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$(CT)_n$ and $(GT)_n$ microsatellites: a new class of genetic markers for *Salmo trutta* L. (brown trout)

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Thirteen $(GT)_n$ and four $(CT)_n$ microsatellite loci ($n = 10$ or more and $n = 20$ or more, respectively) have been isolated from a partial genomic library of brown trout and sequenced. On average, a $(GT)_n$ repeat sequence occurs approximately every 23 kb and a $(CT)_n$ repeat sequence every 76 kb in brown trout genome. Primers for DNA amplifications using the polymerase chain reaction (PCR) were synthesized for three single locus microsatellites. Mendelian inheritance of the observed polymorphisms was confirmed in full-sib families. Four brown trout populations (10 unrelated individuals per population) were screened for polymorphism with these three microsatellite loci. The total number of alleles detected in the four populations is five at one locus, six at the other two microsatellite loci and is three, on average, per population. Heterozygosities range from 0.18 to 0.74. The largest differences in allelic frequencies occurred between the Mediterranean and the Atlantic populations: this result is congruent with previous allozymic data. The gene–centromere distances of the three microsatellite markers were determined on gynogenetic lines: post-reduction rates range from 0.17 to 0.60. For all the three microsatellite loci, the primers designed from brown trout sequences can be used in another closely related species of salmonid, the rainbow trout (*Oncorhynchus mykiss*). This last aspect supports the view that microsatellite markers may have wide application in genetic studies in salmonid species and fishes in general.

Keywords: brown trout, gene–centromere distances, interspecific priming, intraspecific genetic variation, microsatellite markers, *S. trutta*.

Introduction

The existence of repetitive DNA sequences in eukaryotes has been evidenced by DNA reassociation experiments for more than two decades (Britten & Kohne, 1968). This repetitive DNA is usually classified into two categories: (i) satellite DNA which is composed of large numbers of tandemly repeated sequences and can therefore be isolated from the bulk DNA by centrifugation in a cesium chloride gradient, and (ii) interspersed repeated DNA. Mini and microsatellite sequences both belong to this second category. Minisatellites refer to sequences which are composed of tandem repeats of 9–64 base pair motives and have a total length ranging from 0.1 to 7 kb (Jeffreys *et al.*, 1985). They seem to be frequently located near the centromeric and telomeric regions. Microsatellite sequences correspond to tandemly repeated motifs of one to five base pairs for a total length usually lower than 0.2 kb (Rassman *et al.*, 1991); they seem to be more randomly distributed along the chromosomes than minisatellites (Hearne *et al.*, 1992), but are under-represented in the centromeric and telomeric regions of chromosomes (Wong *et al.*, 1990; Wintero *et al.*, 1992). Both kinds of tandemly repeated sequences have been found in all the eukaryotic genomes so far studied (Epplen *et al.*, 1991; Jarman & Wells, 1989).

The restriction fragment length polymorphism obtained with probes homologous to these mini and microsatellite sequences reflects allelic variation at many loci (Jeffreys *et al.*, 1985; Epplen *et al.*, 1991; Taggart & Ferguson, 1991). These highly polymorphic 'fingerprints' are convenient in situations requiring paternity testing and in forensic medicine, but are inadequate for many population genetics purposes

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because of the inability to assign the observed bands to specific loci. More recently, this problem has been overcome by the development of methods allowing visualization of allelic variation at single mini and microsatellite loci (Jeffreys *et al.*, 1988; Tautz, 1989; Weber & May, 1989). In both tandemly repeated sequences, the specificity for a given locus is determined by the flanking regions and its polymorphism by variations in the number of basic motives in the repeated sequence.

Variation at single locus microsatellite has been detected in polyacrylamide sequencing gels after specific PCR amplication with primers homologous to short sequences of the flanking regions. This method has been successfully applied to as distantly related organisms as *Drosophila* (Tautz, 1989) and humans (Weber & May, 1989). Most microsatellite loci investigated so far are composed of GT or, to a lesser extent, of CT motifs. These types of repeat sequences form two of the most abundant classes of microsatellites in higher vertebrates. In rat and human genomes, $(GT)_n$ and $(CT)_n$ repeat sequences occur every 15 kb and 30 kb, and every 50 kb and 113 kb, respectively (Stallings *et al.*, 1991; Beckmann & Weber, 1992). We have recently applied the aforementioned procedure to $(GT)_n$ and $(CT)_n$ microsatellites isolated from a partial genomic library of brown trout.

A genetic characterization was performed for three of these microsatellite loci. After analysing the segregation of the observed polymorphisms in full-sib families, the level of polymorphism of the three markers was studied in brown trout populations to evaluate their efficiency for population studies and genetic mapping. To assess the random location of microsatellites on brown trout chromosomes, gene–centromere distances were determined in gynogenetic lines. The examination of diploid gynogens can also provide basic information about meiosis, such as the amount of chiasma interference. Finally, the similarity between salmonid species of the flanking sequences was tested for the three microsatellite loci on the genomic DNA of rainbow trout.

Materials and methods

Biological material

Microsatellite and protein polymorphisms were analysed in brown trout samples of full-sib families, gynogenetic lines and populations.

The four brown trout populations (10 individuals per population) consisted of two domesticated stocks (Gournay, France and Cuneo, Italy), one natural Atlantic stock (Bresles river, Normandie, France) and one natural Mediterranean stock (Artesiaga river, basin of Ebro, Spain).

The individuals used to produce gynogenetic lines and full-sib families consisted in mature brown and rainbow trout males and mature brown trout females, which originated from domesticated strains kept in our experimental facilities (Gournay strains, France). Gynogenetic lines, denoted G lines, were obtained by applying early heat shocks to brown trout eggs fertilized with U.V. irradiated sperm of rainbow trout (*Oncorhynchus mykiss*), as described in Quillet *et al.* (1991). For each female three controls were produced: a haploid control, denoted B (fertilization with U.V. irradiated rainbow trout sperm), a diploid hybrid control, denoted BR (fertilization with rainbow trout sperm), and a triploid hybrid control, denoted B_2R (fertilization with rainbow trout sperm and early heat shocks on eggs). In addition, one full-sib family was produced for each female. All these groups were reared at $10°C \pm 1°C$ in a recirculating water system, killed at the age of 6 months and sampled (50 individuals per group).

For all analysed individuals, blood was collected for DNA extraction and fishes were frozen at $-30°C$ for the protein electrophoresis studies. A rapid procedure was applied for DNA extraction: 100 μl of blood were homogenized in 4 ml of a TNES-Urea solution (Tris-HCl 10 mM, NaCl 0.3 M, SDS 2%, EDTA 10 mM, urea 4 M, pH 8) and incubated at 55°C for 2 h with proteinase K (200 $\mu g\ ml^{-1}$ final concentration). The DNA precipitate, which appeared after the addition of 1 V of isopropanol and a slow agitation, was transferred to 80% isopropanol for washing. The DNA precipitate was then quickly transferred into 2 ml of TE buffer (Tris-HCl 10 mM, EDTA 1 mM), warmed at 50°C until complete dissolution and stored at $-20°C$.

Cloning and sequencing of single locus microsatellites

Blood samples were taken from two brown trout individuals of the domesticated strain of Gournay. Genomic DNA was isolated and purified as described elsewhere (Sambrook *et al.*, 1989) and digested to completion with *Msp*I. A selective recovery of DNA fragments ranging in size from 0.2 to 0.5 kb was processed in a 1.5% agarose gel followed by electroelution. PBluescript II SK+ vector (Stratagene) was digested by *Cla*I and dephosphorylated. XL1-blue competent cells (Stratagene) were prepared according to standard protocol (Sambrook *et al.*, 1989). Ligation and transformation were carried out following the protocols of Stratagene. Colonies containing an insert were chosen by colour discrimination depending on the polylinker

disruption. After the transfer of white colonies on nitrocellulose membranes (Millipore), the sublibrary was screened using an equal mix of $(GT)_{10}$ and $(CT)_{10}$ oligonucleotides. These oligonucleotides were 5′end-labelled with γ^{32}P-ATP, according to the protocol of the 5′end-labelling Boehringer kit. Positive clones were directly analysed by sequencing. DNA sequencing was performed on alkaline denaturated plasmid DNA (Sambrook *et al.*, 1989) using the dideoxynucleotide chain termination method (Sanger *et al.*, 1977) with T3 and T7 primers and Sequenase (USB). Two primers were designed in the regions flanking the core sequences of the microsatellites, the size of the fragments to be amplified ranging from 100 to 200 bp.

Microsatellite and protein polymorphism

Microsatellite polymorphism was analysed by specific polymerase chain reaction (PCR) performed in a PREM TM III thermocycler (LEP Scientific Limited). The 25 μl reaction mixture contained 100 ng of genomic DNA template, 25 pmol of each oligodeoxinucleotide primer, 75 μM each dCTP, dGTP and dTTP, 2.5 μM dATP, 0.2 μl (approximately 40 pM) α^{35}S-dATP at 1000 Ci/mmol, 1.5 mM $MgCl_2$, 1X Promega reaction buffer, and one unit of Promega *Taq* polymerase. After a denaturating step of 5 min at 94°C, samples were processed through 30 cycles consisting of 30 s at 94°C, 30 s at 58°C and 30 s at 72°C. The last elongation step was lengthened to 10 min. After DNA amplification, aliquots of the reaction mixtures were mixed with one volume of formamide loading buffer, heated 5 min at 85°C and electrophoresed on standard DNA sequencing gels (5 M urea, 6% acrylamide). Fixation, drying and autoradiography were processed as usual. A sequencing reaction was used as a size marker. Allele sizes were measured as the midpoint between the two single strand bands (Weber & May, 1989). The error in this procedure is estimated to be one nucleotide.

Tissue extraction and protein electrophoresis procedures are described in Guyomard & Krieg (1983) and Krieg & Guyomard (1985).

Heterozygosity and PIC values (Polymorphism Information Content; Botstein *et al.*, 1980) were calculated for each population sample analysed with microsatellite markers.

Results

Characteristics of $(GT)_n$ and $(CT)_n$ microsatellites

A total of 13 $(GT)_n$ and four $(CT)_n$ microsatellites were isolated from a partial genomic library composed of 863 clones. The theoretical average size of the cloned insert being equal to 350 bp, we have estimated the total number of analysed base pairs to be $350 \times 863 = 302\,000$ bp. Assuming that $(GT)_n$ and $(CT)_n$ microsatellites are evenly distributed in the cloned fraction of the genome, the average distance between neighbouring microsatellites can be estimated by dividing the total length of screened DNA by the total number of $(GT)_n$ or $(CT)_n$ repeat sequences isolated. Also, we will consider that our partial library is representative of the whole genome. This implies that *Msp*I restriction sites and $(GT)_n$ or $(CT)_n$ microsatellites have independent distributions. Under these assumptions, $(GT)_n$ and $(CT)_n$ microsatellites occur on average every 23 kb and 76 kb, respectively. Considering that the haploid genome of brown trout consists of 2500 Mb (Atkin *et al.*, 1965), rough estimates of the total number of $(GT)_n$ and $(CT)_n$ microsatellites amount to 109 000 and 33 000 loci, respectively in this species.

The three categories of microsatellites described by Weber (1990), namely perfect, imperfect and compound repeat sequences, are represented in the $(GT)_n$ and $(CT)_n$ microsatellites cloned. The total number of repeated motifs of the core sequence ranges from 10 to 40 for $(GT)_n$ microsatellites and from 20 to 87 for $(CT)_n$ microsatellites. Genetic variation, gene segregation and gene–centromere distances were analysed for three microsatellite loci denoted microsatellites 15, 60 and 73. These three repeat sequences were those first tested which showed polymorphism in our full-sib families and gynogenetic lines. The core sequences of microsatellites 15, 60 and 73 are composed of a total number of repeated motifs equal to 18, 13 and 19, respectively and the length of the longest run of uninterrupted repeats is 26 bp (13 dinucleotide repeats) for each of these satellites. Their core sequences and the primers designed for PCR amplification are listed in Table 1.

Genetic variation and gene segregation analysis

The different alleles found for the above three microsatellite loci and the allelic frequencies in the four populations are reported in Table 2. The three loci displayed a high polymorphism: despite the small size of the analysed samples, five alleles were found at microsatellite locus 15 and six alleles at microsatellite loci 60 and 73. The length of the allelic forms ranged from 214 and 224 bp for microsatellite 15, from 97 to 111 bp for microsatellite 60 and from 140 to 158 bp for microsatellite 73. The phenotypes of individuals from Gournay, Cuneo, Bresles and Artesiaga populations (two individuals per population) are shown in Fig. 1 for

Table 1 Core sequences of microsatellite loci 15, 60 and 73 and primers designed for amplification

Microsatellite locus	Core sequence	Primers
15	$(GT)_{13}$	5'TGCAGGCAGACGGATCAGGC3' 5'AATCCTCTACGTAAGGGATTTGC3'
60	$(CT)_{13}ACCA(CT)_3$	5'CGGTGTGCTTGTCAGGTTTC3' 5'GTCAAGTCAGCAAGCCTCAC3'
73	$(GT)_{13}TTATCT(GT)_3$	5'CCTGGAGATCCTCCAGCAGGA3' 5'CTATTCTGCTTGTAACTAGACCTA3'

Table 2 Observed allele frequencies at three microsatellite loci in four brown trout populations. The nomenclature of alleles is the following: (μSat.code) – (size of each allele in base pairs). Population size is 10 individuals per population

Locus μSat.	Allele	Gournay	Cuneo	Bresle	Artesiaga
μ 60	60–97	0.00	0.00	0.05	0.00
	60–101	0.60	0.65	0.65	0.85
	60–103	0.05	0.00	0.00	0.00
	60–105	0.35	0.30	0.30	0.10
	60–107	0.00	0.05	0.00	0.00
	60–111	0.00	0.00	0.00	0.05
H*		0.51	0.49	0.49	0.26
PIC**		0.42	0.41	0.41	0.24
μ 73	73–140	0.15	0.15	0.10	0.90
	73–144	0.00	0.00	0.15	0.00
	73–146	0.75	0.30	0.10	0.00
	73–148	0.10	0.45	0.65	0.10
	73–150	0.00	0.05	0.00	0.00
	73–158	0.00	0.05	0.00	0.00
H		0.41	0.68	0.53	0.18
PIC		0.37	0.63	0.49	0.16
μ 15	15–214	0.00	0.05	0.00	0.10
	15–218	0.05	0.10	0.30	0.90
	15–220	0.20	0.10	0.25	0.00
	15–222	0.50	0.60	0.15	0.00
	15–224	0.25	0.15	0.30	0.00
H		0.65	0.60	0.74	0.18
PIC		0.59	0.57	0.69	0.16
H averaged		0.52	0.59	0.59	0.21

*H: heterozygosity; **PIC: polymorphic information content.

microsatellite loci 15 and 73. Similar allelic frequencies were observed among populations for microsatellite 60 but substantial differences were found for microsatellites 15 and 73. The largest differences occurred between the Mediterranean and the Atlantic populations. The Artesiaga population also displayed the lowest heterozygosity and PIC values.

Gene segregations at these three microsatellite loci were analysed in three full-sib families. The presumed genotypes of the parents and the genotype frequencies observed in each progeny are reported in Table 3 for these loci and for three enzymatic loci. All the genotype frequencies observed in the progenies were in agreement with the expected mendelian proportions

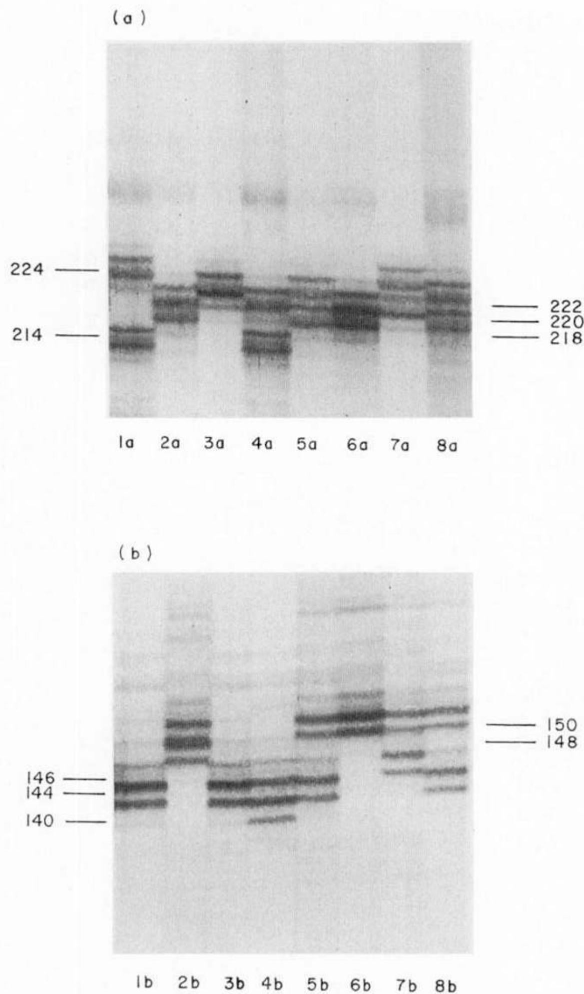

Fig. 1 Genetic variability for microsatellite loci 15 (a) and 73 (b). For both markers, PCR amplifications were performed with α^{35}S-dATP on the genomic DNA of individuals from four brown trout populations (two individuals per population): Gournay population in lanes 7a, 8a, 2b and 8b, Cuneo population in lanes 1a, 4a, 4b and 5b, Bresles population in lanes 3a, 5a, 1b and 7b and Artesiaga population in lanes 2a, 6a, 3b and 6b. Numbers on the sides of the figure refer to the length of allelic forms.

deduced from the parental genotypes proposed, except in full-sib family 1 for microsatellite 73 (chi-squared = 9.16; d.f. = 3; $0.01 < P < 0.05$). This significant value resulted from an excess of A′A′ genotypes. Contamination of this family by other groups could be also excluded as no abnormal segregation was found at the five other microsatellite and protein loci examined. The genotype frequencies of microsatellite 73 in full-sib family 2 being in agreement with the expected mendelian proportions, it is reasonable to assume that the slightly significant deviation from mendelian proportions observed in full-sib family 1 was from random variation.

Gene–centromere distances

Two steps are necessary to produce gynogenetic lines: (i) activation of the embryo development by egg fertilization with U.V. irradiated sperm; and (ii) restoration of diploidy by retention of the second polar body through an early heat shock. The complete mortality in the first two controls, B and BR, clearly shows that only individuals in which the second polar body was retained have survived in G groups (Table 4). The third control (B_2R) shows that rainbow trout sperm cannot be used to produce viable groups for the study of post-reduction rates in brown trout and also suggests that the G groups have no paternal contamination. This absence of paternal contamination is demonstrated for *FH-1**, *FH-2** protein loci as the brown trout and rainbow trout species have no common allele. In addition, the allelic form *MDH-3*75* has never been found in rainbow trout species. Hence, the gynogenetic lines exhibited only maternally inherited alleles and post-reduction rates can be directly estimated from the proportion of heterozygous genotypes observed in the gynogenetic lines. The values obtained are 0.44 for microsatellite 15, 0.68 and 0.49 for microsatellites 60, 0.18 and 0.17 for microsatellite 73; no significant difference was found between females (Table 3). At *MDH-3** and *4**, the post-reduction rates were 0.97 and 1.00 in lines 2 and 3, respectively, values commonly found for this locus in several salmonid species, including brown trout (Thorgaard *et al*., 1983; Guyomard, 1984, 1986; Allendorf *et al*., 1986; Seeb & Seeb, 1986).

Cross priming with other species

The primers of the brown trout microsatellites 15, 60 and 73 were tested for PCR amplifications on the DNA of individuals of a species of a closely related genus, that is on rainbow trout (*Oncorhynchus mykiss*) DNA. We obtained correct PCR amplifications for all these microsatellite markers without changing PCR conditions. Although these reactions were performed on the DNA of related individuals, we obtained several allelic forms for each locus which differed from those obtained in the four brown trout populations. The length differences between alleles were large enough to be distinguished in a 4 per cent NuSieve agarose gel stained by ethidium bromide (Fig. 2). Note that any allelic variation of a minimum of four bp for microsatellites 60 and 73 could be detected in a 4 per cent NuSieve agarose gel.

Table 3 Genotype distribution of three microsatellite and five enzymatic loci in the progenies of three full-sib families and three gynogenetic lines in brown trout

Full-sib family	Locus	Female	Male	Offspring					
				AA	AA′	AA″	A′A′	A′A″	A″A″
1	*MDH-3*,4**	100/100 (AA)	100/100 (AA)	48	—	—	—	—	—
	*MPI-2**	105/105 (AA)	105/105 (AA)	47	—	—	—	—	—
	μSat-60	101/101 (AA)	105/105 (A′A′)	—	42	—	—	—	—
	μSat-73	146/148 (A′A″)	140/146 (AA′)		8	9	21	10	—
	μSat-15	220/224 (AA″)	222/222 (A′A′)	—	28	—	—	19	—
2	*μSat-60*	101/105 (AA′)	101/101 (AA)	26	31	—	—	—	—
	μSat-73	140/146 (AA′)	140/146 (AA′)	15	26	—	11	—	—
3	*MDH-3**	100/75 (AA)	100/75 (AA)	17	24	—	13	—	—
	*MDH-4**	100/100 (AA′)	100/100 (AA′)	54		—		—	—
	μSat-60	101/105 (AA′)	101/101 (AA)	27	26	—	—	—	—

Gynogenetic line	Locus	Female	Offspring						
			AA	AA′	AA″	A′A′	A′A″	A″A″	*r*
1	*FH-1*,2**	100/100 (AA)	50	—	—	—	—	—	—
	MDH-3,4**	100/100 (AA)	50	—	—	—	—	—	—
	*MPI-2**	105/105 (AA)	50	—	—	—	—	—	—
	μSat-73	146/148 (A′A″)	—	—	—	26	9	15	0.18
	μSat-15	220/224 (AA″)	16	—	22	—	—	12	0.44
2	*MDH-3**	100/75 (AA′)	2	49	—	0	—	—	0.97
	*MDH-4**	100/100 (AA′)	51	—	—	—	—	—	—
	μSat-60	101/105 (AA′)	7	35	—	9	—	—	0.68
	μSat-73	140/146 (AA′)	21	8	—	18	—	—	0.17
3	*MDH-3**	100/75 (AA′)	0	35	—	0	—	—	1.00
	*MDH-4**	100/100 (AA)	35	—	—	—	—	—	—
	FH-1,2**	100/100 (AA)	35	—	—	—	—	—	—
	μSat-60	101/105 (AA′)	14	28	—	15	—	—	0.49

r: post-reductional rate.

Discussion

This study provides a first attempt to identify single locus microsatellites and evaluate their polymorphism in a fish species which has been intensively studied with enzymatic (Ryman, 1983; Ferguson, 1989) and mitochondrial markers (Bernatchez *et al.*, 1992).

Quantitative aspects

Brown trout genome turned out to contain numerous $(GT)_n$ and $(CT)_n$ microsatellites which occur, on average, approximately every 23 kb and 76 kb, respectively. These values are dependent on several assumptions (see Results) and, hence, must be treated with caution. However, they are close to those found for rat and human, namely one $(GT)_n$ microsatellite every 15 kb and 50 kb and one $(CT)_n$ microsatellite every 30 kb and 113 kb, respectively (Stalling *et al.*, 1991; Beckmann & Weber, 1992). The estimate of 109000 $(GT)_n$ microsatellite loci in the brown trout haploid genome is also of the same order of magnitude as the number of 200000 copies estimated in the haploid genome of salmon (similar size) by spot hybridization analysis (Hamada *et al.*, 1982).

The conditions of high stringency used for the screening of our partial genomic library probably result in the detection of microsatellites with a relatively large minimum number of repeated motives. As a matter of fact, the shortest $(GT)_n$ and $(CT)_n$ microsatellites detected contain a total number of 10 and 20 repeated motives, respectively. Results from Genbank search or from genomic libraries with conditions of lower stringency indicate that $(GT)_n$ microsatellites with fewer than 12 repeated motives are common within human and porcine genomes (Weber, 1990; Wintero *et*

Table 4 Survival rate at 21 days and 6 months (stage of analysis) after fertilization in the three brown trout gynogenetic lines

G. Line	*N*†	Day 21 (%)	Six months (%)	H‡ (%)	BR§	B2R††
1	820	43.9	41.1 (67.0)	0	0	0
2	850	39.5	33.7 (59.6)	0	0	0
3	790	35.7	17.0 (32.5)	0	0	0

Percentage of survival rate at 6 months, related to that observed in the full-sib family (same female) is given in parentheses.
†*N*: initial number of eggs used for the production of the gynogenetic lines.
‡Haploid control.
§Diploid brown trout/rainbow trout control.
††Triploid brown trout (female = $2n$)/rainbow trout (male = n) control.

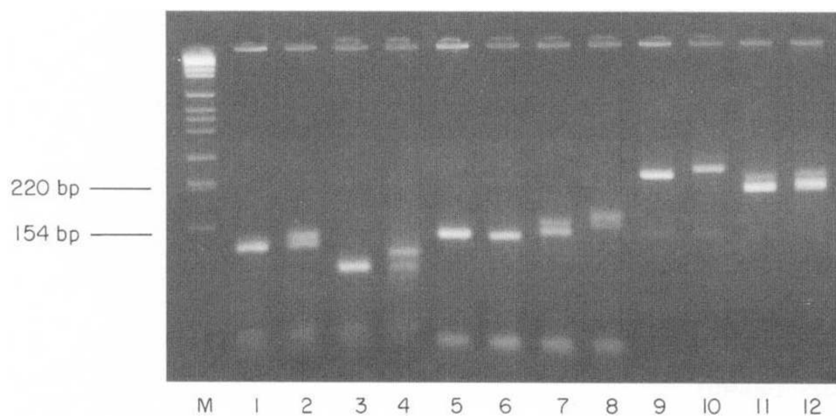

Fig. 2 Interspecific conservation of the flanking regions of microsatellite loci 15, 60 and 73. For each locus, PCR amplifications were performed on the genomic DNA of two related rainbow trout individuals and of the two brown trout individuals which showed the shortest or the longest allele in the four populations studied. Amplification products of microsatellite loci 60 (lanes 1–4), 73 (lanes 5–8) and 15 (lanes 9–12) were electrophoresed in a 4 per cent NuSieve agarose gel stained by ethidium bromide. The size in bp of each allele was precisely determined in DNA sequencing gel after α^{35}S-dATP PCR amplifications (data not shown). The individuals and their genotypes are as follows; rainbow trout: lanes 1-2-5-6-9-10 and genotypes 127/127, 131/135, 138/138, 136/136, 230/230, 240/240, respectively, brown trout: lanes 3-4-7-8-11-12 and genotypes 97/97, 101/111, 140/148, 150/158, 214/218, 214/224, respectively.

al., 1992). Consequently, the average distances between neighbouring $(GT)_n$ or $(CT)_n$ microsatellites are certainly overestimated in the brown trout genome.

Another point is the ratio of $(GT)_n$ and $(CT)_n$ microsatellites in brown trout: that is, approximately three $(GT)_n$ for one $(CT)_n$ repeat sequence. This relative prevalence of $(GT)_n$ microsatellites is also apparent in rat and human genomes, with very similar ratios.

Intraspecific genetic variation

This preliminary study shows that a high polymorphism can be detected at microsatellite loci of brown trout and that this polymorphism is inherited in a Mendelian way. The total number of alleles is five for one locus, six for other loci, giving an average of three per population. This is more than for protein loci, which exhibit generally one or two alleles per population and a total number of alleles rarely higher than three or four.

Previous electrophoretic studies with allozymes have shown that French natural populations of brown trout are divided in two major groups, one including all the Atlantic and domesticated stocks, and the other one encompassing all Mediterranean populations (Guyomard, 1989). The four populations considered in

this paper reflect this pattern: the domesticated stocks (Gournay and Cuneo) and the Bresles population all possess the allozymatic features of the Atlantic group, while the Artesiaga stock is very close to the Mediterranean populations found in the French Mediterranean drainage (R. Guyomard, unpublished data). Consistent with these data is the fact that the largest differences of allelic frequencies for two of the three microsatellite loci, were observed between the Mediterranean and the three other populations. It is likely that microsatellite variation will magnify the differentiation observed in enzyme surveys. This has been recently observed in intraspecific studies with microsatellites or other hypervariable markers in human and other primates (Edwards *et al.*, 1992; Ely *et al.*, 1992), turtle (Karl *et al.*, 1992) and oyster (Karl & Avise, 1992).

Gene–centromere distances and genetic mapping

This study shows that $(GT)_n$ and $(CT)_n$ microsatellites should be appropriate markers for gene mapping of the brown trout genome for several reasons.

1 Because of their high density in the genome, these repeat sequences should provide a number of markers sufficient to allow any region of the genome to be probed at increasing degrees of saturation.

2 The level of polymorphism of these markers is generally very high. The moderately high PIC values obtained for the three microsatellite loci analysed here are, however, not particularly conclusive in that respect. It is noteworthy that these microsatellites contain a relatively short uninterrupted run of dinucleotide repeats (13 repeats) and hence could not belong to the most polymorphic classes of loci. As a matter of fact, a positive correlation between the PIC values of a microsatellite and the number of repeats composing its longest uninterrupted run was evidenced in human by Weber (1990) and Hazan *et al.* (1992). It should nonetheless be very easy to have heterozygous individuals for the three microsatellite markers of this study by crossing individuals from genetically different populations (e.g. Cuneo × Artesiaga). Concerning brown trout genetic mapping in general, the crossing of individuals from the *Salmo trutta marmoratus* subspecies (North-Italian populations) with individuals from the *Salmo trutta trutta* subspecies (Atlantic populations) should be a suitable solution. Definitely, studies with enzymatic and mitochondrial markers have shown that the subspecies *marmoratus* is genetically very distant from Atlantic populations (Giuffra, unpublished data; Bernatchez *et al.*, 1992).

3 Analysis of post-reduction rates in gynogenetic lines issued from different females gives reproducible values. Gene–centromere distances can be estimated from the fraction of heterozygous half-tetrads (y). When interference is complete (no double cross-over), the distance in centimorgans will be equal to $100(y/2)$. Owing to the extremely high level of interference shown for enzyme loci in several species, including brown trout (Thorgaard *et al.*, 1983; Guyomard, 1984, 1986; Allendorf *et al.*, 1986; Seeb & Seeb, 1986), it is reasonable to use this relationship for calculating the gene–centromere distances for the three microsatellite loci studied. The gene–centromere distances range from 9 cM for microsatellite 73 to 25 and 34 cM for microsatellite 60, with an intermediate value of 24 cM for microsatellite 15. These values show that brown trout microsatellites can be located close as well as far from the centromere. These results have to be compared with post-reduction rates for protein loci which were found higher than 0.6 at all the 12 brown trout loci studied by Guyomard (1986).

4 Finally, it seems easy to find alleles which can be distinguished in NuSieve agarose minigel or polyacrylamide non-denaturating gel stained by ethidium bromide, a method that substantially reduces both cost and time required for mapping.

Conclusion

This study reveals that brown trout possesses hypervariable $(GT)_n$ and $(CT)_n$ microsatellites, with qualitative and quantitative characteristics very similar to those of $(GT)_n$ and $(CT)_n$ repeat sequences investigated so far in other vertebrate species. These microsatellites will provide powerful nuclear markers, which could be used in a large scope of genetic studies such as identity testing, population studies, linkage analysis and genome mapping. Important in that respect is that primers designed for brown trout (*S. trutta*) can be used in another salmonid species of economical interest, the rainbow trout (*O. mykiss*) and certainly in other related species such as salmon (*S. salar*). This property accounts for the methodology applied and should contribute to a rapid diffusion and use of microsatellite markers in salmonid species and fishes in general.

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