Population genetics of plaice (pleuronectes platessa L.) in Northern Europe
Hoarau, Galice Guillaume

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Chapter 2

NEW MICROSATELLITE PRIMERS FOR PLAICE, *Pleuronectes platessa* L. (TELEOSTEI : PLEURONECTIDAE)

Hoarau G., Cook D., Stam W.T., Olsen J.L.

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Abstract

Seven microsatellite loci were developed for *Pleuronectes platessa*, a commercially important flatfish found throughout northern European waters. The number of alleles per locus ranged from 11-33 and heterozygosities from 0.74-0.96. Cross reactivity was tested against six other flatfish species. Significant amplification was found in all species from one to all seven of the loci.
Plaice (*Pleuronectes platessa*) is one of the most important flatfish for North Sea fisheries. Its life history (e.g. Van Der Veer 1986, Rijnsdorp and Pastoors 1995) is of interest because high dispersal of pelagic eggs and larvae, as well as adult migration are expected to reduce population differentiation; whereas adults faithful to several discrete spawning grounds could promote population isolation. Previous studies of plaice using allozymes did not reveal any evidence for population differentiation (Purdom *et al.* 1976, Ward and Beardmore 1977). Microsatellite markers may, therefore, be more appropriate genetic makers for exploring population structure and the mating system.

A size selected library (300-800 bp) was prepared and screened simultaneously with synthetic (GT)$_{15}$ and (GA)$_{15}$ oligonucleotides radiolabeled with [$^{32}$P]ATP, as described in Brooker *et al.* (1994). Approximately 250 positive clones were selected after two rounds of screening and the 50 showing the stronger signal were sequenced. Fifteen sets of primers were designed of which 7 were selected according to the clarity of the banding patterns. Polymorphism testing was done against a panel of 48 individuals collected from a broad geographical range (Table 1).

Total genomic DNA was extracted using a CTAB protocol modified from De Jong *et al.* (1998). PCR amplification was carried out in 10-µl volumes containing 1 µl of 1/10 diluted DNA, 1X reaction buffer (Promega), 0.2 mM of each dNTP, 2 mM MgCl$_2$, 0.25 U *Taq* DNA polymerase (Promega) and each primer at the concentration indicated in Table 1. One primer of each pair was end-labelled with a fluorescent dye.

PCR amplification was performed in either a PTC100 (MJ Research) or a Mastercycler gradient (Eppendorf) thermal cycler and consisted of an initial denaturation at 94 °C for 1 min; 3 cycles of denaturation at 94 °C for 1 min, annealing at 48-58 °C for 1 min, and extension at 72 °C for 30 s. This was followed by 30 cycles of denaturation at 94 °C for 20 s, annealing at 48-58 °C for 15 s, and extension at 72 °C for 12 s; and a final extension at 72°C for 10 min. In order to reduce genotyping error, the PCR products of loci PL52, PL142 and PL167, were treated with T4 DNA polymerase to remove the extra bases added by the *Taq* polymerase (Ginot *et al.* 1996). After PCR amplification, 0.4 U T4 polymerase (Promega) was added to the PCR products and the mixture was incubated at 37 °C for 30 min. PCR products were visualized using an ABI Prism-377 automatic
Table 1. Characteristics of 7 microsatellites loci developed for *Pleuronectes platessa*. N = 48. HE and HO are expected and observed heterozygosity. T4 indicate the use of T4 polymerase treatment of PCR product (see text).

<table>
<thead>
<tr>
<th>Locus</th>
<th>Repeat motif</th>
<th>Primer sequences (5’-3’)</th>
<th>Size range (bp)</th>
<th>No. of alleles</th>
<th>HE</th>
<th>H0</th>
<th>Accession number</th>
<th>Annealing temp.</th>
<th>Primer concentration</th>
<th>Special</th>
</tr>
</thead>
<tbody>
<tr>
<td>PL06</td>
<td>(GT)$<em>7$TT(GT)$</em>{25}$</td>
<td>F: AACTGATTTACTTTACTTTTTGTC R:*GTCGTCCTCTCCAAATTGAC</td>
<td>166-256</td>
<td>28</td>
<td>0.95</td>
<td>0.61</td>
<td>AF406745</td>
<td>50°C</td>
<td>0.2µM</td>
<td></td>
</tr>
<tr>
<td>PL09</td>
<td>(CA)$_{30}$</td>
<td>F: GTAATTTTCTAACATATGGAGG R:*TCACCCCAAAGCTGCGT</td>
<td>126-234</td>
<td>24</td>
<td>0.74</td>
<td>0.49</td>
<td>AF406746</td>
<td>50°C</td>
<td>0.5µM</td>
<td></td>
</tr>
<tr>
<td>PL52</td>
<td>(GT)$_{21}$</td>
<td>F:*GAACCTGGATCCTGCCTGCT R: CTTTTTGACCAGTGTTACCCAC</td>
<td>72-166</td>
<td>21</td>
<td>0.92</td>
<td>0.55</td>
<td>AF406747</td>
<td>58°C</td>
<td>0.2µM</td>
<td>T4</td>
</tr>
<tr>
<td>PL92</td>
<td>(CA)$<em>{10}$CGAAAA(CA)$</em>{2}$</td>
<td>F: TCTGGCAAAGTCTCTGTCG R:*GTATTGCTCAGGCTATCTTG</td>
<td>74-210</td>
<td>21</td>
<td>0.91</td>
<td>0.87</td>
<td>AF406748</td>
<td>48°C</td>
<td>0.1µM</td>
<td></td>
</tr>
<tr>
<td>PL115</td>
<td>(CA)$_{33}$</td>
<td>F:*ACCTTCCTATGGATGCTGG R: TTCATGAAAGCTCTCCTGGA</td>
<td>136-168</td>
<td>11</td>
<td>0.75</td>
<td>0.77</td>
<td>AF406749</td>
<td>53°C</td>
<td>0.2µM</td>
<td></td>
</tr>
<tr>
<td>PL142</td>
<td>(GT)$<em>6$TT(GT)$</em>{22}$</td>
<td>F: ACTGTTACCTCGCTCTAGTG R:*GAGATGAAAAAGACTGGTTGCC</td>
<td>122-190</td>
<td>24</td>
<td>0.92</td>
<td>0.86</td>
<td>AF406750</td>
<td>51.5°C</td>
<td>0.2µM</td>
<td>T4</td>
</tr>
<tr>
<td>PL167</td>
<td>(GT)$_{24}$</td>
<td>F:*CAGACAAAAATGAATGACAGG R: CAAGCAGCAGTCCCCAGCA</td>
<td>116-222</td>
<td>33</td>
<td>0.96</td>
<td>0.79</td>
<td>AF406751</td>
<td>51°C</td>
<td>0.2µM</td>
<td>T4</td>
</tr>
</tbody>
</table>

Table 2. Cross species amplification of the microsatellites developed for Pleuronectes platessa. + : amplification, - : no amplification or unreadable peaks, ? : not tested.

<table>
<thead>
<tr>
<th></th>
<th>PL06</th>
<th>PL09</th>
<th>PL52</th>
<th>PL92</th>
<th>PL115</th>
<th>PL142</th>
<th>PL167</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pseudopleuronectes americanus</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Platichthys flesus</em></td>
<td>?</td>
<td>+</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td><em>Microstomus kit</em></td>
<td>?</td>
<td>-</td>
<td>?</td>
<td>+</td>
<td>+</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td><em>Paralichthys lethostigma</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><em>Solea solea</em></td>
<td>?</td>
<td>-</td>
<td>?</td>
<td>+</td>
<td>-</td>
<td>?</td>
<td>?</td>
</tr>
</tbody>
</table>
sequencer (Applied Biosystems) with an internal lane standard and GENESCAN™ software (Applied Biosystems) for determination of allele sizes.

Observed and expected heterozygosity was computed using GENETIX software (Belkhir et al. 2000). The characteristics of the 7 loci are described in Table 1. No linkage disequilibrium was detected among the 21 pairwise comparisons. Cross species amplification was tested on 6 other flatfish species (Table 2).

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References

