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Evolutionary biology

Mitogenomes and relatedness do not predict frequency of tool-use by sea otters

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Many ecological aspects of tool-use in sea otters are similar to those in Indo-Pacific bottlenose dolphins. Within an area, most tool-using dolphins share a single mitochondrial haplotype and are more related to each other than to the population as a whole. We asked whether sea otters in California showed similar genetic patterns by sequencing mitogenomes of 43 otters and genotyping 154 otters at 38 microsatellite loci. There were six variable sites in the mitogenome that yielded three haplotypes, one found in only a single individual. The other two haplotypes contained similar percentages (33 and 36%) of frequent tool-users and a variety of diet types. Microsatellite analyses showed that snail specialists, the diet specialist group that most frequently used tools, were no more related to each other than to the population as a whole. The lack of genetic association among tool-using sea otters compared with dolphins may result from the length of time each species has been using tools. Tool-use in dolphins appears to be a relatively recent innovation (less than 200 years) but sea otters have probably been using tools for many thousands or even millions of years.

1. Introduction

Relatively little is known about tool-use in marine animals: the best-studied species are sea otters (*Enhydra lutris*) and Indo-Pacific bottlenose dolphins (*Tursiops aduncus*), both of which bring tools to the ocean's surface where they can be easily observed [1]. There are several intriguing parallels between the ecology of tool-use in California sea otters and dolphins. First, not all individuals in a population use tools [2,3]. Second, tool-use is related to consumption of prey that are difficult to access. Sea otters use rocks or other hard objects to break open well armoured prey such as marine snails [4]. Dolphins use conical sponges as tools to protect their sensitive snouts while probing among rocks for small, burrowing fish that live at the bottom of deep ocean trenches [3,5]. Third, tool-use appears to be a response to resource limitation from high population density, which leads to the development of dietary specialization [6,7]. In California sea otters, food limitation results in individuals in the same area specializing on different prey. Some eat mainly large prey such as abalones and crabs, others mainly urchins and mussels, and others mainly small marine snails [7]. Individuals belonging to every diet type sometimes use tools but tool-use is most frequent in those that prey heavily on snails [4]. Tool-use in dolphins occurs in a subset of individuals that specialize on small fish that cannot be accessed without the use of tools and only individuals feeding on these fish use tools [8–10]. Finally, diet preferences appear to be transmitted by mothers to their female offspring in both sea otters [11] and dolphins [12].

Table 1. Mitogenomes in southern sea otters. The three complete mitogenome haplotypes and the four D-loop haplotypes found by Larson *et al.* [15] are shown for comparison. Individual genotypes are in table S1, electronic supplementary material.

snp location:	1491 bp	11679 bp	13547 bp	14101 bp	15365 bp	15592 bp	15593 bp	15609 bp	
gene:	tRNA	tRNA-Ser	NDS	ND6	tRNA-Thr	D-loop	D-loop	D-loop	
HAPLOTYPE	T/C	A/G	A/G	A/G	C/T	A/G	C/T	C/T	N
1	C	A	A	A	C	G	C	T	9
2	C	G	A	A	C	G	C	T	1
3	T	G	G	G	T	G	T	T	33
A						G	T	C	Larson <i>et al.</i> [15]
B						A	T	T	Larson <i>et al.</i> [15]
C						G	T	T	Larson <i>et al.</i> [15]
D						G	C	C	Larson <i>et al.</i> [15]

Analyses of maternally inherited mtDNA haplotypes revealed that almost all the tool-using dolphins in an area belong to a single matriline [12,13] and nuclear markers indicated that tool-users were more related to other tool-users than expected by chance (although, this finding was not significant for the western gulf of Shark Bay). We asked whether sea otters showed similar genetic patterns by analysing mitochondrial genomes and nuclear microsatellite genotypes of individual sea otters together with behavioural data on their diet and frequency of tool-use.

2. Material and methods

Individual sea otters were captured and tagged from 2000 to 2014 along the California coast (electronic supplemental material, table 1). We used focal animal sampling [14] to opportunistically record foraging data on individual otters. For each feeding dive, we recorded whether or not prey was captured, prey identification and the presence or absence of tool-use (see Tinker *et al.* [7] for detailed methods). Each otter was assigned to one of six diet specialist groups—ABALONE, CRAB, MUSSEL, CLAM, URCHIN or SNAIL—using fractional composition analysis as detailed in Tinker *et al.* [7]. We excluded abalone captures from analyses of tool-use because we could not consistently determine the frequency of underwater tool-use to obtain abalone [2]. Individuals were considered frequent tool-users if they used tools for at least 40% of observed prey captures, based on a gap in the distribution of tool-use frequency between the individuals that used tools on 1–27% of prey captures and those that used them on 44–90% of prey captures (electronic supplementary material). Dependent pups were not considered in any analyses.

We used massively parallel multiplexed sequencing to obtain complete mitochondrial genomes from 43 otters (electronic supplementary material) in the hopes of finding new haplotypes, as only three are known from D-loop [15]. We performed a χ^2 test and a Goodman–Kruskal τ test to assess the relationship between the mitochondrial haplotype and diet type. In addition, we genotyped 38 microsatellite loci from 154 otters. Mitochondrial haplotypes were identified and compared with diet type and whether individuals used tools frequently or infrequently. Microsatellite genotypes were used to determine if frequent tool-users and otters belonging to the same diet specialist group were more likely to be related to each other than to the population as

a whole using a permutation test implemented in the ‘related’ package in R [16] (electronic supplementary material).

3. Results

Mitochondrial sequencing revealed a 16 431 bp mitogenome with a mean read depth ranging from 21 to 1100. The cytosine homopolymer in the 16 s rRNA starting at position 2644 bp was excluded from analyses because it could not be aligned accurately. The mitogenome contained six variable sites revealing three mitogenome haplotypes (table 1). One haplotype was found in only a single individual. The other two haplotypes contained similar percentages of frequent tool-users (33% in haplotype 1 and 36% in haplotype 3) and there was no significant relationship with diet type ($\chi^2 = 18.3$, $p = 0.405$; Goodman–Kruskal τ value = 0.055) (table 2). Locations of individual haplotypes are shown in electronic supplementary material, figure S1. We recovered one new D-loop haplotype (GCT) in 23% of the samples ($n = 10$). The rest of the samples (77%) had D-loop haplotype GTT, congruent with the Larson *et al.* [15] finding that ‘C’ haplotype for D-loop was most common in California. We did not find their haplotypes ‘A’ or ‘D’, which were less common in their sample ($A < 10\%$ and $D < 20\%$), probably by chance. Larson *et al.* [15] found haplotype ‘B’ only in Alaska.

The microsatellite data did not have any geographical structuring (R. B. Gagne 2017, unpublished data). A summary of locus information (e.g. average number of alleles) is included in the electronic supplementary material. Neither the Queller & Goodnight (Q&G) [17] nor the Lynch and Ritland (L&R) [18] estimator found that frequent tool-users ($n = 21$) were more likely to be related to each other than to the population as a whole ($n = 133$; L&R expected $r = -0.0066$, observed $r = -0.0067$; Q&G $r = -0.031$, 95% CI 0.021, $p > 0.4$) (see electronic supplementary material). The Q&G method found no association with any diet type and relatedness, whereas the L&R estimator found the otters specializing upon clams (expected $r = -0.0065$, observed $r = -0.0016$, $p < 0.05$) and crabs (expected $r = -0.0066$, observed $r = -0.0040$, $p < 0.05$) were significantly more likely to be related to those with the same diet type than to those with other diet types (electronic supplementary material, figure S3). However, these values of

Table 2. Number of sea otters by diet type and mitogenome haplotype. Number of frequent tool-users (more than or equal to 40% of dives) in parentheses.

diet type	mitogenome haplotype			total
	1	2	3	
snail	3(3)	0	9(9)	12(12)
clam	2	1	4	7
mussel	2	0	3	5
abalone	1	0	1	2
crab	1	0	13(3)	14(3)
urchin	0	0	3	3
total	9(3)	1	33(12)	43(15)

relatedness are close to zero (i.e. negative and in the third decimal place) and can likely be interpreted as zero or no relatedness. On a sample of 11 known mothers and pups, the mean value of the Q&G estimator was closer to the expected value of 0.5 (0.46 ± 0.04) than that of the L&R estimator (0.37 ± 0.03).

4. Discussion

Despite the many ecological similarities between tool-use in dolphins and sea otters, we found that the genetic patterns in these two species are different. In otters, diet types and tool-use are dispersed across both common mitogenome haplotypes and neither otters that specialize on snails (the diet type that contains most of the frequent tool-users) nor frequent tool-users are more related to each other than to the population as a whole. By contrast, tool-use in the dolphins and its associated diet type (small cryptic fish) are predominantly confined to a single mitochondrial matriline in each of the two geographical areas in which it occurs and individuals that use tools are more related to each other than expected (however, relatedness was not statistically significant for the western gulf of Shark Bay) [12,13,19].

The lack of genetic association with tool-use in sea otters, compared with dolphins, may result from the length of time each species has been using tools. Tool-use in dolphins is thought to be a recent innovation [20]; however, it is likely a much older behaviour in sea otters. This is supported by evidence that, as in some tool-using birds [21] but unlike dolphins, all young otters appear to be innately predisposed to use tools; orphaned otter pups raised in captivity exhibit rudimentary pounding behaviour without training or previous experience [4,22] and wild pups develop tool-use behaviour

before weaning, regardless of their mother's diet type [22]. However, this behaviour only becomes a regular part of adult foraging behaviour under certain ecological conditions [2,4]. Moreover, similar tool-use behaviours occur in all three sea otter sub-species [23], so it is likely that tool-use developed prior to sub-speciation [2]. Conversely, tool-use in dolphins has only been regularly observed within Shark Bay in western Australia. The Miocene ancestors of modern sea otters, *Enhydriodon* and *Enhydritherium*, had already developed large, flat molars suitable for crushing shells and exoskeletons of macro invertebrates [24]. Very hard-shelled prey, such as snails and large clams, are not easily accessed without the use of tools: recent research on sea otter bite-force indicates that the armouring of marine snails gives them a hardness that is at the upper limits of what a typical otter can crush with its jaws (C. Law 2017, personal communication). Thus, if the ancestors of modern sea otters were eating hard-shelled molluscs, they were likely using tools to open them. It may be possible not only to confirm that the ancestors of modern sea otters used tools but to estimate the approximate period when this behaviour emerged if some fossil sea otters exhibit morphological signs of tool-use, such as the depressed sternum seen in some modern individuals [24].

Ethics. All fieldwork was conducted with authorization by the US Fish and Wildlife Service under permits issued to M. T. Tinker, and with oversight by the Institutional Animal Care and Use Committee at the University of California Santa Cruz.

Data accessibility. Mitogenomes for individual otters are in the electronic supplementary material file. Mitogenomes and microsatellite data were submitted to the DRYAD repository with the accession number #([doi:10.5061/dryad.6vtf7j](https://doi.org/10.5061/dryad.6vtf7j)), URL:<http://datadryad.org/review?doi=doi:10.5061/dryad.6vtf7j>) [25]. Field data have been archived in USGS Sciencebase, DOI <http://doi.org/10.5066/F78050S9>

Authors' contributions. K.R. conceived the study and wrote the first draft. N.R.M., R.B.G., H.B.E. and J.M. supervised and conducted genetic laboratory work and analyses. J.F. and M.T.T. supervised and conducted fieldwork and assigned the otters to diet types. All authors edited and approved the final draft of the manuscript and agree to be accountable for all content.

Competing interests. We have no competing interests.

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