P. Koteja et al.: ***Experimental evolution of physiological and behavioral adaptations in the bank vole: molecular background and alimentary system bacterial symbionts***

Description for the general public

One of the main challenges in biology is to explain the evolution of complex adaptations, allowing realization of the astonishing variety of "lifestyles". Even within a closely related group of animals, such as mammals, particular species represent extremely distinct lifestyles: the huge whales entirely associated with aquatic environment, 10 million times smaller shrews spending most of their life underground, flying bats, sluggish herbivores sloths, predatory cheetahs speeding up easily to 100 km/h – to name just a few examples. Comparative biology tries to use this diversity to explain both biological "sense" and the evolution of such complex adaptation by examining the patterns of the relationship between morphological and anatomical characteristics, organ function, food habits, and type of habitat. Our research program is based on an alternative – actually even an opposite – approach: the "experimental evolution". Instead of trying to explain the variation among species shaped by millions years of evolution, we create additional variation – but under controlled laboratory conditions. In this way we can find out what are the effects of selection acting on specific traits or under certain conditions.

In our unique experiment, carried out on a non-laboratory rodent, the bank vole (*Myodes glareolus*), we simulate the evolution in three directions that have played an important role in the evolution of mammals: the ability to achieve high aerobic exercise metabolism (“Aerobic”), propensity towards predatory behavior ("Predatory"), and improved capability to sustain on a low-quality, herbivorous food ("Herbivorous"). As in any proper experiment, we have a control - animals that are not subjected to a deliberate selection ("Control"). An important methodological requirement of experiments is appropriate replication, and therefore for each of these four selection groups we maintain four independent lines (populations). This allows us to answer the question whether the changes observed in subsequent generations result from random factors (e.g., the so-called genetic drift) or are indeed a response to the specific selection factors.

Within the available time scale, infinitesimally small from the evolutionary perspective, we surely cannot expect an emergence of new species. However, in the course of already more than 20 generations of our selection experiment, substantial differences between the distinctly selected lines have already appeared, not only in the selected traits, but also in several other. In this project we attempt answer two main questions:

1) What is the molecular-genetic basis of the evolution of increased aerobic capacity (Aerobic lines) and of increased propensity to predatory behavior (Predatory lines)? To answer this question we will investigate genetic variation on the "deepest" level: differences in the DNA specific "words" that describe genes, and even more specifically – particular “letters” of these words (i.e., SNPs – the *Single Nucleotide Polymorphism*s). We will examine about 2,000 SNPs to detect those, for which their variants (alleles) are correlated with the physiological and behavioral traits of interest, as well as those for which the frequency of variants has changed systematically in response to the selection. In this way we can find out which genes are involved in the processes resulting in differentiation of our focal traits – the aerobic capacity and predatory behavior.

2) Do the voles from lines selected for improved herbivorous capability (Herbivorous lines) evolved the ability to maintain a modified composition of the gut symbiotic bacteria, which support digestion of low-quality, herbivorous food? From our previous project we already know that the composition of symbiotic bacteria in the Herbivorous lines differs from that in other lines of voles. In this project we will check whether the difference is resistant to the influence of environmental factors, and whether it indeed contributes to the observed difference in the ability to digest the herbivorous food. If this is the case, the experiment will provide a unique example of gripping the evolution of the so called “holobiont”, i.e., the system consisting of a host and all its micro-symbionts (the huge number of various microorganisms inhabiting the body of the animal). This result will provide an elegant support to the concept of "hologenome", which assumes that the relevant objects of natural selection and evolution are the whole holobionts, rather than animals (or plants) detached from the symbiotic microorganisms.

Finally, the experiment offers also a valuable educational tool in the context of public (non-academic) debate concerning Darwinian theory of evolution, because it offers a clear demonstration of the effectiveness of selection acting on organisms biologically similar to humans.