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TECHNICAL NOTE

Development of new microsatellite markers derived from expressed sequence tags for the great scallop (*Pecten maximus*)

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Abstract Nine microsatellites were developed from expressed sequence tags sequences for the great scallop (*Pecten maximus*), and were tested in three natural populations. These markers displayed low to moderate levels of variability. On average across all populations, the number of alleles per locus ranged from 1.333 to 8.667, and the observed and expected heterozygosities ranged from 0.029 to 0.703 and 0.028 to 0.711, respectively. All loci conformed to Hardy–Weinberg equilibrium, except PmGC20 in one single population. These new loci might be very useful for the genetic monitoring of the possible impact of aquaculture on the genetic diversity of native scallop populations.

Keywords Great scallop · *Pecten maximus* · Microsatellites · Expressed sequence tags (EST)

The great scallop (*Pecten maximus*) is a highly valuable shellfish resource that is widely exploited in France and United-Kingdom (Beaumont and Gjedrem 2007). This bivalve has been significantly manipulated with many transfers of individuals reported among natural populations since the 1970's (Beaumont 2000). In particular, some stocks have been regularly enhanced with hatchery-raised spat in order to ensure the sustainability of scallop fisheries. Such manipulations necessitate a good knowledge of the population genetics of the species to prevent any alteration of the genetic diversity of wild populations. However, the genetic structure of great scallop populations remains poorly documented, with studies based only on allozymes or mitochondrial DNA (Beaumont et al. 1993; Rigaa et al. 1997; Wilding et al. 1997; Saavedra and Peña 2005). Only nine microsatellites have been isolated so far (Watts et al. 2005), but two of these markers do not seem usable because of significant heterozygote deficiencies and possible null alleles. As a consequence, new microsatellites associated with expressed sequence tags (ESTs) were developed to complete the existing set of markers.

A total of 1,122 EST sequences (DN793124–DN794245) were downloaded from the Genebank database (http://www.ncbi.nlm.nih.gov). Firstly, overlapping and contiguous sequences were assembled with CAP3 (Huang and Madan 1999), using the online interface available on the PBIL website (http://pbil.univ-lyon1.fr/cap3.php). Microsatellites were identified with TANDEM REPEAT FINDER (Benson 1999), and primers were designed manually for 22 loci showing a minimum of five repeats. A M13 tail (5′-CACGACGTTGTAAAACGAC) was added to the 5′-end of each forward primer to reduce the genotyping cost (Schuelke 2000). In addition a PIG-tail (5′-GTTTCTT) was added to the 5′-end of each reverse primer to avoid genotyping errors due to "plus-A" PCR artefacts (Brownstein et al. 1996).

The markers were tested in three populations: Baie de Seine (BS; France), Rade de Brest (RB; France) and Galicia (GA; Spain). Genomic DNA was extracted from the adductor muscle (GA) using a classical phenol–chloroform method, or from the gills (BS and RB) with a CTAB phenol–chloroform protocol (Jolly et al. 2003). Each marker was amplified by polymerase chain reaction (PCR)

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Table 1 Polymorphic EST-linked microsatellites for *P. maximus*

Locus	Primers	Primer sequence (5′ > 3′)	Nb of PCR cycles	Ta[a] (°C)	$MgCl_2$ (mM)	Size range[b] (bp)	Motif	GenBank accession number(s)	Gene annotation
PmGC01	PmGC01Fb	CAAAAATGCAAAGCAGAAACAAC	25	55	1.5	125–134	$(ATG)_8$	DN793480, DN793845, DN794145	
	PmGC01R	ATATCATAGAAGAGCTTTATTAAGG							
PmGC03	PmGC03Fb	TGTAAGACTTTCCCTTCACTC	25	56	1.5	242–246	$(AT)_7$	DN793150, DN793164, DN793241, DN793282, DN793321, DN793333, DN793430, DN793546, DN793714, DN793747, DN793799, DN793823, DN793835, DN793929, DN794061, DN794245	Similar to myosin essential light chain
	PmGC03Rb	GTTCACATAGTCCTCAACCTC							
PmGC05	PmGC05Fb	AATTGTACTTTCAATCATAAACTGAG	25	55	2.5	175–201	$(TA)_8$	DN793893	
	PmGC05Rb	ACAGTAATCTAGGAAACACAATG							
PmGC06	PmGC06F	ACAAAGGTTTCTTGGCAGAAG	25	56	2.0	90–94	$(TG)_6$	DN793464	
	PmGC06Rb	AAGTCAGGATGTATCTACACC							
PmGC10	PmGC10Fb	TGTGACACTATATGACGTCAC	25	55	2.0	187–193	$(AT)_7$	DN793535, DN793863	
	PmGC10Rb	AACCTTCTTTCATCAGTGTAAAC							
PmGC14	PmGC14F	AGGCGAATATTGTATCCATCC	25	56	2.5	293–297	$(AT)_2$ $(TGT)(AT)_4$	DN794143	
	PmGC14R	TATCCATTTCATCACTGACCG							
PmGC16	PmGC16Fb	TTGTTTGTTCAGTGAATAACCAC	25	55	1.5	182–187	$(TG)_2$ $(TA)(TG)_4$	DN793491	
	PmGC16Rb	CCCTTACACATCTGATAACTG							
PmGC18	PmGC18F	TCCACTCCTTTTCTGTGACG	25	55	2.0	219–223	$(AT)_3T(AT)_3$	DN793553	
	PmGC18Rb	TGAGAAATATATGAGTTGACTATAG							
PmGC20	PmGC20Fb	GTAAATGTTTTTTACTTGTCTCTTG	30	56	2.0	105–107	$(AT)_4T(AT)$	DN793441	
	PmGC20Rb	GGTATACATGTGTAAAGCGTG							

[a] Annealing temperature for the first cycle of the touchdown PCR; [b] Including the PIG-tail and the M13 tail

in 10 μL volume, containing 1× reaction buffer (Uptima), 1.5–2.5 mM $MgCl_2$, 0.074 mM each dNTP, 0.05 μM M13 primer end-labelled with IRDye 800 (Li-Cor), 0.05 μM forward primer, 0.4 μM reverse primer, 0.025 U μL^{-1} Uptitherm DNA polymerase (Uptima) and 1 μL DNA template. PCR amplifications were performed in a Gene-amp PCR System 9700 (Perkin Elmer). A touchdown procedure was incorporated in the thermal cycling regime to increase the stringency of the PCRs: 94 °C for 3 min, T_a for 2 min, 72 °C for 30 s, (94 °C for 30 s, $T_a - 1$ °C for 30 s [−1 °C per cycle until 50 °C], 72 °C for 30 s) × 5–6 cycles, (94 °C for 30 s, 50 °C for 30 s, 72 °C for 30 s) × 19–23 cycles, 72 °C for 5 min. Five μL of denaturing loading buffer were added to the PCR products and 1 μL was loaded on 8 % denaturing polyacrylamide gels of 25 cm with 1 % TBE buffer. The gels were run on an automated sequencer IR2 (Li-Cor) during 1–3 h at 1,500 V and 50 °C, and were analysed with GENE PROFILER (Scan Analytics). Each gel was scored by two independent readers to limit genotyping errors. Seventeen of the 22 tested markers produced clear bands that were confidently scored, and nine of the loci were polymorphic (Table 1).

The number of alleles (N_a), and the level of observed (H_o) and expected (H_e) heterozygosities were calculated for each polymorphic locus in each population with GENETIX v.4.05 (Belkhir et al. 1996). The variability was low to moderate, with the total N_a ranging from two to 13 (Table 2). All loci were polymorphic in each population, except PmGC06 in GA, and PmGC18 in BS and GA. On average, accoss all three populations, N_a varied from 1.333 to 8.667, and H_o and H_e ranged respectively from 0.029 to 0.703 and 0.028 to 0.711. The lowest heterozygosity was observed for PmGC06 in RB ($H_o = 0.040$; $H_e = 0.040$) and the highest was displayed by PmGC05 in BS ($H_o = 0.844$; $H_e = 0.832$). Departures from Hardy–Weinberg equilibrium were tested for each locus and location by estimating F_{IS} values according to Weir and Cockerham with GENEPOP v.4.0.11 (Rousset 2008) and using the Markov chain method (10,000 dememorisation steps, 100 batches, 5,000 iterations). A significant deficit in heterozygotes was found only for PmGC20 in RB after applying a sequential Bonferroni correction for multiple comparisons (Rice 1989), and the other eight loci conformed to Hardy–Weinberg equilibrium. No significant linkage disequilibrium was detected with GENEPOP v.4.0.11 using the Markov chain method (10,000 dememorisation steps, 100 batches, 5000 iterations) and Fisher's exact test.

This new set of microsatellites will be useful for further population genetics studies on *P. maximus*. Moreover, given the aquaculture perspectives for this species, combining these new markers with the existing ones might provide a valuable tool for the genetic monitoring of the possible impacts of hatchery practices on the genetic variability of native scallop populations.

Table 2 Number of alleles (N_a), observed (H_o) and unbiased expected (H_e) heterozygosities, and F_{IS} for each location and each polymorphic locus

Loci		Populations			Mean
		BS (N = 39)	RB (N = 28)	GA (N = 23)	
PmGC01	N_a	3	3	2	2.667
	H_o	0.436	0.200	0.046	0.227
	H_e	0.363	0.334	0.046	0.247
	F_{IS}	−0.205	0.405*	0.000	
PmGC03	N_a	3	3	3	3.000
	H_o	0.222	0.227	0.318	0.256
	H_e	0.206	0.312	0.413	0.310
	F_{IS}	−0.079	0.276	0.234	
PmGC05	N_a	9	11	6	8.667
	H_o	0.844	0.815	0.450	0.703
	H_e	0.832	0.797	0.504	0.711
	F_{IS}	−0.015	−0.022	0.109	
PmGC06	N_a	3	2	1	2.000
	H_o	0.077	0.040	–	0.039
	H_e	0.123	0.040	–	0.054
	F_{IS}	0.377	0.000	–	
PmGC10	N_a	4	3	2	3.000
	H_o	0.387	0.391	0.500	0.426
	H_e	0.609	0.552	0.429	0.530
	F_{IS}	0.368*	0.295	−0.200	
PmGC14	N_a	3	2	2	2.333
	H_o	0.294	0.261	0.500	0.352
	H_e	0.300	0.348	0.500	0.383
	F_{IS}	0.019	0.254	0.000	
PmGC16	N_a	2	2	2	2.000
	H_o	0.316	0.158	0.143	0.206
	H_e	0.305	0.309	0.143	0.252
	F_{IS}	−0.037	0.495	0.000	
PmGC18	N_a	1	2	1	1.333
	H_o	–	0.087	–	0.029
	H_e	–	0.085	–	0.028
	F_{IS}	–	−0.023	–	
PmGC20	N_a	2	2	2	2.000
	H_o	0.278	0.071	0.227	0.192
	H_e	0.318	0.486	0.460	0.421
	F_{IS}	0.127	**0.855*** **	0.512*	

F_{IS} was estimated according to Weir and Cockerham and was tested using the Markov chain method with 5,000 iterations (* $p < 0.05$, *** $p < 0.001$). Bold print indicates F_{IS} values that remained significant after sequential Bonferroni correction

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