Appendix/Dryad 1

Is MHC diversity a better marker for conservation than neutral genetic diversity? A case 2

study of two contrasting dolphin populations 3

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Authors: 5

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49	Variant:	1		10			20	0		3	30			40				50			60				70			79
50	Tu-DQB-1	C ACG	GAG	CGG G	GTG (CGG C	TC G	TG	GAC A	GA <mark>T</mark>	CC A	TC TZ	AT A	AC C	CGG	GAG	GAG	TTA	GTG	CGC	TTC	GA	C AG	GC GA	C GI	rg gg	C GA	G
51	Tu-DQB-2	• •••	• • •	•••	•••	• • • •	•• •	••	••• •	•• •	•• •	•• •	•• •	••••	••	• • •	•••	• • G	• • •	•••	• • •	••	• ••	• ••	• •	• • •	• ••	•
52	Tu-DQB-3	• •••	•••	•••	•••	• • • •	A• •	••	A•• •	•• • 2	A• •	•• •	•• •	••••	•••	•••	•••	• A •	• • •	•••	•••	••	• ••	• ••	• •	• ••	• ••	•
53	Tu-DQB-4	• •••	• • •	•••	•••	• • • •	•• A	••	A•• •	•• • 2	A••	•• •	•• •	••••	•••	•••	•••	• A •	A••	•••	•••	••	• ••	• ••	• • •	• • •	• ••	•
54	Variant:	80		90)		10	00			110			120)			130			14	0			150		157	,
55	Tu-DQB-1	TTC CG	G GC	G GTO	ACC	GAG	CTG	GG	C CGG	CCG	GAC	GCC	GAG	TAC	TG	G A	AC AG	GC CA	AG AA	AG GA	AC A	TC	CTG	GAG	CGG	AAA	CGG	
56	Tu-DOB-2	••• ••		с •••	• • •		• • •	••		TG•	AT•	• • •	• • •	• • •	• •	• •	••••		• • •	• • •	• T	• •	• • •	• • •	• • •		• • •	
57	Tu-DOB-3	••• ••			• • •		• • •	••		• • •	• • •	• • •	• • •	• • •	• T	c • ·	••••				• •	••	• • •	• • •	GA•	G••	• • •	
58	Tu-DOB-4	••• ••			• • •		• • •	••			• • •		• • •	• • •	• T	c • ·	• • •				• •	••		• • •	• A •	G••	• • •	
	~ ~																											
59	Variant	160			1	72																						
60			C CT	CCAC																								
61	TU DQD I TU-DOB-2	GCC GA		GGAC		•																						
62	IU-DQB-2																											
02	TU-DQB-3		C G•	• •••	• • • •	•																						
63	Tu-DQB-4	••• ••	• G•	• • • •	•••	•																						
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Fig. A1 Alignment of MHC II DQB sequence variants detected as homozygous. Numbers above sequence alignment (first row)

66 indicate the number of basepairs (i.e. position). The most prevalent sequence variant (Tu-DQB-1), which was found homozygous in

67 individuals of the two *Tursiops* populations is listed first as reference. A dot (•) indicates an identical basepair with reference to Tu-

68 DQB-1. Following Heimeier *et al.*, 2009, MHC II DQB codons that encode peptide binding sites are indicated according to: Murray *et*

al., 1995; Hoelzel *et al.*, 1999; Seddon & Ellegren, 2002; Hayashi *et al.*, 2003 and Baker *et al.*, 2006. The peptide binding sites are

70 highlighted in turquoise color. Primer sequences are not included in this alignment or the analysis.

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Table A1 Microsatellite loci and primer sequences for Shark Bay and Bunbury population

	Primer s	sequences			Allele		
Locus	Forward (5'-3')	Reverse (5'-3')	Multiplex	Label	size range (bp)	No. of Alleles	References
D22	ACATTCCCCAATAAAAGTTAAAGT	CTCTTTGACATGCCCTCACC	2	NED	110-124	5	(a)
KWM12*	CTGGGCACTGTCCTCTGAACATC	AGGAACGGCACATAAAGCACTGA	3	VIC	156-190	15	(b)
MK3	TGCATTCATGTAAAGGTGCG	CTGCAACTAGAGAAAGCC CG	3	NED	147-171	10	(c)
MK5	CTCAGAGGGAAATGAGGCTG	TGTCTAGAGGTCAAAGCCTTCC	3	6-FAM	205-219	6	(c)
MK6	GTCCTCTTTCCAGGTGTAGCC	GCCCACTAAGTATGTTGCAGC	1	PET	152-190	16	(c)
MK8	TCCTGGAGCATCTTATAGTGGC	CTCTTTGACATGCCCTCACC	3	PET	87-117	11	(c)
MK9	CATAACAAAGTGGGATGACTCC	TTATCCTGTTGGCTGCAGTG	3	6-FAM	168-178	6	(c)
Tur4_E12	CTGGGCACTGTCCTCTGAACATC	AGGAACGGCACATAAAGCACTGA	1	PET	256-280	6	(d)
Tur4_F10	TCTTGATGGCTCAGAGGATGATTTTAC	AGCCAAACTGAAGATGCAACTGACTAC	2	6-FAM	374-398	7	(d)
Tur4_66	GAGGGTGGAATGGGGACAAAAAT	TGAAGCCAGGAGACTAGGACAGGTT	1	VIC	185-205	6	(d)
Tur4_80	AGCCAATGTCAGGGTGCTGGAT	GGGGCTTCTTGGCCTCTGTAA	3	NED	287-331	9	(d)
Tur4_87	CCCCATATGATGCCTTTGTAAGTCC	AATTCCTTGTAACAAACCTCTTTATCT	2	VIC	178-194	5	(d)
Tur4_91	GTTGGCTCTCCAGCTCTCAGGT	CAGTGGCTCCCATCTGTATTAGTCA	2	PET	207-235	8	(d)
Tur4_98*	GTCCCCAGAACTTAGCACACTGTC	CAACTGGGGTCCAAAGAAAGAAG	1	NED	192-196	2	(d)
Tur4_105	CCCCGGCCTGCTTACCTCTG	CCGCCCCTCCCCAAGTC	1	6-FAM	367-403	9	(d)
Tur4_108	ACAGGGACCTGAGTGGGTGTAAG	CTTCCCTGGGTCTCTAGGCTACC	1	6-FAM	258-270	2	(d)
Tur4_111	CTCTGTAAGCACCCGTCCTGTGTA	TTCCCGCAGAATTCTGTGAACC	1	VIC	287-307	5	(d)
Tur4_117	TTGCAGTCAGCGTTTTCCAGAGA	GCCAGCCCATCCTTCAGATTTC	1	6-FAM	175-191	5	(d)
Tur4_128	ACGTGCGCATGTCTTTGTCTTAT	CTTTGGACGGGGGAGTAGAACCTA	1	NED	295-311	5	(d)
Tur4_132	CTTCCATGCGCCAGACAACCT	TGGCAAGATGAGAGGGAAAGAGG	3	VIC	326-334	3	(d)
Tur4_138	GTGGCTTACCATGGTGGATTCAG	GCATGGCCATAAAGGGAGGAG	2	6-FAM	207-231	7	(d)
Tur4_141	CACAAGCCTCAACCCTGGTGT	CTAGTCTGCCAATCTGCCCTACAG	2	NED	218-286	13	(d)

Tur4_142	GGCCCCCTTTTCCATCCTCA	CCAGCCCCCAAAATCACGAGT	3	PET	330-346	5	(d)
Tur4_153	TGAGTAACCCCAATCTCGGTCTCT	CCAGCCCCCAAAATCACGAGT	3	PET	215-219	2	(d)
Tur4_162	GCCAACCTCCAGGCAAACACTC	TGCAGTCAACCTGAGGCAAGTCTC	3	6-FAM	365-419	6	(d)

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79 Primer sequences for 25 microsatellite loci used for genotyping Shark Bay and Bunbury bottlenose dolphins. *Loci KWM12 and

80 Tur4_98 were removed from final analysis because they departed from Hardy-Weinberg-Equilibrium expectations. Annealing

81 temperature for polymerase chain reaction was 60 °C for all three multiplex PCR reactions. Fluorescent labels for forward primers are

82 listed in the column "Labels". The range of allele sizes and the total number of alleles ('No. Alleles') for each locus of all individuals

83 sampled in Shark Bay and Bunbury are listed. References for the primer sequences are (a): Shinohara et al. 1997; (b): Hoelzel et al.,

84 1998; (c): Krützen et al., 2001; (d): Nater et al., 2009. This table is modified from supplementary Table S1, Manlik et al. (2018):

85 Manlik, O., Chabanne, D., Daniel, C., Bejder, L., Allen, S.J., Sherwin, W.B. 2018. Demography and genetics suggest reversal of dolphin

source-sink dynamics, with implications for conservation. Mar. Mammal Sci., doi:10.1111/mms.12555.

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97 Table A2 Conservative sampling of individuals with MHC and microsatellite data, which
98 included equal numbers of males, females, calves (calf), juveniles (juv.) and adults (adu.) for
99 each of the two populations. Age classes, i.e. calves, juveniles and adults are defined as in
100 Manlik *et al.* (2016).

	Cons. Sampling	calf	juv.	adu.	males	females	Total
	BB cons.	2	15	38	32	23	55
	SB sample 1	2	15	38	32	23	55
	SB sample 2	2	15	38	32	23	55
	SB sample 3	2	15	38	32	23	55
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- 120 **Table A3** Microsatellite and MHC II DQB diversity measures of the two sampling locations
- 121 of Shark Bay. Measures of microsatellite diversity (A_e = effective number of alleles; ${}^{1}H$ =
- 122 Shannon index; F_{IS} = fixation index) and MHC II DQB nucleotide diversity (π) are listed for
- the two sampling locations in Shark Bay: East Shark Bay (ESB) and West Shark Bay (WSB).
- 124 Subpopulation fixation index (F_{ST}) indicates the degree of differentiation between ESB and
- 125 WSB.

		Microsatellite diversity						MHC II DQB nucleotide diversity			
	Location		n	A _e	1 H	F _{IS}	F st		n	π	
	ESB	Mean SE	409	3.131 0.365	1.205 0.102	0.030 0.009		Mean SD	231	0.06548 0.00150	
	ESB vs WSB						0.006				
	WSB	Mean SE	258	3.180 0.334	1.225 0.099	0.026 0.010		Mean SD	45	0.06197 0.00213	
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Table A4 MHC II DQB variants* and BLASTN hits. Names of MHC II DQB sequence
 variants found in the Shark Bay and Bunbury Tursiops aduncus populations are listed in order from Tu-DQB-1 to Tu-DQB-3. Only the four sequence variants of thirty-nine individuals that are homozygous for all 172 nucleotide sites are listed here. Sequence identities to alleles that closely match these sequence variants and are published on GenBank are shown along with allele names and species in which they were found. Note that Tu-DQB-4 is identical (100% identity at 100% coverage) to the MHC II DQB exon 2 allele Dede-a, which was reported in the common dolphin (Delphinus delphis). Additional sequence

149 variants, i.e. haplotypes, which were inferred by haplotype reconstruction, are not shown here

150 (see main text).

Variant	Identity	Allele	Species
Tuad-DQB*1	99%	Tutr-DQB*27	Tursiops truncatus
	100%	Tutr-DQB*06 (Note: 98% coverage)	Tursiops truncatus
	98%	Stco-DQB*04	Stenella coeruleoalba
	98%	Orbr-a	Orcaella brevirostris
	97%	Cehe-DQB*02	Cephalorhynchus hectori
	97%	Tutr-DQB*25	Tursiops truncatus
Tuad-DQB*2	100%	Tutr-DQB*03 (Note: 98% coverage)	Tursiops truncatus
	98%	Stco-DQB*04	Stenella coeruleoalba
	98%	Cehe-DQB*02	Cephalorhynchus hectori
	97%	Tutr-DQB*01; Tutr-DQB*15	Tursiops truncatus
Tuad-DQB*3	99%	Stco-DQB*06	Stenella coeruleoalba
	99%	Tutr-DQB*24 (Note: 98% coverage)	Tursiops truncatus
	99%	Tutr-DQB*13 (Note: 98% coverage)	Tursiops truncatus
	98%	Stco-DQB*05	Stenella coeruleoalba
Tuad-DQB*4	100%	Dede-a	Delphinus delphis
	99%	Tutr-DQB*01	Tursiops truncatus
	98%	DQB*0101 (LOC101278540)	Orcinus orca
	98%	Tutr-DQB*02	Tursiops truncatus

- **Table A5** Comparison of microsatellite measures within three SB subsamples.
- 160 Mean values for microsatellite observed heterozygosity (H_o), expected heterozygosity relative
- 161 to Hardy-Weinberg Equilibrium expectations (H_e) , effective number of alleles (A_e) and
- 162 Shannon indices $({}^{1}H)$ are shown for each of the subsamples (SB sample 1-3). *F* and *p*-values
- 163 for analysis of variance (ANOVA) test are tabulated comparing the three subsamples.
- 164 *ANOVA test on A_e was based on log-transformed values because the original values were
- 165 not normally distributed.

	SB sample 1	SB sample 2	SB sample 3	F	р	Sig.
H。	0.551	0.566	0.576	0.0919	0.9124	ns
He	0.561	0.587	0.585	0.130	0.8793	ns
Ae	2.931	3.094	3.051	0.0788*	0.9243*	ns*
1 H	1.141	1.194	1.185	2.886	0.0655	ns

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