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Molecular Evidence of a Captive-Born Intergeneric Hybridization Between Bottlenose and Risso's Dolphins: *Tursiops truncatus* × *Grampus griseus*

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Abstract

Natural hybridization occurs rarely in mammals, but it is thought to have the potential to produce viable hybrid offspring in cetaceans more easily than in other mammals. Among cases of cetacean hybridization, hybrids between bottlenose dolphins (*Tursiops truncatus)* and Risso's dolphins (*Grampus griseus*) have been documented in both the wild and in captivity. However, until now, no molecular evidence has been reported for these cases, and little molecular evidence is available for other cetacean species hybrids. Herein, we examined and documented a hybridization case between a female bottlenose dolphin and a male Risso's dolphin held in captivity at Fushun Royal Polar Ocean World in China. We used microsatellite DNA markers, which makes our study the first molecular evidence of hybridization between *T. truncatus* and *G. griseus*. Furthermore, we confirmed the usefulness of using microsatellite DNA markers to identify hybrids in other species of captive-born cetaceans.

Key Words: hybridization, bottlenose dolphin, *Tursiops truncatus*, Risso's dolphin, *Grampus griseus*, microsatellite, captivity

Introduction

Natural hybridization occurs rarely in mammals (Gray, 1972), although it can be widespread in other taxonomic groups (Hubbs, 1955; Chevassus, 1983; Grant & Grant, 1992; Arnold, 1997). Cetaceans are thought to have the potential to produce viable hybrid offspring more easily than other mammals (Arnason et al., 1991), and cases of their hybridization both in the wild and in captivity have been well-documented by scientists (Dohl et al., 1974; Nishiwaki & Tobayama, 1982; Sylvestre & Tasaka, 1985; Baird et al., 1998; Zornetzer & Duffield, 2003; Willis et al., 2004; Caballero & Baker, 2010; Glover et al., 2010). Most of the reported cases of hybridization were inferred by external morphology or skull measurements, making it difficult and time consuming to reach a decision, and sometimes it was even impossible to measure live individuals. More recently, molecular methods based on mitochondrial DNA markers and nuclear autosomal genes have been introduced (Willis et al., 2004; Caballero & Baker, 2010). These increased the feasibility and accuracy of identifying hybrids, but were also time consuming.

Microsatellite DNA analyses have been developed to provide a useful tool with which to establish genetic relationships. Microsatellites are repetitive sequences with very short (1 to 6 bp) repeat units that are single-locus markers exhibiting codominant inheritance (Tautz, 1989). These features, along with the hypervariability of microsatellite loci, make them extremely useful for inferring paternity (Queller et al., 1993; Ashley & Dow, 1994; Haig, 1998). On 26 June 2011, a dolphin calf was born to a female bottlenose dolphin (*Tursiops truncatus*) in Fushun Royal Polar Ocean World, China. The mother had previously lived together with a 5-y-old male bottlenose dolphin and a mature male Risso's dolphin (*Grampus griseus*). The age of the male bottlenose dolphin would suggest it had not yet reached sexual maturity (> 9 y; Jefferson et al., 2008). The calf appeared similar to a normal bottlenose dolphin by external morphology and had no special or abnormal features. As hybridization between bottlenose dolphins and Risso's dolphins has been previously reported (Sylvestre & Tasaka, 1985), there were two possibilities: (1) hybridization or (2) early maturation—either of which would be considered a bit unusual. As morphological comparisons were inconclusive in this case, we used molecular evidence to sort out which possibility applied.

Methods

We sampled four individuals: (1) the calf, (2) the mother, (3) the male bottlenose dolphin, and (4) the male Risso's dolphin. For each individual, we used venipuncture to collect a blood sample into EDTA-K_2 tubes and stored the samples at -20° C. We extracted genomic DNA from the blood samples using a Wizard Genomic DNA Purification Kit (SBS, Shanghai, China) according to the manufacturer's instructions. To amplify the alleles, we employed 17 pairs of microsatellite DNA primers with previously shown to display specificity for captive cetaceans in Chinese aquariums (Zhang, 2011).

We conducted all PCR reactions on a Techne® TC-512 (Bibby Scientific, Chelmsford, UK) machine with a final volume of 15 μL containing 1× PCR buffer (10 mM Tris-Cl, 50 mM KCl, pH 8.3), 0.5 μM of each primer, 2.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.25 U Taq DNA polymerase, and 10 to 100 ng genomic DNA. For all PCRs, we made an initial denaturation step (94° C for 5 min); followed by 36 cycles of denaturation (94° C for 30 s), annealing (53 to 62° C for 30 s) (Table 1), and extension (72° C for 45 s); and then a final extension (72° C for 10 min). We size-fractionated amplified fragments on 8% non-denaturing polyacrylamide gels running at 5 V for about 180 min. We stained products using silver and visualized them with an Ultraviolet Gel Document System (Sambrook & Russell, 2001). We repeated the experiment with each promising primer pair at least twice to ensure its alleles can be amplified stably to minimize errors. We identified the genotypes manually and then confirmed paternity using the direct elimination method.

Table 1. Characterization of the 17 primer pairs employed

Primer pair	GenBank accession no.	Primer sequence (5' to 3')	Annealing temperature (°C)
1	AF416503	GAGAAAGCTGCTGCCAAACT	58.5
		CTGCATTAGGAGCACGGAGT	
2	AF416506	GTCCAGTTTCCTCCAATGGT	58.5
		TCGGTATCTGACTGCTGTGG	
3	AF416507	CTGGGTCAAAAAGGAAGAGC	58
		CCGCTGGGAAGAAACAATAG	
4	DQ018981	CTTTCAACCTGGCCTTTCTG	60
		GTTTGGCCACTACAAGGGAGTGAA	
5	DQ018982	TGGGTGGACCTCATCAAATC	56
		GTTTAAGGGCTGTAAGAGG	
6	DQ018984	AAGAGGATGCAAATGGCAAG	56
		GTTTGGTAAGAAAATACCAAAGTCC	
7	DQ018985	TGGGTCTTGAGGGGTCTG	60
		GTTTGCTGAGGCTCCTTGTTGG	
8	DQ018986	CAGCTTACAGCCAAATGAGAG	59
		GTTTCTCCATGGCTGAGTCATCA	
9	DQ018987	AAGTAAGTGCTCCTTTGACTGG	60
		GTTTGGCAGAGAGATATTAGGACAGC	
10	DQ018988	GAAAAATGCTTCATGCAAC	53
		GTTTCATGATGGCAAAATGATAC	
11	DQ018989	AAGGGGTCTGGAGCAAATGT	59
		GTTTCCACACCTTCTTTGGGGTAA	
12	EU431966	AGCCAATGTCAGGGTGCTGGAT	62
		GGGGCTTCTTGGCCTCTGTAA	
13	EU431968	GTTGGCTCTCCAGCTCTCAGGT	62
		CAGTGGCTCCCATCTGTATTAGTCA	
14	EU431973	TTGCAGTCAGCGTTTTCCAGAGA	61
		GCCAGCCCATCCTTCAGATTTC	
15	EU431983	TCTTGATGGCTCAGAGGATGATTTTAC	61
		AGCCAAACTGAAGATGCAACTGACTAC	
16	EU431974	ACGTGCGCATGTCTTTGTCTTAT	61
		CTTTGGACGGGGAGTAGAACCTA	
17	EU431976	GTGGCTTACCATGGTGGATTCAG	62
		GCATGGCCATAAAGGGAGGAG	

Results

Of the 17 pairs of primers employed, seven were polymorphic and therefore useful for paternity determination, whereas the remaining loci were either monomorphic or invalid in this study. The electrophoresis results and genotypes for the seven working primer pairs are given in Figure 1 and Table 2. In this study, the primer pairs 2, 3, 4, 5, and 6 are di- or trinucleotide microsatellites, while the primer pairs 16 and 17 are tetranucleotide microsatellites. The latter produced few shadow bands and typed more clearly during the electrophoresis (Figure 1). Mammalian offspring have heteroallelic genes—one from the dam and the other one from the sire. Herein, the allelic genes of the dolphin calf were all from the mother and from the male Risso's dolphin (Table 2 & Figure 1), which, thus, was confirmed as the sire.

Discussion

Hybridizations between *Tursiops* and *Grampus* were reported in 1933 in the wild and in 1978 for animals in captivity. The wild hybrids were found after stranding events, and their hybridizations were identified during rescue efforts or necropsies by either external morphology or skull measurements (Van Gelder, 1977). The captive hybrids were usually confirmed because the female bottlenose dolphin lived with a male Risso's dolphin and the resulting offspring showed an intermediate morphology between the two species (Sylvestre & Tasaka, 1985). To date, none of the reported cases of hybridization have been based on molecular evidence. If hybrids are morphologically similar to *Tursiops*, then they are very hard to identify. In our study, we confirmed the hybridization using microsatellite DNA markers, which eliminated the problem of similar external morphology when identifying cetacean hybrids.

Microsatellite loci unique to cetaceans have been reported to contain a high degree of conserved sequences flanking the simple-sequence repeats. Primers designed for one species will often identify variability in other species (Schlotteröer et al., 1991). Results of our study also supported this opinion in that primers we used herein had not

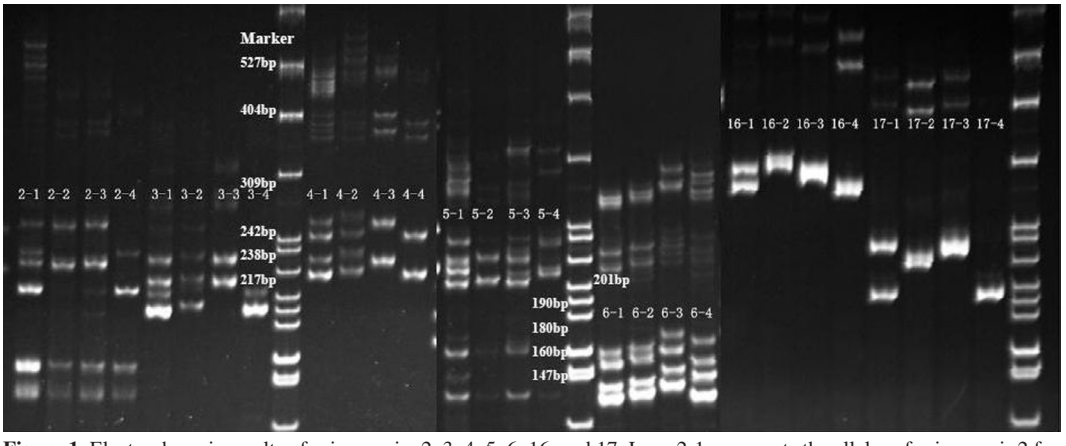

Figure 1. Electrophoresis results of primer pairs 2, 3, 4, 5, 6, 16, and 17. Lane 2-1 represents the alleles of primer pair 2 for the calf (1); Lane 2-2 represents the alleles of primer pair 2 for the male bottlenose dolphin (2); Lane 2-3 represents these alleles for the mother (3); and Lane 2-4 represents these alleles for the male Risso's dolphin (4).

Table 2. Microsatellite genotypes of dolphins used in this study for the seven working primer pairs

	Animal							
Primer pair	Calf (1)		Male bottlenose dolphin (2)		Mother (3)		Male Risso's dolphin (4)	
2	A	C	B	B	A	A	C	C
3	B	D	A	C	B	B	D	D
4	B	D	A	C	B	B	D	D
5	A	C	B	B	C	C	A	A
6	D	F	C	E	A	D	B	F
16	B	C	A	A	B	B	C	C
17	A	C	B	B	A	A	C	C

Note: The letters A, B, C, etc., represent alleles of different size, from large to small, for each primer pair.

been utilized for Risso's dolphin before, and they worked as we expected. We cloned and sequenced PCR products to ensure the reliability of our results (data not shown). Thus, it is easy to employ polymorphic microsatellite primers to determine the paternity of suspected hybrids using paternity exclusion principles. Our study has demonstrated the potential for wide use of this method of hybrid identification in other cetacean species.

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Literature Cited

Arnason, U., Spilliaert, R., Palsdottir, A., & Arnason, A. (1991). Molecular identification of hybrids between the two largest whale species, the blue whale (*Balaenoptera musculus*) and the fin whale (*B. physalus*). *Hereditas*, *115*(2), 183-189.

Arnold, M. L. (Ed.). (1997). *Natural hybridization and evolution*. Oxford, UK: Oxford University Press. http://dx.doi.org/10.1038/36762

Ashley, M., & Dow, B. (1994). The use of microsatellite analysis in population biology: Background, methods and potential applications. *EXS*, *69*, 185-201. http://dx.doi.org/10.1007/978-3-0348-7527-1_10

Baird, R. W., Willis, P. M., Guenther, T. J., Wilson, P. J., & White, B. N. (1998). An intergeneric hybrid in the family Phocoenidae. *Canadian Journal of Zoology*, *76*(1), 198-204. http://dx.doi.org/10.1139/z97-175a

Caballero, S., & Baker, C. (2010). Captive-born intergeneric hybrid of a Guiana and bottlenose dolphin: *Sotalia guianensis* × *Tursiops truncatus*. *Zoo Biology*, *29*(5), 647-657. http://dx.doi.org/10.1002/zoo.20299

Chevassus, B. (1983). Hybridization in fish. *Aquaculture*, *33*(1), 245-262. http://dx.doi.org/10.1016/0044-8486(83)90405-2

Dohl, T. P., Norris, K. S., & Kang, I. (1974). A porpoise hybrid: *Tursiops* × *Steno*. *Journal of Mammalogy*, *55*(1), 217-221. http://dx.doi.org/10.2307/1379276

Glover, K. A., Kanda, N., Haug, T., Pastene, L. A., Øien, N., Goto, M., . . . Skaug, H. J. (2010). Migration of Antarctic minke whales to the Arctic. *PLOS ONE*, *5*(12), e15197. http://dx.doi.org/10.1371/journal.pone.0015197

Grant, P. R., & Grant, B. R. (1992). Hybridization of bird species. *Science*, *256*(5054), 193-197. http://dx.doi.org/10.1126/science.256.5054.193

Gray, A. P. (Ed.). (1972). *Mammalian hybrids* (2nd ed.). Buckinghamshire, UK: Commonwealth Agricultural Bureaux, Farnham Royal.

Haig, S. M. (1998). Molecular contributions to conservation. *Ecology*, *79*(2), 413-425. http://dx.doi.org/10.1890/0012-9658(1998)079[0413:MCTC]2.0.CO;2

Hubbs, C. L. (1955). Hybridization between fish species in nature. *Systematic Zoology*, *4*(1), 1-20. http://dx.doi.org/10.2307/2411933

Jefferson, T. A., Webber, M. A., & Pitman, R. L. (Eds.). (2008). *Marine mammals of the world: A comprehensive guide to their identification.* Waltham, UK: Academic Press.

Nishiwaki, M., & Tobayama, T. (1982). Morphological study on the hybrid between *Tursiops* and *Pseudorca*. *Scientific Reports of the Whales Research Institute*, *34*, 109-121.

Queller, D. C., Strassmann, J. E., & Hughes, C. R. (1993). Microsatellites and kinship. *Trends in Ecology & Evolution*, *8*(8), 285-288. http://dx.doi.org/10.1016/0169-5347(93)90256-O

Sambrook, J., & Russell, D. W. (Eds.). (2001). *Molecular cloning: A laboratory manual* (3rd ed.). New York: Cold Spring Harbor Laboratory Press.

Schlotteröer, C., Amos, B., & Tautz, D. (1991). Conservation of polymorphic simple sequence loci in cetacean species. *Nature*, *354*, 63-67. http://dx.doi.org/10.1038/354063a0

Sylvestre, J., & Tasaka, S. (1985). On the intergeneric hybrids in cetaceans. *Aquatic Mammals*, *11*(3), 101-108.

Tautz, D. (1989). Hypervariability of simple sequences as a general source for polymorphic DNA markers. *Nucleic Acids Research*, *17*(16), 6463-6471. http://dx.doi.org/10.1093/nar/17.16.6463

Van Gelder, R. G. (Ed.). (1977). Mammalian hybrids and generic limits. *American Museum Novitates* (No. 2635). New York: The American Museum of Natural History.

Willis, P. M., Crespi, B. J., Dill, L. M., Baird, R. W., & Hanson, M. B. (2004). Natural hybridization between Dall's porpoises (*Phocoenoides dalli*) and harbour porpoises (*Phocoena phocoena*). *Canadian Journal of Zoology*, *82*(5), 828-834. http://dx.doi.org/10.1139/z04-059

Zhang, P. (2011). *Study on population survey, genetics evaluation and studbook establishment of cetaceans housed in Chinese aquaria* (Doctoral dissertation). Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, China.

Zornetzer, H. R., & Duffield, D. A. (2003). Captive-born bottlenose dolphin × common dolphin (*Tursiops truncatus* × *Delphinus capensis*) intergeneric hybrids. *Canadian Journal of Zoology*, *81*(10), 1755-1762. http://dx.doi.org/10.1139/z03-150