

Supplementary information 2

Genotyping methods

Subantarctic fur seal

A total of 88 *Arctocephalus tropicalis* samples were collected from Macquarie Island. Total genomic DNA was extracted from each sample using a standard phenol-chloroform protocol (Sambrook et al 1989) and genotyped at 36 microsatellite loci (see Supplementary Table 9 for details). These were PCR amplified in 5 separate multiplexed reactions using a Type It Kit (Qiagen) as described in Supplementary Table 9. The following PCR profile was used: one cycle of 5 min at 94 °C; 24 cycles of 30 s at 94 °C, 90 s at T_a °C and 30 s at 72 °C; and one final cycle of 15 min at 72 °C (see Table S1 for T_a). Fluorescently labeled PCR products were then resolved by electrophoresis on an ABI 3730xl capillary sequencer and allele sizes were scored automatically using GeneMarker v1.95. To ensure high genotype quality, all traces were manually inspected and any incorrect calls were adjusted accordingly.

Locus	Literature source	Multiplex	T _a (°C)
Pv9	Allen et al. (1995)	1	53
Hg6.3	Allen et al. (1995)	1	53
Hg8.10	Allen et al. (1995)	1	53
Hg1.3	Gemmell et al. (1997)	1	53
M11a	Hoelzel et al. (1999)	1	53
PvcA	Coltman et al. (1996)	1	53
ZcwB07	Hoffman et al. (2007)	1	53
Agaz2	Hoffman (2009)	1	53
Ag3	Hoffman et al. (2008)	2	60
Agaz6	Hoffman (2009)	2	60
Ag2	Hoffman et al. (2008)	2	60
OrrFCB2	Buchanan et al. (1998)	2	60
Lw10	Davis et al. (2002)	2	60
ZcwCo1	Hoffman et al. (2007)	2	60
Agaz5	Hoffman (2009)	2	60
ZcCgDhB.14	Hernandez-Velazquez et al. (2005)	2	60
Ag7	Hoffman et al. (2008)	3	60
Agt10	Hoffman and Nichols (2011)	3	60
ZcCgDh4.7	Hernandez-Velazquez et al. (2005)	3	60
ZcwE05	unpublished	3	60
Ag1	Hoffman et al. (2008)	3	60
OrrFCB8	Buchanan et al. (1998)	3	60
Agt47	Hoffman and Nichols (2011)	3	60
ZcwFo7	Hoffman et al. (2007)	4	53
ZcwDo2	Wolf et al. (2006)	4	53
ZcCgDh1.8	Hernandez-Velazquez et al. (2005)	4	53
Aa4	Hoelzel et al. (1999)	4	53
ZcCgDh5.8	Hernandez-Velazquez et al. (2005)	4	53
Agaz3	Hoffman (2009)	4	53
962-1	unpublished	5	60
554-6	unpublished	5	60

ZcwA12	Hoffman et al. (2007)	5	60
PvcE	Coltman et al. (1996)	5	60
ZcwBo9	Wolf et al. (2006)	5	60
Agaz10	Hoffman (2009)	5	60
Mang36	Sanvito et al. (2013)	5	60

Supplementary Table 9: Microsatellite loci of the Subantarctic fur seal. “Multiplex” denotes the PCR mastermix into which each locus was multiplexed and “T_a” denotes the annealing temperature used.

- Allen, P. J., W. Amos, P. P. Pomeroy, and S. D. Twiss. 1995. “Microsatellite Variation in Grey Seals (*Halichoerus Grypus*) Shows Evidence of Genetic Differentiation between Two British Breeding Colonies.” *Molecular Ecology* 4 (6): 653–62. <https://doi.org/10.1111/j.1365-294X.1995.tb00266.x>.
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- Hernandez-Velazquez, F. D., C. E. Galindo-Sanchez, M. I. Taylor, J. De La Rosa-Velez, I. M. Cote, Y. Schramm, D. Auriolles-Gamboa, and C. Rico. 2005. “New Polymorphic Microsatellite Markers for California Sea Lions (*Zalophus Californianus*).” *Molecular Ecology Notes* 5 (1): 140–42. <https://doi.org/10.1111/j.1471-8286.2004.00858.x>.
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- Hoffman, Joseph I. 2009. “A Panel of New Microsatellite Loci for Genetic Studies of Antarctic Fur Seals and Other Otariids.” *Conservation Genetics* 10 (4): 989–92. <https://doi.org/10.1007/s10592-008-9669-z>.
- Hoffman, Joseph I., Kanchon K. Dasmahapatra, and Hazel J. Nichols. 2008. “Ten Novel Polymorphic Dinucleotide Microsatellite Loci Cloned from the Antarctic Fur Seal *Arctocephalus Gazella*.” *Molecular Ecology Resources* 8 (2): 459–61. <https://doi.org/10.1111/j.1471-8286.2007.01993.x>.
- Hoffman, Joseph I., and Hazel J. Nichols. 2011. “A Novel Approach for Mining Polymorphic Microsatellite Markers In Silico.” *PLOS ONE* 6 (8): e23283. <https://doi.org/10.1371/journal.pone.0023283>.
- Hoffman, Joseph I., Sebastian Steinfartz, and Jochen B. W. Wolf. 2007. “Ten Novel Dinucleotide Microsatellite Loci Cloned from the Galápagos Sea Lion (*Zalophus Californianus Wollebaeki*) Are Polymorphic in Other Pinniped Species.” *Molecular Ecology Notes* 7 (1): 103–5. <https://doi.org/10.1111/j.1471-8286.2006.01544.x>.
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Northern elephant seal

A total of 260 *Mirounga angustirostris* samples were collected in the southernmost breeding colony of the species, the Islas San Benito (Baja California, Mexico). Total genomic DNA was extracted from each sample using silica-gel-membrane technology (DNeasy Blood and Tissue kit, Qiagen; details in Sanvito et al. 2014) and genotyped at 35 microsatellite loci (see Supplementary Table Supplementary Table 10 for details). Amplification by PCR was carried out using the “universal tag” method of Schuelke (2000). The microsatellite loci were amplified in singleplex or multiplex reactions as described in Supplementary Table 10. The following PCR profile was used: one cycle of 3 min at 94 °C; 30 cycles of 30 s at 94 °C, 30 s at T_a °C and 40 s at 72 °C; 8 cycles of 30 s at 94 °C, 30 s at 47 °C and 40 s at 72 °C; and one final cycle of 10 min at 72 °C (see Supplementary Table 10 for T_a). Magnesium concentrations varied among the mastermixes as shown in Supplementary Table 10. Fluorescently labelled PCR products were resolved by electrophoresis on an ABI 3730xl capillary sequencer and allele sizes were scored automatically using GeneMarker v1.85. To ensure high genotype quality, all traces were manually inspected and any incorrect calls were adjusted accordingly.

Locus	Literature source	Multiplex	Mg (mM)	T_a (°C)
71HDZ441	Huebinger et al. (2007)	–	1.5	54
Hg4.2	Allen et al. (1995)	–	1.5	56
Hg8.9	Allen et al. (1995)	–	2	48
Lw-16	Davis et al. (2002)	–	1.5	55
Lw-20	Davis et al. (2002)	–	1.5	49
Lw-8	Davis et al. (2002)	–	1.5	47
PVC26	Coltman et al. (1996)	–	2	40
PVC74	Coltman et al. (1996)	–	2	53
ZcCgDh4.7	Hernandez-Velazquez et al. (2005)	–	1.75	56
ZcCgDh7tg	Hernandez-Velazquez et al. (2005)	–	2	46
ZcwCo3	Wolf et al. (2006)	–	1.5	56
ZcwEo3	Wolf et al. (2006)	–	1.5	54
Hg1.4	Gemmel et al. (1997)	1	1.5	53
Lw-18	Davis et al. (2002)	1	1.5	53
BG	R. Slade, pers. comm. in Gemmel et al (1997)	2	2	53
PV9	Goodman (1997)	2	2	53
Hg3.6	Allen et al. (1995)	3	1.75	56
Hg8.10	Allen et al. (1995)	3	1.75	56
Hl10	Gelatt et al. (2010)	4	2	39
ZzCgDh3.6	Hernandez-Velazquez et al. (2005)	4	2	39
Hg2.3	Garza (1998)	5	2	53
Hl-8	Davis et al. (2002)	5	2	53
MA11A	Hoelzel (unpubl) in Gemmel et al. (1997)	5	2	53
CORT	Garza (1998)	6	1.75	51
PVC43	Garza (1998)	6	1.75	51
Lw-10	Davis et al. (2002)	7	1.5	52
PVC1	Garza (1998)	7	1.5	52
71HDZ301	Huebinger et al. (2007)	8	1.5	42
ZzCgDh1.8	Hernandez-Velazquez et al. (2005)	8	1.5	42
ZcwA12	Hoffman et al. (2007)	9	1.75	49
ZcwFo7	Hoffman et al. (2007)	9	1.75	49
Ag-9	Hoffman et al. (2008)	10	2	57
ZcwCo1	Hoffman et al. (2007)	10	2	57
ZcwEo4	Hoffman et al. (2007)	11	2	52
ZcwGo4	Hoffman et al. (2007)	11	2	52

Supplementary Table 10: Microsatellite loci of the Northern elephant seal. “Multiplex” denotes the PCR mastermix into which each locus was multiplexed, “Mg” denotes the concentration of magnesium used in the

PCR mastermix and “T_a” denotes the annealing temperature used. Loci not assigned to PCR multiplexes were amplified individually.

- Allen, P. J., W. Amos, P. P. Pomeroy, and S. D. Twiss. 1995. “Microsatellite Variation in Grey Seals (*Halichoerus grypus*) Shows Evidence of Genetic Differentiation between Two British Breeding Colonies.” *Molecular Ecology* 4 (6): 653–62. <https://doi.org/10.1111/j.1365-294X.1995.tb00266.x>.
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- Hernandez-Velazquez, F. D., C. E. Galindo-Sanchez, M. I. Taylor, J. De La Rosa-Velez, I. M. Cote, Y. Schramm, D. Auriolos-Gamboa and C. Rico. 2005. “New polymorphic microsatellite markers for California sea lions (*Zalophus californianus*).” *Molecular Ecology Notes* 5(1): 140-2. <https://doi.org/10.1111/j.1471-8286.2004.00858.x>.
- Hoffman, J. I., S. Steinfartz and J. B. W. Wolf. 2007. “Ten novel dinucleotide microsatellite loci cloned from the Galapagos sea lion (*Zalophus californianus wollebaeki*) are polymorphic in other pinniped species.” *Molecular Ecology Notes* 7(1): 103-5. <https://doi.org/10.1111/j.1471-8286.2006.01544.x>.
- Hoffman, J. I., K. K. Dasmahapatra and H. J. Nichols. 2008. “Ten novel polymorphic dinucleotide microsatellite loci cloned from the Antarctic fur seal *Arctocephalus gazella*.” *Molecular Ecology Resources* 8(2): 459-61. <https://doi.org/10.1111/j.1471-8286.2007.01993.x>.
- Huebinger, R. M., E. E. Louis, T. Gelatt, L. D. Rea and J. W. Bickham. 2007. “Characterization of eight microsatellite loci in Steller sea lions (*Eumetopias jubatus*).” *Molecular Ecology Notes* 7(6): 1097-99. <https://doi.org/10.1111/j.1471-8286.2007.01790.x>.
- Sanvito, S., A. Fabiani and F. Galimberti. 2014. “Sex Determination in the Near Threatened Guadalupe Fur Seal: Molecular Markers and Their Potential Applications.” *Open Journal of Animal Sciences* 4: 270-7. <http://dx.doi.org/10.4236/ojas.2014.45034>.
- Schuelke, M. 2000. “An economic method for the fluorescent labeling of PCR fragments.” *Nature Biotechnology* 18(2): 233-4. <http://dx.doi.org/10.1038/72708>.
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Southern elephant seal

A total of 260 *Mirounga leonina* samples were collected at Sea Lion Island, the main breeding colony of the species in the Falkland Islands. Total genomic DNA was extracted from each sample using silica-gel-membrane technology (DNeasy Blood and Tissue kit, Qiagen; details in Sanvito et al. 2014) and genotyped at 13 microsatellite loci (see Supplementary Table 11 for details). Amplification by PCR was carried out using the “universal tag” method of Schuelke (2000). The microsatellite loci were amplified in singleplex or multiplex reactions as described in Supplementary Table 11. The following PCR profile was used: one cycle of 3 min at 94 °C; 30 cycles of 30 s at 94 °C, 30 s at T_a °C and 40 s at 72 °C; 8 cycles of 30 s at 94 °C, 30 s at 47 °C and 40 s at 72 °C; and one final cycle of 10 min at 72 °C (see Supplementary Table 11 for T_a). Magnesium concentrations varied among the mastermixes as shown in Supplementary Table 11. Fluorescently labelled PCR products were then resolved by electrophoresis on an ABI 3730xl capillary sequencer and allele sizes were scored automatically using GeneMarker v1.85. To ensure high genotype quality, all traces were manually inspected and any incorrect calls were adjusted accordingly.

Locus	Literature source	Multiplex	Mg (mM)	T _a (°C)
ZcwGo4	Hoffman et al. (2007)		1.5	54
Lw-20	Davis et al. (2002)	1	2	49
OrrFCB9	Buchanan et al. (1998)	1	2	49
71HDZ441	Huebinger et al. (2007)	2	1.8	56
Ag-8	Hoffman et al. (2008)	2	1.8	56
Hg3.6	Allen et al. (1995)	3	1.75	58
Hg8.10	Allen et al. (1995)	3	1.75	58
ZcwA12	Hoffman et al. (2007)	4	2	54
ZcwFo7	Hoffman et al. (2007)	4	2	54
71HDZ301	Huebinger et al. (2007)	5	1.5	42
ZzCgDh1.8	Hernandez-Velazquez et al. (2005)	5	1.5	42
ZcCgDh4.7	Hernandez-Velazquez et al. (2005)	6	1.9	48
ZcwCo1	Hoffman et al. (2007)	6	1.9	48

Supplementary Table 11: Microsatellite loci of the Southern elephant seal. “Multiplex” denotes the PCR mastermix into which each locus was multiplexed, “Mg” denotes the concentration of magnesium used in the PCR mastermix and “T_a” denotes the annealing temperature used. Loci not assigned to PCR multiplexes were amplified individually.

Allen, P. J., W. Amos, P. P. Pomeroy, and S. D. Twiss. 1995. “Microsatellite Variation in Grey Seals (*Halichoerus grypus*) Shows Evidence of Genetic Differentiation between Two British Breeding Colonies.” *Molecular Ecology* 4 (6): 653–62. <https://doi.org/10.1111/j.1365-294X.1995.tb00266.x>.

Buchanan, F. C., L. D. Maiers, T. D. Thue, B. G. De March, and R. E. Stewart. 1998. “Microsatellites from the Atlantic Walrus *Odobenus rosmarus rosmarus*.” *Molecular Ecology* 7 (8): 1083–85. <http://dx.doi.org/10.1046/j.1365-294X.1998.00401.x>.

Davis, C. S., T. S. Gelatt, D. Siniff, and C. Strobeck. 2002. “Dinucleotide Microsatellite Markers from the Antarctic Seals and Their Use in Other Pinnipeds.” *Molecular Ecology Notes* 2 (3): 203–8. <https://doi.org/10.1046/j.1471-8286.2002.00187.x-i2>.

Hernandez-Velazquez, F. D., C. E. Galindo-Sanchez, M. I. Taylor, J. De La Rosa-Velez, I. M. Cote, Y. Schramm, D. Auriolos-Gamboa and C. Rico. 2005. “New polymorphic microsatellite markers for California sea lions (*Zalophus californianus*).” *Molecular Ecology Notes* 5(1): 140-2. <https://doi.org/10.1111/j.1471-8286.2004.00858.x>.

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<https://doi.org/10.1111/j.1471-8286.2007.01790.x>.

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<http://dx.doi.org/10.4236/ojas.2014.45034>.

Schuelke, M. 2000. "An economic method for the fluorescent labeling of PCR fragments.” *Nature Biotechnology* 18(2): 233-4. <http://dx.doi.org/10.1038/72708>.

Guadalupe fur seal

A total of 224 *Arctocephalus townsendii* samples were collected from pups of the main breeding colony of the species, Isla Guadalupe (Baja California, Mexico). Total genomic DNA was extracted from each sample using silica-gel-membrane technology (DNeasy Blood and Tissue kit, Qiagen; details in Sanvito et al. 2014) and genotyped at 15 microsatellite loci (see Supplementary Table 12 for details). Amplification by PCR was carried out using the “universal tag” method of Schuelke (2000). The following PCR profile was used: one cycle of 3 min at 94 °C; 30 cycles of 30 s at 94 °C, 30 s at T_a °C and 40 s at 72 °C; 8 cycles of 30 s at 94 °C, 30 s at 47 °C and 40 s at 72 °C; and one final cycle of 10 min at 72 °C (see Table 12 for T_a). Magnesium concentration used in the PCR mastermix was different for the different primers, as detailed in Table 12. Fluorescently labelled PCR products were resolved by electrophoresis on an ABI 3730xl capillary sequencer and allele sizes were scored automatically using GeneMarker v1.85. To ensure high genotype quality, all traces were manually inspected and any incorrect call was adjusted accordingly.

Locus	Literature source	Mg (mM)	T _a (°C)
71HDZ2x	Huebinger et al. (2007)	1	45
71HDZ301	Huebinger et al. (2007)	2	57
71HDZ441	Huebinger et al. (2007)	1.5	56
71HDZ5A	Huebinger et al. (2007)	1.5	56
71HDZ5x	Huebinger et al. (2007)	1.5	50
Ag-10	Hoffman et al. (2008)	1.5	56
Ag-4	Hoffman et al. (2008)	1.75	54
Ag-7	Hoffman et al. (2008)	1.5	56
ZcCgDh7tg	Hernandez-Velazquez et al. (2005)	2	54
ZcwA05	Hoffman et al. (2007)	1.5	53
ZcwA12	Hoffman et al. (2007)	2	54
ZcwE03	Wolf et al. (2006)	1.5	56
ZcwE12	Hoffman et al. (2007)	1.5	54
ZcwG04	Hoffman et al. (2007)	1.5	53
ZzCgDh5.8	Hernandez-Velazquez et al. (2005)	1	47

Supplementary Table 12: Microsatellite loci of the Guadalupe fur seal. “Mg” denotes the concentration of magnesium used in the PCR mastermix and “T_a” denotes the annealing temperature used.

- Hernandez-Velazquez, F. D., C. E. Galindo-Sanchez, M. I. Taylor, J. De La Rosa-Velez, I. M. Cote, Y. Schramm, D. Auriolos-Gamboa and C. Rico. 2005. “New polymorphic microsatellite markers for California sea lions (*Zalophus californianus*).” *Molecular Ecology Notes* 5(1): 140-2. <https://doi.org/10.1111/j.1471-8286.2004.00858.x>.
- Hoffman, J. I., S. Steinfartz and J. B. W. Wolf. 2007. “Ten novel dinucleotide microsatellite loci cloned from the Galapagos sea lion (*Zalophus californianus wollebaeki*) are polymorphic in other pinniped species.” *Molecular Ecology Notes* 7(1): 103-5. <https://doi.org/10.1111/j.1471-8286.2006.01544.x>.
- Hoffman, J. I., K. K. Dasmahapatra and H. J. Nichols. 2008. “Ten novel polymorphic dinucleotide microsatellite loci cloned from the Antarctic fur seal *Arctocephalus gazella*.” *Molecular Ecology Resources* 8(2): 459-61. <https://doi.org/10.1111/j.1471-8286.2007.01993.x>.
- Huebinger, R. M., E. E. Louis, T. Gelatt, L. D. Rea and J. W. Bickham. 2007. “Characterization of eight microsatellite loci in Steller sea lions (*Eumetopias jubatus*).” *Molecular Ecology Notes* 7(6): 1097-99. <https://doi.org/10.1111/j.1471-8286.2007.01790.x>.
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- Schuelke, M. 2000. “An economic method for the fluorescent labeling of PCR fragments.” *Nature Biotechnology* 18(2): 233-4. <http://dx.doi.org/10.1038/72708>.
- Wolf, J., D. Tautz, A. Caccone and S. Steinfartz. 2006. Development of new microsatellite loci and evaluation of loci from other pinniped species for the Galápagos sea lion (*Zalophus californianus wollebaeki*). *Conservation Genetics* 7(3): 461-5. <http://dx.doi.org/10.1007/s10592-005-9045-1>.

Galápagos sea lion

A total of 781 samples of *Zalophus wollebaeki* pups were collected as part of a long-term study on the Galápagos islet of Caamaño (0.45_S, 90.16_W) during 2003–2010 inclusive (Wolf *et al.* 2005). Small skin samples were obtained during capture under permission of the Galápagos National Park (PC-001-03 Ext 01, 02, 03-06, 06-08 and PC-043-09). Tissue was stored in 100% ethanol and DNA was subsequently extracted using a DNeasy® tissue kit from Qiagen™. 22 microsatellite loci were PCR amplified and genotyped in four multiplex reactions on an ABI 3730XL capillary sequencer as specified in Supplementary Table 13 using the Qiagen™ Multiplex PCR kit (for details see Wolf *et al.* (2006) and Hoffman *et al.* (2007)). Genotypes were scored automatically with the MegaBACE® Genetic Profiler and GeneMarker software. To ensure consistency and high quality of genotypes, replicate samples were included for each 96 well plates and all traces were manually curated. A subset of the data has been used in previous studies including (Wolf *et al.* 2007, 2008; Wolf & Trillmich 2008; Pörschmann *et al.* 2010; Lenz *et al.* 2013).

Locus	Literature source	Multiplex	T _a (°C)
ZcwAo5	Hoffman <i>et al.</i> (2007)	1	60
ZcwA12	Hoffman <i>et al.</i> (2007)	1	60
ZcwDo1	Wolf <i>et al.</i> (2006)	1	60
ZcwEo5	Wolf <i>et al.</i> (2007)	1	60
Hg4.2.	Allen <i>et al.</i> (1995)	1	60
SGPv9	Allen <i>et al.</i> (1995)	1	60
ZcwAo7	Wolf <i>et al.</i> (2006)	2	60
ZcwBo9	Wolf <i>et al.</i> (2006)	2	60
ZcwCo3	Wolf <i>et al.</i> (2006)	2	60
ZcwC11	Wolf <i>et al.</i> (2006)	2	60
ZcwDo2	Wolf <i>et al.</i> (2006)	2	60
ZcwHo9	Wolf <i>et al.</i> (2006)	2	60
ZcCgDh5.8	Hernandez-Velazquez <i>et al.</i> (2005)	2	60
ZcwEo3	Wolf <i>et al.</i> (2006)	3	60
ZcwFo7	Hoffman <i>et al.</i> (2007)	3	60
Hg6.1	Allen <i>et al.</i> (1995)	3	60
Hg8.10	Allen <i>et al.</i> (1995)	3	60
ZcCgDh7tg	Hernandez-Velazquez <i>et al.</i> (2005)	3	60
ZcwBo7	Hoffman <i>et al.</i> (2007)	4	60
ZcwEo4	Hoffman <i>et al.</i> (2007)	4	60
ZcwE12	Hoffman <i>et al.</i> (2007)	4	60
SGPv11	Goodman SJ (1997)	4	60

Supplementary Table 13: Microsatellite loci of the Galápagos sea lion. “Multiplex” denotes the PCR mastermix into which each locus was multiplexed and “T_a” denotes the annealing temperature used.

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