Title	Genetic verification of induced gynogenesis and microsatellite-centromere mapping in the barfin flounder, <i>Verasper moseri</i>
Author(s)	Lahrech, Zineb; Kishioka, Chiharu; Morishima, Kagayaki; Mori, Tatsunari; Saito, Setsuo; Arai, Katsutoshi
Citation	Aquaculture, 272(Supplement 1), S115-S124 https://doi.org/10.1016/j.aquaculture.2007.08.005
Issue Date	2007
Doc URL	http://hdl.handle.net/2115/35216
Type	article (author version)
File Information	arai-123.pdf

[Instructions for use](#)

ISGA921 Revised Version

**Genetic verification of induced gynogenesis and
microsatellite-centromere mapping in the barfin flounder
*Verasper moseri***

**Zineb Lahrech ^{a*}, Chiharu Kishioka ^a, Kagayaki Morishima ^a, Tatsunari
Mori ^b, Setsuo Saito ^c, Katsutoshi Arai ^a**

^aGraduate School of Fisheries Sciences, Hokkaido University, Hakodate,
Hokkaido 041-8611, Japan.

^bHokkaido Central Fisheries Experiment Station, Yoichi, Hokkaido 041-8555,
Japan.

^cHokkaido Mariculture Fisheries Experiment Station, Muroran, Hokkaido
051-0013, Japan.

*Corresponding author

Email address: barid006@fish.hokudai.ac.jp

Abstract

Primer sets were newly developed for 34 polymorphic microsatellite loci in the barfin flounder *Verasper moseri*. Mendelian inheritance was confirmed by examining the genotypic segregation in 2 normal diploid full-sib families. All the 34 loci showed genotypic segregation according to the Mendelian manner of inheritance; in some cases, null alleles were assumed. The genotypes at 27 loci were also examined in 4 meiotic gynogenetic diploid lines produced by fertilizing eggs with UV-irradiated sperm, followed by inhibition of the second meiotic division by cold shock. The absence of paternal alleles verified the success of gynogenetic development in all 4 meiotic gynogenetic diploid lines; the proportion of heterozygous progeny of a heterozygous mother, i.e., the frequency of second division segregation (y), was used to estimate the map distance of each microsatellite locus in relation to the centromere. Marker-centromere distances were estimated to be in the range of 0 to approximately 50 centiMorgan (cM) under the assumption of complete interference. Using 8 diagnostic loci located at the telomeric region of the chromosome, complete homozygosity was confirmed in 1 mitotic gynogenetic diploid line that was produced by suppressing the first cleavage via hydrostatic pressure shock.

Keywords: *Verasper moseri*, microsatellite, gynogenesis, genetic map, half-tetrad analysis.

1. Introduction

The barfin flounder *Verasper moseri* is a large flatfish species that inhabits cold sea basins near the east coast of Hokkaido, Japan. This species is a promising candidate for aquaculture and stocking due to its high commercial value and stable growth in cold water. Similar to the growth of other flatfishes, the barfin flounder exhibits sexually dimorphic growth, and the females grow to adult size faster than males (Mori et al., 1999). Thus, sex manipulation is required in order to achieve an all-female population, especially in aquaculture. The induction of gynogenetic diploids is considered a useful technique to identify the sex determination system in the barfin flounder and to generate an all-female population in a male heterogametic species. Meiotic gynogenetic diploids can be produced by inhibiting the extrusion of the second polar body, while completely homozygous mitotic gynogenetic diploids are produced by the inhibition of the first cleavage after the activation of eggs with genetically inert UV-irradiated sperm.

In order to produce an all-female population and for future cloning of the barfin flounder, meiotic and mitotic gynogenetic diploids have been experimentally produced after the optimization of treating conditions to induce gynogenesis and duplicate the chromosomes (Mori et al., 2004). In other species, gynogenesis is usually induced using UV-irradiated sperm of a different species in order to eliminate sporadic or occasionally appearing diploids by inviable hybridization (Arai, 2001). However, in barfin flounders, meiotic gynogenesis has been induced using irradiated sperm from the same species because it is difficult to collect heterospecific sperm during the spawning season (Mori et al., 2004). Thus, the possible contamination of the

putative gynogenetic progeny with sporadic diploids due to insufficient genetic inactivation of the irradiated sperm cannot be ruled out. Genetic verification of gynogenesis, i.e., all-female inheritance, is an important step in chromosome manipulation in order to evaluate the success of induction.

At the level of homozygosity, meiotic and mitotic gynogenesis differ genetically (Arai, 2001; Nagy and Csani, 1984; Palti et al., 2002). Complete homozygosity can be achieved by mitotic gynogenesis in a single generation. Thus, mitotic gynogenesis is an effective technique for producing inbred lines of fish in a substantially shorter time. Such completely homozygous fish that spawn genetically uniform eggs are indispensable for cloning via a second cycle of gynogenesis. However, contamination by meiotic gynogenetic diploids due to the spontaneous inhibition of the release of the second polar body should be eliminated (Arai, 2001). Therefore, precise separation of the 2 kinds of gynogenetic diploids is necessary for successful cloning of a target aquaculture species. In the meiotic gynogenetic diploids of a heterozygous mother, the locus at the distal portion of the chromosome is considered heterozygous because of its high gene or marker-centromere recombination rate, as reported for other fish species (Thorgaard et al., 1983; Guyomard, 1984; Estoup et al., 1993; Kauffman et al., 1995; Lindner et al., 2000; Matsuoka et al., 2004). Thus, putative mitotic gynogens must be identified by their complete homozygosity at such diagnostic markers (Suwa et al., 1994; Morishima et al., 2001; Ezaz et al., 2004).

Codominant microsatellite markers are superior for use in parentage studies because of their numerous polymorphisms, ubiquitous distribution in the genome, and simple sampling and preservation requirements (Ferguson

et al., 1995). In the present study, new microsatellite markers were developed, and their Mendelian inheritance was examined by observing genotypic segregation in 2 normal diploid full-sib families generated by normal fertilization. We used 27 microsatellite markers to confirm the success of gynogenetic development in 4 meiotic gynogenetic diploid lines. The microsatellite-centromere map distance for a polymorphic locus was estimated from the frequency of second division segregation (y) as the proportion of heterozygous meiotic gynogenetic diploid progeny of a heterozygous mother. Microsatellite loci with high marker-centromere recombination frequencies, located in the telomeric region of chromosomes, were then used to verify complete homozygosity of the mitotic gynogenetic diploids.

2. Materials and methods

2.1. Induction of gynogenetic fish

A barfin flounder broodstock was transported from the Hokkaido Institute of Mariculture, Shikabe (reorganized in 2006 to the Hokkaido Mariculture Fisheries Experiment Station, Muroran), to the Hokkaido Central Fisheries Experiment Station, Yoichi, and was reared in 4-kL tanks. Eggs and sperms were obtained from 5 different females and males (Table 1), respectively. In this study, the parental fishes and their progenies from 2 normal diploid full-sib families (ND-1 and ND-2), 4 meiotic gynogenetic diploid lines (MEI-1, MEI-2, MEI-3, and MEI-4), and 1 mitotic gynogenetic diploid line (MIT-1) were used for the genetic analyses. Meiotic gynogenetic diploids were obtained by fertilizing the eggs with UV-irradiated sperm

(40–45 mJ/cm²) and subsequent cold shock (–1.5 °C) for a duration of 70 min at 7 min after insemination (Mori et al., 2004). The same set of parents (F1 and M1) were used to produce the normal diploid full-sib family ND-1 and the meiotic gynogenetic line MEI-1. The female F2 and male M2 parents were used to produce the full-sib family ND-2 and the meiotic gynogenetic line MEI-2. The meiotic gynogenetic lines MEI-3 (parents: F3 and M3) and MEI-4 (parents: F4 and M4) comprised only meiotic gynogenetic diploids, and no counterpart normal diploids were produced. The mitotic gynogenetic diploid line MIT-1 was produced from F5 and M5 by inhibiting the first cleavage via hydrostatic pressure treatment (650 kg/cm²) for a duration of 6 min at 180–240 min after fertilization (Mori et al., 2004).

2.2. Isolation of microsatellite markers

Diploid barfin flounders cultured at the Hokkaido Central Fisheries Experiment Station were the source of DNA for the isolation of microsatellite regions. DNA was isolated and purified using the routine phenol-chloroform procedure. Microsatellite regions were isolated in a manner similar to that used for the loach *Misgurnus anguillicaudatus* (Morishima et al., 2001). Forward and reverse primers were designed based on the unique regions flanking each microsatellite repeat. Sequence data were deposited in DDBJ, and the accession numbers are shown in Table 2.

2.3. Microsatellite genotyping

DNA was extracted from the blood or muscle and purified by the phenol-chloroform method. The DNA samples were adjusted to a

concentration of 100 ng/ μ L and used for PCR. Microsatellite genotyping was performed using an automated sequencer and M13-tailed primers (Zhou et al., 2002). The reaction mixture (10 μ l) used for the amplifications contained 100 ng template DNA, 40 μ M dNTPs, 0.3 pM M13-tailed forward primer, 3.0 pM reverse primer, 3.0 pM fluorescence-labeled M13 primer, and 0.025 U *Taq* polymerase (TaKaRa, Otsu, Japan), and the amplification conditions were as follows: denaturation for 1 min at 94 °C; 30–35 cycles of denaturation for 15 s at 94 °C, annealing for 15 s at 56 °C, and extension for 30 s at 72 °C; followed by final extension for 1 h at 72 °C. Following PCR, 1 μ l of each product and 0.1 μ l of LIZ size standard (Gene Scan 500 LIZ size standard, Applied Biosystems, Foster City, CA, USA) were added to 10 μ l of HiDi Formamide for electrophoresis on an ABI PRISM 3130 Genetic Analyzer (Applied Biosystems). Electrophoretograms were analyzed using the Gene Mapper 3.7 software (Applied Biosystems). The Mendelian inheritance at each locus was verified by observing the genotypes of the parent fish and their progenies in the 2 full-sib families ND-1 and ND-2, and it was statistically confirmed using the chi-square (X^2) test ($\alpha = 0.05$). The significance level for multiple tests was adjusted to confirm the overall statistical significance ($\alpha = 0.05/n$; n = number of tests performed) (Cooper, 1968). The microsatellite loci in which the paternal alleles could be distinguished from the maternal ones were useful for the genetic confirmation of successful meiotic gynogenesis. For microsatellite-centromere mapping, microsatellite loci showing heterozygosity with regard to the female parent were screened in the 4 meiotic gynogenetic lines (MEI-1–MEI-4).

2.4. Microsatellite-centromere mapping

The microsatellite-centromere (M-C) recombination rate (frequency of second meiotic division segregation = y) was estimated from the proportion of recombinant heterozygous genotypes in the half-tetrad meiotic gynogenetic progeny of the heterozygous mother at a specific locus. Assuming complete chiasma interference (only a single cross-over event between non-sister chromatids), the gene (marker)-centromere distance in centiMorgans (cM) may be obtained as $100(y/2)$, where y is the percentage of heterozygous gynogenetic diploid progeny observed in the total number of samples (Thorgaard et al., 1983.)

3. Results

3.1. Development of microsatellite markers

A total of 3290 colonies from the genomic library were screened by hybridization with the probe (GT)₂₅. Consequently, a positive signal was obtained from 172 clones, and 124 of them were sequenced. These clones contained at least one (GT)_n/(CA)_n repeat. However, many such sequences were not used due to the proximity of the microsatellite to the cloning site, degenerate repetitive sequences in one of the flanking regions, extremely small or large microsatellites, and/or unreadable sequences. Finally, specific primers were designed for 34 polymorphic microsatellite regions at a temperature of 56 °C, and fragments of 100–400 base pairs were amplified. Table 2 shows the core sequences, forward and reverse primer sequences, annealing temperatures, number and size of alleles, and the DDBJ accession

numbers of these loci.

3.2. Verification of Mendelian segregation

Genotypic segregation was examined at 34 microsatellite loci in both full-sib families (Table 3). Among these, 30 exhibited good conformation with Mendelian segregation. For example, in the full-sib family ND-2, a cross between the female (152/154) and male (150/154) at the *Vemos1* locus produced 4 genotypes (152/150, 152/154, 150/154, and 154/154) in the progeny, and the genotypic frequencies were in good accordance with the expected ratio (1:1:1:1); this indicated Mendelian inheritance. Significant deviation from the expected Mendelian ratio was detected at the *Vemos1*, *Vemos13*, *Vemos26*, and *Vemos31* loci in ND-1 or ND-2 (χ^2 test, $\alpha = 0.05$). However, after multiple test corrections ($\alpha/30$), this deviation was not significant.

At *Vemos44* and *Vemos66*, inconsistent genotypic segregations were observed between the parents and progenies in ND-1. The observed parental genotypes at *Vemos44* in ND-1 were 262/262 in the female and 262/264 in the male. Therefore, the genotypic frequencies in the progeny were expected to be equal between these genotypes. However, in addition to these 2 expected genotypes, genotype 264/264 was also observed in the progeny. This result was explained by the involvement of a null allele in the female genotype (262/null). By considering this null allele, the genotypic frequencies fit well to the Mendelian inheritance model (Table 3). A similar distortion observed at *Vemos66* in ND-1 was also explained by the assumption of a null allele in the male parent (Table 3).

3.3. Verification of meiotic gynogenesis

In order to examine all-female inheritance in the gynogenetic diploid lines, 27 loci in which the paternally derived alleles were distinguished from the maternally derived ones were selected. The lack of contribution by the paternal alleles was confirmed in all the offspring from all 4 meiotic gynogenetic diploid lines examined in the present study. Thus, successful meiotic gynogenesis was achieved at least in the 4 meiotic gynogenetic lines examined (Table 4).

3.4. *M-C map distance*

The proportions of the heterozygous genotypes at the 27 microsatellite loci in the 4 meiotic gynogenetic diploid lines (MEI-1, MEI-2, MEI-3, and MEI-4) are given in Table 4. Unequal frequencies of the 2 homozygous classes were significant in 6 loci in the four meiotic gynogenetic diploid lines analyzed (Table 4; *Vemos6* in MEI-3, *Vemos11* in MEI-1, *Vemos29* in MEI-1, *Vemos39* in MEI-2, *Vemos60* in MEI-1, and *Vemos62* in MEI-4). The deviation from the equal number of homozygotes remained significant at *Vemos11*, *Vemos39*, and *Vemos60*, although multiple test corrections were considered ($n = 30$; n , number of tests performed). We also tested for homogeneity in the proportion of heterozygotes between the gynogenetic lines at each locus based on the contingency χ^2 analysis. *Vemos43* ($\chi^2 = 4.83$; $df = 1$), *Vemos57* ($\chi^2 = 4.31$; $df = 1$), and *Vemos76* ($\chi^2 = 10.42$; $df = 1$) showed significant differences between the gynogenetic diploid lines (Table 4). However, none of these differences were significant when the number of tests

($n = 19$) was considered. Thus, pooled results from different families were used to estimate the recombination frequency (y). The M-C recombination frequencies for the 27 loci ranged from $y = 0$ at *Vemos6* and *Vemos29* to $y = 0.99$ at *Vemos68*. Therefore, the marker-centromere map distance was estimated to be between 0 and 49.5 cM in the microsatellite loci of the barfin flounder. Of the 27 loci, 14 were estimated to be located in the telomeric region; 10, in the centromeric region; and 3, in intermediate region of the chromosome.

3.5. Verification of mitotic gynogenesis

Vemos1, *Vemos10*, *Vemos18*, *Vemos25*, *Vemos26*, *Vemos42*, *Vemos44*, *Vemos47*, *Vemos57*, *Vemos65*, *Vemos66*, *Vemos68*, and *Vemos76* were used as diagnostic loci because they showed a very high M-C recombination rate, and they were selected to identify mitotic gynogenetic diploids produced by the inhibition of the first cleavage. Putative mitotic gynogenetic progeny were completely homozygous at all the 8 diagnostic loci (*Vemos10*, *Vemos25*, *Vemos26*, *Vemos42*, *Vemos57*, *Vemos65*, *Vemos66*, and *Vemos68*), in which the mother fish showed heterozygosity (Table 5).

4. Discussion

4.1. Polymorphism and inheritance

The 34 microsatellite loci analyzed were polymorphic because 2 to 10 alleles per locus were detected in the 9 barfin flounder parents used in this study. Genetic analyses of the control crosses of the 2 normal diploid full-sib families ND-1 and ND-2 indicated that genotypic segregation at all 34 loci

conformed to Mendelian expectations. Although the allelic segregation at *Vemos11*, *Vemos39*, and *Vemos60* in the control normal diploid full-sib family (Table 3) conformed to Mendelian expectations, the unequal frequencies of 2 homozygotes were observed in 1 of the 4 meiotic gynogenetic diploid lines even after multiple test corrections (Table 4). The aberrant segregation in these loci might have resulted from the reduced viability of homozygous individuals, probably due to the unmasking of a recessive lethal or semi-lethal gene tightly linked to a specific locus.

From the analyses of the normal diploid full-sib families, the presence of null alleles was considered at the *Vemos44* and *Vemos66* loci based on the fact that the genotypic segregation was well explained by this assumption. The null alleles detected in the present study might have resulted from mutations such as substitutions, insertions, or deletions in one or both priming sites, thus preventing the binding of DNA strands and oligoprimers, as suggested by Callen et al. (1993).

4.2. Confirmation of meiotic gynogenetic development

Meiotic gynogenesis is generally easier to induce than mitotic gynogenesis, and it has been widely used to study sex determination and manipulation (Arai, 2001). If the male heterogametic (XX female-XY male) sex determination system is involved, the gynogenetic diploid progeny will be all female because there is no contribution of the sperm with the Y chromosome. Based on the sex ratio in gynogenetic diploid progeny, the involvement of male heterogamety has been suggested in the Japanese flounder (Yamamoto, 1999), marbled sole (Kakimoto et al., 1994), and other fish species (see review Pandian et al., 1998; Felip et al., 2001; Arai, 2001). In

contrast, if the female heterogamety (ZW female-ZZ male) system is involved, the gynogenetic diploids will exhibit various proportions of male (ZZ), super female (WW), and sometimes female (ZW) progeny depending on the cross-over or recombination between the centromeres and the sex-determining element. Based on the sex ratios in gynogenetic diploid progeny, the female heterogametic sex model has been strongly suggested in the rosy bitterling (Kawamura et al., 1998) and some species of sturgeons (Van Eenennaam et al., 1999; Omoto et al., 2005; Flynn et al., 2006).

To elucidate sex determination, complete success of gynogenetic development is required because sporadic diploids, due to the inadequate inactivation of sperm, will contaminate the gynogenetic lines and cause a deviation from the true sex ratio. In particular, in the barfin flounder, sex was reported to fluctuate depending on the water temperature at the stage of gonadal differentiation, and such spontaneous sex reversal from genetic females to physiological males is normally inhibited by rearing the fish at 14 °C during the critical period (Goto et al., 1999). The microsatellite analyses showed no genetic contribution of the paternal parent in all the induced gynogenetic diploid progeny examined in this study. The sex ratio in genetically verified gynogenetic diploids must be examined in order to identify an accurate temperature-dependent sex determination system in the barfin flounder.

4.3. Mapping of microsatellite loci in relation to the centromere

Using the meiotic gynogenetic diploids, the recombination rates of the microsatellite loci were estimated in relation to the centromere, and they

were found to range from $y = 0$ to 0.99. At a locus, the maximum recombination frequency in relation to the centromere is theoretically expected to be 0.667 (2/3) if there is no chiasma interference (Perkins, 1962). However, the recombination rates of 25 loci were higher than the theoretical value (Table 4). This strongly suggests the existence of positive chiasma interference after a single chiasma in the barfin flounder. Such a complete interference has been also reported in other fish species (Thorgaard et al., 1983; Guyomard, 1984; Arai et al., 1991; Liu et al., 1992; Estoup et al., 1993; Suwa et al., 1994; Kauffman et al., 1995; Lindner et al., 1999; Morishima et al., 2001; Matsuoka et al., 2004). Thus, in the barfin flounder, the M-C map distances varied between 0 and 49.5 cM. These mapping data are useful to differentiate the type of gynogenetic diploids for cloning using the chromosome manipulation technique, as described in the next section. In addition, gene (marker)-centromere mapping data are also informative for the determination of the centromere location in a linkage group (Johnson et al., 1996; Sakamoto et al., 2000).

4.4. Complete homozygosity of mitotic gynogenetic diploids

Mitotic gynogenesis is the most important chromosome manipulation technique because it produces a completely homozygous generation that is indispensable for the generation of a clone lineage (Arai, 2001). In the present study, all the mitotic gynogenetic progeny were confirmed to be genetically homozygous at the diagnostic loci, which yielded high recombination rates in relation to the centromere. Thus, this result indicated the successful induction of mitotic gynogenesis in the barfin flounder. These

completely homozygous gynogenetic progeny will be available as mother fish for the production of cloned barfin flounders by the second cycle of gynogenesis in the next generation. Cloned fish have been successfully produced in Japan, for example, the fresh water fish species *ayu Plecoglossus altivelis* (Han et al., 1991) and the amago salmon *Oncorhynchus rhodurus* (Kobayashi et al., 1994) as well as the marine fish species the Japanese flounder *Paralichthys olivaceus* (Yamamoto, 1999) and the red sea bream *Pagrus major* (Kato et al., 2001). Thus, cloning the barfin flounder in the near future appears promising.

Acknowledgments

This study was supported in part by a Grant-in-Aid for the 21st Century COE (Center Of Excellence) Program from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan and the Hokusui Society Foundation..

References

- Arai, K., 2001. Genetic improvement of aquaculture finfish species by chromosome manipulation techniques in Japan. *Aquaculture* 197, 205–228.
- Arai, K., Fujino, K., Sei, N., Chiba, T., Kawamura, M., 1991. Estimating rate of gene-centromere recombination at 11 isozyme loci in the *Salvelinus* species. *Nippon Suisan Gakkaishi* 57, 1043–1055.
- Callen, D.F., Thompson, A.R., Shen, Y., Phillips, H.A., Richard, R.I., Mulley, J.C., 1993. Incidence and origin of null alleles in the (AC)_n microsatellite

- markers. *Am. J. Hum Genet.* 52, 922–927.
- Cooper, D.W., 1968. The significance level in multiple tests made simultaneously. *Heredity* 23, 614–617.
- Estoup, A., Presa, P., Krieg, F., Vaiman, D., Guyonmard, R., 1993. (CT)_n and (GT)_n microsatellites: a new class of genetic markers for *Salmo trutta* L.(brown trout). *Heredity* 71, 488–496.
- Ezaz, M.T., Sayeed, S., McAndrew, B.J., Penman, D.J., 2004. Use of microsatellite loci and AFLP markers to verify gynogenesis and clonal lines in Nile tilapia *Oreochromis niloticus* L. *Aquaculture Res.* 35, 1472–1481.
- Felip, A., Zanuy, S., Carrillo, M., Piferrer, F., 2001. Induction of triploidy and gynogenesis in teleost fish with emphasis on marine species. *Genetica* 111, 175–195.
- Ferguson, A.A., Taggart, J.B., Prodohl, P.A., McMeel, O., Thompson, C., Stone, C., McGinnity, P., Hynes, R.A., 1995. The application of molecular markers to the study and conservation of fish populations, with special reference to *Salmo*. *J. Fish. Biol.* 47, 103–126.
- Flynn, S.R., Matsuoka, M., Reith, M., Martin-Robichaud, D.J., Benfey, T.J., 2006. Gynogenesis and sex determination in shortnose sturgeon, *Acipenser brevirostrum* Lesuere. *Aquaculture* 253, 721–727.
- Goto, R., Mori, T., Kawamata, K., Matsubata, T., Mizumo, S., Adachi, S., Yamauchi, K., 1999. Effects of temperature on gonadal sex determination in barfin flounder *Verasper moseri*. *Fish. Sci.* 65, 884–887.
- Guyomard, R., 1984. High level of residual heterozygosity in gynogenetic rainbow trout, *Salmo gairdneri*, Richardson. *Theor. Appl. Genet.* 67,

307–316.

- Han, H.S., Taniguchi, N., Tsujimura, A., 1991. Production of clonal ayu by chromosome manipulation and confirmation by isosyme marker and tissue grafting. *Nippon Suisan Gakkaishi* 57, 825–832.
- Kakimoto, Y., Aida, S., Arai, K., Suzuki, R., 1994. Induction of gynogenetic diploids by temperature and pressure treatments and sex reversal by immersion in methyltestosterone in marbled sole *Limanda yokohamae*. *J. Fac. Appl. Bio. Sci., Hiroshima Univ.* 33, 113–124.
- Kato, K., Hayashi, R., Yuasa, D., Yamamoto, S., Miyashita, S., Murata, O., Kumai, H., 2001. Production of cloned red sea bream, *Pagrus major*, by chromosome manipulation. *Aquaculture* 207, 19–27.
- Kauffman, E.J., Gestl, E.E., Kim, D.J., Walker, C., Hite, J.M., Yan, G., Rogan, P.K., Johnson, S.L., Cheng, K.C., 1995. Microsatellite-centromere mapping in the zebrafish (*Danio rerio*). *Genomics* 30, 337–341.
- Kawamura, K., 1998. Sex determination system of the rosy bitterling, *Rhodeus ocellatus ocellatus*. *Environ. Biol. Fishes.* 52, 251–260.
- Kobayashi, T., Ide, A., Hiasa, T., Fushiki, S., Ueno, K., 1994. Production of cloned amago salmon *Oncorhynchus rhodurus*. *Fish. Sci.* 60, 275–281.
- Linder, K.R., Seeb, J.E., Habicht, C., Knudsen, K.L., Kretschmer, E., Reedy, D.J., Spruell, P., Allendorf, F.W., 2000. Gene-centromere mapping of 312 loci in pink salmon by half-tetrad analysis. *Genome* 43, 538–549.
- Liu, Q., Goudie, C.A., Simco, B.A., Davis K.B., Morizot, D.C., Gene-centromere mapping of six enzyme loci in gynogenetic channel catfish. *Heredity* 83, 245–248.
- Matsuoka, M.P., Gharrett, A.J., Wimot, R.L., Smoker, W.W., 2004.

- Gene-centromere distances of allozyme loci in even- and odd-year pink salmon, (*Oncorhynchus gorbuscha*). *Genetica* 121, 1–11.
- Mori, T., Saito, S., Kayaba, T., 1999. Differences in growth rate among sexes of barfin flounder *Verasper moseri*. *Sci. Rep. Hokkaido. Fish. Exp. Sta.* 56, 137–141.
- Mori, T., Saito, S., Kishioka, C., Arai, K., 2004. Induction of triploids and gynogenetic diploids in barfin flounder *Verasper moseri*. *Nippon Suisan Gakkaishi* 70, 145–151.
- Morishima, K., Nakayama, I., Arai, K., 2001. Microsatellite-centromere mapping in the loach, *Misgurnus anguillicaudatus*. *Genetica* 111, 59–69.
- Nagy, A., Csanyi, V., 1984. A new breeding system using gynogenesis and sex reversal for fast inbreeding in carps. *Theor. Appl. Genet.* 67, 485–490.
- Omoto, N., Maebayashi, M., Adachi, S., Arai, K., Yamauchi, K., 2005. Sex ratios of triploids and gynogenetic diploids induced in the hybrid sturgeon, the bester (*Huso huso* female $\times$ *Acipenser ruthenus* male). *Aquaculture* 245, 39–47.
- Palti, Y., Shirak, A., Cnaani, A., Hulata, G., Avtalion, R.R., Ron, M., 2002. Detection of genes with deleterious alleles in an inbred line of tilapia (*Oreochromis aureus*). *Aquaculture* 206, 151–164.
- Pandian, T.J., Koteeswaran, R., 1998. Ploidy induction and sex control in fish. *Hydrobiologia* 384, 167–243.
- Perkins, D.D., 1962. Crossing over and interference in a multiply marked chromosome arm of neurospora. *Genetics* 47, 1253–1274.
- Sakamoto, T., Danzmann, R.G., Gharbi, K., Howard, P., Ozaki, A., Khoo, S.K., Woran, R.A., Okamoto, N., Ferguson, M.M., Holm, L.L., Guyomard, R.,

- Hoyheim, B., 2000. A microsatellite linkage map of rainbow trout (*Oncorhynchus mykiss*) characterized by large sex-specific differences in recombination rates. *Genetics* 155, 1331–1345.
- Suwa, M., Arai, K., Suzuki, R., 1994. Suppression of the first cleavage and cytogenetic studies on the gynogenetic loach. *Fish. Sci.* 60, 673–681.
- Thorgaard, G.H., Allendorf, F.W., Knudsen, K.L., 1983. Gene-centromere mapping in rainbow trout: high interference over long map distance. *Genetics* 103, 771–783.
- Van Eenennaam, A.L., Van Eenennaam, J.P., Medrano, J.F., Doroshov, S.I., 1999. Evidence of female heterogametic genetic sex determination in white sturgeon. *J. Hered.* 90, 231–233.
- Yamamoto, E., 1999. Studies on sex manipulation and production of cloned populations in hirame, *Paralichthys olivaceus*. *Aquaculture* 173, 235–246.
- Zhou, Y., Bui, T., Auckland, L.D., Williams, C.G., 2002. Direct fluorescent primers are superior to M13-tailed primers for *Pinus taeda* microsatellites. *Biotechniques* 32, 46–52.

Table1. Full-sib families and gynogenetic diploid lines analyzed in the present study.

Family/ Line	Abbreviation	Parental fish		No.of progeny
		Female	Male	
Normal diploid full-sib family	ND-1	F1	M1	48
	ND-2	F2	M2	30
Meiotic gynogenetic diploid line	MEI-1	F1	M1	40
	MEI-2	F2	M2	40
	MEI-3	F3	M3	40
	MEI-4	F4	M4 ^a	40
Mitotic gynogenetic diploid line	MIT-1	F5	M5	10

^a = unknown due to the loss of sample.

Table2. Core sequences, forward and reverse primer sequences, annealing temperatures, number and size of allele, allele sizes and DDBJ accession numbers of 34 microsatellite regions newly developed

Microsatellite locus	Core sequence	Primer sets (5'-3') (F: forward;R: reverse)	Annealing temperature ()	Number of alleles ^a	Allele size(bp)	DDBJ access number
<i>Vemos1</i>	(GT) ₇ GA(GT) ₁₂	F:TAAATGTGCTGTAGGGTGGGA R:TTGGACCACTGACTCATCTG	56	4	138-154	AB237644
<i>Vemos4</i>	(GT) ₁₂	F:GGAGCACCACATTCTTCTCA R:CTCCCAGCCAAATGCACTAA	56	8	159-199	AB237645
<i>Vemos6</i>	(TG) ₃₈	F:CGTGATACTGTGCTGATTGC R:CTTTGGTAGTTCACTCTCCC	56	7	238-254	AB237646
<i>Vemos7</i>	(GT) ₁₂	F:TATCTGCCAAACCCCCACT R:CCAGCACATTTGCGACAGCT	56	2	214-216	AB237647
<i>Vemos10</i>	(TG) ₁₈	F:CGGGCATCCGTGTGATATTA R:AGCTGTTGGTAITTCGCTCC	56	5	179-196	AB237648
<i>Vemos11</i>	(GT) ₉ GC(GT) ₁₀	F:CCTTATCTCTGCTGTGCAGT R:CATGTGATTGATTCTGTCTCC	56	6	125-159	AB237649
<i>Vemos13</i>	(CA) ₁₉	F:AGAAGGCAACCGCTGATATC R:AACGCTTACAGCAGCTGGGA	56	7	199-249	AB250358
<i>Vemos18</i>	(CA) ₈	F:CTCATTCAACCCAAACCTG R:CACTCGATACCTGCAAGGCA	56	2	136-138	AB237650
<i>Vemos19</i>	(CA) ₈ GCACG(CA) ₁₉	F:CCACCATCTCACTCTCTCAC R:GGAGAGGGGGTCAAAAGAAA	56	4	181-203	AB237451
<i>Vemos25</i>	(GT) ₁₄ TT(GT) ₆	F:CCGTTCTCTAAGCAGTTTCC R:AAGCTCGGCGCAGCACAAT	56	4	161-165	AB237652
<i>Vemos26</i>	(GT) ₈ GG(GT) ₁₂	F:CTCTATCTCTCTTTCCCGTC R:GGATAAGCTGAGAAGTGTGG	56	4	151-159	AB237453
<i>Vemos 29</i>	(GT) ₂₂	F:AAGTCACCTCCTATGGTAAG R:TAACCTAAATCCAGACTCCA	56	2	216-218	AB241059
<i>Vemos 31</i>	(GT) ₂₆	F:GAGGATACATGTGCTGGTTG R:GCTCATCTGGATGATGGAC	56	5	283-311	AB241254
<i>Vemos 35</i>	(TG) ₅	F:AGCAGCCATGTAAGAGACAC R:CCACCAGAAACAGGATCCAC	56	3	351-381	AB241255
<i>Vemos 37</i>	(TG) ₁₆	F:CCACTAAATCACCTGTCACT R:GATGAAAGGCAACGTTGCT	56	3	158-164	AB241256
<i>Vemos 39</i>	(TG) ₂₉	F: GAAGCAGAGCTTATAGGAAC R:GACGATCAATAACCTGAGAG	56	7	244-264	AB241257
<i>Vemos42</i>	(TA) ₂ TCA(TG) ₁₂	F: GAATGTCTCAGCTCTGGAAG R: GAAACAATCTGGGGAAGGAG	56	5	136-142	AB250359
<i>Vemos 43</i>	(CA) ₄ CG(CA) ₆	F:CCTCACTCTAATGAAGACA R:TAAGAGGAAAAGGGGAAGAC	56	3	255-259	AB241258
<i>Vemos 44</i>	(GT) ₈ AAGTCCATGG(GT) ₈	F:CCGTGTTCATAATAGTGTGTAG R:CCGTGTTCATAATAGTGTGTAG	56	2	198-200	AB241259
<i>Vemos 46</i>	(CA) ₁₇	F:GTTGGGGATGAATCCAGT R:GCATGATAAAGGCAGAGGT	56	2	176-180	AB241260
<i>Vemos 47</i>	(CA) ₁₅	F:TTGTCCCCACGTTACACAAC R:CAAGAGGTGCTGGTACTCTG	56	3	157-168	AB241261
<i>Vemos 55</i>	(CA) ₁₇	F:CATAACCTGTTTCTTAGCAC R:CGCTGAGATTAAAGAGGAA	56	4	323-405	AB241262
<i>Vemos 57</i>	(CA) ₂₈	F:CAAACAGAGATACACTGATGG R:TACTGCTTCCTGTGACAAG	56	7	242-299	AB241263
<i>Vemos 60</i>	(TG) ₂₃	F:AAACCTCTGTAAGACGACA R:GCAACATGCATCTTACCAC	56	3	320-324	AB241264
<i>Vemos 61</i>	(GT) ₂₁	F:AGACAGGGTTGTTGTCTCA R:CTTCTGTGACCTTGGTCTCT	56	5	164-204	AB241265
<i>Vemos 62</i>	(GT) ₁₈	F:TGTTGCCCGTAACCTCTCT R:GGAGGAATAACTATGATCACCA	56	7	223-226	AB241266
<i>Vemos 64</i>	(CA) ₅ CG(CA) ₈	F:CAATTGTTGATGACTGCTGA R:CATCAAAGAGCTTGCAGAA	56	2	186-188	AB241267
<i>Vemos65</i>	(GT) ₂₅	F: CATGAGTTCCAAGCAAACC R: CGTTTACTGTTTGATCACCA	56	10	162-208	AB290199
<i>Vemos 66</i>	(CA) ₁₆	F:TCCAGCTAACGTCATGTC R:TCTGAGCTGATGATCACTTAGG	56	4	340-344	AB241268
<i>Vemos 68</i>	(TG) ₁₀	F:CCAGTGTGTCACCTCTACAC R:AAAACACAGCTGATGAAAC	56	3	363-372	AB241270
<i>Vemos 69</i>	(CA) ₄ AA(CA) ₈	F:CTAAAGGGAACAAAGCTG R:AGACAATTCTCCCTTTCC	56	2	267-271	AB241269
<i>Vemos 71</i>	(GT) ₁₆	F:TTCAATCAGGTACGACACA R:TTCATATCCAGAGCGGGTTT	56	4	338-350	AB241271
<i>Vemos 76</i>	(CA) ₁₈	F:GACAAAAGGCTGTCTGCT R:CACAACATGTCGATTTTCTGTC	56	4	178-182	AB241272
<i>Vemos 77</i>	(CT) ₈ (CA) ₁₀	F:GCCTCAAAGATCAGCAGTT R:GCTCCTTCATGTTCACTC	56	4	245-255	AB241273

^a In nine individuals (5 females, 4males)

Table 3. Genotypic segregation in two full-sib families ND1 and ND2 of normally fertilized diploids at 34 microsatellite loci.

Normalized diploids at 1 microsatellite loci.

Locus	Full-sib Family	Parents		Progeny								Total	df	χ ²
		Female	Male	aa	ab	ac	ad	bb	bc	bd				
<i>Vemos1</i>	ND-1	154/154(aa)	138/152(bc)	-	31	16	-	-	-	-	47	1	4.78*	
	ND-2	152/154(ab)	150/154(cb)	-	7	6	-	9	2	-	24	3	4.33	
<i>Vemos4</i>	ND-1	159/185(ab)	199/199(cc)	-	-	23	-	-	17	-	40	1	0.90	
	ND-2	185/189(ab)	159/165(cd)	-	-	9	2	-	8	7	26	3	4.46	
<i>Vemos6</i>	ND-1	244/246(ab)	238/238(cc)	-	-	25	-	-	21	-	46	1	0.35	
	ND-2	246/254(ab)	246/250(ac)	4	10	6	-	-	9	-	29	3	3.14	
<i>Vemos7</i>	ND-1	214/214(aa)	214/214(aa)	43	-	-	-	-	-	-	43	-	-	
	ND-2	214/214(aa)	214/216(ab)	10	20	-	-	-	-	-	30	1	3.33	
<i>Vemos10</i>	ND-1	180/180(aa)	180/180(aa)	45	-	-	-	-	-	-	45	-	-	
	ND-2	180/182(ab)	186/196(cd)	-	-	7	8	-	9	6	30	3	0.67	
<i>Vemos11</i>	ND-1	125/157(ab)	157/159(bc)	-	8	14	-	11	4	-	37	3	5.94	
	ND-2	145/151(ab)	155/157(cd)	-	-	5	6	-	7	6	24	3	0.33	
<i>Vemos13</i>	ND-1	200/210(ab)	200/238(ac)	6	11	12	-	-	15	-	30	3	3.82	
	ND-2	228/249(ab)	200/244(cd)	-	-	5	13	-	3	8	29	3	7.83*	
<i>Vemos18</i>	ND-1	136/138(ab)	136/136(aa)	23	23	-	-	-	-	-	46	1	0.00	
	ND-2	136/138(ab)	136/136(aa)	16	13	-	-	-	-	-	29	1	0.31	
<i>Vemos19</i>	ND-1	180/180(aa)	180/202(ac)	22	-	21	-	-	-	-	43	1	0.02	
	ND-2	180/194(ab)	184/184(cc)	-	-	13	-	-	17	-	30	1	0.53	
<i>Vemos25</i>	ND-1	163/165(ab)	161/165(cb)	-	7	16	-	7	12	-	41	3	5.43	
	ND-2	163/165(ab)	161/161(cc)	-	-	16	-	-	11	-	27	1	0.93	
<i>Vemos26</i>	ND-1	155/159(ab)	153/153(cc)	-	-	17	-	-	25	-	42	1	1.52	
	ND-2	155/159(ab)	151/153(cd)	-	-	5	3	-	4	12	24	3	8.33*	
<i>Vemos29</i>	ND-1	216/218(ab)	216/218(ab)	13	25	-	-	5	-	-	43	2	4.12	
<i>Vemos31</i>	ND-1	283/311(ab)	283/299(ac)	17	11	11	-	-	4	-	43	3	7.88*	
<i>Vemos35</i>	ND-1	373/373(aa)	351/381(bc)	-	18	22	-	-	-	-	40	1	0.40	
<i>Vemos37</i>	ND-1	158/158(aa)	162/164(bc)	-	26	17	-	-	-	-	43	1	1.88	
<i>Vemos39</i>	ND-1	256/264(ab)	244/264(ca)	9	10	11	-	-	12	-	42	3	0.48	
<i>Vemos42</i>	ND-1	138/142(ab)	136/138(ca)	11	6	14	-	-	7	-	26	3	4.32	
<i>Vemos43</i>	ND-1	255/257(ab)	257/259(bc)	-	7	17	-	10	11	-	45	3	4.69	
<i>Vemos44</i>	ND-1	262/n(ab)	262/264(ac)	(18) ^a	15	-	-	5	-	-	38	2	5.37	
<i>Vemos46</i>	ND-1	176/176(aa)	176/180(ab)	24	22	-	-	-	-	-	46	1	0.09	
<i>Vemos47</i>	ND-1	157/159(ab)	168/168(cc)	-	-	21	-	-	19	-	40	1	0.10	
<i>Vemos55</i>	ND-1	323/393(ab)	391/405(cd)	-	-	13	10	-	10	9	42	3	0.86	
<i>Vemos57</i>	ND-1	263/299(ab)	242/260(cd)	-	-	16	5	-	9	7	37	3	7.43	
<i>Vemos60</i>	ND-1	322/324(ab)	320/320(cc)	-	-	17	-	-	22	-	39	1	0.64	
<i>Vemos61</i>	ND-1	164/204(ab)	172/178(cd)	-	-	10	13	-	11	11		3	0.42	
<i>Vemos62</i>	ND-1	226/226(aa)	222/224(bc)	25	-	-	-	-	17	-	42	1	1.52	
<i>Vemos64</i>	ND-1	186/186(aa)	186/188(ab)	28	17	-	-	-	-	-	40	1	2.69	
<i>Vemos65</i>	ND-1	172/182(ab)	202/202(cc)	-	15	-	-	-	23	-	38	1	1.68	
<i>Vemos66</i>	ND-1	340/344(ab)	344/n(bc)	7	11	-	-	(24) ^b	-	-	42	3	1.62	
<i>Vemos68</i>	ND-1	363/371(ab)	363/371(ab)	11	21	-	-	12	-	-	44	3	2.36	
<i>Vemos69</i>	ND-1	267/267(aa)	267/271(ab)	19	22	-	-	-	-	-	41	1	0.22	
<i>Vemos71</i>	ND-1	338/350(ab)	340/340(cc)	-	20	-	-	-	23	-	43	1	0.21	
<i>Vemos76</i>	ND-1	178/184(ab)	182/182(cc)	-	-	23	-	-	19	-	42	3	0.38	
<i>Vemos77</i>	ND-1	245/255(ab)	247/253(cd)	-	-	11	9	-	8	8	36	3	0.67	

* $p < 0.05$

^a =(aa+an), ^b =(bb+bn); n = null allele

Table4. Microsatellite genotypes at 27 microsatellite loci in four meiotic gynogenetic diploid lines.

Locus	Gynogenetic line	Parent genotype		Genotypes of gynogens								Total	Recombination frequency (ν)	M-C distance (cM)	df	χ^2_{2a}
		Female	Sperm donor													
				aa	ab	bb	ac	ad	be	bd						
<i>Vemos1</i>	MEI-2	152/154 (<i>ab</i>)	150/154 (<i>cb</i>)	2	33	0	-	-	-	-	35	0.94	47.1			
	MEI-3	152/154 (<i>ab</i>)	154/154 (<i>bb</i>)	6	27	1	-	-	-	-	34	0.79	39.5			
	Total			8	60	1					69	0.87	43.5	1	3.36	
<i>Vemos4</i>	MEI-1	159/185 (<i>ab</i>)	199/199 (<i>cc</i>)	15	2	17	-	-	-	-	34	0.06	2.9	1	0.13	
	MEI-2	185/189 (<i>ab</i>)	159/165 (<i>cd</i>)	18	8	11	-	-	-	-	37	0.22	10.8	1	1.69	
	MEI-3	157/175 (<i>ab</i>)	181/185 (<i>cd</i>)	18	6	10	-	-	-	-	34	0.18	8.8	1	2.28	
	MEI-4	175/199 (<i>ab</i>)	U	17	9	10	-	-	-	-	36	0.25	12.5	1	1.81	
	Total			68	25	48					141	0.18	9.0	3	4.96	
<i>Vemos6</i>	MEI-1	244/246 (<i>ab</i>)	238/238 (<i>cc</i>)	18	0	21	-	-	-	-	39	0.00	0.0	1	0.23	
	MEI-2	246/254 (<i>ab</i>)	246/250 (<i>ac</i>)	20	0	20	-	-	-	-	40	0.00	0.0	1	0.00	
	MEI-3	232/238 (<i>ab</i>)	244/246 (<i>cd</i>)	23	0	10	-	-	-	-	33	0.00	0.0	1	5.12*	
	Total			61	0	51					112	0.00	0.0			
<i>Vemos10</i>	MEI-2	180/182 (<i>ab</i>)	186/196 (<i>cd</i>)	0	34	1	-	-	-	-	35	0.97	48.6			
<i>Vemos11</i>	MEI-1	125/157 (<i>ab</i>)	157/159 (<i>bc</i>)	0	3	35	-	-	-	-	38	0.08	3.9	1	35.00* ^b	
	MEI-2	145/151 (<i>ab</i>)	155/157 (<i>cd</i>)	13	2	20	-	-	-	-	35	0.06	2.9	1	1.48	
	Total			13	5	55					73	0.07	3.5	1	0.13	
<i>Vemos13</i>	MEI-1	200/210(<i>ab</i>)	200/238 (<i>ac</i>)	13	1	21	-	-	-	-	35	0.03	1.4	1	1.88	
	MEI-2	228/249(<i>ab</i>)	200/244 (<i>cd</i>)	10	0	12	-	-	-	-	22	0.00	0.0	1	0.18	
	MEI-3	200/204(<i>ab</i>)	200/228 (<i>ac</i>)	13	2	19	-	-	-	-	34	0.06	2.9	1	1.12	
	Total			26	3	42					91	0.03	1.5	2	1.48	
<i>Vemos18</i>	MEI-1	136/138 (<i>ab</i>)	136/136 (<i>aa</i>)	0	39	1	-	-	-	-	40	0.98	48.8			
	MEI-2	136/138 (<i>ab</i>)	136/136 (<i>aa</i>)	1	38	1	-	-	-	-	40	0.95	47.5			
	MEI-3	136/138 (<i>ab</i>)	136/136 (<i>aa</i>)	0	34	1	-	-	-	-	35	0.97	48.6			
	MEI-4	136/138 (<i>ab</i>)	U	0	40	0	-	-	-	-	40	1.00	50.0			
	Total			1	151	3					155	0.97	48.5	3	2.00	
<i>Vemos19</i>	MEI-2	180/194 (<i>ab</i>)	184/184 (<i>cc</i>)	8	19	11	-	-	-	-	38	0.50	25.0	1	0.47	
<i>Vemos25</i>	MEI-1	163/165 (<i>ab</i>)	161/165 (<i>ca</i>)	0	36	3	-	-	-	-	39	0.92	46.2			
	MEI-2	163/165 (<i>ab</i>)	161/161 (<i>cc</i>)	0	34	4	-	-	-	-	38	0.89	44.7			
	MEI-3	161/165 (<i>ab</i>)	163/165 (<i>cb</i>)	0	35	1	-	-	-	-	36	0.97	48.6			
	MEI-4	163/167 (<i>ab</i>)	U	1	39	0	-	-	-	-	40	0.97	48.6			
	Total			1	144	8					153	0.94	47.0	3	3.16	
<i>Vemos26</i>	MEI-1	155/159 (<i>ab</i>)	153/153 (<i>cc</i>)	1	35	1	-	-	-	-	37	0.95	47.3			
	MEI-2	155/159 (<i>ab</i>)	151/153 (<i>cd</i>)	0	35	0	-	-	-	-	35	1.00	50.0			
	MEI-3	153/153 (<i>aa</i>)	159/159 (<i>bb</i>)	0	38	0	-	-	-	-	38	1.00	50.0			
	Total			1	108	1					110	0.98	49.0	2	4.02	
<i>Vemos29</i>	MEI-1	216/218 (<i>ab</i>)	216/218 (<i>ab</i>)	24	0	9	-	-	-	-	33	0.00	0.0	1	6.82*	
<i>Vemos31</i>	MEI-1	283/311 (<i>ab</i>)	283/299 (<i>ac</i>)	21	3	13	-	-	-	-	37	0.08	4.1	1	1.88	
	MEI-3	275/299 (<i>ab</i>)	289/311 (<i>cd</i>)	19	3	12	-	-	-	-	34	0.09	4.4	1	1.58	
	Total			40	6	25					71	0.08	4.0	1	0.01	
<i>Vemos39</i>	MEI-1	256/264 (<i>ab</i>)	244/264 (<i>ca</i>)	17	7	14	-	-	-	-	38	0.18	9.2	1	0.29	
	MEI-2	252/260 (<i>ab</i>)	242/254 (<i>cd</i>)	25	7	2	-	-	-	-	34	0.20	10.3	1	19.59* ^b	
	MEI-3	256/260 (<i>ab</i>)	256/264 (<i>ac</i>)	12	13	14	-	-	-	-	39	0.33	16.6	1	0.15	
	Total			54	27	30					111	0.24	12.0	2	2.70	
<i>Vemos42</i>	MEI-1	138/142 (<i>ab</i>)	136/138 (<i>ca</i>)	1	37	1	-	-	-	-	39	0.95	47.4			
	MEI-2	130/138 (<i>ab</i>)	138/140 (<i>bc</i>)	0	38	0	-	-	-	-	38	1.00	50.0			
	Total			1	75	1					77	0.97	48.5	1	2.00	
<i>Vemos43</i>	MEI-1	255/257 (<i>ab</i>)	257/259 (<i>bc</i>)	15	8	15	-	-	-	-	38	0.21	10.5	1	1.00	
	MEI-2	255/257 (<i>ab</i>)	259/259 (<i>cc</i>)	11	17	10	-	-	-	-	38	0.45	22.4	1	0.05	
	Total			26	25	25					76	0.33	16.5	1	4.83*	

Table4 (continued)

Locus	Family	Parent genotype		Genotypes of gynogens								Total	Recombination frequency (γ)	M-C distance (cM)	D _f	χ ² aa:bb =1:1
		Female	Sperm donor	aa	ab	bb	ac	ad	be	bd						
<i>Vemos44</i>	MEI-4	262/264 (<i>ab</i>)	U	1	32	1	-	-	-	-	34	0.94	47.1			
<i>Vemos47</i>	MEI-1	157/159 (<i>ab</i>)	168/168 (<i>cc</i>)	1	33	0	-	-	-	-	34	0.97	48.5			
<i>Vemos55</i>	MEI-1	323/393 (<i>ab</i>)	391/405 (<i>cd</i>)	14	8	9	-	-	-	-	31	0.26	12.9	1	1.09	
<i>Vemos57</i>	MEI-1	263/299 (<i>ab</i>)	242/260 (<i>cd</i>)	3	24	2	-	-	-	-	29	0.83	41.3			
	MEI-3	242/260 (<i>ab</i>)	274/299 (<i>cd</i>)	0	37	1	-	-	-	-	38	0.97	48.5			
	Total			3	61	3					67	0.91	45.5	1	4.31*	
<i>Vemos60</i>	MEI-1	322/324 (<i>ab</i>)	320/320 (<i>cc</i>)	0	21	12	-	-	-	-	33	0.64	32.3	1	12.00 ^{ab}	
<i>Vemos61</i>	MEI-1	164/204 (<i>ab</i>)	172/178 (<i>cd</i>)	9	17	8	-	-	-	-	34	0.50	25.0	1	0.06	
	MEI-3	158/178 (<i>ab</i>)	164/204 (<i>cd</i>)	9	22	8	-	-	-	-	39	0.56	28.2	1	0.06	
	Total			18	39	16					73	0.53	26.5	1	0.30	
<i>Vemos62</i>	MEI-3	212/226 (<i>ab</i>)	220/228 (<i>cd</i>)	15	15	9	-	-	-	-	39	0.38	19.0	1	1.50	
	MEI-4	208/222 (<i>ab</i>)	U	18	13	5	-	-	-	-	36	0.36	18.0	1	7.35*	
	Total			33	28	14					75	0.37	18.5	1	0.04	
<i>Vemos65</i>	MEI-1	172/182 (<i>ab</i>)	202/202 (<i>cc</i>)	5	29	4	-	-	-	-	38	0.76	38.0			
	MEI-2	190/200 (<i>ab</i>)	162/208 (<i>cd</i>)	4	29	1	-	-	-	-	34	0.85	42.6			
	MEI-3	162/206 (<i>ab</i>)	170/170 (<i>cc</i>)	3	34	1	-	-	-	-	38	0.89	44.5			
	Total			12	92	6					110	0.84	42.0	2	2.50	
<i>Vemos66</i>	MEI-1	340/344 (<i>ab</i>)	344/n (<i>bc</i>)	1	33	0	-	-	-	-	34	0.97	48.5			
	MEI-4	344/346 (<i>ab</i>)	U	0	29	0	-	-	-	-	29	1.00	50.0			
	Total			1	62	0					63	0.98	49.0	1	0.87	
<i>Vemos68</i>	MEI-1	363/371 (<i>ab</i>)	363/371 (<i>ab</i>)	1	32	0	-	-	-	-	33	0.97	48.5			
	MEI-2	365/371 (<i>ab</i>)	371/371 (<i>bb</i>)	0	38	0	-	-	-	-	38	1.00	50.0			
	MEI-4	363/371 (<i>ab</i>)	U	0	29	0	-	-	-	-	29	1.00	50.0			
	Total			1	99	0					100	0.99	49.5	2	2.05	
<i>Vemos71</i>	MEI-1	338/350 (<i>ab</i>)	340/340 (<i>cc</i>)	12	11	7	-	-	-	-	30	0.36	18.3	1	1.31	
	MEI-3	338/340 (<i>ab</i>)	338/344 (<i>ac</i>)	7	20	10	-	-	-	-	37	0.54	27.0	1	0.53	
	MEI-4	338/340 (<i>ab</i>)	U	5	19	9	-	-	-	-	33	0.58	28.8	1	1.14	
	Total			24	50	26					100	0.50	25.0	2	3.13	
<i>Vemos76</i>	MEI-1	178/184 (<i>ab</i>)	182/182 (<i>cc</i>)	9	24	0	-	-	-	-	33	0.72	36.4			
	MEI-4	180/182 (<i>ab</i>)	U	0	33	0	-	-	-	-	33	1.00	50.0			
	Total			9	57	0					66	0.86	43.0	1	10.42*	

U= unknown due to the loss of sample

^a Chi square test for equal numbers of homozygotes (1d.f.) is performed when number of homozygotes is superior to10. Chi-square value in the total row is contingency chi-square for differences in *y* between gynogenetic lines. *p<0.05^b Significance after multiple test corrections

Table5. Verification of mitotic gynogenetic MIT-1 line at 8 microsatellite loci with high recombination rates.

Locus	y	Parents		Progeny			Total (N)
		Female	Male	<i>aa</i>	<i>ab</i>	<i>bb</i>	
<i>Vemos10</i>	<i>0.97</i>	174/182(<i>ab</i>)	174/182(<i>ab</i>)	9	0	1	10
<i>Vemos25</i>	<i>0.94</i>	163/165(<i>ab</i>)	165/165(<i>bb</i>)	2	0	6	8
<i>Vemos26</i>	<i>0.98</i>	153/155(<i>ab</i>)	155/155(<i>bb</i>)	6	0	2	8
<i>Vemos42</i>	<i>0.97</i>	136/138(<i>ab</i>)	136/138(<i>ab</i>)	6	0	4	10
<i>Vemos57</i>	<i>0.91</i>	263/265(<i>ab</i>)	289/289(<i>cc</i>)	2	0	8	10
<i>Vemos65</i>	<i>0.84</i>	162/180(<i>ab</i>)	180/202(<i>bc</i>)	5	0	5	10
<i>Vemos66</i>	<i>0.98</i>	340/344(<i>ab</i>)	340/344(<i>ab</i>)	7	0	3	10
<i>Vemos68</i>	<i>0.99</i>	363/371(<i>ab</i>)	363/371(<i>ab</i>)	4	0	5	9

(y) = Meiotic second division segregation frequency (see Table 4)