

Interspecific Hybridization between Triploid Hybrid Fish, *Cobitis sinensis* - *longicarpus* and Two Diploid Species from Korea

Ik - Soo Kim and Eun - Hee Lee

Dept. of Biology, College of Natural Sciences, Chonbuk National University,
Chonju 560 - 756, Korea

Cobitis sinensis - *longicarpus* complex were commonly found in the upper stream of the Nakdong River in Korea and consisted of mostly with diploid and triploid karyotype forms. Among them, the triploid females of *C. sinensis* - *longicarpus* were artificially crossed with diploid males from *C. sinensis* and diploid males from *C. longicarpus*, respectively. The progenies from each cross produced the diploid individuals that possessed the paternal characters in their karyotypes and their body color patterns. Based on this results, we can assume that the triploid females, *C. sinensis* - *longicarpus* have a peculiar reproductive mode eliminating an uneven genome by the primary meiotic division and then producing a haploid ovum by secondary meiosis.

Introduction

Approximately 50 unisexual "species" of vertebrates have been recognized during the past half century (Vrizenhoek, 1989). Generally, unisexual species reported had four characteristics as follows : first, they are of interspecific hybrid origin ; second, they are exclusively female or nearly so ; thirdly, they reproduce by means of aberrant gametogenetic mechanism (meiotic or premeiotic) that inhibits genetic recombination and causes clonal inheritance ; and fourthly, they often include polyploids (Dawley, 1989). The genus *Cobitis* is divided into 9 species in Korea of which 5 are endemic in Korea. In the Nakdong drainage of Korea, the hybrid complex populations between *C. sinensis* and *C. longicarpus* were commonly

found (Kim and Lee, 1990). These hybrid complex were mostly females with both diploidy and triploidy in the karyotypes and were recognized as unisexual lineage originated from hybrid between *C. sinensis* and *C. longicarpus* (Kim and Lee, 1990).

In order to examine the reproductive mode of this hybrid complex, triploid females of the complex were artificially crossed with diploid males from *C. sinensis* or from *C. longicarpus*, respectively. Here we report the results of karyotypes and body color patterns in the parental hybrid species and their progenies focusing on the reproductive mode of the triploid females.

Materials and Methods

Cobitis sinensis, *C. longicarpus* and triploid

Table 1. Sex ratio of *C. sinensis*, *C. longicarpus* and *C. sinensis* - *longicarpus* complex collected from Namwon - gun, Chollabuk - do, Korea in 1992 - 1993.

Date	<i>C. sinensis</i>		<i>C. longicarpus</i>		<i>C. sinensis</i> - <i>longicarpus</i>	
	female	male	female	male	female	male
Apr. 17, 1992	33	37	16	22	58	1
May 8, 1992	69	42	106	86	202	1
Jun. 9, 1992	14	17	15	4	37	1
Jul. 9, 1992	-	-	-	-	3	-
Aug. 7, 1992	3	2	4	3	7	-
May 9, 1993	12	13	7	9	20	2
Jun. 6, 1993	16	23	50	25	61	4
Total number (%)	281(27.4)		347(33.9)		397(39.2)	
Ratios of females (%)	52.3		57.1		97.7	

Table 2. Frequencies of diploid and triploid forms of *C. sinensis* - *longicarpus* collected from Dong - myon, Namwon - gun, Chollabuk - do, Korea in 1992 - 1993.

Species	No. of females sampled
<i>C. sinensis</i> - <i>longicarpus</i>	
diploid form	91
triploid form	141

of *C. sinensis* - *longicarpus* used in this experiment were collected by seines or electrofishing from the upper stream of the Nakdong River at the Namwon - gun (35° 13' N, 128° 27' E) Chollabuk - do, from April in 1992 to June in 1993 (Table 1 and 2). Female triploid *C. sinensis* - *longicarpus* was artificially fertilized with the male of the bisexual species, *C. sinensis* or *C. longicarpus*. In each breeding experiment, ripe eggs obtained from a female injected with human chorionic gonadotropin were divided into two groups and then fertilized with sperm from *C. sinensis* and *C. longicarpus* respectively diluted nearly 100 fold with the Ringer's solution according to Suzuki(1957). Fry hatched from each crossing were separately kept in filtered and aerated glass aquaria at about 18 - 20°C. Fry were at first fed with rotifers and brine shrimps (larvae of *Artemia nauplius*)

and later commercial feed, Tetra bits (Tetra Co.). Color patterns and chromosome number of the progenies were examined following Kim and Lee (1990). Classification of chromosome was followed Levan *et al.* (1964).

Results

Early development : Table 3 shows the fertilization, hatching and early survival rate observed in each crossing. The crossing between the female triploid of *C. sinensis* - *longicarpus* and the male from *C. sinensis* or *C. longicarpus* was mostly successful, and the larvae showed no difficulty in developing periods ; they grew well into the same size as those of purebred *C. sinensis* or *C. longicarpus*. More than 88% of them were survived in the early developmental stages.

Color pattern : Triploid female, *Cobitis sinensis* - *longicarpus* showed a peculiar cloudy color patterns on the upper part of the body sides. However, the color patterns of the middle part on the body sides consisted of mixed forms with rectangular and vertical bands. The band patterns of progenies from the crossing with *C. sinensis* male were similar to those of *C. sinensis*. On the other hand, the progeny from the

Table 3. Rates of fertilization, hatching, abnormality, early survival of experimental groups.

Crosses female	male	Fertilization rate(%)	Hatching rate(%)	Abnormality rate(%)	Early survival* rate(%)
<i>C. sinensis</i> - × <i>longicarpus</i>	<i>C. sinensis</i>	90.7	88.2	4.5	88.4
(triploid, n=7) ×	<i>C. longicarpus</i>	93.9	91.2	3.0	89.3

* From hatching to 6 days

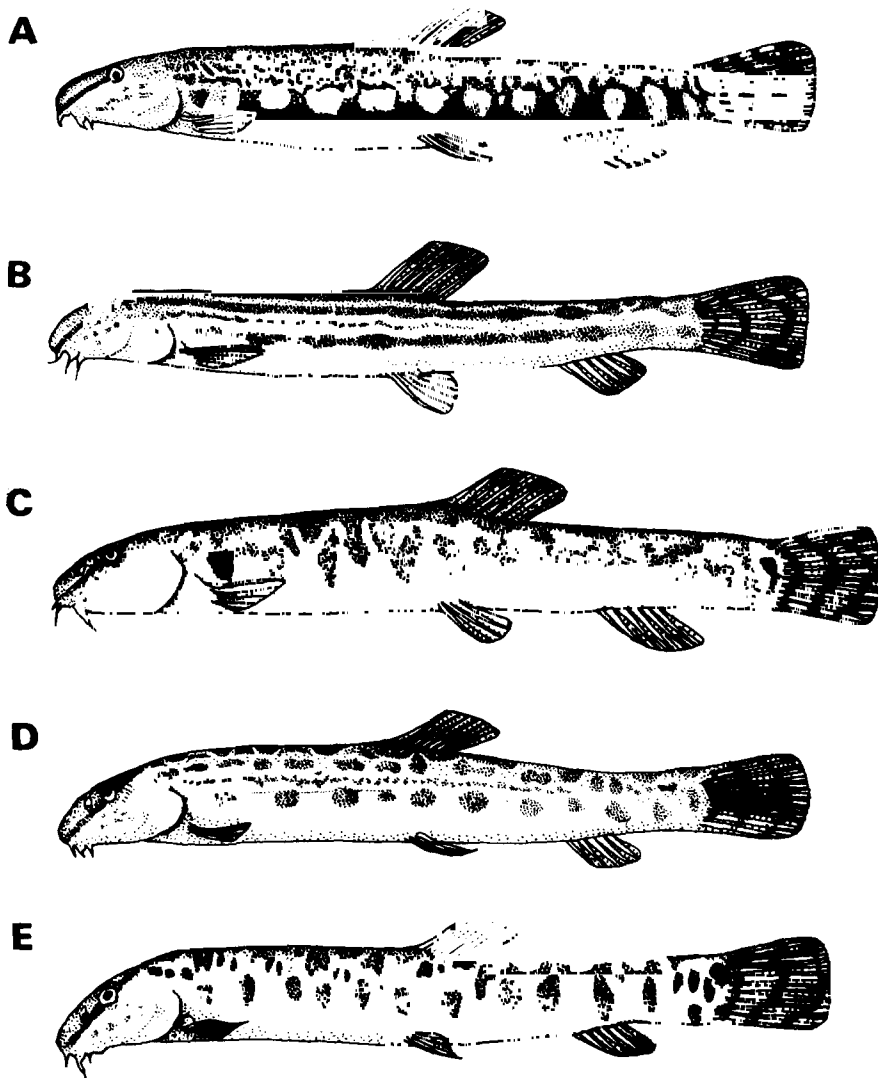


Fig.1. Color patterns of progenies obtained from laboratory breeding experiments and their parents.

A : *Cobitis sinensis* - *longicarpus* (♀), 99.5mm SLB : *Cobitis sinensis* (♂), 99.0mm SLC : *Cobitis longicarpus* (♂), 118.9mm SLD : *Cobitis sinensis* - *longicarpus* (♀, triploid) × *C. sinensis* (♂), 98.5mm SLE : *Cobitis sinensis* - *longicarpus* (♀, triploid) × *C. longicarpus* (♂), 111.9mm SL.

Table 4. Phenotypes and karyotypes of progenies obtained from laboratory breeding experiments.

Crosses		No. of specimens examined	No. of phenotype*	No. of modal chromosome
female	male			
<i>C. sinensis</i> - × <i>longicarpus</i> (triploid, n=7)	<i>C. sinensis</i>	16	S	48
	× <i>C. longicarpus</i>	11	SL	49

* S type : color patterns resembling *C. sinensis* ;

SL (diploidy) type : color patterns resembling wild triploidy *C. sinensis* - *longicarpus*.

Table 5. Frequency distributions of diploid chromosome counts of progenies from laboratory breeding experiments. The numbers in parentheses indicate percentage.

Crosses		No. of chromosome							
female	male	44	45	46	47	48	49	50	51
<i>C. sinensis</i> - × <i>longicarpus</i> (triploid, n=7) ×	<i>C. sinensis</i> (2.3)	9 (2.6)	10 (2.1)	8 (21.8)	85 (54.9)	214 (9.2)	36 (7.2)	28	-
	<i>C. longicarpus</i>	-	6 (2.1)	11 (3.8)	55 (18.8)	58 (19.9)	134 (45.9)	27 (9.2)	1 (2.8)

cross *C. sinensis* - *longicarpus* triploid female × *C. longicarpus* male consisted of the peculiar cloudy patterns in the upper parts of the body and the thick vertical bands in the middle of the body which was typical characteristics of the parental species, *C. longicarpus* (Fig. 1).

Karyotype : In triploid *C. sinensis* - *longicarpus*, somatic chromosome number was $3n=73$ composed of 28 meta - submetacentrics and 45 subtelo - telocentric chromosomes. When the females of triploidy, *C. sinensis* - *longicarpus* were crossed to the male *C. sinensis*, the chromosome number of the progenies was $2n=48$, composed of 18 meta - submetacentrics and 30 subtelo - telocentric chromosomes (Table 4, 5 and Fig. 2A). In the crossing with the male *C. longicarpus*, the chromosome number of the progenies have $2n=49$, composed of 19 meta - submetacentric and 30 subtelo - telocentric chromosome (Table 4, 5 and Fig. 2B). And the diploid - triploid mosaicism was not observed in the all experimental groups. This result suggests that the karyotypes of progenies include one haploid genome of female and one haploid

genome of male.

Discussion

Among the vertebrates, viable triploids are very rare in nature and with few exceptions. They have been found only in certain unisexual forms among the fishes (Schultz, 1971). The triploid from the wild *C. sinensis* - *longicarpus* reported here is presumed to be unisexual lineage originating from hybrid between *C. sinensis* and *C. longicarpus* (Kim and Lee, 1990). In the present study the triploid females were artificially mated with males from the related sympatric bisexual species, *C. sinensis* or *C. longicarpus*. From the breeding experiment, color patterns of F_1 progenies showed the paternal characteristics. These results clearly suggest that their offsprings might be produced through the fusion of the egg nucleus and the sperm nucleus. Karyotypic analysis revealed that the resulting F_1 progenies were all diploid having 48 or 49 chromosomes depending upon male species. These results suggest that the

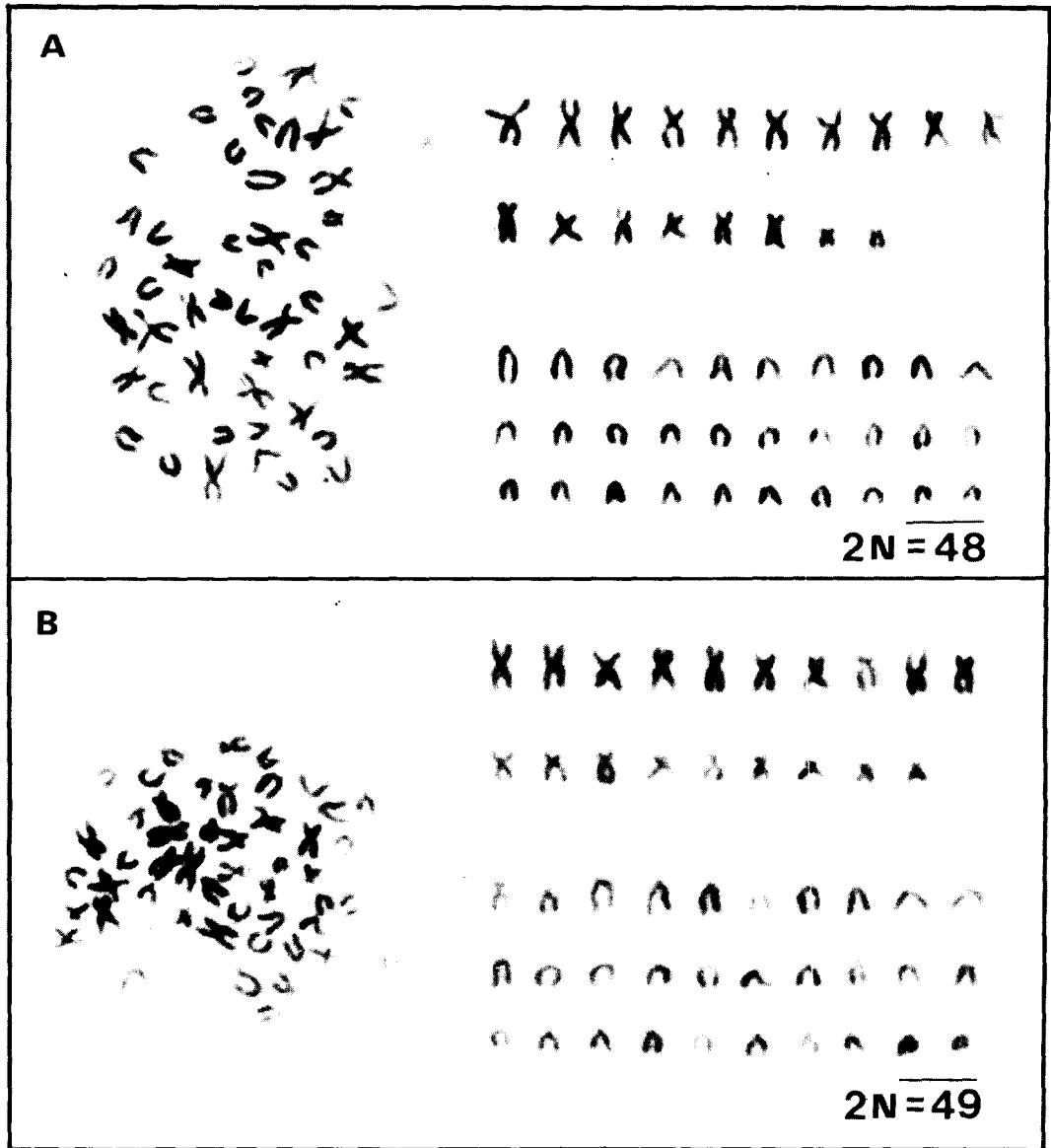


Fig. 2. Metaphases and karyotypes of progenies resulting from breeding experiments. Bars indicate 5 μ m.

A : Karyotype of triploid *C. sinensis* - *longicarpus* (♀) × *C. sinensis* (♂) (2n=48, 18msm - 30stt).

B : Karyotype of triploid *C. sinensis* - *longicarpus* (♀) × *C. longicarpus* (♂) (2n=49, 19 msm - 30stt)

haploid ova would be made diploid offspring by fertilization with haploid sperm. For possible explanations of our observations, we hypothesized that an uneven genome of *C. longicarpus* from triploidy (SSL) genome of *C. sinensis* - *longicarpus* was eliminated during the primary

meiotic division, and then were divided into the haploid ovum by secondary meiosis (Fig. 3). Recently, Goddard and Schultz (1993) reported that triploid female of *Phoxinus eos* - *neogaeus* might have a peculiar reproduction similar to this hypothetical mode. The elimination of a



C. 2sinensis – longicorpus

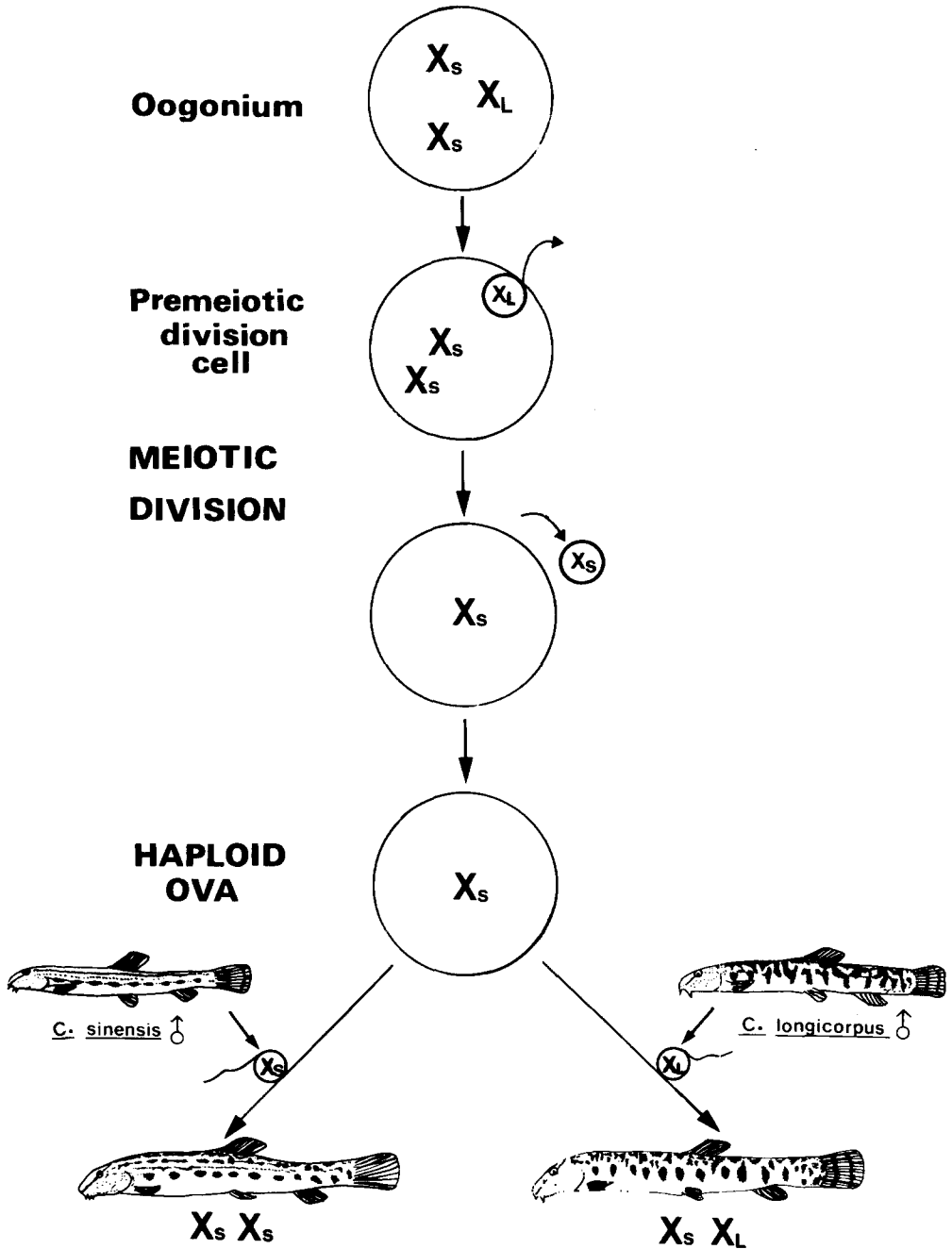


Fig. 3. The hypothetical mode of reproduction of the triploid *Cobitis sinensis - longicorpus* observed in this study.

genome during the meiosis was reported in hybridogenetic topminnow, *Poeciliopsis monacha - lucida* (Cimino, 1971). This reproductive hypothesis of triploid female of *C. sinensis - longicarpus* is different from that of gynogenesis, parthenogenesis or even hybridogenesis in the vertebrate reported already. To verify the hypothesis, the molecular genetic studies of parental species and their progeny should be conducted in the future.

References

- Cimino, M. C. 1971. Meiosis in triploid all female fish (*Poeciliopsis*, Poeciliidae). *Science* 175 : 1484 - 1486.
- Dawley, R. M. 1989. An introduction to unisexual vertebrates. pp. 1 - 18. In : Evolution and ecology of unisexual vertebrates. Ed. by R. M. Dawley and J. P. Bogart, Bulletin 466, New York State Museum, Albany, New York, USA.
- Goddard, K. A., R. M. Dawley and T. E. Dowling. 1989. Origin and genetic relationships of diploid, triploid, and diploid - triploid mosaics in the *Phoxinus eos - neogaeus* unisexual complex, p. 268 - 280. In : Evolution and ecology of unisexual vertebrates. R. M. Dawley and J. P. Bogart (eds). New York State Museum of Natural History Press, Albany.
- Goddard, K. A. and R. J. Schultz. 1993. Aclonal reproduction by polyploid members of the clonal hybrid species *Phoxinus eos - neogaeus* (Cyprinidae). *Copeia* 3 : 650 - 660.
- Kim, I. S. and J. H. Lee. 1990. Diploid - triploid hybrid complex of the spined loach *Cobitis sinensis* and *C. longicarpus* (Pisces, Cobitidae). *Kor. J. Ichthyol.* 2(2) : 203 - 210.
- Levan, A., K. Fredga, and A. A. Sandbag. 1964. Nomenclature for centromeric position on chromosome. *Hereditas* 52 : 201 - 220.
- Schultz, R. J. 1971. Special adaptive problems associated unisexual fish. *Am. Zool.* 11 : 351 - 360.
- Suzuki, R. 1957. Hybridization experiments in Cobitid fishes. *Japan J. Genetics* 32(1) : 8 - 14.
- Vrijenhoek, R. C. 1989. Genetic and ecological constraints on the origins and establishment of unisexual vertebrates. pp. 24 - 31. In : Evolution and ecology of unisexual vertebrates, edited by R. M. Dawley and J. P. Bogart, Bull. 466, New York State Museum, Albany, New York.

잡종 3배체 어류 기름종개 - 왕종개 잡종군의 중간 교잡실험

김 익 수 · 이 은 희
전북대학교 자연대 생물학과

낙동강 상류의 여러 수계에서 거의 암컷으로 구성된 *Cobitis sinensis - longicarpus* complex 2배체와 3배체 집단이 높은 빈도로 출현하였다. 그 가운데, 수컷의 *C. sinensis* ($2n=48$) 및 *C. longicarpus* ($2n=50$)와 암컷 3배체 *C. sinensis - longicarpus* ($3n=73$)를 실험실내에서 인공 교잡 실험을 하였다. 그 결과 각 조합에서 얻은 자손의 체측 반문에는 부계 형질이 발현되었고 그들의 염색체는 모두 부계의 haploid genome이 포함된 diploid form ($2n=48, 49$)이었다. 이상의 결과로 볼때 3배체 암컷인 *Cobitis sinensis - longicarpus* complex는 제1 감수분열에서 uneven genome을 배제하고 제2감수분열에 의한 반수체 ovum을 형성하는 독특한 생식 양상을 갖는 것으로 사료된다.