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ORIGINAL RESEARCH

Genes or Culture: Are Mitochondrial Genes Associated with Tool Use in Bottlenose Dolphins (*Tursiops* sp.)?

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Abstract Some bottlenose dolphins use marine sponges as foraging tools ('sponging'), which appears to be socially transmitted from mothers mainly to their female offspring. Yet, explanations alternative to social transmission have been proposed. Firstly, the propensity to engage in sponging might be due to differences in diving ability caused by variation of mitochondrial genes coding for proteins of the respiratory chain. Secondly, the cultural technique of sponging may have selected for changes in these same genes (or other autosomal ones) among its possessors. We tested whether sponging can be predicted by mitochondrial coding genes and whether these genes are under selection. In 29 spongers and 54 non-spongers from two study sites, the non-coding haplotype at the HVRI locus was a significant predictor of sponging, whereas the coding mitochondrial genes were not. There was no evidence of selection in the investigated genes. Our study shows that mitochondrial gene variation is unlikely to be a viable alternative to cultural transmission as a primary driver of tool use in dolphins.

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Introduction

Culture in wild animals has been broadly defined as socially transmitted innovations that are stable over multiple generations (Whiten and van Schaik 2007). This field has attracted widespread interest, especially as it might serve as a model to explain the more fully developed cultures in humans. However, opinions about the importance of social learning of information or innovations among animals in nature vary dramatically. While some researchers see it as a ubiquitous phenomenon (Dugatkin 2000; De Waal 2001), others are not convinced, arguing that social learning is invoked spuriously to explain patterns of behavioral variation among animal populations (Galef 1992; Heyes 1993; Tomasello 1993; Laland and Hoppitt 2003; Laland and Janik 2006). In early studies, social transmission was invoked by excluding potential ecological and genetic explanations for observed behavioral variants among wild animal populations. For instance, research on chimpanzees (*Pan troglodytes*, Whiten et al. 1999) and orangutans (*Pongo* spp., van Schaik et al. 2003) illustrated striking cultural complexity in great ape species by identifying behaviors that were most likely socially transmitted within and between generations.

These studies have been criticized, however, for both conceptual and interpretative problems (Laland and Janik 2006; but see Krützen et al. 2007). Indeed, a shortcoming of the past approaches is that alternative models have not been explicitly tested (Laland and Janik 2006; Krützen et al. 2007; Krützen 2009; Whitehead 2009). Without doing so, researchers cannot assess the existence or relative

importance of any genetic predispositions underlying these seemingly innovative behaviors. Further, these behaviors may have been subject to gene-culture co-evolution, as genetic predispositions may influence the ability to acquire new skills by social learning (Feldman and Laland 1996; Boyd and Richerson 2005). In both cases, a correlation between behavioral and genetic variation is to be expected that does not, or at least not exclusively, reflect culture (Laland and Janik 2006). Nevertheless, correlations between genetic and behavioral variation may also arise through purely cultural processes. This occurs through parallel matrilineal transmission of socially learned behaviors and mitochondrial DNA (mtDNA), a phenomenon known as "cultural hitchhiking" (Whitehead 1998). Hence, vertical matrilineal transmission patterns may resemble those of genetic inheritance, even though genes do not play a role.

Social transmission of tool-use in bottlenose dolphins provides an interesting example of vertical matrilineal transmission (Krützen et al. 2005). In Shark Bay, Western Australia, bottlenose dolphins show remarkable intra-population variation in foraging tactics (Mann and Sargeant 2003). Most of these tactics are thought to be propagated through social transmission (Mann and Sargeant 2003), although ecological influences on the observed patterns have been shown (Sargeant et al. 2007). One particular foraging tactic, referred to as "sponging", involves individual dolphins carrying conical marine sponges over their rostra and is the first documented case of tool use in a cetacean species (Smolker et al. 1997). It is predominately adult females that engage in sponging, during which they swim slowly just above the sea floor and probe the substrate with a sponge covering their rostra like a protective glove. In the Eastern Gulf of Shark Bay, all but one "spongers" were found to belong to the same matriline, as revealed by sequencing the non-coding hypervariable region I (HVRI) of the mitochondrial DNA (Krützen et al. 2005). A more recent study showed that all sponging individuals at a geographically separate site in the Western Gulf of Shark Bay belong to a single matriline that is different from that of spongers in the Eastern Gulf (Ackermann 2008).

Straightforward genetic inheritance and expression patterns did not explain the observed variation in sponging within the Eastern Gulf (Krützen et al. 2005). However, this study did not exclude alternative genetic explanations. Laland and Janik (2006) suggested that genes in the mitochondrial genome could influence sponging behavior. Under such a scenario, variation in sponging behavior, at least among females, might be due to differences in diving ability caused by genetic variation of mitochondrial genes coding for proteins of the respiratory chain, such as *cytochrome b* (*cytb*) or *cytochrome c oxidase II* (*coxII*). Since mtDNA is passed on maternally, this could result in the same phenotypic pattern being expressed (Laland and Janik 2006) as that which was interpreted as vertical transmission in Krützen et al. (2005).

In order to distinguish between the cultural and genetic interpretation, we tested whether coding mtDNA genes or the non-coding HVRI are better predictors of the observed pattern of tool use within the Shark Bay population. Furthermore, we also tested whether coding mtDNA genes are under positive selection within the Shark Bay population.

Materials and methods

Study site

This study was conducted in Shark Bay, 850 km north of Perth in Western Australia. A long-term study of bottlenose dolphins (*Tursiops* sp.) was established in 1984 off Monkey Mia in the Eastern Gulf of Shark Bay (Connor and Smolker 1985). In 2007, we set up a new study site off Useless Loop in the Western Gulf of Shark Bay, southwest of Monkey Mia at a distance through the water of 110 km (Fig. 1). Both photo-identification and genetic data (Krützen et al. 2004a) suggest there are no direct movements of animals between the two sites.

In the Western Gulf, pre-determined transects of 6 nm across depth contours were conducted using a small (5.5 m) vessel at speeds of 7–8 knots in search of dolphins. When dolphin groups were sighted, transect lines were temporarily broken to conduct ad libitum behavioral surveys (Altmann 1974; Mann 1999). In the Eastern Gulf, only ad libitum behavioral surveys were conducted for this study. During the first 5 min of such surveys, observers recorded behavioral and ecological data, such as group membership, predominant group activity, GPS locations and water depth. At both sites, dolphin identities were determined using long-term photographic databases (Wursig and Wursig 1977). In the Eastern Gulf, spongers were known from previous studies (Krützen et al. 2005). In the Western Gulf, all dolphins sighted using a sponge at least once were included in the dataset.

Biopsy sampling

Tissue samples of free-ranging dolphins were obtained through remote biopsy sampling (Krützen et al. 2002) on an opportunistic basis from various locations across Shark Bay between 1994 and 2008. We recorded the position of each biopsy-sampling event using a Magellan Meridian Marine GPS device. Biopsy samples were stored in a saturated NaCl/20% dimethyl-sulfoxide solution (Amos and Hoelzel 1991) at −20°C in the field and −80°C in the laboratory.

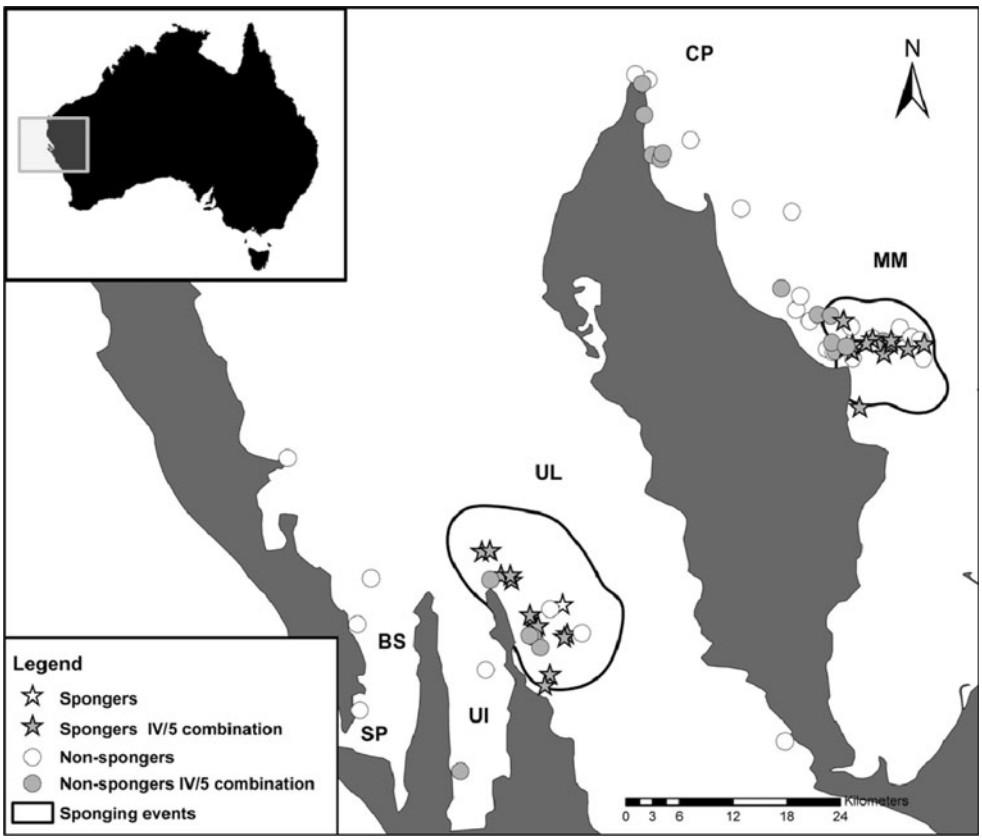

Fig. 1 Sampling locations within Shark Bay. Sponging areas represent the 95% kernel home range from all location points where sponging has been observed off Useless Loop and Monkey Mia. *Grey stars* represent spongers, which show haplotype IV for coxII and 5 for cytb, while *white stars* represent spongers that show another haplotype combination on these gene regions. The same holds for non-spongers, which are represented by *grey* and *white circles*

We included a total of 83 dolphins (59 females and 24 males) from several different sampling sites (Fig. 1) in this study. The dataset consisted of 29 spongers and 54 non-spongers. Sponging behavior is strongly female biased, but a few male spongers have been observed in both gulfs (Ackermann 2008; Mann et al. 2008). We therefore included females and males in our analysis in order to investigate the influence of sex on sponging. In the Eastern Gulf, animals were sampled around Monkey Mia (MM) and Cape Peron (CP), and in the Western Gulf, at Useless Loop (UL), Useless Inlet (UI), Blind Straight (BS) and South Passage (SP). The chosen individuals represented all known HVRI haplotypes in order to increase the likelihood of finding discrete *cytb* and *coxII* haplotypes or combinations thereof.

Choice of mtDNA loci

Single amino acid changes within conserved regions may impair or slightly alter enzymatic functions of proteins encoded by mtDNA genes (Andreu et al. 1999). Due to the clonal inheritance of the mtDNA molecule, this would provide a simple and straightforward mechanism to create and maintain differences between matrilines within populations. Hence, we chose two mitochondrial genes, *coxII* and *cytb*, because of their crucial role in the respiratory chain, which is directly linked to adenosine triphosphate production and metabolic energy (Howell 1989; Prusak and Grzybowski 2004). *CoxII* contains the redox centre cupper (Cu_A), which binds the electron carrier cytochrome c at a loop that contains two conserved Cystine $Cys_{196/200}$ and two conserved Histidine $His_{161/204}$ residues (Capaldi 1990; Michel et al. 1998). *Cytb* is believed to contain two highly conserved redox centers Q_0 and Q_i, which transfer electrons to the heme groups (Prusak and Grzybowski 2004). The heme-ligating sites are at position $His_{83/196}$ and $His_{97/182}$ (Howell et al. 1987). Other highly conserved *cytb* regions include the residues 130–150 within Q_0 and the region spanning residues 270–290 (Howell 1989).

Sequencing

We performed DNA extractions with the Gentra Tissue Kit™ (Gentra) according to manufacturer's instructions. A mtDNA fragment of 468 base-pairs (bp) length, comprising parts of the proline transfer RNA gene and HVRI, was amplified using the polymerase chain reaction (PCR). Sequences were amplified using primers dlp1.5 and dlp5 (Baker et al. 1993). The haplotypes of the *cytb* gene were determined by sequencing a mtDNA fragment of 1140 bp, using flanking primers on the transfer RNA (tRNA) genes on either side. The forward primer *cytb*-L14724 was from Palumbi et al. (1991), and the reverse primer

cytb-L14724R from Southern et al. (1988). To amplify 684 bp of the *coxII* gene, primers CO2 LCET F (5′-TAAARTCTTACATAACTTTGTC-3′) and CO2 RCET R (5′-TCTCAATCTTTAACTTAAAAGG-3′), developed by Gatesy (unpublished) were used. PCRs contained 20 ng template DNA, 0.05 u *Taq* DNA Polymerase (Sigma–Aldrich), 0.2 mM dNTPs, PCR buffer, 2.5 $MgCl_2$ mM final concentration, 0.3 µM of each primer and double-distilled water to add up to a 20-µl volume. PCR amplifications were performed in a PTC-220 thermocycler (MJ Research) with the following profile: initial activation at 94°C for 3 min, 39 cycles of 45 s at 93°C, 60 s at 48°C and 90 s at 72°C, followed by a final extension step of 3 min at 72°C. PCR purification was conducted using silica membrane spin columns (QIAquick®, Quiagen).

Cycle sequencing was performed with the ABI PRISM® BigDye™ Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems). The cycle sequencing reaction contained 20–25 ng template DNA, 0.4 µl of forward or reverse primer, 1.75 µl PCR buffer, 0.5 µl BigDye (Applied Biosystems) and ddH_2O to add up to a 10-µl volume. Reaction conditions were as follows: initial activation at 95°C for 45 s, 29 cycles of 30 s at 95°C, 20 s at 52°C and 2 min at 60°C, followed by a final extension step of 3 min at 72°C. Sequencing reactions were cleaned up by adding 75 µl of 0.2 mM $MgSO_4$ in 70% v/v Ethanol, incubation at room temperature for 15 min, centrifuging at $3{,}000 \times g$ for 30 min, and aspirating off the supernatant. Products were then re-suspended in 20 µl of dH_2O and run on an ABI 3730 DNA Sequencer (Applied Biosystems). Sequences were quality-controlled using the software Sequencing Analysis (version 5.2). Alignment of the forward and reverse sequences was carried out by eye using the software SeqMan (Lasergene version 7.1). Consensus sequences obtained for all three mitochondrial regions were aligned using the software BioEdit 7.0.5.3 (http://www.mbio.ncsu.edu/BioEdit/bioedit.html). Nucleotide sequences of the two coding genes were translated into amino acid sequences using SeqBuilder (Lasergene version 7.1) and BioEdit. For *cytb* and *coxII*, the correct open reading frame (ORF) was determined with the reference sequence AF084092 from *Tursiops aduncus* (http://www.ncbi.nlm.nih.gov), and Q70RQ7 from the white beaked dolphin *Lagenorhynchus albirostris* (UniProtKB), respectively.

Statistical analyses

Generalized linear models (GLM) provide one possible framework in which to analyze the contribution of genetic factors to cultural patterns (Laland and Janik 2006). Analyses of effects of genotype at the HVRI region, as well as *cytb* and *coxII* genes on sponging were conducted using GLMs with binomial errors and a logit link function in R (R Core Development Team 2009). Sex was included as a fixed effect in the model. A binomial error structure fit the data reasonably well, with no over-dispersion. The response variable was comprised of two vectors, number of spongers and the number of non-spongers, for each unique combination of the categorical variables. We could not include interactions between haplotypes in the model as there were too few unique combinations at the three loci, causing a failure of model convergence. To assess significance of effects, we used likelihood ratio tests with Chi-squared significance tests in which the full model including HVRI, *cytb*, *coxII* and sex was compared to models with each factor removed in turn (Crawley 2007). As an alternative inference test, we compared models by penalized log-likelihood using the small sample size correction of the Aikaike Information Criterion (Akaike 1973), the AICc (Hurvich and Tsai 1989).

Tests for selection

Due to the respiratory role of mitochondria, it is conceivable that selection on mitochondrial genes may play a role in the diving ability of dolphins. In this case, a link between mtDNA genes and diving ability in cetaceans may lead to a detectable signature of selection in these genes. We tested this hypothesis by applying Tajima's D statistic (Tajima 1989) and Fu's test (Fu and Li 1993) to both genes using Arlequin, version 2.000 (Schneider et al. 2000).

Results

In a previous study in Shark Bay, eight different HVRI haplotypes (A to H) with 18 polymorphic sites have been identified based on a 426 bp long HVRI fragment (Krützen et al. 2004a). A recent study identified two additional HVRI haplotypes in the Western Gulf of Shark Bay (Ackermann 2008), labeled I and K. All 10 HVRI haplotypes were aligned in a 468 bp final fragment, showing 25 polymorphic sites. Of the 25 polymorphic sites, twenty were transitions, five transversions, and one was an insertion-deletion polymorphism.

The *coxII* amino acid sequence revealed that, within our study population, amino acid replacements did not affect conserved regions crucial for the function of the protein and the ligand-binding of Cu_A and Mg (Michel et al. 1998). As expected, the number of *coxII* haplotypes was much lower than the number of HVRI haplotypes. We found four different *coxII* haplotypes (I–IV) with 22 polymorphic sites. Two amino acid replacements separated the *coxII* haplotype IV and the three other *coxII* haplotypes I–III. Only one replacement occurred between the reference

sequence of *L. albirostris* and the *Tursiops* haplotypes, indicating that these genes are highly conserved between species.

For *cytb*, we obtained a similar picture, as the amino acid substitutions in the different *cytb* haplotypes do not affect conserved regions (Howell 1989). We obtained seven *cytb* haplotypes (1–7) with 45 polymorphic sites. The protein sequences revealed nine amino acid substitutions compared to the reference sequence AF084092 of *T. aduncus*. Due to some problematic sequencing reads in the C-terminus of the *cytb* gene, the full length (1140 bp) was not obtained for all sequences. Therefore, the alignment of our data with the reference sequence started at base 22 (CAC codon). In the resulting 1119 bp fragment, we found one frame shift mutation. However, this mutation affected only the last four amino acids at the *N*-terminus, and is therefore not likely to lead to an overall change in the protein structure and function.

The haplotype of the non-coding HVRI locus was the only significant predictor of sponging (Table 1), thus model simplification using likelihood ratios resulted in a univariate model containing only HVRI. This model also gave the lowest AICc value of all possible additive models (Table 2).

Table 1 Likelihood ratio tests of effects of a single predictor variable removed from the full model

Variable	Change in d.f.	Change in deviance	*P*
HVRI	−4	−11.813	0.019
cytb	−2	−0.327	0.849
coxII	−1	−0.001	0.993
Sex	−1	−1.859	0.173

Table 2 Model AICc for all combinations of factors

Model	Residual d.f.	AICc
Sex	21	69.2
Null	22	68.9
coxII	19	52.7
coxII + sex	18	52.6
cytb + *coxII*	14	47.8
cytb + *coxII* + sex	13	47.4
HVRI + *cytb* + *coxII* + sex	9	46.7
HVRI + *cytb* + *coxII*	10	45.6
HVRI + *cytb* + sex	10	43.8
cytb	16	42.8
HVRI + *cytb*	11	42.8
cytb + sex	15	42.3
HVRI + *coxII* + sex	11	41.3
HVRI + *coxII*	12	40.6
HVRI + sex	12	38.6
HVRI	13	37.9

Table 3 Haplotypes of spongers and non-spongers for the three mitochondrial markers

Haplotype combinations			Number of individuals	
HVRI	*coxII*	*cytb*	Non-spongers	Spongers
A	I	1	7	0
B	I	2	4	0
C	II	3	6	0
D	III	4	4	0
	IV		3	0
E[a]	**IV**	**5**	**6**	**14**
		6	**1**	**1**
F	IV	5	1	0
		3	5	0
G	IV	5	5	0
H[a]	**IV**	**5**	**9**	**14**
I	IV	7	1	0
K	IV	3	2	0

[a] The HVRI haplotypes E and H are shown in *boldface*, as these include all spongers

In the Western Gulf, all spongers share the same HVRI haplotype E (Ackermann 2008), while almost all spongers in Monkey Mia show HVRI haplotype H (Krützen et al. 2005; Ackermann 2008). All spongers with HVRI haplotype E or H share *coxII* haplotype IV and *cytb* haplotype 5. There is one exception: in the Western Gulf, one sponger shows *cytb* haplotype 6 instead of *cytb* haplotype 5 (Table 3, Fig. 1). The fact that the *coxII/cytb* haplotype combination IV/5 is found in all but one sponger may suggest that this particular combination is somehow predictive of sponging. However, the same combination was also found in more than one-third (38%) of all non-spongers.

Both genes investigated in this study appear to evolve under a neutral model of evolution with no selection on them, as both Tajima's D (Tajima 1989) and Fu's test (Fu and Li 1993) revealed no evidence of selection (Tajima's D: $coxII = 0.63$, $P = 0.80$, $cytb = 0.65$, $P = 0.81$; Fu's test Fs: $coxII = 12.19$, $P = 0.99$, $cytb = 15.94$, $P = 0.99$).

Discussion

We provide novel and significant evidence that tool use in bottlenose dolphins is culturally transmitted, as previously assumed (Krützen et al. 2005). The only significant predictor of sponging was the haplotype at the hypervariable region I in the mitochondrial control region. This locus will not lead to any phenotypic differences between

non-spongers and spongers as it is non-coding and has only been used as a proxy to determine matrilineal membership of dolphins. Mitochondrial coding genes investigated in this study do not predict tool use in bottlenose dolphins as well as the non-coding HVRI. Amino acid replacements in these genes do not affect conservative residues thought to be crucial for the function of the proteins. Within-population heterogeneity at both genes can therefore not be responsible for differences in diving ability among different matrilines, as previously hypothesized (Laland and Janik 2006). There is also no signature of selection in the investigated coding genes, as neither test for selection was significant, indicating that the genes under investigation follow a neutral model of evolution and are in mutation-drift equilibrium (Kimura 1985).

Our findings support previous notions that special genetic or physiological adaptations may not be required to exhibit sponging behavior (Smolker et al. 1997; Krützen et al. 2005). Dolphins typically stay submerged for only one to three minutes between surface bouts when sponging, which is not significantly different from foraging dolphins not exhibiting this foraging tactic, but living in the same habitat (Smolker et al. 1997; Mann et al. 2008). Diving ability in marine mammals depends on oxygen storage in skeletal muscles, which is facilitated through myoglobin (Castellini and Somero 1981), rather than on enzymes involved in the respiratory chain, as proposed by Laland and Janik (2006). Myoglobin has been found to correlate positively with body mass and maximum dive duration in toothed whales (Noren and Williams 2000). Different myoglobin alleles, however, are unlikely to contribute to the observed vertical transmission pattern of sponging, as autosomal inheritance patterns are not concordant with the matrilineal inheritance pattern found in sponging dolphins (Krützen et al. 2005). In cetaceans, the myoglobin locus should also be autosomal, as suggested by annotated genomic data from the closest relative of cetaceans with a fully annotated genome (*Bos taurus* genome built Btau_4.0; available from http://www.ncbi.nlm.nih.gov/).

Our results rule out that the investigated mtDNA coding genes alone predict sponging behavior. In our study, we considered with *coxII* and *cytb* only two of the 13 genes encoded on mtDNA, preventing us from completely ruling out any effect from other mtDNA genes, such as *NADH*, on sponging. Furthermore, our model does not exclude other potential, ever more complex genetic explanations for sponging, such as epistatic interactions between mtDNA and nuclear genes. For instance, a previous study showed that the expression of different mtDNA-encoded *NADH* dehydrogenase and *cox* subunits in the central nervous system of congenic mice strains had an effect on their cognitive abilities, due to the interaction with the nuclear genome (Roubertoux et al. 2003). We are unable to test for such effects, given small sample sizes and genetically diverse study individuals. However, we argue that high levels of promiscuity exhibited by both males and females in this population (Krützen et al. 2004b), along with extensive gene flow within the study area (Krützen et al. 2004a), would render linkage disequilibrium between mtDNA and nuclear genes, or other processes such as assortative mating, unlikely to the extreme. Moreover, in Shark Bay bottlenose dolphins, many foraging tactics co-exist within a single population (Mann and Sargeant 2003), of which sponging is only one. This pattern is also found in other cetaceans (Connor 2001), questioning the plausibility of models positing genetic interactions for each foraging tactic in the first place. Nonetheless, these kinds of models are almost impossible to disprove. One solution to this problem is to invoke the parsimony principle in the case of studying culture in wild animal populations. Given the numerous studies on captive and wild cetaceans showing a remarkable capacity for social and vocal learning (Kuczaj et al. 1998; Janik 2000), social transmission of sponging should be the least complex explanation for the observed behavioral variation in our study population.

Bottlenose dolphins are capable of vocal and motor imitation (Bauer and Johnson 1994; Kuczaj et al. 1998), which are prerequistes for social learning to occur. Further support for social transmission of sponging behavior is provided by the observation that only dolphins born to spongers have ever been known to become spongers (Mann and Sargeant 2003). The youngest calf ever observed sponging was at the age of about 20 months significantly older than young dolphins starting to catch fish, suggesting that sponging is a difficult foraging technique to learn (Mann and Sargeant 2003). Dolphins whose mothers do not sponge may lack the social learning experience for this specific behavior, perhaps during a sensitive phase. The sponging foraging tactic seems to be similar to the skilful foraging behaviors documented in chimpanzees, aye-ayes, orangutans and killer whales (Nishida 1973; Bard 1992; Guinet and Bouvier 1995; Krakauer and van Schaik 2005; Jaeggi et al. 2008). All these species have a relatively late weaning age, providing a prolonged mother-offspring phase for more learning opportunities. Indicators for social learning in these skilled foragers include an extreme parental tolerance at feeding, offspring peering at foraging adults, which is also observed in dolphins (Mann et al. 2007), and even food sharing (Bard 1992).

Behavioral variation within populations is one of the most distinctive elements of cetacean behavioral ecology (Rendell and Whitehead 2001). Indeed this sets them apart from the great apes, in which behavioral variation is found primarily between populations (Whiten et al. 1999; van Schaik et al. 2003). Over the past decade, several field studies have documented a remarkable range of variation in

vocal dialects, foraging sites, as well as foraging and feeding tactics (reviewed in Rendell and Whitehead 2001). The occurrence of innovations and their social transmission is underpinned by advanced social learning abilities, one of the characteristics that at least some cetacean species have in common with humans and great apes. For example, killer whale populations of the eastern North Pacific are structured into several social levels with distinctive features in vocal and social behavior, as well as foraging tactics (Ford et al. 1998; Yurk et al. 2002; Yurk 2003). Vertical transmission of socially acquired traits is also found in sperm whales (*Physeter macrocephalus*), which produce distinctive patterns of clicks for acoustic communication (Weilgart and Whitehead 1997). These animals live in stable, matrilineal groups and use socially acquired dialects that are distinct from those of other groups occupying the same habitat. These distinct dialects appear to be transmitted vertically between mothers and their offspring (Weilgart and Whitehead 1997; Whitehead 1998). Not surprisingly, a strong correlation between dialect and mitochondrial DNA was found in a study of six sperm whale groups (Whitehead 1998).

These observations raise the question of whether such stable vertical transmission patterns, like those observed in dolphins, can lead to changes in the genetic makeup of populations. Gene–culture co-evolutionary theory is the appropriate framework in which to analyze the observed patterns. This theory builds on conventional population genetics models. However, in contrast to describing allele proportion changes in response to evolutionary processes solely due to selection or genetic drift effects, gene-culture co-evolutionary analyses also incorporate cultural transmission. Using a simulation approach, Whitehead (1998) showed that low mtDNA variation found in matrilineal whales could be explained by a hitchhiking effect of neutral mtDNA. Under such a scenario, a selective sweep for a particular mtDNA haplotype took place once vertically transmitted cultural traits conferring certain fitness advantages were introduced into the simulation, replacing most other lines. Whitehead's model provides a basic framework for explaining the changes in the genetic make-up of a population due to culture. There appears to be no fitness differences between spongers and non-spongers (Mann et al. 2008). However, even without conferring a selective advantage, it looks as if tool use enabled spongers to exploit a niche that would not be available otherwise (Kreicker 2010). Hence, social transmission can lead to haplotype frequency changes on very small geographic scales, such as foraging niches within populations. In Shark Bay, this is corroborated by findings that in a non-sponging context, matriline membership appears to correlate highly with different habitat types (A. Kopps, unpublished data). This departure from random haplotype distributions in certain ecological niches or habitats suggests that vertically transmitted foraging specializations provide a relatively simple mechanism by which social learning can alter the genetic make-up of a population. We deem it therefore conceivable that these matrilineal transmission patterns described herein provide the foundation from which more complex gene-culture co-evolutionary pathways could have evolved. In highly cultured species such as humans, gene-culture co-evolution has been documented for several genes and human behaviors (Laland et al. 2010). We would expect similar, albeit less obvious, patterns in highly cultured great ape species. The advent of affordable genomic tools will enable researchers to decouple variation in behavior caused by selection and drift from that generated through cultural processes, allowing the investigation of gene-culture co-evolution in non-model species.

In summary, our findings demonstrate that mitochondrial coding genes are inadequate to explain the observed variation in sponging, further strengthening the case for cultural transmission of tool use in dolphins. Shark Bay dolphins provide an ideal system to study the combined effects of genetics, ecology and sociality on the variation of behavior. Transmission of sponging, for instance, is found at least in at least two distinct matrilines. These matrilines occur in allopatry in a single population characterized by weak autosomal substructure (Krützen et al. 2004a, b), allowing for the inclusion of nuclear relatedness into models predicting that certain behaviors are genetically manifested. Rejection of such models would strengthen the case for cultural transmission.

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