KOREAN JOURNAL OF ICHTHYOLOGY, Vol. 22, No. 1, 56-60, March 2010
ISSN: 1225-8598

Received : February 5, 2010
Revised : February 27, 2010
Accepted : March 12, 2010

Fine Structure of Oocyte Envelopes of Diploid and Triploid Biotypes in *Cobitis hankugensis-Iksookimia longicorpa* Complex

*By Meong-Hun Ko and Jong-Young Park**

Faculty of Biological Sciences and Biodiversity for Institute, College of Natural Sciences, Chonbuk National University

ABSTRACT The *Cobitis hankugensis-Iksookimia longicorpa* complex from Korea has been presumed to be a unisexual lineage, originating from *C. hankugensis* and *I. longicorpa* and having almost all females. Recently, it was confirmed that the complex consisted of one diploid and two triploid complexes. From observation of their oocyte envelopes, three forms could be classified: a villous projection in *I. longicorpa*, a granular one in *C. hankugensis* and two triploid complexes, and a granule with villous one in the diploid complex. Even within the same granular projection, they showed specific features in length and density number from each other. These architectures are first observed in cobitid complexes and may play a role in identification of diploids and triploids.

Key words : Oocyte, envelope, *Cobitis hankugensis-Iksookimia longicorpa* complex, diplopid, triploid

INTRODUCTION

The structure of the egg envelope (=adhesive coat, adhesive membrane, secondary egg envelope, chorion) surrounding the entire oocyte has been studied in some fish (Yorke and McMillan, 1979; Hart *et al.*, 1984; Erickson and Pikitch, 1993; Riehl and Greven, 1993; Kim and Park, 1995, 1996; Thiaw and Mattei, 1996; Park and Kim, 1997, 2001, 2003; Baek *et al.*, 2007). Those structures have been also used for the identification of eggs from different taxa and for determining the phylogenetic relationships (Laale, 1980; Groot and Alderdice, 1985; Johnson and Werner, 1986; Riehl and Greven, 1993; Britz *et al.*, 1995; Thiaw and Mattei, 1996; Park and Kim, 1997, 2001, 2003).

Natural populations of unisexual species (all or almost all female) are rare in vertebrate species; the normal modes of reproduction are gynogenesis, hybridogenesis, or parthenogenesis (Dawley, 1989; Goddard and Schultz, 1993). Such an unisexual population was reported in about 90 biotypes , which have been known as mostly polyploidy (Dawley, 1989; Vrijenhoek *et al.*, 1989; Janko *et al.*, 2005; Vasil'ev *et al.*, 2005). *Cobitis hankugensis-Iksookimia longicorpa* complex from Korea has been presumed to be a unisexual lineage originating from *C. hankugensis* and *I. longicorpa*, having nomenclature as formerly *C. sinensis-C. longicorpus* complex (Kim and Lee, 1995, 2000). They are almost all females with rare males, comprising a diploid and triploid complex that coexists and interacts reproductively with one or both of the bisexual parental species, *C. hankugensis* and *I. longicorpa* (Kim and Lee, 1995, 2000; Saithoh *et al.*, 2004). From the previous study, two complex populations were found; a diploid complex of HL type (haploid *C. hankugensis* x haploid *I. longicorpa*) and a triploid complex of HHL type (haploid *C. hankugensi*-haploid *I. longicorpa* x haploid *hankugensis*). Recently another triploid complex, HLL (haploid *C. hankugensis*-haploid *I. longicorpa* x haploid *I. longicorpa*), was known by Ko (2009). Until now, little has been know about egg's envelope on a unisexual complex population. Therefore, we are going to compare the structure of their envelopes in regarding to five kinds of cobitid fishes including their parents and three complex populations, and discuss ecological aspects on their habitat-related features.

MATERIALS AND METHODS

Three types (one diploid and two triploids) of *C. hankugensis-I. longicorpa* complex and two bisexual species, *C. hankugensis* and *I. longicorpa* were collected at Ram Stream of Nakdong River, South Korea in spawning

*Corresponding author: Jong-Young Park Tel: 82-63-270-3344, Fax: 82-63-270-3362, E-mail: park7877@chonbuk.ac.kr

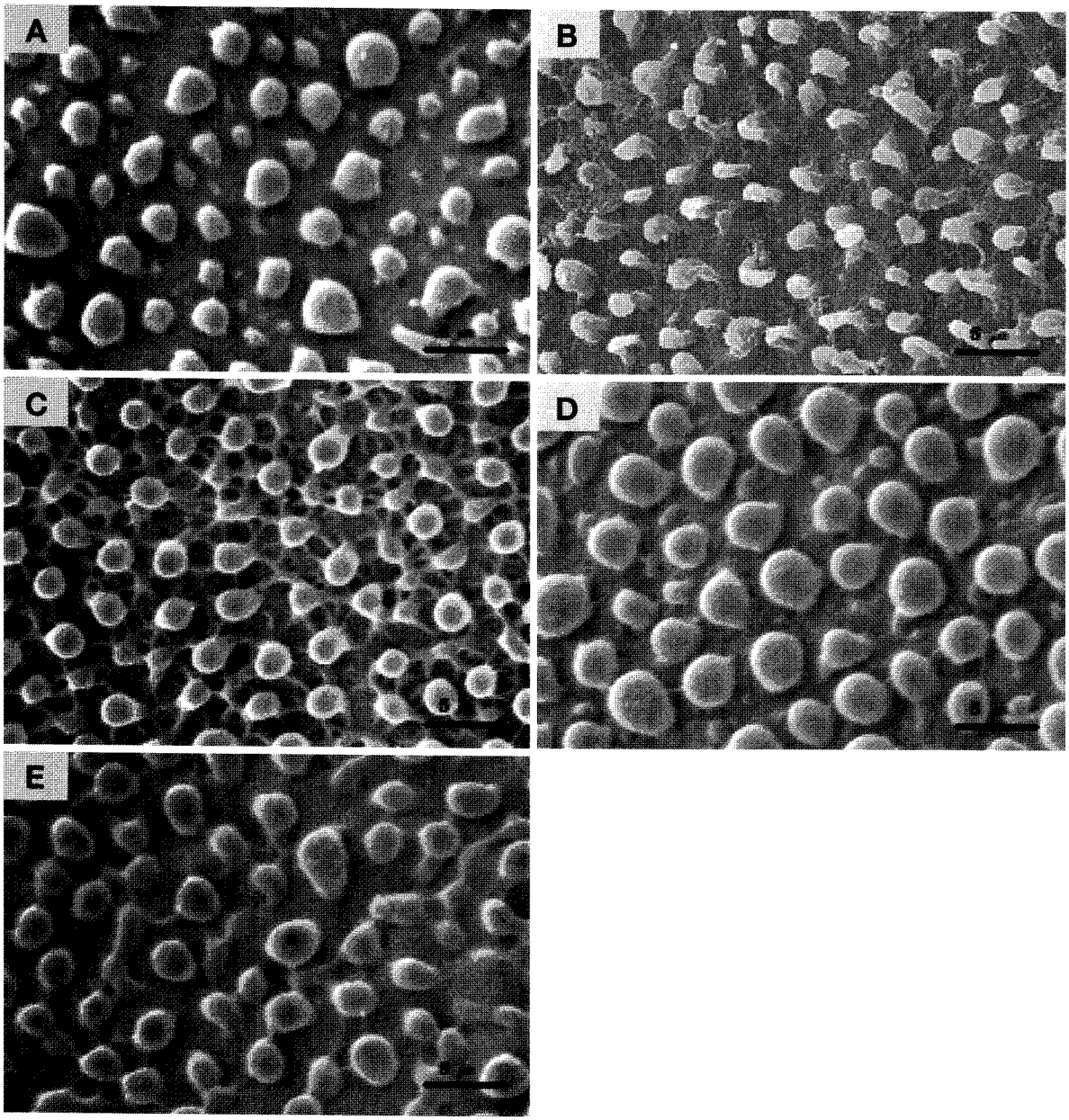

Fig. 1. Scanning electron micrographs (SEM) on oocyte envelopes of *Cobitis hankugensis* (A), *Iksookimia longicorpa* (B) and *C. hankugensis-longicorpa* complex (C: HL type, D: HHL type, E: HLL type).

Table 1. Morphometric data of egg envelopes in the *Cobitis hankugensis*, *Iksookimia longicorpa* and *C. hankugensis-longicorpa* complex

		Cobitis hankugensis	*Iksookimia longicorpa*	*C. hankugensis-longicorpa* complex		
		(HH)	(LL)	HL	HHL	HLL
Adhesive membrane	Form	Granule	Villus	Granule with a villus	Granule	Granule
	Length (μm, n=30)	2.4±0.59 (1.4~3.5)	2.3±0.47 (1.4~3.2)	2.2±0.31 (1.6~3.0)	3.3±0.44 (2.4~4.1)	2.7±0.44 (1.9~4.2)
	Number (10×10 μm)	7~9	11~14	9~12	6~7	7~9

season, April to May, 2008. For the scanning electron microscopy (SEM), their ovaries were excised and pre-fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer at pH 7.2. Postfixation was performed in 1% osmium tetroxide in the same buffer. The samples were dehydrated in a graded alcohol series and dried to critical point in

CO_2. The dried samples were coated with gold-palladium and observed with JEOL JSM-T330A scanning electron microscope. The egg envelope means lots of projections forming in the zona radiata of the full-grown oocyte. The evaluation of the length and the number per 10×10 μm were calculated by Carl Zeiss vision (LE REL. 4.4, Germany).

RESULTS AND DISCUSSION

The surface structures of their egg envelopes on three types of *C. hankugensis-I. longicorpa* complex and their bisexual parents were largely classified into three features; a granule, villus and a granule with villus .

Firstly, a granular projection is granular or round in appearance and founded in *C. hankugensis* living in mostly slow or stagnant waters with a sandy bottom, which is about 2.4 ± 0.59 μm in length and $7 \sim 9$ in number (Fig. 1A). These results were well consistent with the previous study (Park and Kim, 2003). Moreover, a HHL type and HLL type of triploid complex have also such a granular form, which is different from *C. hankugensis* as well as each other; HHL type with 3.3 ± 0.44 μm long and $6 \sim 7$ in density, whereas HLL type having 2.7 ± 0.44 μm and $7 \sim 9$ (Fig. 1D and 1E; Table 1) ($p < 0.001$). HLL type was similar to that of *C. hankugensis*. Secondly, a villous projection was seen in *I. longicorpa* preferring more or less rapidly flowing water with a pebbly bottom, with about 2.3 ± 0.47 μm in length and $11 \sim 14$ in number (Fig. 1B) and it showed the same structure form to the previous study (Park and Kim, 2003). Thirdly, a granule with villous projection was confirmed in a diploid HL type, which resembles a lollipop having a round toward the upper region and a stick or villus to the lower region. It is estimated to be about 2.2 ± 0.31 μm in length and $9 \sim 12$ in number (Fig. 1C; Table 1). This feature is considered as owing both parental structures, villous and granular projection. According to Park and Kim (1997, 2001, 2003) and Kim and Park (1995, 1996), the granular form in architecture of oocyte envelope was revealed in cobitid fishes such a genus *Cobitis* inhabiting sandy bottoms, whereas the villous form was in pebbly bottoms with somewhat rapidly flowing water, genus *Iksookimia*. In this sense, a granule from with villous projections of HL type is expected to be living in the bottom where the sand and pebble were mixed. Although it was very difficult for HL type to prove it clearly in the field, it was certain that other two types, HHL and HLL, were distributed over mostly stagnant sandy bottoms rather than pebbly bottom.

In many fishes, the structure of the egg envelopes closely related to its spawning sites or habitats, and which is known as being specific (Blaxter, 1969; Laale, 1980; Riehl and Greven, 1990, 1993; Park and Kim, 2001, 2003). Occasionally their morphology has been used for taxonomic purpose (Laale, 1980; Groot and Alderdice, 1985; Hirai, 1993; Britz *et al.*, 1995; Kim and Park, 1995, 1996; Thiaw and Mattei, 1996; Park and Kim, 1997, 2001, 2003). Therefore, we could think that such various structures on the three types of *C. hankugenis-I. longicorpa* complex are able to help their taxonomical identification each other.

REFERENCES

Baek, H.A., J.Y. Park, C.H. Kim and J.H. Kim. 2007. Development and transformation of oocyte' follicle cell for Korean four sillurid fishes of *Liobagrus* and *Pseudobagrus* (Pisces: Siluriformes). Korean J. Ichthyol., 19: 112-119. (in Korean)

Blaxter, J.H.S. 1969. Development: eggs and larvae. In: Hoar, W.S., D.J. Randall and E.M. Donalson (eds.), Fish physiology III. Academic Press, New York, pp. 177-252.

Britz, R., M. Kokoscha and R. Riehl. 1995. The anabantoid genera *Ctenops*, *Luciocephalus*, *Parasphaerichthys*, and *Sphaerichthys* (Teleostei: Perciformes) as a monophyletic group: evidence from egg surface structure and reproductive behaviour. Japanese J. Ichthyol., 42: 71-79.

Dawley, R.M. 1989. An introduction to unisexual vertebrates. In: Dawley, R.M. and J.P. Bogart (ed.). Evolution and ecology of unisexual vertebrates. Bulletin 466, New York, USA, pp. 1-11.

Erickson, D.L. and E.K. Pikitch. 1993. A histological description of shortspine thornyhead, *Sebastolobus alascanus*, ovaries: structures associated with the production of gelatinous egg masses. Env. Biol. Fish., 36: 273-282.

Goddard, K.A. and R.J. Schultz. 1993. A clonal reproduction by polyploid members of the clonal hybrid species *Phoxinus eos-neogaeus* (Cyprinidae). Copeia, 3: 650-660.

Groot, E.P. and D.F. Alderdice. 1985. Fine structure of the external egg membranes of five species of Pacific salmon and steelhead trout. Can. J. Zool., 63: 552-566.

Hart, N.H., R. Abraham and M. Donovan. 1984. The structure of the chorion and associated surface filaments in *Oryzias*; evidence for the presence of extracellular tubules. J. Exp. Zool., 230: 273-296.

Hirai, A. 1993. Fine structure of the egg membrane in four species of Pleuronectinae. Japanese J. Ichthyol., 40: 227-235.

Janko, K., P. Ráb, M.A. Culling and P. Kotlík. 2005. Ice age cloning-comparison of quaternary evolutionary histories of sexual and clonal forms of European loaches (Cobitis: Teleostie) using the analysis of mitochond-

rial DNA variation. Mol. Ecol., 14: 2991-3004.

Johnson, E.Z. and R.G. Werner. 1986. Scanning electron microscopy of the chorion of selected freshwater fishes. J. Fish. Bio., 29: 257-265.

Ko, M.H. 2009. Reproductive mechanisms of the unisexual diploid-triploid hybrid complex between the spined loach *Cobitis hankugensis* and *Iksookimia longicorpa* (Teleostei, Cobitidae) in Korea. Unpul. Doctoral Thesis. Chonbuk National Univ. 160pp. (in Korean with English abstract).

Kim, I.S. and E.H. Lee. 1995. Interspecific hybridization between triploid hybrid fish, *Cobitis sinensis-longicorpus* and two diploid species from Korea. Korean J. Ichthyol., 7: 71-78.

Kim, I.S. and E.H. Lee. 2000. Hybrization experiment of diploid-triploid cobitid fishes, *Cobitis sinensis-longicorpus* complex (Pisces: Cobitidae). Folia Zool., 49(1): 17-22.

Kim, I.S. and J.Y. Park. 1995. Adhesive membranes of oocyte in Korean cobitid species (Pisces, Cobitidae). Korean J. Zool., 38: 212-219.

Kim, I.S. and J.Y. Park. 1996. Adhesive membrane of oocyte in four loaches (Pisces: Cobitidae) of Korea. Korean J. Zool., 39: 198-206.

Laale, H.W. 1980. The perivitelline space and egg envelopes of bony fishes; a review. Copeia, 1980: 210-226.

Park, J.Y. and I.S. Kim. 1997. Egg membrane in five cobitid species of *Cobitis* (Pisces: Cobitidae). Korean J. Ichthyol., 9: 121-129.

Park, J.Y. and I.S. Kim. 2001. Fine structure of oocyte envelopes of the related 3 cobitid species in the genus *Iksookimia* (Pisces, Cobitidae). Ichthyol. Res., 48: 71-75.

Park, J.Y. and I.S. Kim. 2003. Variability of egg envelopes in Korean spined loaches (Cobitidae). Folia Biologica, 51(supplement): 187-192.

Riehl, R. and H. Greven. 1990. Electron microscopical studies on oogenesis and development of egg envelopes in two viviparous teleosts, *Heterandria formosa* (Poeciliidae) and *Ameca splenens* (Goodeidae). Zool. Beitr., 33: 247-252.

Riehl, R. and H. Greven. 1993. Fine structure of egg envelopes in some viviparous goodeid fishes, with comments on the relation of envelope thinness to viviparity. Can. J. Zool., 71: 91-97.

Saitoh, K., I.S. Kim and E.H. Lee. 2004. Mitochondrial gene introgression between spined loaches via hybridogenesis. Zool. Sci., 21: 795-798.

Thiaw, O.T. and X. Mattei. 1996. Ultrastructure of the secondary egg envelope of Cyprinodontidae of the genus *Epiplatys* Gill, 1862 (Pisces, Teleostei). Acta Zool., 77: 161-166.

Vasil'ev, V.P., E.B. Lebedeva, E.D. Vasil'eva and E.S. Levenkova. 2005. A unique diploid-tetraploid unisexual-bisexual fish complex (Pisces, Cobitidae). Doklady Biological Science, 404: 364-366.

Vrijenhoek, R.C., R.M. Dawley, C.J. Cole and J.P. Bogart. 1989. A list of the known unisexual vertevrats. In: Evolution and ecology of unisexual vertebrates. In: Dawley, R.M. and J.P. Bogart (eds.). Evolution and ecology of unisexual vertebrates. Bulletin 466, New York, USA, pp. 19-23.

Yorke, M.A. and D.B. McMillan. 1979. Nature and cellular origin of the adhesive coats of the lamprey egg (*Petromyzon marinus*). J. Morphol., 162: 313-326.

Cobitis hankugensis-Iksookimia longicorpa Complex의 2배체, 3배체집단의 난막 미세구조

고명훈 · 박종영

전북대학교 자연과학대학 생물과학부 · 생물다양성연구소

요 약 : 기름종개와 왕종개로부터 기원한 단성생식집단인 *Cobitis hankugensis-Iksookimia longicorpa* complex는 대부분 암컷으로만 구성되어 있으며 최근에 1종류의 2배체와 2종류의 3배체집단이 확인되었다. 이들의 난막 구조를 조사한 결과 3가지 종류로 구분될 수 있었다. 융모형은 이들의 부모종인 왕종개에서 확인되었으며, 과립형은 기름종개와 3배체집단, 그리고 과립을 가진 융모형은 2배체집단에서 나타났다. 특히 과립형을 가지는 3집단에서는 그들의 길이와 밀도에서 서로 차이를 보였다. 이러한 구조들은 cobitid complex에서 처음 보고될 뿐 아니라 2배체, 3배체 complex집단을 분류하는 데 이용될 수 있다.

찾아보기 낱말 : 난모세포, 난막, *Cobitis hankugensis-Iksookimia longicorpa* complex, 이배체, 삼배체, diplopid, triploid