Corso di dottorato nazionale in Biodiversity - Ciclo XL

Compensatory mechanisms for a deleterious mutation in mtND5 of the Critically Endangered Marsican bear population

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Marsican Bear population

The **Marsican** or **Apennine bear** consists in an isolated population of approximately 50 individuals living in the Apennine Mountains in central Italy (National Park). It is one of the five endemic species included in the **Endemixit**[1] project, which aims to study the genomic consequences of thriving at small population size. It is classified as **Critically Endangered** species.

A new mutation discovered

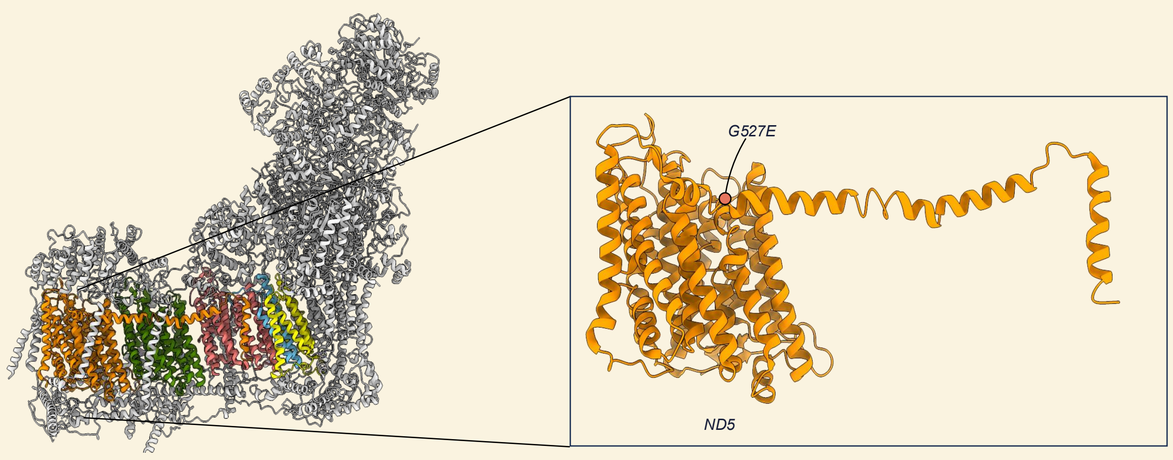
In a recent study[2], the genome of six marsican bears and six additional European bears has been analysed. A total of 40 deleterious fixed mutation were infered in the marsican bear population. A more detailed analysis revealed that three of these are located in the ND5 gene (Respiratory Complex I), one of which has never been observed in other species.

RESPIRATORY COMPLEX I – ND5 structure*

Respiratory Complex I is a crucial component of the mitochondrial electron transport chain. Its primary function is to oxidize NADH and reduce quinone, coupled with proton (H^+) translocation across the inner mitochondrial membrane, thereby generating the proton motive force essential for ATP synthesis. Complex I plays a central role in cellular oxidative metabolism.

Three deleterious mutations, **P448S, T556A,** and **G527E,** have been identified, with **G527E**, located at the base of the horizontal helix, having the most significant deleterious effect[3]. Expression experiment of the mutated isoform ND5 gene and molecular dynamics (MD) simulations show[3]:

- ROS production
- Proton flux (low ATP production)
- Lower hydration of the cavity



AIM OF THE PROJECT

My project aims to investigate any potential compensatory mechanisms that may counterbalance the cytotoxic effects of elevated reactive oxygen species (ROS) production caused by a deleterious mitochondrial mutation (G527A, localized in the ND5 subunit) fixed in the Marsican brown bear population. To achieve this, a three-step analytical pipeline has been designed to comprehensively explore possible compensatory responses at multiple levels.

Biological question: Is there a compensatory mechanism for the overproduction of ROS in the Marsican bear population?

GENOMIC ANALYSIS

Explore potential adaptive events throughout evolution by assessing the structural impact of positively selected sites.

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Collecting all genes involved with ROS molecules in Marsican bear and related species

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Multi sequence alignment (MSA) and phylogeny

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Infer sites under positive selection[4]

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Mapping positive selection sites in structure

GENE EXPRESSION LEVEL ANALYSIS

Identification and quantification of the expression levels of antioxidant-related genes.

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Collecting blood sasmples from Marsican bears and related species

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RNAseq analysis

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Differential gene expression[5]

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Comparing the antioxidant expression profiles between species

TOTAL ANTIOXIDANT CAPACITY ANALYSIS

The bear's diet is predominantly seasonal and omnivorous:

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Spring: It feeds on herbaceous vegetation and, to a lesser extent, on wild ungulates

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Summer: the diet is dominated by wild berries (*Rhamnus* spp.)

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Autumn: It primarily eats hard mast, supplemented by fleshy fruits

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Quantification and comparison of antioxidant concentrations in the bear's diet with those of related species (HPLC technique)

[1] https://endemixit.com/
[2] Benazzo, A., Trucchi, E., Cahill, J. A., Maisano Delser, P., Mona, S., Fumagalli, M., ... & Bertorelle, G. (2017). Survival and divergence in a small group: The extraordinary genomic history of the endangered Apennine brown bear stragglers. *Proceedings of the National Academy of Sciences*, *114*(45), E9589-E9597.
[3] A fixed mutation in the respiratory complex I impairs mitochondrial bioenergetics in the endangered Apennine brown bear (*submitted*)
[4] Kosakovsky Pond, S. L., Poon, A. F., Velazquez, R., Weaver, S., Hepler, N. L., Murrell, B., ... & Muse, S. V. (2020). HyPhy 2.5—a customizable platform for evolutionary hypothesis testing using phylogenies. *Molecular biology and evolution*, *37*(1), 295-299.
[5] Varet, H., Brillet-Guéguen, L., Coppée, J. Y., & Dillies, M. A. (2016). SARTools: a DESeq2-and EdgeR-based R pipeline for comprehensive differential analysis of RNA-Seq data. *PloS one*, *11*(6), e0157022.