Three species of Cobitid fishes, *Cobitis biwae*, *C. taenia taenia* and *C. taenia striata*, are phenotypically indistinguishable from each other at various levels. The color patterns on body side and lamina circularis of pectral fins have been employed as the major characters for classification. But, the color patterns show considerable aberrations, and the lamina circularis of pectral fins appears only in adult males. Cytogenetic pictures of fishes serve as important tools useful not only for species identification but also for the analysis of their evolutional processes. We wish to present here some karyological features of the genus *Cobitis* (Cobitidae, Cyprinida), with special regard to the diploid-tetraploid complex.

**Materials and methods.** Kidney tissues were removed, minced with scissors and suspended in fish-BSS. They were treated with colchicine (0.5 μg/ml) for 30 minutes at room temperature. Following KCl-hypotonization (0.075 M) for 15–30 minutes, and Carnoy-fixation as usual, slides were air-dried and Giemsa-stained. Nomenclature of chromosomes was made according to Levan *et al.* (1964), i.e.: arm ratios up to 1.7 as metacentrics; those up to 3 as submetacentrics; those up to 7 as subtelo-centrics; and those over 7 as acrocentrics.

**Results.** (1) *Cobitis biwae*. Two types of the chromosome number, 48 and 96, were obtained in this species. Specimens collected in the waters of the Kinki district and its adjacent regions, Tottori prefecture and Shimane prefecture belonging to the Chugoku district, the Echi-river, Ado-river, Nabari-river, Ibi-river, Inashi-river, and Hino-river were characterized by 2n, 48 chromosomes. The karyotype consisted of 10 pairs of metacentrics, 11 pairs of submeta- or subtelo-centrics and 3 pairs of acrocentrics (Fig. 1). Sometimes 1 pair of submetacentrics carried a small satellite on their short arms.

On the other hand, tetraploid specimens were found to inhabit in the waters of western Honshu, such as the Yodo-river, Minami-river, Tonda-river, Yoshino-river, Takahashi-river and Ohta-river. They were characterized by 96 chromosomes which consisted of 16

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pairs of metacentrics, 27 submeta- or subtelo-centrics and 5 pairs of acrocentrics (Fig. 2). Apparently they are tetraploid variants referring to the chromosome number. The arm number (AN) of the tetraploid and diploid specimens are indicated in Table I. The AN value of the tetraploid specimens showed twice as much as the diploid one.

(2) Cobitis taenia taenia. Three types of the chromosome number, i.e., 2n=50, 2n=86, and 2n=94, were obtained in this species. Specimens with 50 chromosomes were collected in lower reaches of the Yabe-river (Fukuoka pref.). The chromosomes constitution
showed 6 pairs of metacentrics, 2 pairs of submetacentrics and 17 pairs of acrocentrics (Fig. 3). Tetraploid specimens having 2n=86 came from the midstreams of the Yabe-river and Kikuchi-river (Kumamoto pref.). The karyotype consisted of 16 pairs of metacentrics, 16 pairs of submeta- or subtelocentrics and 11 pairs of acrocentrics (Fig. 4). In most cases, one pair of the small acrocentrics had satellites.

Another tetraploid form with 2n=94 was obtained in the Yamakuni-river and Inumaru-river (Oita pref.). The complement was made up of 13 pairs of metacentrics, 16 pairs of submetacentrics and 18 pairs of acrocentrics (Fig. 5). The AN value in the 4n forms was over twice that of the 2n ones (Table I).

(3) Cobitis taenia striata. This species showed two chromosomal types having 50 and 98 chromosomes. The diploid forms with 50 chromosomes, which came from the Southern basin of Lake Biwa, the Yodo-river (Osaka pref.) and Chigusa-river (Hyogo pref.), were characterized by 6 pairs of metacentrics, 2 pairs of submetacentrics and 17 pairs of acrocentrics (Fig. 6). The tetraploid forms characterized by 2n, 98 were collected from the Amano-river, and Yasu-river (Shiga pref.). They comprised 10 pairs of metacentrics, 11 pairs of submetacentrics and 28 pairs of acrocentrics (Fig. 7). The AN value of the tetraploid type was slightly higher than that of the diploid (Table I).

Discussion. Hitotsumachi et al. (1969) showed that, 5 species of loaches, Misgurnus anguillicaudatus, Barbatula toni, Leflu nikkonis, Cobitis delicata and Cobitis biwae, possessed a diploid number of 50. Cobitis biwae was, however, exceptional in having 96 chromosomes. Two species, Misgurnus anguillicaudatus and Cobitis delicata, had the same diploid number, 50. Their chromosome morphology, however, differed from each other, but they showed a similar amount of DNA. The DNA amount of Cobitis biwae was also approximately twice as much as the others.

The present study revealed that Cobitis biwae showed 2 types of the chromosome number, 48 and 96, C. taenia taenia had 3 types, 50, 86 and 94, and that C. taenia striata showed 2 chromosomal forms having 50 and 98 chromosomes. It appears that a diploid-tetraploid relationship might have existed in these three species. This fact will throw a light to analyse the problem in which the diploidized tetraploidy becomes endowed with a far greater possibility than its diploid ancestor to sponsor a large evolutional change (Ohno 1974).

Very recently Kobayashi (1976), dealing with the relationship between the body size and polyploidy in Cobitis biwae, reported that the small size race collected in the waters of Tokyo and Saitama
prefecture was chromosomally diploid, while the large size race from Wakayama and Gifu prefectures were of a tetraploid nature, although it is not an exact duplication of chromosomes. No visible relationship between the body size and chromosomal ploidy was observed in the present study (Table I). Raicu and Taisescu (1972) reported the diploid number of 100 in *Misgurnus fossilis* from Rumania. Very probably the chromosome number of *M. fossilis* would be tetraploid differentiated from the diploid one. So far as the arm number of chromosomes is concerned, the evidence obtained by us suggests that karyotypic changes of the three species studied can not be explained solely by a Robertsonian principle.

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