68. Further Cytogenetical Studies on the Origin of the Gold-Fish

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On the basis of banding analysis, chromosomal evidence for the origin of the Gold-fish has been reported in part in the Proceedings of the Japan Academy, vol. 55, no. 2, 1979. The present paper reports further cytogenetic evidences on the origin of the Gold-fish which were derived from a banding study of the Chinese Funa (*Carassius auratus auratus*) in comparison with Japanese Funa varieties.

A new illustrate book on freshwater teleosts published in China, "Chinese Cyprinid Fishes", described that there are three kinds of Funa, such as the Black-Funa (*Carassius carassius*), the Silver-Funa (*C. auratus gibelio*) commonly known as Amur-Funa, and the Funa (*C. auratus auratus*). The former two subspecies are distributed in the northern water systems of the Shin-chan district. The third subspecies, the Funa has a wide distribution in island waters through China.

The history of the Gold-fish can be traced back to A.D. 265–419. In some Chinese old literature we can find some records describing that a certain prototypic variety of the Gold-fish appeared due to mutation at the southern part of China about sixteen hundred years ago. Taking these records into consideration, we cannot but conclude that it is impossible to say the Black-Funa and the Silver-Funa, distributing in the northern district, as the origin of the Gold-fish.

Fortunately we had an opportunity to study the chromosomes of the Chinese Funa (*C. a. a.*) in some detail, the results of which served to support our former findings on the origin of the Gold-fish.

Materials and methods. Two females and two males of the Chinese Funa furnished materials for the present chromosome studies. Kidney tissues were removed, minced with scissors and suspended in fish-BSS. They were treated with colchicine (0.5 μ g/ml) for 30 min at room temperature. Following KCl-hypotonization (0.075 M) for 15–20 min and Carnoy-fixation as usual, the Giemsa C-banding technique was applied to them for the banding analysis.

For the electrophoretic analysis of muscle protein in the Chinese Funa, the Gold-fish and the Japanese Funa subspecies involving

Gengoro-Funa (*C. a. cuvieri*), Kin-Funa (*C. a. subsp.*), Gin-Funa (*C. a. langsdorffi*), Nigoro-Funa (*C. a. grandoculis*) and Naga-Funa (*C. a. buergeri*), the horizontal starch-gel method was employed. The muscle samples were homogenized with an equal volume of deionized water, and the homogenates were centrifuged at 10000 g for 10 min. The supernatant thus obtained applied for electrophoresis. The starch-gel condition was 10%. The starch-gel electrophoresis was carried out for 6 hrs at a constant current of 1 mA/cm at about 4°C in discontinuous buffer system. The gel buffer was 0.076 M tris (hydroxymethyl) aminomethane-0.005 M citric acid (pH 8.6), and the electrode buffer was 0.3 M boric acid-0.05 M NaOH (pH 8.45). At the end of run, the gel was sliced and stained for protein with amid black 10B.

Results and remarks. Figs. 1 and 2 show the karyotypes in both sexes of the Chinese Funa (*C. a. a.*), which indicate the chromosome number of $2n=100$ consisting of 6 pairs of metacentrics, 18 pairs of submetacentrics, and 26 pairs of telo- or subtelocentrics. The second largest chromosomes were remarkable amongst them as markers on account of their conspicuous C-banded short arms. Noticeable is the occurrence of two such markers in the female, while one in the male.

The above findings from the Chinese Funa are fairly consistent with those from many varieties of the Gold-fish, as well as from the Japanese Kin-Funa (*C. a. subsp.*), as already presented by Ueda and Ojima (1978) and Ojima *et al.* (1979).

The marker chromosomes found in the Gold-fish, Japanese Funa's subspecies and the Chinese Funa, are shown in Fig. 3.

Fig. 4 shows the starch-gel patterns of muscle protein from the Chinese Funa, varieties of the Gold-fish, and five subspecies of the Japanese Funa.

As seen in Fig. 4-1, the starch-gel pattern of the Chinese Funa was shown by the deep-stained 2nd and 4th bands, and the light-stained 1st band. The patterns of the Gold-fish varieties were of the same type as that of the Chinese Funa (Fig. 4-2), no specific difference having been observed among them.

The pattern of the Japanese Funa was divided into two types. One was represented by the Gengoro-Funa (*C. a. cuvieri*) with the deep-stained 2nd and 3rd bands and the light-stained 1st band (Fig. 4-3). Another type was observed in the Kin-Funa (*C. a. subsp.*), Gin-Funa (*C. a. langsdorffi*), Nigoro-Funa (*C. a. grandoculis*), and Naga-Funa (*C. a. buergeri*) (Fig. 4, 4-A, -AB, -B). The latter type was characterized by the wide-stained 5th band, the deep-stained 3rd or 4th bands and the light-stained 1st and 2nd bands. With regard to the 3rd and the 4th bands, this type was subdivided into three subtypes, such as A with the 4th band, AB with the 3rd and 4th bands, and B

with the 3rd band. According to Taniguchi and Ishiwatari (1972), these three subtypes were controlled by two codominant alleles, A and B.

On the basis of the above findings, it can be stated with certainty that the Chinese Funa and the Gold-fish are fairly identical with each other with respect to the chromosome morphology following the C-banding analysis, as well as to the electrophoretic pattern of muscle protein. Such a kinship was not observed in other subspecies of the

Fig. 1. C-banding karyotype of a female of the Chinese Funa (*Carassius auratus auratus*) (the lower row). The arrows denote the second largest two submetacentrics having intensely C-banded short arms.

Fig. 2. C-banding karyotype of a male of the Chinese Funa (*C. a. a.*) (the lower row). The arrow denotes one submetacentrics of the second largest chromosomes having intensely C-banded short arms.

Fig. 3. Summarized illustrations of second submetacentric markers with C-banded short arms in varieties of the Gold-fish, Chinese Funa, Chinese Gold-fish and subspecies of Japanese Funa.

Fig. 4. Electrophoretic patterns of muscle protein. 1: The Chinese Funa (*C. a. a.*). 2: Varieties of the Gold-fish. 3: *C. a. cuvieri*. 4-A, -AB, -B: *C. a.* subsp., *C. a. langsdorfii*, *C. a. grandoculis*, and *C. a. buergeri*.

Japanese Funa. The above findings provide further evidence to show that the origin of the Gold-fish will be the Chinese Funa (*C. a. a.*).

Summary. In the Chinese Funa (*Carassius auratus auratus*), the C-banding pattern of chromosomes is investigated. The Chinese Funa is characterized by the second largest chromosomes with remarkable C-bands on their short arms as markers. There are two such markers in the female and one in the male. These findings are consistent with those derived from the Gold-fish varieties.

Further, the Gold-fish and the Chinese Funa (*C. a. a.*) are the same in their electrophoretic patterns of muscle protein. No such feature is not observed in five subspecies of the Japanese Funa.

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References

- Ojima, Y., T. Ueda, and T. Narikawa (1979): A cytogenetic assessment on the origin of the Gold-fish. *Proc. Japan Acad.*, **55B**, 58–63.
- Ueda, T., and Y. Ojima (1978): Differential chromosomal characteristics in the Funa subspecies (*Carassius*). *Proc. Japan Acad.*, **54B**, 283–288.
- Taniguchi, N., and T. Ishiwatari (1972): Inter- and intraspecific variations of muscle proteins in the Japanese crucian carp-I. *Japan. J. Ichthyol.*, **19**, 217–222.