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Polymorphic microsatellite loci in the European plaice, *Pleuronectes platessa*, and their utility in flounder, lemon sole and Dover sole

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Microsatellite loci are one category of genetic markers that are frequently highly polymorphic. As such they have proved to be extremely useful tools for examining the population biology of many different taxa, and offer the potential to discriminate among fish stocks. Here, four new polymorphic microsatellite loci in the European plaice, *Pleuronectes platessa* are reported. In addition, data are provided on the utility of all published plaice microsatellite loci in flounder (*P. flesus*), lemon sole (*Microstomus kitt*) and Dover sole (*Solea solea*).

The European plaice, *Pleuronectes platessa* L., is an economically important flatfish that inhabits the shelf waters of the north-eastern Atlantic. Plaice annually migrate and have specific spawning grounds within regional seas. Phenotypic characteristics suggest that this species comprises several stocks (e.g. Nash et al., 2000), but the amount of interbreeding among fish from different areas is not known. For many years, allozymes have been used to attempt to elucidate the stock structure and population dynamics of marine teleosts (e.g. Borsa et al., 1997; Exadactylos et al., 1998). Allozymes have not always proved useful for stock discrimination, however, because they often suffer from low variability; furthermore, most samples for allozyme electrophoresis must be collected by killing the study organism, stored frozen immediately upon collection, and degrade relatively quickly. By contrast, high-quality DNA can be extracted non-destructively from small tissue samples and conveniently maintained in ethanol at ambient temperature; once extracted, DNA may be stored almost indefinitely without a significant loss in quality and easily transferred between laboratories. Thus there has been an increasing focus on using DNA markers for population analysis. In particular, the high variability of microsatellites has made them the 'markers of choice' for investigations into many aspects of closely related populations (see Jarne & Lagoda, 1996; Sunnucks, 2000), and are likely to prove useful for fish stock discrimination (see Carvalho & Pitcher, 1995). Microsatellites have been used to investigate the population-genetic structuring of several marine teleost species (e.g. McConnell et al., 1997; Rico et al., 1997; O'Connell et al., 1998), but, in general, the initial difficulty in establishing microsatellite loci has impeded the widespread use of this class of genetic markers for ecological research. To date only seven variable microsatellite loci have been described for European plaice (Watts et al., 1999), and they were not examined for cross-species amplification. Here we report an additional four polymorphic microsatellite loci for the European plaice as well as information on the usefulness of all of these markers in flounder (*P. flesus* L.), lemon sole (*Microstomus kitt* Walbaum) and Dover sole (*Solea solea* Quensel).

A size selected genomic library (400–900 base pairs) was constructed from DNA isolated from a single European plaice collected from Port Erin Bay, Isle of Man. DNA was digested with *Sau*IIIA, ligated into pUC18 plasmid digested with *Bam*H1 and then transformed into *Escherichia coli* competent cells (Stratagene). The library was screened using γ^{33}P-ATP labelled $(CA)_{10}$, $(CAG)_7$ and $(AAG)_7$ oligonucleotide probes; positive clones were sequenced using an ABI373 automated sequencer. Primers flanking the repeat regions were designed using Primer3 (http://www-genome.wi.mit.edu/cgi-bin/primer/primer3.www.cgi).

LIST1009 and LIST1010 are situated 319 bases apart along a continuous 520 base pair region of DNA. All DNA sequences have been deposited in Genbank (http://www3.ncbi.nlm.nih. gov/) and may be examined using their accession numbers (Table 1).

Polymerase chain reaction (PCR) was undertaken in a 10 μl final volume using Reddy-Load PCR Mix (ABgene) on a PTC-100–96V MJ thermal cycler (MJ Research Inc.). PCR conditions were: (i) an initial denaturation for 1 min at 95°C; (ii) six cycles of denaturation for 30 s at 95°C, 30 s at 50°C and extension for 45 s at 72°C; (iii) a subsequent 26 cycles of 30 s denaturation at 92°C, 30 s at 50°C and 55 s at 72°C; and (iv) a final extension at 72°C for 30 min. The annealing temperature was increased to 55°C for LIST1009 and LIST1010. Each reaction contained 50 ng of template DNA, 75 mM Tris-HCl, 20 mM $(NH)_4SO_4$, 0.01% (v/v) Tween 20, 0.2 mM of each dNTP, 1.5 mM $MgCl_2$, 1.4 pmol of each primer and 0.25 units of *Taq* polymerase (ABgene). The forward primers were labelled with either IRD800 or IRD700 fluorescent dye (MWG Biotech, Germany). PCR products were visualized by electrophoresis through an 8% denaturing polyacrylamide gel on a LiCor 4200 DNA sequencer.

At each locus polymorphisms were studied on 18 juvenile European plaice caught from the coasts of North Wales and Anglesey on 16 June 2000. All loci were variable in *P. platessa*, although the two tri-nucleotide repeat loci were less heterozygous than the two loci with di-nucleotide repeat arrays (Table 1). With the exception of LIST1006, which has a lack of variability, the observed heterozygosities for the microsatellites described here fall within the range of observed heterozygosity values (H_o=0.14–0.82) previously described by Watts et al. (1999) for seven plaice microsatellites.

Using the same PCR conditions described above or in Watts et al. (1999), the amplification of all published microsatellite loci

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Table 1. *Characterization of four polymorphic microsatellite loci in the European plaice,* Pleuronectes platessa *L.*

Locus	Primer sequences (5′→3′)	Repeat array	N_a	Size range (bp)	H_o	H_e	Genbank accession number
LIST1006	CTCATGGGATTGCTGTGCT TTTCAAGCCCTGCTACAACA	$(TGC)_2TGT$ $(TGC)_4TGG$	2	107–110	0.06	0.05	AF314049
LIST1007	CACGCTTTCTCCTGCTTGAT GACACCACAGGTTGCCATTT	$(GAA)_7$	3	119–123	0.44	0.41	AF314050
LIST1009	ATGAGATTTGGGCTCGTGTC AGGCAGAGATGCGAGGTG	$(AC)_{10}$	2	108–110	0.56	0.44	AF315078
LIST1010	TCCATTTCTCTCCTTCTCCTTG CCAGACAGCCCTGGTGAG	$(AC)_{10}$	2	93–95	0.65	0.50	AF315078

Sample size is 18 individuals at each locus; N_a, number of alleles; H_o, observed heterozygosity; H_e, expected heterozygosity.

Table 2. *Characterization of successful cross-species amplification for four microsatellite loci in flounder (*Pleuronectes flesus *L.), lemon sole (*Microstomus kitt *Walbaum) and Dover sole (*Solea solea *Quensel).*

Locus	Species	N_a	Size range (bp)	H_o	H_e
LIST1001	Flounder	2	86–88	0.40	0.48
	Lemon sole	5	150–200	1.00	0.74
LIST1003	Flounder	1	140	0.00	0.00
	Lemon sole	3	142–180	0.70	0.66
LIST1006	Flounder	1	110	0.00	0.00
	Lemon sole	2	110–113	0.40	0.32
	Dover sole	1	137	0.00	0.00
LIST1007	Flounder	3	115–122	0.50	0.62
LIST1009	Flounder	4	120–126	0.90	0.69
LIST1010	Flounder	1	91	0.00	0.00
	Lemon sole	1	87	0.00	0.00
Pplgst2	Flounder	2	119–121	0.40	0.48
	Lemon sole	1	117	0.00	0.00

Sample size is ten individuals for all species at each locus; N_a, number of alleles; H_o, observed heterozygosity; H_e, expected heterozygosity.

in flounder was examined, lemon sole and Dover sole using DNA extracted from the fin clips of ten hatchery-reared animals for each species. Data on microsatellite loci that amplified but were invariable are included in this report as they may prove to be polymorphic in natural populations. In flounder, seven microsatellite loci amplified PCR products that were close to size of the corresponding plaice loci, although only LIST1001, LIST1007, LIST1009 and *Ppl*gst2 were polymorphic (Table 2). Of the five loci that amplified for lemon sole, LIST1001, LIST1003 and LIST1006 were variable. In Dover sole a single locus, LIST1006, produced unambiguous PCR products, but only amplified a single allele (Table 2).

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REFERENCES

Borsa, P., Blanquer, A. & Berrebi, P., 1997. Genetic structure of the flounders *Platichthys flesus* and *P. stellatus* at different geographic scales. *Marine Biology*, **129**, 233–246.

Carvalho, G. & Pitcher, T.J., 1995. *Molecular genetics in fisheries.* London: Chapman & Hall.

Exadactylos, A., Geffen, A.J. & Thorpe, J.P., 1998. Population structure of the Dover sole, *Solea solea* L., in a background of high gene flow. *Journal of Sea Research*, **40**, 117–129.

Jarne, P. & Lagoda, P.J.-L., 1996. Microsatellites, from molecules to populations and back. *Trends in Ecology and Evolution*, **11**, 424–429.

McConnell, S.K.J., Ruzzante, D.E., O'Reilly, P.T., Hamilton, L. & Wright, J.M., 1997. Microsatellite loci reveal highly significant differentiation among Atlantic salmon (*Salmo salar* L.) from the east coast of Canada. *Molecular Ecology*, **6**, 1075–1089.

Nash, R.D.M., Witthames, P.R., Pawson, M. & Alesworth, E., 2000. Regional variability in the dynamics of reproduction and growth of Irish Sea plaice, *Pleuronectes platessa* L. *Journal of Sea Research*, **44**, 55–64.

O'Connell, M., Dillon, M.C., Wright, J.M., Bentzen, P., Merkouris, S. & Seeb, J., 1998. Genetic structuring among Alaskan Pacific herring populations identified using microsatellite variation. *Journal of Fish Biology*, **53**, 150–163.

Rico, C., Ibrahim, K.M., Rico, I. & Hewitt, G.M., 1997. Stock composition in North Atlantic populations of whiting using microsatellite markers. *Journal of Fish Biology*, **51**, 462–475.

Sunnucks, P., 2000. Efficient genetic markers for population biology. *Trends in Ecology and Evolution*, **15**, 199–203.

Watts, P.C., Nash, R.D.M., George, S.G. & Kemp, S.J., 1999. Isolation and characterization of microsatellite loci in the European plaice, *Pleuronectes platessa* L. (Teleostei: Pleuronectidae). *Molecular Ecology*, **8**, 2151–2152.

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