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Ambiguous evidence of a recent bottleneck in the Adriatic bottlenose dolphins (*Tursiops truncatus*)

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Introduction

Bottlenose dolphin (Tursiops truncatus):

- the only resident marine mammal species in Croatian part of the Adriatic Sea with number estimated at around 250 individuals (Gomerčić et al., 2002)
- the main target of exterminations campaigns from 19th century until 1960s - culling was promoted as a means of mitigating conflict with fisheries (Bearzi et al., 2004)
- in 1995 dolphin killings became illegal, when all marine mammals became protected by law in Croatia

The aim of the study:

- to test the hypothesis that the bottlenose dolphin population from the Croatian part of the Adriatic Sea has experienced recent effective population size reduction, i.e. a bottleneck

Materials and methods

- tissue samples of 30 bottlenose dolphin carcasses found on Croatian coast of the Adriatic Sea (Fig. 1), collected from 1994 until 2003
- total genomic DNA extracted using Wizard Genomic DNA Purification Kit, Promega
- genotyping at 12 dinucleotide microsatellite loci: EV1Pm, EV14Pm derived from *Physeter macrocephalus*, EV37Mn, EV94Mn from *Megaptera novaeangliae* (Valsecchi and Amos 1996) and D08, D14, D18, D22, D28, TexVet3, TexVet5, TexVet7 from *Tursiops truncatus* (Shinohara et al. 1997, Rooney et al. 1999)
- ABI PRISM, 310 Genetic Analyzer (Applied Biosystems), GeneScan Analysis Software 3.1 and Genotyper 2.5.2. (Applied Biosystems) softwares to determine allele sizes

Testing for evidence of recent bottleneck events - 3 approaches

- 1.) "Bottleneck, A program for detecting recent effective population size reductions from allele frequency data", v. 1.2.02. (Piry et al., 1999)
 - assumption: in a recently reduced population the gene diversity will be higher than that expected at equilibrium
 - gene diversity estimated under three models of molecular evolution: the stepwise mutation model (SMM), the infinite allele model (IAM), and the two-phase model (TPM) with 95% single-step mutations and 5% multiple-step mutations
 - a variance among multiple steps: 12
 - 10,000 iterations for each mutation model
 - the one-tailed Wilcoxon signed rank test for heterozygote excess (to determine if the number of loci exhibiting heterozygosity excess was significant)

- 2.) "Bottleneck, A program for detecting recent effective population size reductions from allele frequency data", v. 1.2.02. (Piry et al., 1999)
 - distribution of allele frequencies tested to determine whether a bottleneck-induced mode shift has recently occurred
 - mode shift is a transient distortion in the distribution of allele frequencies such that the frequency of alleles at low frequency becomes lower than the frequency of alleles in an intermediate allele frequency class (Luikart et al., 1998)

- 3.) Critical-M and M-P-Val programs (Garza and Williamson, 2001)

- M-ratio test - based on the ratio of the observed number of microsatellite alleles to the range of allele sizes
- M-ratio is expected to decrease in bottlenecked populations since alleles are randomly lost as a result of genetic drift
- two sets of parameter values simulated: $\mu_s = 0.9$ and $\Delta_g = 3.5$; and $\mu_s = 0.88$ and $\Delta_g = 2.8$
- each parameter set tested for four values of θ parameter (0.01, 0.1, 1 and 2)

Results

- 1.) Wilcoxon sign-rank tests were not significant under two mutational models: TPM (with 95% single-step mutations) and SMM ($p = 0.898$ and 0.961 , respectively)
 - p-values were still >0.05 when alternative proportions (range 70-95%) were attributed to the single-step mutations in TPM
 - Wilcoxon sign-rank test was significant only under the IAM ($p = 0.0461$) - however, all loci will follow a mutation model somewhere in-between IAM and SMM
 - the null hypothesis of the Wilcoxon's test (no significant heterozygosity excess on average across loci) can not be rejected - there is no sufficient evidence for a recent bottleneck in the Adriatic bottlenose dolphin population

- 2.) analysis of allele frequency distribution failed to detect a mode-shifted distribution of allele frequencies (Fig. 2) - bottleneck is not likely to have occurred in the recent past

- 3.) the sample M ratio was calculated to be 0.736
 - sample M ratio value was smaller than the simulated critical and average M ratio values in each parameter set and for each value of θ (Fig. 3) - a recent bottleneck in the investigated bottlenose dolphin population is suggested, contrary to the results obtained using above approaches

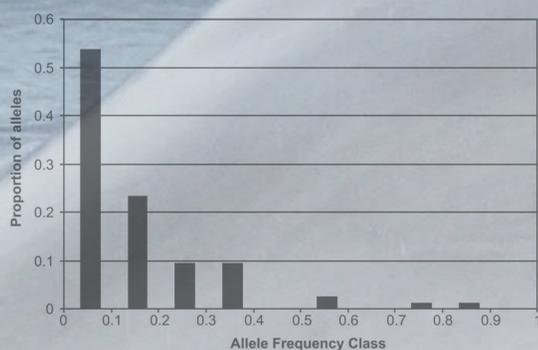


Fig. 2.

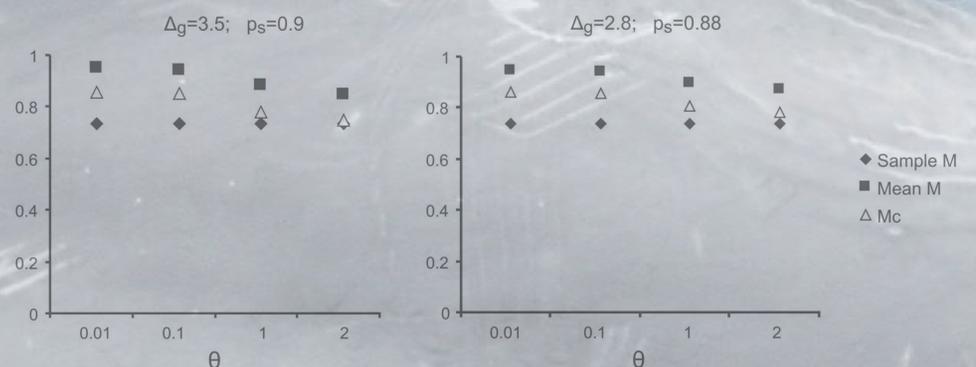


Fig. 3.

Conclusions

- we are more inclined to accept the evidence of recent bottleneck found by M ratio test, taking a more conservative approach to the interpretation of ambiguous bottleneck results
- we suggest maintaining the current level of bottlenose dolphin protection in the area and careful monitoring of the population in the future
- further research is needed to confirm a possible recent bottleneck, and to investigate regional fine-scale population structuring and gene flow among bottlenose dolphins in the eastern Mediterranean Sea

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