Breeding Shrimp for Disease Resistance: Challenges and Opportunities for Improvement

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ABSTRACT

After the 1992 Taura syndrome virus (TSV) epizootic in Ecuador, shrimp farmers in the Western Hemisphere began implementing a variety of strategies to mitigate crop loss from this pathogen, including selective breeding programs to develop TSV-resistant shrimp using between-family selection. Although TSV-resistant shrimp initially improved production and profitability for those farmers who were experiencing a TSV outbreak, genetically improved stocks were not a panacea for the broader disease problems plaguing the industry. In fact, breeding shrimp for resistance to a single viral pathogen, using current breeding strategies, may not be the most effective course of action for the long-term viability of the shrimp farming industry. There are a number of concerns associated with current breeding strategies. As in other organisms, there appears to be a trade-off between disease resistance and growth. In addition, disease-challenge tests typically used to estimate breeding values are based on a threshold character (dead or alive) and such tests are inefficient and low in statistical power. Also, there are concerns that performance in laboratory challenge tests may not be predictive of survival in commercial ponds. Importantly, there are growing concerns about viral mutations, whereby previously resistant shrimp strains may become susceptible to evolving viruses. To mitigate some of these concerns, we suggest that the current practice of selecting survivors of challenge tests (or the relatives of survivors) be replaced by selection based on an index which includes both survival and viral load. The selection objective would be to reduce the viral load in surviving shrimp, with viral strain or serotype being defined with whatever specificity that the situation requires. Viral load is both a cause and indicator of survival, so the practical result of the genetic improvement program would be a line of shrimp resistant to disease, just as it is now. This can be achieved more effectively when the selection objective is re-defined to include a continuous indicator variable (viral load), in addition to survival. In the future, we anticipate that more effective breeding strategies will emerge as we gain a better understanding about the genetic basis for disease resistance.

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INTRODUCTION

In the aftermath of the 1992 Taura syndrome virus (TSV) epizootic in Ecuador, shrimp farmers in the Western Hemisphere began implementing a variety of strategies to mitigate crop loss from this pathogen. Low-cost, practical solutions included increasing shrimp stocking densities to compensate for higher mortalities (Stern, 1995), decreasing stocking densities to minimize horizontal transmission of the virus (Stern, 1995; Brock *et al*., 1995), and polyculture of shrimp with tilapia (Brock *et al*., 1997). In addition, the shrimp farming industry witnessed an emergence of breeding programs designed to develop disease-resistant strains of shrimp (Clifford, 1998; Lightner and Redman, 1998; Moss *et al*., 1998; Bienfang and Sweeney, 1999; CENIACUA, 1999; Goyard *et al*., 1999; Lightner, 1999; Wyban, 1999). This approach seemed reasonable, especially in light of the tremendous improvements made through selective breeding of commercially important agriculture crops and animals (see Boyle, 1999 for a review on the benefits of chicken breeding).

Based on research conducted at the Oceanic Institute (OI, Waimanalo, Hawaii, USA) since 1995, there appears to be additive genetic variation for resistance to TSV in the Pacific white shrimp, *Litopenaeus vannamei*, and significant improvement in TSV resistance has been made. For example, in a recent research trial, shrimp selected for TSV resistance exhibited a mean family survival that was 18.4% higher than unselected control shrimp after a TSV-challenge test (Argue *et al*., 2002). Similar challenge tests conducted at the University of Arizona (UAZ, Tucson, Arizona, USA) from 1998-2000 revealed that mean survival of all TSV-challenged families increased from 24% to 39% during this period (White *et al*., 2002). In addition, mean survival of the best performing families increased from 65% in 1998 to 100% in 2000, and there are now commercial broodstock suppliers who claim to have families of *L. vannamei* that exhibit > 90% survival to TSV (e.g. Wyban, 2000). Although there is no doubt that TSV-resistant shrimp can improve production and profitability for those farmers who experience a TSV outbreak, these genetically improved stocks are not a panacea for the broader disease problems plaguing the industry. In fact, there are compelling reasons why breeding shrimp for resistance to a single viral pathogen, using current selective breeding strategies, may not be the most efficacious course of action for the long-term viability of the shrimp farming industry. Several of these are identified below.

TRADE-OFF BETWEEN TRAITS

Based on performance data from 587 full-sib families of *L. vannamei*, researchers at OI observed a significant negative phenotypic correlation between mean family survival in a TSV-challenge test and mean family harvest weight from a growout pond in Hawaii ($r = -0.15$, $P < 0.001$; Fig. 1). Similarly, researchers from CENIACUA in Colombia reported that shrimp harvest weight from commercial farms was negatively correlated with survival to White spot syndrome virus (WSSV) in laboratory challenges (CENIACUA and AKVAFORSK, 2002). Additional evidence illustrating the negative relationship between TSV resistance and growth was reported by OI researchers who determined that TSV-susceptible *L. vannamei* exhibited a greater mean weight gain, molted more frequently, and had a shorter molt cycle than TSV-resistant *L. vannamei* during a 26-day experiment (Table 1, Moss *et al*., 2002). These results suggest that there may be a gut-mediated immune response to TSV in *L. vannamei* and reinforce the notion of a trade-off between disease

resistance and growth (or molting) in shrimp. This observation is not unprecedented for aquaculture species and has been reported in fish (Chevassus and Dorson, 1990; Henryon *et al*., 2002).

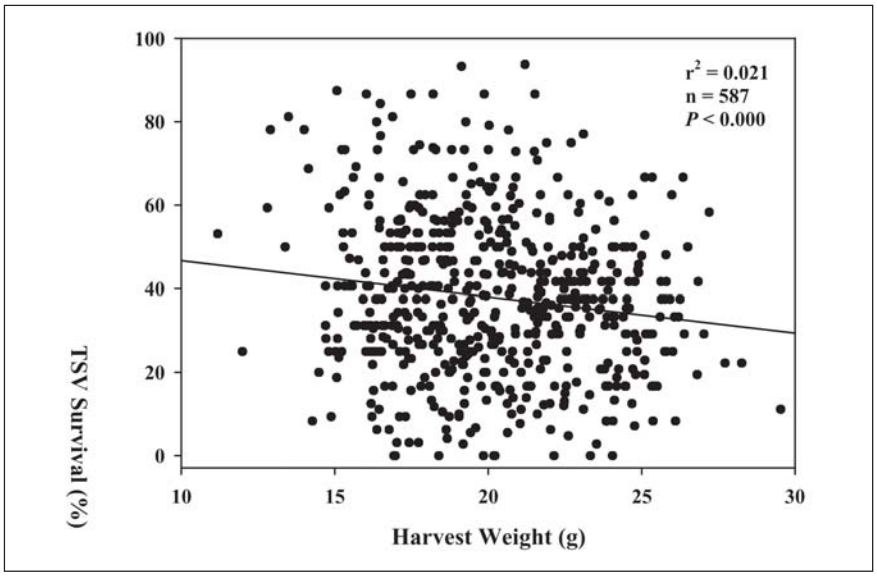

Figure 1. Phenotypic correlation between mean family survival of *L. vannamei* exposed to TSV in a *per os* challenge test and mean family harvest weight from a growout pond in Hawaii.

Table 1. Weight (g), number of molts, molt cycle duration (days), and TSV survival (%) for TSV-susceptible and TSV-resistant strains of the Pacific white shrimp, *Litopenaeus vannamei*. Growth and molt data were collected during a 26-day trail. Except for TSV survival data, values represent means (± SD). As indicated in the last row, there was a significant strain effect on final weight, weight gain, number of molts, and molt cycle duration.

Shrimp Strain	Initial Weight (g)	Final Weight (g)	Weight Gain (g)	Number of Molts Duration (days)	Molt Cycle	Survival to TSV (%)
TSV susceptible	0.93 ± 0.21	3.37 ± 0.57	2.44 ± 0.48	3.25 ± 0.72	7.49 ± 1.71	15
TSV resistant	0.90 ± 0.17	2.83 ± 0.67	1.93 ± 0.57	2.60 ± 1.10	9.64 ± 3.41	81
P	0.55	0.008	0.004	0.03	0.02	

The results described above suggest that pleiotropic genes (genes that directly affect two or more traits) may be responsible for the observed trade-off between disease resistance and growth in shrimp. Alternatively, the correlated response reported by shrimp researchers may have resulted from other factors including sampling error, the genetic makeup of the population under study, and environmental correlations. Environmental correlations are caused by un-analyzed variation among or within ponds, resulting from variable feed supply, population density, temperature, or other factors that affect shrimp performance. The negative relationship between disease resistance and growth is not a universal feature but varies with pathogen and host, and there are reports of positive correlations between growth and

disease resistance in salmonids (Gjedrem *et al*., 1991; Beacham and Evelyn, 1992) and halibut (Imsland *et al*., 2002). In addition to growth, disease resistance in shrimp may be negatively correlated with other commercially important traits, such as carcass quality or fecundity, although such data are lacking. Clearly, additional research is needed to investigate the genetic basis of these relationships so that more effective breeding strategies can be developed, if disease resistance is targeted for selection. With a properly designed selection program, researchers and farmers can improve multiple traits even if they are negatively correlated.

NO COLLATERAL BENEFITS

It would be advantageous for shrimp breeders to realize collateral benefits of multiple disease resistance when selecting shrimp for resistance to a single viral pathogen. However, available data for shrimp do not support this outcome. Researchers from the Waddell Mariculture Center (WMC, Charleston, South Carolina, USA) and OI injected *L. vannamei* from selected families with TSV and WSSV in individual bioassays and found no significant phenotypic correlation in family survival to these two pathogens (r = 0.02, *P* = 0.92, n = 28, Fig. 2). A similar observation was made by a commercial broodstock supplier in Hawaii who reported that TSV- and WSSV-challenge survivals were not correlated among families (Wyban, 2000). It is unclear if these observations are the result of an intrinsic property of the genes or caused by other factors.

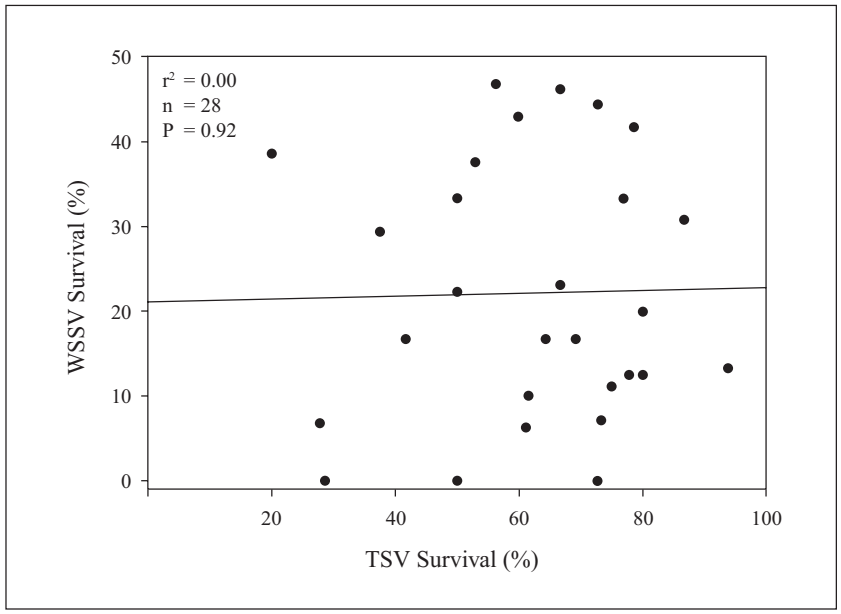

Figure 2. Phenotypic correlation between mean family survival of *L. vannamei* exposed to TSV by injection and WSSV by injection.

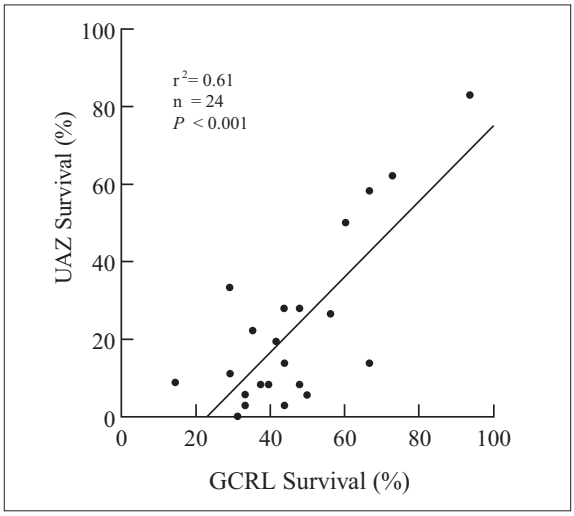

Figure 3. Phenotypic correlation between mean family survival of *L. vannamei* exposed to TSV in a *per os* challenge test conducted at the University of Arizona (UAZ) and a *per os* challenge test conducted at the Gulf Coast Research Laboratory (GCRL).

There is evidence from other organisms that different mechanisms of immunity may be negatively correlated (Biozzi *et al*., 1982; Read and Allen, 2000). In fish, Ehlinger (1977) reported enhanced susceptibility to gill diseases in a strain of brook trout that were selected for resistance to furunculosis. It is interesting to note that researchers from Tufts University (North Grafton, Massachusetts, USA), working with *L. vannamei*, reported that genetic markers (RAPD polymorphisms and mitochondrial DNA RFLPs) associated with susceptibility to *Baculovirus penaei* did not correspond with genetic markers associated with susceptibility to Infectious hypodermal and hematopoietic necrosis virus (IHHNV) and suggested that different loci control the susceptibility of *L. vannamei* to these two viruses (Alcivar-Warren *et al*., 1997). Identification of appropriate quantitative trait loci (QTL) would add significantly to our understanding about the genetics of disease resistance and immunity, and may provide breeders with the tools necessary to exploit multiple disease resistance in shrimp. Although such QTLs currently are unavailable for shrimp, this is an area of active research (e.g. Supungul *et al*., 2002; Luo *et al*., 2003) and significant progress should be forthcoming. Recently, Okamoto and Ozaki (2000) reported using QTLs associated with viral disease resistance in fish, and demonstrated enhanced resistance to Infectious pancreatic necrosis (IPN) virus in rainbow trout using marker-assisted selection.

EFFICACY OF CHALLENGE TESTS

In shrimp breeding programs, disease resistance typically is assessed by exposing different families to viable virus (either through feeding of infected tissue or by injection of a homogenate containing infected tissue) under controlled, laboratory conditions and estimating family survival after a specified amount of time, usually days to weeks (Prior *et al*., 2002; White *et al*., 2002). This information is then used to determine which families

should contribute to the next generation of shrimp. However, survival under these conditions may not be predictive of survival in commercial ponds. In addition, family survival may differ among labs because of procedural differences in the challenge test. In an attempt to address these concerns, researchers from the U.S. Marine Shrimp Farming Program (USMSFP) compared family survival of juvenile *L. vannamei* after exposure to TSV in three USMSFP labs and one commercial shrimp farm where TSV was enzootic (Moss *et al*., 2001). There was a significant positive correlation for mean family survival between each of the two labs where per os challenges were used (Fig. 3) and between these two labs and the commercial shrimp farm (Fig. 4a, Fig. 4b). These results suggest that per os laboratory challenges may be predictive of farm performance. However, TSV was the only pathogen identified in the commercial ponds used in this study, and pond management strategies precluded significant water quality problems. This is atypical of many commercial shrimp farms where multiple pathogens (including viruses, bacteria, protozoa, and metazoan parasites), as well as chemical and physical stressors (including low dissolved oxygen concentrations) are prevalent. Under these conditions, the predictive value of a single-pathogen, *per os* laboratory challenge test may break down.

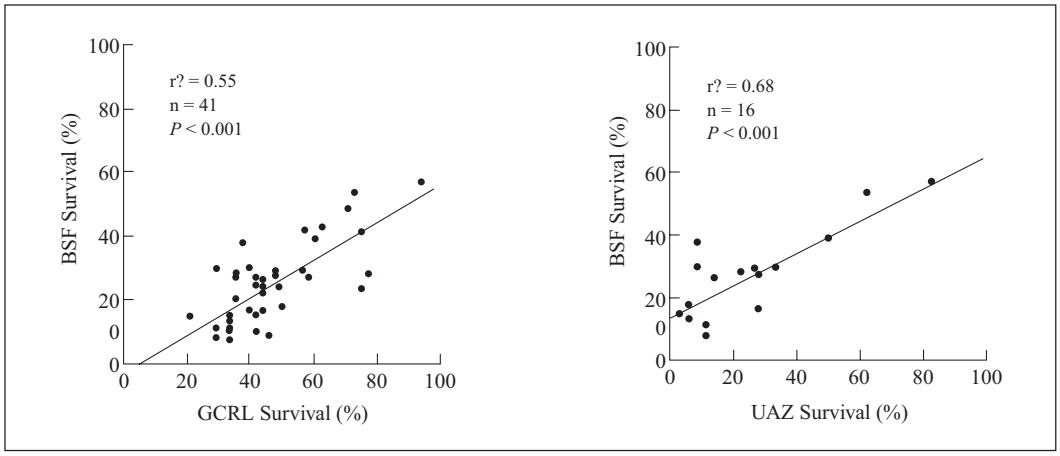

Figure 4. a. Phenotypic correlation between mean family survival of *L. vannamei* exposed to TSV in a *per os* challenge test conducted at the Gulf Coast Research Laboratory (GCRL) and a commercial shrimp farm in Texas (Bowers Shrimp Farm, BSF). b. Phenotypic correlation between mean family survival of *L. vannamei* exposed to TSV in a *per os* challenge test conducted at the University of Arizona (UAZ) and a commercial shrimp farm in Texas (Bowers Shrimp Farm, BSF).

A recent analysis of OI's breeding program has revealed large differences in inbreeding levels among families in the same generation. The coefficient of inbreeding, F, typically ranges from 0.0 to greater than 0.375. This variation is due, in large part, to the differential mating of closely-related shrimp during two or more successive generations prior to the current generation, and similar variation is likely to be found in many commercial shrimp breeding programs. Ongoing experiments at OI, as well as a re-analysis of previous data, indicate that there are significant effects of inbreeding on shrimp performance in disease-challenge tests. To our knowledge, inbreeding has not been included as a covariate in the analysis of disease-challenge data and it was not considered in the experiments referred to

above. It is possible that variable and inconsistent results of challenge tests in different laboratories and among different shrimp populations are significantly affected by un-analyzed inbreeding effects.

With regard to mode of viral exposure, Lotz (1997) reported that mortality from TSV was higher when shrimp were injected with the virus, as opposed to *per os* exposure. This result is consistent with data from other arthropods and suggests that the gut plays an important role as a first line of defense against viral pathogens (Thomas, 1996; Fuxa and Richter, 1998). Interestingly, researchers from the USMSFP found that there was no significant phenotypic correlation in family survival to TSV when shrimp were exposed by injection versus ingestion (r = 0.28, *P* = 0.14; n = 28; Fig. 5). These results highlight the importance of standardizing laboratory protocols used in disease-challenge tests. In addition, they indicate that injection assays may bypass a primary line of defense in shrimp and may be inappropriate for selective breeding programs interested in horizontally transmitted viruses. Additionally, current methods to assess disease resistance in shrimp are based on the determination of a threshold character (dead or alive) which is expressed only at the family level and is influenced by factors that may have little or no relevance in a selection program, such as environmental effects and inbreeding. There is a need to develop disease-challenge measurements that will lead to individual characterization of the shrimp so that more powerful and sophisticated selection strategies can be used. It is theoretically possible to separate mortality factors in a genetic analysis if all the factors have indicator variables associated with them. Viral load would be an appropriate indicator variable, and this concept is developed later in the manuscript.

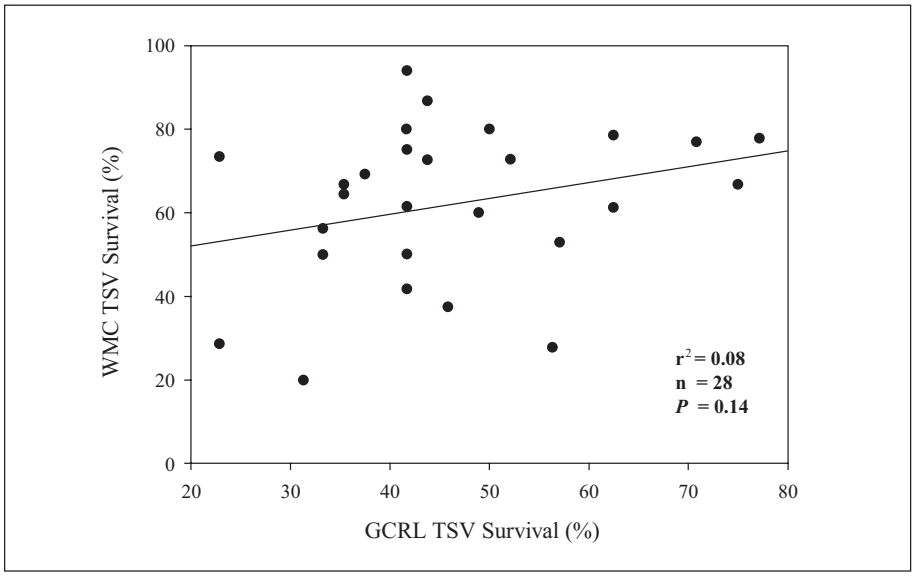

Figure 5. Phenotypic correlation between mean family survival of *L. vannamei* exposed to TSV by injection at the Waddell Mariculture Center (WMC) and a *per os* challenge test conducted at the Gulf Coast Research Laboratory (GCRL).

VIRAL MUTATION

In 1999, significant mortalities of a TSV-tolerant strain of the Western blue shrimp, *L. stylirostris*, occurred at shrimp farms in Mexico (Erickson and Lightner, 2001). These mortalities resulted from TSV epizootics that were accompanied by a slight deviation in the gross signs typical of TSV infection, and there were concerns that a new TSV strain had emerged. Since 2000, researchers at UAZ have analyzed TSV geographic and year isolates in relation to the reference isolate from Hawaii (HI94TSV) to determine possible differences in virulence and host range, using selected OIE (Office of International de Epizooties) diagnostic methods (OIE, 2000) and sequence analysis of nucleotides and amino acids in the TSV VP1 region of the viral genome. Initially, UAZ researchers compared the reference isolate with three TSV isolates from Mexico (SIN98TSV from *L. vannamei*, MX99TSV and SON2KTSV from *L. stylirostris*), and concluded that there are similar but distinct isolates of TSV (Erickson *et al*., 2002). Specifically, the SIN98TSV isolate exhibited characteristics that warranted a different serotype designation (TSV Serotype B, Table 2) from the reference isolate (based on VP1 serological properties), whereas the MX99TSV and SON2KTSV isolates were sufficiently similar to the reference isolate to be included in the same serotype (TSV Serotype A).

In 2001, significant mortalities of *L. vannamei* occurred at shrimp farms in Belize, resulting from TSV epizootics (Rosenberry, 2001), and there were concerns that another TSV strain had emerged which was different from the SIN98TSV isolate. The Belize 2002 isolate of TSV (BLZ02TSV, Table 2) was purified from cultured *L. vannamei* by UAZ researchers, and the virions were found to be slightly but significantly larger (32.693 nm) than those from Hawaii (31.384 nm) or Mexico (32.038 nm). Again, using OIE diagnostic methods and sequence analysis, UAZ researchers concluded that the BLZ02TSV isolate represented a new serotype, TSV Serotype C (Table 2). Importantly, broodstock suppliers to Belize reported that shrimp bred for resistance to the "old" Taura strain (TSV Serotype A) succumbed to the "new" Belize strain (TSV Serotype C, Rosenberry, 2001). In response to these concerns, researchers from the USMSFP compared family survival of juvenile *L. vannamei* after exposure to both TSV serotypes. Shrimp from these families were selectively bred for resistance to the "old" Taura strain. Specifically, researchers at the Gulf Coast Research Laboratory (GCRL, Ocean Springs, Mississippi, USA) exposed shrimp from 80 full-sib families produced at OI to the two TSV serotypes in a *per* os challenge. Mean (±SD) family survival to Serotype A and Serotype C was 43.78 ± 18.94% and 35.64 ± 20.18%, respectively. There was a significant ($P < 0.05$) positive linear correlation in mean family survival between the two serotypes, but the coefficient of determination was low at $r^2 = 0.27$ (Fig. 6).

Table 2. TSV serotype designations based on VP1 serological properties.

Serotype Designation	Isolate Designation	Place of Origin	Year	Species of Origin
TSV Serotype A	HI94TSV	Hawaii, USA	1994	*L. vannamei*
	MX99TSV	Sonora, Mexico	1999	*L. stylirostris*
	SON2KTSV	Sonora, Mexico	2000	*L. stylirostris*
TSV Serotype B	SIN98TSV	Sinaloa, Mexico	1998	*L. vannamei*
TSV Serotype C	BLZ02TSV	Belize	2002	*L. vannamei*

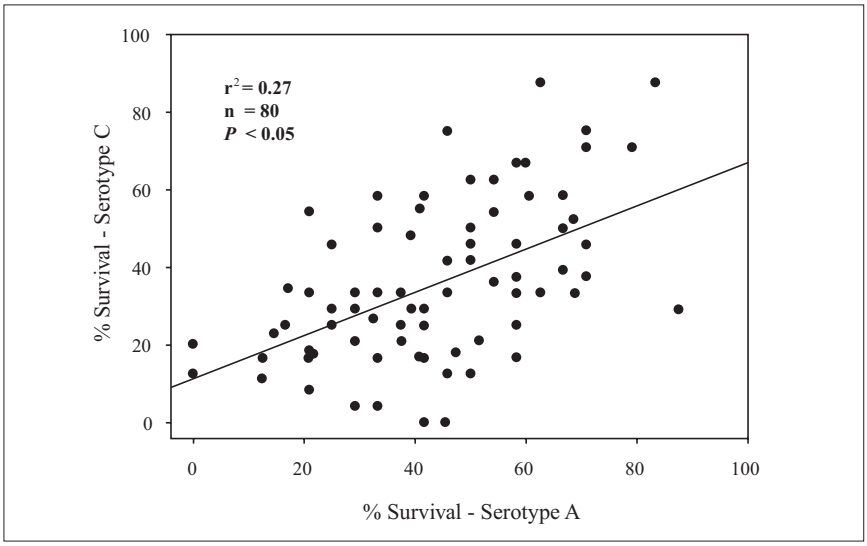

Figure 6. Phenotypic correlation between mean family survival of *L. vannamei* exposed to two different TSV serotypes.

In the trial referred to above, there were four families that exhibited relatively high survival to both TSV serotypes. Offspring from these unique families were produced and subsequently exposed to TSV Serotypes A and C (Moss *et al*., 2003). In addition to evaluating the performance of selectively bred shrimp, a positive control group was evaluated. This group consisted of the OI "Kona" reference strain of *L. vannamei* exposed to both TSV serotypes. These "Kona" shrimp are TSV susceptible and exhibit consistently low survival (0 – 40%) after TSV exposure (White *et al*., 2002; Hennig *et al*., 2004). The positive controls were evaluated to ensure that the virus-laden tissue used in the challenges was infectious. After 14-days post-exposure, selectively bred shrimp exhibited 95% survival to the Hawaii TSV serotype. This was 75% higher than the "Kona" reference strain, which exhibited 20% survival after exposure to the same virus. Importantly, selectively bred shrimp exhibited 63% survival after exposure to the Belize TSV serotype, whereas all of the "Kona" shrimp died by day 4 of the challenge test. These results indicate that the Belize TSV serotype was more lethal than the Hawaii serotype, especially for the "Kona" reference shrimp.

The results discussed above indicate that viruses can mutate and may render previously resistant shrimp incapable of defending themselves against new viral strains. Viral mutations can arise from a number of mechanisms and viruses differ markedly in their mutation rates. This is due largely to differences in the fidelity of transcriptases to replicate nucleic acids. DNA viruses have mutation rates similar to those of eukaryotic cells, and because their replicatory enzymes have a proofreading function, these rates are characteristically low. The error rate for DNA viruses is estimated to be 10^{-8} to 10^{-11} errors per incorporated nucleotide (Fleischmann, 2002). In contrast, RNA viruses, such as TSV, lack a proofreading function, and the error rate for these types of viruses is estimated to be 10^{-3} to 10^{-4} errors per incorporated nucleotide. In light of this, it is not surprising that different strains of TSV exist. Interestingly, sequence information from various geographical isolates of IHHNV (a DNA virus) suggests that there are at least three strains of this virus, although it is not clear if these strains differ in virulence and host range (Tang and Lightner, 2002).

ALTERNATIVE STRATEGIES TO ASSESS DISEASE RESISTANCE

In breeding shrimp for disease resistance, the selection objective has been to increase survival after exposure to a particular pathogen. To date, there have been at least three selection strategies designed to increase survival, including natural selection on the farm, selection of survivors in an artificial challenge, and selection of shrimp whose siblings exhibit superior survival in an artificial challenge. Selecting on-farm survivors of a disease outbreak is an inexpensive, practical approach if shrimp are cultured under non-biosecure conditions, but is inappropriate in a specific pathogen free (SPF) program (Lotz, 1997). For example, Colombian shrimp farmers bred survivors of a TSV outbreak and, after five generations of selection, pond survival increased from 10% to 70% (Santamaría, 1999). However, these shrimp were carriers of TSV and when they were co-cultured with naive shrimp that originated in a TSV-free environment, massive mortalities occurred (Flegel, 2001). Similarly, selection of survivors in an artificial challenge is impractical in an SPF program because of the cost associated with ensuring that survivors are free of specifically listed pathogens. Selection based on information from relatives who have been exposed to pathogens (e.g. family selection) is appropriate for an SPF program because infected or exposed shrimp are not brought back into the breeding program. However, we suggest that increased survival *per se* may not be the best or only selection objective for a breeding program designed to improve disease resistance. This is because survival alone may it not be a good measure of a shrimp's innate (genetic) ability to cope with a particular pathogen. In addition, survival is a categorical variable with only two values and has limited use in genetic models. We suggest that appropriate, continuous variables should be considered for inclusion in a breeding program to improve the accuracy and intensity of selection.

Under farm conditions, all relevant modes of viral transmission will be encountered and all modes of resistance (behavioral, anatomical, and physiological) will have an opportunity to be expressed. Laboratory-challenge tests are used as an expedient alternative to on-farm exposure because of difficulties in controlling and replicating experimental units on the farm. Unfortunately, the convenience of these artificial-challenge tests comes at the cost of reduced relevance and realism, as noted previously. From a geneticist's perspective, this cost means that some types of genetic variation, which could improve farm performance, are never expressed and thus never analyzed or selected when relying on laboratory challenges.

In addition to these concerns, there are other problems with conventional challenge tests when they are used to generate genetic information. Although several types of challenge tests can be used, none are particularly well suited to genetic analysis. Either data do not conform to the assumptions of conventional genetic models, or family-level challenge tests lack statistical power because the number of observations required to reach definitive conclusions becomes impractical. For example, there is a type of challenge in which the measurement variable is *time to reach a pre-determined mortality level* (e.g. 50% of the population dies). These data are not suited to quantitative genetic analysis because a plausible genetic model has not, to our knowledge, been formulated for this variable. However, this approach could be used in practical, between-family selection programs in which no statistical analyses are required.

Challenge tests in which *percent mortality is observed at standardized intervals* can be analyzed by genetic threshold liability models. Each individual is categorized into one or more categories ("alive at one week", "alive at three weeks", etc.) in the analysis. Statistical procedures that accommodate such data have reached a high degree of sophistication, and data of this type can be included in BLUP and REML estimations and QTL mapping algorithms. Unfortunately, the statistical power of threshold analysis is low and a significant number of observations are needed to achieve an acceptable level of statistical power. This is in contrast to what can be achieved with the monitoring of continuous variables, such as weight or length.

Challenge tests in which the *survival time of individuals is continuously observed* are more appropriate for genetic analysis, including linear proportional hazards models with random effects. A recent aquaculture example using this approach is reported in a viral challenge with rainbow trout (Henryon *et al*., 2002). One problem associated with this measure is that some animals may survive to the end of the experiment, so all that is known about their survival time is that it exceeded some particular value. In practice, it is difficult to design and conduct a disease-challenge test which is sufficiently natural for all relevant exposure routes and defense mechanisms to be expressed, but controlled enough so that individual survival times can be observed and recorded.

We believe that some of these problems can be solved by re-defining the selection objective in terms of *resistance to a specific viral pathogen* by combining a measure of viral load and survival in a selection index. Thus, the goal of the breeding program would be to develop shrimp strains that have both higher survival and a lower viral load in farm environments where these pathogens are problematic. Researchers at UAZ reported that a line of *L. stylirostris*, produced by Super Shrimp®, exhibited resistance to IHHNV (Tang *et al*. 2000). Although resistance to the effects of IHHNV infection has been reported in a different domesticated line of *L. stylirostris* (Weppe *et al*., 1992), the Super Shrimp(r) line was completely refractory to infection, as IHHNV did not replicate in postlarvae and juveniles from this line after *per os* exposure. Thus, in a shrimp line such as this, real-time PCR, coupled with per os and injection assays, could be used to evaluate viral load with the ultimate goal of producing a resistant and refractory line of shrimp where the viral load is zero. It is important to note that for viral diseases where horizontal transmission (i.e. cannibalism, water, contact, etc.) is the most important mode of infection, *per os* assays are the most appropriate way to expose shrimp in order to estimate viral load and/or survival in the laboratory. However, for diseases like IHHNV (and TSV to a lesser extent) where vertical transmission is also important, evaluation by injection (in addition to *per os* challenge) may also be necessary.

Viral load, measured by real-time PCR, is a continuous variable highly suited to genetic analysis. Challenge tests would be re-designed so that the output variable used in the selection program is a viral assay. Percent survival and/or survival time also would be measured and incorporated into the selection index. It may be necessary to be highly specific in defining the viral genome used in the challenge tests. Researchers at UAZ have been investigating differences in virulence of different WSSV isolates and have found that some isolates are more lethal than others at the same viral copy number/gram tissue. Hence, the virulence of different viruses or viral strains also will affect the virus load number, survival, and time to

death, which makes all three of these values potentially important indicators of resistance. Another consideration in designing the viral assay is when to quantify viral load. Depending on how shrimp defend against infection, the time at which viral load is quantified could be a critical factor. If viral load is measured too early, before a defense mechanism is activated, selection would not be effective.

In summary, we suggest that the development of disease-resistant shrimp can be achieved more effectively when the selection objective is re-defined to include a continuous indicator variable, as well as survival. In addition to viral load, there are other metrics that may provide geneticists with selection criteria to further improve the accuracy and intensity of selection. For example, the development of DNA microarrays now offers the promise of genome-wide monitoring of gene expression (Dhar *et al*., 2003; Knibb *et al*., 2004). This genome-wide approach may not only provide a means of identifying key genes to target for therapeutics, but may enable selection based on expression intensity. As we look towards the future, we can anticipate that more effective breeding strategies will emerge as we gain a better understanding about the genetic basis for disease resistance.

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