

Mitochondria are the powerhouses of the cell providing the energy needed to sustain life processes in most organisms. Even slight deficiencies in the mitochondrial machinery lead to disease and sometimes death in humans and other species. By a twist of evolutionary fate, the DNA code for the proteins working in mitochondria is found in two cellular compartments: the nuclear genome (nDNA) and the mitochondrial genome (mtDNA). The products of these two genomes must work together smoothly to sustain aerobic metabolism. Because the two genomes evolve in the same cells, tissues, organisms and species, they eventually co-adapt to each other. However, if two different species mate and produce offspring (a common phenomenon in nature), chances are their mtDNAs and nDNAs are not co-adapted and may even be incompatible. The hybrid organisms are then less efficient at producing energy and this may manifest itself by lower performance in other aspects of life, such as obtaining food, escaping from enemies, finding a mate or producing high-quality offspring. These mitonuclear incompatibilities have been proposed as a major reproductive isolation mechanism keeping distinct species on separate evolutionary paths. However, several basic aspects of this potentially universal mechanism remain unknown.

Pairs of species that have not yet attained complete reproductive isolation offer potential to test some key assumptions and consequences of mitonuclear co-adaptation. Amphibians are well-suited for this task because even genetically divergent species mate and produce hybrid offspring in nature. In this project, I first ask: how common is mitonuclear co-adaptation in amphibians? I will look at the specific signatures of adaptation and divergence etched into the DNA of closely related species to answer this question. I then ask: is there a statistical association between co-adapted mtDNA and nDNA genes in areas of overlap of hybridizing species pairs? I will answer this question by examining the spatial relationships of mtDNA and nDNA genes that interact with proteins encoded by mtDNA across contact zones of 30 different hybridizing pairs of amphibian taxa.

In some specific cases, mtDNA may break through species boundaries and invade a closely related but distinct species (introgression), seemingly paving the way for mitonuclear incompatibilities and the demise of the recipient species. However, we know of several apparently viable species with introgressed foreign mtDNA, challenging the mitonuclear incompatibility hypothesis of reproductive isolation. There are several potential explanations for this phenomenon. For instance, large differences in demographic parameters of the two species may lead to asymmetric and widespread introgression. On the other hand, introgression may be beneficial (adaptive), and consistent with mitonuclear co-adaptation, in cases in which the fitness of two mtDNA genomes differs in a particular environmental context. Demographic modelling of species history and introgression can be used to sort between these hypotheses. I plan to examine three exceptional amphibian species in which this type of mtDNA replacement has occurred, using information from the mtDNA and nDNA genomes to look for patterns consistent with these hypotheses.

Molecular evolutionary research has one major disadvantage – it is indirect and can only trace events that happened in the past. In the next stage of the project I will turn to real life experiments using an interesting group of amphibians – European waterfrogs. Some populations of the marsh frog (*Pelophylax ridibundus*) carry the foreign mtDNA of a distantly related species – the pool frog (*P. lessonae*). For reasons outlined above, these individuals should not function as well as their counterparts with native mtDNA. These natural “experimental” animals could provide some of the best, and acutely needed, evidence for the importance of mitonuclear co-adaptation. Under laboratory conditions, my team will compare the survival of marsh frog tadpoles carrying native vs. foreign mtDNA and measure their aerobic respiration to see if there are differences between the two groups.

This project will contribute a quantitative assessment of mitonuclear incompatibility as a basis for reproductive isolation in closely related species, and will also bring new insight on the conundrum of mtDNA introgression. The experiments on introgressed tadpoles will provide a direct test of the functional effects of mitonuclear mismatch. By conducting a comparative analysis of many species at multiple genes, this project will determine the significance of mitonuclear co-adaptation in amphibians and contribute to a greater understanding of the evolutionary genomics of speciation.