

**Supplementary Figure 1.** Distance (y-axis) in base pairs (bp) between sites on the same scaffold used in the estimation of covariance and identity-by-state, ordered from smallest distance to largest. The majority (>98%) of sites were separated from each other by >100,000 bp.



## Supplementary Figure 2. Distributions of depth of coverage per genome.

Counts of sites (y-axis) at different depths of coverage (x-axis) for all samples after marking duplicates with samtools markdup, masking repeats with bedtools intersect and removing the x-chromosome using samtools view. Depths were estimated using angsd -doDepth, considering only reads that uniquely mapped to one region of the genome with a minimum mapping quality of 20, minimum base quality score of 30, after adjusting q-scores around indels (-baq 1) and mapQ for excessive mismatches (-C 50), and discarding bad reads (those with flag scores  $\geq$ 256).



**Supplementary Figure 3. DNA damage patterns.** DNA mismatches at the 5' (left) and 3' (right) read termini sequence data generated from the 1955 museum specimen relative to the modern killer whale reference genome (Oorca1.1). The two distributions for post mortem damage signatures (C>T and G>A) are shown in red and blue respectively. The distribution of soft-clipped bases, *i.e.* bases at the read extremities which did not align with the reference, are shown in orange. These are not taken into account in the computation of damage patterns. The frequency of insertions and deletions relative to the reference sequence are shown in purple and green respectively; all other substitutions are shown in grey. Nucleotide frequencies are shown for 25 bases upstream and downstream of the 5' and 3' read termini.



Supplementary Figure 4. mtDNA variation. Visual inspection of the new mitogenome sequences (generated by mapping) in an alignment of 159 unique haplotypes from 455 killer whales (available at: https://doi.org/10.5061/dryad.wpzgmsbr7) encompassing the known geographic and genetic variation within the species (Morin et al. 2015). Individual branches are shown for clades 10-12 and type D killer whales, all other clades are collapsed. At position 8232 in the alignment the museum haplotype (and the other 158 killer whale haplotypes) have a G nucleotide, the modern type D haplotype has an A nucleotide, resulting in a synonymous variant in the ATP6 gene. The remaining three nucleotide differences occur in the hypervariable d-loop region. At position 15774 in the alignment, the museum haplotype has a G nucleotide, the modern type D haplotype has an A nucleotide. This site is triallelic (A, G & T) when considering the reference global killer whale dataset (Morin et al. 2015). The G nucleotide found in the museum haplotype is also found in six out of nine haplotypes in the sister clade (clade 12 in Morin et al. 2015) and four mitogenomes in clade 11. At position 15927 in the alignment, the museum haplotype and all but two other killer whale haplotypes have a T nucleotide. The modern type D haplotype has a C nucleotide at position 15927, which it shares with mitogenome\_118 in clade 12 and with mitogenome 85 (Morin et al. 2015). The widespread paraphyly throughout the killer whale mitogenome tree of these sites within the d-loop that differ between the modern and museum type D specimens suggests these sites are either mutational hotspots (Stoneking 2000), heteroplasmies (Aquadro & Greenberg 1983) or NUMTs (Lopez et al. 1994). Lastly, at position 15996 in the alignment, the museum haplotype and all haplotypes in sister clade 12 except for mitogenome 2 have a C nucleotide, the modern haplotype and all other killer whales have a T nucleotide. In each of these positions, the consensus nucleotide was found in >90% of the mapped reads, suggesting that they were not due to heteroplasmy or NUMTs.



**Supplementary Figure 5.** Principal component analysis (PCA) of genetic covariance among a global dataset, based upon 5,960 transversions. PC1 explains 9.0% and PC2 explains 6.5% of the variation in the dataset. Inset map shows the locations of samples with colors matching the markers on the PCA. Illustrations by Uko Gorter (www.ukogorter.com).



**Supplementary Figure 6.** Pairwise sequential Markovian coalescent plot of the median estimated effective population size ( $N_e$ ) through time, estimated from the high coverage genome sequences of type D killer whales 202035 and 202036.



Supplementary Figure 7. (Previous Page). Kernel density (violin) plots of the length of individual ROH in each genome in the global dataset from Foote et al. (2021) and the three type D genomes (plots 3-5). White rectangle shows the interquartile range, and the black bar the median of the data. X-axis labels correspond to the following genomes: 1. Southern Ocean (labID 17221), 2. Southern Ocean (labID 17222), 3. type D (labID 202035), 4. type D (labID 202036), 5. type D (labID 202037), 6. Antarctic Peninsula, 7. Brazil, 8. Bremer Canyon, 9. Chatham Islands, 10. Clipperton Island, 11. Crozet, 12. Gabon, 13. Gibraltar, 14. Gulf of Mexico, 15. Hawaii, 16. Iceland, 17. Maldives, 18. Mexico, 19. Newfoundland, 20. Ningaloo, 21. Norway, 22. New Zealand, 23. Alaskan offshore ecotype, 24. Alaskan resident ecotype, 25. Scotland, 26. Alaskan transient ecotype, 27. type B1, 28. type B2, 29. type C.



**Supplementary Figure 8.** Number of ROH compared to the sum of the length of ROH across the autosomes. Type D killer whale genomes are represented by black data points. Blue and red markers indicate the Foote et al. (2021) dataset, where the red marker represents the Scotland sample which had the highest inbreeding coefficient ( $F_{ROH}$ ) in that study.

Southern Resident 126158		0.065	0.092	0.062	0.035	0.008	0.038	-0.520	-0.453
Southern Resident 126161	0.204		0.063	0.061	0.039	0.033	0.041	-0.545	-0.472
Southern Resident 126163	0.197	0.210		0.032	0.024	0.003	0.041	-0.546	-0.463
Southern Resident 126165	0.203	0.207	0.222		0.065	0.042	0.068	-0.540	-0.480
Southern Resident 126167	0.212	0.213	0.222	0.204		0.078	0.025	-0.506	-0.454
Southern Resident 126169	0.228	0.223	0.237	0.219	0.204		0.036	-0.503	-0.432
Southern Resident 126178	0.218	0.220	0.224	0.210	0.223	0.229		-0.529	-0.480
type D 202035 (Chile, 2019)	0.564	0.578	0.580	0.576	0.561	0.566	0.577		1.468
	0 - 4 -	0 - 20	0 5 0 0	0 5 0 0	0 540	0 - 4 -	0 5 9 6		
type D 1077 (New Zealand, 1955)	0.517	0.529	0.528	0.530	0.519	0.517	0.536	0.096	

**Supplementary Table 1.** Covariance (above diagonal) and distance (below diagonal) estimates from pseudo-haploid genotypes at 2,233 transversions covered by at least one read in all samples between one modern type D, one museum specimen type D and five Southern Resident killer whales. Pairwise comparisons including a type D killer whale are shaded grey, pairwise comparisons between the two type D genomes are in bold text.

## **References cited in Supplementary Materials:**

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