Adaptive skull shape changes in bottlenose dolphins (*Tursiops spp*.): inference from combined morphological and genomic analyses

Mammals are very diverse animals, exhibiting large differences in their lifestyles, behaviours and body shapes. A striking example of this is the diversity of their skulls, which can in many groups be extremely modified. Dolphins are one such group, where some of the snout bones are extremely elongated relative to closely related terrestrial mammals. Some of these modifications are thought to be related to different feeding requirements in the ocean, and therefore result from the direct action of natural selection, but this has never been formally tested. It is important to do so, because morphological changes can arise for reasons other than natural selection. In the case of skull shape changes, even if they occur due to natural selection, they might not result from adaptation to different food types, given that the head is involved in many important functions (e.g. breathing, brain activity, sensory activities).

This project is designed to test whether skull shape changes in dolphins are driven by natural selection resulting from different prey types. It will do this by focusing on bottlenose dolphins (genus *Tursiops*), as this genus includes a large diversity of prey types across multiple species and populations within each species. In addition to this, bottlenose dolphins have been subject to extensive studies, and because of this numerous museum specimens and tissue samples are available from around the world, allowing a comprehensive study to be made without invasive sampling of animals in the wild. Bottlenose dolphins from the wild commonly show differences between populations which mostly live in deep oceanic waters, and others that live mostly near the coast. These populations have been shown to be characterised by consistent differences in skull shape.

This project will first quantify the differences in skull shape between bottlenose dolphins around the world, by creating 3 dimensional models of museum specimens from several high quality digital photographs. Specialized software will then be used to mark key features in the 3D models of each skull, and then compare the shape between animals of different environments. This will provide an indication of which skull features contribute the most to differences in shape between environments, but won't test for the effects of selection. To test this, we will analyse the variation in genes known to affect skull shape in mammals, for each of the same dolphin populations that are analysed for skull shape differences. Because natural selection can only act on features that have a strong genetic basis, selection can affect an animal's genetic composition in predictable ways. Therefore, by comparing genes known to affect skull shape in mammals, against the observed changes in skull shape in dolphins, we can test for associations between the specific skull shape changes and selection in genes underlying these changes.

Preliminary research by the project PI has demonstrated the feasibility of this approach . A study on 2D skull shape changes in dolphins showed differences between populations living in different environments. Another study on genome wide genetic variation found that selection could be detected in genes affecting skull length, between dolphin populations which differed mostly in the length of their skulls. The planned study will build upon these preliminary observations, by providing a more comprehensive and realistic analyses of three dimensional shapes and by analysing genes associated with morphology more comprehensively. For the genetic analyses, a technique called exon-capture will be used to isolate only the genes of interest, and then determine their sequence for several samples of dolphin round the world using genomic technologies.

This project will thus enable a formal test of the hypothesis that selection is a driver of skull shape change in bottlenose dolphins, by combining state-of-the-art morphological and genomic analyses. The protocol developed in this project, can easily be adapted to any other morphological or physiological traits as well as other species, and will therefore be of use to a broad range of research questions. Therefore, this project will not only allow us to understand the evolutionary history of bottlenose dolphins, but will also provide valuable contributions to the study of evolutionary history of mammals. Finally, if a local population is shown to be adapted to its environment, then it is less likely to be replaced by animals from elsewhere if it became extinct. Therefore, the project will also inform conservation efforts with these animals, by identifying populations that have greater conservation priority because they are characterised by specific morphological traits that are likely adaptive.