Gene-centromere Mapping at Four Microsatellite and Five Isozyme Loci in *Clarias macrocephalus* Based on Meiotic Gynogenetic Offspring

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**Abstract:** Gene-centromere distance was estimated for 4 microsatellite loci, Cma-2*, Cma-3*, Cma-4* and Cga-3*, and 5 isozyme loci, sMDH-1*, PGM*, GPI-1*, ADH*, and EST*, based on gynogenetic families of *Clarias macrocephalus*. Differences of genotypic ratio between families were observed at Cma-3*, sMDH-1* and GPI-1*. We observed different frequencies between the two homozygous classes at sMDH-1* and GPI-1*. The recombination rates (y) of three loci exceeded the maximum 67%, thus support the evidence for complete interference along chromosomes of *C. macrocephalus*. The gene-centromere map distances ranged from 0.6 cM for the Cga-3* to 49 cM for EST*. Three loci, Cma-4*, Cga-3* and GPI-1*, were closely linked to their centromeres. We observed linkage between sMDH-1* and Cma-3* in one out of the three gynogenetic families. Fixation index, estimated by (1–y), was 0.570.

**Key words:** gynogenesis, gene-centromere distance, *Clarias macrocephalus*, fixation index

Clariid fishes contribute significantly to annual freshwater fish production of countries in South and Southeast Asia and Africa (Na-Nakorn 1999). Among a few popular species for culture, *C. macrocephalus* has received much attention from scientists due to its significant economic value in Southeast Asia. Several genetic studies have been conducted in this species, including selection for disease resistance (Na-Nakorn et al. 1994), induction of polyploidy (Na-Nakorn and Lekaanantar 1993; Fast et al. 1995) and gynogenesis (Na-Nakorn et al. 1993; Na-Nakorn 1995), and assessment of genetic diversity (Daud et al. 1989; Na-Nakorn et al. 1998; Na-Nakorn et al. 1999; Na-Nakorn et al. 2002).

Gynogenesis was induced in attempting to produce all-female stocks of *C. macrocephalus*. UV-irradiated sperm of *Pangasius hypophthalmus* (previous name *P. sutchi*) were used to fertilize *C. macrocephalus* eggs followed by meiotic disruption by cold shock (Na-Nakorn et al. 1993). Although the contributions of these gynogenetics to aquaculture were insignificant (Na-Nakorn 2002), the technique is useful for further genetic studies especially gene-centromere mapping. Diploidization of gynogenetic individuals by retention of the second polar body allows restoration of one set of homologous chromosome after crossing-over. Therefore, the proportions of heterozygous genotypes reflect rates of crossing-over between individual loci and centromeres, thus allowing estimation of gene-centromere distance (Thorgaard et al. 1983; Allendorf et al. 1986; Liu et al. 1992; Johnson et al. 1996). Meiotic gynogenesis has been used as a tool for gene-centromere mapping in several species of fish and shellfish including rainbow trout (Thorgaard et al. 1983; Allendorf et al. 1986), chum salmon, *Oncorhynchus keta*...

Recently, Johnson et al. (1996) was the first group who used gene-centromere mapping, based on analyses of half-tetrads, as a novel and rapid method to assign mutations to a specific linkage group. They identified centromere-linked markers and then mapped them with the markers included in the linkage groups. Hence, they were able to consolidate the 29 linkage groups to 25 haploid chromosomes of zebrafish. Similarly, Mohideen et al. 2000 identified centromere-linked microsatellite markers for 6 linkage groups of zebrafish and will use these markers for locating the available linkage groups to the centromeres.

Moreover, the gene-centromere distance can be used to estimate the rate of inbreeding resulting from the induction of gynogenesis (Thompson 1983; Allendorf and Leary 1984). The induction of diploid gynogenesis has been recognized as a method of producing inbred lines of fish. The efficiency of meiotic gynogenesis induction in inbreeding can be presented based on the inbreeding coefficient (Thompson 1983) or a fixation index (Allendorf and Leary 1984). Allendorf and Leary 1984 clarified that inbreeding coefficient predicts the expected increase in homozygosity at a selectively neutral locus while the fixation index measures the actual increase in homozygosity and thus takes into account both inbreeding and selection. They suggested estimating the fixation index based on a formula 1-y, where y is the frequency of heterozygotes (Allendorf and Leary 1984). Based on gene-centromere recombination rate in rainbow trout (Thorgaard et al. 1983), the fixation index was estimated to be 0.403 and was almost double the inbreeding coefficient obtained from a generation of sib-mating (0.25).

The objectives of this study are: 1) to estimate gene-centromere distances of 4 microsatellite loci and five isozyme loci in *C. macrocephalus*; 2) to determine the increase of homozygosity after one generation of meiotic gynogenesis in this species.

**Materials and methods**

**Fish examined**

Two batches of gynogenetic families of *C. macrocephalus* were produced. The first batch comprised five families, AQ-1 to AQ-5, which were scored for four microsatellite loci, *Cma-2*, 3*, 4*, and *Cga-3*, and three isozyme loci, *GPI*, *sMDH* and *PGM*. The second batch comprised seven gynogenetic families, AQ-6 to AQ-12, which were screened for an additional two isozymes (ADH and EST*).

**Induction of gynogenetic diploids**

Female brooders (designated as AQ-1 to AQ-12) were obtained from a domesticated stock kept at the Department of Aquaculture, Faculty of Fisheries, Kasetsart University. This stock originated from crossing of several local stocks collected across Thailand. Gynogenetic offspring of *C. macrocephalus* were produced using irradiated sperm of *Pangasius hypophthalmus*. Induced ovulation of the female fish followed a standard protocol where gravid females (200–250 g body weight) were injected with 25µg/kg LH-RH-analogue (Suprefact, HOECHST AG, Germany) plus 5 mg/kg domparidone (Motilium-M, JANSSEN-CILAG) 10 hours prior to stripping. A male *P. hypophthalmus* (2 kg body weight) was injected to dorsal skeletal muscle with 10 µg/kg LH-RH-analogue plus 5 mg/kg Domparidone, 4 hours prior to stripping. Muscle and liver samples were taken from each female and stored at −30°C for isozyme analysis. Fin samples were collected from each female and stored in 70% alcohol for DNA extraction.

The protocol for sperm irradiation followed Na-Nakorn et al. (1993) where sperm was diluted with Ringer’s solution (8 g NaCl, 0.2 g NaHCO3, 0.2 g KCl, 0.2 g CaCl2, distilled water to 1,000 ml)
Gene-centromere distance in *Clarias macrocephalus*

at a ratio of 1:9 (sperm: diluent). The sperm dilution was poured into a petri dish to reach approximately 2 mm deep. Irradiation was done using a 30 W ultraviolet lamp placed 30 cm above the sperm. Irradiation lasted 2 minutes. This resulted in a UV dosage of 10.8 x 10⁴ ergs/mm². The irradiated sperm and the eggs obtained from each female were mixed and subsequently subjected to a cold shock (Na-Nakorn et al. 1993). The fertilized eggs were immersed in cold water (7°C) starting at 4 minutes after water activation of eggs and lasting for 14 minutes. After the cold shock, eggs were incubated in hatching trays with water flow until hatching, which occurred within 24 – 27 hours (water temperature of 28°C). Fry were reared in concrete tanks to 100 days old when they reached the average size of 14 cm (approximately 26 g). Tissues and fin samples were collected from each fish and stored for isozyme analyses and DNA extraction as described for the female fish.

**DNA analyses**

DNA extraction and analyses were performed at the DNA Technology Laboratory, Kochi University, Japan. DNA extraction followed standard phenol-chloroform method (Taggart et al. 1992). Three microsatellite primers developed from the *C. macrocephalus* genome (Na-Nakorn et al. 1999), Cma-2*, Cma-3* and Cma-4*, and one *C. gariepinus* primer (Galbusera et al. 1996), Cga-3* were used to amplify DNA from each sample. The reverse primers were 5' end-labeled with γ ³²P. PCR was carried out using a Thermal Cyclers-MP TP 3000 (TaKaRa). Each 6 µl reaction contained 10 – 50 ng template DNA, 2.5 µM unlabeled primer, 0.25 µM labeled reversed primer, 8.8 mM Tris-HCl (pH 8.3), 1.3 mM MgCl₂, 43.9 mM KCl, 1.75 µM of each dNTP and 0.25 unit of Taq Polymerase (TaKaRa). The PCR cycles were 7 cycles of 1 min at 94°C, 30 sec at the appropriate annealing temperature, 30 sec at 72°C, and 33 cycles of 30 sec at 90°C, 30 sec at annealing temperature, and 30 sec at 72°C. PCR products were separated by electrophoresis on polyacrylamide gels followed by autoradiography (Na-Nakorn et al. 1999).

**Isozyme analyses**

Isozyme analyses were performed at Fish Genetics Laboratory, Department of Aquaculture, Faculty of Fisheries, Kasetsart University, Bangkok, Thailand. Eight isozymes which were polymorphic in natural populations of *C. macrocephalus* were analyzed following protocols described by Na-Nakorn et al. 1998. Chemical visualization followed Morizot and Schmidt 1990. Only 5 loci, *ADH*, *GPI-1*, sMDH-1*, EST* and *PGM*, of 5 isozyme systems were heterozygous in female parents used in the study. Isozyme nomenclature followed Shaklee et al. 1990.

**Data analyses**

Data on individual genotypes were collected and analyzed statistically. Differences of genotype frequencies at each locus between families was tested by Markov chain test (dememorization=1,000; batches=100 and iterations per batch=1,000) using the GENEPOP version 3.1c (Raymond and Rousset 1998). Differences of frequencies of two homozygous classes at each locus were tested by x²-tests. A linkage disequilibrium test was performed between each loci pair using Markov chain test (dememorization=1,000; batches=100 and iterations per batch=1,000). Likewise, joint segregation between two loci was tested by comparing the observed number of individuals with the number expected if the loci were inherited independently, using a 3 x 3 contingency table (Thorgaard et al. 1983). It was not possible to test for joint segregation between *ADH* or *EST* and other loci because they were not scored in the same individuals. The proportion of heterozygotes was calculated by γ =1 – (number of homozygotes)/total (Thorgaard et al. 1983). In cases where the proportion of the two homozygous classes was significantly different, the proportion of recombinants was calculated by 1-(2 x number of most common homozygotes).

Gene-centromere distance was estimated from pooled data from undifferentiated families by (1/2) γ x 100, assuming complete interference (Thorgaard et al. 1983; Alldorf et al. 1986). Fixation index was calculated using a
The four microsatellite loci used in this study were scored in a mapping families used by Poompuang and Na-Nakorn (Poompuang and Na-Nakorn 2003) which comprised a female and 79 haploid embryos. Only Cma-2* was heterozygous in the female. Thus only this loci was mapped to a preliminary genetic map of *C. macrocephalus* previously created by Poompuang and Na-Nakorn 2003. Analyses were done following Poompuang and Na-Nakorn 2003.

### Table 1. Progeny genotypic ratio, proportion of gene-centromere recombination (y) and gene-centromere distance \([(1/2) y \times 100]\) in centimorgans (cM) at 9 loci in *C. macrocephalus*

| Locus | Family | Maternal genotype | Progeny genotypes \(^1\) | Progeny genotypes \(y\) | Gene-centromere distance (cM) |
|-------|--------|-------------------|--------------------------|--------------------------|
| GPI-1* | AQ-1 | *100/*100*27 | 47 | 5 | 0 | 0.050\(^4\) |
|       | AQ-2 | *100/*100*27 | 44 | 5 | 2 | 0.045\(^4\) |
|       | AQ-3 | *100/*100*27 | 45 | 2 | 1 | 0.022\(^4\) |
|       | AQ-4 | *100/*100*27 | 14 | 6 | 9 | 0.022\(^4\) |
|       | Total | | 136 | 12 | 3 | 0.042 |
| sMDH-1* | AQ-1 | *100/*50 | 12 | 36 | 4 | 0.600\(^4\) |
|         | AQ-2 | *100/*50 | 1 | 46 | 4 | 0.600\(^4\) |
|         | AQ-3 | *100/*50 | 13 | 27 | 8 | 0.509 |
|         | AQ-4 | *100/*50 | 7 | 22 | 0 | 0.011\(^4\) |
|         | Total | | 32 | 85 | 12 | 0.570\(^3\) |
| PGM* | AQ-1 | *100/*50 | 4 | 43 | 5 | 0.827 |
|       | AQ-2 | *100/*50 | 6 | 35 | 10 | 0.686 |
|       | AQ-3 | *100/*50 | 4 | 38 | 6 | 0.792 |
|       | AQ-4 | *100/*50 | 28 | 0 | 1 | 0.792 |
|       | Total | | 14 | 116 | 21 | 0.768 |
| ADH* | AQ-5 | *-100/*15 | 13 | 1 | 16 | 0.050 |
|       | AQ-7 | *-100/*15 | 10 | 4 | 6 | 0.200 |
|       | AQ-8 | *-100/*15 | 8 | 3 | 9 | 0.15 |
|       | AQ-10 | *-100/*15 | 5 | 5 | 9 | 0.26 |
|       | Total | | 36 | 13 | 40 | 0.146 |
| EST* | AQ-6 | *130/*100 | 0 | 20 | 0 | 1.0 |
|       | AQ-7 | *130/*100 | 0 | 20 | 0 | 1.0 |
|       | AQ-8 | *130/*100 | 0 | 20 | 0 | 1.0 |
|       | Total | | 2 | 96 | 0 | 0.979 |
| Cma-2* | AQ-1 | *117/*120 | 3 | 39 | 5 | 0.830 |
|       | AQ-2 | *117/*120 | 6 | 38 | 4 | 0.792 |
|       | AQ-3 | *117/*120 | 6 | 24 | 5 | 0.686 |
|       | AQ-4 | *123/*120 | 0 | 27 | 1 | 0.964 |
|       | Total | | 15 | 128 | 16 | 0.805 |
| Cma-3* | AQ-1 | *194/*202 | 10 | 30 | 7 | 0.640 |
|       | AQ-2 | *206/*212 | 5 | 36 | 3 | 0.640 |
|       | AQ-3 | *194/*202 | 14 | 21 | 15 | 0.420 |
|       | Total | | 24 | 51 | 22 | 0.526 |
| Cma-4* | AQ-1 | *214/*216 | 19 | 0 | 25 | 0 |
|       | AQ-2 | *214/*216 | 18 | 2 | 27 | 0.043 |
|       | AQ-3 | *214/*215 | 23 | 1 | 19 | 0.023 |
|       | Total | | 60 | 3 | 71 | 0.022 |
| Cga-3* | AQ-1 | *164/*176 | 23 | 0 | 21 | 0 |
|       | AQ-2 | *149/*202 | 26 | 0 | 21 | 0 |
|       | AQ-3 | *164/*176 | 22 | 1 | 27 | 0.02 |
|       | AQ-4 | *202/*222 | 14 | 1 | 13 | 0.036 |
|       | Total | | 85 | 2 | 82 | 0.012 |

\(^1\) 11 \& 22 are homozygotes for the first and second maternal allele respectively, and 12 are heterozygotes.

\(^2\) Family possesses differential genotypic ratio, whose data are not included in the gene-centromere estimation.

\(^3\) Data from families with different homozygote frequency are not included.

\(^4\) The proportion of recombinants was calculated by 1/(2 x number of most common homozygotes) due to differences of the frequencies of the two homozygotes.
**Results**

**Genetic variation**

Detailed results are presented in Table 1. In the first family batch, four out of five females (AQ-1,2,3,4) were heterozygous at Cma-2*, Cga-3*, sMDH-1*, PGM* and GPI-1*, whereas only three females, AQ-1, AQ-2, and AQ-3, were heterozygous at Cma-3* and Cma-4*. At every microsatellite loci, more than 2 alleles were observed: 3 alleles at Cma-2*, 4 alleles at Cma-3* and Cma-4*, and 5 alleles at Cga-3*. At all of the isozyme loci, only 2 alleles were observed.

In the second family batch which was screened for two additional isozymes, four females (AQ-5, AQ-7, AQ-8 and AQ-10) were heterozygous at ADH* while AQ-6, AQ-7, AQ-8, AQ-11 and AQ-12 were heterozygous at EST*. Only two alleles were observed at each locus. These results allowed gene-centromere distance estimation for 9 loci.

**Differences in genotype frequencies between families**

Differences in genotype frequencies between families were significant at 4 loci, Cma-3*, sMDH-1*, GPI-1* and PGM*, among which the AQ-2 family contributed to the differences occurred at Cma-3* and sMDH-1*, while the AQ-4 family contributed to the difference at PGM* and GPI-1*. Therefore, these families were not included for estimation of map-distances at the respective loci.

**Differences between homozygous classes**

Frequencies of two homozygous classes were significantly different in three families at the locus sMDH-1* and in all families at GPI-1*. The proportion of sMDH-1* 100/*100 individuals was higher than the *50/*50 individuals, likewise at GPI-1*, the proportion of *−27/*−27 individuals was significantly lower than *100/*100. The gene-centromere distances at these loci were estimated by assuming the frequency of the two homozygous classes is equal. Therefore, y is equal to one minus twice the frequency of the most common homozygotes.

**Gene-centromere map distances and joint segregation**

Gene-centromere recombination estimates (y) for each locus ranged from 0.012 to 0.979. Three loci, Cma-2*, PGM* and EST*, had recombination rates exceeding 67%, while Cma-4*, Cga-3* and GPI-1* had very low recombination rates (1.2–4.2%). The gene-centromere map distances ranged from 0.6 cM for Cga-3* to 48.9 cM for EST*. The maternal genotypes, numbers of heterozygotes and each homozygote classes, proportions of heterozygotes and gene-centromere distances are shown in Table 1. Genotype disequilibrium was observed between sMDH-1* and Cma-3* (P=0.005) in the family AQ-3. The same result was obtained using the $\chi^2$ test (Table 2).

**Linkage group observed**

We tried to detect linkage group among the loci used in this paper and AFLP loci examined former study (Poompuang and Na-Nakorn 2003). Cma-2* was placed in a linkage group.
LG21 at 27.5 cM distal to AACACT2 AFLP locus. Consequently, a centromere could be placed at 1.7 cM below the marker AACCAT20, 12.7 cM above AACACT2 (Figure 1a).

**Fixation index**

The average recombination frequency across the 9 loci was 0.430 (+0.380), thus allowing estimation of fixation index of 0.570.

**Discussion**

The alleles observed at all loci conformed to those observed in our previous studies (Na-Nakorn et al. 1998; Na-Nakorn et al. 1999). No extra alleles that could have contributed from a parental genome were detected. Together with this evidence, there were no hybrid individuals which can be easily identified from the walking catfish by the morphological appearances (Na-Nakorn 1995). These evidences confirmed successful induction of gynogenetic fish.

The gene-centromere recombination estimates for Cma-2*, PGM* and EST* exceeded the maximum expected 67% crossover rate (Thorgaard et al. 1983; Allendorf et al. 1986; Liu et al. 1992; Bastiaanssen et al. 1996) thus supporting the evidence for complete interference in C. macrocephalus genomes. This fulfilled the assumption for using the formula \((1/2) \times 100\) to estimate gene-centromere distance (Thorgaard et al. 1983; Allendorf et al. 1986). Complete interference has been observed in several fish species including rainbow trout (Thorgaard et al. 1983; Allendorf et al. 1986), ayu (Taniguchi et al. 1987; Seki et al. 1989), Nile tilapia, Oreochromis niloticus (Hussain et al. 1994), channel catfish (Liu et al. 1992) and fancy carp (Aliah and Taniguchi 2000).

Three loci, Cma-4*, Cga-3*, and GPI-1*, were closely linked to their centromeres as indicated by gene-centromere distances of 1.1, 0.6 and 2.1 cM, respectively. At present a preliminary genetic map of C. macrocephalus based on haploid mapping panels is being constructed (Poompuang and Na-Nakorn 2003). The information on these centromere markers is useful for constructing a genetic map of C. macrocephalus by consolidating the number of linkage groups to the number of chromosomes (Johnson et al. 1996; Mohideen et al. 2000). However, there were prerequisites that relationship of genetic maps obtaining from 2 types of mapping panels, haploid, and meiotic gynogens, was established (Johnson et al.1996) and enough markers evenly distributed on chromosomes were identified.

Joint segregation was tested based on the fact that if two loci were not linked they would independently segregate during meiosis II. Thus, the proportions of the four classes of double homozygotes at both loci should be equal. Joint segregation was found between sMDH-1* and Cma-3* in one out of the three families included in this study. A few linkage pairs of isozyme loci were reported in rainbow trout,
Gene-centromere distance in Clarias macrocephalus

IDH-3* and ME-2*, ADA-1* and G3P-3* (May et al. 1979), LDH-4* and ACO-2* (Allendorf et al. 1986), and IDH-2* and EST-1* (Thorgaard et al. 1983; Allendorf et al. 1986). In chum salmon, Oncorhynchus keta, the isoloci AAT-1* or 2* is linked to GPT* (Seeb and Seeb 1986).

Differences in genotypic ratios of offspring between gynogenetic families were significant at four loci. Altered gene-centromere distance was observed in zebrafish where a mutant gol-1 (b1) had a lower recombination rate than a γ-ray induced mutant [gol-1 (b7)] due to different genetic background (Streisinger et al. 1986). Such a difference was also observed in rainbow trout (Allendorf et al. 1986). The authors proposed several possible reasons; differences in rate of recombination, difference in the amount of interference, chromosomal rearrangements, differential survival of genotypes, or statistical chances. Aliah and Taniguchi 2000 reported differences in recombination rates between families of Cyprinus carpio, where they claimed translocation between acrocentric chromosomes (Robertsonian translocation) was the cause. However, they did not provide further evidence, such as rate of occurrence for the Robertsonian translocation. We were unable to explain the differential genotypic ratio among gynogenetic families of C. macrocephalus due to insufficient relevant information.

We observed significant differences between homozygous progeny types at two loci. Such differences could have been the result of differential survival of the two homozygous classes. This difference was not unexpected. As a result of gynogenesis, the chromosome segment containing the locus being studied became homozygous by descent, thus allowing expression of any recessive lethal genes that linked to the marker loci (Thompson 1983; Allendorf et al. 1986; Liu et al. 1992). In plaice, Pleuronectes platessa, Thompson 1983 also reported different frequencies between homozygous classes at sMDH-1*, GPI-1*, and PGM*. Nevertheless, such differences were not observed in gynogenetic rainbow trout (Allendorf et al. 1986), channel catfish (Liu et al. 1992), red sea bream (Sugama et al. 1990; Taniguchi 1996), and Nile tilapia (Hussain et al. 1994). Allendorf et al. 1986 suggested that tetraploid ancestry of rainbow trout might provide alternate loci for shielding effects of deleterious genes. There was no explanation suitable for failure to detect differential survival of homozygote classes in channel catfish. One might suspect the timing of sampling partially contributed to this discrepancy. If the gynogenetic offspring were collected for isozyme analysis at very young age as in the study conducted by Liu et al. 1992, it may not have allowed delayed expression of the deleterious alleles and resulted in equal survival of homozygotes. However, Liu et al. 1996 showed that there was no differential survival between two homozygotes in the 1–2 month-old and 1-year-old fish.

A few reports suggested association of GPI* loci with growth of fish (Van der Walt et al. 1993; Goudie et al. 1995), whereby it may imply that this locus possibly associates with fitness. However, attempts to prove this relatedness were unsuccessful. Van der Walt et al. 1993 claimed that they observed positive association of particular GPI* alleles with growth performance in African catfish, Clarias gariepinus, but their experimental design and statistical analyses failed to support the conclusion. Similarly, Goudie et al. 1995 proposed the relationship between growth, sex and GPI-B phenotype in channel catfish, but they made a final conclusion that no evidence supported direct involvement of the GPI-B in growth.

The fixation index of 0.57 implies that one generation of meiotic gynogenesis in C. macrocephalus increased total homozygosity by 57%. A moderate range of fixation indices has been reported from one generation of gynogenesis in different species: 0.595 in plaice (Thompson 1983), 0.440 in rainbow trout (Allendorf et al. 1986), 0.784 in common carp (Linhart et al. 1986), 0.669 in brook trout (Fujino et al. 1989b), 0.68 in red sea bream (Sugama et al. 1990), 0.53 in Nile tilapia (Hussain et al. 1994), 0.400 in channel catfish (Liu et al. 1996), and 0.678 in fancy carp (Aliah and Taniguchi 2000). All of these exceed the fixation index of 0.25 obtained from a single generation of sib-mating.
The assignment of Cma-2 enabled us to place a centromere to the linkage group LG21. The map distance of 41.7 cM from TAGCTA2 to the centromere and 40.2 cM from the centromere to Cma-2 suggested that this linkage group covered almost the whole length of a chromosome. In this paper we could not map other genes with known gene-centromere distance, especially centromere-linked genes, to the linkage groups mapped by Poompuang and Na-Nakorn 2003 due to lacking of polymorphism of these genes in the maternal fish. Therefore we would suggest mapping those genes using new mapping families so that the information on gene-centromere distances could help consolidation of families so that the information on gene-centromere distances could help consolidation of the thirty-one linkage groups to twenty-seven centromere distances. We will attempt to study gene-centromere distance of more marker loci in the genome of C. macrocephalus. This will greatly benefit gene mapping and more importantly, enhance knowledge on genomics of this species.

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Gene-centromere distance in *Clarias macrocephalus*


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*Clarias macrocephalus*の雌性発生二倍体を用いて*Clarias macrocephalus*におけるマイクロサテライト4座およびアイソサイム5座のG－Cマッピング

*Clarias macrocephalus*の雌性発生二倍体を用いてアイソサイム遺伝子座およびマイクロサテライトマーカー座のそれぞれについて動原体との間の図距離を推定した。対象としたアイソサイム遺伝子はsMDH-1*, PGM*, GPI-1*, ADH*およびEST*の5座であり、マイクロサテライトマーカーはCma-2*, Cma-3*, Cma-4*およびCga-3の4座である。PGM* EST*およびCma-2*は組み替え率が高く、動原体に対し遺伝子座があることが示唆された。また、本稿においては遺伝子座間の強い干渉が発していることが示唆された。他方、Cma-4*, Cga-3*およびGPI-1*は動原体に対し近位にあることが示唆された。また、sMDH-1*とCma-3*は連鎖していることが示唆された。固定指数(1－y)は0.570であった。