

Josh Jeffery
BIO 206, Sec. 1
Dr. Buratovich
November 9, 2007

Speciation Paper on Population Structure and Speciation of Bottlenose Dolphins Based on Microsatellite and Mitochondrial DNA Analyses

The article this paper summarizes is titled “Population structure and speciation in the genus *Tursiops* based on microsatellite and mitochondrial DNA analyses”. In this article, the authors talk about the possibility of speciation among various populations of the bottlenose dolphin. This was a study verifying the reality of whether or not speciation has or is currently occurring in the bottlenose dolphin. The results of this study were quite interesting.

The bottlenose dolphin *Tursiops truncatus* is a species that has a worldwide distribution being found in both hemispheres of the earth and can thrive in tropical and cold temperature waters. The genus that the bottlenose dolphin belongs to (*Tursiops*) consists of as many as twenty species. This article was about a study on the population structure and speciation of bottlenose dolphins based on microsatellite and mitochondrial DNA analyses. The group of researchers involved in this study looked at samples from populations of *T. truncatus* as well as *Tursiops aduncus*, which is an Indo-Pacific variant of the bottlenose dolphin, in several regions around the world¹.

Speciation by definition is the process by which new species are created. Speciation can occur through barriers set on the organism such as a river or mountains. However, a marine environment has very few barriers that would cause speciation based on this definition. Some marine species like some fish can show genetic variation that

¹ Natoli, pg. 363-375

can be panmixia over large geographic areas. Marine mammals on the other hand, have a fine-scale population structure, but the extent of the population structure varies among each species. It has been argued that such a population structure is due to a combination of behavioral specializations for local resources, the social structure of the animal and even cases of historical environmental changes¹.

As mentioned earlier, the genus *Tursiops* contains up to around twenty different species. However, this is often based on very limited data. The more persistent species other than *T. truncatus* include *T. gilli* and *T. nuuanu* which are found in the eastern North Pacific and *T. aduncus* which are found around Australia, the Indian Ocean, China, and South Africa. The differences between these species include color pattern, body dimension, and cranial structure. These characteristics do overlap into each species territories. As a result of this, *T. truncatus* was the only largely recognized species out of the genus *Tursiops*. It was not until molecular data supported the classification of *T. aduncus* as a separate species. The authors of this article point out that such a pattern is not uncommon among members of the delphinidae family and uses the killer whale (*Orcinus orca*), the common dolphin (*Delphinus delphis*), and the spotted dolphin (*Stenella longirostris*) as examples because it has been shown that similar morphotypic diversity exists within these species¹.

There have been previous studies where the species *T. truncatus* and *T. aduncus* have been compared. In one study, samples were retrieved from coastal and pelagic populations of the two species around China. Samples from *T. truncatus* were collected from individuals found in pelagic regions while samples from *T. aduncus* were collected from individuals found in coastal regions. 5' mitochondrial DNA (mtDNA) was used,

and a nucleotide divergence of 4.4%, six fixed nucleotide differences, and reciprocal monophyly were found. That particular study helped confirm that *T. aduncus* was its own separate species¹.

A similar study was conducted in the western North Atlantic which spans from Florida to Nova Scotia. The coastal and pelagic populations of the genus *Tursiops* were compared to morphology, feeding ecology, parasite load, hemoglobin profile, microsatellite DNA, and mtDNA control region diversity. There were evident distinctions between the populations, but the genetic differentiation was not as much as what was found in China between *T. truncatus* and *T. aduncus* individuals found there. A comparison between populations on either side of the state of Florida was also conducted and it was found that there was differentiation. However, it was not made clear if all of the samples were from the same morphotype (all pelagic or all coastal)¹.

In the study that this article is about, researchers tested the hypothesis of whether the local fine-scale population structure for *T. truncatus* that are found in the western North Atlantic is characteristic of all of the other populations in the genus *Tursiops* throughout their range. Representation of the regional populations was greatly extended to include a comparison of *T. aduncus* in South Africa with the data from the China study and with the common dolphin (*D. delphis*). Also included were *T. truncatus* populations from the eastern North Pacific¹.

269 *Tursiops* sp. samples from seven geographic regions were collected for this study. Samples were collected in a large part from stranded dolphins or ones that were caught in nets. DNA samples collected from these dolphins were extracted from tissue samples that were preserved in a salt saturated 20% DMSO solution by a standard

phenol/chloroform extraction method. The China study's data was also used in this study as well as western Africa and the Bahamas¹.

There were nine microsatellite loci that were analyzed for every sample. The only exceptions were the western North Atlantic coastal and western North Atlantic pelagic populations. For those populations, only five of the nine microsatellites were analyzed. These microsatellites were KWM1b, KWM2a, KWM2b, KWM9b, and KWM12a. In this study, these five were derived from killer whales (*O. orca*); EV37Mn came from *Megaptera novaenglinae*, and TexVet5, TexVet7, and D08 came from *T. truncatus*¹.

The use of an automated ABI PRISM 377 DNA sequencer was done to analyze DNA for length variation. This DNA was amplified and put in 6% polyacrylamide gels. This was done after the incorporation of 1/10 fluorescent-labeled primer. Polymerase chain reaction (PCR) conditions occurred. The annealing temperatures (T°ann) were recorded for each microsatellite. The T°ann of KWM1b was 45°C, KWM2a was 43°C, KWM2b was 44°C, KWM9b was 55°C, KWM12a was 46°C, EV37Mn was 57°C, TexVet5 was 54°C, TexVet7 and D08 were both 57°C. To determine the allele size, an internal standard marker was used¹.

To help clarify the relationship between the *T. truncatus* and *T. aduncus* populations, the common dolphin (*D. delphis*), a close relative, was also analyzed. Samples from thirty individuals were collected. These individuals were from the eastern North Atlantic, Mediterranean Sea, and the eastern North Pacific. These samples were compared with the other 269 samples from the genus *Turisops* population¹.

The level of polymorphism for the microsatellite loci was estimated as the number of alleles per locus, observed heterozygosity, expected heterozygosity, and allelic

richness. Using the program FSTAT 2.9.3 determined the allelic richness. Hardy-Weinberg equilibrium was also evaluated for any deviation from the expected genotypic frequencies and linkage disequilibrium using Fisher's exact test and the Markov chain method. Analysis of these numbers was performed using GENEPOP 3.1d¹.

186 sequences of mtDNA were available for analysis. Universal primers MTCRf (5'-TTC CCC GGT GTA AAC C) and MTCRr (5'-ATT TTC AGT GTC TTG CTT T) were used to amplify the mtDNA control region. The use of SEQUENCHER 3.0 aligned the DNA sequences. ARLEQUIN 2.0 estimated the degree of differentiation and Tajima's D. PAUP* 4.0b10 compared individual haplotypes phylogenetically with the neighbor-joining method and was rooted with a homologous sequence from killer whales. Network 2.0 generated a median-joining network that inferred phylogenetic relationships between the Atlantic and Mediterranean mtDNA haplotypes¹.

Microsatellite results revealed a significant Hardy-Weinberg deviation in the Mediterranean population as well as a heterozygote deficiency in two loci. There was no excessive heterozygosity in any of the populations. It was also found that the Mediterranean and western North Atlantic pelagic populations had a higher level of average allelic diversity and heterozygosity than any other population studied. The western North Atlantic pelagic population had a significantly greater allelic richness than the coastal population of the same region, Gulf of Mexico population, and the eastern North Atlantic populations combined. Genetic differentiation was also observed in pairwise comparisons. The results showed a significant differentiation among all comparisons¹.

In mtDNA, sixty-six haplotypes were identified and found fifty-six polymorphic sites. Finding shared haplotypes between any of the populations was not common. The study also found that diversities were rather high for each population. The results of the mtDNA analysis showed great differentiation and diversification¹.

This study found that the genus *Tursiops* has a considerable amount of genetic differentiation and diversity among all of the populations studied. It was discovered that all of the individual populations had their own alleles and had significant differentiation among other populations for both mtDNA and microsatellite DNA loci. It was also found that genetic diversity was the highest wherever the population was known to be pelagic. The high level of differentiation that has been found in all of the populations that were studied has suggested the high potential for speciation within a genus¹.

This article was very interesting as it incorporated some of what has been covered in class. It was also an interesting article because it involved cetaceans namely the bottlenose dolphin. It was a well written article, but some of the terminology was a little confusing. In spite of that little problem, this article was an interesting read.

Works Cited

Natoli, Ada; Peddemors, Victor M.; A. Rus. "Population structure and speciation in the genus *Tursiops* based on microsatellite and mitochondrial DNA analyses". *Journal of Evolutionary Biology* vol. 17, issue 2, pg. 363-375, March 2004. Blackwell Publishing LTD; 2003. Retrieved from <<http://www.blackwell-synergy.com/doi/abs/10.1046/j.1420-9101.2003.00672.x>> 11/6/07.