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An approach to the phylogeny of the order Echinoida using interfamily hybrids

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Order Echinoida in Japan is now classified into the following five families: Temnopleuridae, Toxopneustidae, Strongylocentrotidae, Echinometridae and Parasaleniidae. With the exception of the Parasaleniidae, four of the families are commonly found in shallow waters in Japan. Interrelationships among these four families have been extensively studied by many workers using morphological criteria or at the biochemical and molecular levels, and four different theories have been proposed (Jensen, 1981; Smith, 1984; Mortensen, 1928–1951; Shigei, 1986; Matsuoka, 1988). To examine these theories, I attempted to obtain hybrids using 14 species (4 species of the family Temnopleuridae: *Temnopleurus toreumaticus, Temnopleurus hardwickii, Temnopleurus reeversii* and *Mespilia globulus*; 3 species of the family Toxopneustidae: *Toxopneustes pileolus, Tripneustes gratilla* and *Pseudoboletia maculata*; 4 species of the family Strongylocentrotidae: *Strongylocentrotus intermedius, Strongylocentrotus nudus, Hemicentrotus pulcherrimus* and *Pseudocentrotus depressus*; and 3 species of the family Echinometridae: *Anthocidaris crassispina, Echinostrephus aciculatus* and *Echinometra mathaei*).

Observations were focused on: (1) the final stages to

Table 1 Final stage hybrids developed

Egg \ Sperm	Tt	Th	Tr	Mg	Tp	Tg	Pm	Si	Sn	Hp	Pd	Ac	Ea	Em
Tt		0	ImH	ImH	Ga	0	0	0	Ga	0	–	Pr	–	Ga
Th	ImH		ImH	4a	4a	0	0	4a	4a	Ga	–	4a	4a	4a
Tr	ImH	0		ImH	–	–	–	–	–	Ga	–	–	–	–
Mg	4a	ImH	ImH		4a	0	0	2a	4a	0	–	4a	4a	4a
Tp	–	4a	4a	2a		0	0	2a	4a	–	0	4a	4a	Ga
Tg	0	0	0	0	ImH		0	0	Ga	0	2a	0	0	0
Pm	0	0	0	0	ImH	ImH		0	0	2a	0	0	0	0
Si	Pr	2a	2a	–	Ga	Ga	0		ImH	ImH	ImH	–	–	–
Sn	2a	4a	4a	Pr	Pr	Ga	–	ImH		–	ImH	ImH	ImH	ImH
Hp	–	–	–	–	Ga	Ga	Ga	ImH	ImH		ImH	ImH	–	–
Pd	–	–	–	–	Ga	Ga	Ga	ImH	ImH	ImH		ImH	–	4a
Ac	0	Pr	Pr	Pr	4a	Pr	0	ImH	ImH	–	–		ImH	ImH
Ea	Pr	Pr	Pr	Pr	4a	Pr	0	ImH	ImH	Pr	–	ImH		ImH
Em	0	0	0	0	0	0	0	0	0	–	–	0	ImH	

0, unfertilised; Ga, gastrulae; Pr, prism larvae; 2a, two-armed plutei; 4a, four-armed plutei; ImH, hybrid imagos; –, experiment not done.
Tt, *Temnopleurus toreumaticus*; **Th**, *Temnopleurus hardwickii*; **Tr**, *Temnopleurus reeversii*; **Mg**, *Mespilia globulus*; **Tp**, *Toxopneustes pileolus*; **Tg**, *Tripneustes gratilla*; **Pm**, *Pseudoboletia maculata*; **Si**, *Strongylocentrotus intermedius*; **Sn**, *Strongylocentrotus nudus*; **Hp**, *Hemicentrotus pulcherrimus*; **Pd**, *Pseudocentrotus depressus*; **Ac**, *Anthocidaris crassispina*; **Ea**, *Echinostrephus aciculatus*; **Em**, *Echinometra mathaei*.

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which they developed and (2) morphological characters at the pluteus stage. The morphological characters were followed up to imagos to analyse their mode of inheritance. The gametes were obtained by the KCl method. Sperm concentration was generally 10^{-5} dilution of 'dry' sperm. Fertilised eggs were allowed to develop at 18–25 °C. For rearing the pluteus larvae until the completion of metamorphosis, a 2 litre glass vessel 20 cm deep was used, and the seawater in it was constantly stirred at the rate of 30 rpm. The larvae were fed on *Chaetocerus gracilis* for culturing. Crossing experiments of the 14 species were done from 1993 to 1999, one to five times for each combination.

In 18 interspecies and intergenus combinations, hybrid pluteus larvae were obtained in all the combinations, and most of them developed to imagos. In the interfamily hybridisation, imagos were obtained from a combination between Strongylocentrotidae and Echinometridae alone (Table 1). These results suggest the close affinity between Strongylocentrotidae and Echinometridae, and do not support any of the four current theories. Attention was paid to eight characters that appeared during late development: three characters of four-armed pluteus larvae (the pattern of the body skeleton, the form of the post-oral rods and the form of the body rods end part), two characters of six-armed pluteus larvae (the form of the postero-dorsal rods and the collapse style of the body rods) and three characters of eight-armed pluteus larvae (the form of the posterior transverse rod, the form of the anterior ciliated band and the formation stage of the genital plate). All eight characters observed were common among the conspecific larvae from three families except for Strongylocentrotidae. There were only two common characters in the pluteus larvae of four species of the Strongylocentrotidae; in particular *S. intermedius* was found to have many different characters. Among the species in Strongylocentrotidae, *S. intermedius* was found to have four dominant characters of the six observed.

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