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Unisexual and bisexual types of ginbuna, Carassius auratus langsdorfii in Aichi Prefecture

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JORDAN and FOWLER (1903) gave Japanese crucian carp a scientific name *Carassius auratus* which is the same as that of the goldfish and different from that of the European crucian carp, *Carassius carassius*. According to NAKAMURA (1969) five subspecies of funa inhabit Japan, viz., the kinbuna,*Carassius auratus subsp.*; the ginbuna, *C. auratus langsdorfii*; the nagabuna, *C. auratus bürgeri*; the nigorobuna, *C. auratus grandoculis* and the gengorobuna, *C. auratus cuvieri*.

Of these, the ginbuna is most widely distributed, covering all Japan. It has been known that the relative proportion of males of the ginbuna inhabiting the following districts is very small as compared with females: Sendai and the Tohoku district (SASAKI, 1926; KATO, 1932, EGASHIRA, 1935), Toyohashi (MATSUI, 1931, 1934) and Hiroshima (KINOSHITA, 1935). It is remarkable that the ginbuna obtained from the kanto district consisted solely of females (OKADA and NAKAMURA, 1948, NAKAMURA, 1969).

Gynogenetic reproduction in the ginbuna inhabiting the Kanto plains has been suggested by KOBAYASHI (1967) and NAKAMURA (1969). KOBAYASHI (1970) cytologically showed that the population of the ginbuna in the Kanto district consisted of triploid (rarely tetraploid) females, strongly suggesting their triploid gynogenetic reproduction. Later, he showed by a cytological study that gynogenesis took place in the eggs of the triploid ginbuna (KOBAYASHI, 1971).

The original impetus of the present study which began in 1968 by the authers at Biological Institute of Nagoya University, was to elucidate the reason of telegony of the ginbuna. The method of approach has been reciprocal crosses between the ginbuna (*C. auratus langsdorfii*) and goldfish (*C. auratus auratus*) whose sex ratio is known to be 1 male to 1 female (MATSUI, 1934).

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Table 1 Andric index and sex ratio in the crucian carp (Ginbuna), *Carassius auratus langsdorfii*, collected from localities in Aichi Prefecture

Localities	Drainge basin	Symbol	sex distribution			Andric index $\frac{♂♂}{♀♀+♂♂}\times 100$	Sex ratio $\frac{♂♂}{♀♀}\times 100$
			♀♀	♂♂	total		
Ôtagawa, Tôkai City	——	OT	185	9	194	4.6	5.4
Shimono-isshiki, Nagoya City	Shônai river	SH	95	24	119	20.2	25.3
Kanie, Ama-gun	Nikkô river	KA	173	56	229	24.5	32.4
Ueda, Nagoya City	Tempaku river	TH	82	30	112	26.8	36.6
Okazaki City*	Yahagi river	OK	305	195	500	39.0	63.8
Hekinan City**	Yahagi river	HE	42	28	70	40.0	66.8

*Data of Kobayashi, H. and K. Mizutori (1960).

**Chromosomal number examined was 100 (2n).

Table 2 Straight matings of female ginbuna (C) with male ginbuna (C)

Symbol and mating no.	Parents		Year of breeding	Offspring			Chromosomal number of offsprings
				♀♀	♂♂	total	
$(CC)_1$	OT ♀^{1}	SA ♂^{3}	'64	90	0	90	—
$(CC)_2$	OT ♀^{1}	SA ♂^{10}	'66	62	0	62	156 (3n)
$(CC)_3$	OT ♀^{6}	SA ♂^{1}	'64	550	0	550	—
$(CC)_4$	MI ♀^{3}	SA ♂^{2}	'66	17	0	17	—
$(CC)_5$	OT ♀^{1}	SA ♂^{6}	'70	103	0	103	—
$(CC)_6$	MI ♀^{4}	SA ♂^{6}	'70	99	0	99	156 (3n)*

* Diploid nucleus (chromosomal number, 100) was rarely found among triploid nuclei. MI ; the symbol of individuals collected from Midoriga-ike in Nagoya City.

Materials and Methods

Materials used are ginbuna collected from various localities of Aichi Prefecture situated in the central district of Japan. For convenience in referring to crosses, sources of materials are symbolized OT, SH, KA, TE, HE, designating drainage basins as listed in Table 1. Other sources are SA (Sakurayama market), MI (Midoriga-ike) and TO (Togasa-ike) all in Nagoya-city. Superscript numerals on ♀ or ♂ in lists of Tables are fish number.

The varieties of the goldfish used as mates of ginbuna are Wakin, Ryukin, Calico and Shubunkin which has been mainly used in later phase of the study. Shubunkin has an incomplete dominant *T*-gene responsible for transparent scales eiter herozygously *Tt* or homozygously *TT* whereas ginbuna posses recessive *t*-gene governing normal scales homozygously (*tt*). When a gynogenetic ginbuna female is mated with a shubunkin male, either *Tt* or *TT*, all F_1 offspring with mosaic transparent scales (*Tt*) and normal scales in a 1 : 1 ratio. When a homozygous shubunkin male (*TT*) is used as a mate to a gonochoristic ginbuna female, all F_1 offspring have mosaic transparent scales (*Tt*). Genetics of scale transparency in the goldfish has been worked out by CHEN (1928), MATSUI (1933,1934) and KAJISHIMA (1977), and we adopted the gene symbols used in the last.

In mature fish, sex has been discriminated by examing the cloacal region. In the male this area is elliptic, small and the urogenital papilla between the anus and urogenital pore does not usually project; furthermore pearl organs appear on the opeculum, the dorsal and pectoral fins. From spring to autum, milky semen ooze out by stripping the belley. In the female, on the other hand, the cloacal region is oval, large, and the urogenital papilla projects noticeably. By stripping belley, a few oocytes ooze out. Any immature fish, less than 5 cm SL, sex has been determined by autopsy.

Results

1. *Sex-ratio of ginbuna in localities of Aichi Prefecture.*

Sex ratio of ginbuna collected from various drainage basins of four rivers are shown in Table 1. It is found that sex-ratios are considerably different in localities of Aichi Prefecture especially depending on river basins, although all being less than 100. It is the lowest in the population of a pond of Otagawa (OT) and highest in popurations of the Yahagi river (OK and HE). These results show that ginbuna males in various sex ratios are inhabiting in Aichi Prefecture contrasting to Kanto district where no male ginbuna are found (NAKAMURA, 1969).

2. *Straight matings between ginbuna.*

Six straight matings of ginbuna have been performed (Table 2). Male and female parents collected from OT and MI districts were used and fish Nos. are denoted as

Table 3 Matings of goldfish females with ginbuna males

Symbol and mating no.	Parents	Year of breeding	Sexes of offspring				Scaleness* of offspring		
			♀♀	♂♂	total		matt	nacreous	metallic
$(GC)_1$	Calico *matt* ♀[1] SA ♂[10]	'64	67	75	142	$X^2_{(1)}=0.45$, $P=0.50$	0	142	0
$(GC)_2$	Wakin metallic ♀[6] SA ♂[2]	'64	42	40	82		0	0	82
$(GC)_3$	Shub. *matt* ♀ SA ♂[10]	'66	49	68	117	$X^2_{(1)}=3.4$, $P=0.1-0.05$	0	117	0
$(GC)_4$	Shub. *matt* ♀[66−1]SA ♂[6]	'70	5	11	16		0	16	0

* Scaleness: *matt*, total transparent, genotype being *TT*; *nacreous*, mosaic transparent, genotype being *Tt*; metallic, normal, genotype being *tt*.

Table 4 F_2 and back-crosses from matings of goldfish females and ginbuna males.

Symbol and mating no.	Parents	Year of breeding	Sexes of offspring			Scaleness* of offspring			Caudal fin of offspring	
			♀♀	♂♂	total	matt	nacreous	metallic	single	double
$(GC)^2_1$	$(GC)_1F_1$ ♀ $(GC)_1F_1$ ♂	'66, '67	11	19	30	20**		10	23	7
$(GC)_1$ G	$(GC)_1F_1$ ♀ $(GC)_1F_1$ ♂	'66, '67	18	31	49	16	33	0	49	0
$(GC)^2_2$	$(GC)_2F_1$ ♀ $(GC)_2F_1$ ♂	'70	15	13	28	28	0	0	28	0

* See Table 3 for abbreviations.**

Table 5 Matings of ginbuna females with goldfish males

Symbol and mating no.	Parents	Year of breeding	Sexes of offspring			Scaleness* of offspring			Category of ♀ - parent
			♀♀	♂♂	total	matt	nacreous	metallic	
$(CG)_1$	OT ♀5 Ryukin metal. ♂	'60	133**	0	133	0	0	133	Unisexual
$(CG)_2$	OT ♀1 Wakin metal. ♂	'63	48	0	48	0	0	48	Unisexual
$(CG)_3$	OT ♀1 Shub. matt ♂	'66	146**	0	146	0	1	146	Unisexual
$(CG)_4$	MI ♀3 Wakin metal. ♂	'67	50	0	50	0	0	50	Unisexual
$(CG)_5$	MI ♀3 Shub. matt ♂	'68	161	0	161	0	2	159	Unisexual
$(CG)_6$	KA ♀2 Shub. nacreous ♂	'70	180	0	180	0	0	180	Unisexual
$(CG)_7$	SH ♀3 Shub. nacreous ♂	'71	58	0	58	0	1	57	Unisexual
$(CG)_8$	TO ♀1 Shub. nacreous ♂	'71	10	0	10	0	0	10	Unisexual
$(CG)_9$	TO ♀4 Shub. nacreous ♂	'71	24	0	24	0	0	24	Unisexual
$(CG)_{10}$	SH ♀5 Shub. nacreous ♂	'72	19	0	19	0	0	19	Unisexual
$(CG)_{11}$	SH ♀6 Shub. nacreous ♂	'72	21	0	21	0	0	21	Unisexual
$(CG)_{12}$	TO ♀3 Shub. nacreous ♂	'73	23	0	23	0	0	23	Unisexual
$(CG)_{13}$	OK ♀3 Shub. nacreous ♂	'73	20	23	43	0	19	24	Bisexual
$(CG)_{14}$	OK ♀5 Shub. nacreous ♂	'73	88	0	88	0	2	86	Unisexual
$(CG)_{15}$	OK ♀8 Shub. nacreous ♂	'73	12	13	25	0	9	16	Bisexual
$(CG)_{16}$	HE ♀3 Shub. nacreous ♂	'73	25	26	51	0	29	34	Bisexual
$(CG)_{17}$	HE ♀4 Shub. nacreous ♂	'73	31	0	31	0	2	29	Unisexual
$(CG)_{18}$	HE ♀7 Shub. nacreous ♂	'73	21	0	21	0	0	21	Unisexual
$(CG)_{19}$	OK ♀7 Shub. nacreous ♂	'74	13	15	28	0	21	30	Bisexual

* See Table 4 for abbreviation. ** Triploid cytologically examined.

superscripts. The results show that all the four females used were gynogenetic producing all female offspring.

3. *Matings of goldfish females with ginbuna males.*

Four matings of goldfish females with ginbuna males (GC series) have been performed (Table 3). All the four matings produced both sexes which can be statistically regarded as the 1 : 1 sex ratio.

The results indicate that the reproductive function of ginbuna males are quite normal and the union of male and female gametic nuclei takes place. It may be remarked that SA ♂^{2} and SA ♂^{6} which rendered ginbuna females all female offspring as listed in Table 2 fathered both daughters and sons. As to scaleness, ginbuna males (*tt*) in mating with homozygous transparent scaled (matt) goldfish females (*TT*) produce all heterozygous transparent scaled (nacreous) offspring (*Tt*) and in mating with normal scaled (metallic) goldfish female (*tt*) yielded all normal scaled progeny (*tt*), as expected.

4. *F_2 and backcross offspring of goldfish females with ginbuna males.*

Matings of a $(GC)_1$ female with a $(GC)_1$ male, a $(GC)_2$ female with a $(GC)_2$ male and a $(GC)_1$ female with a goldfish male (shubunkin) have been performed and the results are listed in Table 4. In all these three mating, both females and males were produced in approximately 1 : 1 ratio.

5. *Matings of ginbuna females with goldfish males.*

The sex ratio of ginbuna in various district of Aich Prefecture suggests the presence of the two types of females, unisexual and bisexual, since all males in this district tested are bisexual in mating with goldfish females. In order to actualize this by breeding, a number of females from various localities have been taken at random, numbered and mated each with a goldfish male.

Discussion

The sex ratio of ginbuna, *Carassius auratus langsdorfii,* in Otagawa (Tokai City) is about 5 percent. In the case of goldfish × ginbuna, typical hybridization takes place. Male ginbuna always produce F_1, F_2 and F_R hybrids normally in mating with goldfish females indicating that they are all normally bisexual. In the reciprocal cross, ginbuna × goldfish, paternal characters are not usually transmitted to offspring, and all female progenies are resulted. The majority of females in this district seems to be gynogenetic. However, presence of rare bisexual females (producing both males and females) is suspected, since males behaved normally on cross with goldfish females. Namely, there are two types of females in the ginbuna in Aichi Prefecture, viz., unisexual (gynogenetic) and bisexual (gonochoristic). The unisexual females produced all female progenies in mating either the goldfish or the ginbuna males. Bisexual females, on the other hand, produced both males and females in the ratio of one female

to one male in mating with goldfish males. The cytological observation showed that unisexual females are triploid having somatic chromosomes of 156 which bisexual females and males are all diploid with chromosomes of 100 in number. These results in cross experiments indicate that triploid ginbuna are produced gynogenetically. According to KOBAYASHI (1976), the triploid eggs may be formed through a single homoeotype meiosis during maturation process. In fertilized eggs of triploid ginbuna artificially inseminated with diploid kinbuna (*C. a. subsp.*), the sperm nucleus is not involved in the development (KOBAYASHI, 1976). Thus, the triploid lines of the ginbuna seems to keep by gynogenetic reproduction.

References

AFFLECK, R. J. (1952) The nacreous (mottled) group of the goldfish (*Carassius auratus L.*), with an analysis of the colour seen in these fish. *Aust. J. Mar. Fresh-W. Res.*, **3** : 126-139.

CHEN, S. C. (1928) Transparency and mottling, a case of mendelian inheritance in the goldfish (*Carassius auratus*). *Genetics,* **13** : 434-452.

EGASHIRA, M. (1935) On the survival powers of males and females of *Carassius auratus* (*L.*) under some harmful external condition. *Sci. Rep. Tohoku Univ., Ser. 4,* **9** : 415-426.

JORDAN, D. S. and H. W. FOWIER, (1903) A review of the cyprinoid fishes of Japan. *Proc. U. S. Nat. Mus.,* **26** : 811-862.

KAJISHIMA, T. (1977) Genetic and developmental analysis of some new color mutants in goldfish, *Carassius auratus. Genetics,* **86** : 161-174.

KATO, G. (1932) On the sex ratio and the growth of body in *Carassius auratus* and its variety "the Iron-fish". *Sci. Rep. Tohoku Imp. Univ., Ser. 4,* **7** : 365-381.

KINOSHITA, T. (1933) A new case of hermaphroditism in *Carassius auratus* (*L.*). *J. Sci. Hiroshima Univ.,* **2** : 1-7.

KOBAYASHI, H. (1967) On the kinbuna and ginbuna of Kanto district studied by hybridization. *Zool. Mag.* **76** : 375.

——— (1970) Comparative chromosome studies in the genus *Carassius,* especially with a finding of poliploidy in the ginbuna (*C. auratus langsdorfii*). *Jap. Jour. Icht.,* **17** : 153-160.

——— (1971) A cytological study on gynogenesis of the triploid ginbuna (*Carassius auratus langsdorfii*). *Zool. Mag.,* **80** : 316-322.

——— (1976) A cytological study on the maturation division in the oogenic process of the triploid ginbuna (*Carassius auratus langsdorfii*). *Jap. Jour. Icht.,* **22** : 234-240.

MATSUI, Y. (1931) Genetical studies of fresh water fish. 2 : On the hybrid of *Cyprinus carpio L.* and *Carassius carassius* (*L.*). *Jour. Imp. Fish. Exp. Station,* **2** : 129-137.

——— (1934) Genetical studies on goldfish of Japan. *J. Fish. Inst. Tokyo,* **30** : 1-57.

NAKAMURA, M. (1969) Cyprinid fishes of Japan. *Special publication of the research institute for natural resources,* No. **4**.

OKADA, Y. and M. NAKAMURA (1948) Studies on the races of *Carassius auratus* (*L.*) in Japan. 1. *Carassius auratus* (*L.*) in Jo-numa, a small lake in Gumma Prefecture. *Bull. Jap. Soc. Sci. Fish.,* **13** : 279-284.

SASAKI, K. (1926) On the sex ratio in *Carassius auratus. Sci. Rep. Tohoku Imp. Univ., Ser. 4,* **1** : 229-238.