Zygote 8 (Supplement), p. S90. © 2000 Cambridge University Press Printed in the United Kingdom

Experimental hybridisation between two tropical species of sea urchins (genus *Echinometra*) in Okinawa

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Recent morphological, biochemical, ecological and reproductive studies have revealed that there are four sympatric biological species of sea urchins in *Echinometra* from Okinawa, which are currently referred to as *Echinometra* species A, B, C and D (e.g. Uehara *et al.*, 1990, 1991; Arakaki & Uehara, 1991; Matsuoka & Hatanaka, 1991; Palumbi & Metz, 1991; Nishihira *et al.*, 1991). Of these four species, *E.* sp. A (Ea) and *E.* sp. C (Ec), while being most closely related to each other genetically (Matsuoka & Hatanaka, 1991), can also be distinguished from each other by differences in adult morphology and microhabitat preference. No studies have addressed the mechanisms that maintain the independence of these species despite their close affinity and sympatry.

Experimental hybridisation conducted between two genetically very divergent species of Okinawan *Echinometra*, *E.* sp. A (Ea) and *E.* sp. D (Ed), revealed the presence of prezygotic isolation (Aslan & Uehara, 1997). To test whether the closely related Okinawan *Echinometra* spp. are isolated by pre- or postzygotic mechanisms, we carried out experimental hybridisation by using much closer species, Ea and Ec, through a series of cross-fertilisation experiments and rearing of the resulting hybrids. The percentage of successful heterogametic fertilisation was high when eggs of Ec and sperms of Ea were involved, whereas it was considerably lower with eggs of Ea and sperms of Ec. This reduction, at least among some heterogametic crosses, indicates the presence of a gamete recognition protein binding system, as reported by Metz *et al.* (1994) and Metz & Palumbi (1996), which might eventually lead to gametic incompatibility and reproductive isolation.

Hybrids produced from crosses in both directions developed normally through larval and juvenile stages to sexually mature adults. In adults, Ea × Ea were largest in test size, followed by Ec (ova) × Ea (sperms), Ea (ova) × Ec (sperms) and Ec × Ec in that order. Colour patterns of the hybrids were closer to the maternal colorations, whereas other characters such as relative test dimensions and spine lengths, morphology of tubefeet and gonad spicules, and gamete sizes were intermediate. Fertilisation rates in backcrosses using the gametes of F_1 progeny were high, revealing their closer genetic affinity. On the other hand, intensive surveys by us and others have failed to find individuals with such hybrid characteristics in the field, despite their physiological potential to hybridise. This further suggests the presence of other, most likely prezygotic, isolating mechanisms (e.g. gametic incompatibility) between Ea and Ec.

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