# ALLOZYME MARKER BASED COMPARISON ON GENETIC VARIATION AMONG *MACROBRACHIUM ROSENBERGII* POPULATIONS PRODUCED FROM A CROSS-BREEDING SYSTEM OF THREE DIFFERENT STOCKS IN THAILAND

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## INTRODUCTION

The giant freshwater prawn, *Macrobrachium rosenbergii* has been domesticated in Thailand for decades, but appropriate selective breeding program of this species has not yet been achieved. Thus, a good quality seed for the *Machrobrachium* industry is therefore not regularly produced. A selective breeding program which includes improvement of growth performance on the domesticated strain was carried out at the Aquatic Animal Genetics Research and Development Institute (AAGRDI), Department of Fisheries. The institute has now developed a domesticated and genetically improved stock of *Macrobrachium rosenbergii* for 2 generations.

A wild stock was also domesticated under hatchery conditions at AAGRDI for 1 generation. Meanwhile, domesticated stocks from private hatcheries have also been sourced. There is therefore a need to develop another improved stock of the species basically from the two stocks of AAGRDI, the genetically improved and the wild, together with the domesticated stock from a good private hatchery. This is because the new created stock, which will be used as base population for further selective breeding program, should be developed with higher genetic diversity.

Generally, a good base population for a genetic improvement program requires high genetic variation as well as an ideally suitable stock that can be well adapted for different local environments. Therefore, all proper crosses of these three stocks should be cultured in different areas of the country and their performance and genetic variation evaluated before a selective breeding program will have taken place.

Molecular technology at enzyme/protein level known as “allozyme marker” is a widely accepted powerful technique to study genetic variation (Ward and Grewe, 1995) as well as intra-specific population studies (Sodsuk, 1996; Sodsuk and Sodsuk, 1998a & 1998b; Sodsuk *et al*., 2001). Due to the availability of the allozyme technique, it is basically and initially applied for the genetic variation evaluation.

The objectives of this research were to: (1) evaluate genetic variation (in terms of genetic variabilities as per locus averages of observable heterozygosities and number of alleles) of nine crosses from the above three mentioned stocks (the genetically improved by AAGRDI, the wild, and the private farm) of *Macrobrachium rosenbergii*; (2) apply polymorphism system of allozyme markers in the evaluation; (3) compare the evaluated genetic variation among the nine crosses for differences; and (4) use of the information on genetic variation evaluated, together with the performances, to choose the best cross for further selective breeding program in an appropriate area.

## MATERIALS AND METHODS

### Sample Analysis

About 40-60 individuals of both sexes of each of the three stocks (the AAGRDI, wild, and private farm) and each progeny population of 9 crosses were sampled. Pleopods from each individual were cut and collected in separate microtubes. All pleopod samples in microtubes were preserved at -70 ºC in deep freezer for further molecular analysis of allozyme markers. The preserved samples were electrophoretically analysed at 19-25 allozyme loci (see Tables in appendix) following the procedure already studied before in *Macrobrachium rosenbergii* by Sodsuk and Sodsuk (1998b)

### Data Analysis

All allozyme data from the laboratory analyses were collected and calculated as per locus averages of heterozygosities (H) and number of alleles (NoA) for genetic variation evaluation. The work made use of particular software for population genetics studies known as BIOSYS release 1.7 of Swofford and Selander (1989).

The genetic variations, as per locus averages of heterozygosities and number of alleles, of 9 crosses (see Tables in appendix) were statistically compared following the methods of Sokal and Rohlf (1981) and Ward *et al*. (1994). This procedure was done using statistical software known as SYSTAT of Wilkinson *et al*. (1992).

## RESULTS AND DISCUSSION

The genetic variation, evaluated as per locus averages of heterozygosities and number of alleles, of three initial stocks and all 9 crosses, is shown respectively in Tables 1 and 2. There were no significant differences among the three initial stocks as well as the 9 cross, both by heterozygosities and number of alleles. The appearance of heterozygosities and number of alleles both in the three initial stocks (H = 0.023 – 0.043, NoA = 1.20 – 1.44) and in the 9 crosses (H = 0.010 – 0.042, NoA = 1.11 – 1.53) were close to those of the natural stocks (H = 0.027 – 0.036, NoA = 1.29 – 1.33) studied before by Sodsuk and Sodsuk (1998b).

**Table 1.** Per locus averages of heterozygosities (H) and number of alleles (NoA) of the three initial stocks

| Stock | H | NoA |
|---|---|---|
| AAGRDI | 0.043 (±0.018)$^{A}$ | 1.36 (±0.11)$^{a}$ |
| Wild | 0.023 (±0.014)$^{A}$ | 1.20 (±0.10)$^{a}$ |
| Farm | 0.036 (±0.016)$^{A}$ | 1.44 (±0.13)$^{a}$ |

Values in parentheses are standard errors (±S.E.)
Same superscripts in the same column means no significant differences ($p$>0.05)

**Table 2.** Per locus averages of heterozygosities (H) and number of alleles (NoA) of all 9 crosses

| | Cross (male x female) | H (Average ±S.E.) | NoA (Average ±S.E.) |
|---|---|---|---|
| $T_1$ | (Wild x AAGRDI) | 0.011 (±0.008)$^{A}$ | 1.11 (±0.07)$^{a}$ |
| $T_2^1$ | (AAGRDI x Wild) | 0.042 (±0.027)$^{A}$ | 1.26 (±0.10)$^{a}$ |
| $T_3^2$ | (AAGRDI x Farm) | 0.010 (±0.007)$^{A}$ | 1.16 (±0.09)$^{a}$ |
| $T_4^3$ | (Farm x AAGRDI) | 0.016 (±0.007)$^{A}$ | 1.32 (±0.13)$^{a}$ |
| $T_5^4$ | (Wild x Farm) | 0.030 (±0.010)$^{A}$ | 1.53 (±0.14)$^{a}$ |
| $T_6^5$ | (Farm x Wild) | 0.026 (±0.013)$^{A}$ | 1.26 (±0.13)$^{a}$ |
| $T_7^6$ | (Farm x Farm) | 0.024 (±0.010)$^{A}$ | 1.37 (±0.11)$^{a}$ |
| $T_8^7$ | (Wild x Wild) | 0.018 (±0.009)$^{A}$ | 1.21 (±0.10)$^{a}$ |
| $T_9^8$ | (AAGRDI x AAGRDI) | 0.015 (±0.009)$^{A}$ | 1.16 (±0.09)$^{a}$ |

Same superscripts in the same column means no significant differences ($p$>0.05)

Table 3 shows the genetic information of the resulting heterozygosities and number of alleles, together with those resulting from growth performance (Uraiwan *et al*., 2005). This genetically informative table is very helpful for choosing the best breeding-pair for further selection program in an appropriate area.

## CONCLUSION

1. Amounts of genetic variation, as per locus averages of heterozygosities and the number of alleles, of three initial stocks and all 9 crosses were evaluated and statistically compared but non-significant differences were obtained among the stocks.

2. Heterozygosities and number of alleles obtained from this study together with growth performances from the study of Uraiwan *et al*. (2005), would help in choosing the best cross for an appropriate area.

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**Table 3.** Genetic informations by heterozygositiies (H), number of alleles (NoA), growth performances and % heterosis of all crosses in four different areas

| Environment (months) | Mate pair | Crosses | Sodsuk *et al.* (2005) | | Uraiwan *et al.* (2005) | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Per formances | | % heterosis | |
| | | | H | NoA | Length | Weigth | Length | Weigth |
| Uttaradit (5) | AAGRDI & Wild | $T_1$ | 0.011[A] | 1.11[a] | 12.982 | 24.354 | 2.28 | 15.47 |
| | | $T_2$ | 0.042[A] | 1.26[a] | 12.671 | 24.449 | | |
| | AAGRDI & Farm | $T_3$* | 0.010[A] | 1.16[a] | 13.140 | 23.977* | 20.28* | 9.16* |
| | | $T_4$* | 0.016[A] | 1.32[a] | 13.822* | 22.083 | | |
| | Wild & Farm | $T_5$ | 0.030[A] | 1.53[a] | 12.500 | 22.908 | 1.01 | 3.13 |
| | | $T_6$ | 0.026[A] | 1.26[a] | 12.002 | 20.681 | | |
| | Farm & Farm | $T_7$ | 0.024[A] | 1.37[a] | 12.212 | 21.965 | - | - |
| | Wild & Wild | $T_8$ | 0.018[A] | 1.21[a] | 12.044 | 22.035 | - | - |
| | AAGRDI & AAGRDI | $T_9$ | 0.015[A] | 1.16[a] | 12.267 | 20.230 | - | - |
| Buriram (4) | AAGRDI & Wild | $T_1$ | 0.011[A] | 1.11[a] | 10.430 | 17.220 | 0.61 | 0.48 |
| | | $T_2$ | 0.042[A] | 1.26[a] | 10.783 | 16.140 | | |
| | AAGRDI & Farm | $T_3$* | 0.010[A] | 1.16[a] | 11.061* | 20.709* | 1.58* | 19.86* |
| | | $T_4$ | 0.016[A] | 1.32[a] | 10.447 | 16.710 | | |
| | Wild & Farm | $T_5$ | 0.030[A] | 1.53[a] | 10.618 | 17.740 | -2.85 | -0.30 |
| | | $T_6$ | 0.026[A] | 1.26[a] | 10.049 | 15.040 | | |
| | Farm & Farm | $T_7$ | 0.024[A] | 1.37[a] | 10.687 | 15.450 | - | - |
| | Wild & Wild | $T_8$ | 0.018[A] | 1.21[a] | 10.589 | 17.430 | - | - |
| | AAGRDI & AAGRDI | $T_9$ | 0.015[A] | 1.16[a] | 10.496 | 15.770 | - | - |
| Pathumthani (2) | AAGRDI & Wild | $T_1$ | 0.011[A] | 1.11[a] | 7.516 | 3.905 | -6.67 | -23.61 |
| | | $T_2$ | 0.042[A] | 1.26[a] | 7.244 | 3.588 | | |
| | AAGRDI & Farm | $T_3$ | 0.010[A] | 1.16[a] | 7.922 | 4.963 | 1.97 | 18.39 |
| | | $T_4$ | 0.016[A] | 1.32[a] | 6.706 | 3.156 | | |
| | Wild & Farm | $T_5$ | 0.030[A] | 1.53[a] | 7.628 | 4.546 | 1.66 | 6.96 |
| | | $T_6$* | 0.026[A] | 1.26[a] | 8.329* | 5.244* | | |
| | Farm & Farm | $T_7$ | 0.024[A] | 1.37[a] | 7.113 | 3.299 | - | - |
| | Wild & Wild | $T_8$* | 0.018[A] | 1.21[a] | 8.583* | 5.854* | - | - |
| | AAGRDI & AAGRDI | $T_9$ | 0.015[A] | 1.16[a] | 7.232 | 3.559 | - | - |
| Chumphon (2) | AAGRDI & Wild | $T_1$* | 0.011[A] | 1.11[a] | 8.122* | 4.681* | 4.54* | 14.48* |
| | | $T_2$ | 0.042[A] | 1.26[a] | 7.576 | 4.036 | | |
| | AAGRDI & Farm | $T_3$ | 0.010[A] | 1.16[a] | 7.30 | 3.69 | 2.74 | 13.21 |
| | | $T_4$ | 0.016[A] | 1.32[a] | 7.506 | 4.102 | | |
| | Wild & Farm | $T_5$ | 0.030[A] | 1.53[a] | 7.456 | 3.634 | -1.35 | -1.98 |
| | | $T_6$ | 0.026[A] | 1.26[a] | 7.210 | 3.502 | | |
| | Farm & Farm | $T_7$ | 0.024[A] | 1.37[a] | 7.131 | 3.274 | - | - |
| | Wild & Wild | $T_8$ | 0.018[A] | 1.21[a] | 7.736 | 4.006 | - | - |
| | AAGRDI & AAGRDI | $T_9$ | 0.015[A] | 1.16[a] | 7.280 | 3.609 | - | - |

Asterisks (*) identify the best crosses with the best genetic informations to be chosen in appropriate areas.

# APPENDIX

**Appendix Table 1.** Observable heterozygosities (H) and number of alleles (NoA) of the three stocks (the AAGRDI, wild and private farm)

| Allozyme locus/ci | Heterozygosities (H) | | | Number of alleles (NoA) | | |
|---|---|---|---|---|---|---|
| | AAGRDI | Wild | Private farm | AAGRDI | Wild | Private farm |
| 1. AAT-1 | 0 | 0 | 0 | 1 | 1 | 1 |
| 2. AAT-2 | 0.033 | 0.050 | 0.034 | 2 | 2 | 2 |
| 3. ACP-1 | 0 | 0 | 0 | 1 | 1 | 1 |
| 4. ACP-2 | 0 | 0 | 0 | 1 | 1 | 1 |
| 5. ALAT | 0.037 | 0 | 0 | 2 | 1 | 1 |
| 6. EST | 0 | 0 | 0 | 1 | 1 | 1 |
| 7. ESD | 0.080 | 0 | 0 | 2 | 1 | 2 |
| 8. FBALD-1 | 0 | 0 | 0 | 1 | 1 | 1 |
| 9. FBALD-2 | 0 | 0 | 0 | 1 | 1 | 1 |
| 10. G3PDH-1 | 0 | 0 | 0 | 1 | 1 | 1 |
| 11. G3PDH-2 | 0 | 0 | 0 | 1 | 1 | 1 |
| 12. G6PDH | 0 | 0 | 0.037 | 1 | 1 | 2 |
| 13. GPI | 0.100 | 0.050 | 0.067 | 2 | 2 | 3 |
| 14. HK-1 | 0 | 0 | 0 | 1 | 1 | 1 |
| 15. HK-2 | 0 | 0 | 0 | 1 | 1 | 1 |
| 16. IDHP | 0.250 | 0.316 | 0.069 | 3 | 3 | 2 |
| 17. LDH | 0 | 0 | 0 | 1 | 1 | 1 |
| 18. MDH-1 | 0 | 0 | 0 | 1 | 1 | 1 |
| 19. MDH-2 | 0.367 | 0 | 0.233 | 2 | 1 | 2 |
| 20. MEP | 0.100 | 0.158 | 0.333 | 2 | 2 | 3 |
| 21. MPI | 0 | 0 | 0.033 | 1 | 1 | 2 |
| 22. PGDH | 0 | 0 | 0 | 1 | 1 | 1 |
| 23. PGM | 0.100 | 0 | 0.103 | 2 | 1 | 2 |
| 24. XDH | 0 | 0 | 0 | 1 | 1 | 1 |
| 25. ODH | 0 | 0 | 0 | 1 | 1 | 1 |
| Average (±S.E.) | 0.043 (±0.018) | 0.023 (±0.014) | 0.036 (±0.016) | 1.36 (±0.11) | 1.20 (±0.10) | 1.44 (±0.13) |

**Appendix Table 2.** Observable heterozygosities (H) of each progeny population of 9 crosses

| Allozyme Locus/ci | Heterozygosities | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | $T_1$ | $T_2$ | $T_3$ | $T_4$ | $T_5$ | $T_6$ | $T_7$ | $T_8$ | $T_9$ |
| 1. AAT-1 | 0 | 0.067 | 0 | 0 | 0 | 0 | 0 | 0 | 0.111 |
| 2. AAT-2 | 0 | 0 | 0.100 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3. ACP | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4. AK | 0 | 0 | 0 | 0 | 0.100 | 0 | 0.053 | 0 | 0 |
| 5. EST | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6. ESD-1 | 0 | 0 | 0 | 0.050 | 0 | 0 | 0 | 0 | 0 |
| 7. ESD-2 | 0 | 0 | 0 | 0 | 0 | 0 | 0.053 | 0 | 0 |
| 8. GPI | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 9. MPI | 0.060 | 0 | 0 | 0 | 0.050 | 0 | 0.150 | 0.060 | 0 |
| 10. PGDH | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 11. XDH | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 12. IDHP | 0 | 0 | 0 | 0 | 0.050 | 0 | 0.100 | 0 | 0.067 |
| 13. G3PDH | 0 | 0.500 | 0 | 0.050 | 0.118 | 0.200 | 0 | 0.133 | 0 |
| 14. G6PDH | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 15. HK | 0 | 0 | 0 | 0 | 0.105 | 0.050 | 0 | 0 | 0 |
| 16. MDH-1 | 0 | 0.118 | 0 | 0.056 | 0 | 0 | 0 | 0 | 0 |
| 17. MDH-2 | 0.143 | 0.059 | 0 | 0.100 | 0.105 | 0.150 | 0.050 | 0.067 | 0.111 |
| 18. LDH | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 19. PGM | 0 | 0.059 | 0.083 | 0.056 | 0.050 | 0.100 | 0.050 | 0.067 | 0 |
| Average (±S.E.) | 0.011 (±0.008) | 0.042 (±0.027) | 0.010 (±0.007) | 0.011 (±0.008) | 0.030 (±0.010) | 0.026 (±0.013) | 0.024 (±0.010) | 0.018 (±0.009) | 0.015 (±0.009) |

**Appendix Table 3.** Number of alleles (NoA) of each progeny population of 9 crosses

| Allozyme Locus/ci | Number of alleles | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | $T_1$ | $T_2$ | $T_3$ | $T_4$ | $T_5$ | $T_6$ | $T_7$ | $T_8$ | $T_9$ |
| 1. AAT-1 | 1 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 2 |
| 2. AAT-2 | 1 | 1 | 2 | 1 | 1 | 1 | 1 | 1 | 1 |
| 3. ACP | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 4. AK | 1 | 1 | 1 | 1 | 2 | 1 | 2 | 1 | 1 |
| 5. EST | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 6. ESD-1 | 1 | 1 | 1 | 2 | 2 | 1 | 1 | 1 | 1 |
| 7. ESD-2 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 1 |
| 8. GPI | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 9. MPI | 2 | 1 | 1 | 1 | 2 | 1 | 2 | 2 | 1 |
| 10. PGDH | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 11. XDH | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 12. IDHP | 1 | 1 | 1 | 1 | 2 | 1 | 2 | 1 | 2 |
| 13. G3PDH | 1 | 2 | 2 | 3 | 3 | 3 | 2 | 2 | 1 |
| 14. G6PDH | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 15. HK | 1 | 1 | 1 | 1 | 2 | 2 | 1 | 1 | 1 |
| 16. MDH-1 | 1 | 2 | 1 | 2 | 2 | 1 | 1 | 1 | 1 |
| 17. MDH-2 | 2 | 2 | 1 | 2 | 2 | 2 | 2 | 2 | 2 |
| 18. LDH | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 19. PGM | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 1 |
| Average (±S.E.) | 1.11 (±0.07) | 1.26 (±0.10) | 1.16 (±0.09) | 1.32 (±0.13) | 1.53 (±0.14) | 1.26 (±0.13) | 1.37 (±0.11) | 1.21 (±0.10) | 1.16 (±0.09) |