similar trend was observed in cross IV (L × H). The 12s rRNA expression levels in cross III (H × L) were relatively high throughout the developmental stages examined, with higher levels in M1, M3, PL1, PL2, and PL4, a trend more similar to early developmental stages seen in cross I (L × L).

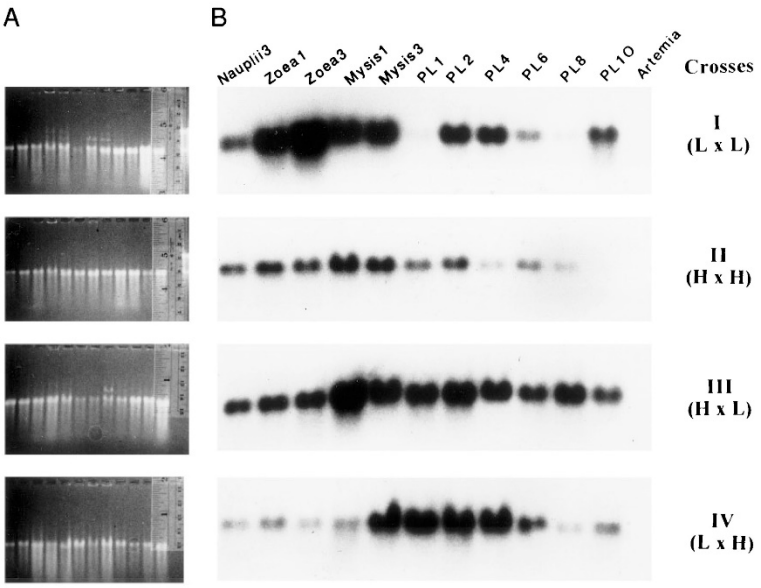


**Figure 1.** Autoradiogram of a Northern blot showing differential expression of mitochondrial 12s rRNA from nauplii stage 3 to postlarvae stage 10 (PL10) in four high health *Penaeus vannamei* family crosses. Aliquots of denatured total RNA were electrophoresed in 1% formaldehyde-agarose gels, blotted onto nitrocellulose membranes, and hybridized with a $^{32}$P-labeled PCR-amplified mitochondrial 12s rRNA probe.

## Discussion

The susceptibility of shrimp to IHHNV appeared to be dependent on the genetic background of the parental crosses. Of four crosses, cross I was the most susceptible and cross III was the most resistant to IHHNV infection. Cross I originated from mating both paternal and maternal broodstock from family 1.6, which has ranked for three generations as the lowest growth performer (Carr, unpublished data). The most resistant cross, III, was made using a female from the high-growth family 1.3 and a male from the low-growth family 1.6. The genetic effects observed could be specific either to the individual broodstock used to perform the crosses or to the families to which they belong. Because the males used in both crosses I and III originated from the same family, 1.6, the results suggested that IHHNV resistance may be influenced by the genetic background of the maternal broodstock. Alternatively, IHHNV susceptibility could be influenced directly, or in combination, by the growth status and differential expression of metabolic (or other) genes in the families from which the broodstock were chosen.

The results suggested that resistance to BP is genetically regulated and is an age-related phenomenon. It has been reported that mortality due to BP infection occurs mostly at larval and young postlarval stages (Overstreet et al., 1988; LeBlanc and Overstreet, 1990). With increasing age, the intensity of BP infection decreased, the length of time needed for infection to be detected increased, and the ability to lose the BP infection increased (Stuck and Overstreet, 1994; Overstreet, 1994). A recent study has shown that susceptibility of

shrimp to BP appears to be at least partially dependent on lipid (triacylglycerides, TAG) reserves (Stuck et al., 1996) with high levels associated with increased susceptibility to BP infection in larval and postlarval shrimp (Stuck et al., 1996; Stuck and Overstreet, 1996). The differences in TAG levels were not only due to nutrition but may have been influenced by the genetic makeup (Overstreet, 1994). Crosses I and III showed highest mortalities after exposure to BP than did crosses II and IV. In cross IV, most of the survivors (85%) retained their infection until Day 26 PE, whereas only 52% of animals in cross II had the infection at the same time, indicating that cross II had greater resistance. Although survivability of cross IV was slightly higher than that of cross II, the later cross could be considered the best cross for BP resistance because (1) it cleared infection at a faster rate than the others and (2) it had relatively high survival.

The genetic parameters associated with BP susceptibility did not correspond with the susceptibility to IHHNV, suggesting that different loci are involved in resistance to IHHNV and BP infection. The levels of genetic diversity determined by RAPD technique did not show any trend with the prevalence of BP infection or survival rate. However, it is tempting to speculate a relationship between the prevalence of IHHNV infection and the levels of genetic diversity. Possibly, genetic diversity is more crucial in monogenic disease resistance than in polygenic resistance. In the case of monogenic resistance, for instance, if the gene for susceptibility is widely prevalent in the population, then a reduction in polymorphisms makes the population highly susceptible to disease. In contrast, for polygenic resistance, multiple loci are involved in resistance phenomena. Therefore, even when a population is relatively less polymorphic, it can still show resistance or tolerance to any particular disease. Evidence correlating genetic diversity of survivors with pollution or disease resistance has been presented for other marine species (Nevo et al., 1986; Ferguson and Drahushchak, 1990). Examples of genetic variation for disease resistance within and between fish populations have also been reported for viruses in sockeye salmon, rainbow trout, and channel catfish (reviewed in Chevassus and Dorson, 1990). Perhaps genotype-genotype and genotype-environment interactions may occur not only at the family or population levels but also at the level of the individual host (Teale, 1994).

We observed that at least for BP, the homologous cross involving high-growth animals (cross II) showed much more survivability (77%) than the homologous cross I involving low-growth animals (19%). Animals with higher growth have been shown to be more resistant to disease in rainbow trout (Ferguson and Drahushchak, 1990). Genetic heterogeneity and size of survivors were correlated with resistance to bacterial gill disease in freshwater rainbow trout, with survivors being more heterozygous and larger than nonsurvivors (Ferguson and Drahushchak, 1990). These authors also hypothesized that survivors may have resisted disease because of the larger size (as seen in cross II in this study) rather than higher heterozygosity per se, although heterozygosity was considered an important factor in disease resistance because of its association with size (Ferguson and Drahuschark, 1990).

Although no mtDNA haplotype was found to be associated with growth or disease resistance, the RAPD assay provided some potential markers (OPB-13 and OPM-09) that could be associated with IHHNV infection in crosses I and II, while other markers (OPB-19 and OPZ-09) could be considered specific for crosses I and IV. It is unknown if these po-

tential cross-specific markers are associated with growth performance of these crosses. Indeed, two of the families (1.6 and 1.4) used to produce crosses I and IV were the lowest growth performers in the breeding program for the past few years (data not shown). Additional testing of these markers using inbred lines will help to determine if they are indeed related to disease susceptibility or growth.

The differential expression of 12s rRNA detected during development of penaeid shrimp was influenced by the genetic background of the animals. Perhaps all, or most, mitochondrial genes are needed in early stages of shrimp development, as demonstrated for shrimp COI mRNA (Alcivar-Warren et al., 1995) and for various mitochondrial genes in the mouse (Taylor and Piko, 1995, and references therein). A possible relationship between growth performance and mitochondrial gene expression has been reported in other agriculturally important species (Smith and Alcivar, 1993; Danzmann and Ferguson, 1996). It is possible that the paternal mitochondrial genome or its interaction with nuclear genes influenced expression of the 12s rRNA, as seen in offspring derived from cross III (H × L) which expressed higher levels of 12s RNA than cross II (H × H). Our current efforts aimed at developing a linkage map for shrimp will help to elucidate the mechanisms involved in growth and viral disease resistance (Alcivar-Warren et al., 1996).

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