Inter-populational difference in microsatellite-centromere map distances in the loach,

Misgurnus anguillicaudatus

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Abstract Microsatellite–centromere recombination rates were estimated at 21 loci in relation to centromere of chromosomes in gynogenetic diploid lines induced from loaches of two different populations in Japan. All the microsatellite loci gave allelic segregation according to the Mendelian manner of inheritance in normal diploid families. Since loaches from Kita population in the southern area of Hokkaido Island and those from Memanbetsu population in the northern area, Hokkaido, Japan, were reported to be genetically diversified by previous genetic studies, map distances were compared between loaches from the two different populations. Three (*Mado7*, *Mac3* and *Mac49*) of five loci, which could be compared inter-populationally, gave significantly different recombination rates, i.e., map distances. The results support the presence of genetic difference between the two populations.

Keywords Clone · Comparative genomics · Cobitidae · Polyploidy · Unisexual

Introduction

In the loach *Misgurnus anguillicaudatus*, most individuals in Japanese wild populations are diploids which reproduce sexually, but a small proportion of natural clonal diploids have been found in the northern area of Hokkaido Island, Japan (Morishima et al. 2002). These natural clonal individuals generate genetically identical diploid eggs by the cytological mechanism called premeiotic endomitosis (Itono et al. 2006). Most of such eggs develop by sperm-dependent parthenogenesis, i.e., gynogenesis (Itono et al. 2007). Triploids accidentally appear in nature by incorporation of a haploid sperm nucleus of normal diploid loach into unreduced diploid egg of the clone (Morishima et al. 2002; Itono et al. 2007). Such clone-derived triploids generally produce haploid gametes by atypical reproductive system of meiotic hybridogenesis: meiosis after the preferential pairing between one genome (chromosome set) of the clone and a counterpart genome of the sperm donor as well as the elimination of unmatched one genome of the clone (Morishima et al. 2008c). This previous result suggested the presence of two distinct genomes in the clone, and its hybrid origin in the loach.

Although the loach *M. anguillicaudatus* has been recognized as a single species entity in Japan (Saitoh 1989), recent analyses on sequences of the control region of mtDNA demonstrated the presence of diversified two clades in the phylogenic tree, probably

corresponding to cryptic species which exist in *Misgurnus* loaches (Morishima et al. 2008a). One of such genetic groups inhabits in the northern area of Hokkaido Island, while the second corresponds to those in the other part of Japanese territory including the southern area of Hokkaido Island (Morishima et al. 2008a). Genetic differences between northern Hokkaido and others have also been suggested by earlier allozyme studies (Khan and Arai 2000) and recent microsatellite analysis (Arias-Rodriguez et al. 2007). Although these previous results suggest the presence of distinction in genomic or chromosome structure between genetically different groups, karyological variations have not been detected in these diploid loaches so far examined by conventional method (Arai et al. 1991b; Zhang and Arai 1999; Itono et al. 2006). Molecular cytogenetic tools to realize fluorescence *in situ* hybridization have not been used for the understanding of genomic structure on *Misgurnus* loaches chromosomes.

Comparative mapping is considered a powerful approach to clarify structure of the genome of target species, but linkage mapping is generally much laborious, expensive and time-consuming. In the *Misgurnus* loach, only first-generation linkage map including 159 microsatellite markers and one color gene is available at present (Morishima et al. 2008b). In contrast to linkage analyses, mapping genes or markers on chromosomes in relation to its centromere can be relatively easily achieved by half-tetrad analysis based on measurements of the frequency of second meiotic division segregation (*y*) in triploid and gynogenetic diploid lines induced by chromosome manipulation (Thorgaard et al. 1983). In salmonid species in

which gene-centromere (G-C) recombination rates were measured for relatively large number of allozyme loci, map distances to the centromere were reported to be well conserved in most enzyme loci with some minor differences (Guyomard 1984; Allendorf et al. 1986; Seeb and Seeb 1986; Arai et al. 1991a; Lindner et al. 2000; Matsuoka et al. 2004). While map distances were different in the two allozyme loci between odd-year and even-year populations of the pink salmon *Oncorhynchus gorbuscha,* which species shows larger year-group genetic differentiation than geographic groups (Matsuoka et al. 2004).

In the loach, map distances of allozyme genes and microsatellite markers in relation to the centromere have been estimated in artificially induced gynogenetic diploid lines (Suwa et al. 1994; Morishima et al. 2001, 2008b), but inter-populational microsatellite–centromere (M-C) map difference was not detected between a population from the southern area of Hokkaido Island and that from Hiroshima, Honshu Island. In the present study, we produced normal diploid families and gynogenetic diploid lines in loaches collected from the genetically different populations of the northern and southern area of Hokkaido Island (Khan and Arai 2000; Arias-Rodriguez et al. 2007; Morishima et al. 2008a). For comparative half-tetrad analysis, we estimated M-C map distances in microsatellite loci between these highly diversified populations of the loach to verify the presence of inter-populational genetic differentiation again by gene or microsatellite–centromere mapping approach.

Materials and methods

Fish specimens. Bisexually reproducing normal diploid loaches were collected from Memanbetsu (M) town (Present locality name: Ozora Town after municipal consolidation at 2006), northern area of Hokkaido Island and Kita (K) Village (Present locality name: Iwamizawa city), southern area of Hokkaido Island, Japan. Mature goldfish males were provided from the brood stock reared in the Nanae Fresh-Water Laboratory, Field Science Center for Northern Biosphere, Hokkaido University, Nanae town, Kameda County, Hokkaido, Japan.

Normal diploid families and gynogenetic diploid lines. Mature eggs were obtained from seven females, two from M (1M and 2M) and five from K (1K to 5K) according to Morishima et al. (2002). Sperm was collected from loach for normal fertilization and from goldfish for gynogenesis. Goldfish sperm was UV-irradiated according to Suwa et al. (1994). Eggs from each female were fertilized with UV-irradiated sperm to induce gynogenetic development and then eggs were heat-shocked at 42.0 ± 0.1°C for 2 min duration or cold-shocked at 0.0 ± 0.2°C for 32 min duration at 5 min after activation with aged tap water to duplicate chromosomes by inhibiting the second polar body release. Normal diploid families were produced from same females used for induction of gynogenesis and eggs were

fertilized with either male from M or K population. Hatched larvae were reared individually and fed three times a day with *Artemia* for 3 weeks.

Genetic analysis and microsatellite–centromere mapping. Eighteen to twenty larvae from each normal diploid family and 30 to 60 larvae from each gynogenetic diploid line were genotyped for microsatellite DNA marker loci developed by Morishima et al. (2001) and Arias-Rodriguez et al. (2007). Genomic DNA of each sample was extracted and purified according to Arai and Mukaino (1997). Microsatellite markers were amplified and analyzed according to Morishima et al. (2001) and Arias-Rodriguez et al. (2007).

Mendelian manner of segregation at each microsatellite loci was tested in normal diploid families by chi-square statistics ($P < 0.05$). M-C recombination rate, i.e., frequency of the second meiotic division segregation (y), of each locus was estimated by scoring recombinant heterozygous and non-recombinant homozygous genotypes in each gynogenetic diploid line induced from a heterozygous female. M-C map distance was estimated from y value under the assumption of complete chiasma interference suggested by Thorgaard et al. (1983). Map distance in centiMorgan (cM) is equivalent to $100(y/2)$. Theoretically equal frequencies of two homozygous genotypes in gynogenetic diploid line were tested by chi-square ($P < 0.05$). The contingency chi-square test was also made for the frequencies of heterozygous and homozygous genotypes between or among lines from each population.

Results

In normal diploid families showing genetic variation in parental fish, microsatellite alleles were segregated according to the Mendelian manner of inheritance at 21 loci examined (electronic supplementary material).

M-C recombination rates were estimated at 21 loci using at most seven gynogenetic diploid lines, comprising five lines from Kita population (1K to 5K) and two lines from Memanbetsu population (1M and 2M) (Table 1). Frequencies of the second meiotic division segregation (*y*) for *Mado1*, *Mado4* and *Mado30* loci were only estimated in 1M and/or 2M lines. Those for *Mado3, Mado6*, *Mado8*, *Mado14*, *Mado21*, *Mado27*, *Mac2*, *Mac24*, *Mac35*, *Mac37*, *Mac40*, *Mac44* and *Mac45* were exclusively assessed in gynogenetic diploid lines from females in Kita population (K1 to 5K). In these loci, map distances could not be compared between the two populations. *Mado3*, *Mado27*, and *Mac45* gave low *y* values, while *Mado14, Mado21*, *Mac40* and *Mac44* showed high *y* values (Table 1). Other loci demonstrated intermediate *y*-values (Table 1).

Recombination rates could be compared between the two populations in other *Mado7*, *Mado18*, *Mac3*, *Mac36* and *Mac49* loci (Table 1). In *Mado7* locus, no or very few recombinant progeny appeared in gynogenetic lines 1M and 2M ($y = 0$ or nearly 0), but

gynogens from the different Kita population (1K, 2K, 4K and 5K) gave *y*-values from 0.27 to 0.45 (0.39 in average).

Inter-populational differences were statistically significant. In *Mac3* locus, gynogens from females of Kita population showed $y = 0.02$ to 0.17 (average 0.10), but those from a Memanbetsu female gave $y = 0.25$. Contrastive *y* values were also observed in *Mac49* locus: $y = 0.06$ in gynogens from Kita population and $y = 0.32$ in those from Memanbetsu population. However, similar recombination rates were detected at *Mado18* ($y = 0$) and *Mac36* ($y =$ 0.38–0.48) in both two populations.

Inter-populational comparison of microsatellite–centromere map distances between Memanbetsu and Kita populations was shown in Table 2, together with the previous results by Morishima et al. (2001, 2008b). In loaches from Kita population, *Mado3*, *Mado18*, *Mado27*, *Mac2*, *Mac3, Mac45* and *Mac49* loci were concluded to be located at proximal or centromeric region of chromosomes, 0 to 5.5cM from the centromere. While *Mado14, Mado21*, *Mac40* and *Mac44* loci were located at distal or near telomeric region (46.3 to 50cM) from the centromere. These estimations in *Mac* series loci are similar to the previous estimations in loaches from Ohno population, southern area of Hokkaido Island (Morishima et al. 2001, 2008b) and Sera population, Hiroshima Prefecture, Honshu Island (Morishima et al. 2001). In loaches from Memanbetsu population, *Mado18* and *Mac36* loci gave similar map distances from the centromere with those estimated in Kita loaches. However, map distances of *Mado7*,

Mac3 and *Mac49* loci were different from those estimated in Kita loaches and in the previous studies (Morishima et al. 2001, 2008b).

Discussion

Allelic segregation at the analyzed *Mac* and *Mado* microsatellite marker loci followed the Mendelian manner of inheritance in the normal diploid loach families from the two populations, as previously reported by Morishima et al. (2001) for the *Mac* markers.

Segregation distortion was not observed in the families analyzed in the present study. Seven out of ten *Mado* loci were fixed to alleles specific to the Kita or Memanbetsu population as already reported by Arias-Rodriguez et al. (2007). Thus, the segregation in these loci could not be examined in one of the two populations, in which both female and male were homozygous. Homozygous genotypes were observed at *Mac2*, *Mac24*, *Mac35*, *Mac37*, *Mac40*, *Mac44* and *Mac45* loci in Kita population.

G-C or M-C map distance was estimated from the fraction of recombinant heterozygous half-tetrads (y) and microsatellite loci were distributed from the proximal or centromeric (y = 0) to distal or telomeric (y = 1) region on the loach chromosome, as reported in previous studies (Morishima et al. 2001, 2008b). In Kita population, *Mado14*, *Mado21*, *Mac40* and

Mac44 gave very high recombination rates (y = 0.93–1). Second meiotic division segregation frequencies larger than two-third (y = 0.67) have been noticed in gene and marker loci in previous studies in the loach (Suwa et al. 1994; Morishima et al. 2001, 2008b) and other fish species (Streisinger et al. 1986; Liu et al. 1992; Kauffman et al. 1995; Johnson et al. 1996; Nomura et al. 2006; Lahrech et al. 2007), suggesting the presence of strong positive chiasma interference (Thorgaard et al. 1983).

When compared M-C map distances of *Mac* loci, similar distances were observed between loaches from Kita population in the present study and those from Ohno and Sera populations reported in Morishima et al. (2001, 2008b) (Table 2). The comparison suggests little difference among loaches from these three localities. Recent population genetic result shows genetic similarities among loaches in these localities (Khan and Arai 2000; Morishima et al. 2008a). On the other hand, map distances at three of the five microsatellite loci, which were able to compare, gave a difference between the two populations (Table 2). *Mado7* locus was close to the centromere in loaches from Memanbetsu, while that was located at about intermediate position of a chromosome in loaches from Kita population. Tightly linked *Mac2* and *Mac3* were located at near the centromere of short arm of the same chromosome (linkage group 12) (Morishima et al. 2008b). The locus *Mac3* (and *Mac2* probably) of Kita loaches are located at centromeric region, but those of Memanbetsu loach were located at the region, about 8 to 10 cM shifted to telomere. Similar map difference was also found at the *Mac49*

locus of a different linkage group 11 (Morishima et al. 2008b): centromeric position in Kita loaches and intermediate position in Memanbetsu loach. At present, it is very difficult to verify the involvement of chromosome rearrangement such as inversion of markers in these linkage groups (i.e., chromosomes) on the inter-populational difference in M-C map distances, due to the shortage of linkage-mapped loci examined.

However, the present results support the previous population genetic conclusion based on mtDNA which showed the possible presence of two diversified groups in the *Misgurnus* loaches (Morishima et al. 2008a). The relationship among the presence of cryptic species, occurrence of clonal lineages and natural polyploids in *Misgurnus* loaches remains to be solved.

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Electronic Supplementary Material

Additional Table Genotypic segregation in 21 microsatellites Mado and Mac loci in normal diploid full-sib families from the Memanbetsu (1M–2M) and the Kita (1K–5K) populations crosses

		Parental genotype						
Locus	Family	Female	Male	Genotype of progeny : Observed				χ^2
Mado1	1M	*143/169*	*133/155*	*133/143*:4	*133/169*:5	*143/155*:7	*155/169*:4	1.20
	2M	*133/169*	*133/155*	*133/133*:5	*133/155*:6	*133/169*:6	*155/169*:3	1.20
Mado3	1K	*78/80*	*78/80*	*78/78*:2	*78/80*:14	*80/80*:3		4.85
	2K	*78/80*	*78/80*	*78/78*:5	*78/80*:14	*80/80*:1		5.25
	3K	*78/80*	*80/80*	*78/80*:8	*80/80*:12			0.80
	5K	*78/80*	*85/85*	*78/85*:11	*80/85*:9			0.20
Mado4	1M	*86/89*	*90/90*	*86/90*:13	*89/90*:7			1.80
	2M	*90/90*	*86/86*	*86/90*:20				–
Mado6	1K	*100/100*	*100/107*	*100/100*:12	*100/107*:8			0.80
	5K	*100/107*	*100/100*	*100/100*:12	*100/107*:8			0.80
	2M	*100/100*	*100/103*	*100/100*:10	*100/103*:10			0.00
Mado7	1K	*138/140*	*138/148*	*138/138*:4	*138/140*:6	*138/148*:5	*140/148:*5	0.40
	2K	*138/140*	*138/148*	138/138:12	*138/148*:8			0.80
	4K	*114/138*	*136/144*	*114/136*:6	*114/144*:5	*136/138:*4	*138/144:*5	0.40
	5K	*140/148*	*144/144*	140/144:11	*144/148*:9			0.20
	1M	*134/148*	*126/146*	*126/134*:4	*126/148*:7	*134/146:3*	*146/148:6*	2.00
	2M	*140/148*	*126/146*	*126/140*:6	*126/148*:5	*140/146:*6	*146/148:*3	1.20
Mado8	1K	*210/218*	*210/218*	*210/210*:4	*210/218*:12	*218/218*:3		2.67
	2K	*210/218*	*210/210*	*210/210*:10	*210/218*:10			0.00
	3K	*218/218*	*210/210*	*210/218*:20				–
	4K	*218/238*	*218/218*	*218/218*:10	*218/238*:10			0.00
	5K	*210/218*	*210/210*	*210/210*:10	*210/218*:10			0.00
	2M	*218/218*	*223/223*	218/223:20				–
Mado14	3K	*122/126*	*126/126*	*122/126*:9	*126/126*:11			0.20
	4K	*122/126*	*126/126*	*122/126*:10	*126/126*:10			0.00
Mado18	1K	*165/180*	*186/186*	*165/186*:11	*180/186*:9			0.20
	2K	*165/165*	*162/166*	*162/165*:11	*165/166*:8			0.47
	3K	*157/170*	*165/165*	*157/165*:10	*165/170*:10			0.00
	4K	*170/170*	*186/186*	*170/186*:20				–
	5K	*157/165*	*165/165*	*157/165*:9	*165/165:*11			0.20
	1M	*176/196*	*184/196*	*176/184*:5	*176/196*:5	*196/196*:5	*184/196*:5	0.00
	2M	*196/196*	*176/176*	*196/176*:20				–

Mado21	1K	*101/101*	*101/103*	*101/103*:12	*101/101*:7			1.31
	2K	*100/100*	*101/116*	*100/101*:9	*100/116*:11			0.20
	4K	*100/103*	*100/100*	*100/100*:9	*100/103*:11			0.20
	5K	*103/103*	*101/101*	*101/103*:20				–
	2M	*73/73*	*67/73*	*67/73*:10	*73/73*:10			0.00
Mado27	1K	*111/124*	*108/111*	*108/111*:7	*108/124*:3	*111/111*:3	*111/124*:6	2.68
	2K	*111/111*	*111/124*	*111/111*:8	*111/124*:12			0.80
	4K	*108/108*	*124/124*	*108/124*:20				–
	5K	*108/124*	*111/111*	*108/111*:8	*111/124*:12			0.80
	1M	*117/117*	*117/117*	*117/117*:20				–
	2M	*108/108*	*111/11*	*108/111*:20				–
Mado30	1M	*121/133*	*121/125*	*121/121*:7	*121/133*:4	*121/125*:3	*125/133*:6	2.00
	2M	*125/125*	*121/125*	*125/125*:20				–
Mac2	1K	*78/98*	*96/140*	*78/140*:7	*98/140*:4	*96/98*:5	*78/96*:3	1.84
	2K	*98/98*	*96/108*	*96/98*:13	*98/108*:7			1.80
	4K	*96/116*	*98/98*	*96/98*:8	*98/116*:12			0.80
	5K	*78/96*	*108/108*	*78/108*:10	*96/108*:10			0.00
Mac3	1K	*82/90*	*84/94*	*84/94*:5	*82/90*:7	*82/84*:3	*90/94*:5	1.60
	2K	*82/90*	*80/96*	*80/82*:4	*80/90*:5	*82/96*:5	*90/96*:5	0.20
	3K	*84/86*	*80/80*	*80/84*:10	*80/86*:10			0.00
	4K	*82/90*	*84/84*	*82/84*:8	*84/90*:12			0.80
	5K	*84/86*	*84/86*	*84/84*:7	*84/86*:7	*86/86*:6		0.10
	1M	*96/140*	*96/96*	*96/96*:9	*96/140*:11			0.20
Mac24	2K	*102/104*	*104/120*	*102/120*:7	*102/104*:7	*104/104*:4	*104/120*:2	3.60
	3K	*106/116*	*100/100*	*100/106*:10	*100/116*:10			0.00
	5K	*98/100*	*100/100*	*98/100*:10	*100/100*:10			0.00
Mac35	1K	*124/142*	*122/122*	*122/124*:10	*122/142*:8			0.22
	2K	*122/142*	*122/122*	*122/142*:14	*122/122*:6			3.20
	3K	*114/122*	*114/114*	*114/114*:10	*114/122*:10			0.00
	4K	*120/142*	*120/120*	*120/120*:12	*120/142*:8			0.80
	5K	*116/142*	*142/142*	*116/142:9*	*142/142:11*			0.20
Mac36	1K	*114/130*	*114/122*	*114/122*:4	*114/130*:4	*122/130*:6	*114/114*:5	0.24
	2K	*114/130*	*110/114*	*110/114*:4	*110/130*:4	*114/114*:6	*114/130*:6	0.80
	3K	*122/136*	*122/136*	*122/122*:4	*122/136*:10	*136/136*:6		0.50
	4K	*116/122*	*122/122*	*122/122*:11	*116/122*:9			0.20
	5K	*118/126*	*118/118*	*118/118*:10	*118/126*:10			0.00
	1M	*128/166*	*128/166*	*128/128*:5	*128/166*:9	*166/166*:6		0.20
Mac37	1K	*88/94*	*76/88*	*76/88*:6	*76/94*:5	*88/88*:6	*88/94*:3	1.40
	2K	*88/94*	*94/104*	*88/94*:4	*88/104*:5	*94/94*:2	*94/104*:9	5.40

	3K	*78/98*	*88/88*	*78/88*:8	*88/98*:12			0.80
	4K	*78/88*	*88/88*	*78/88*:10	*88/88*:10			0.00
Mac40	1K	*128/158*	*128/130*	*128/130*:5	*128/158*:3	*128/128*:8	*130/158*:4	3.00
	2K	*128/158*	*128/128*	*128/128*:10	*128/158*:10			0.00
	4K	*126/130*	*128/128*	*126/128*:10	*128/130*:10			0.00
Mac44	1K	*96/96*	*96/98*	*96/96*:10	*96/98*:10			0.00
	4K	*96/98*	*98/98*	*96/98*:10	*98/98*:10			0.00
	5K	*96/98*	*98/98*	*96/98*:9	*98/98*:11			0.20
Mac45	2K	*90/90*	*90/98*	*90/90*:11	*90/98*:8			0.47
	3K	*90/92*	*90/90*	*90/90*:10	*90/92*:10			0.00
	4K	*88/90*	*90/90*	*88/90*:9	*90/90*:11			0.20
	5K	*90/90*	*92/90*	*90/90*:10	*90/92*:10			0.00
Mac49	1K	*88/98*	*84/88*	*84/88*:5	*84/98*:2	*88/98*:9	*88/88*:3	6.04
	2K	*86/102*	*84/106*	*84/86*:2	*84/102*:6	*86/106*:6	*102/106*:4	2.43
	3K	*106/108*	*84/84*	*84/106*:10	*84/108*:10			0.00
	4K	*102/110*	*102/110*	*102/102*:5	*102/110*:9	*110/110*:6		0.40
	5K	*88/96*	*88/88*	*88/88*:10	*88/96*:10			0.00
	1M	*78/84*	*78/78*	*78/78*:10	*78/84*:10			0.00
	2M	*100/102*	*78/78*	*78/100*:11	*78/102*:9			0.20

Table 1 Microsatellite–centromere recombination rate (second meiotic division segregation frequency = *y*) and map distance (cM) of microsatellites loci examined in gynogenetic lines from Kita (1K–5K) and from Memanbetsu (1M–2M) populations

		Maternal	Genotype			Recombination	M–C	
Locus	Family	genotype	1/1	1/2	2/2	frequency	distance	χ^2
Mado1	1M	*143/169*	25	7	27	0.12	5.9	0.08
	2M	*133/169*	26	10	22	0.17	8.6	0.33
		Sum	*51*	*17*	*49*	*Ave 0.15*	*Ave. 7.3*	*0.68*
Mado3	1K	*78/80*	31	6	23	0.10	5.0	1.19
	2K	*78/80*	29	3	28	0.05	2.5	0.02
	3K	*78/80*	16	3	25	0.07	3.4	1.98
	5K	*78/80*	23	5	32	0.08	4.2	1.47
		Sum	*99*	*17*	*108*	*Ave. 0.08*	*Ave. 3.8*	*1.16*
Mado4	1M	*86/89*	10	44	6	0.73	36.7	1.00
Mado6	5K	*100/107*	14	24	22	0.40	20.0	1.78
Mado7	1K	*138/140*	24	22	14	0.37	18.3	2.63
	2K	*138/140*	16	26	18	0.43	21.7	0.12
	4K	*114/138*	17	9	8	0.27	13.2	3.24
	5K	*140/148*	14	27	19	0.45	22.5	0.76
		Sum	*71*	*84*	*59*	*Ave. 0.39*	*Ave. 19.6*	*3.75*
	1M	*134/148*	30	1	29	0.02	0.8	0.02
	2M	*140/148*	27	0	30	0.00	0.0	0.16
		Sum	*57*	*1*	*59*	*Ave. 0.01*	*Ave. 0.4*	*0.96*
Mado8	1K	*210/218*	23	22	15	0.36	18.3	1.68
	2K	*210/218*	15	27	18	0.45	22.5	0.27
	4K	*218/238*	17	9	8	0.26	13.2	3.24
	5K	*210/218*	25	23	12	0.38	19.2	4.57
		Sum	*80*	*81*	*53*	*Ave. 0.37*	*Ave. 18.9*	*3.22*

Mado14	3K	*122/126*	0	44	0	1.00	50.0	–
	4K	*122/126*	0	33	0	1.00	50.0	–
		Sum	*0*	*77*	*0*	*Ave. 1.00*	*Ave. 50.0*	–
Mado18	1K	*165/180*	31	0	29	0.00	0.0	0.07
	3K	*157/170*	25	0	19	0.00	0.0	0.82
	5K	*157/165*	27	0	28	0.00	0.0	0.02
		Sum	*83*	*0*	*76*	*Ave. 0.00*	*Ave. 0.0*	–
	1M	*176/196*	33	0	27	0.00	0.0	0.60
Mado21	4K	*100/103*	0	34	0	1.00	50.0	–
Mado27	1K	*111/124*	35	3	22	0.05	2.5	2.96
	5K	*108/124*	29	0	31	0.00	0.0	0.07
		Sum	*64*	*3*	*53*	*Ave. 0.03*	*Ave. 1.3*	*3.08*
Mado30	1M	*121/133*	9	33	17	0.56	28.0	2.46
Mac2	1K	*78/98*	29	5	23	0.09	4.4	0.69
	4K	*96/116*	13	4	14	0.13	6.5	0.04
	5K	*78/96*	31	7	20	0.12	6.0	2.37
		Sum	*73*	*16*	*57*	*Ave. 0.11*	*Ave. 5.5*	*0.47*
Mac3	1K	*82/90*	28	5	26	0.08	4.2	0.07
	2K	*82/90*	22	1	32	0.02	0.9	1.85
	3K	*84/86*	7	7	28	0.17	8.4	12.6*
	4K	*82/90*	13	5	13	0.16	8.1	0.00
	5K	*84/86*	21	6	30	0.11	5.3	1.59
		Sum	*91*	*24*	*129*	*Ave. 0.10*	*Ave. 4.9*	*7.74*
	1M	*96/140*	23	14	19	0.25	12.5	0.38
Mac24	2K	*102/104*	6	40	10	0.71	35.5	1.00
	3K	*106/116*	8	28	3	0.72	35.5	2.27
	5K	*98/100*	9	42	9	0.70	35.0	0.00
		Sum	*23*	*110*	*22*	*Ave. 0.71*	*Ave. 35.5*	*0.05*
Mac35	1K	*124/142*	10	37	10	0.65	32.5	0.00

	2K	*122/142*	3	51	4	0.88	44.0	0.14
	3K	*114/122*	9	23	6	0.61	30.3	0.60
	4K	*120/142*	3	18	9	0.60	30.0	3.00
	5K	*116/142*	5	43	8	0.76	38.4	0.69
		Sum	*30*	*172*	*37*	*Ave. 0.72*	*Ave. 36.0*	*14.0*
Mac36	1K	*114/130*	17	22	19	0.38	19.0	0.11
	2K	*114/130*	20	24	15	0.41	20.0	0.71
	3K	*122/136*	13	20	9	0.48	23.8	0.72
	4K	*116/122*	5	15	11	0.48	24.2	2.25
	5K	*118/126*	16	28	14	0.48	24.1	0.13
		Sum	*71*	*109*	*68*	*Ave. 0.44*	*Ave. 22.0*	*2.03*
	1M	*128/166*	14	24	20	0.42	20.7	1.06
Mac37	1K	*88/94*	18	24	18	0.40	20.0	0.00
	2K	*88/94*	17	24	19	0.40	20.0	0.11
	3K	*78/98*	10	20	12	0.48	23.8	0.18
	4K	*78/88*	20	25	15	0.42	20.8	0.71
		Sum	*65*	*93*	*64*	*Ave. 0.41*	*Ave. 20.9*	*0.74*
Mac40	1K	*128/158*	3	47	3	0.89	44.3	0.00
	2K	*128/158*	2	56	2	0.93	46.7	0.00
	4K	*126/130*	0	32	0	1.00	50.0	–
		Sum	*5*	*135*	*5*	*Ave. 0.93*	*Ave. 46.6*	*3.99*
Mac44	4K	*96/98*	0	30	0	1.00	50.0	–
	5K	*96/98*	4	53	3	0.88	44.2	0.14
		Sum	*4*	*88*	*3*	*Ave. 0.93*	*Ave. 46.3*	*3.80*
Mac45	3K	*90/92*	18	4	19	0.10	4.9	0.03
	4K	*88/90*	18	3	13	0.09	4.4	0.81
		Sum	*36*	*7*	*32*	*Ave. 0.09*	*Ave. 4.7*	*0.02*
Mac49	1K	*88/98*	26	2	29	0.04	1.8	0.16
	2K	*88/102*	28	2	28	0.03	1.7	0.00
	3K	*106/108*	23	4	17	0.09	4.5	0.90
	4K	*102/110*	12	1	19	0.03	1.6	1.58
	5K	*88/96*	32	5	23	0.08	4.2	1.47
		Sum	*121*	*14*	*116*	*Ave. 0.06*	*Ave. 2.8*	*3.22*

1M	*78/84*	13	12	27	0.23	11.5	4.90
2M	*100/102*	15	23	21	0.39	19.5	1.00
	Sum	*28*	*35*	*48*	*Ave. 0.32*	*Ave. 15.8*	*3.24*

Genotypes 1/1 and 2/2 indicate non-recombinant homozygotes and 1/2 indicates recombinant heterozygotes

Stand χ^2value at each gynogenetic line denotes that between the two homozygotes

Italic χ^2 value at *sum* line denotes that between or among gynogens from the same population

asterisk significant difference at $P < 0.05$

Ave. averaged value

cM centiMorgans.

Table 2 Comparison of microsatellites-centromere map distances estimated in gynogenetic families produced from the Kita population, Memanbetsu population and the ones from previous works

		Microsatellite-centromere map distance (cM) (*n*)			
		Present study		Morishima et al.	
Locus	LG	Kita	Memanbetsu	2001*	2008b**
Mado1			7.3 (2)		
Mado3		3.8 (4)			
Mado4			36.7 (1)		
Mado6		20.0 (1)			
Mado7		19.6 (4)[a]	0.4 (2)[b]		
Mado8		18.9 (2)			
Mado14		50.0 (2)			
Mado18		0.0 (4)[a]	0.0 (1)[a]		
Mado21		50.0 (1)			
Mado27		1.3 (2)			
Mado30			28.0 (1)		
Mac2	LG12	5.5 (3)[a]		4.0 (2)[a]	1.3 (1)[a]
Mac3	LG12	4.9 (5)[a]	12.5 (1)[b]	3.0 (3)[a]	
Mac24		35.5 (3)[a]		33.0 (1)[a]	
Mac35		36.0 (5)[a]		45.0 (2)[a]	
Mac36	LG3	22.0 (5)[a]	20.7 (1)[a]	23.0 (2)[a]	23.8 (1)[a]
Mac37	LG10	20.9 (4)[a]		24.0 (3)[a]	21.3 (1)[a]
Mac40	LG12	46.6 (3)[a]		47.0 (2)[a]	47.6 (1)[a]
Mac44		46.3 (2)[a]		46.0 (1)[a]	
Mac45	LG10	4.7 (2)[a]		6.0 (2)[a]	
Mac49	LG11	2.8 (5)[a]	15.8 (2)[b]	3.0 (2)[a]	7.3 (1)[a]

LG linkage group (Morishima et al. 2008b), *cM* centiMorgan, *n* number of gynogenetic lines analyzed

*2001** based on genotyped data in gynogenetic lines produced from Ohno population in the southern area, Hokkaido, and from Sera population in Hiroshima Prefecture, Honshu island, described in Morishima et al. (2001)

*2008b*** based on row genotyped data in gynogenetic line produced from Ohno populaiton in Hokkaido provided by the authors of Morishima et al. (2008b)

Different *superscript letters* mean significant difference (χ^2 test, $P < 0.05$)