UNIVERSIDADE FEDERAL DE PERNAMBUCO
CENTRO DE BIOCIÊNCIAS
PROGRAMA DE PÓS-GRADUAÇÃO EM BIOLOGIA ANIMAL

MARIA GISLAINE PEREIRA

ESTUDO DA RESISTÊNCIA À RADIAÇÃO IONIZANTE NATURAL E INDUZIDA EM *DROSOPHILA MELANOGASTER* RESIDENTES DE CIDADES DO NORDESTE BRASILEIRO COM ALTO NÍVEL DE ^{222}RN

Recife
2025

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Tese apresentada ao Programa de Pós-Graduação em Biologia Animal da Universidade Federal de Pernambuco como requisito para obtenção do título de Doutora em Biologia Animal.

Orientador: Dr. André Morgado Esteves

Coorientadora: Dra. Claudia Rohde

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RESUMO

A radiação ionizante é capaz de provocar alterações biológicas graves nos organismos expostos, decorrentes de mutações no material genético que podem culminar em danos irreparáveis e morte celular. Contudo, os seres vivos possuem sistemas de reparo genéticos para manutenção da integridade do DNA, a depender da dose e tempo de exposição a agentes mutagênicos. Este trabalho buscou investigar como a radiação ionizante presente em dois municípios da região semiárida do Nordeste do Brasil afeta o organismo-modelo *Drosophila melanogaster* (Insecta, Diptera). Após a busca por locais com níveis alterados de radiação natural, monitorados pela presença de partículas alfa emanadas do solo, foram escolhidos os municípios de Cerro Corá e Parelhas, no Rio Grande do Norte, para serem obtidas amostras de indivíduos *D. melanogaster*, denominadas CC-res (Cerro Corá residente) e PAR-res (Parelhas residente). Em laboratório, essas linhagens foram mantidas nas mesmas condições de cultivo que a linhagem controle Oregon-R. Decorridos sete e 13 meses, três réplicas de cada linhagem foram expostas por seis dias em Cerro Corá e em Parelhas, junto com Oregon-R. Larvas e adultos expostos retornaram ao laboratório para serem processados pelo Ensaio cometa, que quantifica os danos no DNA (efeito genotóxico) pelo Índice de Dano (ID) e Frequência de Dano (FD%). Para ampliar os resultados obtidos em campo (exposição crônica), uma segunda abordagem foi feita com as mesmas populações, e com a linhagem controle ambiental procedente de Vitória de Santo Antão (VSA-res), local com níveis normais de radiação (< 200 $Bq.m^{-3}$). Três réplicas de larvas CC-res, PAR-res, Oregon-R e VSA-res foram expostas a doses absorvidas de radiação gama (10, 30, 50 e 70 Gy), induzida por fonte de ^{60}Cobalto. Os efeitos genotóxicos foram avaliados pelo Ensaio cometa após 1 hora da irradiação, e após 24 horas, visando observar o efeito celular de recuperação aos danos. Os resultados dos experimentos crônicos e agudos estão apresentados nos Artigos 1 e 2 desta Tese. O primeiro artigo apresenta as medidas de radiação alfa em Cerro Corá (acima de 2800 $Bq.m^{-3}$), quantificadas em duas estações do ano (seca e chuvosa), juntamente com os resultados da exposição crônica e aguda de CC-res e Oregon-R (OR_1). São apresentadas evidências da presença de adaptação genética em CC-res, com baixas medidas de ID e FD% em comparação com OR_1. Os resultados de radiorresistência foram corroborados pelos testes de exposição aguda, com baixos ID e FD% em CC-res (exceto na dose 10 Gray), ao contrário de OR_1, com danos crescentes (efeito dose-dependente). Esses resultados se mantiveram mesmo após 24 horas da irradiação gama, sugerindo que os danos são permanentes em OR_1 (efeito mutagênico). No artigo 2 foi avaliada a população natural de *D. melanogaster* proveniente de Parelhas, um local com níveis moderados de radiação natural (entre 400 e 900 $Bq.m^{-3}$). Como os experimentos foram realizados nas mesmas datas em Parelhas e Cerro Corá, o Artigo 2 apresenta, além dos resultados da exposição crônica e aguda, a comparação entre todas as populações, incluindo Vitória de Santo Antão (VSA-res), que se comportou como grupo controle ambiental após exposição à radiação gama. Os resultados confirmam a hipótese inicial de que apenas as populações *D. melanogaster* residentes de locais com níveis anômalos de radiação natural desenvolveram a capacidade adaptativa de controlar os danos ao DNA, provavelmente devido a um aprimoramento das vias de reparo do DNA. Os resultados apontam para a necessidade de serem realizados mais estudos nos ambientes da Caatinga onde há elevados níveis de radiação, e avaliar outros organismos, visando compreender como ocorrem os processos genético-adaptativos decorrentes da exposição à radiação natural.

Palavras-chave: Caatinga; *D. melanogaster*; Radiação ionizante; Resposta radioadaptativa.

ABSTRACT

Ionizing radiation can cause serious biological changes in exposed organisms, resulting from mutations in genetic material that can culminate in irreparable damage and cell death. However, living organisms have genetic repair systems to maintain DNA integrity, depending on the dose and time of exposure to mutagenic agents. This study sought to investigate how ionizing radiation present in two municipalities in the semiarid region of Northeastern Brazil affects the model organism *Drosophila melanogaster* (Insecta, Diptera). After searching for locations with altered levels of natural radiation, monitored by the presence of alpha particles emanating from the soil, the municipalities of Cerro Corá and Parelhas, in Rio Grande do Norte, were chosen to obtain samples of *D. melanogaster* individuals, called CC-res (Cerro Corá resident) and PAR-res (Parelhas resident). In the laboratory, these strains were maintained under the same culture conditions as the control strain Oregon-R. After seven and 13 months, three replicates of each lineage were exposed for six days in Cerro Corá and Parelhas, together with Oregon-R. Larvae and adults exposed were returned to the laboratory to be processed by the Comet Assay, which quantifies DNA damage (genotoxic effect) by the Damage Index (DI) and Damage Frequency (DF%). To expand the results obtained in the field (chronic exposure), a second approach was carried out with the same populations, and with the environmental control lineage from Vitória de Santo Antão (VSA-res), a place with normal radiation levels (< 200 $Bq.m^{-3}$). Three replicates of CC-res, PAR-res, Oregon-R and VSA-res larvae were exposed to absorbed doses of gamma radiation (10, 30, 50 and 70 Gy), induced by a ^{60}Cobalt source. The genotoxic effects were evaluated by the Comet assay after 1 hour of irradiation and after 24 hours, aiming to observe the cellular recovery effect to the damage. The results of the chronic and acute experiments are presented in Articles 1 and 2 of this Thesis. The first article presents the alpha radiation measurements in Cerro Corá (above 2,800 $Bq.m^{-3}$), quantified in two seasons of the year (dry and rainy), together with the results of the chronic and acute exposure of CC-res and Oregon-R (OR_1). Evidence of the presence of genetic adaptation in CC-res is presented, with low DI and DF% measurements compared to OR_1. The radioresistance results were corroborated by the acute exposure tests, with low DI and DF% in CC-res (except at the dose of 10 Gray), unlike OR_1, with increasing damage (dose-dependent effect). These results were maintained even after 24 hours of gamma irradiation, suggesting that the damage is permanent in OR_1 (mutagenic effect). In article 2, the natural population of *D. melanogaster* from Parelhas, a place with moderate levels of natural radiation (between 400 and 900 $Bq.m^{-3}$), was evaluated. Since the experiments were carried out on the same dates in Parelhas and Cerro Corá, Article 2 presents, in addition to the results of chronic and acute exposure, the comparison between all populations, including Vitória de Santo Antão (VSA-res), which behaved as an environmental control group after exposure to gamma radiation. The results confirm the initial hypothesis that only populations *D. melanogaster* resident in places with anomalous levels of natural radiation developed the adaptive capacity to control DNA damage, probably due to an improvement in DNA repair pathways. These results indicate the need for further studies in Caatinga environments where there are high levels of radiation, and with other organisms to understand how the genetic-adaptive processes resulting from exposure to natural radiation occur.

Keywords: Caatinga; *D. melanogaster*; Ionizing radiation; Radioadaptive response.

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SUMÁRIO

1 INTRODUÇÃO

Os seres vivos estão expostos a diferentes níveis de radiação natural de fundo (Yushkova, 2022), sendo o gás radônio-222 (^{222}Rn) o principal contribuinte para a exposição humana a essa radiação em todo o mundo, configurando-se como a segunda principal causa de câncer de pulmão, atrás apenas do tabagismo (Robertson et al., 2013). O ^{222}Rn possui considerável impacto no ambiente devido à sua meia-vida de aproximadamente quatro dias, o que permite ampla dispersão antes de seu decaimento. Esse isótopo origina-se naturalmente do decaimento radioativo do urânio-238 (^{238}U) e é o principal emissor de partículas alfa (α) (Gillmore; Phillips; Denman, 2005), uma das mais perigosas fontes de mutações, com potencial para comprometer a integridade genômica dos organismos (Stanley et al., 2019; Walczak et al., 2020).

No Brasil, estudos realizados na região Nordeste constatam a presença de altos índices de radiação natural, com destaque para os estados de Pernambuco e Paraíba, que abrangem uma camada não contínua de fosforito uranífero conhecida como Bacia Sedimentar da Paraíba (Souza, 2006), e no estado do Rio Grande do Norte, sobretudo em sua porção ocidental, que está inserida na Província Pegmatítica da Borborema (PPB), com elevados níveis de urânio, conforme Silva et al. (2010). A região PPB reúne corpos pegmatíticos em aproximadamente 6000 km^2, uma das províncias minerais mais críticas do nordeste brasileiro (Pastura; Campos, 2016). Nesses afloramentos rochosos e terrenos constituídos por rochas ígneas e metamórficas foram encontrados componentes radioativos, como o urânio, cujos isótopos e seus produtos de decaimento contribuem para o aumento da radiação de fundo na região.

A exposição ao ^{222}Rn é capaz de induzir danos ao material genético, como a quebra de fitas de DNA, podendo ocasionar doenças como o câncer (Stanley et al., 2019, Eidy; Angela; Tishkowski, 2024). No entanto, é possível que determinados organismos, mesmo estando expostos a elevados índices de radiação ionizante, se adaptem a esta condição e não sofram danos consideráveis em seu material genético. Tal adaptação à radiação, denominada radiorresistência, foi observada no organismo-modelo *Drosophila melanogaster* (Koval et al., 2020; Yushkova, 2022). É importante destacar que os experimentos destes autores, ou se restringem a investigação de linhagens laboratoriais expostas apenas aos efeitos da radiação gama induzida, algumas das linhagens sendo geneticamente modificadas para conter genes marcadores; ou que refletem resultados decorrentes do efeito da radiação após acidentes nucleares, tal como de Chernobyl e Fukushima. Logo, essas contribuições sobre a radiorresistência podem não refletir os efeitos reais da radiação natural, como os que podem

ser estudados em populações residentes de locais com níveis anômalos de radiação. A espécie *Drosophila melanogaster* tem se mostrado um excelente organismo-modelo para estas variadas abordagens, por ser cosmopolita e desenvolver-se muito bem tanto em ambientes naturais quanto em condições laboratoriais.

Drosophila melanogaster tem sido amplamente utilizada nas mais diversas áreas da biologia devido a sua facilidade de manipulação laboratorial, rápido ciclo de vida, elevada fecundidade e conhecimento detalhado acerca de seu genoma (Baenas; Wagner, 2019; Flatt, 2020), além de apresentar mecanismos celulares e moleculares semelhantes aos seres humanos (Ugur, Chen, Bellen, 2016). E dentre os testes biológicos capazes de avaliar a extensão dos danos genéticos causados pela radiação ionizante nesta espécie, destaca-se o Ensaio cometa (Ostling; Johanson, 1984; Singh et al. 1988), uma técnica que reúne sensibilidade, rapidez e eficácia para determinar e mensurar a intensidade dos danos no DNA em células somáticas (Azqueta et al., 2020; Møller et al., 2020).

Neste viés, este estudo se propôs a avaliar o efeito tóxico-genético da radiação natural sobre o material genético de larvas e adultos de *D. melanogaster* descendentes de organismos residentes de duas cidades do Nordeste Brasileiro, onde medidas de radiação alfa são elevadas. A primeira hipótese deste estudo é que as linhagens de *D. melanogaster* residentes são adaptadas para correção dos efeitos genotóxicos causados pela radiação natural, diferente de uma linhagem controle nunca exposta à radiação. Uma segunda hipótese, é de que esta adaptação é genética e se perpetua nos descentes das linhagens, mesmo após muitas gerações de manutenção em laboratório, que é um local sem radiação. Por fim, a terceira hipótese deste estudo é de que a radiorresistência adquirida pelas populações residentes se evidencia também quando os organismos são expostos a doses absorvidas de radiação gama, de forma que os organismos não sofrem danos, mesmo com aumento crescente das doses de irradiação, ao contrário de uma linhagem controle.

2 FUNDAMENTAÇÃO TEÓRICA

2.1 Radiação Ionizante Natural

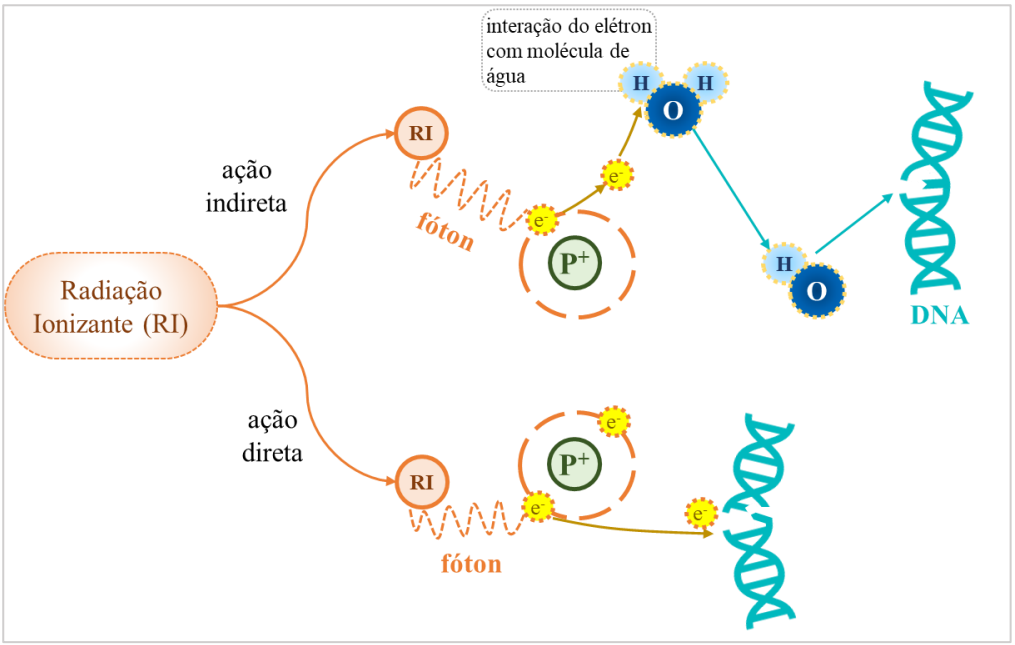
Organismos vivos estão constantemente expostos à radiação, derivada de fontes artificiais ou naturais (Balonov, 2008, Desouky; Ding; Zhou, 2015, Santos Júnior et al., 2017). A radiação de origem natural corresponde aos radionuclídeos cosmogênicos renováveis como o ^{3}H, ^{14}C e o ^{22}Na, que se mantém contínuos devido a frequência equilibrada entre seus níveis

de produção e decadência radioativa, e aos radionuclídeos primordiais de vida longa como o ^{238}U, ^{235}U e o ^{232}Th, que reduz gradativamente com o passar do tempo em decorrência de seu processo de decaimento (Balonov, 2008) e cuja distribuição ocorre em variadas concentrações nas rochas e solo da Terra (Agbalagba et al., 2014).

Elementos radioativos com núcleos atômicos instáveis liberam o excesso de energia de forma natural por meio do processo de decaimento radioativo, emitindo radiação na forma de partículas, como alfa e beta, ou como ondas eletromagnéticas, a exemplo dos raios gama (Lee et al., 2012). Isso gera riscos à saúde, que varia de acordo com a natureza do radionuclídeo, o grau de contaminação e a extensão de sua propagação, podendo afetar tanto o ambiente quanto os indivíduos (Ogundare, Adekoya, 2015). A exposição à radiação ionizante pode causar efeitos biológicos classificados em estocásticos e não-estocásticos, além de efeitos genéticos, que podem se manifestar de forma somática ou germinativa (Calegaro, Casulari, Orlando, 2024). Historicamente, as explosões nucleares de Hiroshima e Nagasaki, ocorridas no final da Segunda Guerra Mundial, representaram o maior impacto da radiação ionizante sobre os seres humanos. Essas explosões resultaram na morte de milhares de pessoas devido ao intenso calor gerado e aos altos níveis de radiação emitidos pela fissão do material radioativo (Calegaro, Casulari, Orlando, 2024).

Em relação aos danos à molécula de DNA, a radiação pode atuar por meio de dois mecanismos: ação direta e indireta (**Figura 1**). A ação direta ocorre quando a radiação interage diretamente com o DNA, provocando rupturas estruturais que podem resultar em morte celular ou em alterações genéticas, contribuindo para o desenvolvimento de câncer. Por sua vez, a ação indireta ocorre quando a radiação interage primeiramente com moléculas de água ou outras biomoléculas da célula, gerando radicais livres, como hidroxila (HO) e alcoxi (RO_2), que subsequentemente reagem com o DNA, causando danos à sua estrutura (Desouky; Ding; Zhou, 2015).

Figura 1. Esquema representativo dos mecanismos de ação direta e indireta da radiação ionizante no DNA.



Fonte: Adaptado de Silva (2022).

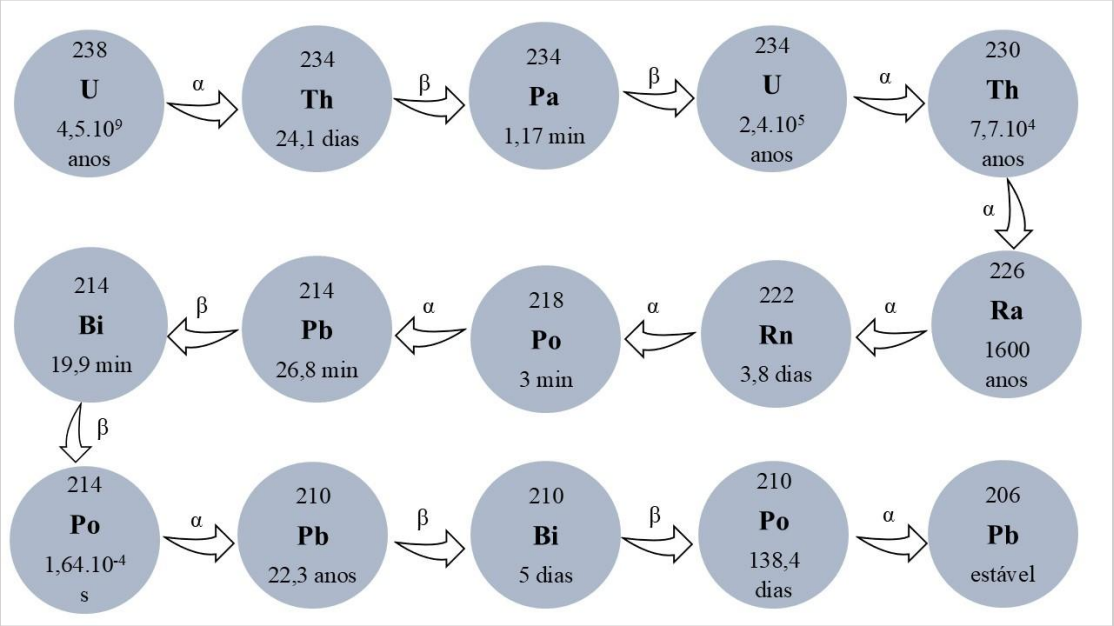
O urânio é um dos radioelementos mais importantes devido à sua abundância no ambiente e ao seu longo período de meia-vida, de 4,5 bilhões de anos (Salahel Din; Vesterbacka, 2012). Ocorre naturalmente em três isótopos radioativos, ^{238}U, ^{235}U e ^{234}U, sendo que os dois primeiros iniciam séries de decaimento radioativo independentes, enquanto o último é produto do decaimento da série do ^{238}U. Durante esse processo, os isótopos de urânio, assim como a maioria de seus subprodutos de decaimento, liberam radiação por meio da emissão de partículas alfa, beta e raios gama. No entanto, a radiação alfa é predominante e representa um risco à saúde quando inalada ou ingerida (Wise, 2024a).

A partícula alfa possui baixa capacidade de penetração devido ao seu núcleo pesado, composto por dois prótons e dois nêutrons, o que faz com que percam rapidamente sua energia cinética. Em razão disso, não conseguem, por exemplo, atravessar a epiderme da pele humana (Bleise; Danesi; Burkart, 2003). No entanto, essas partículas são altamente ionizantes e em contato com tecidos e células podem desencadear efeitos deletérios (Karmaker et al., 2021).

O isótopo de urânio-238 (^{238}U) é um dos elementos radioativos mais presentes na crosta terrestre, sendo encontrado em rochas e solo (Dantas et al., 2020). A partir do seu decaimento radioativo, origina-se o rádio-226 (^{226}Ra), que por sua vez dá origem ao radônio-222 (^{222}Rn) (**Figura 2**). O radônio é um gás nobre inodoro, incolor e insípido, com meia-vida

de 3,8 dias e que se configura como um dos principais contribuintes da radiação ionizante de origem natural (WHO, 2009, Dobrzyńska; Gajowik; Wieprzowski, 2023). A exposição a este gás pode causar instabilidade no genoma, pois as partículas alfa emitidas durante seu decaimento são capazes de induzir danos a molécula de DNA, que quando não reparados levam a mutações (WHO, 2009, UNSCEAR, 2010, Meenakshi; Sivasubramanian; Venkatraman, 2017, Walczak et al., 2020).

Figura 2. Representação do decaimento radioativo do urânio. Cada elemento contém símbolo, massa atômica, tempo de meia-vida e tipo de partícula ionizante emitida.



Fonte: Adaptado de Dantas et al. (2020).

O território brasileiro possui muitas áreas contendo material radioativo de ocorrência natural (NORM) (Souza Pereira et al., 2020), ocupando o sexto lugar entre os países que abrigam os principais reservatórios de urânio do mundo (CNEN, 2016), tais como os depósitos situados em Rio Cristalino (PA), Pitinga (AM), Poços de Caldas (MG), Lagoa Real /Caetité (BA), Santa Quitéria (CE) (Wise, 2024b) e São José de Espinharas (PB) (Santos Júnior et al., 2017). Algumas dessas regiões podem apresentar valores mais elevados de radiação em relação a outras áreas no Brasil. No entanto, níveis exacerbados de radioatividade natural são comumente detectados em locais próximos a anomalias geológicas (Costa Júnior et al., 2013).

Particularmente no Nordeste do Brasil situa-se a Província Pegmatítica da Borborema (PPB), que abrange os estados do Rio Grande do Norte e Paraíba, e apresenta elevadas concentrações de radioelementos, como Urânio, Tório e Potássio associados aos granitos

pegmatíticos da região (Silva et al., 2010). A província está localizada na Mesorregião do Seridó e compreende uma área de aproximadamente 380.000 km^2, consistindo em um complexo sistema de zonas falhadas. Ela está entre as províncias minerais de destaque no país e dispõe de uma quantidade significativa de corpos pegmatíticos que contribuem para economia de diversos municípios do estado, sendo berílio (Be), tântalo (Ta) e lítio (Li) os principais minerais associados a pegmatitos como a magnesita, grafita e mármore (Almeida et al., 1981; Campos et al., 2013).

Populações que vivem em áreas geologicamente ricas em minerais como o urânio, estão expostas aos efeitos deletérios dos radionuclídeos derivados, como o ^{222}Rn. Um estudo realizado por Marcon et al. (2017) identificou lesões mutagênicas em células da mucosa oral de indivíduos expostos a altos níveis de radônio *indoor* no município de Lucrécia, no Rio Grande do Norte. Essa região é conhecida por seus depósitos minerais e apresenta elevados índices de casos de câncer entre seus habitantes. Além disso, a água da Barragem Boqueirão do município de Parelhas, localizada na Província Pegmatítica da Borborema, demonstrou potencial mutagênico para células humanas e para o organismo-modelo *Oreochromis niloticus* (Chaves et al., 2016). Mais recentemente, Silva et al. (2021) realizaram um estudo com lagartos da espécie *Phyllopezus periosus*, que vivem sobre rochas emissoras de elementos radioativos. O estudo identificou frequências altas de micronúcleos e anormalidades nucleares, evidenciando a relação entre a radioatividade natural presente nos locais e os danos causados ao DNA.

2.2 Radiorresistência

É sabido que os organismos vivos estão continuamente em contato com uma gama de agentes genotóxicos que afetam a integridade do DNA à medida que ocasionam danos genéticos e mutações, o que pode comprometer a viabilidade das próximas gerações celulares. Contudo, para defesa e manutenção do genoma, os organismos dispõem de um complexo sistema de reparo, capaz de detectar, sinalizar e corrigir esses danos (Jackson; Bartek, 2009), atuando por meio de uma rede de proteínas envolvidas na resposta ao dano ao DNA, incluindo a regulação do ciclo celular e a apoptose (Moreno-Villanueva et al., 2017).

A radioadaptação é um mecanismo relacionado à resposta adaptativa das células à radiação. Esse mecanismo é caracterizado por uma maior eficiência no reparo do DNA lesionado e pela diminuição na frequência de mutações após a exposição prévia a uma dose condicionante, que antecede a aplicação de uma dose desafiadora (Fornalski et al., 2022,

Piotrowski; Krasowska; Fornalski, 2023). Estudos demonstraram que alguns organismos conseguem se adaptar a radiação ionizante e não sofrer grandes danos no material genético, mesmo estando expostos a altos níveis (Beam et al., 1954). Espécies como *Deinococcus radiodurans*, *Saccharomyces cerevisiae* e *Drosophila melanogaster* são exemplos, respectivamente, de bactéria, fungo e inseto, nos quais a radiorresistência foi observada (Mattimore; Battista, 1996, Beam et al., 1954, Koval et al., 2020).

Um estudo conduzido por Ruiz-González et al. (2016), no qual foram isolados diferentes morfotipos de bactérias associadas a penas de andorinhas *Hirundo rustica* de três pontos no entorno da cidade de Chernobyl, demonstra que a exposição crônica à radiação do local favoreceu a resistência de comunidades bacterianas. Elas viviam expostas a níveis intermediários de radiação ionizante de fundo, e após serem irradiadas em laboratório, apresentaram melhor sobrevivência em relação às demais comunidades. Os autores sustentam que em populações naturais, o contato a longo prazo com a radiação ionizante pode exercer pressão seletiva, favorecendo traços que protejam os organismos contra os efeitos deletérios deste agente, facilitando a sobrevivência no local.

Em nosso grupo de pesquisa, a primeira observação da resistência à radiação foi realizada por Castro (2016), em *Drosophila melanogaster*. O autor demonstrou que organismos da linhagem Oregon se mostraram mais sensíveis aos efeitos da radiação no ambiente natural de Lajes Pintadas, RN, e índices significativamente menores de danos foram observados nos organismos residentes. O autor manteve as linhagens residentes e a Oregon-R em cultivos laboratoriais durante seis e doze meses, momentos em que expos as linhagens e aplicou o Ensaio cometa para verificar os níveis de danos no DNA dos organismos. Os resultados demonstraram presença de radiorresistência nos organismos originalmente residentes, uma resposta adaptativa em meio a condição de elevada radioatividade de Lajes Pintadas, e efeito genotóxico superior na linhagem controle Oregon-R.

Recentemente, estudos como o de Yushkova (2022), também identificaram traços de radiorresistência em descendentes de *Drosophila melanogaster* cuja geração parental habitava áreas com altos níveis de contaminação radioativa. Entretanto, a fonte da radiação, neste caso, foi o acidente ocorrido na usina nuclear de Chernobyl, ocorrido em 1986, na Ucrânia. Respostas radioadaptativas podem ser interpretadas como mecanismos desenvolvidos para mitigar os efeitos da radiação, visando reduzir os danos ao DNA que comprometem a integridade genômica e a sobrevivência dos organismos (Yu et al., 2011; Morciano et al., 2018).

2.3 *Drosophila melanogaster* Meigen, 1833 (Diptera: Drosophilidae): aspectos gerais e aplicação como organismo-modelo

Organismos-modelo são espécies amplamente investigadas para elucidar processos biológicos. As informações obtidas por meio desses organismos são frequentemente extrapoladas para outros seres, sobretudo aqueles cuja complexidade dificulta o estudo direto, como os humanos. Tais organismos apresentam diversas vantagens experimentais e práticas, dentre as quais destacam-se a facilidade de reprodução e manutenção sob condições laboratoriais (Ankeny; Leonelli, 2020). Dentre as espécies animais conceituadamente usados como organismo modelo estão mamíferos como o camundongo *Mus musculus*, o peixe-zebra *Danio rerio*, o nematoide *Caenorhabditis elegans* e dípteros como *Drosophila melanogaste*r (Irion; Nüsslein-Volhard, 2022).

Drosophila melanogaster tem desempenhado um papel significativo em diversos campos da biologia, apoiando pesquisas em áreas como fisiologia, genética, ecologia e evolução. Isso se deve a várias características vantajosas desse organismo, como seu genoma bem compreendido e altamente versátil, especialmente devido ao uso de cromossomos balanceadores e linhagens mutantes; à sua fácil manipulação e rápida reprodução em laboratório, além de seu tamanho pequeno, que requer pouco espaço para criação. O conhecimento detalhado sobre seu desenvolvimento inclui um ciclo de vida rápido (cerca de 12 dias), alta fecundidade e curto tempo de vida útil (em torno de 80 dias). Além disso, sua distribuição cosmopolita e a simplicidade de coleta contribuem para seu uso extensivo em variados estudos (Baenas; Wagner, 2019; Flatt, 2020).

D. melanogaster é comumente conhecida como "mosca da fruta", embora não se alimente diretamente de frutas, e sim de microrganismos, como fungos e bactérias, que se desenvolvem nas frutas em decomposição. Essas moscas são atraídas pelo ácido acético, composto responsável pelo odor do vinagre, que se acumula à medida que a fruta fermenta. Por isso, o termo "mosca do vinagre" também é utilizado para se referir a espécie. Curiosamente, *D. melanogaster* tende a evitar tanto níveis baixos quanto altos de ácido acético, o que indica que a fruta ainda não está madura o suficiente ou está podre, respectivamente (Jouandet; Gallio, 2015; Flatt, 2020).

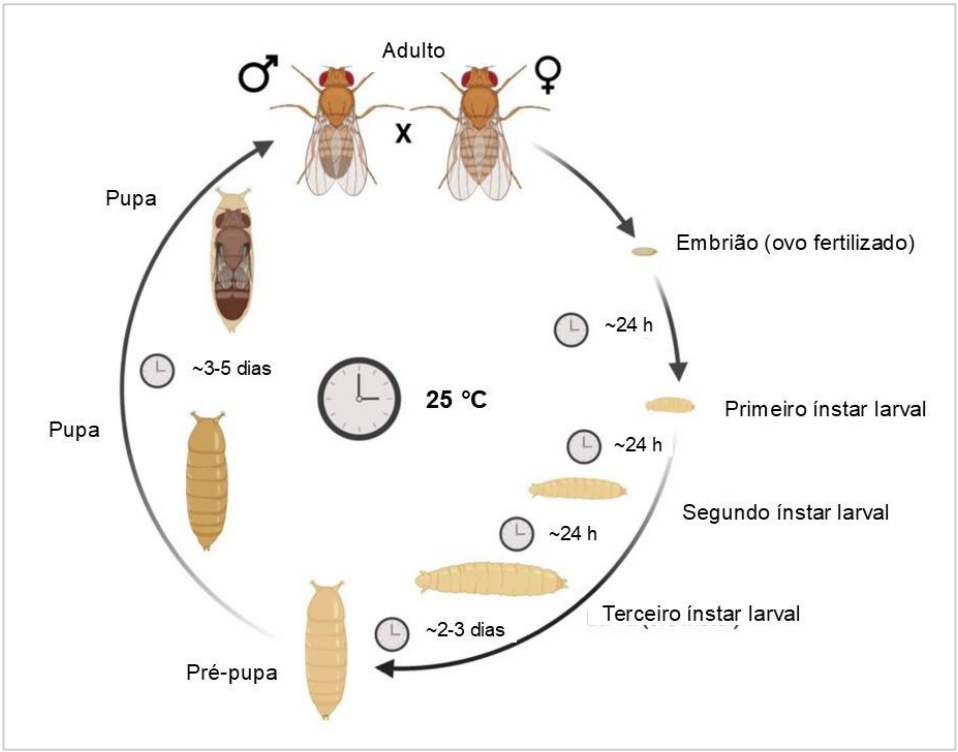
O ciclo de vida de *Drosophila melanogaster* é holometábolo e caracteriza-se por quatro estádios distintos durante o desenvolvimento: ovo, larva, pupa e adulto (imago). Ovos e pupas são organismos sésseis, e, embora as larvas se movimentem, esta locomoção está limitada ao substrato onde se encontram, enquanto os adultos apresentam capacidade de

dispersão aérea. O tempo de desenvolvimento depende das condições ambientais. Em condições laboratoriais padronizadas, com temperatura controlada a 25°C, umidade relativa de 60%, dieta adequada e sem superlotação, o ciclo de vida completo, do ovo ao adulto, é concluído em aproximadamente 10 dias. Em linhagens selvagens ou com densidade populacional elevada, esse intervalo pode aumentar para aproximadamente 16 dias (Markow, 2015; Staats et al., 2018; Baenas; Wagner, 2019; Flatt, 2020).

Após o acasalamento entre machos e fêmeas adultas, os ovos fertilizados são depositados e o desenvolvimento embrionário resulta na formação de uma larva de primeiro ínstar em aproximadamente 24 horas. As larvas então progridem por mais dois estádios larvais (segundo e terceiro ínstares), com cada estádio durando em torno de 24 horas. Ao final do terceiro ínstar, que dura em média 72 horas, inicia-se a fase de pré-pupa, seguida pela pupação. Durante o estádio pupal, ocorre a metamorfose, que dura entre 3 e 5 dias, período em que os tecidos da mosca adulta são formados. Posteriormente, as imagos emergem, e os adultos atingem a maturidade sexual cerca de 24 horas após a emergência. A longevidade das moscas varia de 60 a 90 dias, dependendo das condições ambientais e de criação (Brischigliaro; Fernandez-Vizarra; Viscomi, 2023). A duração do ciclo de vida de *Drosophila melanogaster* está representando na **Figura 3**. Na fase adulta, o corpo deste díptero está dividido em três regiões morfologicamente distintas: cabeça, tórax e abdômen (**Figura 4**).

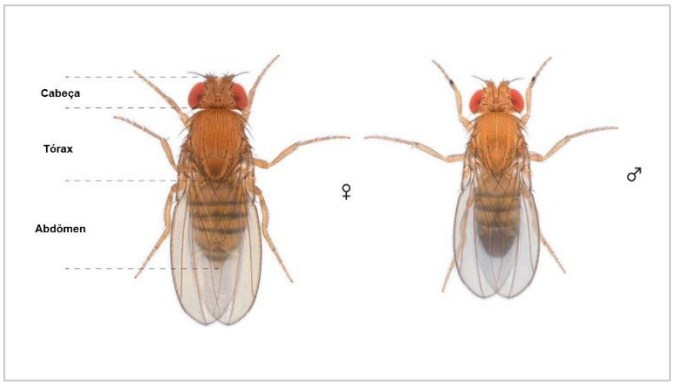
A cabeça abriga estruturas sensoriais importantes, como os olhos compostos, além de antenas e a probóscide. O tórax é segmentado em três partes: o protórax, localizado anteriormente, que possui um par de pernas; o mesotórax, localizado na região mediana, que contém um par de pernas e um par de asas; e o metatórax, na região posterior, que também apresenta um par de pernas e um par de asas modificadas, denominadas halteres. O abdômen é segmentado, com listras escuras e sua extremidade contém o aparelho reprodutor, o epândrio nos machos, e nas fêmeas, o ovipositor (Chyb; Gompel, 2013).

Figura 3. Ciclo de vida da *Drosophila melanogaster* desde o embrião até a emergência da imago.



Fonte: Adaptado de Brischigliaro, Fernandez-Vizarra e Viscomi (2023).

Figura 4. Morfologia do corpo de um adulto de *Drosophila melanogaster*, além diferença de tamanho entre macho e fêmea, indicando dimorfismo sexual.

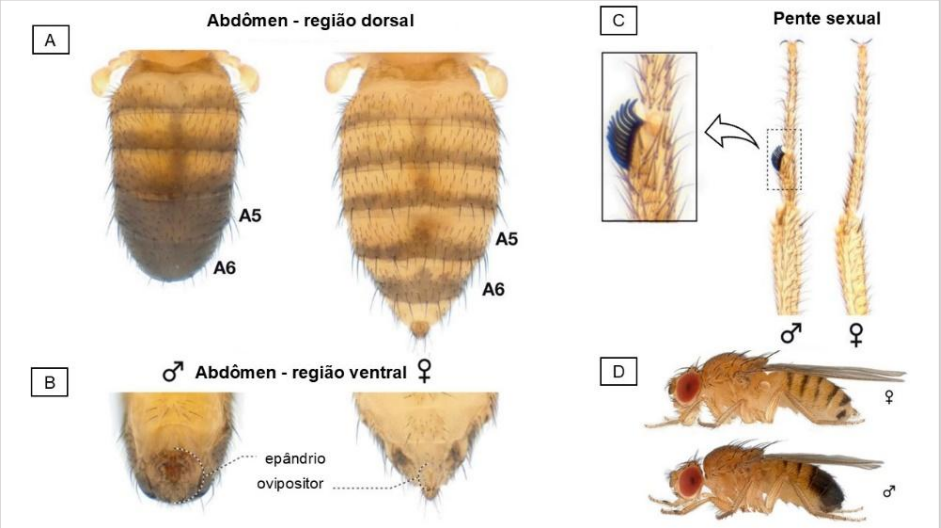


Fonte: Adaptado de Chyb; Gompel (2013).

D. melanogaster apresenta dimorfismo sexual, com as fêmeas sendo normalmente maiores que os machos (**Figura 5**). Além do tamanho, outras características morfológicas diferenciam ambos os sexos: nos machos, os segmentos A5 e A6 do abdômen exibem pigmentação uniforme e escura, sendo a extremidade abdominal arredondada e ligeiramente curvada ventralmente, enquanto nas fêmeas, a porção final do abdômen é pontiaguda (Figura

5A). Os machos também possuem estruturas denominadas "pentes sexuais" no par de pernas anterior, compostas por uma fileira de cerdas grossas e escuras localizadas no primeiro segmento tarsal (Figura 5C) (Chyb; Gompel, 2013).

Figura 5. Diferenças entre machos e fêmeas de *Drosophila melanogaster*. **A**: morfologia e coloração do abdômen. **B**: destaque do aparelho reprodutor. **C**: presença do pente sexual, estrutura exclusiva dos machos utilizada na identificação. **D**: comparação de tamanho entre os sexos.



Fonte: Adaptado de Chyb e Gompel (2013).

Há mais de 100 anos, *D. melanogaster* tem sido utilizada como organismo-modelo em pesquisas científicas, desempenhando um papel crucial em grandes avanços e descobertas, sobretudo, no campo da genética (Jennings, 2011; Rohde, 2012; Stephenson; Metcalfe, 2013; Markow, 2015). O uso da mosca-da-fruta em estudos genéticos começou com Thomas Hunt Morgan, que investigou o papel dos cromossomos na hereditariedade. Suas descobertas renderam-lhe o Prêmio Nobel de Fisiologia ou Medicina em 1933. Em 1946, Hermann Joseph Muller, ex-aluno de Morgan, recebeu o Prêmio Nobel por suas pesquisas que elucidaram os efeitos mutagênicos da radiação ionizante. Anos depois, em 1995, Edward B. Lewis, Christiane Nüsslein-Volhard e Eric F. Wieschaus, compartilharam o prêmio por suas descobertas acerca do controle genético do desenvolvimento inicial dos embriões. A mais recente premiação foi concedida a Jules Hoffmann, que, em 2011, dividiu o prêmio por suas descobertas sobre a ativação da imunidade inata em *Drosophila* (Markow, 2015).

A utilização de *Drosophila melanogaster* como modelo experimental, em substituição a mamíferos como ratos e camundongos, e especialmente a humanos, é mais viável devido a

menores implicações éticas, que poderiam limitar certos estudos (Jennings, 2011; Staats et al., 2018). Isto porque, apesar das diferenças morfológicas e de alguns aspectos celulares entre moscas e humanos, uma parte significativa dos mecanismos moleculares que regulam o desenvolvimento e os processos celulares e fisiológicos, é amplamente conservada entre ambas as espécies (Ugur; Chen; Bellen, 2016). Análises genômicas comparativas demonstram que aproximadamente 75% dos genes associados a doenças humanas possuem homólogos em *Drosophila melanogaster* (Reiter et al., 2001; Bier, 2005).

Embora uma parte dos genes humanos tenha ortólogos em *Drosophila melanogaster*, o genoma da mosca é consideravelmente mais compacto, apresentando famílias de genes menores, com menor redundância, além de possuir menos íntrons e regiões reguladoras não codificantes mais simples. Essas características facilitam a investigação dos genes e a compreensão de suas funções (Lloyd; Taylor, 2010).

2.4 Ensaio Cometa

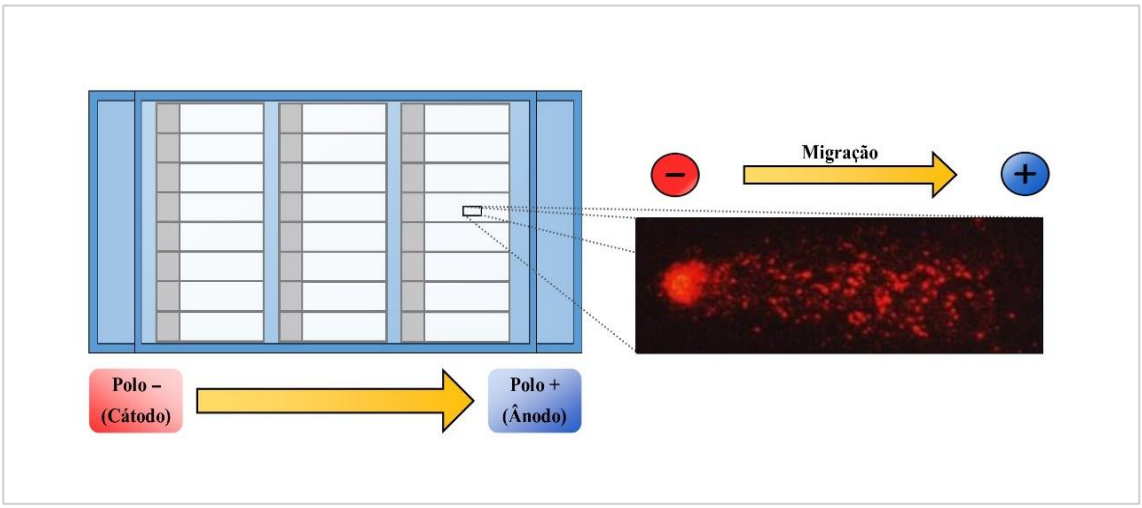
O Ensaio cometa, também conhecido como eletroforese em gel de célula única, foi desenvolvido primordialmente por Ostling e Johanson (1984) e aperfeiçoado por Singh et al. (1988), quando adquiriu uma versão alcalina que é amplamente disseminada em ensaios de genotoxicidade (Collins et al., 2014) e considerada a melhor versão da técnica sob o ponto de vista de especialistas (Tice et al., 2000). Nas últimas décadas, o ensaio cometa tem se mostrado uma ferramenta útil e prática aplicada a estudos que vão desde o campo da ecogenotoxicologia ao biomonitoramento humano (Azqueta; Collins, 2013; Collins, 2015).

Este método reúne simplicidade e eficiência para detecção e quantificação de danos ou mecanismos genoprotetores no DNA em células individualizadas (Azqueta et al., 2019; Gajski et al., 2019; Azqueta et al., 2020; Møller et al., 2020). O ensaio cometa alcalino é capaz de revelar lesões primárias do tipo quebras de fitas simples (SSBs), quebras de fitas duplas (DSBs) e locais alcalinos lábeis em frequências de algumas centenas a vários milhares de quebras por célula, atividades de reticulação (por exemplo, DNA-DNA e DNA-proteína) e quebras de fitas simples vinculadas a regiões de reparo de excisão incompleta (Tice et al., 2000; Møller et al., 2020). O material genético pode ser lesionado diariamente por fontes endógenas ou exógenas, sendo assim, o dano ao DNA é considerado um biomarcador valioso de exposição a agentes genotóxicos e o ensaio cometa é a técnica mais comum para mensurar esses danos (Azqueta et al., 2020). A metodologia apresenta vantagens em comparação a outros métodos, dentre elas a utilização de uma pequena quantidade amostral de células,

elevada sensibilidade para detecção de diferentes níveis de danos ao DNA, agilidade de execução e conclusão do estudo, praticidade de aplicação e viabilidade econômica (Ostling; Johanson, 1984; Singh et al., 1988; Tice et al., 2000; Dhawan et al., 2009).

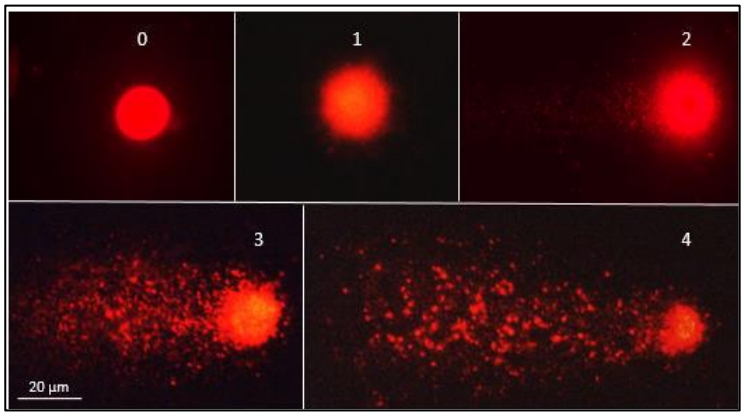
O ensaio cometa revela danos por meio da migração de DNA em gel de agarose devido o relaxamento de seus *loops* ocasionado por quebras de fita. O DNA fica concentrado em uma matriz análoga a um núcleo, após a diluição das membranas celulares e nucleares, e passa a ser denominado de nucleoide. Os nucleoides são submetidos a eletroforese, na qual o DNA danificado é arrastado em direção ao polo positivo (ânodo), formando uma cauda semelhante à de um "cometa" (**Figura 6**) (Shaposhnikov et al., 2008; Afanasieva et al., 2010; Collins, 2015; Azqueta et al., 2020). A integridade da matriz nuclear e extensão da cauda são usados como parâmetros para mensuração dos danos genéticos em 5 níveis (0 a 4) (**Figura 7**).

Figura 6. Disposição das lâminas na cuba de eletroforese para migração dos fragmentos de DNA.



Fonte: Adaptado de Santana (2015).

Figura 7. Padrão visual segundo Verçosa et al. (2017) dos cinco níveis de classificação de dano genético (0 a 4) baseada no comprimento e quantidade de DNA na cauda dos cometas. As classes 0 1, 2, 3 e 4 correspondem, respectivamente, a nucleoides intactos, com dano mínimo, com dano intermediário, com dano intenso e com dano máximo.



3 OBJETIVOS

3.1 Objetivo Geral

Investigar a ocorrência de radiorresistência em populações de *Drosophila melanogaster* residentes de duas cidades do Rio Grande do Norte, por meio da exposição crônica e aguda à radiação natural e induzida, e quantificação de danos pelo Ensaio cometa.

3.2 Objetivos Específicos

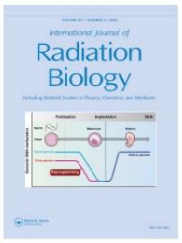
- Estabelecer cultivos em laboratório das linhagens *D. melanogaster* de Cerro Corá e Parelhas (Rio Grande do Norte), Vitória de Santo Antão (Pernambuco), e controle laboratorial, Oregon-R.
- Realizar medidas de partículas alfa associadas ao gás Radônio nos ambientes de Cerro Corá, Parelhas e Vitória de Santo Antão, em duas estações do ano (seca e chuvosa).
- Avaliar o efeito genotóxico por meio do Ensaio cometa, após a exposição crônica à radiação natural de Cerro Cora e Parelhas, em larvas e adultos descendentes, e da linhagem Oregon-R.
- Avaliar o efeito genotóxico da exposição aguda a diferentes doses de radiação gama induzida por ^{60}Cobalto, em larvas descentes das linhagens Cerro Corá, Parelhas, Vitória de Santo Antão e Oregon-R, por meio do Ensaio cometa.
- Evidenciar uma possível adaptação genética (radiorresistência) nas linhagens estudadas.

4 RESULTADOS

CAPÍTULO 1

4.1 Artigo 1

Publicado na *International Journal of Radiation Biology* – Qualis A1 (FI 2,1)



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ORIGINAL ARTICLE

Evidences of radioresistance in *Drosophila melanogaster* from Northeastern Brazil

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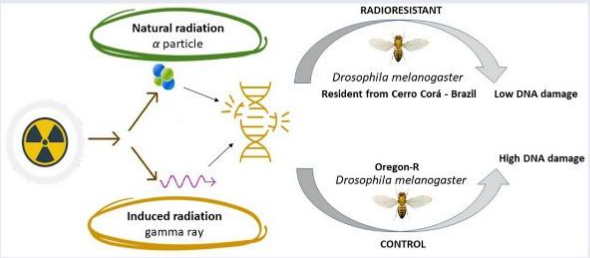
ABSTRACT

Background: Ionizing radiation can inflict cellular damage, the severity of which is determined by the dose, exposure duration, and its capacity to penetrate cells. Some studies have demonstrated that genetic and epigenetic mechanisms have enabled organisms to develop adaptive traits and enhance their ability to repair DNA damage. Northeastern Brazil, a region containing rocky outcrops rich in uranium and thorium, is an ideal scenario to study natural radiation and its effects on natural populations. This study presents evidence of radioresistance in the offspring of a natural strain of *Drosophila melanogaster* resident in the municipality of Cerro Corá (CC-res), an environment with high levels of radon-222.

Material and methods: Genotoxicity was assessed using the comet assay in offspring of the CC-res and Oregon-R (OR), the control group, both reared under the same laboratory conditions for between 7 and 13 months. The adults and their offspring larvae were exposed to the Cerro Corá environment for 6 days during the dry and wet seasons. Low damage index and frequency were observed only in the CC-res. To confirm the radioresistance, the same strains were exposed after 16 months of cultivation to controlled doses of gamma radiation.

Results and conclusions: CC-res exhibited significantly lower levels of damage compared to the OR strain, with a clear dose–response effect to the irradiation observed exclusively in the OR group. The results support the occurrence of radioresistance in the CC-res strain and underscore the need for further in vivo studies investigations into the impact of Brazil's natural environmental radiation.

GRAPHICAL ABSTRACT



HIGHLIGHTS

- The high atmospheric concentrations of Radon-222 (>2800 Bq.m^{-3}) in Cerro Corá, Brazil, make it an ideal natural environment for biological studies.
- *Drosophila melanogaster* Cerro Corá resident (CC-res) do not suffer DNA damage when exposed to Cerro Corá, unlike Oregon-R flies that suffer genetic damage.
- The exposure to doses of gamma radiation supports the hypothesis of radioresistance in CC-res.
- The genetic memory of radioresistance persists even after many generations of maintenance of CC-res.

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Introduction

Organisms face constant exposure to a spectrum of genotoxic agents, which can compromise DNA integrity to a greater or lesser extent (Lam 2022). A key example is ionizing radiation, which pervades all living organisms. This interaction takes place due to the presence of naturally occurring radioactive elements (radionuclides) found in varying concentrations in rocks, soil, water sources, air and even food (Balonov 2008; Agbalagba et al. 2014; Belli and Indovina 2020). This natural radiation, always present on Earth, is known as "background radiation." Sources of this radiation include renewable cosmogenic radionuclides (^{3}H,^{14}C and ^{22}Na), which remain continuous due to the balanced frequency between their production levels and radioactive decay, and long-lived primordial radionuclides (^{238}U,^{235}U and ^{232}Th), which reduce over time as a result of the natural process of radioactive decay (Shahbazi-Gahrouei et al. 2013). Chronic exposure to large amounts of these radionuclides can lead to negative health effects such as damage to genetic material (i.e. deoxyribonucleic acid, or DNA), causing cancer and the degeneration of cells and tissues (Lam 2022). On the same way, chronic exposure to radioactivity, exemplified by the Chernobyl accident, significantly destabilizes the genomes of organisms, leading to genetic damage, mutations, morphological anomalies, and increased offspring mortality (Yushkova 2022).

According to the International Atomic Energy Agency (IAEA 2009; OECD 2021), Brazil is among the six main geological reservoirs of uranium in the world. The Northeastern region of Brazil is known for harboring radionuclides within its geological formations and primarily concentrated in two key locations: the rock layer of the Borborema Pegmatite Province, rich in uranium and thorium (IAEA 2009; Silva et al. 2010; Dantas et al. 2020; Santos et al. 2023); and the Paraíba Sedimentary Basin, a non-continuous layer rich in uraniferous phosphorite in the continental margin (Amaral et al. 2005; Lima et al. 2017). During the decay series of ^{238}U and ^{232}Th, ^{226}Ra is formed, which subsequently decays into radon-222 (^{222}Rn) gas in the soil. With a short half-life of 3.8 days, ^{222}Rn diffuses easily into the atmosphere from spaces between the rocks and soil. Although ^{222}Rn is chemically inert and does not readily react with other substances, its daughter elements are active, such as lead, bismuth and polonium (Moore et al. 2014), which have a great capacity to alter DNA stability, interfering with the cell cycle (checkpoints), replication fidelity, chromosome segregation, and protein regulation and metabolism (Bleise et al. 2003; WHO 2009; Moore et al. 2014; Lam 2022). Therefore, natural ionizing radiation poses a potential genotoxic threat, directly inducing DNA damage such as crosslinks, base exchanges, and single- and double-strand breaks, which are known to contribute to cancer development (UNSCEAR 2010; Vaisnav et al. 2014; Meenakshi et al. 2017; Walczak et al. 2020; Ruano-Ravina et al. 2023). Because of this, inhalation of ^{222}Rn contributes to the majority of human exposure to background ionizing radiation (OMS 2009; Taga et al. 2012; Belli and Indovina 2020; Ruano-Ravina et al. 2023), and prolonged exposure to levels exceeding 100 Bq.m^{-3} is considered a significant environmental health hazard (WHO 2009; UNSCEAR 2010).

In contrast, all organisms possess sophisticated defense systems to safeguard the integrity of their genetic material. These systems include DNA repair mechanisms (Iyama and Wilson 2013; Lam 2022), which act to preserve normal cellular functions and transcriptional and post-translational control mechanisms that regulate gene expression and protein activity (Hafer et al. 2007; Boothby 2019). These intricate DNA repair networks have evolved to remove aberrant chemical modifications or mismatched bases effectively restoring the genome to its pristine state. Consequently, individuals with a superior DNA repair capacity or enhanced expression of repair-associated genes exhibit increased radiation tolerance (Beam et al. 1954; Ogaki and Nakashima-Tanaka 1966; Mattimore and Battista 1996; Jackson and Bartek 2009; Koval et al. 2020; Vaiserman et al. 2021; Zarubin et al. 2023).

Studies carried out in the states of the semiarid region of Northeastern Brazil have revealed concerning levels of ^{222}Rn and other radionuclides in the atmosphere, soil and water sources (Marcon et al. 2010, 2017; Chaves et al. 2016; Dantas et al. 2020; Silva et al. 2021; Nascimento-Silva et al. 2024). These elevated levels pose potential risks to various organisms. However, a significant gap remains since there have been relatively few studies with wildlife populations living in areas of high atmospheric radioactivity and which have been exposed to radiation for many generations (Silva et al. 2021). These populations represent valuable biological resources for genetic and ecological investigations (Kratz 1975; Belli and Indovina 2020).

In this scenario, *Drosophila melanogaster* Meigen (Insecta, Diptera) has been a model organism widely used to demonstrate the effects of induced ionizing radiation (Moskalev et al. 2011; Zhikrevetskaya et al. 2015; Paithankar et al. 2017; Koval et al. 2020; Zarubin et al. 2023) and only more recently, assessed for the effect of environmental radiation (Verçosa et al. 2017; Morciano et al. 2018; Yushkova 2022; Nascimento-Silva et al. 2024). This organism has emerged as a valuable experimental model due to its advantageous traits: ease of cultivation and laboratory manipulation, a short life cycle, and a well-characterized genome (Pandey and Nichols 2011; Mirzoyan et al. 2019; Koval et al. 2020). Furthermore, the comet assay in *D. melanogaster* has offered several advantages, including high sensitivity, repeatability and efficiency, making it a promising tool in ecogenotoxicology research (Godschalk et al. 2013; Verçosa et al. 2017; de Santana et al. 2018; Møller et al. 2020, Nascimento-Silva et al. 2024).

In this study, we seek to assess the genotoxic effects in two strains of *D. melanogaster* (resident and control) in a Brazilian semiarid location with high rates of alpha particles associated with ^{222}Rn (above 2800 Bq.m^{-3}). The present investigation is based on the hypothesis that larvae and adult offspring of *D. melanogaster* collected in Cerro Corá are more radioresistant than the Oregon-R control strain,

even after several generations of being maintained in the laboratory.

Material and methods

Drosophila melanogaster strains and study area

Two *Drosophila melanogaster* strains were exposed to atmospheric radiation in the municipality of Cerro Corá (Figure 1), in the state of Rio Grande do Norte, a semiarid zone in the Caatinga biome, Northeastern Brazil. The municipality of Cerro Corá (coordinates 06°02'45.6" S and 36°20'45.6" W) is located in the Central Potiguar mesoregion and in the Serra de Santana microregion, with the urban region located at an average altitude of 575 m. The location of Cerro Corá was chosen because the alpha (α) particle reaches an average of 3,374 Bq.m^{-3} in the dry season (measured in September 2020), and an average of 2,886 Bq.m^{-3} in the wet season (measured in May 2022). Besides the high environmental radiation, Cerro Corá also has a low level of urbanization—with only 11,000 inhabitants across 393,573 km^2 (IBGE 2022) and is located far from other municipalities, urbanized centers or polluting factories. Moreover, in Cerro Corá, the average temperatures only vary 2.4 °C throughout the year, and the fluctuation in precipitation varies from 77 mm, observed between the month of lowest rainfall (September) and the month of highest rainfall (March). The month of April presents the highest relative humidity (73.18%) and October the lowest relative humidity (60.71%). Variations in temperature and rainfall throughout the months of the year may be observed in the compilation made by Climate-Data (2022) for Cerro Corá.

The alpha radiation levels were recorded with an AlphaGUARD portable monitor (Bertim Instruments) coupled to the top layer of the soil. The equipment connects to a pump (AlphaPUMP) that sucks the air sample contained inside the emanation chamber and directs it to a detection system that quantifies the rate of α-particles emitted by radon-222 (^{222}Rn) (Farias et al. 2016).

The in vivo experiments began with a collection of Drosophilidae species in Cerro Corá, attracted to plastic traps containing 100 g of crushed banana and biological yeast. After 48 h, the adults were collected and transferred to glass tubes containing a standard culture medium (based on corn flour, rye, sugar, agar and nipagin), and taken to the laboratory for taxonomic identification. The individuals classified as *Drosophila melanogaster* were distributed into 10 isolines, each containing one pair of adults (male and female), initiating a strain called Cerro Corá resident (CC-res). A sample of *D. melanogaster* Oregon-R strain was cultivated under the same conditions as the CC-res: a

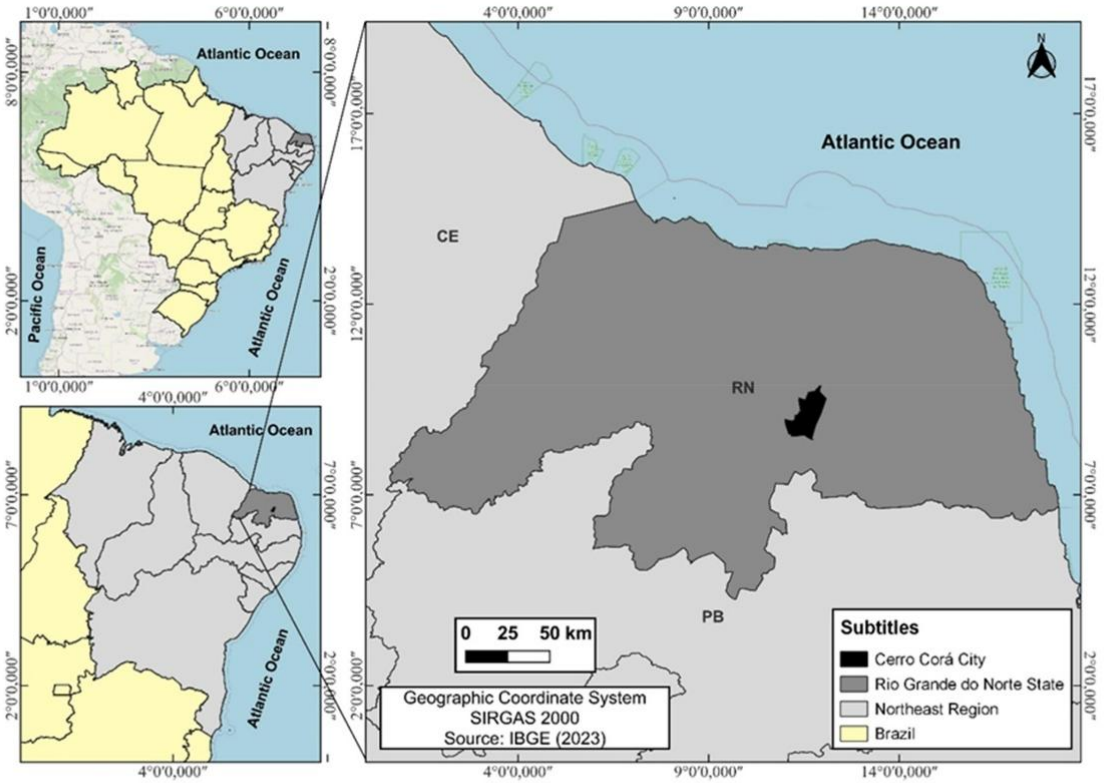

Figure 1. Map of Brazil, highlighting the Northeastern region (light grey). Inset shows the location of Rio Grande Do Norte state (dark grey) and the Cerro Corá city (in dark), where this study was carried out.

standard culture medium (Verçosa et al. 2017), a temperature of 24 ± 2 °C, a 65–70% relative humidity, and a light-dark exposure cycle of 14/10. Oregon-R is a sensitive strain to the genotoxic effect of natural radiation and responds in the same way as a strain not exposed to radon or an environment with low radiation, as previously validated by Verçosa et al. (2017). The Oregon-R strain has also been the control group of several other studies carried out by our research group, on air pollution exposures (de Santana et al. 2018), dose-response effects of plant extracts (Amorim et al. 2020) and plant lectins (de Oliveira dos Santos et al. 2022), and contamination of water sources with radiation in Brazil (Nascimento-Silva et al. 2024).

Three replicates of CC-res and OR offspring were exposed to the environment of Cerro Corá, in two moments: one in the dry season (in August 2022), after maintaining the strains for seven months under laboratory conditions (from January 2022 to August 2022); and another in the wet season (in February 2023), after 13 months (between January 2022 and February 2023). Each replicate was composed of 120 CC-res adults or 120 OR adults. These were transferred into plastic bottles, produced in accordance with Verçosa et al. (2015) (Figure 2(A)). The bottles remained in Cerro Corá for 6 days, sufficient time for adults and their offspring larvae to be exposed to radon in the air (Verçosa et al. 2017). The bottles were arranged in pairs (CC-res and OR), hung 1.5 m above the ground (Figure 2(B)), in three different shaded locations, 30 m apart from one another.

The comet assay in D. melanogaster

Following 6 days of field exposure, the adults and their offspring third-instar larvae were collected and taken to the laboratory. The genotoxic effects were assessed using comet assay in hemolymph cells from each replicate (60 adults and 60 offspring larvae), with a total of 300 adults and 300 larvae processed per each *D. melanogaster* strain, following the procedures of Verçosa et al. (2017). To facilitate manipulation, the individuals were cooled at 4 °C for 1 min, following the hemolymph extraction in the presence of EDTA. Each 60 cell pool (=60 µL) was homogenized in 100 µL of low melting point agarose solution, to be mounted on glass slides treated with standard agarose (1.5% agarose in PBS) and solidified at 4 °C for 10 min. The material was subsequently treated with a lysis solution (2.5 M NaCl; 100 mM EDTA; 1 M NaOH; 1% Tris pH 10; 1% Triton X-100 and 10% DMSO), at 4 °C for 72 h. In a 40 cm electrophoresis vat, the material was stabilized for 20 min in a buffer solution (1 M NaOH; 200 mM EDTA pH 13) and subjected to an electric current (40 V and 300 mA) for 20 min. Following this, the slides were subjected to neutralization treatment (0.4 M Tris-HCl, pH 7.5) for 15 min, and the material was fixed in absolute ethanol for 5 min, air-dried and stored in a laminarium at 4 °C until microscopic analysis.

To analyze comet patterns, which range from 0 (minimum damage) to 4 (maximum damage), the length of the comet's tail and the integrity of the head were viewed in 100 nucleoids per replicate, following visual descriptions of Verçosa et al. (2017) and Amorim et al. (2020). For fluorescent microscope visualization (Zeiss-Imager M2, Alexa-Fluor 546 filter at 400× magnification), the genetic material in each slide was stained with 50 µL of GelRed (Biotium), diluted in purified water (1:500). To calculate the mean damage index (DI) and the mean damage frequency (DF%) parameters, we followed the formulas presented in Nascimento-Silva et al. (2024). The statistical Analysis of Variance (ANOVA) and the Bonferroni *post-test* were run in software Stata 14.2.

Figure 2. (A) Aspect of one of bottle with culture medium (yellow arrow) for feeding adults and its offspring larvae of *Drosophila melanogaster*. (B) Visualization of one of the hanging replicas side by side under natural vegetation of Cerro Corá, Brazil.

Exposure to gamma radiation (γ)

The CC-res population and OR strain were also exposed after 16 months of cultivation to known doses of gamma radiation. To this end, 80 third-stage CC-res larvae and 80 third-stage Oregon-R larvae were separately transferred to acrylic vials containing instant mashed potato, previously hydrated with 1.5 mL of distilled water, and sealed with foam stoppers to allow air circulation. One hour after larvae collection, they were exposed to gamma radiation (γ) using a cobalt-60 (^{60}Co) source from MDS Nordion, Gammacell 220 Excel, with a dose rate of 1.047 KGy/h, at doses of 10, 30, 50, and 70 Gy. All experiments were performed in triplicate and the comet assay was applied 1 h and 24 h after irradiation. Two groups (CC-res Control and Oregon-R Control) remaining under laboratory conditions served as negative controls for no damage, while one CC-res Blank and one Oregon-R Blank served as a control for the stress conditions during the irradiation procedures, but were not irradiated. The comet assay applied 24 h after irradiation served to analyze the differential response of each strain of *D. melanogaster*, after larval recovery. In this case, the larvae irradiated on the first day remained in the laboratory, in acrylic tubes with a culture medium, until proceeding with the second round of the comet assay.

Results

The average DI and DF% results for each group exposed in the natural environment of Cerro Corá are presented in Figure 3. The figure also presents two reference measurements of ^{222}Rn obtained in September 2020 (dry season) and in May 2022 (wet season). The greatest genetic damage was observed in the OR larvae (DI = 128.00 and DF% = 48.67) and in the OR adults (DI = 157.00 and DF% = 55.00), both exposed in the dry season. During the wet season, all groups presented less damage, although the OR larvae (DI = 63.00 and DF% = 34.00) and, especially, the OR adults were still the most affected by local natural radiation (DI = 89.00 and DF% = 42.33). In all tests carried out in both the dry and wet periods, the DI and DF% of the CC-res were lower than the OR ($P \leq 0.05$). Details of the values for each test (mean damage levels, DI, DF% and standard deviation) and the

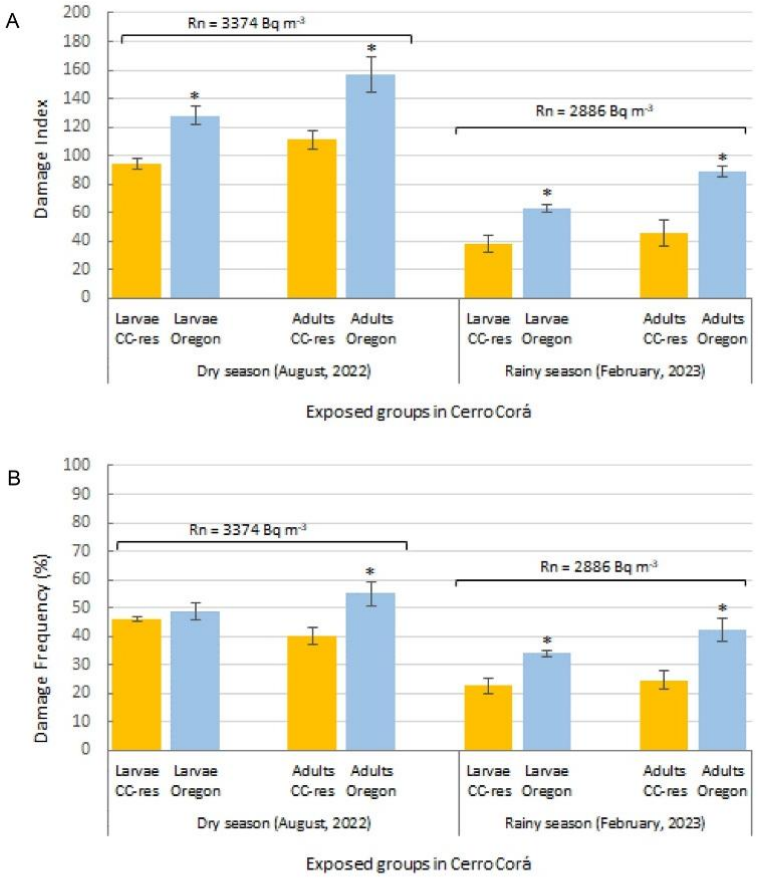

Figure 3. Comparative analyzes of mean values and standard deviation of the damage Index (A) and damage frequency (%) (B) in larvae and adults of *Drosophila melanogaster* Cerro Corá residents (CC-res) and Oregon-R (control group), exposed to the natural radiation of Cerro Corá, at dry (August 2022) and wet (February 2023) seasons. Asteriscs represent statistical differences ($P \leq 0.05$) between the two life stages (CC-res and Oregon-R).

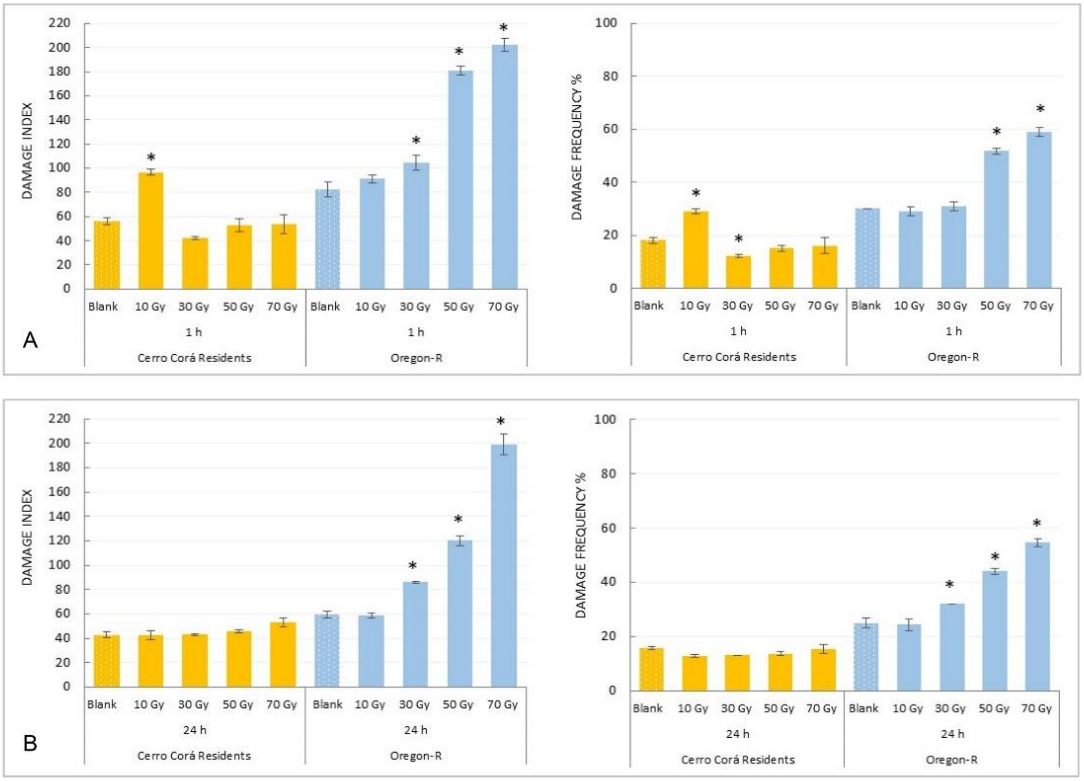

Figure 4. Mean damage Index, damage frequency, and respective standard deviations observed in *Drosophila melanogaster* larvae of Oregon-R and Cerro Corá residents. In (A), the asterisks represent statistical differences ($P \leq 0.05$) between the Blank and the results obtained 1 hour after the irradiation (at 10, 30, 50 and 70 Gy); and in (B), the asterisks represent statistical differences ($P \leq 0.05$) between the Blank and the results obtained 24 h (time to the recover after the same irradiation).

results of the statistical analysis of the pairwise comparisons (Bonferroni *post-hoc* test) are described in Tables S1 and S2 (Supplementary Material).

The results of the comet assay applied to the *D. melanogaster* CC-res and OR larvae, 1 h after gamma exposure are presented in Figure 4. Low DI and DF% measurements were observed in the OR negative control group (DI = 42.33 and DF % = 15.00) and the CC-res negative control (DI = 32.33 and DF% = 11.33), which were both maintained under laboratory conditions and not exposed to irradiation. A progressive increase in genotoxicity was also observed in the OR at doses from 10 to 70 Gy, reflecting a clear dose-response effect. Finally, there was a low level of genotoxicity in CC-res, except in the 10 Gy group (DI = 96.67 and DF% = 29.0).

The results observed 24 h after irradiation demonstrate reduced damage in both *D. melanogaster* strains, although the DI and DF% values in the OR strain were higher than those observed in CC-res, once again demonstrating the adaptation or radioresistance of the CC-res offspring. Another relevant aspect was observed in the 10 Gy group with a significant reduction in damage (DI = 42.33, DF% = 12.67), matching those observed with 30, 50 and 70 Gy. Details of the values of each test (damage levels, DI, DF% and standard deviation) and results of the statistical analysis of all pairwise tests (Bonferroni *post-hoc* test) are described in Tables S3 and S4 (Supplementary Material).

Discussion

According to the data, the CC-res individuals were less sensitive to natural radiation than the Oregon-R individuals, thereby suggesting the presence of radioresistance. These results were corroborated by exposure to low doses of gamma radiation, which demonstrated an absence of dose-response in the CC-res population, with no significant differences compared to the CC-res Blank, which was not exposed. One exception was the CC-res group at a dose of 10 Gy, which demonstrated greater damage compared to the other groups, 1 h after exposure. However, we observed a reduction in damage 24 h after administration of the 10 Gy dose, and the levels were the same as those obtained with the other doses (30, 50 and 70 Gy). On the other hand, the *D. melanogaster* OR revealed increasing damage, with a clear dose-dependent effect (10 to 70 Gy), all above the OR Blank in 1 h or 24 h after exposure.

Our results have demonstrated that resident organisms undergo generational selection for enhanced genetic traits,

probably those related to efficient cellular repair mechanisms in response to natural radiation. These finding further support previous research results about the genes that enhances radioresistance in *D. melanogaster* (Koval et al. 2020; Zarubin et al. 2023). Otherwise, without adaptation mechanisms, populations would struggle to survive long-term in such environments, as their cellular functions would be progressively compromised (Yu et al. 2011; Morciano et al. 2018; Boothby 2019; Koval et al. 2020).

Field exposure of the CC-res and the OR offsprings to ionizing radiation in two different seasons in Cerro Corá revealed contrasting results. Notably, in the wet season exposure were observed a significantly less DNA damage (DI and DF%), coinciding with the lowest recorded alpha particle measurements with AlphaGUARD. It is likely that the ^{222}Rn concentrations in the atmosphere are modulated by environmental factors (López-Coto et al. 2014; Müllerová et al. 2018; Yang et al. 2019), especially precipitation and soil moisture. Thus, this study highlights the need to undertake a more in-depth assessment regarding the influence of seasonal variations on local radiation levels and, consequently, on genotoxicity in living organisms.

As the observed genetic damage in the *D. melanogaster* ORs does not solely correlate with ^{222}Rn concentrations in Cerro Corá, this study necessitates a more comprehensive and precise examination of local radioactivity. This includes obtaining accurate estimates of radiation doses received by local biota and a clearer understanding of the true radiological exposure experienced by living organisms. As highlighted by Beaugelin-Seiller et al. (2020), comprehensive assessment of the radionuclides present is crucial, including detailed measurements of all their emissions (gamma, beta, and alpha) and consideration of both internal and external exposure pathways. Additionally, determining the dose rate of ionizing radiation exposure is essential to assess potential negative or positive health effects (Tang and Loganovsky 2018).

According to Paithankar et al. (2017), the class Insecta has a greater capacity for resistance to radiation compared to vertebrate animals. Insects exhibit substantial variation in radiation resistance, with species undergoing complete metamorphosis demonstrating a heightened ability to withstand radiation. Consequently, insect populations chronically exposed to ionizing radiation and possessing short life cycles are able to rapidly evolve, acquiring resistance that becomes evident over generations. This suggests an efficient selection of specific intracellular mechanisms, such as DNA repair pathways, transposable element activity, and epigenetic control pathways, that likely contribute to their resilience against radiation damage.

This was demonstrated in the study by Yushkova (2022), in which populations of *D. melanogaster* offspring were exposed to the accidental radioactive environment of Chernobyl. These individuals demonstrated radioadaptive potential, possibly attributed to the efficient selection of specific intracellular mechanisms, such as DNA repair pathways, transposable element activity and epigenetic control pathways.

The laboratory gamma radiation exposure experiments here done confirm previous findings about OR strain exhibiting greater sensitivity to radiation compared to the radioresistant or radio tolerant natural population (CC-res), which displayed minimal damage in most tests. In a study by Porrazzo et al. (2022), *D. melanogaster* larvae exposed to chronic, low-dose gamma radiation (0.4 Gy) exhibited a reduced frequency of chromosomal breaks and telomeric fusions when subsequently exposed to a higher dose (10 Gy) administered gradually until the larvae reached the third stage of development. RNA sequences assessed by Porrazzo et al. (2022), indicated that this protective response against chromosomal breaks appears to be linked to a decrease in the expression of a specific isoform of the Loquacious D protein (Loqs-PD), which is known to be involved in the formation of small endoribonucleases. Loqs-PD suppression may represent a mechanism by which cells activate a radioadaptive response, mitigating the detrimental effects of ionizing radiation. However, the genetic underpinnings of this radioprotective response across various organisms remain largely unknown.

In any event, low-dose exposure to ionizing radiation can trigger protective mechanisms and enhance their resilience to subsequent high doses of radiation and other subsequent stressors. In this context, Moskalev et al. (2011) highlighted the radioprotective and hormetic effect related to radiation, and Koval et al. (2020) reinforced the importance of the chronic exposure factor on radioadaptation. This is because the effects on survival after acute exposure to 30 Gy of gamma radiation were attenuated in wild-type strains of *D. melanogaster* after chronic γ radiation of 40 cGy (centigray) during the developmental phases. Ultimately, the authors reported that the activity of genes responsible for repairing genetic material play crucial roles in the radioadaptive response and in the hormesis mechanism. By generating mutations in genes involved in the recognition and repair of DNA damage, Koval et al. (2020) observed a significant decrease in radioprotective effects, compared to non-genetically modified strains.

This study, conducted in Brazil's semiarid region, builds upon the recent findings about populations from Cerro Corá and other high-radiation sites, as Lajes Pintadas (Castro, I.F.A. and Oliveira, R.A. *personal communications*). This work and others coming soon underscores the immense potential of such environments to reveal novel mechanisms of biological adaptation. For example, the genotoxic effects and mutagenic potential of natural radiation in the Northeastern of Brazil appear to be most intense for soil-dwelling species, such as native scorpions (Silva et al. *under preparation*) and much less intense for native cactophilic fly species (Guimarães-Silva et al. *under preparation*). The present study sheds new light on the biological consequences of natural radiation, proposing viable methods to assess how living organisms adapt to this persistent environmental pressure.

Conclusion

This study has revealed evidence on adaptations, such as radioresistance and an increded tolerance to gamma radiation, which are manifested in the offspring of a *Drosophila melanogaster* strain originating from a high-background radiation area in Northeastern Brazil. The genetic memory of radioresistant individuals persisted even after many generations had passed since the initial collection from field-dwelling parents. Furthermore, *D. melanogaster* Oregon-R remains a valuable strain for assessing environmental risks associated with high natural background radioactivity in Brazil.

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Ethics statement

The study did not require any ethics committee approval, according to Brazil's legislation.

Author contributions

M.G.P.: writing, review & editing, resources, methodology, investigation, formal analysis, conceptualization. E.M.A.: conceptualization, resources, methodology, writing, formal analysis. A.A.S.: writing, resources, methodology, formal analysis. D.G.S.: resources, methodology, formal analysis. A.M.E.: writing, review & editing, formal analysis, conceptualization, supervision, funding acquisition. C.R.: writing, review & editing, resources, methodology, formal analysis, conceptualization, project administration, funding acquisition.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Supplementary Material

Table S1. Mean values and standard deviation (Sd) of damage levels (0 to 4) and calculated values of damage index (DI) and damage frequency (FD%) in hemolymph cells of larvae and adults of *Drosophila melanogaster* Cerro Corá residents (CC-res) and Oregon-R (control group) exposed to the environment with natural radiation of Cerro Corá, in the dry (August, 2022) and wet (February, 2023) seasons.

Groups exposed	Damage levels ± Sd						
	0	1	2	3	4	DI ± Sd	DF% ± Sd
Dry season (August, 2022)							
Larvae CC-res	54.00±1.00	17.33±2.08	15.00±4.58	7.67±3.21	6.00±1.00	94.33±3.79	46.00±1.00
Larvae Oregon	51.33±3.06	11.00±3.61	9.67±5.77	14.33±1.53	13.67±5.51	128.00±6.24	48.67±3.06
Adults CC-res	60.00±3.00	7.00±5.29	6.33±2.31	15.00±3.61	11.67±1.53	111.33±6.66	40.00±3.00
Adults Oregon-R	45.00±4.36	3.67±2.08	11.00±1.73	30.00±1.00	10.33±2.52	157.00±12.1	55.00±4.36
Wet season (February, 2023)							
Larvae CC-res	77.33±2.52	12.33±4.04	6.00±2.65	3.33±1.53	1.00±1.73	38.33±5.69	22.67±2.52
Larvae Oregon	66.00±1.00	17.00±1,00	9.00±1.73	4.00±1.00	4.00±2.00	63.00±2.65	34.00±1.00
Adults CC-res	75.33±3.21	12.00±2.00	6.33±2.08	4.00±1.00	2.33±2.08	46.00±8.89	24.67±3.21
Adultos Oregon-R	57.67±4.16	18.67±0.58	8.33±5.86	7.67±1.53	7.67±3.21	89.00±3.61	42.33±4.16

Damage levels: Zero (0) represents no genetic damage and 1-4 are increasing levels of genetic damage.

Table S2. Bonferroni *post-test* comparisons between the mean values of damage index (below the grey diagonal) and damage frequency (above the grey diagonal) observed in larvae and adults of *Drosophila melanogaster* populations (CC-res and Oregon-R) exposed to natural radiation of Cerro Corá.

Groups exposed	Dry season				Wet season			
	Larvae CC-res	Larvae Oregon	Adults CC-res	Adults Oregon	Larvae CC-res	Larvae Oregon	Adults CC-res	Adults Oregon
Dry season								
Larvae CC-res		1.000	0.767	0.062	0.0001*	0.005*	0.0001*	1.000
Larvae Oregon	0.001*		0.082	0.585	0.0001*	0.001*	0.0001*	0.585
Adults CC-res	0.220	0.249		0.0001*	0.0001*	0.767	0.0001*	1.000
Adults Oregon	0.0001*	0.003*	0.0001*		0.0001*	0.0001*	0.0001*	0.003*
Wet season								
Larvae CC-res	0.0001*	0.0001*	0.0001*	0.0001*		0.009*	1.000	0.0001*
Larvae Oregon	0.001*	0.0001*	0.0001*	0.0001*	0.012*		0.046	0.109*
Adults CC-res	0.0001*	0.0001*	0.0001*	0.0001*	1.000	0.220		0.0001*
Adults Oregon	1.000	0.0001*	0.030*	0.0001*	0.0001*	0.008*	0.0001*	

* Significant differences ($P \leq 0.05$).

Table S3. Mean values and standard deviation (Sd) observed in damage levels (0 to 4), damage index (DI) and damage frequency (FD%) in hemolymph cells of *Drosophila melanogaster* Cerro Corá larvae (CC-res) and Oregon-R, besides controls groups. The results of the Comet assay were evaluated 1 hour (1 h) and 24 hours (24 h) after irradiation with a ^{60}Co source, at doses of 10, 30, 50 and 70 Gray (Gy).

Exposured groups	Damage levels ± Sd					DI ± Sd	DF% ± Sd
	0	1	2	3	4		
CC-res (1 h)							
Negative control	88.67±0.58	3.00±1.73	0.33±0.58	3.33±1.15	4.67±2.31	32.33±5.69	11.33±0.58
Blank	82.00±1.00	1.33±1.53	2.67±0.58	6.67±1.15	7.33±0.58	56.00±2.65	18.00±1.00
10 Gy	71.00±1.00	0.33±0,58	3.33±2.08	11.67±2.52	13.67±2.52	96.67±2.08	29.00±1.00
30 Gy	87.67±0.58	0.33±0.58	0.67±1.15	5.00±1.00	6.33±1.15	42.00±1.00	12.33±0.58
50 Gy	85.00±1.00	0.00±0.00	0.33±0.58	6.67±1.15	8.00±2.00	52.67±5.03	15.00±1.00
70 Gy	84.00±3.00	1.00±1.00	0.33±0.58	5.67±2.08	8.67±0.58	53.33±7.77	16.00±3.00
Oregon (1 h)							
Negative control	85.00±2.65	2.00±1.00	3.33±2.52	5.00±0.00	4.67±0.58	42.33±3.51	15.00±2,65
Blank	70.00±0.00	4.00±1.73	7.67±1.15	10.33±0.58	8.00±200	82.33±6.11	30.00±0.00
10 Gy	71.00±1.73	2.00±1.00	4.00±1.00	11.00±1.00	12.00±0.00	91.00±3.46	29.00±1.73
30 Gy	69.00±1.73	1.33±0,58	0.67±0.58	14.00±1.00	15.00±1.00	104.67±6.35	31.00±1.73
50 Gy	48.33±1.15	0.33±0.58	0.33±0.58	24.00±2.65	27.00±1.00	181.00±3.61	51.67±1.15
70 Gy	41.00±1,73	1.67±0.58	0.00±0.00	29.00±1.00	28.33±0.58	202.00±5.29	59.00±1.73
CC-res (24 h)							
Blank	84.33±0.58	1.33±0,58	4.00±1.00	8.00±0.00	2.33±0,58	42.67±2.52	15.67±0.58
10 Gy	87.33±0,58	0.00±0.00	1.00±1.00	6.33±1.53	5.33±1.53	42.33±3.79	12.67±0.58
30 Gy	87.00±0.00	0.33±0.58	1.33±0.58	5.33±0.58	6.00±0.00	43.00±1.00	13.00±0.00
50 Gy	86.33±0.58	0.00±0.00	1.33±0.58	6.33±0.58	6.00±0.00	45.67±1.53	13.67±0.58
70 Gy	84.67±1.53	0.00±0.00	0.00±0.00	7.00±0.00	8.00±1.00	53.00±4.00	15.33±1.53
Oregon (24 h)							
Blank	75.00±1.73	6.67±1.15	5.67±0,58	9.00±0.00	3.67±0.58	59.67±3,06	25.00±1.73
10 Gy	75.67±2.08	5.33±1.53	6.33±3.06	9.67±0.58	3.00±1.00	59.00±2.00	24.33±0.00
30 Gy	68.00±0.00	5.00±1.00	7.00±2.65	13.67±2,65	7.00±1.00	86.00±1.00	32.00±0.00
50 Gy	56.00±1.00	5.67±0.58	10.67±1.15	17.67±0,58	10.00±1.73	120.00±4.00	44.00±1.00
70 Gy	45.33±1.53	1.00±1.00	1.33±0.58	14.33±3.06	38.00±3.46	198.67±8.50	54.67±1.53

Table S4. Bonferroni *post-test* comparisons between the mean values of damage index (below the diagonal) and damage frequency (above the diagonal) observed in larvae of *Drosophila melanogaster* (Oregon-R and CC-res) submitted to comet assay 1 hour and 24 hours after gamma radiation exposure (at 10, 30, 50 and 70 Gy), and respective Control and Blank groups.

Irradiated groups		Cerro Corá residents (CC-res)						Oregon-R						Cerro Corá residents (CC-res)					Oregon-R				
		Comet assay after 1 h						Comet assay after 1 h						Comet assay after 24 h					Comet assay after 24 h				
		Control	Blank	10	30	50	70	Control	Blank	10	30	50	70	Blank	10	30	50	70	Blank	10	30	50	70
CC-res																							
After 1 h	Control		0.0001*	0.0001*	1.000	0.631	0.049*	0.631	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.118	1.000	1.000	1.000	0.277	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*
	Blank	0.0001*		0.0001*	0.003*	1.000	1.000	1.000	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	1.000	0.008*	0.020*	0.118	1.000	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*
	10	0.0001*	0.0001*		0.0001*	0.0001*	0.0001*	0.0001*	1.000	1.000	1.000	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.277	0.049*	1.000	0.0001*	0.0001*
	30	1.000	0.066	0.0001*		1.000	0.631	1.000	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	1.000	1.000	1.000	1.000	1.000	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*
	50	0.0001*	1.000	0.0001*	1.000		1.000	1.000	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	1.000	1.000	1.000	1.000	1.000	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*
	70	0.0001*	1.000	0.0001*	0.603	1.000		1.000	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	1.000	1.000	1.000	1.000	1.000	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*
Oregon-R																							
After 1 h	Control	1.000	0.088	0.0001*	1.000	1.000	0.782		0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	1.000	1.000	1.000	1.000	1.000	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*
	Blank	0.0001*	0.0001*	0.049*	0.0001*	0.0001*	0.0001*	0.0001*		1.000	1.000	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.020*	0.003*	1.000	0.0001*	0.0001*
	10	0.0001*	0.0001*	1.000	0.0001*	0.0001*	0.0001*	0.0001*	1.000		1.000	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.277	0.049*	1.000	0.0001*	0.0001*
	30	0.0001*	0.0001*	1.000	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.088		0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.001*	0.0001*	1.000	0.0001*	0.0001*
	50	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*		0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.001*	1.000
	70	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*		0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.118
CC-res																							
After 24 h	Blank	1.000	0.117	0.0001*	1.000	1.000	1.000	1.000	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*		1.000	1.000	1.000	1.000	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*
	10	1.000	0.088	0.0001*	1.000	1.000	0.782	1.000	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	1.000		1.000	1.000	1.000	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*
	30	1.000	0.155	0.0001*	1.000	1.000	1.000	1.000	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	1.000	1.000		1.000	1.000	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*
	50	0.117	1.000	0.0001*	1.000	1.000	1.000	1.000	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	1.000	1.000	1.000		1.000	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*
	70	0.0001*	1.000	0.0001*	0.782	1.000	1.000	1.000	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	1.000	1.000	1.000	1.000		0.0001*	0.0001*	0.0001*	0.0001*	0.0001*
Oregon-R																							
After 24 h	Blank	0.0001*	1.000	0.0001*	0.002*	1.000	1.000	0.003*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.004*	0.003*	0.006*	0.066	1.000		1.000	0.0001*	0.0001*	0.0001*
	10	0.0001*	1.000	0.0001*	0.004*	1.000	1.000	0.006*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.008*	0.006*	0.011*	0.117	1.000	1.000		0.0001*	0.0001*	0.0001*
	30	0.0001*	0.0001*	1.000	0.0001*	0.0001*	0.0001*	0.0001*	1.000	1.000	0.001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*		0.0001*	0.0001*
	50	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.020*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*		0.0001*
	70	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.002*	1.000	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	

* Significant differences ($P \leq 0.05$)

CAPÍTULO 2

4.2 Artigo 2

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Genetics and Molecular Biology

Expanding evidences of the genetic adaptation of Drosophila melanogaster strains from Caatinga to chronic and acute ionizing radiation

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Keyword:	Radioresistance, DNA damage, Radon, Drosophilidae, Brazil

Expanding evidences of the genetic adaptation of *Drosophila melanogaster* strains from Caatinga to chronic and acute ionizing radiation

Running title: **Radiation effect in *D. melanogaster***

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Abstract

Natural radiation of geological origin is a common phenomenon in Brazil, where radioactive agents, such as uranium and thorium, are frequently found. To better understand the extent of radioresistance in the model organism *Drosophila melanogaster*, a strain resident from Parelhas city (PAR-res), Rio Grande do Norte state, with moderate levels of alpha radiation (418 to 957 $Bq.m^{-3}$), was exposed to chronic natural radiation and to acute gamma radiation (^{60}Cobalt). The results were compared with a control strains from the same climatic zone, Vitória de Santo Antão residents (VSA-res), with acceptable radiation levels (< 200 $Bq.m^{-3}$), and with Oregon-R, a strain long maintained under laboratory conditions. Using the Comet assay, it was demonstrated that descendant PAR-res exhibited radioresistance when exposed to gamma radiation, even with the increase doses (10, 30, 50 and 70 Gy), unlike Oregon-R and VSA-res, which responded in a dose-dependent manner, with increased genotoxicity. The radioresistance adaptation persisted across many generations since the initial collection, and corroborate previous chronic tests conducted in Cerro Corá city (> 2800 $Bq.m^{-3}$). The results expand evidences that natural radiation is a powerful selective force shaping the genomes of some Brazilian species in the Caatinga biome.

Key-words: Radioresistance, DNA damage, Radon, Drosophilidae, Brazil.

Introduction

DNA is the molecular carrier of genetic information, and its integrity is essential for inheritance, thereby playing a fundamental role in the evolution of species. However, DNA is inherently reactive and highly susceptible to modifications induced by both endogenous and exogenous agents. Ionizing radiation (IR) is a prime example of a natural physical agent in the environment capable of altering DNA, inducing a range of damage such as base and sugar alterations, the formation of DNA–DNA and DNA–protein cross-links, as well as single-strand breaks (SSBs) and double-strand breaks (DSBs). In natural environments, IR originates from rocks and soils containing unstable radionuclides such as uranium (^{238}U) and thorium (^{232}Th). Over time, these two primordial radionuclides emit alpha and beta particles, as well as gamma rays (Chatterjee and Walker, 2017), giving rise to daughter radionuclides whose concentrations vary geographically, influenced by the local geological composition and other factors (Al-Hamarneh and Awadallah, 2009).

Evidence suggests that chronic stress, particularly at low doses of ionizing radiation, can promote microevolutionary processes by selecting more efficient systems that enhance protective responses to damage (Koval *et al*., 2020; Yushkova, 2022). This occurs because cells are equipped with complex, specialized systems, including DNA repair, damage tolerance, cell cycle checkpoints, and cell death pathways, which operate in a coordinated manner to mitigate the harmful effects of DNA lesions, depending on the radiation dose (Ogura *et al*., 2009; Ciccia and Elledge, 2010; Georgoulis *et al*., 2017; Chatterjee and Walker, 2017; Demir and Demir, 2024).

Areas with elevated ionizing radiation indices are of particular interest in understanding the genetic effects on natural populations of organisms exposed to chronic radiation at various intensities. Cellular responses can vary significantly between low and high radiation doses, with key phenomena in this context including radioadaptive responses, low-dose hyper-radiosensitivity and increased radioresistance (Wodarz *et al*., 2014).

While many studies commonly rely on *in vitro* experiments to assess radiation toxicity, *in vivo* approaches tend to provide more robust evidence that is more relevant to risk assessment (Demir and Demir, 2024). In this regard, Brazil is notable not only for its rich diversity of Drosophilidae species (over 300 species) (TaxoDros, 2024), but also for hosting a unique biome, the Caatinga (dry forest vegetation) (Leal *et al*., 2003), and holding a prominent position as the world's sixth largest uranium reserve (CNEN, 2016). Natural radiation is particularly significant in Northeastern Brazil, where the Borborema Pegmatite Province is located (IAEA,

2009; Dantas *et al*., 2020). This region is rich in radionuclides from the ^{238}U and ^{232}Th decay chains, which pose risks to the health and survival of organisms (Singh *et al*., 2011; Verçosa *et al*., 2017; Cimboláková *et al*., 2019; Silva *et al*., 2021; Slobodian *et al*., 2021; Mitra *et al*., 2022; Morais *et al*., 2022).

Arthropods represent a diverse group of invertebrates and play a pivotal role as model organisms for genetic environmental monitoring related to radiation (Gajski *et al*., 2019). One notable species within this phylum is *Drosophila melanogaster* Meigen (Diptera: *Drosophilidae*), a member of the class Insecta. Widely recognized as a model organism and since the early 1900 is employed in studies spanning from fundamental genetics to tissue and organ development to investigations of adaptation mechanisms to environmental changes and the inheritance of genetic traits. The genetic and ecological characteristics of *D. melanogaster* significantly contribute to its utility in *in vivo* studies on ionizing radiation (Verçosa *et al*., 2017). According to Yushkova and Bashlykova (2020), the early developmental stages of *D. melanogaster* occur in the soil, on substrates undergoing fermentation, which enables them to accumulate heavy metals and radionuclides present in the environment. Another distinctive feature that makes this species particularly compelling is its high fertility, with females producing approximately two hundred embryos each, combined with a short developmental period, ranging from eleven to fourteen days. These characteristics facilitate the efficient generation of reproducible results and the assessment of transgenerational effects (Yushkova, 2022).

In environments with anomalous levels of natural radiation, greater exposure to high levels of natural radioactivity results in more prolonged genetic effects, such as genetic damage or increased activation of damage response systems (Verçosa *et al*., 2017; Silva, 2021; Silva *et al*., 2021). In a recente study, Pereira *et al*. (2025) reported that a *D. melanogaster* strain originating from Cerro Corá, Rio Grande do Norte, Brazil, a municipality characterized by high levels of natural radioactivity, exhibited greater resistance to the genetic effects of natural radiation compared to the control strain, *D. melanogaster* Oregon-R, which is known to be radiation-sensitive (Verçosa *et al*., 2017). The Cerro Corá strain also showed higher radioresistance to acute damage induced by gamma radiation, in contrast to the Oregon-R strain, wich exhibited a clear dose-dependent effect (doses of 30, 50, and 70 Gy).

Given the few *in vivo* studies on organisms constantly exposed to natural radiation in Northeastern Brazil (Verçosa *et al*., 2017; Silva, 2021; Silva *et al*., 2021; Pereira *et al*., 2025), the present study offers new evidences on the genetic effects of radiation on descendants of Parelhas city, a place with moderate natural radiation levels (between 418 and 957 $Bq.m^{-3}$), and

in descendants of Vitória de Santo Antão, a rural place with safe radiation levels (<200 Bq.m^{-3}) and urban pollutants (Santana *et al*., 2018), which behaved as a negative control group.

Material and Methods

Drosophila melanogaster populations and study areas

Drosophilid adults were collected in two natural environments in the Northeastern Brazil (Figure 1). The first site was the rural area of the municipality of Parelhas, a place with anormal radioacitivty (between 418 and 957 Bq.m^{-3} of alpha radiation). Parelhas is a municipality with an area of 513.507 km^2 located in the semi-arid environment of the Caatinga biome (coordinates 6°41'50.4"S, 36°37'49.5"W), in the Central Potiguar mesoregion and the Seridó microrregion in the state of Rio Grande do Norte, with a low population density (21,499 inhabitants) (IBGE, 2022).

The second site was the rural area of Vitória de Santo Antão, previously assessed as being free from heavy metal contamination (Santana *et al*., 2018) and with levels of natural radiation <200 Bq.m^{-3}. Vitória de Santo Antão is a municipality in the Zona da Mata region in the state of Pernambuco (8°06'55.9"S, 35°20'11.2"W), with a larger area (336.573 km^2) and population (134,084 inhabitants), as well as growing urbanization and new industries (Santana *et al*., 2018; IBGE, 2022).

Once collected and taxonomically identified, only specimens of *Drosophila melanogaster* from each location were cultured under laboratory conditions, in the same way as the Oregon-R, the standard lineage of *D. melanogaster* sensitive for environmental mutagenesis studies in Northeastern Brazil (Verçosa *et al*., 2017; Nascimento-Silva *et al*., 2024; Pereira *et al*., 2025). *D. melanogaster* individuals collected in Parelhas were named Parelhas residents (PAR-res), while those collected in Vitória de Santo Antão were named Vitória de Santo Antão residents (VSA-res). The populations were maintained at optimal density and conditions of 25±1°C, 60% relative humidity, and under a 14-hour photoperiod regime. They were fed a cornmeal-based medium containing cornmeal, rye flour, agar, yeast, and nipagin, with regular exchanges and maintenance conducted weekly.

Natural radiation measurements

The study sites were assessed for the concentration of alpha (α) particles emitted from the soil, using a portable AlphaGUARD monitor (Bertim Instruments). The device is placed on the surface layer of the soil and is connected to a pump (AlphaPUMP) that draws air from the

emanation chamber and directs it to a detection system that quantifies the rate of α particles emitted, primarily by Radon gas (^{222}Rn) (Farias *et al*., 2016). The mean radiation levels measured in Parelhas were 418.00 Bq.m^{-3} (in August 2022) and 957.00 Bq.m^{-3} (in March 2024), corresponding to the two seasons of the year, dry and wet, respectively. In the same seasons, radiation levels in Vitória de Santo Antão were 79.28 Bq.m^{-3} (in August 2022) and 169.42 Bq.m^{-3} (in March 2024).

Chronic exposure of organisms to radiation in Parelhas

Three replicates of PAR-res and OR offspring were exposed to the Parelhas environment during the same period described by Pereira *et al*. (2025): August, 2022, after seven months under laboratory cultivation (from January 2022 to August 2022), and February 2023, after 13 months (between January 2022 to February 2023). Each replicate consisted of 120 PAR-res adults or 120 OR adults, maintained in plastic bottles (Verçosa *et al*., 2015). The bottles remained in Parelhas for six days, a sufficient period for adults and their offspring larvae to be exposed to local radiation. The bottles were arranged in pairs (PAR-res with OR), suspended 1.5 m above ground level, in three different shaded locations, 30 m apart. After six days of field exposure, the adults and their third-instar larvae offspring were collected and transported to the laboratory for analysis using the Comet assay.

Acute exposure to gamma radiation

To determine the sensitivity or resistance to ionizing radiation, synchronized third-instar larvae of *Drosophila melanogaster* from the PAR-res, VSA-res, and Oregon-R strains were exposed to gamma (γ) radiation using a ^{60}Co Gammacell source (Excel-MDS Nordion) at a dose rate of 1.047 kGy/h, with doses of 10, 30, 50, and 70 Gray (Gy), as described in Pereira *et al*. (2025). Briefly, 80 larvae were placed in acrylic tubes (3.5 cm in diameter and 9 cm in height) containing 0.5 grams of (Yoki) instant mashed potatoes hydrated with 1.5 mL of distilled water and sealed with foam stoppers. To establish the baseline damage index (DI) and damage frequency (DF%) measurements for populations maintained exclusively under laboratory conditions, synchronized third-instar larvae from the three *D. melanogaster* strains (PAR-res, VSA-res, and OR), were assessed using the Comet assay. A negative control, designated as "Blank," served as a reference for assessing genetic damage in populations subjected to handling and the stress of transport to the ^{60}Co Gammacell but without exposure to radiation. All experiments were performed in triplicate, following the protocols described by Pereira *et al*. (2025). The assessments included the analysis of larvae one hour post-radiation

exposure and 24 hours post-exposure to allow for recovery and the visualization of potential DNA repair mechanisms following acute irradiation.

Comet assay

Individuals exposed to the Parelhas environment or subjected to gamma radiation received cryoanesthesia at 4°C for 1 minute to reduce metabolic activity, followed by hemolymph extraction via a small incision. For the cell pool preparation, 60 larvae per replicate were used, and the hemolymph was processed in the presence of the anticoagulant ethylenediaminetetraacetic acid (EDTA). The collected material was transferred to a microcentrifuge tube and centrifuged once at 3.000 rpm for 9 minutes. Subsequently, 60 µL of the biological material was homogenized in 100 µL of low-melting-point agarose solution (0.5%) and immediately spread onto histological slides pre-coated with standard agarose (1.5%). Lastly, a coverslip (24 mm x 60 mm) was placed over the material, and the slides containing the cellular material were refrigerated at 4°C for 10 minutes to solidify the agarose. The coverslips were then carefully removed, and the slides were immersed in a lysis solution (2.5 M NaCl; 100 mM EDTA; 1 M NaOH; 1% Tris pH 10; 1% Triton X-100 and 10% DMSO) for 72 hours. After this period, the slides were aligned in an electrophoresis tank (40 cm) and covered with buffer solution (1 M NaOH; 200 mM EDTA pH 13) for 20 minutes. During this step, the DNA strands loosen and the damaged regions are exposed. An electric current (40 V and 300 mA) was then applied for 20 minutes, prompting the migration of the fragmented DNA from the negative to the positive pole, forming characteristic tails. After electrophoresis, the slides were neutralized in a 0.4 M Tris-HCl solution (pH 7.5) for 15 minutes, then fixed in absolute ethanol (5 minutes) and air-dried at room temperature. All steps following homogenization were performed under dim light to protect the samples from photodegradation.

To visualize the nucleoids, the samples were stained with 50 µL of GelRed (Biotium) diluted in purified water (1:500) and examined under a Zeiss-Imager M2 fluorescence microscope, equipped with the Alexa-Fluor 546 filter at 400x magnification. Comets were classified according to the integrity of the "head" and the length of the "tail" of the nucleoids, assigning comparative scores from 0 (intact), 1 (minimal damage), 2 (intermediate damage), 3 (intense damage) and class 4 (maximum damage). Visual inspection was based on the classification described by Collins *et al*. (2008), and adhered to the patterns presented in Verçosa *et al*. (2017). A total of 100 cells were analyzed per replicate, yielding 300 nucleoids per treatment. The damage index (DI) was calculated using the formula: $DI = 0 \times (N_0) + 1 \times (N_1) + 2 \times (N_2) + 3 (N_3) + 4 \times (N_4)$, so that it ranges from a minimum of zero, in case no DNA

damage is observed in any cell (level 0) to 400, which corresponds to all cells (N = 100) under the maximum DNA damage (level 4). The damage frequency was calculated as: DF% = [(N_T – N_0) x 100] / N_T, where N_1, N_2, N_3, N_4 represent the total number of damages 0, 1, 2, 3 and 4, respectively, where N_T represents the total number of damaged nucleoids, and N_0 the total number of undamaged nucleoids. The damage frequency varied from 0 to 100%. For graphical representation, the mean DI and DF% of each treatment were calculated across the three replicates, accompanied by the standard deviation (SD). The means were compared using the analysis of variance (ANOVA) and the Bonferroni post-hoc pairwise test to determine the significant differences between individual groups. The analyses were conducted using Stata 14.2, and results were considered statistically significant at $P \leq 0.05$.

Results

Exposure to chronic radiation

The genotoxicity results, based on the mean values of the damage index (DI) and damage frequency (DF%) for the *D. melanogaster* strains exposed to radiation in Parelhas during two seasons, are presented in Table 1. As indicated, no significant (*ns*) differences in DNA damage were observed in the hemolymph cells of either PAR-res larvae or adults compared to their respective OR controls exposed under the same seasonal conditions. These findings indicate that the PAR-res larvae and adults were as sensitive to radiation as the corresponding OR individuals under identical exposure conditions. Conversely, the results in reveal that DNA damage was approximately twice as high during the dry season compared to the wet season across all exposed groups. The Bonferroni post-test for multiple comparisons of mean DI and DF% values is presented in Table S1 (Suplementary Material). Were observed differences in damage levels between the same groups of different seasons (dry and wet) and these results align with the findings of Pereira *et al*. (2025), who also reported significant seasonal variations in *D. melanogaster*, exposed in Cerro Corá during the same periods. Pereira *et al*. (2025) also suggested that the Cerro Corá population possesses a trait of radioresistance that is preserved across generations, possibly due to the efficiency of DNA repair pathways.

Exposure to acute gamma radiation

As PAR-res and OR strains were cultivated under the same laboratory conditions, their genotoxic response to acute exposure to controlled doses of gamma radiation (10, 30, 50, and 70 Gy) were also analyzed by comet assay, to further characterize their genetic profiles. To enhance the study's comparative framework, a third strain was incorporated as an environmental

control group. This strain was derived from the natural environment of Vitória de Santo Antão, a city with rural and sparsely urbanized areas. According to Santana *et al*. (2018), *D. melanogaster* individuals exposed in this region exhibit low DI and DF% values, attributed to the absence of environmental pollution, as corroborated by the authors through the quantification of heavy metals. Thus, this site was selected to establish the environmental control strain (VSA-res), after confirming safe alpha particle emissions from the soil, which measured below 200 $Bq.m^{-3}$.

Before conducting the acute gamma radiation exposure tests, the PAR-res, VSA-res, and OR strains cultured in the laboratory were assessed using the Comet assay to establish reference genetic damage values, called negative control groups. As detailed in Table 2, this preliminary assessment revealed low DI and DF% values for Parelhas-res (DI = 24.00, DF% = 7.00), Vitória de Santo Antão-res (DI = 40.67, DF% = 15.67), and Oregon-R (DI = 42.33, DF% = 15.00). The table also summarizes the genotoxicity results obtained after acute exposure to varying doses of gamma radiation, followed by the Comet assay, conducted 1 hour and 24 hours post-irradiation, adhering to the previously described methodology. Notably, the "Blank" groups for each strain, while not irradiated, were transported to the cobalt irradiator location alongside the exposed groups to ensure consistent handling conditions. As indicated, the VSA-res strain exhibited a response pattern similar to that of the Oregon-R strain, characterized by elevated and progressively increasing levels of damage, unlike the PAR-res strain.

The results clearly demonstrate a dose-dependent effect in both VSA-res and OR, with damage intensifying as gamma radiation doses increased. This finding is highly significant, as it confirms VSA-res as a valid environmental control group, given its lack of chronic exposure to genotoxic agents, such as natural ionizing radiation. The dose-dependent results are further highlighted in Figure 2 and the comparative Bonferroni statistical analyses are detailed in Table S2 (Suplementary Material), across all doses, strains, and time points (1 hour and 24 hours after acute exposure).

Comparative analysis

To expand the evidences and obtain a deeper understanding of the genotoxic effects of both acute and chronic ionizing radiation exposure in *Drosophila melanogaster* in Brazilian environments, we compared our results of Damage Index with those of Pereira *et al*. (2025) (Figure 3). These comparisons were possible because the field experiments in Cerro Corá and Parelhas were conducted on the same dates (August 2022 and February 2023), as was the Comet assay processing, on samples of hemolymph cells from larvae and adults.

As discussed in Pereira *et al*. (2025), significant differences were observed between the DI and DF% values of larvae and adults from CC-res compared to their respective Oregon control groups (denoted as OR_1). In contrast, the current study of genotoxicity results for larvae and adults from PAR-res did not differ significantly from those of their respective Oregon control group (denoted here as OR_2 to distinguish them from OR_1, which were exposed in Cerro Corá). One interesting aspect of the chronic exposure analysis is that individuals from OR_1 exposed in Cerro Corá, exhibited more damage than those from OR_2, exposed in Parelhas. This discrepancy may be attributed to the differences in radiation levels in the the two places, with Cerro Corá demonstrating levels exceeding 2800 $Bq.m^{-3}$, compared to Parelhas with a maximum of 957.0 $Bq.m^{-3}$.

A comparative overview of the results of the present study with the chronic irradiation results obtained by Pereira *et al*. (2025), evaluated in 1 hour and 24 hours after gamma irradiation, are presented in Figure 4, and the Bonferroni analyses in Table S3 (Suplementary Material). Clearly, all tested *D. melanogaster* strains clustered into two main groups, at all doses: the group of non-radioresistants (OR_1, OR_2 and VSA-res), and the group of radioresistants (PAR-res, CC-res). The only exception was the Cerro Corá at the 10 Gy dose, 1 hour after irradiation, a result previouly discussed in Pereira *et al*. (2025).

Discussion

We assessed DNA damage in two strains of *D. melanogaster* (OR and PAR-res) exposed to natural radiation present in Parelhas, and assessed the damage in three strains (OR, VSA-res and PAR-res) exposed to acute gamma radiation (at 1 hour and 24 hours post-exposure). The analyses provides an overview of the results obtained so far with *D. melanogaster* individuals living in the Caatinga biome, gathering data from different locations in terms of genotoxic damage and local adaptation.

The results revealed low levels of damage and the absence of a dose-dependent effect only in the PAR-res, suggesting resistance to radiation in this strains even after one year of cultivation under laboratory conditions. In contrast, the environmental control VSA-res and OR, exhibited high levels of DI and DF%, with a clear dose-dependent effect when exposed to gamma radiation, particularly 1 hour after irradiation. Although some groups showed signs of recovery after 24 hours, the damage remained higher in VSA-res and OR compared to that observed in Parelhas. It is important to highlight the comparison between the *D. melanogaster* strains from Vitória de Santo Antão, collected from a low-radiation area (within safe human

exposure limits) to serve as an environmental control, and the Oregon-R strain, which was obtained from multiple generations of inbreeding in the laboratory and serves as a standard control in genetic studies. Overall, the wild population exhibited DI and DF% levels similar to those of the Oregon-R strain, with no significant differences in the DI observed 1 hour after exposure, in the 10 and 30 Gy gamma radiation doses. Similarly, Verçosa *et al*. (2017) found that both the Oregon-R strain and a wild *D. melanogaster* population collected from the urban area of Recife, in the state of Pernambuco, Northeast Brazil, were equally sensitive to the natural radiation of the municipality of Lajes Pintadas, located in the same Caatinga biome as Parelhas and Cerro Corá, in the state of Rio Grande do Norte.

Our finding of the increase genotoxicity due to gamma radiation are consistent with the findings of Jiménez *et al*. (2019), who reported an increase in mutation and somatic recombination frequencies in *D. melanogaster*, with values directly correlated to both the dose and dose rate of gamma radiation. As published by Pereira *et al*. (2025), this phenomenon can be interpreted as an adaptive gain, resulting from chronic exposure to environmental radiation, to which the Parelhas population is subjected. This suggests the presence of mechanisms that mitigate genetic damage induced by this physical agent (gamma radiation), thus ensuring the survival and perpetuation of the species.

The negative effects of ionizing radiation on individuals have been well documented, since it can cause damage to genetic material and induce mutations, and these effects can occur through both direct and indirect actions on the DNA of organisms (Desouky *et al*., 2015). According to Jackson and Bartek (2009) and Baonza *et al*. (2022), cells have evolved enhanced response mechanisms to address DNA damage caused by a variety of genotoxic agents, aiming to preserve genomic integrity and ensure the success of future generations. These responses include the regulation of the cell cycle, activation of DNA repair pathways, and induction of cell death, which can act synergistically to mitigate the harmful effects on damaged cells. Additionally, the expression of DNA repair enzymes may also contribute to radioprotective effects (Vidal *et al*., 2020) . Although the cellular and molecular mechanisms that activate these pathways are not yet fully understood, Guéguen *et al*. (2019) highlighted that the repair pathways include mismatch repair, base and nucleotide excision repair, homologous recombination repair, and non-homologous end joining.

A study conducted by Velegzhaninov *et al*. (2020) concluded that the overexpression of the *RPA1* gene in HEK293T cells enhanced resistance to ionizing radiation. However, this resistance was accompanied by increased DNA damage levels, as assessed by the Comet assay. Despite the heightened DNA damage, these overexpressed *RPA1* cells also exhibited a higher

rate of DNA repair, which likely contributed to their radioresistance. In contrast, Koval *et al*. (2020) reported that the overexpression of repair genes in *Drosophila melanogaster* did not enhance resistance to ionizing radiation, but rather exacerbated the negative effects on the organisms' lifespan. And that the inducted mutations in genes involved in DNA damage recognition and repair resulted in a significant reduction in the radioadaptive response, compared to genetically unmodified strains. In a different approach, Yushkova (2019) evaluated the specific role of the transposable elements *hobo* in the formation of radiobiological effects, suggesting that *D. melanogaster* indivduals with full-size hobo-elements with high activity level (less often with a mean activity level) are responsible for delayed deleterious irradiation effects.

According to Maclean et al. (2018), factors such as specific fecundity and development time, lifespan, mobility activity level, standard metabolic rate, desiccation tolerance, hunger, and heat tolerance are maintained in descendant laboratory-cultured, making *D. melanogaster* suitable for comparative ecological, physiological, and evolutionary experiments. As presented by Zarubim et al. (2020), the cultured strains from different geographical origins exhibit differential and specific responses in transcriptome analysis, reflextion the local adaptations that shaped their genomes through positive selection.

Our results further validate the Oregon-R strain as a reliable indicator of genotoxic effects in areas with high levels of natural radiation, as previouly evaluated by Verçosa *et al*., 2017; Pereira *et al*., 2025). As expected, we also showed that individuals from Vitória de Santo Antão behave very similarly to Oregon-R, with increasing genotoxic damage in the face of gamma-ray doses, reinforcing the quality of the results in general. These comparisons were possible because the tests were carried out under the same conditions and times of the year. All genotoxicity findings were supported by measurements of alpha particles emitted from the soil at the study sites, which aligned with the observed genotoxicity data. The highest levels of genetic damage were found in individuals from locations with the highest radiation levels, as Cerro Corá, followed by Parelhas.

The high radiation levels measured in Cerro Corá and Parelhas raise important questions about the necessity of measuring natural radioactivity levels in genetic-ecological study sites, particularly in the Caatinga environments of Northeast Brazil. This is because radiation is a powerful evolutionary force that can shape critical genetic characteristics of local populations. Given that the Caatinga is an exclusively Brazilian biome with high levels of endemism, it is highly likely that natural radiation plays a significant role in generating small genetic variations,

which in turn contribute to the emergence of new species in an environment of intense competition.

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Supplementary Material

Table S1 - Bonferroni post-test comparisons of mean values for Damage Index (below the diagonal) and Damage Frequency % (above the diagonal) in *Drosophila melanogaster* larvae and adults (Oregon-R and PAR-res) exposed to natural radiation in Parelhas.

Groups exposed in Parelhas	Dry season				Wet season			
	Larvae Oregon-R	Larvae PAR-res	Adults Oregon-R	Adults PAR-res	Larvae Oregon-R	Larvae Parres	Adults Oregon-R	Adults PAR-res
Dry season								
Larvae Oregon-R		1.000	1.000	1.000	0.0001*	0.0001*	0.0001*	0.011*
Larvae PAR-res	1.000		1.000	1.000	0.0001*	0.0001*	0.0001*	0.074
Adults Oregon-R	1.000	1.000		1.000	0.0001*	0.0001*	0.0001*	0.188
Adults PAR-res	0.422	0.961	1.000		0.0001*	0.001*	0.0001*	0.741
Wet season								
Larvae Oregon-R	0.0001*	0.0001*	0.0001*	0.0001*		1.000	0.029*	1.000
Larvae PAR-res	0.0001*	0.0001*	0.0001*	0.0001*	1.000		0.188	1.000
Adults Oregon-R	0.0001*	0.0001*	0.0001*	0.0001*	1.000	1.000		0.046*
Adults PAR-res	0.0001*	0.0001*	0.0001*	0.0001*	1.000	0.486	1.000	

* Significant differences ($P \leq 0.05$).

Table S2 - Bonferroni post-hoc comparisons between the mean values of the Damage Index (below the diagonal) and Damage Frequency (above the diagonal) observed in *Drosophila melanogaster* larvae from Parelhas resident, Vitória de Santo Antão (environmental control), and Oregon-R (laboratory control) subjected to the Comet assay 1 hour and 24 hours after gamma irradiation (10, 30, 50, and 70 Gy), and their respective Blank groups. Significant differences between doses for each strain are highlighted in yellow, and not significant (ns) differences are highlighted in green.

	1 hour post-irradiation															24 hours post-irradiation													
Exposured groups	**Parelhas-res**					**Vitória de Santo Antão-res**					**Oregon-R**					**Parelhas-res**					**Vitória de Santo Antão-res**					**Oregon-R**				
	Blank	**10**	**30**	**50**	**70**	**Blank**	**10**	**30**	**50**	**70**	**Blank**	**10**	**30**	**50**	**70**	**Blank**	**10**	**30**	**50**	**70**	**Blank**	**10**	**30**	**50**	**70**	**Blank**	**10**	**30**	**50**	**70**
PAR-res 1 h																													
Blank		ns	ns	ns	ns	***	***	***	***	***	***	***	***	***	***	ns	***	**	ns	ns	ns	***	***	***	***	***	***	***	***	***
10	ns		ns	ns	*	***	***	***	***	***	***	***	***	***	***	ns	**	*	ns	ns	*	***	***	***	***	***	***	***	***	***
30	ns	ns		ns	ns	***	***	***	***	***	***	***	***	***	***	ns	***	**	ns	ns	ns	***	***	***	***	***	***	***	***	***
50	ns	ns	ns		ns	***	***	***	***	***	***	***	***	***	***	ns	***	***	ns	ns	ns	***	***	***	***	***	***	***	***	***
70	ns	*	*	ns		**	***	***	***	***	***	***	***	***	***	ns	***	***	ns	ns	ns	***	***	***	***	***	***	***	***	***
VSA-res 1 h																													
Blank	*	**	**	ns	ns		ns	***	***	***	ns	***	***	***	***	***	***	***	***	***	**	ns	*	***	***	ns	ns	***	***	***
10	***	***	***	***	***	***		***	***	***	ns	***	***	***	***	***	***	***	***	***	***	ns	ns	***	***	ns	ns	***	***	***
30	***	***	***	***	***	***	***		ns	***	***	ns	***	***	***	***	***	***	***	***	***	***	***	ns	***	***	***	ns	ns	***
50	***	***	***	***	***	***	***	**		***	***	ns	ns	***	***	***	***	***	***	***	***	***	***	*	***	***	***	ns	ns	ns
70	***	***	***	***	***	***	***	***	***		***	***	***	ns	**	***	***	***	***	***	***	***	***	***	ns	***	***	***	***	***
Oregon 1 h																													
Blank	***	***	***	***	*	ns	ns	***	***	***		***	***	***	***	***	***	***	***	***	***	ns	ns	***	***	ns	ns	***	***	***
10	***	***	***	***	***	***	ns	ns	***	***	***		**	***	***	***	***	***	***	***	***	***	***	ns	***	***	***	ns	ns	***
30	***	***	***	***	***	***	***	ns	ns	***	***	***		***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	ns	ns
50	***	***	***	***	***	***	***	***	***	ns	***	***	***		**	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
70	***	***	***	***	***	***	***	***	***	***	***	***	***	***		***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
PAR-res 24 h																													
Blank	ns	ns	ns	ns	**	***	***	***	***	***	***	***	***	***	***		***	**	ns	ns	ns	***	***	***	***	***	***	***	***	***
10	***	**	**	***	***	***	***	***	***	***	***	***	***	***	***	*		ns	***	***	***	***	***	***	***	***	***	***	***	***
30	***	**	**	***	***	***	***	***	***	***	***	***	***	***	***	*	ns		***	***	***	***	***	***	***	***	***	***	***	***
50	ns	ns	ns	ns	ns	ns	***	***	***	***	***	***	***	***	***	ns	***	***		ns	ns	***	***	***	***	***	***	***	***	***
70	ns	ns	ns	ns	ns	ns	***	***	***	***	***	***	***	***	***	ns	***	***	ns		ns	***	***	***	***	***	***	***	***	***
VSA-res 24 h																													
Blank	ns	ns	ns	ns	ns	**	***	***	***	***	***	***	***	***	***	ns	***	***	ns	ns		***	***	***	***	***	***	***	***	***
10	***	***	***	***	***	**	ns	***	***	***	ns	**	***	***	***	***	***	***	***	***	***		ns	***	ns	ns	ns	***	***	***
30	***	***	***	***	***	***	ns	***	***	***	ns	*	***	***	***	***	***	***	***	***	***	ns		ns	ns	ns	ns	***	***	***
50	***	***	***	***	***	***	***	ns	**	***	***	ns	ns	***	***	***	***	***	***	***	***	ns	ns		***	***	***	ns	ns	***
70	***	***	***	***	***	***	***	***	ns		***	***	***	ns	***	***	***	***	***	***	***	ns	ns	ns		***	***	***	***	**
Oregon 24 h																													
Blank	*	**	**	ns	ns	ns	***	***	***	***	ns	***	***	***	***	***	***	***	ns	ns	**	**	***	***	***		ns	***	***	***
10	***	***	***	***	***	***	ns	***	***	***	ns	ns	***	***	***	***	***	***	***	***	***	ns	ns	***	***	***		***	***	***
30	***	***	***	***	***	***	ns	ns	***	***	***	ns	***	***	***	***	***	***	***	***	***	***	*	ns	***	***	ns		ns	***
50	***	***	***	***	***	***	***	ns	ns	***	***	**	ns	***	***	***	***	***	***	***	***	***	***	ns	***	***	**	ns		**
70	***	***	***	***	***	***	***	***	*	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	**	***	***	***	***	

ns (not significant), * (statistical difference between 0.01 and 0.05), ** (statistical difference between 0.001 and 0.009), *** (statistical difference of 0.0001).

Table S3 - Comparisons of Bonferroni post-test between the mean values of Damage Index (below diagonal) and Damage Frequency % (above diagonal) observed in larvae and adults of *Drosophila melanogaster* populations (Oregon-R_1 and CC-res; Oregon-R_2 and PAR-res) exposed to natural radiation of Cerro Corá (data from Pereira *et al*., 2025) and Parelhas (this study), in dry season (A) and wet season (B).

(A)

Groups exposed in Cerro Corá and Parelhas	**Dry season**						
	Larvae OR_1	Larvae CC-res	Adults OR_1	Adults CC-res	Larvae OR_2	Larvae PAR-res	Adults OR_2
Dry season							
Larvae OR_1	-						
Larvae CC-res	0.001*	-					
Adults OR_1	0.005*	0.005*	-				
Adults CC-res	0.388	0.346	0.0001*	-			
Larvae OR_2	0.038*	1.000	0.0001*	1.000	-		
Larvae PAR-res	0.077	1.000	0.0001*	1.000	1.000	-	
Adults OR_2	0.097	1.000	0.0001*	1.000	1.000	1.000	-
Adults PAR-res	1.000	0.034*	0.0001*	1.000	1.000	1.000	1.000

* Significant differences ($P \leq 0.05$).

(B)

Groups exposed in Cerro Corá and Parelhas	**Wet season**						
	Larvae OR_1	Larvae CC-res	Adults OR_1	Adults CC-res	Larvae OR_2	Larvae PAR-res	Adults OR_2
Wet season							
Larvae OR_1	-						
Larvae CC-res	0.001*	-					
Adults OR_1	0.001*	0.0001*	-				
Adults CC-res	0.035*	1.000	0.0001*	-			
Larvae OR_2	0.627	0.026*	0.0001*	0.992	-		
Larvae PAR-res	1.000	0.177	0.0001*	1.000	1.000	-	
Adults OR_2	1.000	0.002*	0.0001*	0.093	1.000	1.000	-
Adults PAR-res	1.000	0.0001*	0.001*	0.013*	1.000	0.244	1.000

* Significant differences ($P \leq 0.05$).

Conflict of Interest

The authors have no conflicts of interest to declare.

Author Contributions

MGP and CR conceived, designed, and performed the research. MGP, SLS, LFS, MDSF and CR, performed genotoxic experiments and data analysis. MGP, LFS, MDSF, AME and CR designed the experiments and contributed to the writing of the manuscript. All authors read and approved the manuscript.

Data Availability

All the data supporting the findings are included in this published article and its supplementary information files.

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Figure Legends

Figure 1 - Map of Brazil, highlighting the states of Rio Grande do Norte and Pernambuco (dark gray) and cities of origin of the *Drosophila melanogaster* strains studied: Parelhas (in brown) and Vitória de Santo Antão (in green).

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Figure 3 - Comparative analysis of Damage Index results after exposure of *Drosophila melanogaster* Cerro Corá residents (CC-res) and Oregon strains in the Cerro Corá environment (Pereira *et al*., 2025) and results for *D. melanogaster* Parelhas residents (PAR-res) and Oregon strain (this study), conducted on the same dates and during the same seasons (dry and wet). Asterisks indicate significant differences ($P \leq 0.05$) between each resident and its control strain.

Figure 4 – Comparative analysis of Damage Index after exposure of *Drosophila melanogaster* Oregon-1 and Cerro Corá resident strains (Pereira *et al*., 2025), *D. melanogaster* Oregon-2, Vitória de Santo Antão resident, and Parelhas resident strains (this study), and their respective laboratory negative Control groups, all assessed by the Comet assay. Damage Index (A) evaluated 1 hour and (B) 24 hours after exposure to gamma radiation doses (10, 30, 50, and 70 Gy).

Table 1 - Mean values and standard deviations (Sd) of genetic damage levels (0 to 4), Damage Index and Damage Frequency % in the hemolymph cells of *Drosophila melanogaster* larvae and adults from the Oregon-R control group and Parelhas residents (PAR-res), exposed to natural environmental radiation in Parelhas during the dry and wet seasons.

Groups exposed	Damage levels (mean number ± Sd)					Damage Index ± Sd	Damage Frequency % ± Sd
	0	1	2	3	4		
Dry season (August, 2022)							
Larvae Oregon-R	58.33±2.08	8.33±6.81	11.00±3.46	15.00±7.00	7.33±1.15	104.67 ±8.74	41.67 ±2.08
Larvae PAR-res	59.67±2.52	7.67±0.58	6.33±0.58	19.00±3.00	7.33±0.58	106.67 ±7.02 *ns*	40.33 ±2.52 *ns*
Adults Oregon-R	60.33±0.58	5.67±1.53	7.33±1.53	19.67±0.58	7.00±0.00	107.33 ±2.31	39.67 ±0.58
Adults PAR-res	61.33±2.52	4.33±0.58	4.67±2.08	14.33±2.08	15.33±0.58	118.00 ±7.81 *ns*	38.67 ±2.52 *ns*
Wet season (February, 2023)							
Larvae Oregon-R	71.00±1.73	13.00±1.00	8.33±1.15	4.33±0.58	3.33±0.58	56.00 ±5.00	29.00 ±1.73
Larvae PAR-res	69.67±2.08	17.33±2.08	7.00±4.00	3.33±0.58	2.67±0.58	52.00 ±5.29 *ns*	30.33 ±2.08 *ns*
Adults Oregon-R	70.67±1.15	15.00±1.00	3.33±1.53	4.67±1.53	6.33±0.58	61.00 ±3.46	29.33 ±1.15
Adults PAR-res	65.00±1.00	17.00±3.00	9.33±3.21	5.33±0.58	3.33±1.53	65.00 ±5.57 *ns*	35.00 ±1.00 *ns*

ns means not significant differences in relation to the mean ID or FD% of Parelhas exposed individuals compared to respective Oregon-R control group (larvae x larvae; adults x adults).

Table 2 - Mean values and standard deviations (Sd) observed for damage levels (0 to 4), Damage Index and Damage Frequency % (± Sd) in hemolymph cells of *Drosophila melanogaster* larvae from the Parelhas resident, the Vitória de Santo Antão resident, and the Oregon-R control, after 1 hour and 24 hours of irradiation with a ^{60}Co source, at doses of 10, 30, 50, and 70 Gy. Negative controls refer to strains maintained under laboratory conditions, while the "Blank" groups consist of control strains sent to the cobalt irradiator, but not exposed to gamma radiation. Asterisks indicate significant differences between the doses and their respective "Blank" group for each strain.

Exposured groups	Damage levels (mean number ± Sd)					Damage Index ± Sd	Damage Frequency % ± Sd
	0	1	2	3	4		
Parelhas							
Negative control	93.00±1.00	0.33±0.58	0.00±0.00	3.00±1.00	3.67±0.58	24.00±3.46	7.00±1.00
Vitória de Santo Antão							
Negative control	84.33±1.53	2.67±1.53	5.00±1.00	4.00±0.00	4.00±1.00	40.67±3.21	15.67±1.53
Oregon-R							
Negative control	85.00±2.65	2.00±1.00	3.33±2.52	5.00±0.00	4.67±0.58	42.33±3.51	15.00±2.65
Parelhas (1 h)							
Blank	87.67±0.58	0	0	7.00±1.00	5.33±1.53	42.33±3.21	12.33±0.58
10 Gy	88.67±0.58	1.00±1.00	0	2.67±0.58	7.67±0.58	39.67±1.53	11.33±0.58
30 Gy	87.67±2.31	1.00±1.00	0	6.67±1.53	4.67±2.31	39.67±7.02	12.33±2.31
50 Gy	85.67±0.58	1.33±1.15	0	7.33±1.53	5.67±3.06	46.00±7.00	14.33±0.58
70 Gy	83.67±0.58	0.67±1.15	0	7.33±2.52	8.33±2.08	56.00±4.36	16.33±0.58
Vitória de Santo Antão (1 h)							
Blank	78.33±1.53	3.33±0.58	4.33±1.15	8.00±0.00	6.00±0.00	60.00±2.65	21.67±1.53
10 Gy	74.00±1.00	0.00±0.00	2.67±1.53	11.33±0.58	12.00±2.00	87.33±4.73*	26.00±1.00
30 Gy	67.67±0.58	0.33±0.58	2.67±0.58	15.00±2.00	15.33±2.89	112.00±5.57*	33.33±0.58*
50 Gy	62.33±1.53	0.67±0.68	0.33±0.58	15.33±1.53	21.33±0.58	132.67±4.93*	37.67±1.53*
70 Gy	49.67±0.58	1.67±0.58	1.33±0.58	15.67±0.58	31.67±0.58	178.00±2.65*	50.33±0.58*
Oregon-R (1 h)							
Blank	75.33±1.15	4.00±1.73	3.00±1.00	8.00±1.00	9.67±2.08	72.67±5.03	24.67±1.15
10 Gy	66.00±2.65	2.67±1.15	7.67±2.08	13.33±2.08	10.33±1.15	99.33±6.03*	34.00±2.65*
30 Gy	60.33±1.53	3.00±1.00	3.00±1.00	19.67±2.08	14.00±2.00	124.00±7.81*	39.67±1.53*
50 Gy	49.67±0.58	2.67±1.53	1.00±1.00	20.00±2.00	26.67±0.58	171.33±2.08*	50.33±0.58*
70 Gy	44.00±1.00	0.00±0.00	0.33±0.58	19.00±1.00	36.67±2.08	204.33±5.51*	56.00±1.00*
Parelhas (24 h)							
Blank	88.00±1.73	2.00±1.00	0.33 ±0.58	5.00±1.73	4.67±0.58	36.33±3.06	12.00±1.73
10 Gy	94.67±0.58	0.33±0.58	0.00±0.00	2.00±1.00	3.00±1.73	18.33±4.04*	5.33±0.58*
30 Gy	93.33±0.58	1.00±1.00	0.67±1.15	4.00±2.00	1.00±1.00	18.33±2.08*	6.67±0.58*
50 Gy	85.33±1.73	2.00±1.00	1.67±0.58	6.00±0.00	5.33±1.15	44.67±4.51	15.00±1.73
70 Gy	85.33±1.53	1.00±0.00	0.33±0.58	6.67±0.58	6.67±1.53	48.33±6.66	14.67±1.53
Vitória de Santo Antão (24 h)							
Blank	84.00±1.00	4.00±1.00	3.33±0.58	4.33±0.58	4.33±1.53	41.00±5.29	16.00±1.00
10 Gy	75.67±2.08	1.33±0.58	1.67±2.08	11.00±1.73	10.33±1.15	79.00±5.29*	24.33±2.08*
30 Gy	73.33±2.08	2.33±1.53	2.67±2.89	11.33±2.52	10.33±1.53	83.00±2.00*	26.67±2.08*
50 Gy	67.00±1.73	0.33±0.58	0.00±0.00	17.00±1.00	15.67±1.53	114.00±5.29*	33.00±1.73*
70 Gy	52.00±0.00	0.00±0.00	0.00±0.00	23.67±3.79	24.33±3.79	168.00±3.79*	48.00±0.00*
Oregon-R (24 h)							
Blank	77.00±1.00	4.67±1.53	4.67±2.08	9.00±1.00	4.67±0.58	59.67±3.79	23.00±1.00
10 Gy	75.33±2.08	0.00±0.00	0.33±0.58	11.67±2.08	12.67±0.58	86.33±6.66*	24.67±2.08
30 Gy	66.67±1.15	1.67±1.15	5.67±1.15	16.33±0.58	9.67±2.08	100.67±7.09*	33.33±1.15*
50 Gy	63.33±1.15	0.33±0.58	2.00±1.73	21.67±1.53	12.67±0.58	120.00±1.73*	36.67±1.15*
70 Gy	58.00±1.00	0.33±0.58	0.33±0.58	17.33±2.08	24.00±1.73	149.00±4.58*	42.00±1.00*

* Significant differences ($P \leq 0.05$).

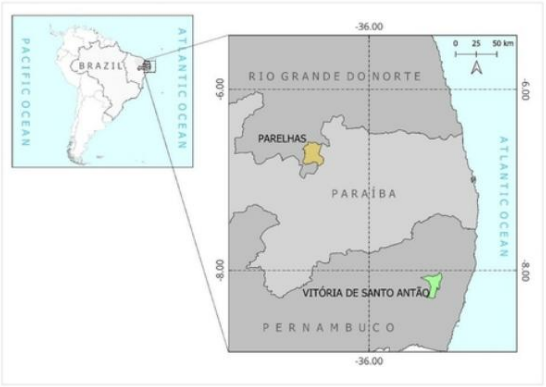

Figure 1 - Map of Brazil, highlighting the states of Rio Grande do Norte and Pernambuco (dark gray) and cities of origin of the Drosophila melanogaster strains studied: Parelhas (in brown) and Vitória de Santo Antão (in green).

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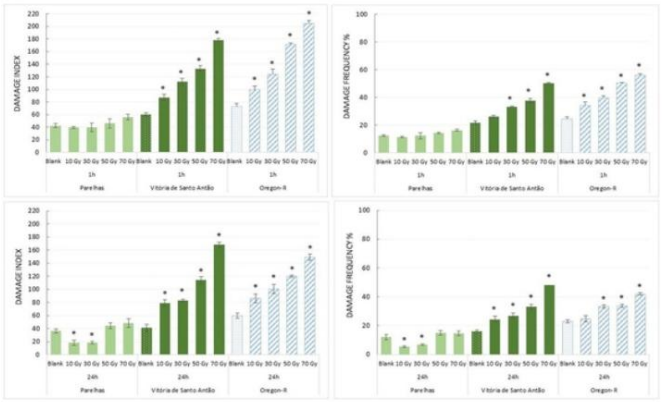

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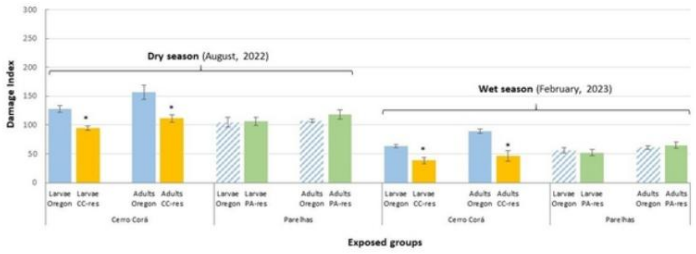

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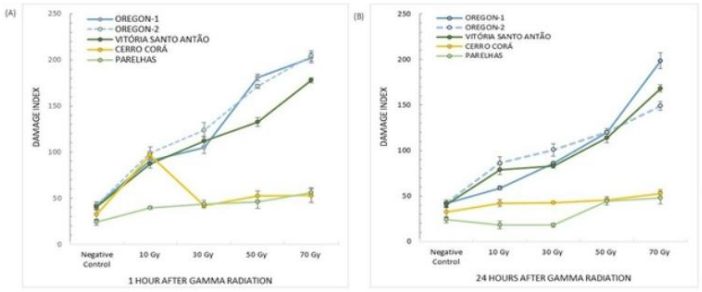

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54x30mm (300 x 300 DPI)

5 CONSIDERAÇÕES FINAIS

Este estudo fornece evidências importantes sobre a adaptação genética de populações de *Drosophila melanogaster* provenientes de cidades com elevados níveis de radiação natural no Nordeste do Brasil. Foi demonstrado que essas populações exibem notável radiorresistência, com tolerância significativa à radiação gama, o que é confirmada pelos baixos índices de danos no DNA nos indivíduos residentes. Além disso, foi observado que essas características persistem por gerações, devido à memória genética das populações radiorresistentes. Os achados reforçam a importância da linhagem Oregon-R como indicador confiável de genotoxicidade, assim como a linhagem Vitória de Santo Antão, recentemente coletada e proveniente de local com radiação abaixo de 200 $Bq.m^{-3}$. As análises comparativas entre as populações (residentes e controles) ampliam a compreensão sobre a evolução de adaptações genéticas em resposta a ambientes com condições adversas, contribuindo para estudos futuros sobre radiorresistência e impactos da radiação natural nos organismos que habitam a Caatinga.

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