**Drosophila comet assay: insights, uses, and future perspectives**

Isabel Gaivão¹ and L. María Sierra² *

¹ Department of Genetics and Biotechnology, Animal and Veterinary Research Centre, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal
² Área de Genética, Departamento de Biología Funcional, and Instituto Universitario de Oncología del Principado de Asturias, Universidad de Oviedo, Oviedo, Spain

*Correspondence:
L. María Sierra, Área de Genética, Departamento de Biología Funcional, and Instituto Universitario de Oncología del Principado de Asturias, Universidad de Oviedo, C/ Julián Clavería s/n, 33006 Oviedo, Asturias, Spain
email: lmsierra@uniovi.es

INTRODUCTION

The single cell gel electrophoresis test, or comet assay, was originally developed by Östling and Johanson (1984) as a microelectrophoretic technique to visualize DNA damage in single cells. Subsequently it was improved by Singh et al. (1988), and since then so extensively used that some working-groups were created to standardize its application to mammal and human cells studies (Burlinson et al., 2007; Karlsson, 2010; Azqueta and Collins, 2013; Burlinson et al., 2007). Its usefulness and easy performance lead to its rapid application to several fields, like genotoxicity analyses (Speit and Hartmann, 1999; Tice et al., 2000; Hartmann et al., 2003; Collins, 2004), human population biomonitoring (Collins et al., 1998; Somorovská et al., 1999; Kassie et al., 2000; Moller et al., 2000; Faust et al., 2004; Hoffmann et al., 2005; Burlinson et al., 2007; Dusinska and Collins, 2008; Uriol et al., 2013) and DNA repair (Collins and Horváthová, 2001; Collins et al., 2001; Collins and Gaivão, 2007; Gaivão et al., 2009; Dusinska and Collins, 2010). Because of this, it was also applied to other organisms, using different cell types (Menke et al., 2001; Dixon et al., 2002; Lee and Steinert, 2003; Jha, 2008; Dhawan et al., 2009; Ventura et al., 2013).

Surprisingly, its application to *Drosophila melanogaster* was rather late, despite the fact that this organism is one of the most valuable higher eukaryotic model organism, for all kind of processes and situations related to human health (Reiter et al., 2001; Koh et al., 2006; Wolf et al., 2006; Khurana et al., 2006; Rand, 2010), including the *in vivo* DNA damage response processes (Søndergaard, 1993; Vogel et al., 1999; Sekelsky et al., 2000; Vecchio, 2014).

The first attempt to apply the comet assay to *Drosophila in vivo* was performed by Gaivão (1999) in her Ph.D. Thesis, checking the availability of imaginal disk and brain ganglia cells. In the first published work, appeared 3 years later, the comet assay was performed with brain ganglia cells from third instar larvae (Bilbao et al., 2002). As with other organisms, several cell types, apart from the brain cells, have been used to carry out this assay in *Drosophila in vivo*, such as midgut cells (Mukhopadhyay et al., 2004; Siddique et al., 2005a; Sharma et al., 2011), hemocytes (Carmona et al., 2011a), and imaginal disk cells (Verma et al., 2012).

Most of these authors used the comet assay for its original purpose, the *in vivo* analyses of genotoxicity and DNA repair. But more recently, this assay has also been used to study genotoxicity *in vitro* (Guanggang et al., 2013), to analyze the influence of protein overexpression on genome integrity and degradation. Although the assay is well established, it could benefit from some research to determine optimal experimental design to standardize it, and then to allow comparisons among laboratories independently of the chosen cell type.

**Keywords:** *Drosophila*, comet assay, neuroblast cells, hemocytes, midgut cells, genotoxicity, DNA repair

INSIGHTS

**BRAIN CELLS**

The *Drosophila* comet assay using brain ganglia cells was developed at the University of Oviedo (Spain) by Isabel Gaivão and the group of L. Maria Sierra and M. A. Comendador (Gaivão, 1999;
Bilbao et al., 2002). Our aim was to develop a tool to study both genotoxicity and in vivo DNA repair in somatic cells.

The developed protocol included the use of third instar larvae (developed 24 h at 24°C and five additional days at 21°C) treated in the food during 12 h. Brain ganglia were extracted, and cells were mechanically individualized, shredding the tissue with tungsten wires, and suspended in Ringer’s buffer (Bilbao et al., 2002; García-Sar et al., 2008, 2012; Rodríguez et al., submitted). Cells were embedded in 0.5% low melting point agarose (LMPA), three agarose layers were prepared, and cells were disrupted during 2 h with a lysis solution containing N-lauroylsarcosine sodium salt (N-LS), 0.77%, and dimethyl sulfoxide (DMSO) 10%. Denaturation was performed at pH 12.6, for 20 min, and electrophoresis was set at 0.9 V/cm, for 20 min. After neutralization and fixation, slides were stained with ethidium bromide (0.4 μg/mL), with Vectashield® fluorescence protector (Vector Laboratories, Inc., Burlingame, CA, USA) to avoid fluorescence decay (Table 1). A very detailed protocol was recently published (Sierra et al., 2014).

Microscope photos were analyzed with the Komet 5 software program (Kinetic, England), collecting data on % tail DNA, tail length, and tail moment, although the analyses were carried out with the tail moment parameter because it increased linearly with the amount of DNA damage and was the best to detect statistically significant differences. The wild-type OregonK Drosophila strain was used as a standard, since it is rather sensitive to the action of DNA damaging agents in somatic cells (Gaivão and Comendador, 1996). Under all these conditions, the comet assay yielded spontaneous DNA damage measurements of 6.5 ± 0.5 for tail moment and of 30 ± 1.25 for % tail DNA.

Recently, we have developed a technical variation of this protocol to be able to quantitate DNA repair activities in vitro. This variant consists on the incubation of nucleoid DNA with cell-free protein extracts from repair-efficient and deficient-strains, after the lysis step (Rodríguez et al., submitted).

Plyusnina et al. (2011) also used brain cells to perform the comet assay. They disaggregated them mechanically in Poels’ salt solution (PSS). Cells were embedded in 0.75% LMPA, lysis was performed for 1 h, with a buffer without N-LS or DMSO. Denaturation was carried out at pH 13 for 10 min, followed by electrophoresis at 15 V–300 mA for 10 min. Nuclei were stained with acridine orange. Comet images were analyzed with the Comet Score™ software (TriTek Corporation, USA), and the parameter for analysis was the Olive tail moment. The wild-type strain was Canton-S and the values of spontaneous DNA damage measurements were approximately 1.2 units of the analyzed parameter.

HEMOCYTES

The comet assay using hemocytes from Drosophila was developed by the group of R. Marcos at the Autonomous University of Barcelona (Spain). In this protocol, 72 ± 2 h old larvae (developed at 24°C) were treated for 24 h. Since hemocytes are individual cells, they were just collected in phosphate buffered saline (PBS), with 0.07% phenylthiourea (Carmona et al., 2011a,b,c; Sabella et al., 2011).

Cells were embedded in 0.75% LMPA, and two agarose layers were prepared. Lysis buffer contained N-LS 1% (Carmona et al., 2011a,b,c), or DMSO 10% (Sabella et al., 2011). Lysis time was 2 h. Small variations on the denaturation time and the electrophoresis conditions were performed (Table 1). Nucleoids were stained with DAPI (1 μg/mL). Detailed protocols for this assay are available (Marcos and Carmona, 2013; Sierra et al., 2014).

Comets were analyzed with the Komet 5 software program, and results were mostly expressed as % tail DNA (Carmona et al., 2011a,b,c), although DNA damage was also measured as percentage of damaged nuclei (Sabella et al., 2011). The standard wild-type strain used was OregonR, an insecticide resistant strain with high levels of cytochrome P450 and xenobiotic metabolism (Hällström et al., 1984). With this protocol, the highest % tail DNA detected for spontaneous DNA damage was 18.93 ± 0.84 (Carmona et al., 2011c).

MIDGUT CELLS

The comet assay with midgut cells was developed by the group of A. Dhawan and D. K. Chowdhuri at the CSIR-Indian Institute of Toxicology Research, formerly Industrial Toxicology Research Center (India). They also developed the enzymatic brain cell disaggregation protocol. Mid-gut tissue, with or without brain ganglia, from third instar larvae treated for different times were explanted in PSS buffer. Cells were enzymatically individualized, incubating 15 min with collagenase (0.5 mg/mL) in PBS. Treatment times varied from 12 to 74 h (Table 1; Mukhopadhyay et al., 2004; Siddique et al., 2005a,b, 2008, 2013; Mishra et al., 2011, 2013, 2014; Sharma et al., 2011, 2012; Shukla et al., 2011).

Cells were embedded in 0.75% LMPA, with two or three agarose layers. Lysis buffer did not contain N-LS, or DMSO, and lysis time was 2 h. As presented in Table 1, the denaturation step was mainly performed at pH > 13 during 10 min, although in two works this step was performed at neutral conditions, pH 8.5 for 60 min (Sharma et al., 2011; Mishra et al., 2013). In these two cases electrophoresis was also set up differently from the more standard 0.7 V/cm during 15 min (Table 1). Staining was carried out with ethidium bromide (20 μg/mL), for 10 min.

Some of the works carried out at the CSIR-Indian Institute of Toxicology Research analyzed three comet parameters, % tail DNA, tail length, and Olive tail moment (Mukhopadhyay et al., 2004; Siddique et al., 2005a,b), and in others only the % tail DNA was used for result analyses. The Komet 5 software program was throughout used for photo analysis, except by Siddique et al. (2013), who used the Comet Score™ software, v1.5, to analyze tail length. The standard wild-type strain was OregonR. With this protocol, % tail DNA varied from 6 to 10%, with errors lower than 1%, and Olive tail moment varied from 0.7 to 1.5, with errors under 0.12.

IMAGINAL DISK CELLS

Imaginal disk cells have also been used to carry out the comet assay in vivo in Drosophila (Verma et al., 2012). In this case, cell disaggregation was performed enzymatically, as described earlier for midgut cells (see Midgut Cells).
Table 1 | Methodological details, analyzed agents, and results of genotoxicity and/or DNA repair studies carried out in *D. melanogaster* with the comet assay.

| Cell type      | Treat. time (h) | % Agarose | Denaturat pH/time (min) | Electrophoresis V/cm/time (min) | Staining        | Strain                        | Agents     | Results | Reference                        |
|----------------|-----------------|-----------|-------------------------|-------------------------------|-----------------|-------------------------------|------------|---------|----------------------------------|
| Brain cells    | 12              | 0.5       | 12.6/20                 | 0.9/20                        | EthBr 40 μL (0.4 μg/mL) | *OregonK, mus201*             | cDDP       | +       | García-Sar et al. (2008, 2012)   |
|                |                 |           |                         |                               | *OregonK, mus201, mus308, double mut* | MMS            | +                     | Rodriguez et al. (submitted) |
|                |                 |           |                         |                               |                  | *OregonK, mus201, mus308*     | MMS        | +       | Bilbao et al. (2002)            |
| Hemocytes      | 24 ± 2          | 0.75      | 13/25                   | 0.7/20                        | DAPI 20 μL (1 μg/mL) | *OregonR*                    | EMS        | +       | Carmona et al. (2011a)          |
|                |                 |           |                         |                               |                  | Cr(VI)-K$_2$Cr$_2$O$_7$       | +          |         | Carmona et al. (2011b)          |
|                |                 |           |                         |                               |                  | γ-rays                        | +          |         | Carmona et al. (2011c)          |
|                |                 |           |                         |                               |                  | PbCl$_2$                      | −          |         | Sabella et al. (2011)           |
| Mid-gut cells  | 74              | 0.75      | > 13/10                 | 0.7/15                        | EthBr 75 μL (20 μg/mL) | *OregonR*                    | Cypermethrin | +       | Mukhopadhyay et al. (2004)      |
|                |                 |           |                         |                               |                  | Industrial waste leachates   | +          |         | Siddique et al. (2005b)         |
|                |                 |           |                         |                               |                  | EMS                           | +          |         | Siddique et al. (2005a)         |
|                | 24              |           |                         |                               |                  | MMS                           | +          |         |                                   |
|                |                 |           |                         |                               |                  | ENU                           | +          |         |                                   |
|                | 24/48           |           |                         |                               |                  | CP                            | +          |         |                                   |
|                |                 |           |                         |                               |                  | *OregonR* plus FPG and EndoIII enzymes | H$_2$O$_2$ | +       | Shukla et al. (2011)            |
|                |                 |           |                         |                               |                  |                               | CdCl$_2$  | +       |                                   |
|                |                 |           |                         |                               |                  |                               | CuSO$_4$  | +       |                                   |

(Continued)
Table 1 | Continued

| Cell Type                      | Treat. time (h) | % Agarose | Denaturant pH/time (min) | Electrophoresis V/cm/time (min) | Staining Strain Agents | Results | Reference |
|-------------------------------|-----------------|-----------|--------------------------|---------------------------------|------------------------|---------|-----------|
| Oregon R, mei9, mus201, mus210, mei41, mus207, mus209 | 48               |           |                          |                                 |                        | −       | Mishra et al. (2011) |
| Oregon R transgenic, hsp70, hsp83, hsp26 plus FPG and EndoIII enzymes | 12–48             |           |                          |                                 |                        | +      | Mishra et al. (2014) |
| Oregon R, mei41, mus201, mus207 | 48/24           |           |                          |                                 |                        | +      | Sharma et al. (2012) |
|Oregon R, ligIV, kud80, sar-A, ofe, mre11 | 48/24           |           |                          |                                 |                        | +      | Siddique et al. (2008) |
|Oregon R | 48              | 8.5/60    | 14V-60mA/60             |                                 |                        | −      | Sharma et al. (2011) |
|Imaginal disk | 48              | 0.75      | Propidium iodide (1 μg/mL) |                                 |                        | +      | Mishra et al. (2013) |
|Oregon R, Act-GAL4/CyO; +/+ | 6/24            | 0.5       | Alkal/30                 |                                 |                        | +      | Sharma et al. (2012) |
|Cultured cells | 24/48           | 1         | EthBr 40 μL (20 μg/mL) |                                 |                        | +      | Verma et al. (2012) |
|Oregon R, Act-GAL4/UAS-Acet-GAL4/Rx, RNAi; +/+ | 48/24           |           |                          |                                 |                        | +      | Radyuk et al. (2006) |
|S2 cell line: standard and transfected | 48/24           |           |                          |                                 |                        | +      | Siddique et al. (2008) |
|Oregon R, Act-GAL4/CyO; +/+ | 24/48           | 13/10     | 1/10 SYBR green          |                                 |                        | +      | Guanggang et al. (2013) |
|Oregon R, ligIV, kud80, sar-A, ofe, mre11 | 48/24           |           |                          |                                 |                        | +      | Siddique et al. (2008) |
|Oregon R | 48              | 8.5/60    | 14V-60mA/60             |                                 |                        | +      | Sharma et al. (2011) |
|Imaginal disk | 48              | 0.75      | Propidium iodide (1 μg/mL) |                                 |                        | +      | Mishra et al. (2013) |
|Oregon R, Act-GAL4/CyO; +/+ | 6/24            | 0.5       | Alkal/30                 |                                 |                        | +      | Sharma et al. (2012) |
|Cultured cells | 24/48           | 1         | EthBr 40 μL (20 μg/mL) |                                 |                        | +      | Verma et al. (2012) |
|Oregon R, Act-GAL4/UAS-Acet-GAL4/Rx, RNAi; +/+ | 48/24           |           |                          |                                 |                        | +      | Radyuk et al. (2006) |
|S2 cell line: standard and transfected | 48/24           |           |                          |                                 |                        | +      | Siddique et al. (2008) |
|Oregon R, Act-GAL4/CyO; +/+ | 24/48           | 13/10     | 1/10 SYBR green          |                                 |                        | +      | Guanggang et al. (2013) |

Frontiers in Genetics | Genomic Assay Technology  August 2014 | Volume 5 | Article 304 | 4
The conditions of the comet assay were also those described above (see Midgut Cells) with two exceptions: the lysis buffer contained DMSO 10%, and nuclei were stained with propidium iodide (1 μg/mL). Photos were analyzed with the Comet Score™ software, and DNA damage was quantified using the % tail DNA parameter. The wild-type strain used was OregonR, and the spontaneous values of % tail DNA were around 7 (only a graph was presented).

OTHER CELLS

Spermatocytes were other cell type chosen to perform the comet assay in vivo, in this case from D. simulans (Brennan et al., 2012). Testes were dissected in PBS. However, with respect to the comet assay, the only information available from this work is that they have used the OxiSelect Comet Assay Kit (from Cell BioLabs, San Diego, CA, USA) to perform it, the Comet Score™ software for image analysis, and a classification of % tail DNA in five categories for the analysis of results.

The comet assay in Drosophila was also performed in vitro using S2 cultured cells (Radyuk et al., 2006; Guanggang et al., 2013). Cells were treated for 24 h, embedded in 0.5% LMPA, lysed for 30 min, denatured in alkaline conditions for 30 min, electrophoresed at 1 V/cm for 10 min, and stained with SYBR green dye; and the DNA damage was measured classifying the damaged cells in four categories (Radyuk et al., 2006).

Alternatively, cells were treated for 24 or 48 h and embedded in 1% LMPA. Lysis buffer contained DMSO 10%, and lysis time was 30 min. Denaturation at pH 13 for 10 min was followed by electrophoresis 1 V/cm for 10 min. Nucleoids were stained with ethidium bromide (20 μg/mL), and comet photos were analyzed with CASP image analysis system, measuring % tail DNA and tail moment. The values of these parameters for spontaneous DNA damage were 11.57 ± 5.84 for % tail DNA and 2.20 ± 1.24 for tail moment (Guanggang et al., 2013).

USES

GENOTOXICITY AND DNA REPAIR ANALYSIS

It is possible to study DNA repair in vivo in Drosophila germ cells, male and female ones, since many years ago (Vogel et al., 1996; Hernando et al., 2004). However, it was not possible to study it in somatic cells, with the available in vivo SMART assays (Vogel and Nivard, 2001). Because of this, our main aim when designing the first comet assay protocol in Drosophila was to develop a tool to study DNA repair in vivo in somatic cells (Gaivão, 1999; Bilbao et al., 2002). Consequently, many (but not all) of the works carried out with this assay in Drosophila were aimed to study genotoxicity, specifically that of the insecticide methomil, was also the aim of the comet assay performed in vitro with S2 culture cells (Guanggang et al., 2013).

In addition to these studies of genotoxicity and DNA repair, the comet assay in vivo in Drosophila had been used to study: (i) the influence of GADD45 protein over-expression on longevity and spontaneous DNA damage, as an indication of increased DNA repair activity (Plyusnina et al., 2011); (ii) chromatin integrity in DNA pol α mutants exposed to bleomycin (Verma et al., 2012); and (iii) oxidative DNA damage in spermatocytes of Wolbachia-infected D. simulans flies (Brennan et al., 2012).

OTHER USES

In addition to these studies of genotoxicity and DNA repair, the comet assay in vitro in Drosophila was used to check the repair activity of cell free protein extracts obtained from wild-type and repair mutant strains in the repair of methyl methanesulfonate induced DNA damage (Rodriguez et al., submitted).

After checking their use with known inducers of DNA strand breaks (Carmona et al., 2011a), hemocytes were used to demonstrate that not all the salts of lead and nickel were genotoxic (Carmona et al., 2011b,d), but that gold nanoparticles were so (Sabella et al., 2011).

Midgut cells, with or without brain cells, have been used to study oxidative DNA damage, using incubations with FPG and Endo III enzymes (Shukla et al., 2011; Sharma et al., 2012), and to demonstrate the genotoxicity of chromium salts (Mishra et al., 2011, 2013; Sharma et al., 2011), pesticides like cypermethrin (Mukhopadhyay et al., 2004), endosulfan (Sharma et al., 2012), and dichlorvos (Mishra et al., 2014), contaminants as industrial waste leachates (Siddique et al., 2005b, 2008), and nanomaterials like graphene copper nanocomposite (Siddique et al., 2013). In addition, some of these genotoxic agents, like chromium salts, dichlorvos, and industrial waste leachates, were analyzed in different repair conditions, with the in vivo comet repair assay (Siddique et al., 2008; Mishra et al., 2011, 2013, 2014), checking the influence of pre- and post-replication DNA repair pathways on their genotoxicity. Other genotoxic agents, like endosulfan and graphene copper nanocomposite, were analyzed in transgenic strains for genes encoding heat shock proteins (hsp), to check responses to xenobiotic stress, and influence of xenobiotic metabolism (Sharma et al., 2012; Siddique et al., 2013).

Analysis of genotoxicity, specifically that of the insecticide methomil, was also the aim of the comet assay performed in vitro with S2 culture cells (Guanggang et al., 2013).

FUTURE PERSPECTIVES

Considering the relevance of D. melanogaster as an established insect model for human diseases and toxicological research, recommended by the European Centre for the Validation of Alternative Methods (ECVAM), all the results of in vivo genotoxicity studies with this organism should be considered as relevant for human health. In this aspect, the comet assay performed in vivo is even more important because, in addition to its high sensitivity, it is the only assay that allows the analysis of DNA repair in somatic cells. And, at least theoretically, the comet
assay results should be more easily and directly compared among species. There is however a possible problem: there are several groups using different protocols, what make comparisons even among Drosophila laboratories impossible. So, it is necessary to standardize the basic comet assay protocol. Azqueta et al. (2011) demonstrated in human cells how small changes in some variables, such as agarose concentration, alkaline unwinding time, or electrophoresis conditions, might significantly affect the results. And these are specifically some of the variables that differ between the protocol for brain cells and the rest: LMPA percentage (0.5 vs. 0.75%), lysis buffer composition (N-LS and DMSO vs. only N-LS or none of them), or denaturation and electrophoresis conditions (more V/cm, compared to the protocol for hemocytes, and more denaturation time and V/cm, compared to the protocol for midgut cells). These differences might explain the higher values of the comet parameters, for spontaneous DNA damage, found with the brain cell protocol, compared to the others, because although some differences might be attributed to the wild-type strain analyzed (Oregon R is more sensitive than Oregon N), at least in the case of human cells differences due to individuals or cell types were not so relevant (Azqueta et al., 2011). It is then necessary to study the effects of these differences and whether a higher sensitivity is an advantage or a disadvantage.

To help with the required standardization, some of the protocols optimizations performed for other cells and organisms can be tested and applied to Drosophila, including its simplification (number of layers, size of gels, or solution compositions) and the high throughput versions, recently developed based on the use of 12 mini-gels on one slide (Shaposhnikov et al., 2010). Additionally, the modified comet assay performed incubating with repair lesion-specific enzymes, as used by Shukla et al. (2011) and Sharma et al. (2012) for oxidative damage, can be extended to other types of damages and repair systems (Collins et al., 2008). This standardization would also clearly help the use of this assay in other types of studies, different from genotoxicity and DNA repair testing.

REFERENCES

Azqueta, A., and Collins, A. R. (2013). The essential comet assay: a comprehensive guide to measuring DNA damage and repair. Arch. Toxicol. 87, 949–968. doi: 10.1007/s00204-013-1070-0

Azqueta, A., Gutzkow, K. B., Brunborg, G., and Collins, A. R. (2011). Towards a more reliable comet assay: Optimising agarose concentration, unwinding time and electrophoresis conditions. Mutat. Res. 724, 41–45. doi: 10.1016/j.mrrev.2011.05.010

Bilbao, C., Ferreiro, J. A., Comendador, M. A., and Sierra, L. M. (2002). Influence of genotoxicity of model chemicals in somatic cells in vivo measured with the comet assay. Mutat. Res. 503, 11–19. doi: 10.1016/S0078-5173(02)00070-2

Brennan, L. J., Haukedal, J. A., Earle, J. C., Keddie, B., and Harris, H. L. (2012). Disruption of redox homeostasis leads to oxidative DNA damage in spermatocytes of Wobblachia-infected Drosophila simulans. Insect Mol. Biol. 21, 510–520. doi: 10.1111/j.1365-2918.2012.01155.x

Burlinson, B., Tice, R. R., Speit, G., Agurell, E., Brendler-Schwaab, S. Y., Collins, A. R., et al. (2007) In vivo comet assay workgroup, part of the fourth international workshop on genotoxicity testing: results of the in vivo comet assay workgroup. Mutat. Res. 627:31–35. doi: 10.1016/j.mrrev.2006.08.011

Carmona, E. R., Guecheva, T. N., Creus, A., and Marcos, R. (2011a). Proposal of an in vivo comet assay using hemocytes of Drosophila melanogaster. Environ. Mol. Mutagen. 52, 165–169. doi: 10.1002/em.20604

Carmona, E. R., Creus, A., and Marcos, R. (2011b). Genotoxic effects of two nickel-compounds in somatic cells of Drosophila melanogaster. Mutat. Res. 718, 33–37. doi: 10.1016/j.mrgentox.2010.10.008

Carmona, E. R., Creus, A., and Marcos, R. (2011c). Genotoxicity testing of two lead-compounds in somatic cells of Drosophila melanogaster. Mutat. Res. 724, 35–40. doi: 10.1016/j.mrgentox.2011.05.008

Collins, A. R. (2004). The Comet assay for DNA damage and repair. principles, applications, and limitations. Mol. Biotechnol. 26, 249–261. doi: 10.1385/MB:26:3:249

Collins, A. R., Azqueta, A., Brunborg, G., Gaivão, I., Giovannelli, L., Kruszewski, M., et al. (2008). The comet assay: topical issues. Mutagenesis 23, 143–151. doi: 10.1093/mutage/gem051

Collins, A. R., Dusinska, M., Horvathova, E., Munro, E., Savio, M., and Stetina, R. (2001). Inter-individual differences in DNA base excision repair activity measured in vitro with the comet assay. Mutagenesis 16, 297–301. doi: 10.1093/mutage/16.4.297

Collins, A. R., and Gaivão, I. (2007). DNA base excision repair as a biomarker in molecular epidemiology studies. Mol. Aspects Med. 28, 307–322. doi: 10.1016/j.mam.2007.05.003

Collins, A. R., Gedik, C. M., Olmedilla, B., Southon, S., and Belizzi, M. (1998). Oxidative DNA damage measured in human lymphocytes: large differences between sexes and between countries, and correlations with heart disease mortality rates. FASEB J. 12, 1397–1400.

Collins, A. R., and Horvathova, E. (2001). Oxidative DNA damage, antioxidants and DNA repair: applications of the comet assay. Biochem. Soc. Trans. 29, 337–341. doi: 10.1042/BST0293037

Collins, A., Koppen, G., Valdiglesias, V., Dusinska, M., Kruszewski, M., Moller, P., et al. (2014). The comet assay as a tool for human biomonitoring studies: the ComNet project. Mutat. Res. Rev. Mutat. Res. 759, 27–39. doi: 10.1016/j.mrrev.2013.10.001

Dhawan, A., Baijapure, M., and Parmar, D. (2009). Comet assay: a reliable tool for the assessment of DNA damage in different models. Cell Biol. Toxicol. 25, 3–52. doi: 10.1007/s10565-008-9072-z

Dixon, D. R., Pruski, A. M., Dixon, L. R. J., and Jha, A. N. (2002). Marine invertebrate eco-genotoxicology: a methodological overview. Mutagenesis 17, 495–507. doi: 10.1093/mutage/17.6.495

Dusinska, M., and Collins, A. R. (2008). The comet assay in human biomonitoring: gene-environment interactions. Mutagenesis 23, 191–205. doi: 10.1093/mutage/gen007

Dusinska, M., and Collins, A. R. (2010) DNA oxidation, antioxidant effects and DNA repair measured with the comet assay,” in Biomarkers for Antioxidant Defense and Oxidative Damage: Principles and Practical Applications, eds G. Aldini, K. J. Yeum, E. Niki, and R. Russell (Oxford: Blackwell Publishing Ltd), 261–282.

Ersson, C., Møller, P., Forchhammer, L., Loft, S., Azqueta, A., Godschalk, R. W., et al. (2013). An ECVAG inter-laboratory validation study of the comet assay: inter-laboratory and intra-laboratory variations of DNA strand breaks and FPG-sensitive sites in human mononuclear cells. Mutagenesis 28, 279–286. doi: 10.1093/mutage/get011

Faust, F., Kassie, F., Knaasmüller, S., Boedercker, R. H., Mann, M., and Mersch-Sundermann, V. (2004). The use of the alkaline comet assay with lymphocytes in human biomonitoring studies. Mutat. Res. 566, 209–229. doi: 10.1016/j.mrrev.2003.09.007

Gaivão, I. (1999) Genotoxic Evaluation of Reactive Oxygen Species Generatig Clumps: A Study in Drosophila melanogaster. Ph.D. thesis, Universidade de Trás- os-Montes and Alto Douro, Vila Real.

Gaivão, I., and Comendador, M. A. (1996) The w/w+ somatic mutation and recombination test (SMART) of Drosophila melanogaster for detecting reactive oxygen species: characterization of 6 strains. Mutat. Res. 360, 145–151. doi: 10.1016/0165-1161(96)00009-3

Gaivão, I., Piasék, A., Brevik, A., Shaposhnikov, S., and Collins, A. R. (2009). Comet assay-based methods for measuring DNA repair in vitro; estimates of inter- and intra-individual variation. Cell. Biol. Toxicol. 25, 45–52. doi: 10.1007/s10565-007-9047-5

Gaivão, I., Rodrigues, R., and Sierra, L. M. (2014). “Use of the comet assay to study DNA repair in Drosophila melanogaster,” in Genotoxicity and DNA Repair: A Practical Approach, Methods in Pharmacology and Toxicology, eds L. M. Sierra and I. Gaivão (New York: Springer). doi: 10.1007/978-1-4398-1068-7_16

García-Sar, D., Aguado, L., Montes-Bayón, M., Comendador, M. A., Blanco González, E., Sanz-Medel, A., et al. (2012). Relationships between
cisplatin-induced adducts and DNA strands breaks, mutation and recombination in vivo in somatic cells of *Drosophila melanogaster*, under different conditions of nucleotide excision repair. *Mutat. Res.* **741**, 81–88. doi: 10.1016/j.mrgentox.2011.11.005

Garcia-Sa, D., Montes-Bayon, M., Aguado Ortiz, L., Blanco-Gonzalez, E., Sierra, I. M., and Sany-Medel, A. (2008) In vivo detection of DNA adducts induced by cisplatin using capillary HPLC-ICP-MS and their correlation with the genotoxic damage in *Drosophila melanogaster*. *Anal. Biochem.** 390**, 37–44. doi: 10.1016/j.sab.2010.02.016

Goddschalk, R. W., Ersson, C., Riso, P., Porrini, M., Langie, S. A., van Schooten, F. J., et al. (2013). DNA-repair measurements by use of the modified comet assay: an inter-laboratory comparison within the European Comet Assay Validation Group (ECVAG). *Mutat. Res.** 757**, 66–67. doi: 10.1016/j.mrgentox.2013.06.020

Guan, X., Diqu, J., Jianzhong, Y., Jingmin, G., HuiFeng, Z., Mingan, S., et al. (2013). Carbamate insecticide methonil confers cytotoxicity through DNA damage induction. *Food Chem. Toxicol.** 53**, 352–358. doi: 10.1016/j.fct.2012.12.020

Hallström, I., Blank, A., and Atuma, S. (1984). Genetic variation in cytochrome P450 and xenobiotic metabolism in *Drosophila melanogaster*. *Biochem. Pharmacol.** 33**, 13–20. doi: 10.1016/0005-2767(84)90364-2

Hartmann, A., Agurell, E., Beevers, C., Brendler-Schwaab, S., Burlinson, B., Clay, P., et al. (2003). Recommendations for conducting the in vivo animal comet assay. *Mutagenesis** 18**, 43–51. doi: 10.1093/mutage/18.1.43

Hernando, J., Alvarez, L., Ferreiro, J. A., Sancho, I., Comendador, M. A., and Sierra, L. M. (2004). Female germ cell mutagenicity of model chemicals in *Drosophila melanogaster*: mechanism based information and analysis of repair systems. *Mutat. Res.** 545**, 59–72. doi: 10.1016/j.mrfmmm.2003.09.013

Hoffmann, H., Högel, J., and Spett, G. (2005). The effect of smoking on DNA effects in the comet assay: a meta-analysis. *Mutagene** 20**, 455–466. doi: 10.1093/mutage/gei064

Jha, A. N. (2008). Ecotoxicological applications and significance of the comet assay. *Mutat. Res.** 675**, 189–197. doi: 10.1016/j.mrgentox.2008.03.016

Karlsson, H. L. (2010). The comet assay in nanotoxicology research. *Anal. Bioanal. Chem.** 398**, 651–666. doi: 10.1007/s00216-010-3977-0

Kassie, F., Parzeffal, W., and Knasmüller, S. (2000). Single cell gel electrophoresis assay: a new technique for human biomonitoring studies. *Mutat. Res.** 463**, 13–31. doi: 10.1016/S0165-5730(00)00411-7

Khurana, V., Lu, Y., Steinhilb, M. L., Oldham, S. A., Steinhilb, M. L., and Carmona, E. R. (2013). The wing-spot and the comet tests as useful for age associated changes in sleep: wake cycles. *Biochem. Biophys. Res. Commun.** 430**, 1667–1672. doi: 10.1016/j.bbrc.2013.03.024

Koh, K., Evans, J. M., Hendricks, J. C., and Sehgal, A. (2006). *Drosophila* model for age associated changes in sleep: wake cycles. *Proc. Natl. Acad. Sci. U.S.A.** 103**, 13843–13847. doi: 10.1073/pnas.0605903103

Lee, R. F., and Steinert, S. (2003). Use of the single cell gel electrophoresis/comet assay for detecting DNA damage in aquatic (marine and freshwater) animals. *Mutat. Res.** 544**, 43–64. doi: 10.1016/S0165-5730(03)00017-6

Marcos, R., and Carmona, E. R. (2013). The wing-spot and the comet tests as useful assays for genotyping accuracy in *Drosophila*. *Methods Mol. Biol.** 1044**, 417–427. doi: 10.1007/978-1-62703-529-3_23

Menke, M., Chen, I.-P., Angelis, K. J., and Schubert, I. (2001). DNA damage and repair in *Arabidopsis thaliana* as measured by the comet assay after treatment with different classes of genotoxins. *Mutat. Res.** 493**, 87–93. doi: 10.1016/S0165-3781(01)00165-6

Mishra, M., Sharma, A., Negi, M. P., Dwivedi, U. N., and Chowdhuri, D. K. (2011). Tracing the tracks of genotoxicity by trivalent and hexavalent chromium in somatic cells of *Drosophila melanogaster*. *Mutat. Res.** 722**, 44–51. doi: 10.1016/j.mrgentox.2011.02.010

Mishra, M., Sharma, A., Shukla, A. K., Kumar, R., Dwivedi, U. N., and Chowdhuri, D. K. (2014) Genotoxicity of dichlorvos in strains of *Drosophila melanogaster* defective in DNA repair. *Mutat. Res.** 766**, 35–41. doi: 10.1016/j.mrgentox.2014.02.004

Mishra, M., Sharma, A., Shukla, A. K., Pragya, P., Murthy, R. C., de Pomerai, D., et al. (2013). Transcriptomic analysis provides insights on hexavalent chromium induced DNA strand breaks and their possible repair in midgut cells of *Drosophila melanogaster* larvae. *Mutat. Res.** 747–748**, 28–39. doi: 10.1016/j.mrfmmm.2013.04.005
Søndergaard, L. (1993). Homology between the mammalian liver and the Drosophila fat body. Trends Genet. 9, 193. doi: 10.1016/0168-9525(93)90113-V
Speer, G., and Hartmann, A. (1999). “The comet assay (single cell gel test). A sensitive genotoxicity test for the detection of DNA damage and repair,” in Methods in Molecular Biology, Vol. 113. DNA Repair Protocols: Eukaryotic Systems, ed. D. S. Henderson (Totowa, NJ: Humana Press Inc.), 203–212.
Tice, R. R., Aquarell, E., Anderson, D., Burlinson, B., Hartmann, A., Kobayashi, H., et al. (2000). The single cell gel/comet assay: guidelines for in vitro and in vivo genetic toxicity testing. Environ. Mol. Mutagen. 35, 206–221. doi: 10.1002/(SICI)1098-2280(2000)35:3<206::AID-EM8>3.0.CO;2-J
Uriol, E., Sierra, M., Comendador, M. A., Fra, J., Martínez-Camblor, P., Lacave, A. J., et al. (2013). Long-term biomonitoring of breast cancer patients under adjuvant chemotherapy: the comet assay as a possible predictive factor. Mutagenesis 28, 39–48. doi: 10.1093/mutage/ges050
Vecchio, G. (2014). A fruit fly in the nanoworld: once again Drosophila contributes to environment and human health. Nanotoxicology doi: 10.3119/17435390.2014.911985 [Epub ahead of print].
Ventura, L., Giovannini, A., Savio, M., Donà, M., Macovei, A., Buttafava, A., et al. (2013). single cells gel electrophoresis (comet) assay with plants: research on DNA repair and ecogenotoxicity testing. Chemosphere 92, 1–9. doi: 10.1016/j.chemosphere.2013.03.006
Verma, A., Sengupta, S., and Lakhotia, S. C. (2012). DNApol-( gene is indispensable for the survival and growth of Drosophila melanogaster. Genesis 50, 86–101. doi: 10.1002/dvg.20791
Vogel, E. W., Graff, U., Frei, H. I., and Nivard, M. M. (1999). The results of assays in Drosophila as indicators of exposure to carcinogens. IARC Sci. Publ. 146, 427–470.
Vogel, E. W., and Nivard, M. J. M. (2001). Phenotypes of Drosophila homologs of human XPF and XPG to chemically-induced DNA modifications. Mutat. Res. 476, 149–165. doi: 10.1016/S0027-5107(01)00121-X
Vogel, E. W., Nivard, M. J. M., Ballering, L. A. B., Bartsch, H., Barbin, A., Nair, J., et al. (1996). DNA damage and repair in mutagenesis and carcinogenesis: implications of structure-activity relationships for cross-species extrapolation. Mutat. Res. 353, 177–218. doi: 10.1016/0027-5107(96)00032-2
Wolf, M. J., Amrein, H., Izatt, J. A., Chorna, M. A., Reedy, M. C., and Rockman, H. A. (2006). Drosophila as a model for the identification of genes causing adult human heart disease. Proc. Natl. Acad. Sci. U.S.A. 103, 1394–1399. doi: 10.1073/pnas.0507359103

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.