

In the manuscript entitled "*Speciation in the face of gene flow within the toothed whale superfamily Delphinoidea*", the authors use available genomic data from 9 cetacean species to reconstruct as best they can the demographic history of the surveyed samples.

While some results seem robust, notably phylogeny and ancestral variations in effective sizes, other analyses still require further controls to convince and reinforce the current interpretations, which are at the origin of the title of the article. In particular, I did not understand how the authors rejected speciation scenarios with cycles of (geographically related) reproductive isolation / secondary contacts?

Such a test seems to me to be missing here or I inadvertently missed it. I doubt whether it is possible to test it with the current data so far, and therefore suggest in this respect to reduce the emphasis on the conclusion of "speciation in the face of gene flow". Certainly there would have been gene flow in the past, but this does not make it a support for widespread sympatric speciation in cetaceans. This gene flow may have been periodic, interspersed with periods of allopatric barrier accumulation. Although I cannot support the title put forward in the current state of the manuscript (and of the available data), the main interest I find in the article is to prepare the next step, which would be the acquisition of population data of the 9 cetacean species studied, and then to use explicit model comparison methods to statistically evaluate alternative speciation scenarios.

My overall opinion on this article is therefore positive and promises exciting future studies on this topic, studies which will provide a better answer to the question currently being asked and which need to temper a little more the conclusions that can be drawn from the current results.

#### **Comments :**

The title is a bit strong. I found nothing in the paper that rejects the scenario of past cycles of repeated geographic isolation followed by secondary contacts. An explicit comparison of different models would be needed to be able to test that the inferred past migrations are not past secondary contacts following older isolations.

The introduction refers a bit too much to a vision of diversification as a series of dichotomies. It has been now accepted that gene flows between species are common and that hybrid zones of semi-isolated species are frequent. Perhaps this emphasis should be reduced a little in the introduction, on a point that is certainly exciting and worth mentioning, but not so new nowadays.

#### Figure 1:

It is possible that phylogenetic discrepancies are linked to the GC content (%GC) of the windows considered. This can be explained by the GC-Biased Gene Conversion (gBGC) known to be very strong in vertebrates. More recombination means more gBGC, and so, more elevated %GC. But more recombination also means less genetic interference (because reduced linkage) as well, and therefore less ILS.

Can the authors look at the proportion of alternative topologies for the 33% of windows that have the highest %GC, the 33% of windows that have an intermediate %GC and the 33% of windows that have the lowest %GC?

#### D-foil :

Same remark as for phylogenetic discordances and D-foil. Do the regions that recombine the most (at least, those that have suffered the most from gBGC) have different D-foil than the AT-rich regions?

#### Cessation of gene flow :

I personally did not understand in the material and method what exactly was done.

This is what I understood :

**Step 1)** Run PSMC on a diploid genome composed of two haploids which are the consensus of 2 different species. This would apparently give the size of the common ancestor.

**Step 2)** Using PSMC's estimated size and predefined divergence times to identify a gene flow stop using coalescent simulations.

I don't understand what are the effective size values between the present time (at the time of sampling) and the past time (just after the split) that have been used.

How simulations with  $m_s$  can be used to estimate parameters. Do the authors use ABC to estimate some parameters while fixing the others?

As I am not familiar with estimating gene flow parameters with PSMC, would it be complicated to imagine a robustness check of this inference?

This could be easily done by :

**i)** a simulation step of the pseudo-F1 hybrid of 2 species using  $m_s$ . This pseudo-F1 can be produced for two types of scenarios: without migration (Strict Isolation model) and with ancestral migration (which takes place between  $T_{split}$  and  $T_{am}$  where  $T_{am}$  is the stop time of the ancestral migration). For this, it is possible to use a few tens (hundreds) of random combinations of  $N_e$ ,  $T_{split}$ ,  $T_{am}$  considered as being the pseudo-F1 hybrids.

**ii)** repeat steps 1 and 2 which have been applied on the empirical data sets but on the pseudo-F1 produced, but for each of the pseudo-F1 hybrids produced using known parameters.

This would make it possible to convince a little more that the practice put in place by the authors makes it possible to estimate  $T_{split}$  and  $T_{am}$  with precision.

The result shown in [Figure 2-A](#) is astonishing. While the estimated split times vary enormously, this is not the case for  $T_{am}$ . The cessation of gene flow seems to take place simultaneously for each pair, and this for pairs of species with different levels of divergence at the precise time of cessation of gene flow. As if molecular divergence in Delphinoidea has no impact on reproductive isolation, which is not intuitive. Is it possible to represent the shown results not in terms of years, but in terms of the expected net divergence? This could be reconstructed for each pair by simulating the scenario inferred by the authors in order to obtain empirically what the level of divergence was at the moment when the gene flow was interrupted. This would make it possible to see whether the Delphinidae make gene flow for levels of divergence greater than 2% (to the right of the grey zone of speciation) or not.

#### Interspecific hybridisation :

I find the proposal of an increased interspecific introgression during periods of low abundance interesting but obviously does not seem to be exclusive. What is suggested here is that for a small  $N_e$ , selection against hybrids would be less efficient and would allow more efficient transfer of alleles between species, facilitated by drift.

However, this may also be the case with large  $N_e$  when, in a sub-region of the species distribution range, the local density of individuals decreases. This process would be found again without observing any  $N_e$  related effect.

An asymmetry in  $N_e$  could also explain an increase in introgression. Notably by pump effects with species with small  $N_e$  (and therefore with many weakly deleterious mutations) which would introgress alleles without burden from a species with large  $N_e$ .

While studying the  $N_e$ -introgression relationship is an interesting idea, the large number of possible and different scenarios makes highly difficult to distinguish between them.

#### Figure 3:

are the effective sizes shown on panels B, C and D really expressed as  $10^4$  individuals?

Conclusions :

The authors insist on cases of speciation with frequent gene flow in Delphinoidea. While they show that there was past gene flow, they did not reject scenarios with past allopatrate/secondary contact cycles.