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Hybridization and Growth of Tropical Sea Urchins

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ABSTRACT

Potential for interspecific hybridization between genetically diverged species of tropical sea urchins, *Echinometra* sp. A (Ea) and *Echinometra mathaei* (Em) was examined through cross fertilization and hybrid rearing experiments. Mean performance traits of fertilization, larval survival, metamorphosis and recovery of juveniles Em (ova)×Ea (sperm) and Ea (ova)×Em (sperm) hybrids were not significantly different from each other but were significantly lower than either of their conspecific control, Ea×Ea and Em×Em. Despite these, hybrids in both directions were developed normally to sexually mature adults. The growth parameters (final weight, weight gain, gonad weight, gonad index and SGR) of 2-year-old adult hybrids were significantly higher than the superior parent (Ea×Ea) and inferior parent (Em×Em). The gonad production showed an increment of 45.49% in F_1 hybrids over mid-parents, while it showed an increase of 33.74%, 62.60% and 46.76% in F_1 hybrid of Em×Ea and 31.42, 59.79 and 44.22% in F_1 hybrid of Ea×Em over the superior, inferior and mid-parents, respectively. Survival was highest in Em×Em followed by Ea×Ea, Em×Ea and Ea×Em in that order. Therefore, body growth, gonad production and survival indicate hybrids in either direction were viable in laboratory conditions. The superiority of these growth traits of the hybrid groups over their parental values indicates positive heterosis (hybrid vigor). This study is the first successful demonstration of hybrid vigor between two diverged species of sea urchins. Hence hybrids in both directions appear to have considerable potential for use in aquaculture.

Key words: Sea urchins, *Echinometra*, hybrids, growth, heterosis, aquaculture

INTRODUCTION

Four genetically and ecologically diverged tropical sea urchins belonging to *Echinometra mathaei* species complex occur sympatrically in adjacent microhabitats on Okinawan reef flats, have been considered as one of the most ubiquitous and abundant sallow-water echinoids in the warm Indo-Pacific region (Palumbi, 1996; Palumbi *et al.*, 1997; McClanahan and Muthiga, 2001). They occur commonly in and around reefs and widely distributed from central Japan in the north, to southeast Australia in the south and from Clarion Island off Mexico in the east and to the gulf of Suez in the west (Kelso, 1970; Clark and Rowe, 1971; Russo, 1977). At first, these four species were regarded as a single species, *Echinometra mathaei* (Shigei, 1987). However, recent studies on morphology, ecology, allozymes, gamete compatibility, DNA-DNA hybridization, mtDNA and the loci coding for gamete recognition molecules showed that there exists four independent gene pools of *Echinometra* in the Indo-west Pacific, distinguished as *Echinometra* spp. A, B, C and D. (Uehara *et al.*, 1991; Palumbi, 1996, 1998; Palumbi *et al.*, 1997; Arakaki, 1989; Rahman *et al.*, 2001, 2005). Molecular phylogenies indicate that *Echinometra* in the central and west Pacific splits from each other in the last 1-3 million years (Palumbi, 1996). *Echinometra* sp. B is now recognized as *Echinometra mathaei* (de Blainville 1825), *sensu stricto* (Arakaki *et al.*, 1998) while *Echinometra* sp. D belongs in the *Echinometra oblonga* (de Blainville 1825) species complex, which may include a cryptic species composed of at least three species (Arakaki and Uehara, 1999). Taxonomic description and designation of the other two species, *Echinometra* sp. A and *Echinometra* sp. C are yet to be made (Rahman *et al.*, 2001).

Among these four morphologically and genetically diverged species of *Echinometra*, *Echinometra* sp. A (Ea) and *Echinometra mathaei* (Em) can be distinguished from each other by differences in adult morphology and habitat preferences. Ea is abundant in more or less protected, constantly submerged habitat which is very calm and situated below the level of MLWS (Mean Low Water Surface), such as tide pools and shallow reef slops or areas protected from strong wave action, whereas Em inhabits in the shallow burrows on reef flat behind reef margin and affected by strong wave action, situated above the level of MLWS (Nishihira *et al.*, 1991). The two species can also be distinguished from each other by differences in adult morphology, distribution pattern and microhabitat preference (Table 1). The reproductive seasons of the two species overlap, extending from April to December with a maximum size of the gonads around September (Arakaki and Uehara, 1991).

Table 1: Summary of characters relevant to identification and species integrity of parental *Echinometra* sp. A (Ea) and *Echinometra* sp. C (Ec)

Characters	Ea	Em	Sources
Habitat	Moat and tide pools	Shallow excavated burrows on reef flat behind reef margin, lower than Ec and broader range of distribution	1, 2 and 6
Bathymetric range	Intertidal, below MLWS	Intertidal, both above and below mean low water level MLWS	1, 2 and 6
Salinity and thermal tolerance	Lower tolerance to sudden temperature and salinity changes	Lower tolerance to sudden temperature and salinity changes	4 and 5
Body size	Biggest among Okinawan *Echinometra*	Bigger among Okinawan *Echinometra*	3
Wet weight (g)	50.79±12.75	44.55±7.38	7
Test size (mm)	47.43±4.59	45.49±5.66	7
Spine length (mm)	22.54±1.11	18.43±1.04	7
Color	Entirely white to greenish or brownish black with white tip and distinct basal white ring	Color very variable, spine mostly brown and greenish brown, tip of spines not white, basal ring of spine unclear	3
Spicules			
Tubefeet	C-like or bihamate	C-like or bihamate	3
Gonad	Spindle	Spindle	3
Breeding season	April-December (max. around late September)	April-September (max. around late September)	4 and 5
Egg size (μm)	66.91±1.27	69.05±1.10	7
Sperm head size (μm)	3.93±0.59	4.92±0.53	7
Thickness of jelly layer (μm)	23.93±3.17	20.98±3.55	7

1: Tsuchiya and Nishihira (1984), 2: Tsnchiya and Nishihira (1985), 3: Uehara and Shingaki (1985), 4: Arakaki (1989), 5: Arakaki and Uehara (1991), 6: Nishihira *et al.* (1991), 7: This study: measurements from 25 adult individuals of each species were examined for each character, Mean±SD

Sea urchins are classic objects of research in different fields of biology, ecology and evolution. Sea urchins are also used as raw material to produce foodstuff, in particular, the product of processing gonads known as "Sea urchin Roe or Uni" and are considered a prized delicacy in Asia, Mediterranean and Western Hemisphere countries such as Barbados and Chile (Lawrence *et al.*, 1997). Gonads of sea urchins have long been a luxury food in Japan (Shimabukuro, 1991). Although, sea urchin gonad has not yet been used as food in Malaysia, it is reported that in Sabah, an indigenous tribe known as 'Bajau Laut' eats sea urchin roe with rice. The body of sea urchins, known as test, is cleaned and the roes removed. The clean test is then filled with rice and roe and after adding spices, the concoctions are steamed and then serve to the guests and customers (Rahman and Yusoff, 2010). In Japan, people dissect gonads from five *Echinometra* spp. at low tide from the shallower intertidal reefs. They consume the gonads and use the test and rest of the body as fertilizer for vegetable cultivation (Rahman *et al.*, 2000). Sea urchin gonads are also rich in valuable bioactive compounds, such as polyunsaturated fatty acids (PUFAs) and β-carotene (Dincer and Cakli, 2007). PUFAs, especially Eicosapentaenoic Acid (EPA, C20:5) (n-3)) and Docosahexaenoic Acid (DHA C22:6 (n-3)), have significant preventive effects on arrhythmia, cardiovascular diseases and cancer (Pulz and Gross, 2004). β-Carotene and some xanthophylls have strong pro-vitamin A activity and can be used to prevent tumor development and light sensitivity (Britton *et al.*, 2004). On the other hand, the high levels of AA and EPA recently detected in *Anthocidaris crassispina*, *Diadema setosum* and *Salmacis sphaeroides*, supported the development of aquaculture of sea urchin (Chen *et al.*, 2010), since PUFAs are important for human nutrition (Lawrence, 2007).

Heterosis is the biological phenomenon whereby an F_1 hybrid of two genetically dissimilar parents shows an increased vigor at least over the mid parents. Interspecific hybridization can also increase productivity through hybrid vigor, produce animals that are sterile, or combine desirable characteristics found in one species with those of another (Hedgecock, 1987; Longwell, 1987; Menzel, 1987; Bartley *et al.*, 2001). In view to produce hybrid vigor (positive heterosis) for maximizing productivity, a large number of studies have been conducted on hybridization in fishes (Hecht *et al.*, 1991; Bartley *et al.*, 2001; Pongthana, 2001; Nakadate *et al.*, 2003) and crustaceans (Lawrence *et al.*, 1984; Lin *et al.*, 1988; Bray *et al.*, 1990; Benzie *et al.*, 1995; Misamore and Browdy, 1997). Attempts to produce such hybrids are largely lacking in sea urchins due to difficulties in rearing of urchins through larval stages to juveniles and adults (Rahman *et al.*, 2000, 2005).

Of the two species of *Echinometra*, Ea is larger and Em is smaller. Because of its small size and slow growth rate compared to Ea, Em is not a good species for culture and exploitation. The objectives of the experiments reported here were (1) to assess the hybridization potential between two genetically diverged Ea and Em and (2) to explore the likelihood of producing desirable traits in the F_1 hybrids compared to conspecific controls through captive rearing condition.

MATERIALS AND METHODS

Sample collection and maintenance: Mature adults of Ea (identified by their brownish dark test and white-tipped spines) and Em (identified on the basis of brownish test and spines) (Arakaki *et al.*, 1998) (Fig. 1) were collected from the Sesoko coast of Okinawa Island at low tide during their natural breeding season. Specimens collected were immediately transported to the laboratory at the University of the Ryukyus, Okinawa, where they were maintained in closed aquaria and spawned within 3-4 days of collection.

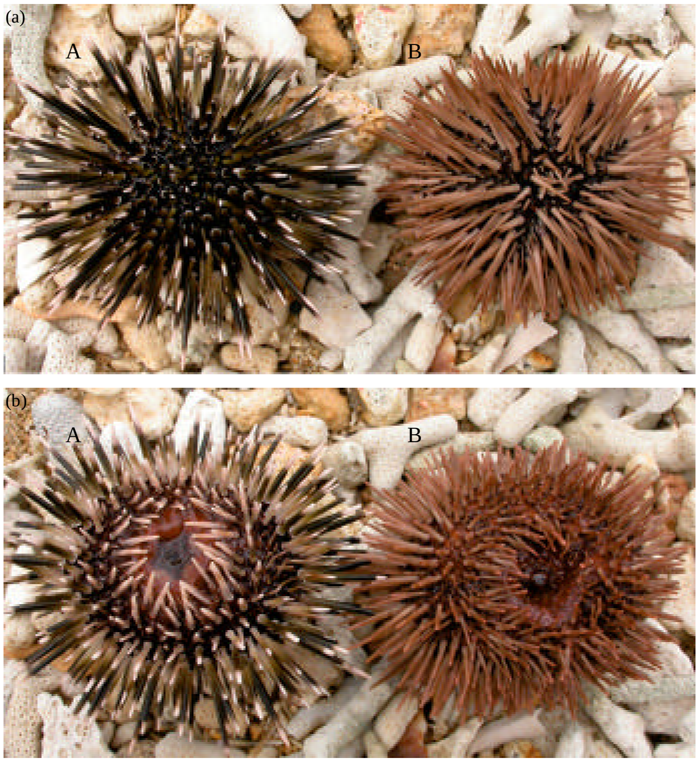

Fig. 1(a-b): (a) Aboral and (b) Oral color patterns of adult *Echinometra* spp., A: *Echinometra* sp., B: *E. mathaei*

Cross-fertilization experiments: Cross-fertilization was done at room temperatures (27-28°C) using all possible combinations of ova and sperm from the two *Echinometra* spp. Sperm were collected at the highest concentrated form ("dry") from the dissected testes and was stored undiluted in a refrigerator at 4-5°C till use. Females were induced by injecting 0.5 M KCl solution into the body cavity and eggs were collected by inverting female urchins over a glass beaker filled with FSW. Sperm concentration was maintained at 10^{-4} dilution of 'dry' sperm for conspecific crosses and 10^{-1} dilution for heterospecific crosses. For consistency, when referring to the heterospecific crosses, the maternal species is named first. For example, Em×Ea means that ova from Em females were fertilized by sperm from Ea males. Fertilization was done by mixing the above diluted sperm into one petri dish containing 15 mL conspecific egg suspensions and another dish with 15 mL of heterospecific egg suspensions. Sperm were allowed to remain with the eggs for 10 min; excess sperm were then removed with three consecutive washes with Filtered Seawater (FSW). In each heterospecific fertilization, a conspecific fertilization by use of ova from the same female was also conducted as a control. In crosses between female Ea and male Em, for example, 9 control cultures derived from the eggs from 9 different females and males were maintained in parallel with 9 cross-fertilized cultures. The first 100 eggs encountered were classified as "fertilized" if they had reached the 2-4 cell stage. The fertilized eggs were then transferred in glass beakers and incubated in FSW at ambient room temperature until they attained free-swimming blastula stage.

Larval rearing: Early stage embryos from the same female were reared in standing cultures in small glass beakers. When blastulae were seen swimming at the surface of the water, they were transferred to glass bottles containing 400 mL of Filtered Sea Water (FSW) which was stirred constantly by 10 rpm rotating motors. Larval densities up to the four-armed pluteus stage were maintained at 2-3 individuals mL^{-1}. When the larvae attained four-armed pluteus stage, they were cultured in the same system (400 or 800 mL glass bottles) with a larval density of 1 individual mL^{-1}. All cultures were carried out in FSW at 27-28°C, approximating ambient water temperature. About 50-75% of the culture water was removed by reverse filtration/siphoning every 3 days and replaced with fresh FSW. Larvae were supplemented with a laboratory cultured phytoplankton, *Chaetoceros gracilis* at concentrations of 1×10^4~2×10^4 cells mL^{-1} by adjusting the food level every 2 days from 4-arm feeding larvae until attaining metamorphic competence.

Metamorphosis: After 20-24 days of rearing, the mature larvae that were deemed competent were used in settlement induction tests. Competence was indicated by the presence of large juvenile rudiments and a high rate of metamorphosis. Induction of metamorphosis for all crosses was performed on Coralline Red Algal Stones (CRAS), which were immersed into FSW in the petri dishes containing 40 mL FSW each. Larval density at this stage was maintained at 1 individual 2 mL^{-1} FSW. The majority of the larvae tended to metamorphose within 1 day after induced to settlement on CRAS.

Culture of juveniles and adults: The newly produced juveniles were reared in small (25×20×10 cm) aquaria with aerated filtered sea water and pieces of coralline red algal skeletons were added as the source of food. Sea water was partially changed once a week with fresh filtered sea water. This was continued for up to three months, by which time the juveniles were 6.0-7.0 mm in test diameter. The juveniles were then transferred to plastic aquarium (46×55×25 cm) supplied with aerated flow through sea water at Sesoko Marine Science Centre, University of the Ryukyus. Coral skeletons covered with encrusting coralline algae were supplied as food. The stocking density was maintained at 1 individual L^{-1} of seawater. The algal stones were changed at weekly intervals for the first year and at 3 days interval for the 2nd year of culturing with new ones to supplement them with adequate algal foods. The cultures were continued for two year by which time the urchins attained sexual maturity and contained mature gametes. Growth performance and health conditions of the cultured urchins were monitored through monthly samplings. The performances of larvae, juvenile and adults were then compared among the hybrid groups and their parental species controls.

Heterosis: Heterosis was assessed as the ratio between the performance of the F_1 hybrid and the mid-parent, i.e.:

$$\text{Heterosis} = F_1 \text{ value/Mid-parental value}$$

where the mid-parental value was the mean performance of the parents (Gomez and Gomez, 1984).

Data analysis: Statistical analysis in this experiment was performed by one way analysis of variance and Student's t-test. Percentage data were arcsine transformed before analysis. This transformation helped to normalized the data and reduce heterogeneity in variances. A "Bartlett's test" was used to analyze the homogeneity of variances (Bartlett, 1937). When variances were not significantly heterogeneous and no major departures from normality, a one way analysis of variance (ANOVA) was done followed by Tukey's multiple comparison test. Data that did not meet the normality assumption of parametric analysis, were analyzed using non-parametric statistics. This was done by transforming values to ranks and then applying one way ANOVA followed by Tukey's multiple comparison tests. In case of heterosis, comparison between two treatment means was done using Student's t-test. The level for statistical significance was set at 0.05.

RESULTS

Cross-fertilization: Fertilization between Ea and Em were highly asymmetrical. In crosses with the eggs of *Echinometra* sp. A, the fertilization rate of the conspecific, Ea×Ea was significantly higher ($p<0.05$) than that obtained from Em×Ea, where Em eggs were fertilized by Ea sperm (Table 2). Similarly, in crosses with the eggs of *Echinometra mathaei*, the percent fertilization of the conspecific (Em×Em) was significantly higher ($p<0.05$) than that obtained from the corresponding Ea×Em crosses, where Ea eggs were fertilized by Em sperm. Both conspecific crosses showed nearly 100% fertilization with a low concentration of sperm (10^{-4} 'dry' sperm dilution) while both heterospecific crosses (Em×Ea and Ea×Em) showed very low percentage of fertilization even at a very high concentration of sperm (10^{-1} dilution) but did not differed significantly.

Larval performance: Survival (%) of competent larvae (transformation of pelagic to benthic phase for settlement induction) of Em×Ea and Ea×Em hybrids was not significantly different ($p>0.05$) but was significantly lower ($p<0.05$) than survival of larvae of conspecific controls (Table 3). However, survival of larvae of parental crosses (Ea×Ea and Em×Em) did not differ significantly from each other (Table 3).

Metamorphosis and Juvenile performance: The larvae of the parental and hybrid groups attained metamorphic competence stage through pelagic (2-, 4-, 6- and 8-arm pluteus) to benthic phase for settlement induction (Fig. 2) at about 20-24 days of age as evidenced by having large rudiment and higher rate of metamorphosis to young juvenile (Rahman and Uehara, 2001). Survival (%) of competent larvae of Em×Ea and Ea×Em hybrids was not significantly different ($p>0.05$) but was significantly lower ($p<0.05$) than survival of larvae of conspecific controls (Table 2). However, survival of larvae of parental crosses (Ea×Ea and Em×Em) did not differ significantly from each other (Table 2). The majority of the larvae were metamorphosed to young juveniles on coralline red algal stone within one day (Fig. 3) and there were no particular deformities/defects observed in the metamorphosed juvenile hybrids, Em×Ea and Ea×Em.

Table 2: Comparison of fertilization rates and larval and juvenile performances of Ea×Ea, Em×Em and their reciprocal hybrids. A total of 12 crosses were done using gametes from new individuals each time

	Fertilization (%)		Survival (%)*		Metamorphosis (%)		Recovery (%)**	
Treatments	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range
Ea×Ea	99.58±0.67^{a}	98.00-100.00	80.08±2.18^{a}	77.25-83.50	88.33±6.06^{a}	80.00-95.00	73.31±2.05^{a}	70.75-75.50
Em×Ea	50.33±7.09^{b}	40.00-62.00	67.75±2.02^{a}	64.75-70.50	75.83±5.85^{a}	70.00-85.00	63.75±1.67^{a}	61.50-65.50
Ea×Em	48.91±6.10^{b}	38.00-600.00	66.67±2.64^{b}	63.50-70.00	73.33±4.08^{b}	70.00-80.00	62.69±1.84^{b}	60.50-64.75
Em×Em	100^{a}		80.50±2.55^{a}	77.75-84.50	89.17±5.84^{a}	80.00-95.00	73.88±1.79^{a}	71.50-75.75

*Matured larvae that were deemed competent for metamorphosis after a 20-24 day culture period in laboratory condition, **Three months old juvenile urchins that were transferred to flow-through sea water system for advanced culture, Values in the same row having the same superscripts are not significantly different at $p>0.05$

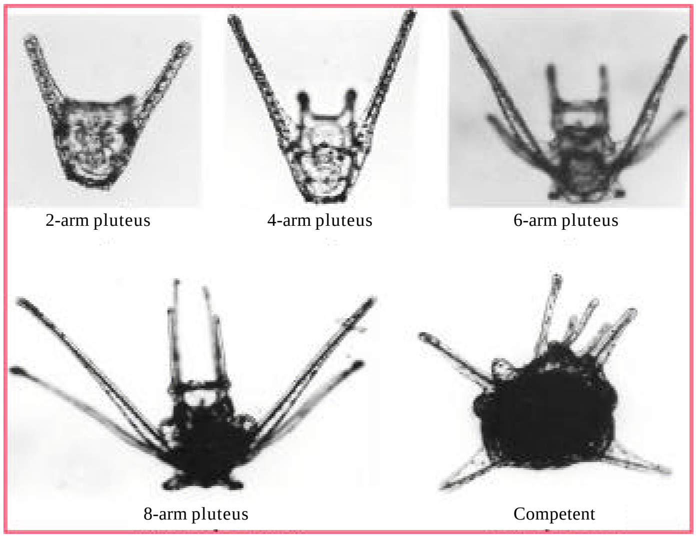

Fig. 2: Developmental stages for larvae of *Echinometra* species

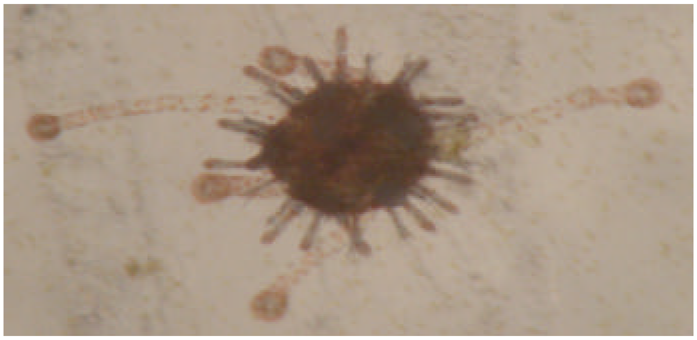

Fig. 3: Newly metamorphosed juvenile, 1 day after settlement on coralline algal substratum

Hybrids from either direction showed significantly lower ($p<0.05$) percent metamorphosis than both conspecific controls. Percent metamorphosis in parental groups did not differ significantly ($p>0.05$). Percent recovery of 3-month-old juveniles of conspecific parents and their reciprocal hybrids followed the same trends as percent metamorphosis (Table 2).

Adult performance: The detailed growth performances (viz. initial weight, final weight, weight gain, specific growth rate, test size, gonad weight) and survival of the hybrids and their parental species at the end of the 2 year culture period are summarized in Table 3, while the growth trend is plotted in Fig. 4. Growth of hybrids from either directions were significantly ($p<0.05$) faster than their parental species, the hybrids did not differ significantly from each other and always showed higher values compared to their parental controls (Fig. 4). The mean initial weights of 0.164±0.004, 0.183±0.005, 0.176±0.004 and 0.140±0.001 g in Ea×Ea, Em×Ea, Ea×Em and Em×Em reached to a mean final weight of 41.31±0.83, 50.66±0.45, 49.97±0.30 and 34.46±0.19 g, respectively. Significantly higher ($p<0.05$) growth was observed in Em×Ea and Ea×Em hybrids than either parents but the hybrid groups did not significantly so (Table 3). The mean weight gain attained

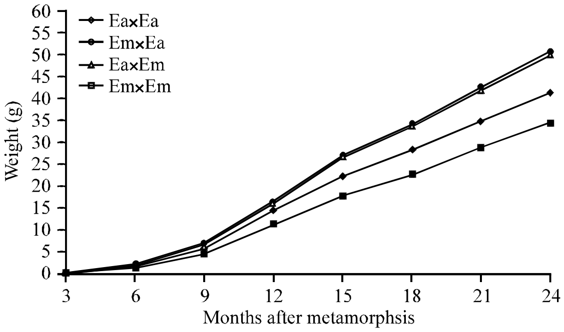

Fig. 4: Mean live weight (g) attained by the parental species controls and hybrids produced experimentally using *Echinometra* sp. A (Ea) and *Echinometra mathaei* (Em) during the culture period of two years; maternal species named first. Thirty specimens were measured every month with 10 randomly selected individuals per replicate for each treatment. Both the reciprocal hybrids exhibited significantly higher ($p<0.05$) growth than the either conspecific parent

Table 3: Comparison of the growth performances and survival of conspecific controls (Ea×Ea, Em×Em) and their reciprocal hybrids (Em×Ea and Ea×Em) at the end of 2 years of culture

	Ea×Ea		Em×Ea		Ea×Em		Em×Em	
Parameters	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range
Mean initial weight (g)	0.164 ± 0.004^b	0.14-0.19	0.183 ± 0.005^a	0.15-0.21	0.176 ± 0.003^a	0.15-0.20	0.142 ± 0.004^c	0.12-0.16
Mean final weight (g)	41.31 ± 0.83^b	39.25-44.00	50.66 ± 0.45^a	48.75-53.65	49.97 ± 0.30^a	48.05-52.56	34.46 ± 0.19^c	32.98-36.25
Weight gain (g)	41.14 ± 0.82^b	40.20-41.73	50.47 ± 0.44^a	50.12-50.97	49.79 ± 0.30^a	49.44-49.94	34.32 ± 0.19^c	34.12-34.50
Weight gain (%)	24719 ± 237^b	24496-24969	28191 ± 572^a	27591-28730	27538 ± 445^a	27112-28000	24396 ± 64^b	24348-24468
SGR (% day^{-1})	0.376 ± 0.001^b	0.375-0.377	0.384 ± 0.002^a	0.383-0.386	0.383 ± 0.001^a	0.382-0.386	0.374 ± 0.001^b	0.374-0.375
Wet gonad weight (g)	9.07 ± 0.42^b	7.97-10.50	12.13 ± 0.68^a	11.06-13.25	11.92 ± 0.64^a	10.85-12.88	7.46 ± 0.03^c	7.42-7.49
Test diameter (mm)	43.21 ± 0.93^b	38.00-47.00	52.02 ± 0.83^a	46.80-59.50	50.44 ± 1.34^a	45.00-58.20	39.07 ± 1.24^c	33.50-46.00
Survival (%)	85.56 ± 1.93^a	83.33-86.67	78.89 ± 3.85^b	76.67-83.33	77.78 ± 3.85^b	73.33-80.00	86.67 ± 3.34^a	83.33-90.00

Thirty specimens were measured for each parameter in each treatment. Mean±SE, ranges in parentheses. Mean values in each row having same superscript are not significantly different at $p>0.05$

by Ea×Ea, Em×Ea, Ea×Em and Em×Em was 41.14±0.82, 50.47±0.44, 49.79±0.30 and 34.32±0.19 g, respectively at the end of the experimental period. Both the reciprocal hybrids, Em×Ea and Ea×Em exhibited faster growth than the better parent (Ea×Ea) and inferior parent (Em×Em). Although the mean weight gained by the parental species differed significantly ($p<0.05$) from the hybrid groups and from each other (Table 3). The increase in weight of Em×Ea and Ea×Em hybrids over Ea×Ea, Em×Em and mid parents were 22.68 and 21.03, 47.06 and 45.08 and 33.77 and 31.96%, respectively. Among the four groups, percent weight gain between Em×Ea and Ea×Em was not significant ($p>0.05$), while parental groups differed significantly and showed lower values in this trait (Table 3).

The reciprocal hybrids showed significantly ($p<0.05$) higher Specific Growth Rate (SGR) than parents, but did not differ significantly between themselves ($p>0.05$) (Table 3). Mean test size of Em×Ea was slightly, but not significantly larger than Ea×Em. The mean test size of hybrids did differ significantly ($p<0.05$) from the values of superior and inferior parents (Table 3). Test size of

Em×Ea and Ea×Em hybrids was 34 and 31% larger than Ea×Ea, 63 and 60% larger than Em×Em and 47 and 44% larger than mid-parents. Test size of hybrids (mean of two hybrids) was 45% larger than mid parents.

Production of fresh edible gonad was significantly ($p<0.05$) lowest in slow-growing Em×Em (7.46±0.03 g) than the fast-growing Ea×Ea (9.07±0.42 g). The reciprocal hybrids contained significantly larger ($p<0.05$) amount of gonads (12.13±0.12 g in Em×Ea and 11.92±0.24 g in Ea×Em) than the parental controls, but did not differ significantly from each other (Table 3). The gonad index (percent gonad weight in respect to total body weight) was higher in hybrids than parents. The gonad production showed an increase of 45% in F_1 hybrids over mid-parents and an increase of 34, 63 and 47% in F_1 hybrid of Em×Ea and 31, 60 and 44% in F_1 hybrid of Ea×Em over the superior (Ea×Ea), inferior (Em×Em) and mid-parents. Survival was highest in Em×Em (86.67%) followed by Ea×Ea (85.56%), Em×Ea (78.89%) and Ea×Em (77.76%) in that order. Both the hybrids did not show significant differences ($p>0.05$) from parental Ea×Ea but the hybrid Ea×Em differed significantly ($p<0.05$) from the parental Em×Em but Em×Ea did not show so (Table 3). Despite the slightly lower survival in the reciprocal hybrids, the values tended to be very closer to their parental controls. Therefore, the growth and survival indicate hybrids in either direction were viable in lab-reared conditions and showed parental heterosis in these traits.

Major phenotypic color patterns of the hybrids and their parental species were examined at the end of the experiment and are shown in Fig. 5. Test and spine color of Ea×Ea was dominated by brownish dark and each spine had a white tip and a clear white basal ring. Em×Em had brownish dark test and each spine was uniformly brown with an unclear basal white ring. Ea×Em hybrid was more similar to Ea×Ea having brownish dark test and spines. On the other hand, Em×Ea hybrid was more similar to Em×Em having brownish dark test and brownish spines. The color of the hybrids was inherited by maternal genomes. The color of the hybrids was inherited by maternal genomes.

Heterosis effects: Heterosis was expressed as the F_1 hybrid/mid parent value. Heterosis values assessed from the adult performance traits of the reciprocal hybrids at the end of the experimental period are summarized in Table 4. Positive heterosis (hybrid vigor) was observed in many traits. The mean heterosis values for final body weight, weight gain, percent weight gain and test size of Em×Ea and Ea×Em hybrids were not differed significantly ($p>0.05$). A significant difference ($p<0.05$) in heterosis was found for SGR values of Em_Ea and Ea_Em, but the values were very similar (Table 4). The gonad weights of Em_Ea and Ea_Em were not significantly different ($p>0.05$). Heterosis for gonad index followed the same trend as gonad weight. The higher values of the hybrids over those of the parents, however, indicate positive heterosis (hybrid vigor), except for adult survival where both hybrids had slightly lower, but not significantly different levels of heterosis ($p>0.05$) (Table 4).

DISCUSSION

The two species in this study, *Echinometra* sp. A and *Echinometra mathaei* have long been recognized as morphologically and genetically distinct species, despite the former one has yet to be described and named (see references in the text). Recent molecular studies reveal they are more diverged than the other closely related pairs of *Echinometra* spp. complex (Matsuoka and Hatanaka, 1991; Palumbi and Metz, 1991; Palumbi, 1996). Fertilization rates in heterospecific

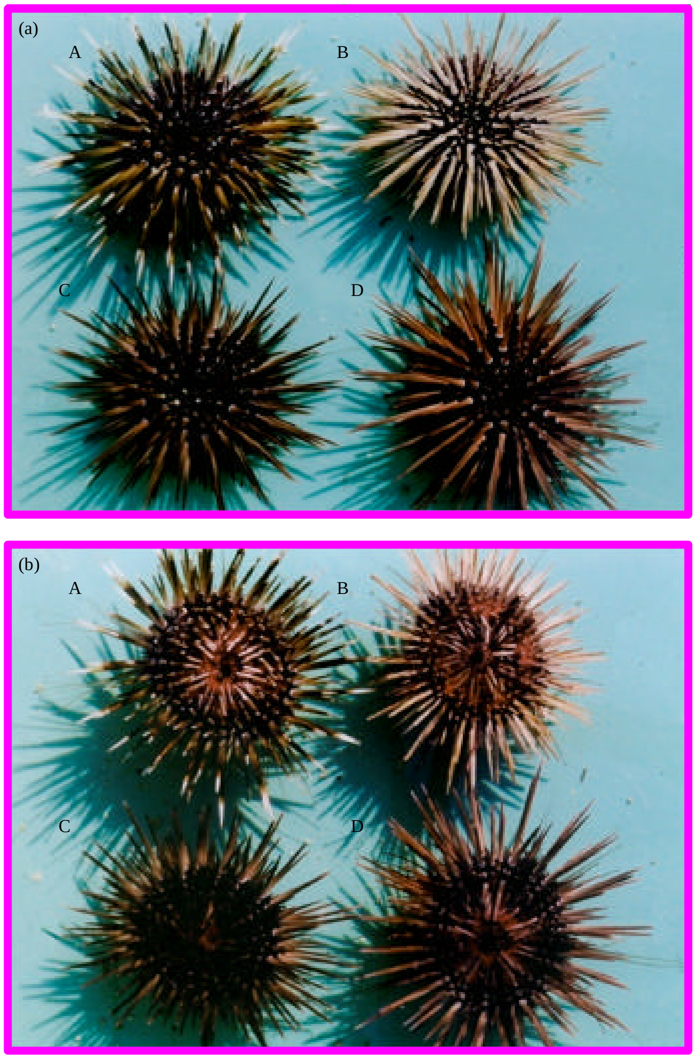

Fig. 5(a-b): (a) Aboral (upper) and (b) Oral (lower) color patterns of Ea, Em and their reciprocal hybrids, 2 year after metamorphosis; maternal species are written first: A: Ea×Ea, B: Em×Em, C: Ea×Em and D: Em×Ea

Table 4: Estimation of heterosis of the F_1 hybrids (Em×Ea and Ea×Em) produced experimentally through interspecific crosses between *Echinometra* sp. A (Ea) and *Echinometra mathaei* (Em) at the end of the culture period of 2 years

	Em×Ea		Ea×Em	
Performance traits/treatments	Mean±SD	Range	Mean±SD	Range
Final body weight	1.34 ± 0.02^a	1.32-1.36	1.31 ± 0.01^a	1.30-1.34
Weight gain	1.34 ± 0.02^a	1.32-1.35	1.30 ± 0.01^a	1.30-1.32
Weight gain (%)	1.15 ± 0.02^a	1.13-1.16	1.12 ± 0.02^a	1.11-1.14
SGR (%/day)	1.03 ± 0.01^a	1.02-1.03	1.01 ± 0.01^b	1.01-1.02
Test diameter	1.26 ± 0.03^a	1.24-1.29	1.23 ± 0.03^a	1.20-1.26
Wet gonad weight	1.47 ± 0.03^a	1.44-1.50	1.44 ± 0.06^a	1.40-1.50
Survival	0.92 ± 0.07^a	0.87-1.00	0.90 ± 0.02^a	0.88-0.92

Mean values in each row having the same superscript are not significantly different (t-test, $p>0.05$)

crosses using the gametes of Ea and Em in either direction at highest sperm dilution were consistently lower (50%) than those from either conspecific crosses, where fertilization rates were 100% at lower sperm concentration. These reduction in fertilization rates in either heterospecific crosses revealed by the presence of gamete recognition protein binding system as reported by Metz *et al.* (1994) and Metz and Palumbi (1996). Similar lower fertilization rates in hybrid crosses (either in one direction or the both) were also reported in fishes (Laywonyawut *et al.*, 1992; Rahman *et al.*, 1995; Bartley *et al.*, 2001), crustaceans (Lawrence *et al.*, 1984; Lin *et al.*, 1988; Bray *et al.*, 1990; Benzie *et al.*, 1995) including in sea urchins (Uehara *et al.*, 1990; Lessios and Cunningham, 1990; Aslan and Uehara, 1997; Rahman *et al.*, 2000, 2001, 2004; McCartney *et al.*, 2000; McCartney and Lessios, 2002; Rahman and Uehara, 2004).

The results from the above study indicated that the proportion of larval survival, metamorphosis and juvenile (3 month old) recovery of Em×Ea and Ea×Em hybrids were similar but comparatively low to those of either parental control. These differences further related with their higher genetic differences compared to other closely related pairs of Okinawan *Echinometra* sp. where hybrids in one direction were as viable as conspecifics while hybrids in the other direction were less viable (Rahman *et al.*, 2000, 2001, 2004; Rahman and Uehara, 2004). The hybrid groups showed slightly inferior performances in the larval and juvenile traits, although they showed better performances in advanced stages. Similar results were observed in fishes (Laywonyawut *et al.*, 1992; Mukhopadhyay and Dehadrai, 1987; Basavaraju *et al.*, 1995; Rahman *et al.*, 1995; Bartley *et al.*, 2001) and in sea urchins (Rahman *et al.*, 2000, 2001, 2004; Rahman and Uehara, 2004). Despite this, there was slightly lower fitness of hybrids than either conspecific in the above stages. This indicates postzygotic isolating mechanisms were not large enough to cause developmental incompatibility or hybrid inviability.

Throughout the 2 year culture period, the hybrid groups exhibited similar but significantly higher growth than the pure lines. Other growth performances such as weight gain, percent weight gain, Specific Growth Rate (SGR) and test sizes of the adult hybrids also showed similar trends in replicate experiments, i.e., the hybrids showed superior performances over their parental siblings. All the growth parameters of the reciprocal hybrids thus showed positive heterosis (hybrid vigor) over parental lines. Hybrids also contained significantly larger amount of edible gonads than either parent. For further study, factors controlling gonad weight or volume such as food, age, temperature should be determined. Despite the slightly lower survival of adults, the overall growth data confirm hybridization between Ea and Em was successful, producing viable-hybrids that showed superior performances (positive heterosis) over the mid-, superior and inferior parents. The color of the hybrids was inherited by maternal genomes. Similar color patterns have also been observed in the hybrids between other cross-combinations of *Echinometra* spp. (Aslan and Uehara, 1997; Rahman *et al.*, 2001, 2004; Rahman and Uehara, 2004).

Interspecific hybrids of penaeid prawns have been successfully achieved for *Penaeus setiferus*×*P. stylirostris* (Lawrence *et al.*, 1984), *P. setiferus*×*P. schmitti* (Bray *et al.*, 1990) and reciprocal crosses of *P. monodon* and *P. penicillatus* (Lin *et al.*, 1988) although spawn rate, hatch rate and the survival of hybrid progeny to post-larval stages were low compared with the intraspecific matings. The occurrence of hybrid vigor has only been specifically addressed by Lin *et al.* (1988) who described hybrid vigor in *Penaeus monodon* and *P. penicillatus* reciprocal crosses. It was stated that the hybrids grew faster than either of the parent species.

Hybridization has been used in numerous species of fish to increase growth rate, manipulate sex ratios, produce sterile animals, improve flesh quality, increase disease resistance, improve environmental tolerance and to improve a variety of other desirable traits to make fish more profitable to raise. Heterosis or hybrid vigor was resulted from the crossing between inbred lines, or between genetically diverged populations (Falconer, 1989). Evidence for superior performances and hybrid vigors has been reported in a wide variety of fishes. The sunshine bass, a cross between white bass (*Morone chrysops*) and the tripped bass (*M. saxatilis*) grows faster, has overall culture characteristics than either parental species under commercial culture conditions and is the preferred product in the USA (Smith, 1988). Crosses of the silver carp×bighead carp (*Hypophthalmichthys molitrix*×*Aristichthys nobilis*) in polyculture systems (Krasnai, 1987), black crappie×white crappie (*Pomoxis nigromaculatus*×*P. annularis*), stocked in small ponds and impoundments (Hooe *et al.*, 1994) and catfish hybrids between the African catfish (*Clarias gariepinus*) and the vindu (*Heterobranchus longifilis* or *H. bidorsalis*) in intensive concrete tanks (Salami *et al.*, 1993; Nwadukwe, 1995) were reported to grow faster (positive heterosis) than parental lines. Similar hybrid vigor was also observed in the hybrids between male African catfish (*Clarias gariepinus*) and female Asian catfish (*C. batrachus*) (Rahman *et al.*, 1995) and between North American female channel catfish (*Ictalurus punctatus*) and male blue catfish (*I. furcatus*) (Masser and Dunham, 1998). The hybrids between catla (*C. catla*) and other Indian carps (such as *L. rohita*, *L. calbasu*, *L. fimbriatus*) also exhibited heterosis with promising potential (Varghese *et al.*, 1984; Bhowmick *et al.*, 1987; Maheshwari *et al.*, 1990; Basavaraju *et al.*, 1995). Progeny from crosses of tambaqui (*Colossoma macropomum*) with the pacu (*Piaractus brachypomus* and *P. mesopotamicus*) grew faster than parental species in Brazil and Venezuelan raceways and ponds (Senhorini *et al.*, 1988). Crosses of the green sunfish (*Lepomis cyanellus*) with bluegill (*L. macrochirus*) (Tidwell *et al.*, 1992; Will *et al.*, 1994) and crosses of the gilthead sea bream (*Sparus aurata*) with red sea bream (*Pagrus major*) reared in Israel (Hulata, 1995) also had positive heterosis for growth and other culture characteristics. Hybrids among various species of tilapia also exhibited positive heterosis in respect of growth, production and other desirable traits (Lahav and Lahav, 1990; Earnst *et al.*, 1991; Hulata *et al.*, 1993; Lim *et al.*, 1993; Head *et al.*, 1994; Wohlfarth, 1994; Verdegem *et al.*, 1997; Bartley *et al.*, 2001).

Evaluation of heterosis and hybrid potential has been studied in two genetically distinct species, *Echinometra* sp. A and *Echinometra* sp. C (Rahman *et al.*, 2000). Hybrids showed better performance over mid and inferior parents but poorer performance than superior parents (Rahman *et al.*, 2000). Growth and heterosis of the hybrids among three commercially important species of temperate sea urchins, *Strongylocentrotus nudus* (Sn), *S. intermedius* (Si) and *Anthocidaris crassispina* (Ac), have recently been evaluated in China (Ding *et al.*, 2007). Among the four hybrid groups obtained, the Si×Ac, Si×Sn and Ac×Sn groups exhibited heterosis with respect to test diameter, test height, wet body weight and gonad index (Ding *et al.*, 2007).

CONCLUSION

Our parent study demonstrated that both the reciprocal adult hybrids between *Echinometra* sp. A and *Echinometra mathaei* outperformed either parent and showed positive heterosis. On the other hand this is the first successful attempt to produce hybrid vigor in tropical sea urchins. However, F_1 hybrids between the two gene pools exhibited higher performances and heterosis for many desirable traits compared to mid parent values and hence these hybrids are of great value towards the development of a sea urchin fishery to a greater extent.

Sea urchin research is quite new in Malaysia. However, very few systematic works have been done on the abundance and distribution patterns of sea urchins in Malaysia but no published information on their breeding, seed production and culture techniques are available. The findings obtained from the above studies would immensely be helpful towards the stock improvement and grow-out culture techniques of commercially important sea urchins and other marine invertebrates in the Malaysian coral reef communities. In addition, development of appropriate breeding, rearing and culture techniques would greatly be helpful to produce adequate quantities for nutraceutical and pharmaceutical product development.

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