Aquaculture 280 (2008) 81–88

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Centromere-linkage in the turbot (*Scophthalmus maximus*) through half-tetrad analysis in diploid meiogynogenetics

Paulino Martínez [a,*], Miguel Hermida [a], Belén G. Pardo [a], Carlos Fernández [a], Jaime Castro [a], Rosa M. Cal [b], José A. Álvarez-Dios [c], Antonio Gómez-Tato [d], Carmen Bouza [a]

[a] Departamento de Genética, Universidad de Santiago de Compostela (USC), Facultad de Veterinaria, Campus de Lugo, 27002 Lugo, Spain
[b] Instituto Español de Oceanografía (IEO), Centro Oceanográfico de Vigo, 36280 Vigo, Spain
[c] Departamento de Matemática Aplicada, (USC), Facultad de Matemáticas, 15782 Santiago de Compostela, Spain
[d] Departamento de Geometría (USC), Facultad de Matemáticas, 15782 Santiago de Compostela, Spain

ARTICLE INFO

Article history:
Received 28 January 2008
Received in revised form 2 May 2008
Accepted 9 May 2008

Keywords:
Turbot
Scophthalmus maximus
Genetic map
Centromere position
Segregation distortion
Deleterious genes
Mapping function

ABSTRACT

Seventy nine microsatellite markers selected across all linkage groups (LG) from a previous turbot genetic map were studied in a diploid meiogynogenetic family for centromere mapping using half-tetrad analysis. Significant deviations from Mendelian segregation were observed at 25% loci analyzed. The clustering of distorted loci at specific LGs, suggested the existence of genes of different deleterious effects. The lack of Mendelian segregation distortion at 1 day and 10 days post-hatching larvae at these loci precluded an explanation based on aberrant meiotic segregation. Heterozygote frequency distribution in gynogenetic offspring showed close to 50% values above 0.667, which suggested high chiasma interference in turbot. Complete interference appeared as the best fitting function when estimating centromere position. However, Kosambi and Haldane functions performed better at specific LGs as a consequence of the variable crossover pattern of centromere-distant markers among LG. Great concordance between half-tetrad data and the positions previously reported in the turbot map was observed. Most centromeres were localized with an error around or below 5 cM and closely linked markers exist now in 8 LGs. Centromere location was mostly in accordance with previous karyotypic information.

1. Introduction

Genetic maps constitute essential organizational tools for genomic research (Sewell et al., 1999). They are being applied to identify QTL or genomic regions related with evolutionary or productive characters, and eventually for positional cloning or candidate gene strategies (Donovan et al., 2000; Mackay, 2001; Blott et al., 2003; Colosimo et al., 2005). Positioning centromeres constitutes an important goal during map construction (Danzmann and Gharbi, 2001; Nichols et al., 2003; Guyomard et al., 2006). The heterogeneity of recombination frequency along chromosome arms disturbs the correspondence between genetic and physical maps (Kauffman et al., 1995). The lower recombination frequency in the vicinity of centromeres influences positional cloning or marker assisted selection strategies, since markers are embracing larger genomic regions at these areas. Markers close to centromeres are particularly useful to detect linkage of mutations to specific linkage groups (LG), especially in fish where high chiasma interference limits the number of multiple crossovers (Johnson et al., 1995; Kauffman et al., 1995; Mohideen et al., 2000).

Half-tetrad analysis using diploid meiogynogenetics has been generally used to locate centromeres in fish (Thorgaard et al., 1983; Allendorf et al., 1986; Kauffman et al., 1995; Sakamoto et al., 2000; Guyomard et al., 2006; Nomura et al., 2006). Crossovers along chromosome arms during meiosis determine that genetic markers close to centromeres segregate mostly during first meiotic division, while distal ones during meiosis II (Johnson et al., 1996). So, heterozygotes in gynogenetic offspring can be used to obtain genetic distance between markers and centromeres (Danzmann and Gharbi, 2001). Different mapping functions can be applied to estimate distances between genetic markers and centromeres (Kauffman et al., 1995). Complete interference, which assumes that one recombinational exchange inhibits the formation of additional crossovers, has been generally applied in fish (Sakamoto et al., 2000; Morishima et al., 2001; O'Malley et al., 2003; Matsuoka et al., 2004; Guyomard et al., 2006).

The turbot (*Scophthalmus maximus*; Scophthalmidae; Pleuronectiformes) is one of the most promising aquaculture species in Europe. Genetic information has increased in the last 15 years in response to the demand of turbot industry for evaluating genetic resources and for parentage analysis (Bouza et al., 2002; Castro et al., 2003, 2004). Recently, a first genetic map of 242 microsatellites distributed across 26 LGs has been published in this species (Bouza et al., 2007).

* Corresponding author. Tel./fax: +34 982254681.
E-mail address: paumarpo@lugo.usc.es (P. Martínez).

0044-8486/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.aquaculture.2008.05.011

In this study, we have analyzed a large sample of microsatellites distributed across all LGs for locating centromeres in the reported turbot map (Bouza et al., 2007) using diploid meiogynogenetic offspring. We were specifically concerned with: i) contrasting previous map information with gene centromere distances and searching for congruence between both data sets; ii) analyzing crossover pattern across linkage groups; iii) locating centromeres at linkage groups as a further step towards consolidating turbot map.

2. Materials and methods

2.1. Diploid gynogenetic offspring

A diploid meiogynogenetic family was obtained at the facilities of the Instituto Español de Oceanografía (IEO, Vigo) using a female and a donor-sperm male coming from a natural population following the procedure by Piferrer et al. (2004). Three different samples from this family were considered attending to the objectives of this study: i) forty eight diploid gynogenetic individuals of 90 days post-hatching (dph) were used for estimating gene centromere (G-C) distances; ii) two additional samples at 1 dph and 10 dph (48 larvae each) from the same female, respectively, were obtained for evaluating Mendelian segregation distortion. Both the mother and the sperm-donor male were genotyped for the same microsatellite set to confirm the exclusive maternal inheritance in all offspring (Castro et al., 2003).

2.2. Microsatellite markers

A specific set of markers were selected at each turbot linkage group to locate centromeres starting from the turbot genetic map previously reported by Bouza et al. (2007). Seventy nine microsatellite markers were finally chosen attending to their distribution across LGs and polymorphism criteria. The markers were amplified following the protocols described by Pardo et al. (2006, 2007).

2.3. Segregation distortion in gynogenetic offspring

All markers were tested for deviation from Mendelian expectations using a chi-square test. The sequential Bonferroni correction (Rice, 1989) was considered for multiple tests. G-C distances at those loci with significant deviation after Bonferroni correction were calculated by counting twice the commonest homozygote class according to Thorgaard et al. (1983). The detection of significant deviations from Mendelian segregation in the 90 dph progeny moved us to study two additional samples of 1 dph and 10 dph (48 offspring each). This permitted to confirm the implication of deleterious alleles in such distortion and to find out the time at which this condition could be operating.

2.4. Location of centromeres in turbot map: evaluating mapping function

Taking into account the majority of acro-subtelocentric chromosomes in turbot karyotype (Bouza et al., 1994; Cuñado et al., 2001), two segregating microsatellite markers located at both extremes of each linkage group were initially selected to ascertain centromere orientation along chromosome axis. Only one locus could be used at LGs 22, 23, 24 and 26 due to their small size and/or availability of segregant markers in the mother. This approach permitted us to localize the ends where the centromere was positioned at uniarmed chromosomes. When large G-C distances were observed with both terminal markers, the centromere was considered internal (biarmed chromosomes). One or more available markers, the closest as possible to the region where the centromere was located, were then selected for a more precise location. The relative position of markers close to centromeres was obtained considering the minimum number of multiple recombinational events. Seventy nine microsatellites were finally analyzed, constituting an average of three markers per LG.

Table 1
Diploid meiogynogenetic segregation for the 79 microsatellite loci used to locate centromeres in turbot genetic map

Linkage group	Locus	Progeny[a]			y^b	P^c
		11	12	22		
LG1	Sma-USC271	2	43	2	0.915	1.000
	Sma-USC13	20	11	16	0.234	0.505
	Sma-USC218	20	0	28	0.000	0.248
	Sma-USC268	12	10	9	0.323	0.513
LG2	Sma-USC90	2	40	4	0.870	0.414
	Sma-USC36	0	17	28	0.233	**0.000**
	Sma-USC185	37	1	0	0.013	**0.000**
	Sma-USC219	0	25	19	0.397	**0.000**
	Sma-USC64	15	31	1	0.508	**0.000**
LG3	Sma-USC30	7	38	3	0.792	0.206
	Sma-USC200	9	25	10	0.568	0.819
	Sma-USC77	18	5	22	0.111	0.527
LG4	Sma-USC205	31	0	16	0.000	**0.029**
	B12-I GT14	27	8	12	0.170	**0.016**
LG5	Sma-USC10	6	28	10	0.636	0.317
	Sma-USC202	2	45	1	0.938	0.564
	Sma-USC270	16	31	0	0.492	**0.000**
	Sma-USC65	0	34	13	0.567	**0.000**
	Sma-USC12	0	46	2	0.958	0.157
LG6	3/3GT	17	0	30	0.000	0.058
	Sma-USC28	17	19	9	0.422	0.117
	Sma-USC132	10	35	2	0.745	**0.021**
	Sma-USC227	1	42	3	0.913	0.317
LG7	Sma-USC37	5	37	6	0.771	0.763
	Sma4-14INRA	8	32	6	0.696	0.593
	Sma-USC238	7	37	4	0.771	0.366
	Sma-USC154	12	31	5	0.646	0.090
	Sma-USC272	20	22	6	0.458	**0.006**
	Sma-USC174	22	17	7	0.370	**0.005**
LG8	Sma-USC194	6	37	3	0.804	0.317
	Sma-USC18	27	10	10	0.213	**0.005**
	Sma-USC208	7	17	23	0.362	**0.003**
LG9	Sma-USC150	8	33	6	0.702	0.593
	Sma-USC21	8	25	12	0.556	0.371
	Sma-USC226	13	14	19	0.304	0.289
LG10	Sma-USC113	1	42	2	0.933	0.564
	Sma-USC281	3	32	9	0.727	0.083
LG11	Sma-USC116	8	27	11	0.587	0.491
	Sma-USC152	6	40	2	0.833	0.157
	Sma-USC275	16	10	21	0.213	0.411
	Sma-USC8	10	32	6	0.667	0.317
	Sma-USC258	2	38	7	0.809	0.096
LG12	Sma-USC183	21	0	26	0.000	0.466
	Sma-USC19	5	33	10	0.688	0.197
LG13	Sma1-125INRA	24	2	22	0.042	0.768
	Sma-USC16	3	42	2	0.894	0.655
	Sma-USC27	4	37	7	0.771	0.366
LG14	Sma-USC220	10	34	2	0.739	**0.021**
	Sma-USC213	6	30	12	0.625	0.157
	Sma-USC253	23	3	22	0.063	0.881
LG15	Sma-USC232	9	33	6	0.688	0.439
	Sma-USC149	6	32	10	0.667	0.317
	Sma-USC45	5	42	0	0.894	**0.025**
	Sma.USC111	5	27	16	0.478	**0.016**
	Smax-01	5	33	9	0.702	0.285
LG16	Sma-USC50	9	32	5	0.696	0.285
	Sma3-8INRA	5	35	6	0.761	0.763
	Sma-USC250	19	16	12	0.340	0.209
LG17	Sma-USC31	4	31	13	0.646	**0.029**
	Sma3-129INRA	0	44	4	0.917	**0.046**
	Smax-02	6	25	17	0.521	**0.022**
	Sma-USC52	9	37	2	0.771	**0.035**
LG18	Sma-USC160	10	27	11	0.563	0.827
	Sma-USC193	8	26	7	0.634	0.796
LG19	Sma-USC23	8	36	4	0.750	0.248
	F1-OCA19	2	44	2	0.917	1.000
	3/20CA17	12	29	7	0.604	0.251
	Sma-USC24	5	39	2	0.848	0.257
LG20	Sma-USC95	21	0	27	0.000	0.386
	Sma-USC284	3	40	5	0.833	0.480
	Sma-USC29	6	41	1	0.854	0.059
LG21	Sma-USC231	3	40	4	0.851	0.705
	Sma-USC117	21	0	27	0.000	0.386