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Polymorphic microsatellite loci isolated from humpback whale, *Megaptera novaeangliae* and fin whale, *balaenoptera physalus*

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In conservation genetics, it is becoming increasingly clear that parameters derived over evolutionary time scales may not apply to much shorter ecological timescales (Palsbøll 1999). Molecular genetic methods can be employed to estimate parameters on an ecological timescale if the focus is aimed only at recently diverged lineages, i.e., among individuals as opposed to among populations. These extensions of current approaches are vital to align the application of molecular genetics to contemporary issues in conservation. In order to estimate the degree of kinship in a reliable manner, an adequate number of loci must be analyzed per individual. Ensuring an adequate number of loci decreases/eliminates the interference of other levels of relatives, and compensates for the exponential increase in the number of pair-wise comparisons when the sample size increases. Towards this end, we presented, in this note, an additional 17 polymorphic microsatellite loci, which, originated from a di-, tri- and tetra-nucleotide microsatellite loci enriched library constructed from humpback whale, *Megaptera novaeangliae*, and fin whale, *Balaenoptera physalus*, genomic DNA, using the protocol previously described in Palsbøll et al. (1997) and Bérubé et al. (2000).

More specifically, the microsatellite loci were isolated from size-selected total-cell DNA extracted from humpback whale and fin whale using the approach described by Rassmann and colleagues (1991). Recombinant colonies (dot blotted to a ZetaProbe^{TM} nylon membrane) were hybridized (under the conditions recommended by the manufacturer) with a variety of oligonucleotides (of different di-, tri-, and tetramer repeat motifs), each end-labeled with γ-P^{32}ATP using T4 kinase (Sambrook et al. 1989). Positive clones were isolated, grown over night, then the plasmid DNA was isolated (Sambrook et al. 1989), dot blotted and re-hybridized to the oligonucleotides. Of the 644 clones resulted in a strong signal after the second round of hybridization, 293 were subsequently sequenced as described in Palsbøll et al. (1995) using SP6 and T7 oligo-nucleotide primers on a Stratagene RoboCycler^{TM}. The PCR^{TM} (Polymerase Chain Reaction, Mullis, Faloona 1987; Saiki et al. 1988) amplification products were cleaned using a simple one-tube enzymatic method using Exonuclease to digest excess PCR primers and Shrimp Alkaline Phosphatase to degrade excess nucleotides carried over from the PCR.

Table 1. PCR conditions

Locus	Species of origin	Primer sequences (5′–3′)	Label	Celsius	Time	Cycles	Thermo-cycler	GenBank access #
GT541	*M. novaeangliae*	CTTCACACTCATTAGGATGCC CCTTTATATCCTTGCCAAGAC	HEX	56	15/15/15	30	MJR DYAD	DQ151644
AC045	*M. novaeangliae*	AGCAGCCCAACACATTCAAGA TGACCACTCACCTTCACACTTC	FAM	55	15/15/15	28	MJR DYAD	DQ151645
AC087	*M. novaeangliae*	ACCAGGGTGGGTTCTTAA ACTA GCTTCCAGAAGCAATGATGGA	TET	55	15/15/15	30	MJR PTC 100	DQ151646
GT129	*B. physalus*	CGCAATGAAGAGTTAAAA GAATG GCACTGGTGAATGCAGATTGA	TET	55	15/15/15	28	MJR DYAD	DQ151655
CA128	*B. physalus*	ATGGGAAGTATTATTTCCTG GCAC GTCCATGAACCCCTAGAGTAT	FAM	55	15/15/15	30	MJR PTC 100	DQ151656
AC137	*M. novaeangliae*	ACCACTTTGTGGAGAATAGAC TAGGTCCCTGTTGTCAGAGAG	HEX	55	15/15/15	30	MJR DYAD	DQ151647
TGAA610	*M. novaeangliae*	CTGCATAGCCTTGATCAAGGA CATCCAGGTGTAGATCAAGGC	FAM	55	15/15/15	30	MJR DYAD	DQ151648
CAAA074	*B. physalus*	ATGCTGGTGTTTCCTGTATCA TTGTCTCCTGCTGGCTGATTA	TET	55	15/15/15	30	MJR DYAD	DQ151657
GT142	*B. physalus*	CTGAGTAATATTCCACCATAC ATGGATAAAGAAGATGTGGGG	FAM	60	15/15/15	33	MJR DYAD	DQ151658
GT001	*M. novaeangliae*	CATGATTTAGTGTGCATACTG CATGATGTGTTAAARACTTGC	TET	56	15/15/15	33	MJR DYAD	DQ151649
CA232	*M. novaeangliae*	GATCACATAATCTTGATCAGA CACTCAGATTAAGACTTCAGA	HEX	55	15/15/15	30	MJR DYAD	DQ151650
CA141	*B. physalus*	CTCTGCATTGGGATGGCTCTG GCGGTAGAACACGTGCCACTG	FAM	61	15/15/15	31	MJR DYAD	DQ151659
GT122	*B. physalus*	CCTTTTAAAACCCAGAATGTAG TGTTTGCAGTGACGAATG AAAGG	FAM	55	15/15/15	30	MJR DYAD	DQ151660
CA234	*M. novaeangliae*	TGGATCCTCTACCTACCTTAG CAACCTTATTCTTGACCTCAT	TET	55	15/15/15	30	MJR DYAD	DQ151651
GT238	*M. novaeangliae*	CTTAAATGCAGTAGGAAGCCA TGCATATCTAATCATGTTAC TTGCCTG	TET	55	15/15/15	30	MJR DYAD	DQ151652
GT227	*M. novaeangliae*	GTAATCATCATGGACACTCA CACTTTACTTTGTCTGTTGGC	FAM	55	15/15/15	30	MJR DYAD	DQ151653
AC082	*M. novaeangliae*	ATTAGTCCTGTTTCTCTGGAG CAGATGTTCTGTGAGTACTTG	TET	63	15/15/15	31	MJR DYAD	DQ151654

Notes: Locus name consists of the repeat sequence (e.g., AC) following by a three-digit number which refers to the clone number. Celsius denotes temperature at the annealing step in each cycle. Time denotes the number of seconds at each step (denaturing/annealing/extension) in each cycle. MJR denotes MJ Research

Fluorescent-labeled single-stranded products were generated by PCR™ using the ABI Big-Dye Terminator Cycle Sequencing Ready Reaction Kit following the manufacturer's protocol (Applied Biosystems, Inc.) using a Stratagene RoboCycler™. The labeled fragments were separated by electrophoresis through 5% denaturing Long Ranger™ (FMC, Inc.) sequencing matrix and the order of fragments detected by an ABI Prism™ 377 automated DNA sequencer. From the 293 sequenced clones, 57 contained repeat inserts from which, 25 sets of primer were designed. Only 17 primer sets showed polymorphisms within the different analyzed species (see Table 2).

Table 2. Levels of polymorphism

Locus	*n*	*K*	Size range	H_{obs}	H_{exp}	*P* H_{obs}	*I*
AC045							
B. acutorostrata	25	5	182–190	0.76	0.75	< 0.57	0.10
B. borealis	8	3	199–207	0.50	0.57	< 0.37	0.26
B. musculus	10	3	178–182	0.70	0.61	< 0.78	0.23
B. physalus	9	6	187–199	1.0	0.74	< 1.08	0.11
E. australis	10	6	185–195	0.90	0.77	< 0.90	0.09
M. novaeangliae	11	7	160–182	0.73	0.66	< 0.80	0.14
AC082							
B. acutorostrata	14	3	128–132	0.64	0.49	< 1.0	0.31
B. borealis	8	3	135–141	0.63	0.65	< 0.46	0.20
B. musculus	10	4	125–131	0.20	0.47	< 0.01	0.33
B. physalus	9	1	123	–	–	–	1.0
E. australis	9	1	105	–	–	–	1.0
M. novaeangliae	10	3	121–127	0.40	0.34	< 1.0	0.46
AC087							
B. acutorostrata	25	3	163–167	0.64	0.53	< 0.88	0.32
B. borealis	8	1	151	–	–	–	1.0
B. musculus	10	4	172–184	0.50	0.51	< 0.53	0.30
B. physalus	10	4	152–160	0.80	0.66	< 0.88	0.18
E. australis	10	4	167–175	0.40	0.53	< 0.18	0.27
M. novaeangliae	10	9	154–180	0.80	0.87	< 0.21	0.03
AC137							
B. acutorostrata	24	7	109–127	0.63	0.68	< 0.27	0.14
B. borealis	8	1	85	–	–	–	1.0
B. musculus	10	6	93–115	0.60	0.66	< 0.31	0.15
B. physalus	9	8	97–149	0.78	0.83	< 0.29	0.05
E. australis	10	5	88–96	0.30	0.48	< 0.05	0.30
M. novaeangliae	11	7	123–139	1.0	0.82	< 1.0	0.06
CA128							
B. acutorostrata	25	5	125–143	0.72	0.69	< 0.68	0.15
B. borealis	7	6	53–77	1.0	0.74	< 1.0	0.10
B. musculus	10	7	57–85	1.0	0.82	< 1.0	0.06
B. physalus	9	7	76–90	0.78	0.77	< 0.55	0.08
E. australis	8	2	58–64	0.75	0.47	< 1.0	0.39
M. novaeangliae	10	8	70–90	0.80	0.85	< 0.30	0.04
CA141							
B. acutorostrata	15	1	179	–	–	–	1.0
B. borealis	4	1	173	–	–	–	1.0
B. musculus	7	5	169–183	1.0	0.68	< 1.0	0.14
B. physalus	8	6	173–187	1.0	0.76	< 1.0	0.09
E. australis	9	3	167–171	0.89	0.61	< 1.0	0.20
M. novaeangliae	8	6	173–187	0.88	0.77	< 0.84	0.09
CA232							
B. acutorostrata	25	3	148–154	0.36	0.51	< 0.06	0.32
B. borealis	8	2	135–137	0.25	0.22	< 1.0	0.63
B. musculus	8	3	168–172	0.25	0.54	< 0.05	0.31
B. physalus	10	6	139–167	0.50	0.67	< 0.12	0.17
E. australis	7	5	145–157	0.71	0.62	< 0.85	0.18
M. novaeangliae	8	5	154–168	0.75	0.63	< 0.91	0.18

Table 2. (Continued)

Locus	*n*	*K*	Size range	H_{obs}	H_{exp}	*P* H_{obs}	*I*
CA234							
B. acutorostrata	17	2	191–193	0.76	0.50	< 0.10	0.38
B. borealis	8	5	200–210	0.75	0.73	< 0.59	0.12
B. musculus	7	5	198–210	0.86	0.71	< 0.85	0.13
B. physalus	7	6	187–219	0.86	0.82	< 0.61	0.06
E. australis	7	3	185–189	0.29	0.61	< 0.05	0.23
M. novaeangliae	6	3	182–194	0.50	0.65	< 0.24	0.19
CAAA074							
B. acutorostrata	25	1	143	–	–	–	1.0
B. borealis	8	2	142–152	0.25	0.22	< 1.0	0.63
B. musculus	10	3	139–147	0.50	0.49	< 0.65	0.34
B. physalus	10	1	143	–	–	–	1.0
E. australis	10	1	139	–	–	–	1.0
M. novaeangliae	11	1	143	–	–	–	1.0
GT001							
B. acutorostrata	25	1	215	–	–	–	1.0
B. borealis	7	6	210–226	0.71	0.76	< 0.40	0.10
B. musculus	10	1	207	–	–	–	1.0
B. physaluss	10	4	215–225	1.0	0.59	< 1.0	0.25
E. australis	10	3	207–215	0.50	0.40	< 1.0	0.41
M. novaeangliae	11	5	213–225	0.91	0.76	< 0.92	0.09
GT122							
B. acutorostrata	25	4	136–142	0.56	0.63	< 0.21	0.19
B. borealis	8	4	131–145	0.63	0.63	< 0.53	0.20
B. musculus	5	2	132–134	0.20	0.18	< 1.0	0.69
B. physalus	7	9	151–167	0.86	0.86	< 0.45	0.04
E. australis	9	6	133–147	0.67	0.68	< 0.48	0.14
M. novaeangliae	4	1	130	–	–	–	1.0
GT129							
B. acutorostrata	25	3	101–105	0.48	0.39	< 1.0	0.42
B. borealis	8	1	91	–	–	–	1.0
B. musculus	10	2	91–93	0.10	0.10	< 1.0	0.82
B. physalus	9	4	96–104	0.56	0.56	< 0.51	0.24
E. australis	10	1	85	–	–	–	1.0
M. novaeangliae	10	5	83–97	0.60	0.48	< 1.0	0.30
GT142							
B. acutorostrata	-amp	–	–	–	–	–	–
B. borealis	8	3	68–78	0.38	0.57	< 0.14	0.26
B. musculus	10	7	66–90	0.90	0.78	< 0.88	0.09
B. physalus	10	8	72–90	0.80	0.80	< 0.51	0.07
E. australis	9	5	72–86	0.78	0.67	< 0.84	0.15
M. novaeangliae	9	8	70–86	0.89	0.81	< 0.80	0.06
GT227							
B. acutorostrata	24	1	117	–	–	–	1.0
B. borealis	8	2	129–137	0.38	0.30	< 1.0	0.53
B. musculus	10	1	117	–	–	–	1.0
B. physalus	10	2	114–120	0.20	0.19	< 1.0	0.67
E. australis	9	3	114–120	0.22	0.20	< 1.0	0.64
M. novaeangliae	10	1	115	–	–	–	1.0

Table 2. (Continued)

Locus	*n*	*K*	Size range	H_{obs}	H_{exp}	$P\ H_{obs}$	*I*
GT238							
B. acutorostrata	24	1	138	–	–	–	1.0
B. borealis	7	2	133–140	0.14	0.13	< 1.0	0.76
B. musculus	9	2	127–131	0.33	0.48	< 0.35	0.39
B. physalus	8	2	127–129	0.38	0.30	< 1.0	0.53
E. australis	6	2	124–126	0.17	0.15	< 1.0	0.73
M. novaeangliae	9	4	131–137	0.44	0.67	< 0.07	0.17
GT541							
B. acutorostrata	23	4	96–104	0.43	0.67	< 0.01	0.17
B. borealis	7	5	95–113	0.71	0.74	< 0.43	0.11
B. musculus	9	4	92–102	0.56	0.67	< 0.25	0.18
B. physalus	8	5	78–94	0.75	0.74	< 0.52	0.11
E. australis	10	4	74–86	0.60	0.48	< 1.0	0.31
M. novaeangliae	11	4	81–101	0.18	0.25	< 0.14	0.57
TGAA610							
B. acutorostrata	25	1	134	–	–	–	1.0
B. borealis	7	1	134	–	–	–	1.0
B. musculus	10	1	134	–	–	–	1.0
B. physalus	9	2	126–138	0.11	0.10	< 1.0	0.81
E. australis	10	1	134	–	–	–	1.0
M. novaeangliae	11	3	126–138	0.45	0.52	< 0.32	0.28

Notes: *n* denotes the number of samples for which a complete genotype was obtained. *K* is the number of alleles detected, and H_{exp} and H_{obs}, expected (assuming panmixis) and observed heterozygosity, respectively. $P\ H_{obs}$, denotes the probability of the observed heterozygosity assuming panmixis (estimated from 1000 permutations of the data). *I* denotes the probability that two unrelated individuals from the same panmictic populations have identical genotypes (Paetkau and Strobeck 1994).

The data presented here are based upon genotypes obtained from humpback whales, fin whales, minke whales (*B. acutorostrata*), right whales (*Eubalaena australis*), sei whales (*B. borealis*) and blue whales (*B. musculus*). Except for the minke whale samples, total-cell DNA was extracted from skin biopsies by standard phenol and chloroform extractions (Sambrook et al. 1989) and the DNA re-suspended in 1 × TE (Sambrook et al., 1989). The minke whale samples were extracted using the Chelex method (Walsh et al., 1991). The fragments at each locus were amplified by PCR under the following conditions in 10 μl volumes, each with 10 ng of genomic DNA, 67 mM Tris–HCl, pH 8.8, 2 mM $MgCl_2$, 16.6 mM $(NH_4)_2SO_4$, 10 mM β-mercaptoethanol, 0.2 mM dNTPs, 1 mM of the unlabeled oligo-nucleotide primer, 40 μM of the end-labeled oligo-nucleotide primer as well as 0.4 units of *Taq* DNA polymerase (New England Biolabs, Inc.). The thermo-cycling profiles and GenBank accession numbers are listed in Table 1.

The amplification products were separated by electrophoresis through a 5% denaturing Long Ranger™ (FMC, Inc.) sequencing matrix. The size of the amplification products was calculated using the size standard GeneScan™ TAMRA-500 (Applied Biosystems, Inc.) and multiple positive control samples (of known genotype) included in each amplification and detection.

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