

Gene expression for paternal forms of lactate dehydrogenase and malate dehydrogenase in horseshoe crab hybrids among three Asian species, *Tachypleus tridentatus*, *T. gigas* and *Carcinoscorpius rotundicauda* (Merostomata: Xiphosura)\*

Hiroaki SUGITA and Koichi SEKIGUCHI

Institute of Biological Sciences  
University of Tsukuba  
Sakura-mura, Ibaraki, 305 Japan

Morphological studies on echinoderm, amphibian, teleost, and other species hybrids show that, in general, only maternal characters are evident until gastrular or postgastrular organogenesis. This conclusion is supported by many studies in which enzymes and other proteins of paternal type are first observed at postgastrular stages (Davidson, 1976; see p. 27). In interspecific hybrids of arthropods, however, no work has been carried out to detect the paternal forms of enzymes during the development of the embryo.

A single embryo at each stage was homogenized in one or two drops of distilled water. Larval extracts were prepared from a single animal at the first-instar stage (just after hatching). To prepare the adult tissue extracts, the hepatopancreas was homogenized in a volume of distilled water approximately equal to the tissue volume, because all isozyme molecules of the lactate dehydrogenase (LHD) were included in horseshoe crab hepatopancreas. After starch-gel electrophoresis of sample homogenates, enzymic band of the LDH and MDH (malate dehydrogenase) were detected with specific enzyme staining.

The larval LHD from *Tachypleus tridentatus*, *T. gigas*, and *Carcinoscorpius rotundicauda* showed only one enzymic band, while the LHD from the hepatopancreas tissues of *T. tridentatus* and *C. rotundicauda* occurred in 3 isozymic forms on starch gel. Furthermore, the LDH from each of interspecific hybrid larvae showed 3 enzymic bands, suggesting that 2 peptides produced from paternal and maternal genes for the LDH could form heterodimers in horseshoe crab hybrids. The adult LDH of *T. tridentatus* and *C. rotundicauda* was monomorphic and that of *T. gigas* was polymorphic. Genetic variants of the LDH could not be detected in larvae developed from eggs of a single female.

In hybrid embryos between *T. tridentatus* ♀ and *C. rotundicauda* ♂, the paternal form of the LDH was first detected at stage 17 (immediately before the 1st embryonic molt), but the paternal form of the LDH was not observed even on the 41st day after insemination (stage 19, after the 2nd embryonic molt) in hybrid embryos between *C. rotundicauda* ♀ and *T. tridentatus* ♂. The LDH from hybrid embryo between *T. gigas* ♀ and *T. tridentatus* ♂ occurred in 3 molecular forms at stage 20 (after the 3rd embryonic molt), suggesting that the LDH of the hybrid embryo consisted of a maternal homodimer, a paternal homodimer, and a hybrid heterodimer. This paternal form of the enzyme was first observed at stage 18 (after the 1st embryonic molt) in hybrid embryo *T. gigas* ♀ and *T. tridentatus* ♂.

On a gel, the MDH from 3 Asian horseshoe crabs showed the slower-migrating system (MDH-1), which was the mitochondrial form, and the faster-migrating system (MDH-2), or the supernatant form. There were electrophoretic variants of both MDHs in 3 Asian horseshoe crabs but genetic variants of the MDHs were not detected in larvae developed from eggs of a single female. The paternal form of the MDH-1 was first detected in the *T. tridentatus* ♀ × *C. rotundicauda* ♂ embryo at stage 14 (stage of appearance of rudimental appendages), while in the hybrid embryo of the reciprocal cross the paternal forms of the MDH-1 and MDH-2 were not expressed even on the 41st day after insemination (stage 19, after the 2nd embryonic molt). The paternal form of the MDH-2 was first observed in the *T. gigas* ♀ × *T. tridentatus* ♂ embryo at stage 17 (immediately before the 1st embryonic molt).

#### References

- Davidson, E. H. (1976) Gene activity in early development, pp. 452, 2nd ed., Academic Press, New York.  
\* Full content of this paper may be seen in the article entitled;  
Sugita, H. and Sekiguchi, K. (1983): The developmental appearance of paternal forms of lactate dehydrogenase and malate dehydrogenase in hybrid horseshoe crabs. *Biol. Bull.*, **165**, 436–443.