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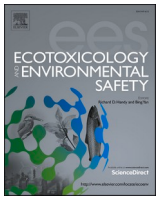

Ionizing radiation affects the demography and the evolution of *Caenorhabditis elegans* populations

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ABSTRACT

Ionizing radiation can reduce survival, reproduction and affect development, and lead to the extinction of populations if their evolutionary response is insufficient. However, demographic and evolutionary studies on the effects of ionizing radiation are still scarce. Using an experimental evolution approach, we analyzed population growth rate and associated change in life history traits across generations in *Caenorhabditis elegans* populations exposed to 0, 1.4, and 50.0 $mGy.h^{-1}$ of ionizing radiation (gamma external irradiation). We found a higher population growth rate in the 1.4 $mGy.h^{-1}$ treatment and a lower in the 50.0 $mGy.h^{-1}$ treatment compared to the control. Realized fecundity was lower in both 1.4 and 50.0 $mGy.h^{-1}$ than control treatment. High irradiation levels decreased brood size from self-fertilized hermaphrodites, specifically early brood size. Finally, high irradiation levels decreased hatching success compared to the control condition. In reciprocal-transplant experiments, we found that life in low irradiation conditions led to the evolution of higher hatching success and late brood size. These changes could provide better tolerance against ionizing radiation, investing more in self-maintenance than in reproduction. These evolutionary changes were with some costs of adaptation. This study shows that ionizing radiation has both demographic and evolutionary consequences on populations.

1. Introduction

Since the end of World War II, radionuclide releases from anthropogenic activities (military or civil) to the environment have increased (Rhodes et al., 2020). For example, the accidents at the Chernobyl (Ukraine) or Fukushima (Japan) nuclear power plants have released respectively 1.4×10^{19} and 5.2×10^{17} Bq of radionuclides into the environment (IAEA, 2006; Okano et al., 2016). Ionizing radiation can affect survival, reproduction and development of plants, mammals, fish, or invertebrates (Dallas et al., 2012; Real et al., 2004). Furthermore, its deleterious effects can last over several generations (i.e., *Eisenia fetida;* 2 generations: Hertel-Aas et al., 2007; *Daphnia magna;* 3 generations: Sarapultseva and Dubrova, 2016; *Oncorhynchus mykiss;* 3 generations: Smith et al., 2016; *Caenorhabditis elegans;* 3 generations: Buisset-Goussen et al., 2014; *Caenorhabditis elegans;* 4 generations: Guédon et al., 2021). These studies have focused on the effect of ionizing radiation on survival and reproduction over very few generations, but have not investigated the consequences on reproduction over 20 generations and especially on population growth over generations as we have down in this study.

Regulatory ecotoxicological risk assessment of pollutants (chemical, ionizing radiation, etc.) focuses mainly on the study of individual effects over a short-term exposure (Zhang et al., 2010; Zhou et al., 2016), neglecting in many cases the study of long-term or multigenerational effects at the population level (Hope, 2006; Kapustka, 2008; Thoré et al., 2021). Thus, this type of approach may underestimate the level of risk to natural populations, which may be exposed for several generations to a pollutant (Thoré et al., 2021). Long-term exposure may induce genetic or non-genetic inheritance change (Danchin et al., 2011), leading to evolutionary consequences on populations, which may alter our ecological risk assessment diagnoses (Coutellec and Barata, 2011; Straub et al., 2020). For example, the new environmental conditions resulting from the presence of the pollutant may induce plastic, genetic or epigenetic responses on life history traits (i.e., all traits that directly contribute to offspring production and survival) (Reznick et al., 2000; Hoffmann and Willi, 2008). If adaptive, this change in life history traits can in turn improve the fitness of the population and lead to higher

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population growth rate and resilience (De Leániz et al., 2007; Dutilleul et al., 2017).

Studies showing adaptation to ionizing radiation are extremely rare. It has for instance been shown in the fungi *Hormoconis resinae* (Tugay et al., 2011), in bacteria (Ruiz-González et al., 2016), or in the arthropod *Chorthippus albomarginatus* (Bonisoli-Alquati et al., 2017). In contrast, the deleterious effects of ionizing radiation were amplified over three generations in *Daphnia magna* (Parisot et al., 2015) and could lead to the extinction of populations (Alonzo et al., 2016). Moreover, increasing the dose rate of ionizing radiation decreased population size and increased risk of extinction in *Daphnia pulex* populations (Marshall, 1966). These results show how multigenerational experiments bring essential knowledge about the demographic and evolutionary dynamics of a population affected by pollution, and therefore improve the assessment of ecological risks caused by pollutants.

Population growth rate, or the temporal change in population size, is fundamental for population ecology and conservation (Wells et al., 1998; Wisdom et al., 2000), and ecotoxicology (Sibly et al., 2002). For Forbes and Calow (1999) population growth rate is a better measure of the responses to toxicants than individual-level effects, because it integrates potentially complex interactions among life-history traits and provides a more relevant measure of ecological impact. Pollutants can adversely affect individual birth rates, growth rates, or mortality rates, and thus reduce population growth rate (Sibly et al., 2002). For example, heavy metal pollution decreased population growth rate in the red mason solitary bee, *Osmia rufa* (Moroń et al., 2014). Similarly, pollution by selenium, antibiotic tetracycline, or by sulfamethoxazole affected the population growth rate of *C. elegans* (Li et al., 2014; Vangheel et al., 2014; Yu et al., 2017). Nevertheless, measuring population growth alone does not provide insight into the underlying biological traits impacted by pollution, and conversely individual responses do not necessarily correctly predict population scale responses (Spromberg and Meador, 2006). Thus, combining the study of population growth with measures of life history traits could be particularly relevant for assessing the ecological risks of pollutants.

Here, we studied the population growth rate and associated change in life history traits of a *C. elegans* population that we chronically exposed to ionizing radiation (gamma irradiation) for 60 days, or around 20 generations, under experimental conditions. The aim of this study was (1) to characterize *C. elegans* population change exposed to gamma radiation, and (2) to determine whether the variations observed are related to phenotypic plasticity or to adaptive processes. We used the nematode *C. elegans* (Nematoda, Rhabditidae) an androdiecious organism because of its short life cycle (reaches sexual maturity within three days at 20 °C), its short lifespan (21 days at 20 °C), and its high fecundity (300 offspring from self-fertilized hermaphrodites, up to 1000 when crossed with males). *C. elegans* is a powerful model for evolutionary experiments (Braendle et al., 2007; Goussen et al., 2013; Gray & Cutter, 2014; Dutilleul et al., 2017). It was previously found that ionizing radiation reduces reproduction and somatic growth in *C. elegans* (Buisset-Goussen et al., 2014; Dufourcq-Sekatcheff et al., 2021; Guédon et al., 2021; Lecomte-Pradines et al., 2017; Maremonti et al., 2019). However, these studies did not examine the impact of these changes on the evolution of the population, in particular on the adaptive response. We hypothesized that chronic exposure to ionizing radiation reduces the population growth rate and slows down life history. In addition, we hypothesized that multigenerational exposure to ionizing radiation leads to local adaptation of populations to this stressor.

2. Material and methods

2.1. Test organism and population maintenance

We used the population of *C. elegans* A6140 created from a mixture of 16 wild isolates (Noble et al., 2017; Teotonio et al., 2012) and characterized by a high genetic diversity and about 20% of males. We placed the nematodes on 6 cm Petri dishes filled with 12 mL of nematode growth medium (NGM). Petri dishes were seeded with *Escherichia coli* bacteria (OP50 strain) ad libitum and exposed to UV radiation (Bio-Link Crosslinker, $\lambda = 254$ nm; intensity = 200 μwatt. m^{-2}) for 15 min to stop bacterial growth and to avoid uncontrolled heterogeneity in food availability (Dutilleul et al., 2013). Nematode populations were cultured at 20 °C and 80% relative humidity to have a generation time of approximately 3 days (Byerly et al., 1976). Before exposure, the stock population was maintained for at least 75 days, or 25 three-day transfers (around 25 generations), in pairs of Petri dishes, with 500 individuals in each dish. Every three days, we transferred nematodes into new dishes to ensure they were fed ad libitum. To do so, we washed nematodes off the two Petri dishes with an M9 solution. We then pooled them in a 15 mL tube Falcon®, homogenized, and the number of individuals, from the egg (i.e., embryo *ex utero*) to the adult stage, was estimated based on six sample drops of 5 μL (Teotonio et al., 2012). Two separate volumes corresponding to 1000 individuals at all developmental stages were then transferred into two new Petri dishes.

2.2. Irradiation conditions

The external gamma radiation exposure was conducted at the Mini Irradiator for Radio Ecology ^{137}Cs irradiation facilities, at the *"Institut de Radioprotection et de Sûreté Nucléaire"* (MIRE, IRSN, Cadarache, France). We used the same irradiation facilities, and the same protocol as previously described by Quevarec et al. (2022). The irradiators were placed in incubators with 80% relative humidity and a temperature set at 20 °C. *C. elegans* populations were exposed to three gamma radiation treatments (5 replicates per treatment): 0, 1.4 and 50.0 $mGy.h^{-1}$, corresponding to the control, low and high irradiation treatments, respectively. We measured dose rates with radiophoto luminescent (RPL) micro-dosimeters twice during the experiment. As explained in Quevarec et al. (2022), the Petri dishes were placed vertically in the irradiator to homogenize the dose received over the entire dish. Placing the plates at different distances from the source and separated by shields (Petri dish filled with lead filings) allowed us to obtain the required dose rates.

2.3. Multigenerational experiment

We used three dose rate gamma radiation treatments: control (0.0 $mGy.h^{-1}$), low irradiation (1.4 $mGy.h^{-1}$), and high irradiation (50.0 $mGy.h^{-1}$). Low irradiation treatment had an environmental reality. In the Chernobyl Exclusion Zone, terrestrial wildlife could be exposed to dose rates up to ~10 $mGy.h^{-1}$ (Garnier-Laplace et al., 2013). High irradiation treatment was selected because studies have shown an impact on *C. elegans* reproduction at a similar dose rate (Buisset-Goussen et al., 2014; Dubois et al., 2018; Dufourcq-Sekatcheff et al., 2021; Guédon et al., 2021; Maremonti et al., 2019). For each treatment, we created five independent replicates taken from the stock population and maintained for over 60 days, with a transfer to new Petri dishes once every three days. Although three days correspond to about one generation in natural conditions, gamma irradiation has been shown to delay growth and reproduction at high dose rates (Lecomte-Pradines et al., 2017). Furthermore, it is possible that this generation time evolves over time and with irradiation conditions. In this context, we cannot guarantee that every three-day transfer corresponds precisely to a generation. However, we assume that 60 days would corresponds to about 20 generations. We thus describe the dynamics of changes during the experiment as a function of the number of days, based on the number of three-day transfers.

Nematode populations were not synchronized during multigenerational experiment, similar to other studies (e.g., Menzel et al., 2001; Goussen et al., 2013; Dutilleul et al., 2014; Dutilleul et al., 2017; Kanzaki et al., 2018; Quevarec et al., 2022). We chose this method to minimize as much as possible the different stressors related to the experiment, with

the exception of ionizing radiation. Because *C. elegans* is relatively resistant to ionizing radiation (Sakashita et al., 2012), the impact of synchronization could partially overshadow that of ionizing radiation. In addition, these synchronization methods take time (Porta-de-la-Riva et al., 2012), during which populations cannot be irradiated. The potential biases of these methods seemed unacceptable to us to properly study the impact of ionizing radiation.

At the beginning of each transfer, each replicate contained initially 1000 worms equally distributed into two Petri dishes (initial density of 500 worms/plate). At the end of each three-day transfer, we estimated the final population density (i.e., from egg to adult stage). We then calculated population growth rate R as:

$$R = \frac{\ln N_t - \ln N_0}{t}$$

Where N_0 and N_t are respectively the initial and final population density, all developmental stages together, and t is the incubation time in days (Yin et al., 2013). We calculated the realized fecundity (i.e., the number of offspring produced in a population in a given environment) as the number of eggs per 1000 hatched individuals (i.e., from larval to adult stage; population estimated after 3 days of growth) per unit time (Tarsi and Tuff, 2012). We estimated hatching success and brood size at transfer 0, 2, 5, 8, 11, 14, 17, and 20. For the determination of hatching success, we transferred 100 eggs per replicate into a new Petri dish (3 cm) with NGM. These eggs came from the Petri dishes containing the populations and were collected after washing the dishes. Forty-eight hours after the transfer, we counted hatched nematodes (L4 and young adults), using a stereomicroscope (Olympus SZX12, 1.6 × 90 magnification). Hatching success was estimated as the ratio of the number of hatched individuals against the number of eggs initially put on the Petri dish. To measure the brood size from self-fertilized hermaphrodites, four hermaphrodites per replicate (i.e., 20 hermaphrodites per treatment), from the hatching success protocol, were isolated outside the irradiator in a 12 well plate. These hermaphrodites were isolated before they started to lay eggs or mate with males. We counted early brood size of each hermaphrodite with a stereomicroscope at 96 h after egg collection on the Petri dish. Then, the hermaphrodite was transferred to a new well in the plate. After 2 days (144 h after egg collection), we counted late brood size. We calculated the total brood size as the sum of early and late brood size.

2.4. Reciprocal-transplant experiments

We performed a reciprocal-transplant experiments between the control and both the low and the high irradiation treatment to test for the hypothesis of irradiation-induced adaptive changes in the treated populations (Fig. 1). We used hatching success and brood size as indexes of fitness at the population level.

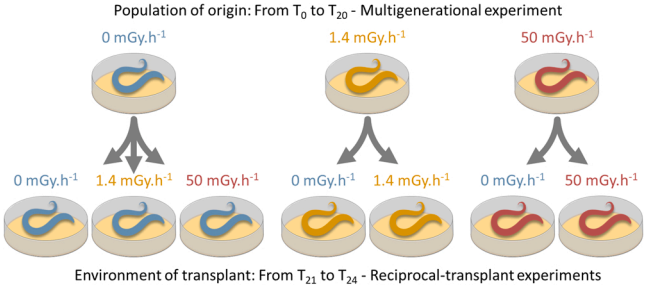

Fig. 1. Schematic overview of the reciprocal-transplant experiment design for *C. elegans* populations in three gamma radiation treatments. After 20 transfers in the initial environment (Population of origin) (0, 1.4 and 50 mGy.h^{-1}) during the multigenerational experiment, populations were placed in a second environment (environment of transplant) for four transfers. This partial reciprocal transplant was performed as shown here. The measurements of hatching success and brood size were made after four transfers to ensure that the differences between populations was due to genetic differentiation and not parental effects.

At transfer 20 (60 days), we transferred individuals from the treated populations into the non-irradiated environment, and individuals from the control populations into either irradiated environment (1.4 and 50 mGy.h^{-1}). Populations that had spent the last 60 days in either the controlled environment or in the two irradiated treatments were called "population of origin", and the environment in which they had spent the last 60 days as the "environment of origin". We called "environment of transplant" the novel environment the populations were transferred into.

We proceeded as described earlier with a new transfer into a new Petri dish, once every three days. We created five replicates for each condition of reciprocal-transplant experiments, for a total of 40 replicates. At the end of the fourth transfer, or 12 days, we estimated hatching success and brood size, as described above. Measuring the traits after four transfers to exclude parental effects on the difference between groups of nematodes, and thus to ensure that the differences between populations reflected genetic differentiation (Badyaev and Uller, 2009; Kawecki et al., 2012; Dutilleul et al., 2014).

2.5. Statistical analysis

Before the analysis, we log-transformed data of realized fecundity. We used a Mixed Generalized Additive Model (GAMM) with R software (Team, 2013) and the Mgcv package (Wood et al., 2016) to analyze population growth rate, realized fecundity and hatching success (data in supplementary informations; Table A.1, Table A.2) with quasi-Poisson (log link function), Gaussian and quasi-binomial (logit link function) distribution, respectively. No overdispersion of the data was observed. Population growth rate, realized fecundity, and hatching success were analyzed as a function of the number of transfers (as a continuous variable), irradiation treatment (i.e., control, low and high irradiation) and their interaction as fixed effects. ID of the replicate and measurement group ID (only for population growth rate and realized fecundity) were added as random effects. The smoothing was performed on the variable transfer in function of treatment.

We analyzed early, late and total brood size data (data in supplementary informations; Table A.3) by considering the effect of different treatments on the number of laid eggs using Kruskal-Wallis rank sum test with R software and the stats package (Team, 2013). Then, a post hoc pairwise comparison with Holm correction was realized between each treatment. We analyzed egg-laying delay as a function of treatment using Spearman's rank correlation between early and late brood size.

For the reciprocal-transplant experiments, we analyzed hatching success and brood size (early, late and total) using Generalized Linear Mixed Model (GLMM) with Ade4 package (Bougeard and Dray, 2018; Dray and Dufour, 2007) and glmmTMB package (Brooks et al., 2017), with environment of origin, environment of transplant, and the interaction between the two as fixed effects, and replicate ID as a random effect (data in supplementary informations; Table A.4, Table A.5). For hatching success, we used a binomial distribution and a logit link function. We used a quasi-Poisson distribution and a log link function for low and high irradiation and for early, late, and total brood size, apart from high irradiation treatment for early brood size, where we used a Gaussian distribution. No overdispersion of data was observed.

Because we used GAMMs and GLMMs with different link functions, we provide the estimated parameters in the text after back transforming the coefficient using the inverse log function or inverse logit function (untransformed coefficient are shown in the Tables). The log-transformed raw data of realized fecundity are also back transformed in the text.

3. Results

3.1. Multigenerational experiment

Control, low and high irradiation treatments showed population growth rates from larval to adult stage equal to 1.51, 1.53 and 1.48, respectively (Table 1). Control populations showed a population growth rate significantly lower than the low irradiation treatment, and significantly higher than the high irradiation treatment (Table 1a; Fig. 2a). In the control populations, population growth rate decreased slightly, but significantly, between transfers 1 and 20. Population growth rate fluctuated significantly without increasing or decreasing in both irradiation treatments, and its frequency increased with the treatment dose (Table 1b; Fig. 2a, Fig. A.1).

Control, low and high treatment populations showed realized fecundity equal to 2779, 2243 and 2053 eggs per 1000 individuals, respectively. Realized fecundity was significantly higher in the control than in the low and high irradiation treatments (Table 2a; Fig. 2b). Realized fecundity in the control populations increased slightly from transfers 1–10, and decreased slightly, but significantly from transfers 10–20. Realized fecundity fluctuated significantly without increasing or decreasing in both irradiated treatments (Table 2b; Fig. 2b, Fig. A.2).

The hatching success rate was estimated at 0.92, 0.90 and 0.85 for control, low and high irradiation treatments, respectively (Table 3). Control populations showed a significantly higher proportion of hatched eggs than high irradiation populations but did not differ significantly from low irradiation populations (Table 3a; Fig. 2c). Hatching success in high treatment fluctuated across time (Table 3b; Fig. 2c, Fig. A.3).

Mean early brood size was at 252, 253 and 231 eggs per individual for control, low and high irradiation treatments, respectively (Fig. 3a). High irradiation treatment showed a significantly smaller proportion of early brood size (Kruskal-Wallis chi-squared = 10.651, df = 2, $p = 0.00487$) than the control ($p = 0.012$, Holm adjustment method) and low irradiation treatment ($p = 0.012$, Holm adjustment method). In contrast, no significant difference was observed for low irradiation treatment compared to control treatment ($p = 0.867$, Holm adjustment method). The mean late brood size was at 18, 23 and 26 for control, low and high irradiation treatments, respectively in the multigenerational experiment (Fig. 3b). No significant difference was observed between treatments (Kruskal-Wallis chi-squared = 0.0442, df = 2, $p = 0.978$). The mean total brood size was at 268, 276 and 252 for control, low and high irradiation treatments, respectively in the multigenerational experiment (Fig. 3c). High irradiation treatment showed a significantly smaller proportion of total brood size (Kruskal-Wallis chi-squared = 7.799, df = 2, $p = 0.0203$) than the control ($p = 0.044$, Holm adjustment method) and low irradiation treatment ($p = 0.044$, Holm adjustment method). In contrast, no significant difference was observed for low irradiation treatment compared to control treatment ($p = 0.824$, Holm adjustment method). Finally, results showed a negative correlation between early and late brood size in individual level for high irradiation treatment only (Spearman's rank correlation: S = 349, $p = 0.0077$, $\rho = -0.243$). No significant correlation was observed for low irradiation treatment (Spearman's rank correlation: S = 243, $p = 0.870$, $\rho = 0.015$) and control treatment (Spearman's rank correlation: S = 362, $p = 0.124$, $\rho = -0.139$).

Table 1
Effects of (a) irradiation condition (Control: 0.0 mGy.h^{-1}, Gamma low: 1.4 mGy.h^{-1}, and Gamma high: 50.0 mGy.h^{-1}) and (b) Time (EDF: Effective degrees of freedom) on *C. elegans* population growth rate during the 20 transfers of a multigeneration experiment. Results from a Generalized Additive Mixed Model (GAMM). * , $P < 0.05$; * *, $P < 0.01$; * ** , $P < 0.001$.

a)	Estimate	Std. Error	t	P	
(Intercept)	0.413	0.003	123.822	2.00 e-16	* **
Gamma Low	0.010	0.005	2.086	0.037	*
Gamma High	-0.020	0.005	-4.182	3.02 e-05	* **
Approximate significance of smooth terms					
b)	edf	Ref.df	F	P	
s (Time): Control	1.980	1.980	6.788	0.003	* *
s (Time): Gamma Low	6.565	6.565	9.238	2.00 e-16	* **
s (Time): Gamma High	11.474	11.474	6.275	2.00 e-16	* **

3.2. Reciprocal transplant experiments

In the experiment comparing control and low irradiation treatment, we found a significant interaction between population of origin and environment of transplant on hatching success (Table 4a; Fig. 4a). Hatching success was maximized when populations have evolved in the same environment of origin and transplant. The transplanted populations in the control treatment had a better hatching success than in low irradiation treatment. In the control to high irradiation treatment transplant, we did not find any significant results between the population of origin and the environment of the transplant on hatching success (Table 4a; Fig. 4b).

The transplant between control and low irradiation treatment showed a significant interaction between population of origin and environment of transplant for late brood size (Table 4c; Fig. 4e). Late brood size decreased from the control to the low irradiation environment, whereas the control population did not change its late brood size after being transplanted into a low irradiation treatment (Table 4c; Fig. 4e). In the control to high irradiation treatment transplant on late brood size and for the transplants on early and total brood size, we did not find any significant result between the population of origin and the environment of the transplant (Table 4; Fig. 3).

4. Discussion

In agreement with our initial hypothesis that ionizing radiation reduces population growth rate, we found a 2% lower growth rate in the highly irradiated populations compared to the control ones. However, the 1% higher growth rate in the low-irradiated populations compared to the control populations did not support our hypothesis for low radiation levels. Besides, irradiation increases fluctuations in population growth. Realized fecundity decreased by 19% in the low irradiation and by 26% in the high irradiation treatment compared to the control condition. We also showed that high doses of ionizing radiation decreased total brood size by 8% and delayed egg laying by decreasing early brood size by 6% compared to the control condition. High doses of ionizing radiation affected embryogenesis and recruitment with a decrease of 7% hatching success compared to the control condition. No significant effects were found on life history traits for the low dose treatment compared to the control.

Consistent with our hypothesis that selective pressure exerted by ionizing radiation would lead to evolutionary change in life history traits, we found some adaptive responses in populations subjected to irradiation. Hatching success and late brood size were significantly higher in the low irradiation conditions, after 60 days (20 transfers), compared to populations newly placed in the irradiated environment, suggesting evolutionary change towards a slower life history. Although not significant, highly irradiated populations showed similar trends toward a slower life history. The absence of significant interaction for early brood size indicates that changes in this trait under irradiation conditions may not be adaptive (Kawecki and Ebert, 2004). Finally, the lower hatching success and late brood size of the irradiated populations back to the control environment reveal some costs of adaptation to gamma radiation (Dutilleul et al., 2017).

4.1. Multigenerational experiment: characterizing demographic change

Previous reviews have found a negative impact of ionizing radiation

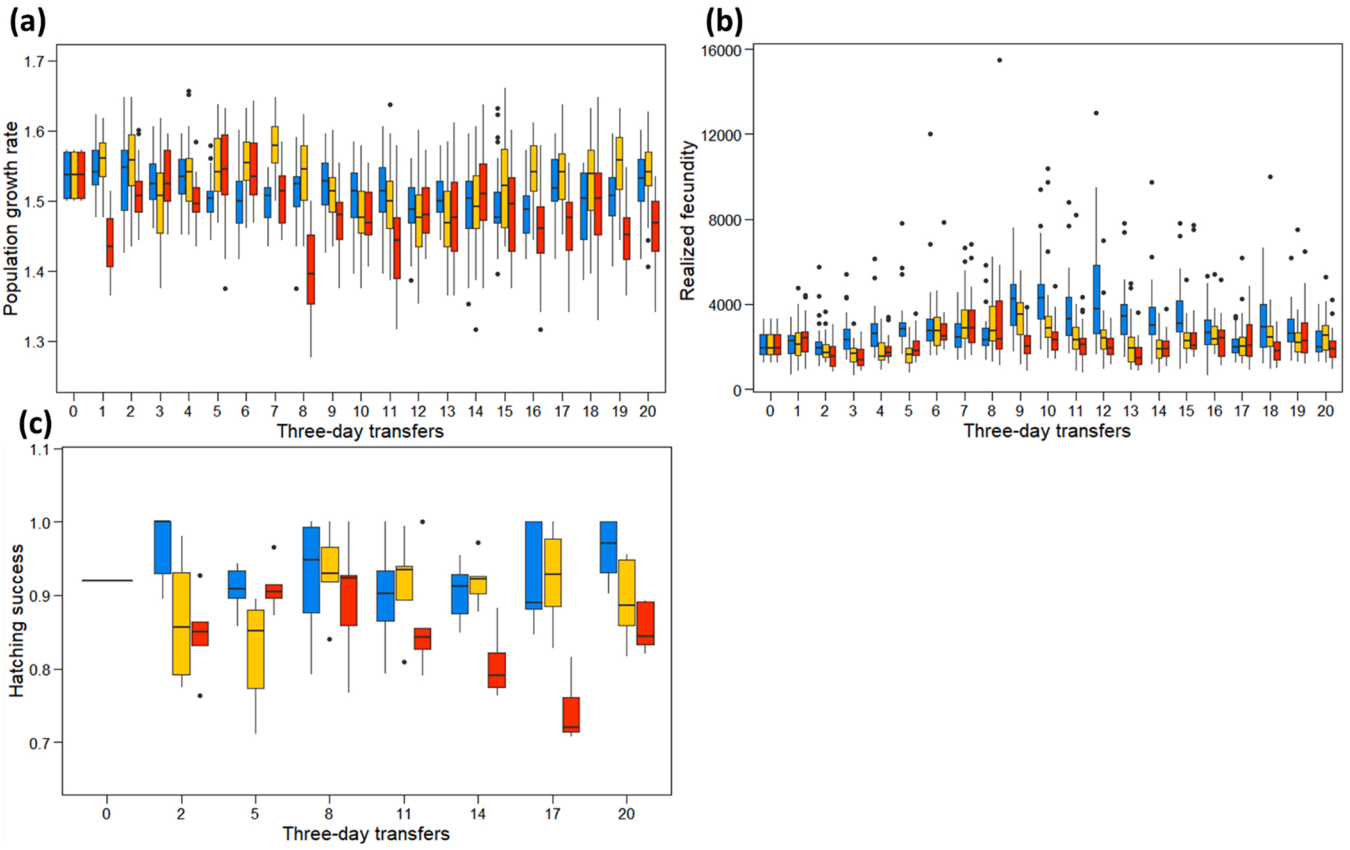

Fig. 2. Boxplot of (a) population growth rate and (b) realized fecundity over time during 20 three-day transfers and (c) hatching success over time (i.e., three-day transfers: 0, 2, 5, 8, 11, 14, 17 and 20) for *C. elegans* populations living in different gamma radiation environments. Blue: control; yellow: low radiation (1.4 mGy.h^{-1}); red: high radiation (50.0 mGy.h^{-1}).

Table 2
Effects of (a) irradiation condition (Control: 0.0 mGy.h^{-1}, Gamma low: 1.4 mGy.h^{-1}, and Gamma high: 50.0 mGy.h^{-1}) and (b) Time (EDF: Effective degrees of freedom) on *C. elegans* realized fecundity during the 20 transfers of a multigeneration experiment. Results from a Generalized Additive Mixed Model (GAMM). * , P < 0.05; * *, P < 0.01; * ** , P < 0.001.

a)	Estimate	Std. Error	T	P	
(Intercept)	7.935	0.028	287.832	2.00 e-16	* **
Gamma Low	-0.214	0.039	-5.477	4.94 e-08	* **
Gamma High	-0.304	0.039	-7.784	1.18 e-14	* **
Approximate significance of smooth terms					
b)	Edf	Ref.df	F	P	
s (Time): Control	6.208	6.208	14.226	2.00 e-16	* **
s (Time): Gamma Low	8.184	8.184	11.330	2.00 e-16	* **
s (Time): Gamma High	9.210	9.210	6.309	2.00 e-16	* **

Table 3
Effects of (a) irradiation (Control: 0.0 mGy.h^{-1}, Gamma low: 1.4 mGy.h^{-1}, and Gamma high: 50.0 mGy.h^{-1}) and **(b)** Time (EDF: Effective degrees of freedom) on *C. elegans* hatching success during the 20 transfers of a multigeneration experiment. * , P < 0.05; * *, P < 0.01; * ** , P < 0.001.

a)	Estimate	Std. Error	t	P	
(Intercept)	2.433	0.170	14.340	2.00 e-16	* **
Gamma Low	-0.194	0.232	-0.837	0.405	*
Gamma High	-0.674	0.226	-2.991	0.004	* *
Approximate significance of smooth terms					
b)	Edf	Ref.df	F	P	
s (Time): Control	1.852	1.852	1.549	0.167	
s (Time): Gamma Low	1.000	1.000	2.737	0.101	
s (Time): Gamma High	2.783	2.783	4.111	0.0102	*

on reproduction and survival on many species (plants, mammals, fish, or invertebrates) (Dallas et al., 2012; Real et al., 2004). These traits can strongly affect the demography of the population (Moyson et al., 2019). In our case, the lower population growth rate of the high irradiation treatment reflects a parallel decrease in realized fecundity, brood size and hatching success. In short-lived species like *C. elegans*, fecundity can impact population growth to a higher extent than survival (Sibly et al., 2002). In the low irradiation treatment, the significant decrease for realized fecundity and the absence of a significant increase for brood size and hatching success cannot explain the increased population growth rate. This decrease in fecundity could be caused by other unmeasured parameters, changing the ratio between the number of eggs and the number of individuals. First, low ionizing radiation could increase developmental speed during the 20 transfers, as has been shown in *C. elegans* exposed to manganese (Lin et al., 2006) or with an increase of temperature (Byerly et al., 1976). Second, higher adult survival or longer lifespan could also decrease the ratio between the number of eggs and the number of individuals. Indeed, Johnson and Hartman (1988) suggested that ionizing radiation could increase *C. elegans* lifespan at acute doses between 100 and 300 Gy. Moreover, some stressors such as a dietary restriction (Kaeberlein et al., 2006) or chemicals (thioallyl compounds: Ogawa et al., 2016; mianserin: Petrascheck et al., 2009) can increase the lifespan in *C. elegans*. More generally, other unmeasured demographic traits in this study may impact population growth rate (Sibly et al., 2002), and could explain our results. For example, age structure (Hoy et al., 2020), population density (Tanner, 1966) or sex ratio (Le Galliard et al., 2005; Lenz et al., 2007) could change population

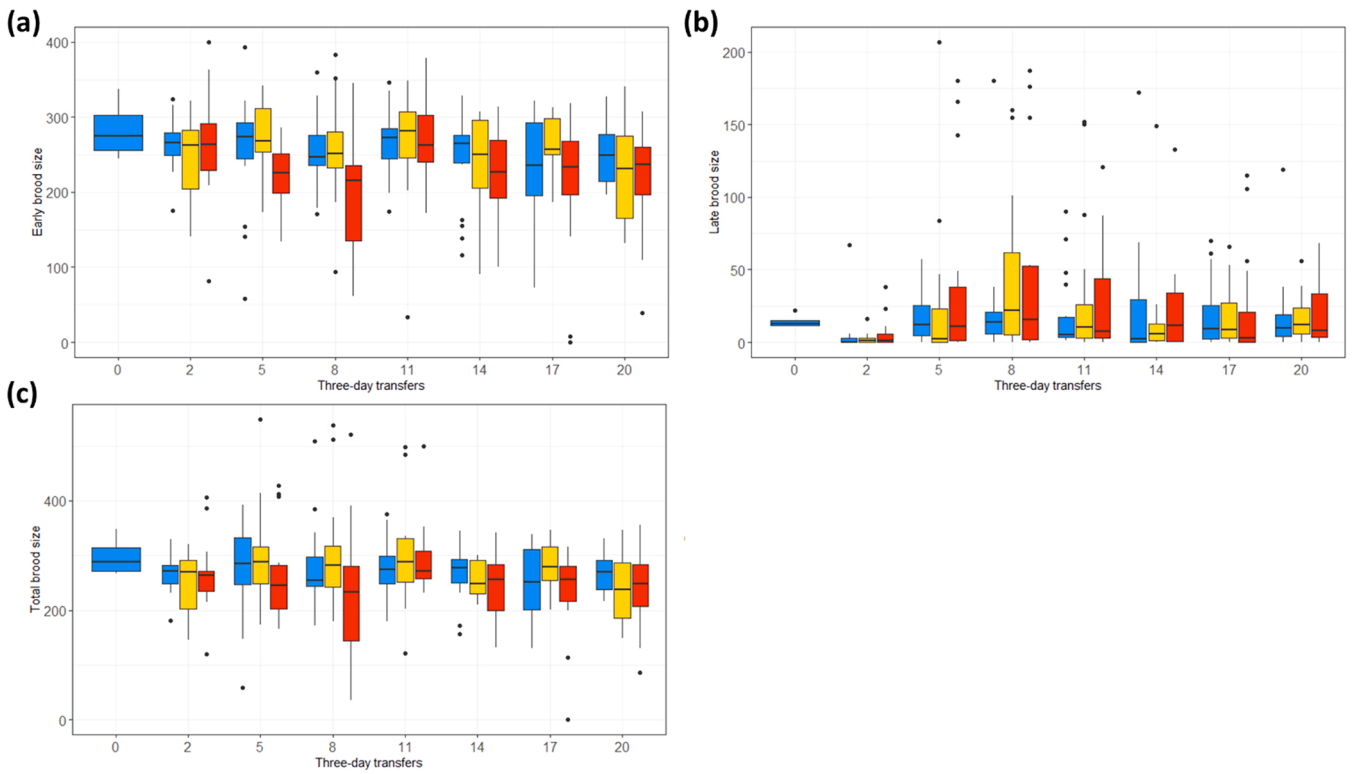

Fig. 3. Boxplot of (a) early, (b) late and (c) total brood size over time (i.e., three-day transfers: 0, 2, 5, 8, 11, 14, 17 and 20) for *C. elegans* populations living in different gamma radiation environments. Blue: control; yellow: low radiation (1.4 mGy.h^{-1}); red: high radiation (50.0 mGy.h^{-1}).

growth rate.

Our results also showed fluctuation in population growth rate over time under irradiated conditions only. This suggests that variation in population growth was caused by this stressor, indicating that ionizing radiation increases the instability in population growth. These results could be related to the fluctuations observed for realized fecundity and hatching success. Other unmeasured traits could also participate in explaining these fluctuations, since variation in population growth is a global response to environmental change. Such variation has already been observed in other studies, reproduction of *C. elegans* continuously exposed for 11 generations to 1-ethyl-3-methylimidazolium bromide showed oscillatory changes between stimulation and inhibition over generations related transgenerational effect (e.g., epigenetic effects) (Yue et al., 2021).

Our results show that at the population level the two irradiated conditions decreased fecundity, and the decrease intensified with dose rate. Other studies on the *C. elegans* N2 strain have found a decrease fecundity of 25%, 35%, 43% and 61% in populations chronically exposed to 42.7, 50.0, 108.0 and 228.0 mGy.h^{-1}, respectively (Buisset-Goussen et al., 2014; Dufourcq-Sekatcheff et al., 2021; Maremonti et al., 2019). Such decrease in fertility may be caused by germ cell apoptosis and reduced spermatids production (Guédon et al., 2021; Maremonti et al., 2019). Interestingly, we found a decrease in fecundity at much lower dose rate than Buisset-Goussen et al. (2014), Maremonti et al. (2019) or Dufourcq-Sekatcheff et al. (2021). First, The A6140 population used in this study may be less resistant to ionizing radiation than the N2 strain. *C. elegans* strains differ in their radioresistance (Clejan et al., 2006). Second, the multigenerational irradiation exposure could amplify deleterious reproductive effects, through for example transgenerational effects, as shown in several studies on invertebrates (*Ophryotrocha diadema:* Knowles and Greenwood, 1994; *Daphnia magna:* Alonzo et al., 2008; *C. elegans*: Buisset-Goussen et al., 2014). As for population growth rate, under irradiated conditions the realized fecundity fluctuated significantly over time, suggesting transgenerational effect of the *C. elegans* reproduction (Yue et al., 2021). Moreover, this result seems logical since the calculation of both indexes is based on the same data set.

Offspring survival is essential in the maintenance and growth of a population over the long term. We, therefore, measured hatching success during the experiment in parallel with population growth rate. Our results showed a lower hatching success in the high irradiation treatment compared to the control treatment. A decrease of the hatching success of about 20% has already been observed with an acute gamma irradiation of 50 Gy of the L4/young adult stage with the N2 strain (Dubois et al., 2018). However, this study showed no decrease in hatching success with a chronic irradiation of 50.0 mGy.h^{-1} between the egg and L4/young adult stages (Dubois et al., 2018). Other studies have shown a decrease in larval survival at much lower chronic dose rates (gamma radiation) in *Neanthes arenaceodentata* (0.19 mGy.h^{-1}; Harrison and Anderson, 1994), *Ophryotrocha diadema* (3.2 mGy.h^{-1}; Knowles and Greenwood, 1994) and *Eisenia fetida* (4.0 mGy.h^{-1}; Hertel-Aas et al., 2007). In our study, the significant decrease in hatching success appeared after several transfers of irradiation (Fig. 2c), which may explain this difference in results. Moreover, for this study we used the A6140 population (Noble et al., 2017; Teotonio et al., 2012) and not the N2 strain, so we can expect a slightly different response. Dubois et al. (2018) showed that the decrease in hatching success was correlated with protein carbonylation and suggests that the decrease in hatching success could be possibly explained by an apoptosis phenomenon. Other work has shown that many genes can cause embryogenic mortality when inactivated in *C. elegans* (Maeda et al., 2001). An accumulation of damage in gametes or gonads caused by chronic exposure to ionizing radiation could decrease hatching success, as has been suggested in the earthworm *Eisenia fetida* after chronic gamma irradiation (Hertel-Aas et al., 2007).

We found a decrease in early and total brood size for high irradiation treatment compared to control treatment. Hermaphrodites having grown and laid eggs outside the irradiator, these results showed that

Table 4
Results of generalized linear mixed models (GLMM) for the (a) hatching success, (b) early, (c) late and **(d)** total brood size in eight reciprocal transplants between the environment of origin and the environment of transplant (between control and 1.4 mGy.h^{-1} and between control and 50.0 mGy.h^{-1}). * , $P < 0.05$; * *, $P < 0.01$; * ** , $P < 0.001$.

Fixed effect	LR Chisq	Df	P
a) **Hatching success**			
Control versus 1.4 mGy.h^{-1}			
population of origin	3.2838	1	0.070
environment of transplant	6.6972	1	0.010 * *
population of origin: environment of transplant	5.6754	1	0.017 *
Control versus 50.0 mGy.h^{-1}			
population of origin	1.3714	1	0.242
environment of transplant	0.9183	1	0.338
population of origin: environment of transplant	0.0231	1	0.879
b) **Early brood size**			
Control versus 1.4 mGy.h^{-1}			
population of origin	0.0834	1	0.773
environment of transplant	0.0144	1	0.905
population of origin: environment of transplant	1.1145	1	0.291
Control versus 50.0 mGy.h^{-1}			
population of origin	0.0355	1	0.851
environment of transplant	0.0030	1	0.956
population of origin: environment of transplant	0.9661	1	0.326
c) **Late brood size**			
Control versus 1.4 mGy.h^{-1}			
population of origin	2.4000	1	0.121
environment of transplant	0.0024	1	0.961
population of origin: environment of transplant	4.9495	1	0.026 *
Control versus 50.0 mGy.h^{-1}			
population of origin	0.1147	1	0.735
environment of transplant	0.0171	1	0.896
population of origin: environment of transplant	0.6864	1	0.407
d) **Total brood size**			
Control versus 1.4 mGy.h^{-1}			
population of origin	0.0146	1	0.904
environment of transplant	0.0692	1	0.793
population of origin: environment of transplant	0.0187	1	0.891
Control versus 50.0 mGy.h^{-1}			
population of origin	0.0504	1	0.822
environment of transplant	0.0067	1	0.935
population of origin: environment of transplant	0.6640	1	0.415

early irradiation at the embryonic stage can have an impact on reproduction once adult. The observed decrease in hermaphrodite fecundity was consistent with several studies showing the deleterious impact of ionizing radiation on *C. elegans* reproduction, by decreasing the number of germ cells by radiation-induced apoptosis, and by decreasing the quantity and quality of gametes (Buisset-Goussen et al., 2014; Dufourcq-Sekatcheff et al., 2021; Guédon et al., 2021; Lecomte-Pradines et al., 2017; Maremonti et al., 2019). In addition, the decrease in early brood size was consistent with Lecomte-Pradines et al. (2017), who showed that external gamma irradiation induced delayed growth and spawning in *C. elegans* at a dose rate of 26.8 mGy.h^{-1}. The authors suggested these ionizing radiation-induced delays were caused by an increase growth and maturation costs, which explains the decrease in early brood size that we observed. Furthermore, the decrease in early brood size and the negative correlation between early and late brood size suggest a trade-off in high irradiation treatment between the both traits. This result showed that this treatment was particularly detrimental to nematodes, as trade-offs are more likely to be detected under stressful conditions, where resources available are limited for the organisms (Buchanan et al., 2018; van Noordwijk and de Jong, 1986; Reznick et al., 2000). The decrease of total brood size was smaller than the decrease of 25% observed by Buisset-Goussen et al., 2014 at 42.7 mGy.h^{-1} with N2 strain. This difference was not surprising, once the nematodes were placed off irradiation, we can expect the impact of ionizing radiation to decrease with time. For example, results have shown that the repair of DNA strand breaks caused by acute gamma irradiation (30 Gy) in *C. elegans* is already observable 6 h after irradiation with comet tests (Park et al., 2016).

4.2. Reciprocal-transplant experiments: study of evolutionary changes

After the multigenerational experiment, reciprocal-transplant experiments were performed to study variations in life history traits and analyze the mechanisms underlying these changes. Our reciprocal-transplant experiments showed some adaptive responses for hatching success and late brood size in populations subjected to irradiation. Results suggested an evolutionary trend towards an improvement of embryo survival and a slower life history in response to low irradiation. The similar but nonsignificant trends observed for hatching success and late brood size for high radiation suggest adaptive process in the same direction but slowed at high doses rate. These changes could provide better resistance and fitness against ionizing radiation. Indeed, organisms with a slower life strategy invest more in self-maintenance than in reproduction, leading to increased stress tolerance, better immune response or lower rates of aging (Tökölyi et al., 2016). For example, Dutilleul et al. (2017) showed an adaptation of *C. elegans* populations exposed to salt over 22 generations towards a slower life history strategy. In *C. elegans*, other studies have shown an extend lifespan and delay in reproductive timing with improved resistance to different stressors (heat, oxygen, and juglone: Cypser and Johnson, 2002; quercetin, caffeic and rosmarinic acid: Pietsch et al., 2011; arsenite: Schmeisser et al., 2013; mitochondrial stress: Maglioni et al., 2014). For high irradiation treatment, populations did not appear to adapt as successfully as populations subjected to low irradiation. In these stressful conditions, local adaptation could be limited by trade-offs among fitness-related traits (Colautti and Lau, 2015; Kraemer and Boynton, 2017), as previously suggested between early and late brood size. In addition, regarding the lack of significant results in reciprocal-transplant experiment for hatching success at high dose rates could be related to the large amplitude of the standard error, indicating great variability in the response between biological replicates (Fig. 4b). Radiation being strongly mutagenic (Breimer, 1988), we can expect a random accumulation of differences (i.e., mutations, genetic drift) in the irradiated populations, resulting in a divergence of phenotypic responses between independent populations after several generations (Teotónio et al., 2017; Seymour et al., 2019).

The lower hatching success and late brood size of the irradiated populations back to the control environment reveal some costs of adaptation or fitness trade-offs to gamma radiation (Hereford, 2009). A previous study suggested the existence of adaptive costs of Chernobyl bacterial communities exposed to background radiation dose of 0.45 μGy.h^{-1}. In an irradiated environment, these bacteria had higher resistance to radiation than control populations, but their performance was worse than control populations in a non-irradiated environment (Ruiz-González et al., 2016). Although this study is the only one to our knowledge to show an adaptive cost in the context of ionizing radiation, many examples exist with other stressors: salt, uranium, pesticide, copper or cadmium (Boivin et al., 2003; Dutilleul et al., 2017; Jansen et al., 2011; Mireji et al., 2010; Ward and Robinson, 2005).

The absence of interaction in reciprocal-transplant experiment for early and total brood size indicates that changes in these traits under irradiated conditions may not be adaptive or that mechanisms have prevented populations from reaching adaptive optima (Hereford, 2009). The decrease of these traits was related to the deleterious impact of ionizing radiation on reproduction (Buisset-Goussen et al., 2014; Dufourcq-Sekatcheff et al., 2021; Guédon et al., 2021; Lecomte-Pradines et al., 2017; Maremonti et al., 2019). Alternatively, the absence of adaptation of these traits could be explained by the evolution to a slower life strategy, constraining nematodes to lay eggs later and in smaller quantities, in order to promote self-maintenance for increased stress tolerance (Tökölyi et al., 2016).

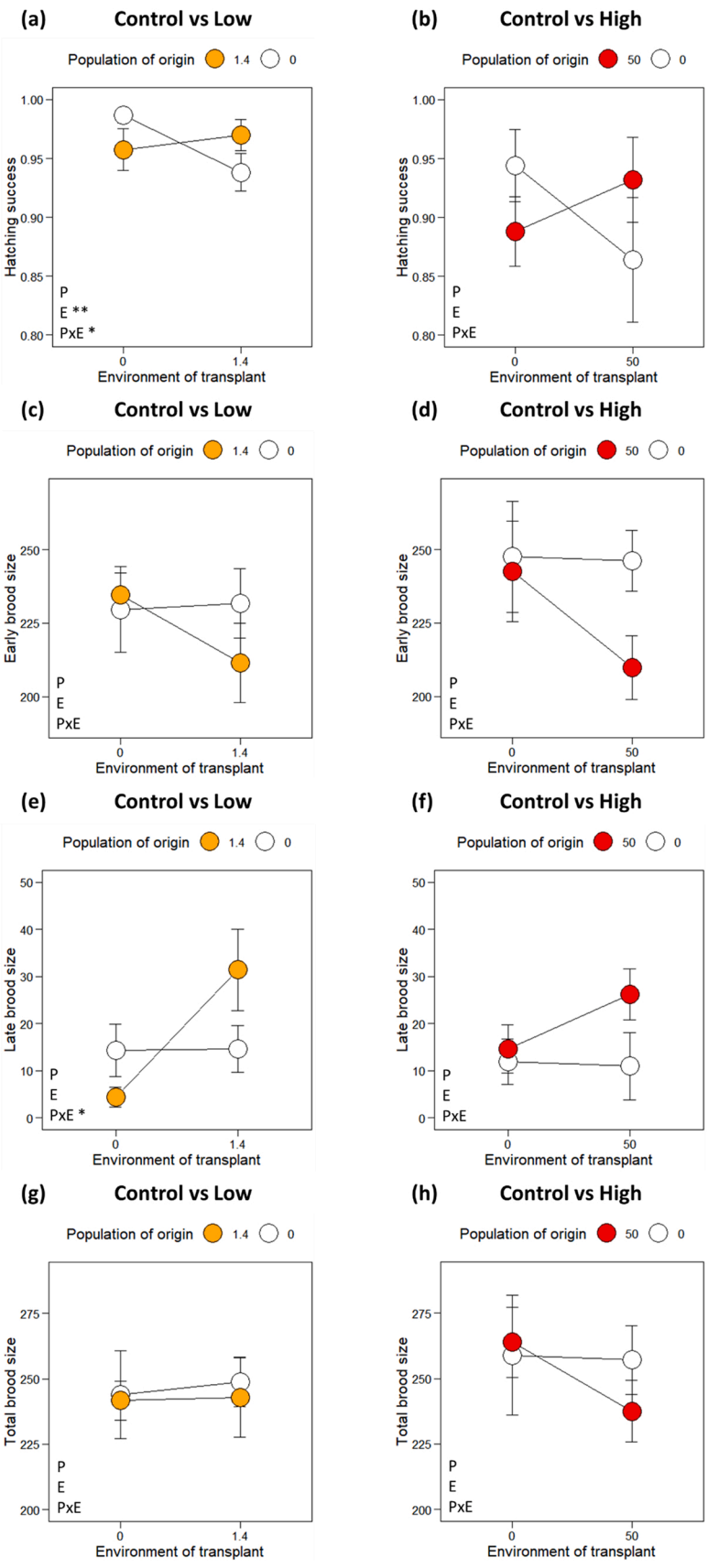

Fig. 4. Hatching success (a, b), early (c, d), late (e, f) and total brood size (g, h) of *C. elegans* populations after four transfers of reciprocal transplant between control and (a, c, e, g) low (1.4 $mGy.h^{-1}$) or (b, d, f, h) high (50.0 $mGy.h^{-1}$) irradiation treatments. Dots represent the mean of life history traits ± S.E for each new treatment. The color of dot represents the populations' treatment during the multigenerational experiment. White: control; yellow: low radiation (1.4 $mGy.h^{-1}$); red: high radiation (50.0 $mGy.h^{-1}$). The significance of each main effect [population of origin (P), environment of transplant (E) and their interaction (P × E)] is indicated in the bottom left of each graph. * , $P < 0.05$; * *, $P < 0.01$; * ** , $P < 0.001$.

5. Conclusion

As far as we know, this study presents one of the first characterization of the effects of chronic exposure to ionizing radiation over so many generations. Our results showed a higher *C. elegans* population growth rate for low irradiation treatment and a lower for high irradiation treatment compared to the control. The results of life history traits show a decrease of realized fecundity for both irradiation treatment. Moreover, we observed a decrease of brood size from self-fertilized hermaphrodites and hatching success for high irradiation treatment. Finally, results suggest an adaptive response of populations that live in low irradiation conditions, showing an improvement of embryo survival and a slower life strategy. These evolutionary changes were with some costs of adaptation. This work has brought a new argument to show the importance of considering demographic and evolutionary changes in ecotoxicology, and for Ecological Risk Assessment, by investigating the effects of a stressor at the population level over several generations.

CRediT authorship contribution statement

LQ, JMB, DR, and OA conceived and designed the study. LQ, JMB and EDS collected the data. LQ and DR performed the statistical analysis. LQ, JMB and DR drafted the manuscript and all authors read, comment and approved the final manuscript. Finally, all the authors have contributed to this study, and without the contribution of each, this manuscript would not be what it is.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Loic Quevarec reports financial support, administrative support, article publishing charges, and equipment, drugs, or supplies were provided by Institute of Radiation Protection and Nuclear Safety.

Data availability

All data are in tables in supplementary files (Table A.1–A.5).

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2022.114353.

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