Description for the general public

Phenotypic plasticity is an important biological phenomenon that allows organisms with the same genotype to respond adaptively to variable biotic and abiotic environments. There are several molecular mechanisms that can contribute to genomic flexibility and thus phenotypic plasticity, including transcriptional regulation, posttranscriptional modification, alternative splicing, and epigenetic modifications of DNA. Epigenetic DNA modifications can take several forms for example methylation, demethylation, deamination or uracilation. Methyl groups can be added directly to nucleotides in a process called DNA methylation. Primarily methylation occurs at the cytosines in CG dinucleotides, but methylation can occur on other cytosines or even other nucleotides. This process involves several DNA methyltransferases (DNMTs). Most organisms with a fully functional DNA methylation system have at least one copy of each of DNMT1, DNMT2, and DNMT3. DNMT1 maintains methyl tags, while DNMT3 is involved in de novo methylation. DNMT2 is not considered a true DNA methyltransferase and may be involved in methylation of tRNAs.

Several key aspects of DNA methylation in mammals remain entirely unexplored in invertebrates and insects. For example, global DNA demethylation occurs during early development in mammals, which allows the 'reprogramming' of the genome essential for proper development, and play an important role in transcriptional cycling of mammalian gene promoters. Whether DNA demethylation is similarly critical during insects (and, more generally, invertebrates) development is unknown. Likewise, the presence of methylation cycling in insects and other invertebrates has yet to be demonstrated, because it is unclear whether large-scale active demethylation events, such as those seen in mammalian development, also occur in non-mammalian animals.

Fruit fly (*D. melanogaster*) is very important model organism. For very long time it was hypothesis that *Drosophila* lost ability to methylation (hasn't 5-methylcytosine). This was due to the fact that it has lost key DNA methyltransferases (has only DNMT2). Use of modern and sensitive methods enabled the determination of low levels of 5-methylcytosine in *Drosophila* embryos and adult flies. It is interesting, that *D. melanogaster* possess an equivalent of TET *(Ten Eleven Translocation*) proteins. These enzymes are involved in the process of active DNA demethylation in mammals. *Drosophila* TET protein (dTet) can oxidase 5-methylcytosine to 5-hydroxymethylcytosine and this modification was observed in adult flies. Another possibility is that the main task of dTet is produced 5-hydroxymetyluracil from thymine. In the light of recent findings it is possible, that a signaling molecule during metamorphosis of *D. melanogaster* may be uracil present in DNA (paired with adenine). The extraordinary situation of tolerance and interpretation of uracil-DNA may not be exclusively present in *Drosophila*, as absence of uracil glycosidase UNG is ubiquitous among *Holometabola* (insects of the which go through distinctive larval, pupal, and adult stages). It is possible that 5-hydroxymethyluracil may be a signaling molecule during *Drosophila* metamorphosis, like uracil.

The main objective of this project is to search for new epigenetic markers in material isolated from fruit fly (*Drosophila melanogaster).* Analysis will be done on the whole-genome level with using the most reliable methodology: UPLC-MS/MS with stable isotope-labeled internal standards. To accomplish specific aims we would like to identify and, if present, to quantify 5-methylcytosine, 5-hydroxymethylcytosine, 5-formylcytosine, 5-carboxylcytosine, 5-hydroxymethyluracil, uracil and 8-oxoguanine (all in deoxynucleosides form) in genomic DNA isolated from insects in various developmental stages, as well as during life time. We will also make experiments on in vitro model. For that reason we will conduct *Drosophila* S2 cell culture.