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Mini-review

The Comet assay in insects—Status, prospects and benefits for science

Maria Augustyniak*, Marcin Gladysz, Marta Dziewięcka

Department of Animal Physiology and Ecotoxicology, University of Silesia, Bankowa 9, PL 40-007 Katowice, Poland

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ABSTRACT

The Comet assay has been recently adapted to investigate DNA damage in insects. The first reports of its use in *Drosophila melanogaster* appeared in 2002. Since then, the interest in the application of the Comet assay to studies of insects has been rapidly increasing. Many authors see substantial potential in the use of the Comet assay in *D. melanogaster* for medical toxicology studies. This application could allow the testing of drugs and result in an understanding of the mechanisms of action of toxins, which could significantly influence the limited research that has been performed on vertebrates. The possible perspectives and benefits for science are considered in this review.

In the last decade, the use of the Comet assay has been described in insects other than *D. melanogaster*. Specifically, methods to prepare a cell suspension from insect tissues, which is a difficult task, were analyzed and compared in detail. Furthermore, attention was paid to any differences and modifications in the research protocols, such as the buffer composition and electrophoresis conditions.

Various scientific fields in addition to toxicological and ecotoxicological research were considered. We expect the Comet assay to be used in environmental risk assessments and to improve our understanding of many important phenomena of insect life, such as metamorphosis, molting, diapause and quiescence. The use of this method to study species that are of key importance to humans, such as pests and beneficial insects, appears to be highly probable and very promising. The use of the Comet assay for DNA stability testing in insects will most likely rapidly increase in the future.

1. Introduction

The single cell gel electrophoresis assay (SCGE), also known as the Comet assay, is a sensitive technique that is used to detect DNA damage. Initially, the process only involved reactions that occur in neutral conditions, thereby only allowing for the detection of double-strand breaks in DNA [1]. The extension of the assay to alkaline conditions resulted in the detection of single-strand DNA damage [2], after which numerous modifications led to significant increases in the assay's applications.

SCGE has many advantages that make this technique a valuable tool, especially in toxicological research. The Comet assay is widely accepted as a simple, quick and inexpensive technique. Various cell types can be used in the test without prior knowledge of their karyotype and genome structure, irrespective of their proliferating status [3]. Although this test only detects DNA damage in the form of strand breaks, it can be modified via various experimental models, making it powerful in the verification of hypotheses. The application of the enzyme-modified Comet assay permits the investigation of the mechanisms of action of genotoxic chemicals, DNA damage that is caused by oxidative stress, the effects of dietary antioxidants and environmental pollution by studying sentinel organisms [4]. Another important modification is the combination of the Comet assay with fluorescent in situ hybridization (Comet-FISH). The use of fluorescently labeled probes in the Comet-FISH assay provides information about DNA damage in specific gene domains. The position of fluorescent hybridization spots (the comet's head or tail) can be used to assess effects on cells, especially if the sequence of interest lies within the damaged region of DNA [5]. This information can be vital to understand the cellular response to damaging factors and to

Abbreviations: MMS, methyl methanesulphonate; EMS, ethyl methanesulphonate; ENU, *N*-ethyl-*N*-nitrosourea; CP, cyclophosphamide; BLM, bleomycin; CPT, cisplatin; PD, potassium dichromate; TBT, tributyltin; BPA, bisphenol A; NP, nonylphenol; PCP, pentachlorophenol; TCS, triclosan; DEPH, bis(2-ethylhexyl) phthalate; PQ, araquat dichloride; SNAP, S-Nitroso-*N*-acetylpenicillamine; GCNC, graphene copper nanocomposite; ZnO NP, zinc oxide nanoparticles; TiO_2 NP, tanium dioxide nanoparticles; SAS NP, synthetic amorphous silica nanoparticles.

* Corresponding author.
E-mail address: maria.augustyniak@us.edu.pl (M. Augustyniak).

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predict biological effects at both the organism and population levels [6].

Although the Comet assay was first developed as a tool to study genotoxicity in mammals and was successfully implemented to study epidemiological profiles in humans [7–22], the use of this technique in other organisms, such as bacteria, plants, fungi or invertebrates, is not limited [23–27]. DNA is a ubiquitous molecule, and its structure, function and repair mechanisms are similar among various organisms. This similarity allows the use of this method to study the genotoxicity of chemicals in various organisms and to assess the influence of genotoxic xenobiotics on the environment.

Invertebrates are an interesting subject of ecotoxicological research due to their significance in ecosystems. The Comet assay has primarily been used for genotoxicity assessment in marine and freshwater invertebrates [28–31]. Insects could partially replace vertebrates in toxicological studies due to the ethical issues that are related to this type of research. Naturally, the extrapolation of the data that are obtained in invertebrates to mammals could be problematic or even sometimes impossible. Nonetheless, model invertebrate species have advantages, which in many cases can compensate for any inconveniences and can overcome some common limitations. Invertebrate breeding is inexpensive and does not require much space or time. Experiments can be conducted on a large scale at a low cost. This type of experimental design minimizes problems associated with inter-individual variability and provides appropriate data for statistical analyses, which allows for the generation of reliable results.

Insects are the largest group of invertebrates and can be widely utilized in both toxicological and ecotoxicological research. In this paper, we highlight the utility of the Comet assay in insect studies to assess the genotoxic properties of various compounds as well as in environmental risk assessments. A detailed analysis of published articles in terms of the SCGE methodology that is used in insects, from the first report in 2002 [32] to the most recent study, is presented in this review.

2. Examined insect species

The Comet assay has been used to measure DNA damage in a few different insect species (Fig. 1). These organisms belong to various systematic groups and inhabit different ecological niches. DNA damage measurements based on the Comet assay have most often been used in *Drosophila melanogaster*[32–64] and have been rarely used in species that are related to *D. melanogaster*, such as *Drosophila simulans* [65] and *Drosophila heteroneura* [66]. This form of application can be explained by the fact that *D. melanogaster* is undoubtedly a model organism that is perfectly suited for genetic studies. The presence of numerous repair deficient/efficient mutants allows for the design of complex experimental models that can be used to understand DNA repair mechanisms [32,39,44,53,56].

Recently, the Comet assay has been applied to other insects, thus creating new possibilities for toxicology, ecotoxicology and environmental monitoring. The Comet assay has become a tool that permits the study of interactions between various xenobiotics. Specifically, it can be useful to explore pesticide resistance or the selection of environmental pollutant immunity, as well as to better understand the aging of insects. To date, terrestrial species of several orders, including insects that are significant to the human economy, are commonly examined using the Comet assay, including Diptera—*Liriomyza trifolii* [67]; Coleoptera—*Tenebrio molitor* [66],*Curculio sikkimensis* [68], *Sitophilus zeamais* [69], and *Lasioderma serricorne* [70]; Lepidoptera—*Plodia interpunctella*[71], *Plutella xylostella* [72], and *Spodoptera litura* [73]; and Orthoptera—*Chorthippus brunneus* [74–77], *Schistocerca gregaria* [78], *Dolichopoda laetitiae* and *Dolichopoda geniculata* [79]. Aquatic species, including *Chironomus riparius* [80–83], *Chironomus kiiensis* [84], and *Chironomus tentans* [85], have also been examined. We expect that the Comet assay will be adjusted to study more insect species. Undoubtedly, evaluations of damage to genetic material will be important in insects that are of substantial importance to humans, such as crop pests, disease vectors and social insects.

D. melanogaster as a suitable model for genetic toxicology

The fruit fly *D. melanogaster* is one of the most widely used model organisms in genetic and developmental research (Table 1). The popularity of this species is mainly based on its simple breeding requirements and short generation time. These same advantages may also be responsible for its growing popularity in toxicology, especially genetic toxicology. *Drosophila* is commonly accepted to play a dual function in the field of genotoxicology—in short-term tests that are designed to identify carcinogens and

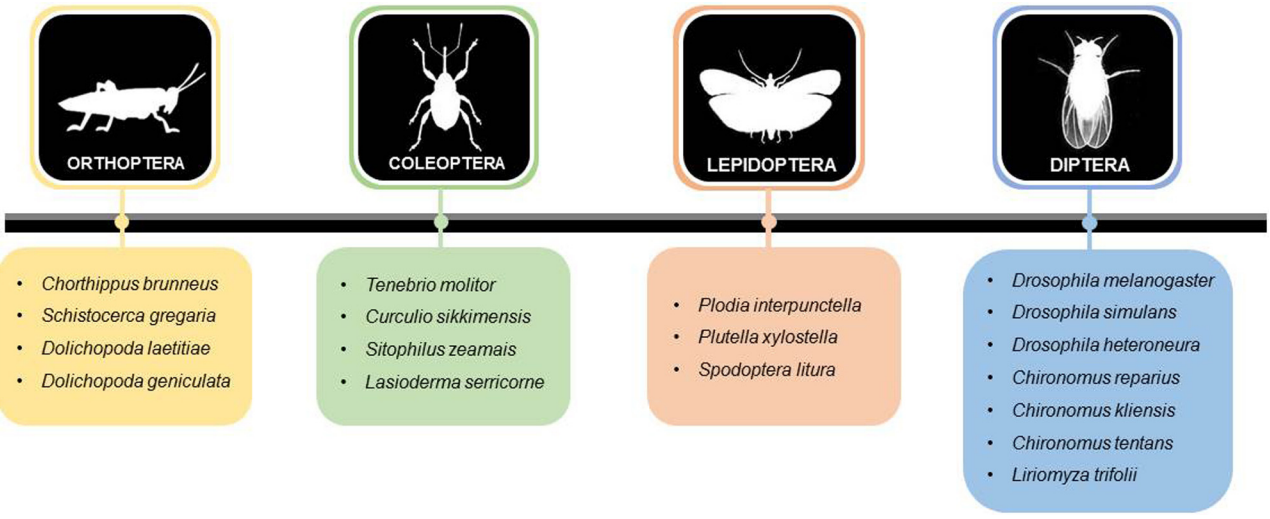

Fig. 1. Comet assay was used in assessment of DNA damage in various insect species belonging to four orders.