Japanese Journal of Ichthyology
Vol. 38, No. 3 1991

魚類学雑誌
38巻3号 1991年

Hybridization Experiment between *Gnathopogon elongatus elongatus* ♀ and *Carassius carassius* ♂

Midoriko Kasama and Hiromu Kobayasi

Department of Biology, Japan Women's University, Mejirodai, Bunkyo-ku, Tokyo 112, Japan

Abstract Hybrids from an intersubfamilial cross between *Gnathopogon elongatus elongatus* ♀ and *Carassius carassius* ♂ were analyzed morphologically and karyologically and compared with the parental species. These hybrids possessed a mosaic of character expressions that showed overall intermediacy though surperficial appearances were similar to *G. elongatus elongatus*. Their karyotype was constituted of one genome of the *Carassius* parent and two genomes of the *Gnathopogon* parent.

Hybridization experiments have been carried out by many investigators. As domestic investigations, Minamori (1949, 1950, 1951a, b, 1952, 1953), Suzuki (1962, 1963a–e, 1964, 1965a, b, 1966, 1968), Ueno (1972) and Arai (1984, 1987) dealt with salmonid, cyprinid, cobitidid and gasterosteid fishes. Suzuki, based on hybridization studies with cyprinid fishes, suggested that the hybrids which could be brought to attainment of adulthood were confined to be intergeneric or interspecific hybrids among members of the same subfamily. However, previous morphological and karyological analyses of various artificial hybrids between cyprinid species have shown that intersubfamilial hybrids between *Carassius carassius* (Linnaeus) ♀ and *Ctenopharyngodon idellus* (Cuvier et Valenciennes) ♂, or *Gnathopogon elongatus elongatus* (Temminck et Schlegel) ♂ reached the adult stage, and that they were allotriploidy (Kasama and Kobayasi, 1989b, 1990a).

This paper, the seventh in this series (Kasama and Kobayasi, 1987, 1988, 1989a, b, 1990a, b), examines intersubfamilial and intergeneric hybrids between *G. elongatus elongatus* ♀ and *C. carassius* ♂.

Materials and methods

Fishes used in this study were *G. elongatus elongatus* collected from Lake Biwa and *C. carassius* in Holland. They had bred in our laboratory, and the second generation in each species was used as parental species. Artificial fertilization was performed as in the foregoing experiment (Kasama and Kobayasi, 1987). Hybrids were used for morphological and karyological studies at about three and a half years old.

Methods for making most meristic counts and morphometric measurements were from Matsubara (1955) and Hubbs and Lagler (1970). Measurements were read from a dial caliper to 0.1 mm. Two measures not typically taken on cyprinid fishes and all meristic and morphometric characters followed Kasama and Kobayasi (1990). The hybrid index computed following Hubbs and Kuronuma (1942) and Hubbs et al. (1943) was used to compare hybrid character states to the parental species. The mean of each character value was set at 0.0 for *G. elongatus elongatus* and 100.0 for *C. carassius*. A hybrid index of 50.0 for any character would indicate exact intermediacy of the hybrid between the parental species for that character. Scales at the region between the anterior base of the dorsal fin and the lateral line were plucked out, and examined after staining with alizarin red S. Pharyngeal bones were cleared in 1% KOH and stained with alizarin red S to examine their morphological features. The terminology for the scales and the pharyngeal bones was that of Chu (1935). Vertebrae were counted from radiographs and included the four Weberian ossicles and the urostylar vertebra. The gonads fixed in dioxan-Bouin's solution were sectioned 6 μm by the usual paraffin method and stained with Delafield's hematoxylin and eosin. Chromosome study was carried out using cells from the primary culture of the scale epithelium. The cells were fixed in Carnoy's fluid, flame-dried on slides and stained with Giemsa.

Results

Development and viability. About 66% of 2634 eggs produced from *G. elongatus elongatus* ♀ × *C.*

carassius ♂ developed into embryos up to the gastrular stage. Embryo mortality was high at this stage. Almost all surviving embryos hatched at 98 to 117 hours after fertilization, and 64% of the eggs produced larvae. But 78% of the larvae suffered from edema, bent bodies and circulatory disorders. Many of them could not feed and died at 1 to 3 days after hatching. The larvae that did feed died with the lapse of time. Over a period of a year, 15 hybrids were raised to the young stage, but most of them died during this stage due to malnutrition caused by digestive system disorders. Only six hybrid reached the adult-like stage.

Morphological characters. The hybrids were generally intermediate between the parental species on the basis of 11 characters (Table 1) although their superficial appearance was similar to *G. elongatus elongatus* (Fig. 1a–c).

The body side was yellowish silver and each fin was yellow. This coloration was very similar to *G. elongatus elongatus* although somewhat darker. The lateral band was distinct and similar to that of *G. elongatus elongatus*. The dark spot at the base of the caudal fin, characteristic of *C. carassius*, was present but much indistinct compared with that of this species. The hybrids possessed barbels as in *G. elongatus elongatus* but they were shorter than that of this species. The ratios of body depth/standard length and caudal peduncle length/caudal peduncle depth were closer to those of *G. elongatus elongatus* than to intermediacy. The ratio of pectoral fin origin to ventral fin origin/ventral fin origin to anal fin origin was closer to that of *G. elongatus elongatus*, and the ratio of dorsal fin base length/longest dorsal fin ray length was much closer to this species. The dorsal fin was very similar to that of *G. elongatus elongatus* in shape, but its outline was round as in *C. carassius*. Forking of the caudal fin was weaker than that of *G. elongatus elongatus*, and rather similar to that of *C. carassius*. The number of dorsal fin rays was closer to that of *G. elongatus elongatus*. The number of lateral line scales and scales below the lateral line approached intermediacy. The number of scales above the lateral line was seven, which was typical number for *C. carassius*. The aspect of the scales was very similar to those of *G. elongatus elongatus*. The basal margin gently swelled out in the middle and the radial grooves extended only to the apical area as in *G. elongatus elongatus*, although there were fewer grooves. The focus was situated by the basal margin. The basolateral ridge was present, although very indistinct compared with that of *G. elongatus elongatus*. The number of gill-rakers was closer to that of *G. elongatus elongatus* than to intermediacy. The

Table 1. Comparison of meristic and morphometric characters of *Gnathopogon elongatus elongatus* ♀ × *Carassius carassius* ♂ with parental species. $\bar{\chi}$, mean; r, range; N, sample size; I, index value.

Character	*G. elongatus*			Hybrids				*C. carassius*		
	$\bar{\chi}$	r	N	$\bar{\chi}$	r	N	I	$\bar{\chi}$	r	N
Body depth/standard length	0.247	0.218–0.288	20	0.287	0.271–0.300	6	25.4	0.404	0.401–0.413	20
Caudal peduncle length/ caudal peduncle depth	1.529	1.303–1.792	20	1.421	1.234–1.568	6	20.1	0.992	0.879–1.137	20
Pectoral fin origin to ventral fin origin/ventral fin origin to anal fin origin	1.177	1.089–1.270	20	1.131	1.116–1.146	6	27.3	1.009	0.938–1.055	20
Dorsal fin base length/ longest dorsal fin ray length	0.704	0.638–0.828	20	0.882	0.659–0.967	6	10.8	2.344	2.243–2.446	20
Dorsal fin rays	8.0	8.0	20	11.2	10.0–12.0	6	29.6	18.8	18.0–21.0	20
Lateral line scales	37.0	35.0–39.0	20	34.0	34.0	6	57.6	31.8	31.0–33.0	20
Scales above lateral line	5.6	5.0–6.0	20	7.0	7.0	6	100.0	7.0	7.0	20
Scales below lateral line	4.1	4.0–5.0	20	5.0	5.0	6	56.0	5.7	5.0–6.0	20
Gill-rakers	9.6	8.0–12.0	20	12.4	11.0–15.0	6	14.8	28.4	28.0–30.0	20
Pharyngeal teeth formula:			17			6	84.8			18
outer row on left side	3.0	3.0		0.8	0.0–1.0			0.0	0.0	
inner row on left side	5.0	5.0		4.0	4.0			4.0	4.0	
inner row on right side	5.0	5.0		3.8	3.0–4.0			4.0	4.0	
outer row on right side	3.0	3.0		1.0	0.0–2.0			0.0	0.0	
Vertebrae	35.4	35.0–37.0	20	33.6	33.0–34.0	6	50.0	31.8	29.0–33.0	20
					Hybrid index = 43.3					

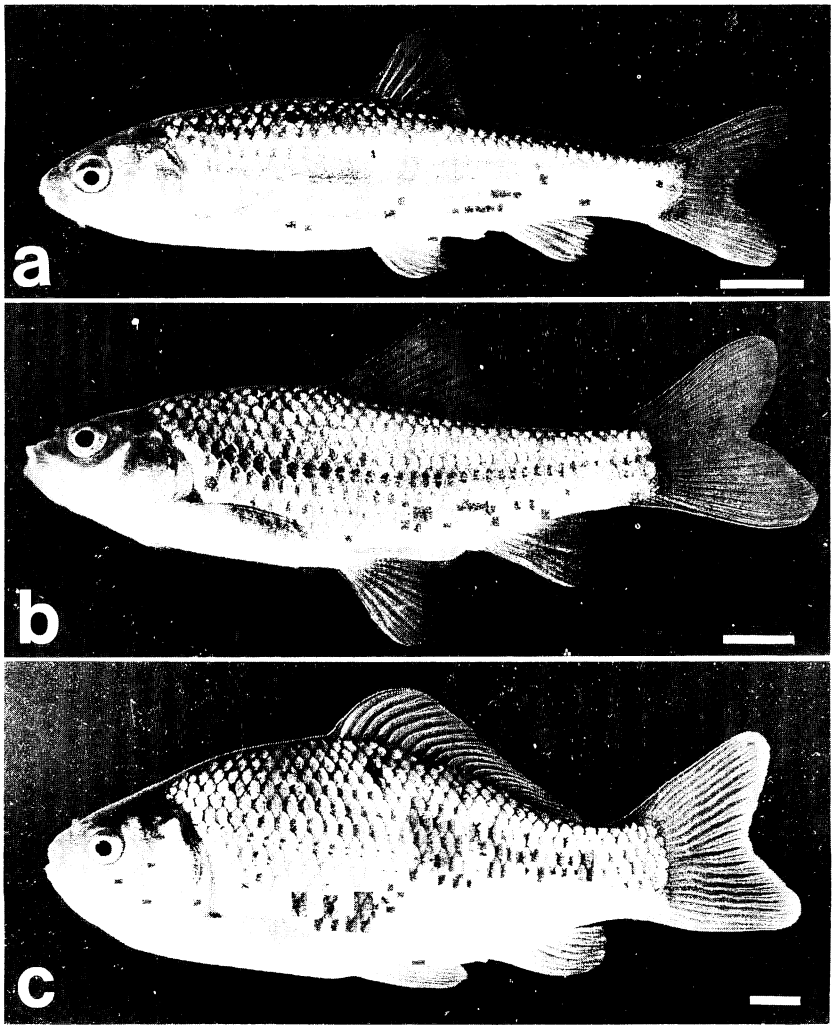

Fig. 1. Hybrid and parental species. *Gnathopogon elongatus elongatus* (a), *G. elongatus elongatus* ♀ × *Carassius carassius* ♂ hybrid (b), *C. carassius* (c). Bars equal 10 mm.

length of the gill-rakers in relation to the gill-filaments was shorter than that of *C. carassius*, very similar to that of *G. elongatus elongatus*. The gill arch angled obtusely and its corner was indistinct, more similar to that of *C. carassius* than *G. elongatus elongatus*. The number of pharyngeal teeth was closer to that of *C. carassius* than to intermediacy. The aspect of the pharyngeal bones was similar to those of *G. elongatus elongatus*, but the tooth platform was somewhat shorter than this species. The number of vertebrae was exactly intermediate. The gas-bladder was similar to that of *G. elongatus elongatus* in shape although somewhat more roundish as in *C. carassius*. The intestine was very simple in its coiling, resembling that of *G. elongatus elongatus*. Anatomical observations showed the gonads to be extremely underdeveloped in the spawning season and to be hidden by fat tissues. Microscopic examination disclosed that the hybrids were neuter (Fig. 2).

Karyotypes. The chromosome number of the hybrids was 100 (Fig. 2). While it was $2n=50$ for *G. elongatus elongatus* and $2n=100$ for *C. carassius*. The karyotype of *G. elongatus elongatus* contained 12 metacentrics, 32 submetacentrics and 6 acrocentrics and that of *C. carassius* involved 20 metacentrics, 40 submetacentrics and 40 acrocentrics. These results for the parental species agreed with those

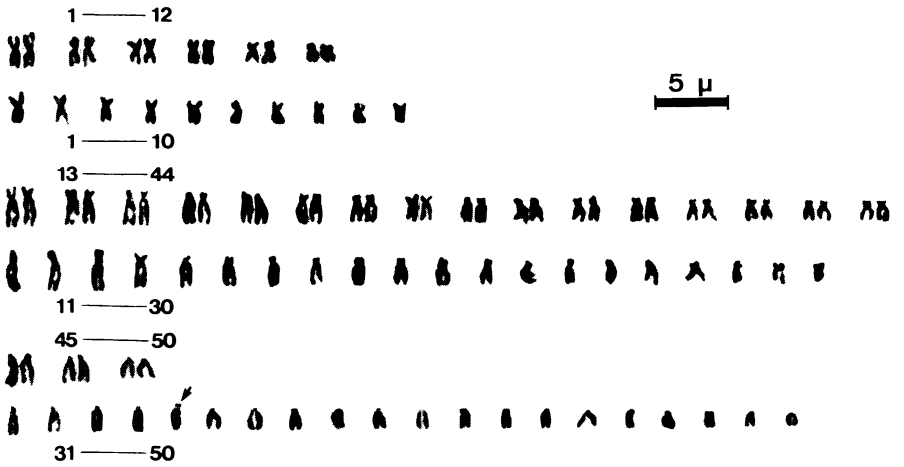

Fig. 2. Karyotype of *Gnathopogon elongatus elongatus* ♀ × *Carassius carassius* ♂ hybrid. Arrow shows chromosome with satellites.

reported by Kobayasi et al. (1970) and Ojima et al. (1972). Chromosomes of the hybrids were distinguished into 22 metacentrics, 52 submetacentrics and 26 acrocentrics, from which 50 chromosomes, i.e. 10 metacentrics, 20 submetacentrics and 20 acrocentrics, were chosen out as a genome belonging to *C. carassius* on the basis of the karyotype. The other 50 chromosomes consisted of 25 pairs of homologous chromosomes, 6 pairs of metacentrics, 16 pairs of submetacentrics and 3 pairs of acrocentrics. These 50 chromosomes were identical to the karyotype of *G. elongatus elongatus* in both number and morphology and could belong to two genomes of this species.

Discussion

In the previous studies in this series, the hybrids bred from female *C. carassius* have been reported to be allotriploidy. *C. carassius* ♀ × *Ctenopharyngodon idellus* ♂ hybrids had the karyotype incorporating two genomes of *C. carassius* and one of *Ct. idellus*, and their phenotype was similar to *C. carassius* (Kasama and Kobayasi, 1989b). In *C. carassius* ♀ × *G. elongatus elongatus* ♂ hybrids, the phenotype could be distinctly distinguished into two types, one closer to *C. carassius* and the other to *G. elongatus elongatus*. The karyotype of the *Carassius*-type hybrids consisted of two genomes of *C. carassius* and one of *G. elongatus elongatus*, while that of the *Gnathopogon*-type hybrids combined one genome of *C. carassius* and two genomes of *G. elongatus elongatus* (Kasama and Kobayasi, 1990a). On the basis of these data, we have cautioned against discussing the interrelationship among parental species of various hybrids on the basis of morphological analsis only, and have suggested that fishes belonging to the genus *Carassius* seem to produce offspring by a specific fertilization mechanism in intergeneric or interspecific crosses.

In the present cross using male *C. carassius*, the karyotype of the hybrids incorporated two genomes of *G. elongatus elongatus* and one of *C. carassius*. These data not only offer our caution and suggestion, but also indicate that spermatozoa as well as oocytes of *C. carassius* occasion a specific fertilization mechanism. The phenotype of the present hybrids showed a trend toward general intermediacy although they had the same karyotype as that of the *Gnathopogon*-type in *C. carassius* ♀ × *G. elongatus elongatus* ♂ hybrids. Generally reciprocal hybrids do not always show correspondense in their phenotypes. However, it may be necessary to consider a difference in the specific fertilization occationed by spermatozoa from that by oocytes in futher studies.

Acknowledgments

We are grateful to Mr. Masayoshi Maehata, Biwako Bunkakan for supplying us with *G. elongatus elongatus*.

Literature cited

Arai, K. 1984. Developmental genetic studies on salmon-

ids. morphogenesis, isozyme phenotypes and chromosomes in hybrids embryos. Mem. Fac. Fish. Hokkaido Univ., 31: 1–94.

Arai, K. 1987. Increased viability of allotriploid pink salmon × Japanese char hybrids. Nippon Suisan Gakkaishi, 59(9): 1477–1481.

Chu, Y. T. 1935. Comparative studies on the scales and on the pharyngeal and their teeth in Chinese cyprinids, with particular reference to taxonomy and evolution. Biol. Bull. St. John's Univ. (Shanghai), 2: 1–225, 30 pls.

Hubbs, C. L. and K. Kuronuma. 1942. Hybridization in nature between two genera of flounders in Japan. Rap. Mich. Acad. Sci. (Arts and Lett.), 27: 267–306.

Hubbs, C. L., L. C. Hubbs, and R. E. Johnson. 1943. Hybridization in nature between species of catostomid fishes. Contr. Lab. Vert. Biol. Univ. Mich., 22: 1–77.

Hubbs, C. L. and K. F. Lagler. 1970. Fishes of the Great Lakes Region. Univ. of Michigan Press, Ann Anbor, Michigan, xv+213 pp., 44 pls., 1 map.

Kasama, M. and H. Kobayasi. 1987. Hybridization experiments in Cyprinida (I). Cross between *Gnathopogon elongatus elongatus* and *Pseudorasbora parva*. Japan Women's Univ. J., 34: 107–112.

Kasama, M. and H. Kobayasi. 1988. Hybridization experiments in Cyprinida (III). Cross between *Gnathopogon elongatus elongatus* and *Sarcocheilichthys biwaensis*. Japan Women's Univ. J., 35: 119–124.

Kasama, M. and H. Kobayasi. 1989a. Hybridization experiments in Cyprinida (IV). Cross between *Gnathopogon elongatus elongatus* and *Squalidus gracilis*. Japan Women's Univ. J., 36: 101–106.

Kasama, M. and H. Kobayasi. 1989b. Hybridization experiment between crucian carp *Carassius carassius* ♀ and grass carp *Ctenopharyngodon idellus* ♂. Hybridization experiments in Cyprinida (II). Nippon Suisan Gakkaishi, 55(6): 1001–1006.

Kasama, M. and H. Kobayasi. 1990a. Hybridization experiment between *Carassius carassius* ♀ and *Gnathopogon elongatus elongatus* ♂. Hybridization experiments in Cyprinida (V). Japan. J. Ichthyol., 36(4): 419–426.

Kasama, M. and H. Kobayasi. 1990b. Hybridization experiments in Cyprinida (VI). Crosses with *Gnathopogon elongatus elongatus*, *Moroco steindachneri*, *Moroco jouyi*, *Zacco temmincki* and *Zacco platypus*. Japan Women's Univ. J., 37: 103–111.

Kobayasi, H. and M. Hashida. 1977. Morphological and cytological studies in back-cross hybrids of F_1 fishes between the kinbuna (*Carassius auratus* subsp.) ♀ and the crucian carp (*Carassius carassius*) ♂. Japan Women's Univ. J., 24: 121–133. (In Japanese with English summary.)

Kobayasi, H. and K. Nakano. 1976. Morphological and cytological studies in back-cross hybrids of F_1 fishes between the ginbuna (*Carassius auratus langsdorfii*) ♀ and the carp (*Cyprinus carpio*) ♂. Japan Women's Univ. J., 23: 113–122. (In Japanese with English summary.)

Kobayasi, H., Y. Kawashima and N. Takeuchi. 1970. Comparative chromosome studies in the genus *Carassius*, especially with a finding of polyploidy in the ginbuna (*C. auratus langsdorfii*). Japan. J. Ichthyol., 17(4): 153–159. (In Japanese with English summary.)

Matsubara, K. 1955. Fish morphology and hierarchy. Part I. Ishizaki Shoten, Tokyo, xi+789 pp. (In Japanese.)

Matsui, Y. 1950. On Japanese carp *Cyprinus carpio*, funa *Carassius carassius* and the hybids between these species. Pages 152–188 *in* The topics in mordern biology. Zoushindo, Tokyo, 589 pp. (In Japanese.)

Minamori, S. 1949. On sterility of hybrids between two local races of the spinous loach, *Cobitis taenia striata*. Japan. J. Genet., 24: 29–30. (In Japanese.)

Minamori, S. 1950. Isolating mechanisms in striated spinous loach, *Cobitis taenia striata*. J. Sci. Hiroshima Univ., Ser. B, Div. 1, 11: 55–59.

Minamori, S. 1951a. The lethal phenomena in the second generation of the spinous loach hybrid. J. Sci. Hiroshima Univ., Ser. B, Div. 1, 12: 57–66.

Minamori, S. 1951b. Hybridization and classification in spinous loaches. Japan. J. Ichthyol., 1: 215–225. (In Japanese with English resume.)

Minamori, S. 1952. Physiological isolation in Cobitidae I. Two races of the striated spinous loach with special reference to the difference in their embryonic respiration. J. Sci. Hiroshima Univ., Ser. B., Div. 1, 13: 199–212.

Minamori, S. 1953. Physiological isolation in Cobitidae II. Inviability of hybrids between the mud loach and some local races of spinous loaches. J. Sci. Hiroshima Univ., Ser. B, Div. 1, 14: 125–149.

Ojima, Y., M. Hayashi and K. Ueno. 1972. Cytogenetic studies in lower vertebrates X. Karyotype and DNA studies in 15 species of Japanese Cyprinidae. Japan. J. Genet., 47(6): 431–440.

Suzuki, R. 1962. Hybridization experiments in cyprinid fishes I. *Gnathopogon elongatus elongatus* ♀ × *Pseudorasbora parva* ♂ and the reciprocal. Bull. Japan. Soc. Sci. Fish., 28(10): 992–997.

Suzuki, R. 1963a. Hybridization experiments in cyprinid fishes II. Reciprocal crosses between *Pseudogobio esocinus* and *Gnathopogon elongatus elongatus*. Japan. J. Genet., 38(2): 89–96.

Suzuki, R. 1963b. Hybridization experiments in cyprinid fishes III. Reciprocal crosses between *Pseudorasbara parva* and *Gnathopogon elongatus elongatus*. Bull. Japan. Soc. Sci. Fish., 29(5): 421–423.

Suzuki, R. 1963c. Hybridization experiments in cyprinid fishes IV. Reciprocal crosses between *Biwia zezera* and *Gnathopogon elongatus elongatus*. Bull. Japan. Soc. Sci. Fish., 29(7): 655–657.

Suzuki, R. 1963d. Hybridization experiments in cyprinid fishes V. Reciprocal crosses between *Carassius carassius auratus* and *Gnathopogon elongatus elongatus*. Annot.

Zool. Jap., 36(4): 203–207.
Suzuki, R. 1963e. Hybridization experiments in cyprinid fishes VI. Reciprocal crosses between *Gnathopogon elongatus elongatus* and *Gnathopogon japonicus*. Japan. J. Ichthyol., 10(2/6): 39–42.
Suzuki, R. 1964. Hybridization experiments in cyprinid fishes VII. Reciprocal crosses between *Pseudogobio esocinus* and *Biwia zezera*. Japan. J. Ichthyol., 12(1/2): 18–22.
Suzuki, R. 1965a. Hybridization experiments in cyprinid fishes VIII. Two kinds of reciprocal crosses, *Pseudogobio esocinus* × *Pseudorasbora parva* and *Biwia zezera* × *Pseudorasbora parva*. Japan. J. Ichthyol., 13(1/3): 64–68.
Suzuki, R. 1965b. Hybridization experiments in cyprinid fishes IX. *Gnathopogon japonicus* ♀ × *Biwia zezera* ♂ and reciprocal crosses between *Pseudorasbora parva pumila* and *Biwia zezera*. Bull. Freshw. Fish. Res. Lab., 14(2): 91–95.
Suzuki, R. 1966. Hybridization experiments in cyprinid fishes X. *Gnathopogon japonicus* ♀ × *Pseudogobio esocinus* ♂. Bull. Freshw. Fish. Res. Lab., 16(1): 65–69.
Suzuki, R. 1968. Hybridization experiments in cyprinid fishes XI. Survival rate of F_1 hybrids with special reference to the closeness of taxonomical position of combined fishes. Bull. Freshw. Fish. Res. Lab., 18(2): 113–155.
Uyeno, T. 1972. Chromosomes of offspring resulting from crossing coho salmon and brook trout. Japan. J. Ichthyol., 19(3): 166–171.

(Received September 27, 1990; accepted August 20, 1991)

タモロコ雌とヨーロッパブナ雄の間の交雑実験

笠間緑子・小林　弘

タモロコ *Gnathopogon elongatus elongatus* 雌とヨーロッパブナ *Carassius carassius* 雄との間で人工的に交雑を試みた．この交雑における発生率，孵化率はそれぞれ約66%, 64% であったが，孵化した仔魚は奇形や栄養障害などが原因で徐々に死亡し，最終的に6個体が生き残って，ほぼ成魚形にまで生長した．これらの雑種を孵化後3年6ヶ月経過したところで形態的，核学的に分析し両親種との比較を行なった．その結果，雑種の形態はほぼ両親種の中間型に近く，また核型は雌親に由来する2ゲノムと雄親に由来する1ゲノムの合体したもので構成されており，これらの雑種は異質3倍体であった．以上の結果から，先に報告した逆交雑の結果(笠間・小林，1990)と考え合わせるとヨーロッパブナを使った異属間交雑では，ヨーロッパブナの雌雄にかかわらず染色体の倍数的変異が引き起こされることがわかった．

(112 東京都文京区目白台　日本女子大学生物学教室)