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[COMMUNICATION]

Gene-Centromere Mapping for 5 Visible Mutant Loci in Multiple Recessive Tester Stock of the Medaka (*Oryzias latipes*)

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ABSTRACT—Five visible mutant loci used for development of the multiple recessive tester Medaka were mapped in relation to their centromeres by the use of the diploid gynogenetic technique. Under complete interference condition, gene-centromere distances were 2cM for *pl*, 13cM for *r*, 32cM for *b*, 40cM for *Da* and 48cM for *gu*, respectively. No joint segregations were observed between the following sets of loci; *b* with *pl*, *b* with *gu*, and *b* with *Da*. The frequency of 0.97 for the heterozygosity at the *gu* locus strongly suggested the presence of chiasma interference. That is, one cross-over most likely inhibited occurrence of another cross-over in the same interval on the chromosome arm carrying the *gu* locus.

INTRODUCTION

The multiple recessive tester stock homozygous for 5 loci was developed [1] to investigate the frequency and mechanism of radiation-induced [2] and/or chemically induced mutations in the fish *Oryzias latipes*. The loci used in this tester fish were *b* (colorless melanophores), *Da* (double anal fins), *gu* (reduced deposition of guanine in iridocytes), *pl* (no pectoral fins) and *r* (colorless xanthophores) [3]. All loci except *Da* were recessive [3]. The *r* locus was sex-linked [4] and others were autosomal [3].

We believe it important for further understanding of the basic biology of the Medaka as a laboratory animal to characterize these loci in terms of linkage relationship between different genes and between the gene and centromere. For this purpose, we used diploid gynogenesis [5]. The phenotypes of diploid gametes in the Medaka, which were produced by blocking the second meiotic division of the eggs, i.e. by diploid gynogenesis, reflect the egg genotypes. Thus, using this gynogenesis linkage analyses in the Medaka were accomplished without backcrosses to double mutants or F_1 inbreeding.

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MATERIALS AND METHODS

Genetic cross to obtain F_1 hybrids

In order to produce F_1 hybrids, females of the HB-12 inbred strain of the Medaka *Oryzias latipes* [6] were mated with males of the multiple recessive tester stock homozygous for the *b*, *pl* and *r* loci or *b*, *gu* and *r* loci [1]. F_1 hybrids with *Da*/+ and *b*/*B* were produced by crossing females of wild stock from Aomori (stock # 1) [7, 8] with males of multiple recessive tester stock homozygous for the *b* and *Da* loci.

Production of gynogenetic diploid Medaka

The method to produce gynogenetic diploid Medaka was described by Naruse *et al.* [5]. Briefly, unfertilized eggs were collected from F_1 hybrids using sham-mating method, and eggs thus obtained were fertilized with UV-irradiated genetically impotent Medaka sperm, and exposed to heat shock or hydrostatic pressure 2 to 3 min after insemination to block the second meiotic division [5]. The yield of gynogenetic diploid Medaka was

over 20% at hatching. The phenotypes of the gynogenetic diploid progeny were visually recognized using a stereoscopic microscope.

The judgement of phenotype of the *b* locus was performed 4 days after fertilization, those of *pl* and *gu* loci just after hatching. The phenotypes of the *Da* and *r* loci were judged at about one month after hatching.

Determination of gene-centromere distances

Recombination frequency (X) between a gene and its centromere can be estimated from the frequency (Y) of heterozygous diploid progeny gynogenetically obtained from heterozygous F_1 hybrids. But it was difficult or impossible to distinguish the heterozygotes from the homozygotes for wild type alleles with regard to the marker genes used for the production of the multiple recessive tester Medaka. So we assumed that the proportions of the homozygotes for mutant alleles and wild-type alleles were identical. Then the fraction of heterozygotes was obtained from the following formula:

$$Y = 1 - 2m$$

where Y= fraction of heterozygotes, and m= fraction of homozygotes for mutant alleles.

In order to convert Y to map distance (X) in cM between the gene and centromere, two formulas were used:

$$X = Y \times 100/2 \quad \text{(with complete interference)} \qquad (1)$$

$$X = -[\ln(1-Y)] \times 100/2 \quad \text{(with zero interference)} \qquad (2)$$

The formula (1) was based on Morgan's summation formula (multiplication with 100 for conversion into cM unit and division by 2 because of using diploid gynogenesis), while formula (2) was in accordance with Haldane [9].

Linkage analysis using diploid gynogenesis

The analyses for joint segregation of two different genes were made by comparing the observed numbers of individuals with the numbers expected if the loci were inherited independently using 2×2 contingency table.

RESULTS AND DISCUSSION

Gene-centromere distance

Table 1 shows the gene-centromere distance under zero and complete interference condition, respectively. When the fraction of heterozygotes was small, map distance between the gene and centromere was almost the same under both conditions. On the other hand, under high heterozygous proportion, the map distances were largely different.

If the occurrence of cross-over is independent of each centromere, the maximum frequency of heterozygotes in gynogenetic diploids is expected to be 0.67 [10]. The frequency of 0.97 for the *gu* locus was significantly different (upper/lower 99% confidence limits: 0.983/0.932) from 0.67. This result suggests that exactly one cross-over occurred between the *gu* locus and the centromere. Such high frequency was not exclusive for the *gu* locus. Indeed in the Medaka the *Ldh-A* and *Amy* loci showed the high heterozygous fraction (1.00 for *Ldh-A* and 0.93 for *Amy*; Naruse and Shima, in preparation). Further, other species of fish with high heterozygous fractions were also reported (e.g., 1.00 for the *Sod* locus of rainbow trout, and 0.89 for *gol-1* gene of zebrafish) [11–13]. Thus, to obtain high heterozygous frequencies for some loci appeared not exceptional for the Medaka but rather frequent in fish. Our result also suggests that the chiasma interference is very high on the chromosome arm carrying the *gu* locus.

Considerable variations in heterozygous fractions were observed in the *b* locus. When maternal genotype was *b*/*B* (DO–AO), heterozygous fraction was low in comparison with other maternal genotype (0.47, 0.63 and 0.67). But the difference among these values were not statistically significant (95% confidence limit). Therefore, we summed up progeny numbers of each phenotypes to estimate the heterozygous fraction and gene-centromere distance for the *b* locus (Table 1).

To the best of our knowledge, there is only one paper on zebrafish (*Brachydanio rerio*) reporting gene-centromere distances of visible mutant loci which were comparable for the *b* locus of the Medaka [12]. Streisinger *et al.* [12] estimated

TABLE 1. Progeny phenotypes in gynogenetic diploid Medaka and gene-centromere distances

Locus	Maternal* genotype	Progeny phenotype		Heterozygous fraction	Gene-centromere distance	
		Mutant	Wild		Interference	
					Complete**	Zero***
pl	*pl*/+(HB-PL)	278	303	0.04	2	2
r	*r*/*R* (HB-PL)	13	22	0.26	13	15
b	*b*/*B* (HB-PL)	107	474	0.63	32	50
	b/*B* (HB-GU)	58	289	0.67	33	55
	b/*B* (DO-AO)	14	39	0.47	24	32
		179†	802§	0.64#	32	51
Da	*Da*/+(DO-AO)	5	48	0.81	40	83
gu	*gu*/+(HB-GU)	6	341	0.97	48	175

* HB; HB-12 inbred strain, PL; multiple recessive Medaka with *b*/*b*, *pl*/*pl* and *r*/*r*, GU; multiple tester Medaka with *b*/*b*, *gu*/*gu* and *r*/*r*, DO; multiple tester Medaka with *b*/*b* and *Da*/*Da*, AO; wild stock collected from Aomori. (HB-PL) indicates crossing of females of HB-12 strain with males of PL stock.
** Heterozygous fraction $\times 100/2$.
*** $-[\ln(1-\text{Heterozygous fraction})]\times 100/2$ [9].
† Total number of mutants at the *b* locus.
§ Total number of wild type progeny at the *b* locus.
# Calculated as $1-2\times[179/(179+802)]$.

gene-centromere distances of 44.5 cM for *gol-1* and 28.5 cM for *gol-2*, respectively. Our study gave 32 cM for the *b* locus in the Medaka. Thus, the gene-centromere distance of the *b* locus was rather similar to that of *gol-2* than *gol-1*. Obviously a simple comparison of distances does not allow to draw any conclusion about the extent of genome conservation among fishes without further accumulation of more information about linkages of not only the visible mutant loci but also the enzymatic polymorphic loci.

Linkage analysis using diploid gynogenesis

We tested joint segregation of the following sets of loci in gynogenetic diploid Medaka: *pl* with *b*; *gu* with *b*; *Da* with *b* (Table 2). The tests were made by comparing the observed numbers of individuals with the numbers expected if the loci were inherited independently using 2×2 contingency table. The results of all tests were nonsignificant ($\chi^2=3.08$ for the *pl* and *b* loci, 1.23 for the *gu* and *b* loci, and 1.98 for the *Da* and *b* loci, respectively). These results indicated that these loci were not closely linked, as suggested by segregation analysis using F_2 progeny [1].

TABLE 2. Linkage analysis in gynogenetic diploid Medaka

Genotype	Genotype	
	gu/*gu*	*gu*/+ and +/+
b/*b*	0 (0.99)*	58 (56.96)*
b/*B* and *B*/*B*	6 (4.91)*	283 (284.14)*
	pl/*pl*	*pl*/+ and +/+
b/*b*	43 (51.10)**	64 (55.80)**
b/*B* and *B*/*B*	235 (226.62)**	239 (247.47)**
	Da/*Da*	*Da*/+ and +/+
b/*b*	0 (1.3)***	14 (12.5)***
b/*B* and *B*/*B*	5 (3.7)***	34 (35.5)***

The number in parentheses is expected number if two loci were independent.
* $\chi^2=1.23$, $P>0.05$, d.f.$=1$.
** $\chi^2=3.08$, $P>0.05$, d.f.$=1$.
*** $\chi^2=1.98$, $P>0.05$, d.f.$=1$.

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