FAO TCP/INT/3502 “Reducing and managing the risk of Acute Hepatopancreatic Necrosis Disease (AHPND) of Cultured Shrimp”

Preliminary results on genetic resistance to AHPND and WSSV in *Penaeus (Litopenaeus) vannamei* in Mexico: the role of inbreeding

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Genetic Nucleus of Maricultura del Pacífico

• Facilities operating since 2006

• Isolated facilities for disease control

• Managing approximately 150 families per year in a Commercial line plus 120 families in a Conservation line (2005 – 2013)

• Located in Sinaloa, in the Pacific coast of Mexico
Selection Objectives & Criteria

Selection Objective

– Obtain a larger biomass (higher economic value)
– Biomass = survival rate x individual average body weight

Selection Criteria

– Individual body weight at 130 d
– Grow-out Survival rate (65-130 d)

– From 2014 disease resistance for AHPND/EMS and WSSV
- Families generated by AI
- Genealogy recording since 2003
- Inbreeding control
Studies

We will present here results from four studies:

1) Genetic parameter estimation for AHPND/EMS and WSSV resistance
   – Challenges of mixed families in 2014 from Ecuadorean, commercial Maricultura, and F1 lines

2) Estimation of differences between lines and crosses for AHPND and WSSV resistance
   – Challenges of mixed families in 2014 from Ecuadorean, commercial Maricultura, and F1 lines

3) Inbreeding effects on survival and growth
   – Conservation Maricultura line 2010 – 2012, planned inbreeding experiment and retrospective data analysis of commercial line

4) Preliminary results on inbreeding effects on AHPND/EMS and WSSV resistance
   – Challenges of pedigreed Maricultura families in 2014 for AHPND and WSSV
Some ideas on small population size, inbreeding and disease resistance

1) Conventional wisdom

2) Some theoretical and simulation results

3) Results form actual populations

4) Conclusions
Populations involved in 2014 in disease resistance challenge tests

Ecuadorean line (28 families)
   – (Originated from 2 hatcheries with WSSV resistance background)

Maricultura Mexican line (100 families)
   – (From merging the 2 lines in 2013)

F1 crosses Ecuador x Maricultura (16 families)
AHPND/EMS resistance challenge test

- Planned experimental challenge of a mixed population to estimate heritability and line differences
- AHPND inoculated by immersion using a *Vibrio parahemolyticus* strain (M0904) at $10^5$ CFU/ml
- Shrimp averaging 1 g of body weight were immersed for 15 hours
- We used the survival time as response at 50% of general survival
- We analized 3,928 shrimp from 144 families. Each family was evaluated in two aquariums
- A linear mixed model was used for genetic parameter estimation
Acute Hepatopancreatic Necrosis Disease (AHPND / EMS) challenge tests studies
White Spot Syndrome Virus (WSSV) challenge test
WSSV resistance challenge test

- Planned experimental challenge of a mixed population to estimate heritability and line differences

- WSSV inoculated by *per os* using an infecting dose $> 10^7$ copies of viral DNA/g

- Shrimp averaging 4 g of body weight were maintained until mortality reached 50%

- We analyzed 3,966 shrimp from 144 families

- A linear mixed model was used for genetic parameter estimation for survival time
Genetic parameter estimates for disease resistance (survival time) for AHPND and WSSV and body weight at 130 days of age in a mixed *P. vannamei* population

<table>
<thead>
<tr>
<th></th>
<th>AHPND-1</th>
<th>AHPND-2</th>
<th>WSSV-1</th>
<th>BW130</th>
</tr>
</thead>
<tbody>
<tr>
<td>AHPND-1</td>
<td>0.06 ± 0.08</td>
<td>0.86</td>
<td>0.33</td>
<td>-0.01</td>
</tr>
<tr>
<td>AHPND-2</td>
<td></td>
<td>0.09 ± 0.04</td>
<td>-0.14</td>
<td>0.16</td>
</tr>
<tr>
<td>WSSV-1</td>
<td></td>
<td></td>
<td>0.17 ± 0.05</td>
<td>-0.21*</td>
</tr>
<tr>
<td>BW130</td>
<td></td>
<td></td>
<td></td>
<td>0.61 ± 0.12</td>
</tr>
</tbody>
</table>

Heritabilities in diagonal (boldtype), genetic correlations above diagonal
Least-Squares means (SE) or the three genetic groups of *P. vannamei* included in the assays performed in 2014

<table>
<thead>
<tr>
<th></th>
<th>Ecuador</th>
<th>F1</th>
<th>Mexico</th>
</tr>
</thead>
<tbody>
<tr>
<td>AHPND-1 (hours)</td>
<td>13.4(^a) (0.3)</td>
<td>13.0(^a) (0.3)</td>
<td>11.6(^b) (0.3)</td>
</tr>
<tr>
<td>AHPND-2 (hours)</td>
<td>77.1 (2.8)</td>
<td>79.8 (2.8)</td>
<td>79.2 (3.1)</td>
</tr>
<tr>
<td>WSSV (hours)</td>
<td>101.0(^a) (2.3)</td>
<td>90.2(^b) (2.4)</td>
<td>87.1(^b) (2.7)</td>
</tr>
<tr>
<td>Weight 130 d (g)</td>
<td>11.1(^a) (2.4)</td>
<td>16.0(^b) (1.5)</td>
<td>20.2(^c) (2.2)</td>
</tr>
</tbody>
</table>
Heritability of resistance conclusions

Genetic component of variation (heritability)

- AHPND: Low?
- WSSV: Moderated
- W130: High > than within line estimates
- No evidence of unfavorable genetic correlated response between these two disease resistances from rg
- Unfavorable genetic relationship between WSSV and W130
Differences between lines conclusions

Between lines response

– Ecuadorean line is more resistant for both diseases at 1 g, but no differences were found at 4 g

  Same genes involved?

– *Negative heterosis* evidence for WSSV resistance

  A single major recessive gene?

– Moderate positive heterosis for AHPND and W130
Study on inbreeding effects

(Designed experiment)

- Planned experimental inbreeding by recurrent sib mating in four generations in the conservation line of Maricultura
- Data on 320 families aprox. 16 thousand shrimp 2010-2012
- Inbreeding coefficients from 0 to 61%
- 41% of families have inbreeding coefficients > 3.25%
- Linear mixed model analysis
Heritability and inbreeding effects for harvest size body weight (130 d) and general survival rate (65-130 d) in *P. vannamei*

<table>
<thead>
<tr>
<th>Trait</th>
<th>Mean</th>
<th>Heritability</th>
<th>Change in the mean per 10% increase on inbreeding coefficient (Experiment)</th>
<th>Change in the mean per 10% increase on inbreeding coefficient (Retrospective analysis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight</td>
<td>19.6 g</td>
<td>0.20</td>
<td>- 2.2 ± 0.4%**</td>
<td>-3.4 ± 0.3%*</td>
</tr>
<tr>
<td>Survival rate</td>
<td>81.7%</td>
<td>0.01</td>
<td>-0.01 ± 0.01%</td>
<td>-1.88 ± 5.87%</td>
</tr>
</tbody>
</table>
Study on inbreeding effects

Conclusions

- Inbreeding depressed body weight at harvest size in *P. vannamei*

- Depression per 10% of inbreeding coefficient increase is about 2.2% of the mean

- Inbreeding did not depress general grow-out survival rate

- Probably harmful genes for survival were purged in this population by natural selection
AHPND/EMS and WSSV inbreeding effects

Results

• Preliminary analysis were made to estimate possible effects of inbreeding on disease resistance using the same data from the 2014 challenge tests and inbreeding coefficient for pedigreed Maricultura families

• Inbreeding coefficients ranged from 1.3 to 6.6%

• Inbreeding linear effects for 10% increase in inbreeding were negative for AHPND-1 resistance (-1.8 hours) and body weight at 130 d (-2.2 g) but were not significant (P > 0.05)

• The estimate for WSSV and AHPND-2 resistance were positive and not significant either
AHPND/EMS and WSSV inbreeding effects

conclusions

• These estimates are clearly inaccurate. The SE for the estimate for body weight at 130 d was 1.7 g, compared to an estimate of 0.08 g from that for the inbreeding experiment (about 21 times larger)

• Experimental data with wider inbreeding values are needed to answer the question on whether may inbreeding be involved in disease susceptibility / resistance. However these effects are by definition different for each population
Conventional wisdom on bottlenecks

Reduction on effective population size (Ne)

Increase in inbreeding (F)

Reduction of genetic variability by genetic drift

Affects
Survival?
Growth?
Reproduction?

Affects
Genetic gain?
Opportunities
to improve new Traits?
Theory and simulations
Average population fitness ($W$) against number of generations ($t$) of maintenance with population size $N_e = 10$ when, in the original population, individuals carried on the average one rare recessive lethal. Evolution of $W$ expected only from inbreeding (red line) or from inbreeding and purging (blue line).

- purging can be very efficient preventing inbreeding depression

- for non-lethal deleterious alleles, the efficiency of purging would be smaller
Mean fitness (W) in purged populations

- Purging can be very efficient preventing inbreeding depression for fitness in small populations
  García-Dorado, A. *Genetics, 2012, 190, 1461-1476*
Changes in gene frequencies in 20 generations for bottleneck populations with or without selection \( Ne = 50 \)

No selection, pure drift

Selection against the homocysygous recessive: \( W_{aa} = 0.5 \)

If bad alleles are really bad, they will be effectively selected against even in small populations
Changes in gene frequencies in 20 generations for a very small bottleneck populations (Ne = 10 -> Ne = 4  gen 4 to 20), with mild selection against a recessive (Waa = 0.5)

Defect genes which are neutral can be fixed, but not genes related to survival
Results from research with actual populations
Effects of inbreeding depression recovery plus crossbreeding on survival rates of *P. stylirostis* to *Vibrio spp.* challenges

Average survival rates in ponds and cages (%) for inbred lines and F1 (Goyard et al., 2008)

<table>
<thead>
<tr>
<th>Inbreed</th>
<th>Hawaii</th>
<th>F1</th>
</tr>
</thead>
<tbody>
<tr>
<td>42.2</td>
<td>45.0</td>
<td>55.2</td>
</tr>
</tbody>
</table>

Average survival rates (%) in challenge tests for inbred lines and F1 in 2005 and 2006 (Goyard et al., 2008)

<table>
<thead>
<tr>
<th>Inbreed</th>
<th>Hawaii</th>
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</tr>
</thead>
<tbody>
<tr>
<td>49.0</td>
<td>47.0</td>
<td>57.0</td>
</tr>
</tbody>
</table>

Maximum differences F1-inbreds 4.6 to 13%
Quotes

Bottlenecks, by purging deleterious alleles, can enable the evolution of invaders (insects) that maintain high fitness even when inbred
Facon et al., *Current Biology* 21, 424–427, 2011

*Drosophila* consistently experience more inbreeding depression in fertility than in viability

The amount of inbreeding depression measured often varies according to life-history stage, trait measured, experimental habitat, environmental conditions, or year of study

Heterozygosity, population size, and quantitative genetic variation—were positively and significantly correlated with population fitness. They explained, however, only 15–20% of the variation in fitness. [however only 10% for population size]

Different populations of a single species, and different inbred lineages from the same population, often exhibit highly variable responses to inbreeding under stressful conditions
Armbruster and Reed. *Heredity* 95, 235–242, 2005
Conclusions
Conclusions about small populations

• Drift is inevitable in populations of limited size
• Any cultured broodstock population will suffer drift
• Existence of bottlenecks will increase drift
• Bottleneck effects on variability can be reversible to a certain extent
• Drift will imply the loss of alleles, however most lost alleles will be neutral (unrelated to disease resistance or productivity)
• Maintenance good fitness after severe bottlenecks (inbreeding) exist in the literature for many populations. This may be caused by selection (purge) and overdominance
• Drift is random, but selection is a strong directional force
• Inbreeding effects are very hard to predict, because depend on the population structure and history
Conclusions on mutations for genetic resistance

• Mutation can create new genetic variability
• Variability caused by new mutations is independent from the history of the population and current Ne
• To capture beneficial mutations, large numbers of animals should be exposed to the pathogen
• The strategy on how to introgress the mutation genes into the breeding populations is important
General ideas about selection for genetic resistance

• Inbreeding will cause depression if bad alleles are not selected against, but bad genes will be maintained at low frequencies by natural/artificial selection even in small populations

• In principle small populations will have more limited options to adapt to new disease challenges, but small populations may be expanded to capture mutations for resistance

• Most diversity measured by genetic markers is neutral and will not make a difference for improving any trait

• A way to increase selection pressure is to do disease resistance challenges.

• Keeping the survivors for breeding is a more efficient way to rapidly develop resistant populations than selecting families based on sib tests, but practical hygienic considerations are important