On the track of DNA damage in insects

DNA is a molecule containing all information necessary for proper function, development, growth, and reproduction of an organism. DNA usually consists of two strands, which are made of nucleotides. Each of them contains one nitrogen nucleobase – cytosine, guanine, adenine or thymine. The sequence of different nucleotides is what constitutes genetic information.

DNA strand breaks may be very dangerous for a cell, and in consequence for the whole organism. Usually cells are well prepared to repair damaged strands. However, when the breaks are numerous, the cell can't keep up with repairing them and might start producing incorrect proteins, or even die. DNA damage is caused by various factors. Many of them result from human activity. Environmental pollutant, such as heavy metals and pesticides, as well as UV radiation, are the most important ones. All organisms, including insects, are susceptible to these kinds of stress.

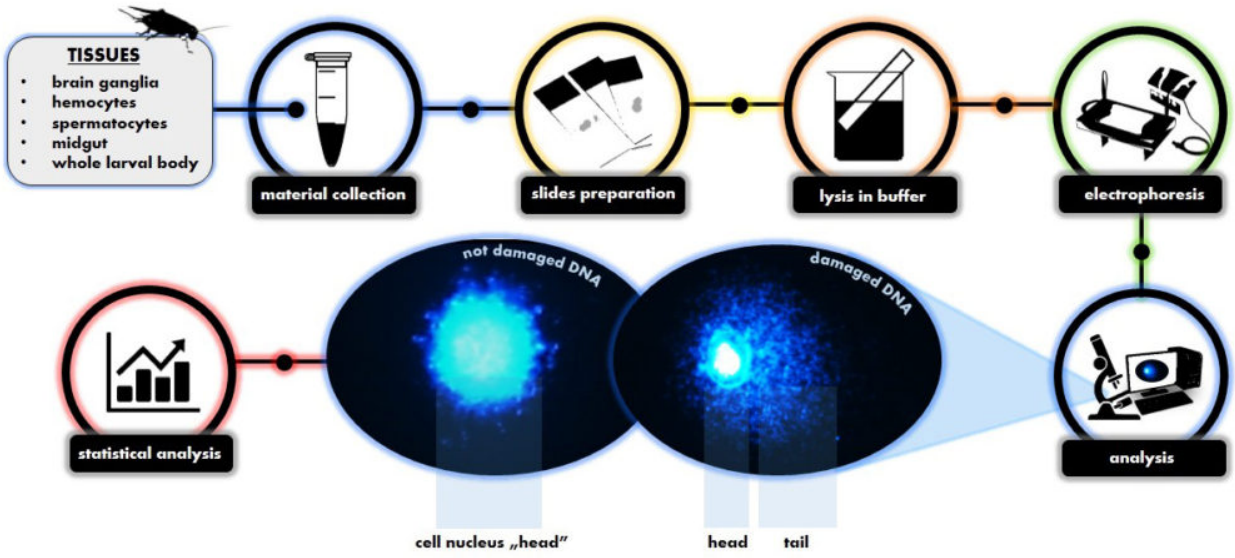

Fig. 1. Steps of the comet assay.

DNA damage level is measurable. One of the easy methods off assessing DNA damage is the comet assay, or more precisely, Single Cell Gel Electrophoresis (SCGE) assay. The comet assay is a very sensitive and reliable method of detecting DNA damage and its repairs at the level of an individual cell. Cells obtained from different tissues are embedded in agarose on a microscope slide, lysed and subjected to an electric field. DNA fragments have a negative charge, so they migrate towards the positive electrode. The shorter the DNA fragments are, the faster they move. The resulting microscopic image reminds a distinctive comet shape, with fragmented DNA in the tail. DNA damage is assessed basing on tail length and amount of DNA in the tail (Fig. 1).

The Comet assay was first developed as a tool to study genotoxicity in mammals. Nowadays, the comet assay can be used to study genotoxicity of many damaging factors in various organisms. Invertebrates are an interesting model of ecotoxicological research due to their significance in ecosystems. Insects are the largest group of invertebrates and can be widely utilized in both toxicological and ecotoxicological research, and could partially replace vertebrates in studies due to the ethical issues that are related to this type of research. Moreover, invertebrate breeding is inexpensive and does not require much space or time. Experiments can be conducted on a large scale and at a low cost. The Comet assay has been used to measure DNA damage in a few different insect species belonging to various systematic groups and inhabiting different ecological niches (Fig. 2).

Fig. 2. Comet assay was used in insect species belonging to four orders.

DNA damage measurements have most often been used in fruit fly (*Drosophila melanogaster*) as it is undoubtedly a model organism perfectly suited for genetic studies. The presence of numerous repair deficient/efficient mutants allows to design complex experimental setups that can be used to understand DNA repair mechanisms. Various strains of *D. melanogaster* are key models in the field of genotoxicology, especially in short-term tests that are designed to identify carcinogens, and in more complex experiments that investigate mutagenesis mechanisms. Recently, the SCGE has been applied to other insects, thus creating new possibilities for environmental monitoring. The Comet assay has become a tool that makes the study of interactions between various toxic/stressing factors (e.g. pesticides, radiation, heavy metals, pollutants) possible. The Comet assay could also be applied to insects to study the aging mechanisms or DNA stability in relation to age and sex. Undoubtedly, evaluations of DNA damage will be increasingly important in insects that are of substantial importance to humans, such as crop pests, disease vectors and social insects.

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