**Linking scales of life-history variation with population structure in Atlantic cod**

**Wright P.J.1\*,a, Doyle A.1,2,3,a, Taggart, J.B.2 & Davie A2**

1 Marine Scotland Science, 375 Victoria Road, Aberdeen, AB11 9DB

2 Institute of Aquaculture, University of Stirling, Stirling FK9 4LA

3Joint Nature Conservation Committee, Inverdee House, Baxter Street, Aberdeen AB11 9QA

**\* Correspondence:**Corresponding Author: P.J. Wright  
[P.J.Wright@marlab.ac.uk](mailto:P.J.Wright@marlab.ac.uk)

a. Wright and Doyle should be considered joint first authors.

**Supplementary Methods and Data**

**Supplementary Methods**

**ddRAD library preparation, screening and SNP selection**

The ddRAD library preparation protocol was based on the methodology originally reported by Peterson et al. (2012), with modifications / refinements as described in Brown et al. (2016) and Manousaki et al. (2016). Each of the 120 cod DNA samples (c. 25 ng) was simultaneously digested by two high fidelity restriction enzymes (RE): SbfI (CCTGCA|GG recognition site), and SphI (GCATG|C recognition site), both sourced from New England Biolabs, (NEB) UK. Digestions were incubated at 37°C for 90 min, using 0.6 U of each RE in 1× CutSmart Buffer (NEB), in a 6 µL total reaction volume. After cooling the reactions to room temperature, 3 µL of a premade barcode / adapter mix was added to the digested DNA, and incubated at room temperature for 10 min. This adapter mix comprised individual-specific barcoded combinations of P1 (SbfI-compatible) and P2 (SphI-compatible) adapters at 6 nM and 72 nM concentrations respectively, in 1× reaction buffer 2 (NEB). Adapters were compatible with Illumina sequencing chemistry (see Peterson et al. 2012 for details). The barcoded adapters were designed such that adapter– genomic DNA ligations did not reconstitute RE sites, while residual RE activity limited concatemerization of genomic fragments during the ligation step. The adapters included an inline five- or seven-base barcode for sample identification. Ligation was performed over 3.25 hr at 22°C by addition of a further 3 µL of a ligation mix comprising 4 mM rATP (Promega, UK), and 2000 cohesive-end units of T4 ligase (NEB) in 1× CutSmart buffer. The ligated samples were then heat denatured at 65°C for 20 min, cooled, and all 120 reactions combined into a single pool. The pooled sample was column-purified (MinElute PCR Purification Kit, Qiagen, UK), and eluted in 70 µL EB buffer (Qiagen, UK). Size selection of fragments was performed by chilled agarose gel separation at 4°C (1.1% gel; 0.5x TAE buffer; 4°C; 10V.cm-1 for 60 min). and excision of gel containing fragments ranging from approximately 400 to 700 base pairs (bp) in size. Following gel purification (MinElute Gel Extraction Kit, Qiagen, UK), the eluted size-selected template DNA (55 µL in EB buffer) was PCR amplified (14 cycles PCR; 32 separate 12.5-µL reactions, each with0.35 µL template DNA) using a high fidelity Taq polymerase (Q5 Hot Start High-Fidelity DNA Polymerase, NEB). The PCR reactions were combined (400 µL total), and column-purified (MinElute PCR Purification Kit). The 60 µL eluate, in EB buffer, was then subjected to a further size-selection cleanup using an equal volume of AMPure magnetic beads (Perkin-Elmer, UK), to maximize removal of small fragments (less than ca. 200 bp). The final library was eluted in 19 µL EB buffer and sequenced in house, over two consecutive full Illumina MiSeq runs (v2 chemistry, 300 cycle kit, 161 base paired-end reads; Illumina, Cambridge, UK). The sequence data are lodged in EBI Project accession number PRJEB41987.

The raw MiSeq generated reads (16.9M & 17.2M paired-end reads for runs 1 & 2 respectively) were processed using a software pipeline designed specifically for RAD analysis, Stacks (v.1. 27; Catchen et al. 2013). First, the ‘process\_radtags’ function was used to demultiplex the samples. Individual specific combinatorial P1 and P2 barcodes are given in Supplemental Table S1). During this step sequence reads with quality scores below 10, missing either restriction site or with ambiguous barcodes were discarded. Barcodes were removed and all sequences 3’ end trimmed to be 144 bases long. This process reduced the dataset to c. 28.6 M paired-end reads assigned among the 120 cod. For the purposes of subsequent analysis paired-end reads were treated as separate entities, read 2 sequences being appended to read 1 sequence files. Given the library size range and trimmed read length, few, if any, paired-end reads would have detectable overlap. Satisfactory reads from all 120 individuals were obtained and were assigned to RAD loci and genotypes using the ‘denovo\_map.pl’ component of Stacks. The key parameter values employed in identifying RAD loci were; a minimum stack depth (m) of 10, a maximum of 2 mismatches allowed in a locus (M) in an individual and up to 1 mismatch between loci when building the catalog (n). An average of c. 7 K stacks (loci) were detected per individual with mean coverage per locus being 34.5 × (range 14.7 ×– 76.7 ×). Further STACKS metrics are provided in Supplemental Table S1). The ‘export’ component of Stacks was used to provide an Excel compatible database file containing those RADtags containing a single SNP only. Within Excel the dataset was further filtered, using inbuilt cell count and sort functions, to include only those RAD loci where the identified SNP resided at least 30 bases from either end of the sequence (to allow for allele specific primer design) and where the SNP was called in at least 18 of the 20 individuals from each of the six population samples. Finally the ‘populations’ component of Stacks was used to export SNP genotype data for this whitelist of candidate RAD loci in Genepop format.

## Supplementary References

Brown, J.K., Taggart, J.B., Bekaert, M., Wehner, S., Palaiokostas, C., Setiawan, A.N., Symonds, J.E. and Penman, D.J., 2016. Mapping the sex determination locus in the hāpuku (Polyprion oxygeneios) using ddRAD sequencing. BMC genomics 17(1), p.448.

Catchen, J., Hohenlohe, P., Bassham, S., Amores, A. & Cresko, W. (2013) Stacks: an analysis tool set for population genomics. Mol Ecol22 (11), 3124–3140.

Manousaki, T., Tsakogiannis, A., Taggart, J.B, Palaiokostas, C., Tsaparis, D., Lagnel, J., Chatziplis, D., Magoulas, A., Papandroulakis, N., Mylonas, C.C. & Tsigenopoulos, C.S. (2016) Exploring a non-model teleost genome through RAD sequencing - Linkage mapping in Common Pandora, *Pagellus erythrinus* and comparative genomic analysis. G3 Genes Genomes Genetics 6, 509-519.

Peterson, B. K., Weber, J. N., Kay, E. H., Fisher, H. S., & Hoekstra, H. E. (2012). Double digest RADseq: an inexpensive method for de novo SNP discovery and genotyping in model and non-model species. PLoS One 7(5), e37135.

**Supplementary Table S1:** Combinatorial barcodes and basics Stacks database statistics for the 120 cod samples

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Name** | **P1 Barcode** | **P2 Barcode** | **Coverage** | **Unique\_Stacks** | **Polymorphic\_Loci** | **SNPs\_Found** |
| ScIW\_001 | TCTCTCA | TAGCA | 34.1 × | 7191 | 1385 | 1807 |
| ScIW\_002 | GTACACA | TAGCA | 38.8 × | 7479 | 1461 | 1949 |
| ScIW\_003 | CTCTTCA | TAGCA | 51.3 × | 8239 | 1611 | 2150 |
| ScIW\_004 | CTAGGAC | TAGCA | 65.3 × | 8622 | 1663 | 2257 |
| ScIW\_005 | ACGTA | TAGCA | 60.3 × | 8925 | 1778 | 2353 |
| ScIW\_006 | AGAGT | TAGCA | 68.7 × | 9564 | 1821 | 2390 |
| ScIW\_007 | ATGCT | TAGCA | 58.2 × | 8820 | 1721 | 2277 |
| ScIW\_008 | GACTA | TAGCA | 61.2 × | 9020 | 1692 | 2228 |
| ScIW\_009 | CAGTCAC | TAGCA | 58.7 × | 8914 | 1741 | 2314 |
| ScIW\_010 | GCTAACA | TAGCA | 76.0 × | 8868 | 1770 | 2378 |
| ScIW\_011 | ACACGAG | TAGCA | 42.9 × | 7447 | 1393 | 1868 |
| ScIW\_012 | AGGACAC | TAGCA | 48.9 × | 8683 | 1681 | 2230 |
| ScIW\_013 | TCAGA | GCATA | 29.5 × | 6473 | 1149 | 1525 |
| ScIW\_014 | GATCG | GCATA | 25.2 × | 5939 | 1104 | 1453 |
| ScIW\_015 | CATGA | GCATA | 40.8 × | 7265 | 1377 | 1850 |
| ScIW\_016 | ATCGA | GCATA | 44.4 × | 7487 | 1452 | 1965 |
| ScIW\_017 | TCGAG | GCATA | 27.7 × | 6197 | 1235 | 1622 |
| ScIW\_018 | GTCAC | GCATA | 29.2 × | 6616 | 1234 | 1646 |
| ScIW\_019 | GCATT | GCATA | 26.0 × | 6134 | 1162 | 1510 |
| ScIW\_020 | CGATA | GCATA | 44.3 × | 7367 | 1469 | 1936 |
| ScOW\_001 | TGCAACA | GCATA | 38.8 × | 7370 | 1433 | 1930 |
| ScOW\_002 | CGTATCA | GCATA | 37.5 × | 7050 | 1321 | 1769 |
| ScOW\_003 | CACAGAC | GCATA | 39.3 × | 7345 | 1380 | 1801 |
| ScOW\_004 | ACTGCAC | GCATA | 58.4 × | 8313 | 1684 | 2244 |
| ScOW\_005 | TCTCTCA | GAGATGT | 23.3 × | 5939 | 1091 | 1462 |
| ScOW\_006 | GTACACA | GAGATGT | 27.7 × | 6411 | 1277 | 1710 |
| ScOW\_007 | CTCTTCA | GAGATGT | 30.9 × | 6758 | 1307 | 1735 |
| ScOW\_008 | CTAGGAC | GAGATGT | 35.2 × | 7103 | 1314 | 1742 |
| ScOW\_009 | ACGTA | GAGATGT | 30.2 × | 7416 | 1459 | 1953 |
| ScOW\_010 | AGAGT | GAGATGT | 30.9 × | 7383 | 1432 | 1894 |
| ScOW\_011 | ATGCT | GAGATGT | 44.5 × | 8136 | 1659 | 2171 |
| ScOW\_012 | GACTA | GAGATGT | 51.7 × | 8419 | 1694 | 2229 |
| ScOW\_013 | CAGTCAC | GAGATGT | 38.6 × | 8068 | 1549 | 2081 |
| ScOW\_014 | GCTAACA | GAGATGT | 46.2 × | 7833 | 1561 | 2088 |
| ScOW\_015 | ACACGAG | GAGATGT | 33.2 × | 6727 | 1269 | 1675 |
| ScOW\_016 | AGGACAC | GAGATGT | 24.7 × | 6789 | 1262 | 1701 |
| ScOW\_017 | TCAGA | CGATC | 22.5 × | 5665 | 1069 | 1408 |
| ScOW\_018 | GATCG | CGATC | 33.6 × | 6928 | 1290 | 1708 |
| ScOW\_019 | CATGA | CGATC | 31.8 × | 6916 | 1317 | 1765 |
| ScOW\_020 | ATCGA | CGATC | 28.0 × | 6412 | 1142 | 1523 |

**Supplementary Table S1** (contin)

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Name** | **P1 Barcode** | **P2 Barcode** | **Coverage** | **Unique\_Stacks** | **Polymorphic\_Loci** | **SNPs\_Found** |
| ShIE\_001 | TCGAG | CGATC | 24.9 × | 6492 | 1664 | 2375 |
| ShIE\_002 | GTCAC | CGATC | 24.1 × | 6114 | 1115 | 1516 |
| ShIE\_003 | GCATT | CGATC | 76.7 × | 9087 | 1805 | 2411 |
| ShIE\_004 | CGATA | CGATC | 30.3 × | 6398 | 1242 | 1626 |
| ShIE\_005 | TGCAACA | CGATC | 33.1 × | 7017 | 1315 | 1716 |
| ShIE\_006 | CGTATCA | CGATC | 21.3 × | 5465 | 999 | 1313 |
| ShIE\_007 | CACAGAC | CGATC | 26.2 × | 6156 | 1213 | 1610 |
| ShIE\_008 | ACTGCAC | CGATC | 21.9 × | 5693 | 1058 | 1395 |
| ShIE\_009 | TCTCTCA | CATCTGT | 30.8 × | 6798 | 1345 | 1771 |
| ShIE\_010 | GTACACA | CATCTGT | 30.9 × | 6884 | 1287 | 1708 |
| ShIE\_011 | CTCTTCA | CATCTGT | 42.6 × | 7605 | 1437 | 1896 |
| ShIE\_012 | CTAGGAC | CATCTGT | 38.8 × | 7592 | 1460 | 1927 |
| ShIE\_013 | ACGTA | CATCTGT | 41.6 × | 8157 | 1600 | 2075 |
| ShIE\_014 | AGAGT | CATCTGT | 39.0 × | 8130 | 1634 | 2135 |
| ShIE\_015 | ATGCT | CATCTGT | 43.5 × | 8396 | 1593 | 2168 |
| ShIE\_016 | GACTA | CATCTGT | 43.3 × | 8248 | 1583 | 2097 |
| ShIE\_017 | CAGTCAC | CATCTGT | 32.4 × | 7788 | 1526 | 2050 |
| ShIE\_018 | GCTAACA | CATCTGT | 43.0 × | 7787 | 1520 | 2074 |
| ShIE\_019 | ACACGAG | CATCTGT | 34.5 × | 6969 | 1343 | 1811 |
| ShIE\_020 | AGGACAC | CATCTGT | 37.0 × | 7809 | 1551 | 2099 |
| ShIW\_001 | TCTCTCA | CTGGT | 21.5 × | 5287 | 946 | 1249 |
| ShIW\_002 | GTACACA | CTGGT | 21.5 × | 5603 | 1017 | 1338 |
| ShIW\_003 | CTCTTCA | CTGGT | 28.5 × | 6344 | 1154 | 1551 |
| ShIW\_004 | CTAGGAC | CTGGT | 26.6 × | 6114 | 1115 | 1453 |
| ShIW\_005 | ACGTA | CTGGT | 23.4 × | 6435 | 1151 | 1531 |
| ShIW\_006 | AGAGT | CTGGT | 22.2 × | 6389 | 1216 | 1609 |
| ShIW\_007 | ATGCT | CTGGT | 29.4 × | 7149 | 1392 | 1839 |
| ShIW\_008 | GACTA | CTGGT | 23.0 × | 6195 | 1210 | 1600 |
| ShIW\_009 | CAGTCAC | CTGGT | 21.2 × | 6248 | 1121 | 1491 |
| ShIW\_010 | GCTAACA | CTGGT | 21.7 × | 5443 | 1015 | 1329 |
| ShIW\_011 | ACACGAG | CTGGT | 17.7 × | 4668 | 813 | 1101 |
| ShIW\_012 | AGGACAC | CTGGT | 15.6 × | 4408 | 804 | 1061 |
| ShIW\_013 | TCAGA | GTCAAGT | 28.8 × | 6343 | 1132 | 1520 |
| ShIW\_014 | GATCG | GTCAAGT | 20.9 × | 5228 | 949 | 1255 |
| ShIW\_015 | CATGA | GTCAAGT | 28.8 × | 6453 | 1196 | 1582 |
| ShIW\_016 | ATCGA | GTCAAGT | 47.6 × | 8003 | 1609 | 2121 |
| ShIW\_017 | TCGAG | GTCAAGT | 42.7 × | 7544 | 1443 | 1912 |
| ShIW\_018 | GTCAC | GTCAAGT | 46.8 × | 7861 | 1557 | 2083 |
| ShIW\_019 | GCATT | GTCAAGT | 47.3 × | 7914 | 1487 | 1994 |
| ShIW\_020 | CGATA | GTCAAGT | 37.9 × | 6847 | 1295 | 1756 |

**Supplementary Table S1** (contin)

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Name** | **P1 Barcode** | **P2 Barcode** | **Coverage** | **Unique\_Stacks** | **Polymorphic\_Loci** | **SNPs\_Found** |
| ShOE\_001 | TGCAACA | GTCAAGT | 46.4 × | 7870 | 1540 | 2064 |
| ShOE\_002 | CGTATCA | GTCAAGT | 68.8 × | 8737 | 1709 | 2299 |
| ShOE\_003 | CACAGAC | GTCAAGT | 48.1 × | 7897 | 1550 | 2084 |
| ShOE\_004 | ACTGCAC | GTCAAGT | 41.8 × | 7602 | 1507 | 2009 |
| ShOE\_005 | TCAGA | GAAGC | 24.8 × | 5686 | 1093 | 1428 |
| ShOE\_006 | GATCG | GAAGC | 32.2 × | 6387 | 1206 | 1629 |
| ShOE\_007 | CATGA | GAAGC | 36.4 × | 6966 | 1281 | 1739 |
| ShOE\_008 | ATCGA | GAAGC | 30.9 × | 6498 | 1255 | 1670 |
| ShOE\_009 | TCGAG | GAAGC | 59.5 × | 8199 | 1534 | 2082 |
| ShOE\_010 | GTCAC | GAAGC | 23.0 × | 5662 | 1038 | 1358 |
| ShOE\_011 | GCATT | GAAGC | 28.7 × | 6272 | 1114 | 1516 |
| ShOE\_012 | CGATA | GAAGC | 66.7 × | 8363 | 1674 | 2228 |
| ShOE\_013 | TGCAACA | GAAGC | 31.3 × | 5869 | 1102 | 1444 |
| ShOE\_014 | CGTATCA | GAAGC | 43.7 × | 7671 | 1463 | 1985 |
| ShOE\_015 | CACAGAC | GAAGC | 36.3 × | 6666 | 1260 | 1675 |
| ShOE\_016 | ACTGCAC | GAAGC | 26.6 × | 5951 | 1201 | 1610 |
| ShOE\_017 | TCTCTCA | GAAGC | 23.4 × | 5564 | 1009 | 1311 |
| ShOE\_018 | GTACACA | GAAGC | 20.1 × | 5149 | 1007 | 1337 |
| ShOE\_019 | CTCTTCA | GAAGC | 21.9 × | 5233 | 992 | 1313 |
| ShOE\_020 | CTAGGAC | GAAGC | 23.0 × | 5454 | 1014 | 1350 |
| ShOW\_001 | ACGTA | GAAGC | 37.4 × | 7322 | 1388 | 1882 |
| ShOW\_002 | AGAGT | GAAGC | 35.3 × | 7576 | 1421 | 1875 |
| ShOW\_003 | ATGCT | GAAGC | 21.7 × | 5711 | 1082 | 1450 |
| ShOW\_004 | GACTA | GAAGC | 18.6 × | 5065 | 922 | 1226 |
| ShOW\_005 | CAGTCAC | GAAGC | 24.6 × | 6807 | 1294 | 1728 |
| ShOW\_006 | GCTAACA | GAAGC | 33.4 × | 6712 | 1248 | 1700 |
| ShOW\_007 | ACACGAG | GAAGC | 29.5 × | 6293 | 1171 | 1579 |
| ShOW\_008 | AGGACAC | GAAGC | 15.8 × | 4696 | 805 | 1058 |
| ShOW\_009 | TCAGA | ATACGGT | 14.7 × | 3958 | 683 | 890 |
| ShOW\_010 | GATCG | ATACGGT | 21.8 × | 5474 | 1018 | 1371 |
| ShOW\_011 | CATGA | ATACGGT | 18.3 × | 4909 | 890 | 1192 |
| ShOW\_012 | ATCGA | ATACGGT | 20.7 × | 5400 | 992 | 1324 |
| ShOW\_013 | TCGAG | ATACGGT | 18.6 × | 5104 | 914 | 1202 |
| ShOW\_014 | GTCAC | ATACGGT | 21.3 × | 5601 | 1047 | 1393 |
| ShOW\_015 | GCATT | ATACGGT | 22.2 × | 5765 | 1078 | 1419 |
| ShOW\_016 | CGATA | ATACGGT | 31.9 × | 6392 | 1208 | 1565 |
| ShOW\_017 | TGCAACA | ATACGGT | 27.5 × | 6300 | 1150 | 1563 |
| ShOW\_018 | CGTATCA | ATACGGT | 20.1 × | 5441 | 969 | 1283 |
| ShOW\_019 | CACAGAC | ATACGGT | 19.3 × | 4957 | 899 | 1173 |
| ShOW\_020 | ACTGCAC | ATACGGT | 20.2 × | 5214 | 956 | 1259 |

**Supplementary Table S2:** Loci used for population structure analysis.

|  |  |
| --- | --- |
| **Locus Name** | **Loci Sequence [SNP annotated]** |
| SNP\_1 | CATGCTTGTTAAGCAGGATTCCCAAAGCG**[T/C]**ACACAGAGTTCAATGGGGCATGCACGTGGACTGTTTTAAAGTCATCGCAGGCCTTTTAGCTTGATTTCCCGCTGCTTGCACTGGTTCACTTATTTATTTACGTTTTTCTGCCTT |
| SNP\_2 | TGCAGGCCAACCTCATCGAGAGCGTCAGCCCAAACACCTTCTGGGAGTGTGTCAACATCGAGAACATAGACCTCTC**[C/A]**ATGAATAGGTACTGGCATGGCAGCATGTGTGAAGCATGTGTGTGATGAGAAAACATAGAATCCTGAG |
| SNP\_3 | CATGCAGGCCTACATATAGAAAATACAGAAAACAGA**[T/C]**GGCTCACAGAGAAAAGGTGAACAACCGATTGAAATGACATGGAATCTGAGCGTATTCATAAAATGTCAAACAGTGTGATAAAGTTAAAACCCACGGGTAAGTTATCT |
| SNP\_4 | CATGCATTTTTTTAACTCTCT**[A/C]**ATCAAAACAAATAAAAATATCATCCCTCATTTTGACAGCACGCGGCGGTGCTGGAGGATTGGGTCCTTGATGGCTCTATAAGGCTACCAGTCTGCCTTGGTTTGTTTGGACTGCGCTTTGGG |
| SNP\_5 | CATGCAAG**[G/A]**CCCCTTGAGAGTTTGACAATTTGTACTTTCTACCTAGCTTTGTGCTATTGTTGCTATGGTCCACTCTGGTCTCCTCATATCACAATGAGTGGATGTTCAGGTGCAGGTCCGACCAGAGCAGAACACGAGCTATAC |
| SNP\_6 | CATGCTAATTTCATATATTTGTGCACTTTAATTAGAGCATATTGAGCATATATTTCCATTAGCATGTAGCTTGGTGTC**[G/T]**TACCTTCATGAGGTCGCGGTGGTGTTCCAGGACACTGCAGGTGGTCTGCCAGGTCTGCTGGTAGC |
| SNP\_7 | CATGCTTGTATTAAAGAATAAAAAAGAAAACATGAATCCTTGTCGTAATTTTTTCACGCATAATTTTAATAAACAAGATAGTAAG**[A/C]**CCTTGTAAGTCCACATCTGTATTTCATATAGGCCTGTATTAAATACATATAGTTATGA |
| SNP\_8 | TGCAGGACTATGAAAGAGCCATCGATTACCATCTCAAACACCTC**[A/C]**TCATCGCCCAGGACCTTGAAGACCGGTACTTACCTCAAACGCAGTGTTTTGTTGTTGTGGTTTCCTCCCAAAGGAGGCTTAGTAACATAGGGAGAATAT |
| SNP\_9 | TGCAGGCGAGATGAATCCAAAGAAAAATAAATATACATCATAGTAGTTATCA**[A/T]**GCAACATATATCAATATAGCCATAGAACAATTGTATTAACCCAGTTACTGAGGAGGTTCACGATGAACTTCAAATTCCCATCTTCTTGACC |
| SNP\_10 | TGCAGGTGCTCCAGAGCAGGCGCTCCAGAGCTGGCAAACCAGATATTTGCCAGCCTCTTGAGGGAGCCTATACCTTTTGCGAGGCAAAGGGCCCCTCCTGG**[A/G]**CATGCAGGCAGTCAATTCCATCTACCAGGCCCTTGTGGCTTC |
| SNP\_11 | CATGCATTCTTTTCTTTAAATGGAGGTATTCAGATGATGAATTATTTCAACGCCAAGTATAGTTTTT**[A/C]**GCCTTTATCTCTAACTTGTGAATGATACCCTTCTTAAGTGTGTTGAAACTTTGTTAAAACTGAGAAACATGTGTGT |
| SNP\_12 | TGCAGGGTTTGTGTTAGTGTTGGGGTGGTTATTCAAAAATC**[G/A]**GACCTTGAATTTGGGTATTGTGGTAGCAGCCATCAGAGCATCCTTTCTATCCAAGATGGAAGCGAATCTGTGTTTGATCGCCTGTCATATGAAATAGTAGGC |
| SNP\_13 | CATGCCAATGTATCACGTCCACTTATTAAAATACCTGCGACAGC**[G/A]**AAAGTGCTACACAGTGCAAGCTACAAAAAAAATCATCTAAACTATGTTTCACATGCCACACAAAAATATCTAAAGGGGTGTTACCCCATCAAGCGGTCG |

**Supplementary Table S3:** Minimum adequate GAM model coefficients and deviance explained for female maturity in relation to length, longitude and latitude based on genetic samples only.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Coefficients | | |  |
| Explanatory variables | Estimate | SE | *p* | Deviance (%) |
| Intercept | -5.186 | 1.208 | <0.001 |  |
| Length | 0.136 | 0.022 | <0.001 | 22.9 |
| s(Longitude) |  |  | <0.001 | 43.2 |
| s(Latitude) |  |  | 0.011 | 48.3 |

**Supplementary Table S4:** Summary information for the markers used in the study for the complete dataset listing for each loci the global observed heterozygosity (Ho) and unbiased expected heterozygosity (uHe), outputs of hierarchical AMOVA per locus as well as Chromonsome (bold) and positional reference (bp) to which they align to the GadMor3 genome construction.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Loci** | **Ho** | **uHe** | **Fct** | ***P* - value** | **Fsc** | ***P* - value** | **Fst** | ***P* - value** | **Chromosome:** Location |
| **SNP\_1** | 0.203 | 0.211 | 0.0003 | 0.3765 | -0.0015 | 0.6397 | -0.0012 | 0.6437 | **3** : 10282576 – 10282433 |
| **SNP\_2** | 0.194 | 0.196 | -0.0011 | 0.6154 | 0.0044 | 0.1355 | 0.0033 | 0.1759 | **3** : 19406219 – 19406361 |
| **SNP\_3** | 0.499 | 0.473 | 0.0100 | 0.0108 | 0.0028 | 0.1777 | 0.0127 | 0.0025 | **4** : 36597569 – 36597426 |
| **SNP\_4** | 0.173 | 0.170 | -0.0012 | 0.7992 | -0.0019 | 0.6555 | -0.0031 | 0.7458 | **5** : 6372661 – 6372518 |
| **SNP\_5** | 0.212 | 0.234 | 0.0028 | 0.1877 | 0.0063 | 0.1257 | 0.0091 | 0.0520 | **5** : 10640377 – 10640520 |
| **SNP\_6** | 0.332 | 0.330 | -0.0007 | 0.6106 | 0.0026 | 0.2117 | 0.0020 | 0.2277 | **8** : 16574032 – 16573889 |
| **SNP\_7** | 0.467 | 0.452 | -0.0013 | 0.6216 | 0.0062 | 0.0561 | 0.0049 | 0.0673 | **9** : 24896503 – 24896646 |
| **SNP\_8** | 0.362 | 0.367 | 0.0055 | 0.2511 | 0.0925 | <0.00001 | 0.0974 | <0.00001 | **12** : 1174999 – 1175142 |
| **SNP\_9** | 0.481 | 0.479 | 0.0112 | 0.0738 | 0.0332 | <0.00001 | 0.0441 | <0.00001 | **12** : 6781635 – 6781492 |
| **SNP\_10** | 0.371 | 0.359 | -0.0007 | 0.4677 | 0.0202 | <0.00001 | 0.0195 | <0.00001 | **12** : 17106073 – 17105930 |
| **SNP\_11** | 0.428 | 0.431 | 0.0031 | 0.0586 | 0.0001 | 0.4484 | 0.0033 | 0.1864 | **18** : 17368128 – 17368271 |
| **SNP\_12** | 0.243 | 0.249 | 0.0008 | 0.3099 | 0.0017 | 0.3522 | 0.0025 | 0.2500 | **21** : 21609862 – 21610005 |
| **SNP\_13** | 0.184 | 0.190 | -0.0011 | 0.7885 | -0.0013 | 0.6605 | -0.0023 | 0.7245 | **22** : 17466965 – 17467108 |