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The long-term effects of acute exposure to ionising radiation on survival and fertility in *Daphnia magna*

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ABSTRACT

The results of recent studies have provided strong evidence for the transgenerational effects of parental exposure to ionising radiation and chemical mutagens. However, the transgenerational effects of parental exposure on survival and fertility remain poorly understood. To establish whether parental irradiation can affect the survival and fertility of directly exposed organisms and their offspring, crustacean *Daphnia magna* were given 10, 100, 1000 and 10,000 mGy of acute γ-rays. Exposure to 1000 and 10,000 mGy significantly compromised the viability of irradiated *Daphnia* and their first-generation progeny, but did not affect the second-generation progeny. The fertility of F_0 and F_1 *Daphnia* gradually declined with the dose of parental exposure and significantly decreased at dose of 100 mGy and at higher doses. The effects of parental irradiation on the number of broods were only observed among the F_0 *Daphnia* exposed to 1000 and 10,000 mGy, whereas the brood size was equally affected in the two consecutive generations. In contrast, the F_2 total fertility was compromised only among progeny of parents that received the highest dose of 10,000 mGy. We propose that the decreased fertility observed among the F_2 progeny of parents exposed to 10,000 mGy is attributed to transgenerational effects of parental irradiation. Our results also indicate a substantial recovery of the F_2 progeny of irradiated F_0 *Daphnia* exposed to the lower doses of acute γ-rays.

1. Introduction

The results of recent studies have clearly demonstrated the existence of non-targeted effects of ionising radiation (Kadhim et al., 2013). This phenomenon describes a number of effects, including transgenerational genomic instability where parental exposure to ionising radiation destabilises the genome of their offspring (Barber and Dubrova, 2006; Dubrova, 2013). As destabilisation of the offspring's genomes results in accumulation of mutations at protein-coding genes and chromosome aberrations, these observations imply that a number of fitness-related traits in the offspring may be affected by this process. Given that the abovementioned effects can manifest over a number of generations, they may therefore be regarded as an additional component of the genetic risk of ionising radiation (Barber and Dubrova, 2006; Dubrova, 2013; Little et al., 2013).

Being initially established in mammals, the long-term effects of parental exposure to a number of environmental factors have also been described in other species – *Arabidopsis thaliana* (Suter and Widmer, 2013; Groot et al., 2016), zebrafish (King Heiden et al., 2009; Baker et al., 2014), rainbow trout (Smith et al., 2016), as well as in some invertebrates, including *Caenorhabditis elegans* (Buisset-Goussen et al., 2014), the earthworm *Eisenia fetida* (Hertel-Aas et al., 2007, 2011) and crustaceans (Alonzo et al., 2008; Plaire et al., 2013; Parisot et al., 2015).

It should be noted that the abovementioned studies on transgenerational instability in mice have so far provided little experimental evidence regarding the multigenerational effects of parental exposure on viability and fertility. As such analysis in mammals is extremely laborious, the introduction of new model organisms characterised by short life span and high fertility is clearly warranted. We and others have previously shown that the parthenogenetic crustacean *Daphnia magna* represents a useful and very sensitive experimental model for the analysis of long-term effects of exposure to ionising radiation (Sarapultseva and Bychkovskaya, 2010; Sarapultseva and Gorski, 2013; Alonzo et al., 2008; Massarin et al., 2010). *Daphnia* is characterised by relatively short life span which seldom exceeds 10–11 weeks. For most of the growth season females produce a clutch of at least 10 eggs every 3–4 days (Ebert, 2005). Moreover, *Daphnia* is an ecologically important organism

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well-studied in the context of evolution, ecology, ecotoxicology (Stollewerk, 2010) and genomics (Colbourne et al., 2011).

Given the abovementioned advantages, *D. magna* has been used in numerous studies aimed to analyse the effects of ionising radiation on mortality and fecundity (reviewed in Dallas et al. (2012) and Fuller et al. (2015)). In a number of studies the effects of chronic exposure have been established across several successive generations of irradiated *D. magna* (Alonzo et al., 2008; Zeman et al., 2008; Massarin et al., 2010; Plaire et al., 2013; Parisot et al., 2015). However in our opinion, their results do not provide a definitive evidence for the manifestation of transgenerational effects in *D. magna*, as daphnids were continuously irradiated across all generations. It should be stressed that the manifestation of transgenerational effects can only be established by analysing the non-exposed progeny of irradiated parents. For example, in our recent study we have shown that acute parental irradiation at doses of 100 and 1000 mGy significantly reduces the life-span of directly exposed *Daphnia* and their F_1 offspring (Sarapultseva and Gorski, 2013). In other words, our data suggest that the phenomenon of radiation-induced transgenerational inheritance manifests in *Daphnia*, affecting the F_1 survival. Here we report that parental irradiation can also compromise the fertility of directly exposed *Daphnia* and their progeny.

2. Materials and methods

2.1. Daphnia maintenance

The strain of *Daphnia magna* Straus used in our experiments was originally collected in the pond of the Moscow Zoo and maintained for several years at the laboratory in continuous parthenogenetic reproduction following the OECD guideline 211 (Organisation for Economic Co-operation and Development, 2012) with modifications. *D. magna* were reared at density of one animal per 50 mL in aerated dechlorinated filtered tap-water (pH 7.5–8.2, O_2 ~9.0 mg/l; total mineralisation ~6.4 mg/l, Ca:Mg 4:1, Fe 0.3 mg/l, Mn 0.1 mg/l) renewed twice a week. *Daphnia* were fed with green algae suspension (*Chlorella vulgaris*) at daily ration of 90 µg C per daphnid (4×10^5 cells/mL). Algae were cultured in high salt Tamiya medium (Anderson et al., 2005). Algal cell concentration was determined using Goryaeva camera (PJSC "Steklopribor" Ukraine) on an optical density meter (IPS-03, Ltd. OMIKRON, Krasnoyarsk, Russia). *D. magna* were incubated at 20 °C (±0.5 °C) on a 12 h/12 h light/dark cycle photoperiod at light intensity 700–1200 lx (Climate Control model R2, LLC Omicron, Krasnoyarsk, Russia). Neonates were removed every weekday.

2.2. Irradiation

One-day-old *Daphnia* from the third broods of at least five females were acutely γ-irradiated at the Lutch Facility (^{60}Co source, Lutch Irradiator, Latenegro, Latvia) at Medical Radiological Research Center, Obninsk, Russia at doses of 10 mGy (for 21 s at 28 mGy min^{-1}), 100 mGy (for 35 s at 170 mGy min^{-1}), 1000 and 10,000 mGy (for 1 and 10 min, respectively, at 1000 mGy min^{-1}). The absorbed dose was assessed using 27,012 and DKS-101 dosimeters. During irradiation, *D. magna* were kept in the plastic tubes containing 15 mL of water with 10 individuals. All corresponding non-treated control groups were placed in the same conditions but without irradiation.

2.3. Survival and fertility

Control and irradiated daphnids (referred to as the exposed generation F_0) were maintained individually, one per test vessel, with 50 mL of water in each glass vials (Apparaturschik Ltd., Moscow Region). Experimental vials were checked daily during 21 days for survival and neonatal removal. Each day, neonates were removed and counted as well as all dead daphnids.

To analyse the effects of parental exposure on the successive generation (F_1), one-day-old neonates from the third broods of generation F_0 were randomly taken from at least three females of irradiated or control groups and transferred to glass vials with 50 mL of water (one *Daphnia* per vial). Using the same protocol, a group of second-generation offspring (F_2) was also established. Generations F_1 and F_2 were maintained as the original samples but without exposure to γ-irradiation. The survival and fecundity of the parthenogenetic progeny from these generations were measured on a daily basis over 21 days.

2.4. Data analysis

The probability of survival was estimated using an algorithm proposed by Breslow and Day (1987). The data were analysed using ANOVA and the Kruskal-Wallis test. All statistical analyses were conducted using SYSTAT 13 version (Systat Software Inc., San Jose, CA, USA).

3. Results

3.1. The effects of parental irradiation on survival

Fig. 1 summarises the effects of parental irradiation on survival of directly exposed daphnids and their first- and second-generation progeny. As the survival of control F_0, F_1 and F_2 daphnids did not significantly differ ($P > 0.74$), we therefore combined the control data for all three generations. The survival of F_0 *Daphnia* exposed to 1000 and 10,000 mGy of acute γ-rays was significantly compromised (Fig. 1A). Parental irradiation with the same doses also affected the survival of their F_1 progeny (Fig. 1B). In the meantime, the survival of F_2 progeny of irradiated parents did not significantly differ from that in controls (Fig. 1C).

3.2. The effects of parental irradiation on fertility

In all irradiated and control groups, daphnids started producing eggs at the age of 9 days independent of F_0 generation dose and released 3–4 successive broods over the 21-day period. As exposure to ionising radiation compromised the viability, here we analysed the fertility of daphnids which survived to the end of the experiment. Table 1 presents the results of ANOVA analysis estimating the effects of parental irradiation on the number of progeny per *Daphnia*. The two-way ANOVA analysis revealed that the magnitude of the effects of parental irradiation on fertility differed across generations. Acute γ-irradiation also differentially affected the number of broods and brood size. According to the one-way ANOVA, the significant effects of irradiation on the number of progeny per *Daphnia* were observed in all generations.

The detailed analysis of the effects of parental irradiation on fertility of daphnids is given in Table 2. According to our data, acute parental exposure to γ-rays equally compromised the total fertility of irradiated F_0 *Daphnia* and their F_1 progeny (Fig. 2A). The fertility of F_0 and F_1 *Daphnia* declined with the dose of parental exposure and significantly decreased at dose of 100 mGy and at higher doses. In contrast, the F_2 total fertility was compromised only among progeny of parents that received the highest dose of 10,000 mGy.

We also analysed the effects of parental irradiation on the brood size and number of broods. The number of broods was only affected among the F_0 *Daphnia* exposed to 1000 and 10,000 mGy, whereas in the F_1 and F_2 progeny of irradiated parents it did not