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# Ant-icipating the fallout: a study on the radioresistance of the black garden ant *Lasius niger*

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**Abstract.** The radioresistance of ants has been a subject of curiosity and fascination, with speculation that they could thrive in radiation-contaminated environments, such as those resulting from nuclear fallout. This study investigates the radioresistance of the black garden ant *Lasius niger*, a widespread species inhabiting many geolocations around the world. Newly mated queens were exposed to varying doses of X-ray radiation (0–250 Gy) prior to colony initiation, and survival, fertility, and offspring development were monitored over a 77-day period. Results showed high survival rates across a broad range of radiation doses, with no significant differences between control and irradiated queens up to 11 weeks post-exposure. However, sterilization of queens was observed from doses of 50 Gy onwards, with only a few workers being produced after exposure to this dose. The specific factors contributing to the observed radioresistance differences among Formicidae species are yet to be elucidated. Further research is therefore needed to better understand these factors and their interplay in determining radioresistance. This study contributes to the understanding of ant radioresistance and provides a more accurate representation of their capacity to withstand radiation exposure.

**Keywords.** Ants, radioresistance, *Lasius niger*, X-rays, sterilization.

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## Introduction

The impact of radiation on various metazoan organisms has long captivated the scientific community, particularly concerning the potential for certain species to endure extreme radiation events. Within this diverse kingdom, while many vertebrate species exhibit significant vulnerability to radiation exposure (Mole 1984; Harrison & Anderson 1996), some invertebrates, such as bdelloid rotifers and tardigrades, demonstrate remarkable resistance (Jönsson *et al.* 2005; Horikawa *et al.* 2006; Gladyshev & Meselson 2008; Hespeels *et al.* 2023). This dichotomy raises intriguing questions about the underlying factors that contribute to these disparities in radiation resistance. Among metazoans, insects have attracted considerable attention in this context due to their potential to survive extreme radiation events, notably because they were observed surviving in radiation-exposed zones (Perrault & Castet 1988; Stockmann *et al.* 2010). Some insect species indeed show remarkable radioresistance, withstanding more than 1000 Gy, exemplified by species like *Polypedilum vanderplanki* (Hinton, 1951) larvae (Diptera, Chironomidae) (Watanabe *et al.* 2006, 2007) or *Hypothenemus hampei* (Ferrari, 1867) (Coleoptera, Curculionidae) (Kiran *et al.* 2019). Gaining insight into the radioresistance of insects is crucial for predicting their survival in radiation-contaminated environments and for deepening our understanding of the fundamental biological mechanisms that underpin resistance to radiation-induced damage. However, while comparisons of radiation tolerance across species are informative, caution must be exercised due to potential differences in experimental methods and measured biological parameters. For example, Kiran *et al.* (2019) observed 100% mortality in *Hypothenemus hampei* at 3200 Gy, while Follett (2018) demonstrated sterilization at much lower doses, around 100 Gy. This highlights the need to consider two distinct perspectives when describing the radioresistance of living organisms: first, the maximum radiation dose that organisms can survive (the limit of life), and second, the dose required to sterilize a population, which may be a more practical measure for predicting population growth and persistence in radiation-exposed environments.

The belief that cockroaches would be the sole survivors of a nuclear war due to their radiation resistance has become a deeply ingrained truism in popular culture (Berenbaum 2001). Interestingly, this idea can also be applied to ants, another highly adaptable and resilient taxon. Like cockroaches, ants have demonstrated remarkable capabilities to survive and thrive in a variety of harsh environments. Their social structure, featuring highly organized colonies and efficient division of labor, allows them to adapt quickly to changing conditions. George Orwell's 1945 essay "*You and the Atomic Bomb*" underscored the potential repercussions of humanity's self-destruction, positing that ants or other social species could rise to dominance in a post-apocalyptic world (Orwell 1945). Understanding the mechanisms that enable ants to survive radiation exposure could not only help predict their survival in radiation-contaminated environments but also provide insights into the fundamental biological processes that underly resistance to radiation-induced damage. However, a comprehensive overview of ants' radioresistance is still lacking. As for roaches, it is difficult to trace the origin of this idea or determine if it is based on an outstanding resistance of these taxa or simply on the life mode of these species underground, which would protect them from radiation. Ultimately, our knowledge of ants' radioresistance remains dramatically incomplete.

To date, only a limited number of studies have examined the radiotolerance of ants, which is striking considering the immense diversity of ant species described, with over 16 000 known species (Gadau *et al.* 2012; https://www.antweb.org/). Moreover, most of these investigations have only focused on invasive ant species that share some ecological traits (Lach 2021) and are thus not representative of the entire ant diversity. The minimum radiation doses required to sterilize queens and prevent reproduction in these species were reported as follows: 90 Gy for *Pheidole megacephala* (Fabricius, 1793) (Follett & Taniguchi 2007 cited in Follett *et al.* 2016), 62 Gy for *Linepithema humile* (Mayr, 1868) (Coulin *et al.* 2014 cited in Follett *et al.* 2016), 72 Gy for *Wasmannia auropunctata* (Roger, 1863) (Calcaterra *et al.* 2012 cited in Follett *et al.* 2016), and 60 Gy for *Solenopsis invicta* (Buren, 1972) (Follett *et al.* 2016). Some data are also available for a few European ant species. In *Temnothorax recedens* (Nylander, 1856), all individuals were killed by a dose of 600 Gy, 82 days post-radiation (Dejean 1975). Queens of *Dolichoderus quadripunctatus* (Linnaeus, 1771) were sterilized with a dose of 125 Gy (Torossian *et al.* 1967). Interestingly, it was proposed that queens were more radioresistant than workers. For example, at 500 Gy, the lifespan was approximately 40 days for workers (compared to 80 days in controls) versus 112 days for irradiated queens (compared to a minimum of 172 days in controls) (Torossian *et al.* 1967). Finally, in contrast to acute radiation resistance, some ant species have reported survival and development of populations exposed to chronic irradiation. For example, in 1988, Perrault and Castet observed the ant population of a Mediterranean ecosystem 18 years after chronic irradiation from a 137Cs source, which exposed the area to approximately 200–300 Gy per year. Over this period, three ant species were recorded near the irradiation source, including two species that had not been present in 1970 (Perrault & Castet 1988).

The black garden ant *Lasius niger* (Linnaeus, 1758) is widely distributed in Europe, Asia and North America, but also occurs in South America and Oceania, and is often found in anthropogenic habitats. With queens living up to 28 years, this species has one of the longest lifespans of queens among ants (Kramer *et al.* 2016). *Lasius niger* queens perform claustral nest foundations, which means they do not collect food themselves, and live on their own body resources until first workers emergence (Keller & Passera 1989; Kramer *et al.* 2016). The flight period of this species typically ranges from May to July in Europe. *Lasius niger* has a generalist diet, and can feed on insect prey as well as on honeydew from aphids (Portha *et al.* 2004).

In the present study, we investigate the radioresistance of *Lasius niger* by exposing newly mated queens to varying doses of X-rays, ranging from 0 to 250 Gy, before colony initiation. Our research specifically differentiates between the effects of radiation on queen mortality, sterility, and the development of their progeny. Considering that the majority of existing data on ant radioresistance has been derived from studies focusing on pest management using ionizing radiation in the United States, this study offers a novel perspective on the radioresistance of a European ant species, thereby contributing to a more comprehensive understanding of ant radiotolerance across diverse geographic locations.

## Material and methods

### Sampling and culture

Wingless queens of *Lasius niger* were collected by hand, at two distinct sites in Namur (Belgium) during a large mating flight on the 29th of June 2022. A total of 49 queens were obtained from site A (50°28′16.6″ N, 4°51′43.7″ E), and 52 were collected from site B (50°27′56.7″ N, 4°51′37.9″ E). These newly mated queens were kept in the laboratory, under dark conditions at room temperature (around 21°C), without food. Individual 15-mL tubes were used, each queen being separated from a water reserve (8 mL Spa water) by a continuously moistened piece of cotton. The tubes were closed by a cotton plug (Fig. 1). These replicates are later called “foundations” or “colonies”.

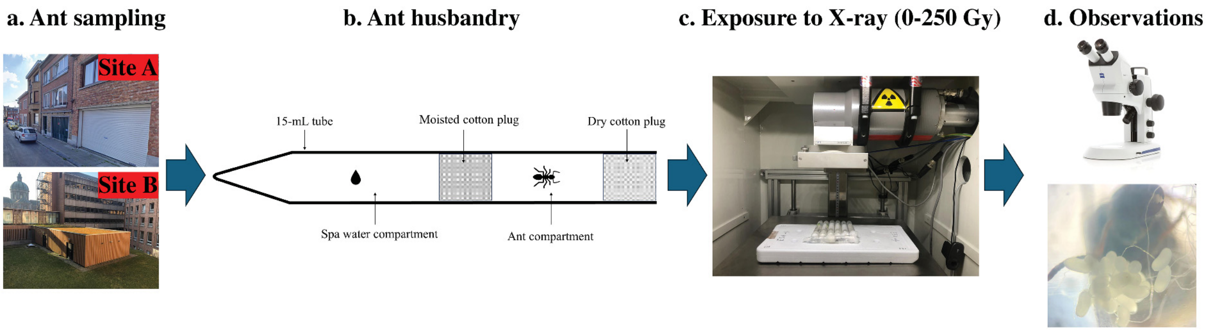


Figure 1 – Overview of the experimental design. The figure outlines the experimental approach for studying radiation effects on *Lasius niger* queens sampled in Namur, Belgium. **a**. Newly mated queens were captured and isolated from two urban sites, designated site A (n = 49) and site B (n = 52). **b**. Isolated queens were cultivated in controlled ant husbandry enclosures, each consisting of an ant compartment adjoined to a water compartment. **c**. The queens were exposed to varying levels of X-ray radiation, with doses spanning from 0 to 250 Gy. Protective measures, including the use of cold pack refrigerators, were employed to prevent thermal damage during irradiation. **d**. Post-exposure, the queens were kept at ambient room temperature, and their viability and reproductive rates – marked by the production of eggs, larvae, and workers – were tracked at multiple time points over a period extending to 77 days using a Stereo Microscope. Image credits: B. Hespeels and M. Vastrade, except for site A (obtained from Google Streetview) and the Stereo Microscope (courtesy of Zeiss.com).

Before irradiation, individuals were kept for 6 days following the protocol described above. Only living and healthy individuals (response to touch) were selected for the experiment. Dead or weak individuals were discarded from the experimental setup.

### X-ray exposure

Active *Lasius niger* queens were irradiated using an X-ray irradiator (PXi X-RAD 225 XL), with a dose rate of ~8 Gy/min (19 mA, 225 kVp, no filter) available at LARN (UNamur, Belgium). Irradiation time was calculated from this dose rate to obtain the different doses. The dose rate was quantified using a PTW Unidos E dosimeter, with validation conducted both before and after irradiation to ensure uniformity between irradiation conditions. However, the dose received by the samples may vary by 1–2%, due to their relative positions to the radiation source within the same irradiation experiment (Hespeels *et al.* 2020). All samples, including control samples, were put on a refrigerated water bag to mitigate heating from soft X-rays. The control samples were put on cold water for the same duration as the 5 Gy-exposed samples, without radiation exposure. In addition to the controls (later called "0 Gy", n = 12), ants were exposed to 5 Gy (n = 12), 10 Gy (n = 13), 25 Gy (n = 13), 50 Gy (n = 13), 100 Gy (n = 6) and 250 Gy (n = 12). For each dose, the 12 or 13 replicates were divided into two batches of 6 or 7 individuals and exposed during two independent exposures (called "1" and "2" in the dataset), except from the ants being exposed to 100 Gy, which were only represented by 6 replicates and exposed in a single exposure due to technical issues.

### Survival, fertility and development

Survival rate was measured for all exposed individuals after 1h of storage (21°C, dark condition) post irradiation (J0), then after one day (J1), one week (J7), two weeks (J14), four weeks (J28), seven weeks (J49) eight weeks (J56), nine weeks (J63) and eleven weeks (J77). The fertility of the queens was quantified by counting the number of laid eggs and their development into larvae, pupae, and workers. These countings were made after 6 days of incubation (J0, eggs laid before irradiation), then one day (J1), one week (J7), two weeks (J14), four weeks (J28), seven weeks (J49), eight weeks (J56), nine weeks (J63) and eleven weeks (J77) after the irradiation.

### Statistical analyses

Statistical analyses were performed using Rstudio software version 2022.12.0+353 (RStudio Team 2015) with version 4.2 of R. The *questionr* package version 0.7.8 was used to analyze survival rates between X-ray doses at 77 days and determine if a significant difference was observed using a chi-2 test. As the data representing the effects of X-ray doses on reproductive capacity and developmental progression did not follow a normal distribution, the package bestNormalize version 1.9.1 was used to find the best transformation. Then, a global linear model (using a gaussian family) was performed at each timepoint for each stage. Post hoc comparisons at the 5% significant level were performed with the general linear hypothesis (Tukey test) and multiple comparisons for GLM were performed with multcomp package version 1.4-22 (Hothorn *et al.* 2008). Significant differences between groups are indicated by different letters: a significant difference (P value $< 0.05$) between two conditions is observed when these conditions share no letter. For all comparisons, the letter "a" was assigned to the higher value.

## Results

### Survival rates across a range of X-ray radiation doses over 77 days

The radioresistance of *Lasius niger* queens, recently fertilized after their nuptial flight, was assessed by exposing them to increasing doses of X-ray radiation over a 77-day period. These newly fertilized queens are responsible for founding new colonies by laying eggs and establishing the initial worker population. Survival rates were observed for various radiation doses (0, 5, 10, 25, 50, 100, and 250 Gy) and at different time points throughout the study (0, 1, 7, 14, 28, 49, 56, 63 and 77 days) (Figure 2). In the control condition (0 Gy), an initial survival rate of 100% was observed, which had decreased to 81.8% by the end of the 77-day test period (Fig. 2, red chart). At the beginning of the experiment, survival rates remained generally high across all radiation doses. Nevertheless, some variations in survival rates emerged by the end of the 77-day test period, although these changes did not result in any significant trends or dose-dependent patterns. The findings suggest that *Lasius niger* queens can withstand a wide range of X-ray radiation doses without experiencing a considerable impact on their survival rates over the 77-day test period when compared to control samples.

### X-ray radiation impact on queen fertility and offspring development in *Lasius niger*

The mere presence of eggs does not guarantee their fertility or their ability to develop into worker ants. The progression of eggs to workers was assessed by tracking the presence of larvae. Then, larvae undergo metamorphosis, transforming into pupae, and ultimately workers in the colonies. In all conditions, the development of microcolonies appears to start with an increase in egg production within the first 14 days, followed by a subsequent decline, as the eggs hatch or die (Fig. 3). In control foundations (0 Gy), the first larvae were observed at 28 days, and their presence is maintained through the experiment by continuous hatching of newly laid eggs. Pupae were observed in most control colonies at the 49$^{th}$ day of experiment, then workers emerged at the 56$^{th}$ day. The 5 Gy-exposed foundations showed a development dynamic very similar to the control. The 10 Gy-exposed colonies also showed a similar pattern, but with a slightly lower larvae production, and a slight increase in egg production at the end of the experiment

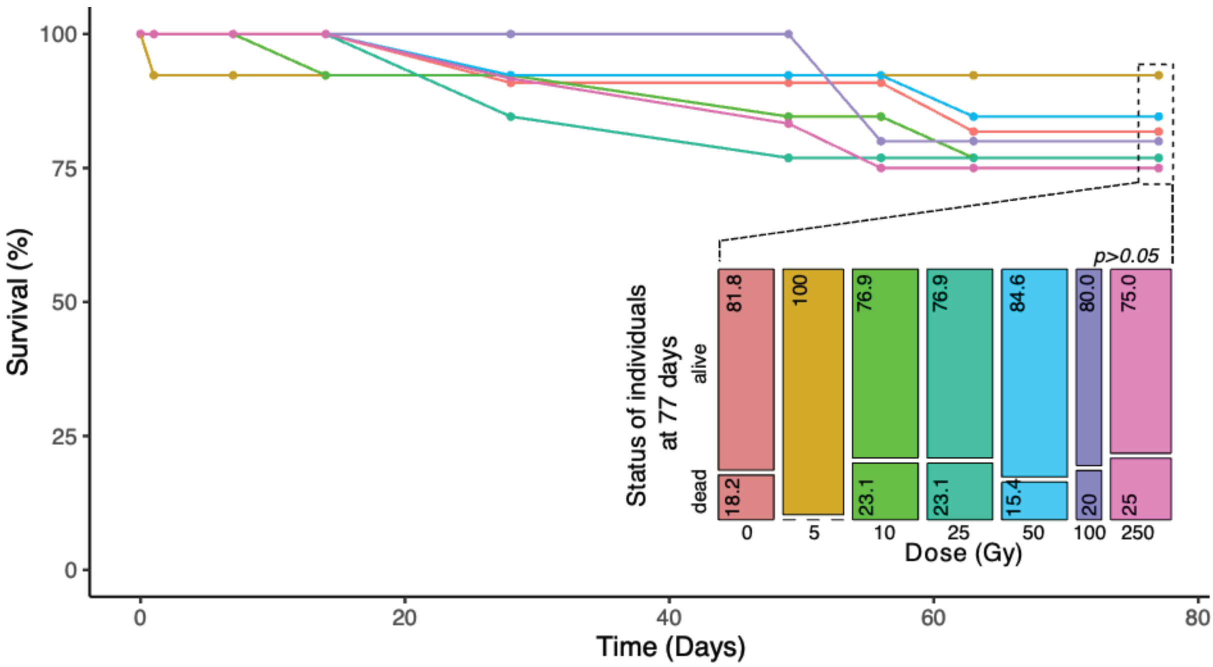


Figure 2 – Survival rates of *Lasius niger* queens subjected to varying X-ray radiation doses (0, 5, 10, 25, 50, 100, and 250 Gy) throughout a 77-day test period. The x-axis indicates the time points (0, 1, 7, 14, 28, 49, 56, 63, and 77 days), while the y-axis displays the survival rate percentages. A separate bar plot provides a detailed view of the data collected at the end of the 77-day period, showing the fraction of deceased versus living individuals. A color-coded scheme differentiates the radiation doses: 0 Gy (red), 5 Gy (yellow), 10 Gy (green), 25 Gy (cyan), 50 Gy (blue), 100 Gy (purple), and 250 Gy (pink).

as compared to 0 and 5 Gy-exposed ants. Foundations being exposed to 25 Gy showed comparable egg production, a sharply lower larvae production compared to the controls, but were still able to produce a similar number of viable pupae and workers. In foundations exposed to 50 Gy and more, the egg count dropped to zero starting from day 49, and only a few larvae and almost no pupa and worker were observed in these colonies.

These findings were statistically analyzed by comparing the development of microcolonies subjected to increasing radiation doses at various time points (Fig. 4, Appendix Figures S1 to S5). The queen egg laying showed no significant difference between the control group and the irradiated individuals in the first 14 days post-irradiation (Figure 4; $p > 0.05$). However, starting from day 28, a significant decrease in the number of living eggs was observed in foundations being exposed to radiation levels of 50 Gy and above (Fig. 4 and Appendix Figure S1; $p < 0.0001$). This drop continued during subsequent time points, until no eggs were observed anymore in these replicates from day 49 onwards until the end of the experiment (Appendix Figures S2 to S5). In many cases, dead eggs were observed in these foundations. In contrast, queens having been exposed to 0 to 25 Gy continued to produce viable eggs.

The first hatching of eggs, leading to the emergence of larvae, was observed on day 28 post-irradiation. At this time point, there was no significant difference in the number of larvae between the control group and replicates exposed to 5 Gy and 10 Gy of radiation (Fig. 4 and Appendix Figure S1; $p > 0.05$). However, a significant difference compared to the control group was observed at exposure levels of 25 Gy and above, with no hatching reported after 50 Gy ($p < 0.0001$). By the end of the experiment (day 77), no difference in larvae number was observed between the control group and colonies exposed to 5 and 10 Gy of radiation (Appendix Figure S5; $p > 0.05$).

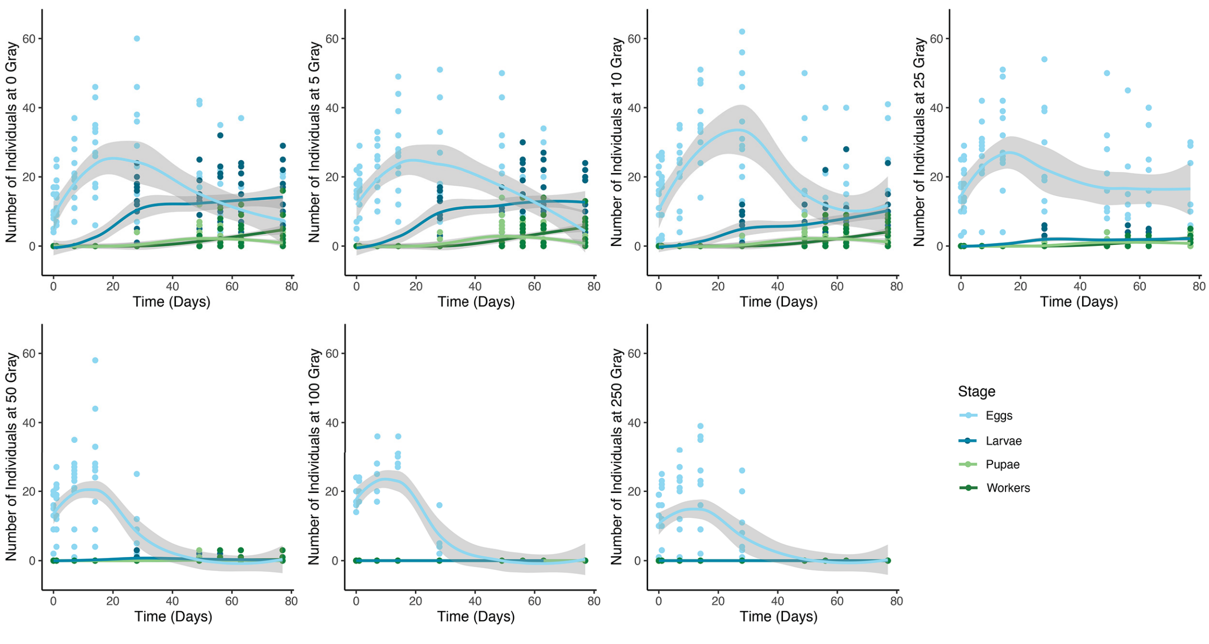


Figure 3 – Evolution of colony foundations over time, from the beginning of the experiment until day 77, for tested radiation doses ranging from 0 to 250 Gy. The curves represent the regression line obtained with a loess smooth method and the surrounding grey band is the confidence interval at 95% (ggplot2 package version 3.4.1). The points indicate the number of individuals of each stage (eggs, larvae, pupae, workers) from each colony.

The first pupae were observed 28 days post-irradiation in foundations having been exposed to radiation levels between 0 and 25 Gy, but with no significant difference between all foundations (Fig. 4 and Appendix Figure S1; $p>0.05$). However, compared to the control, pupa production was significantly lower in foundations having been exposed to 50 Gy and more, from day 49 until the end of the experiment (Appendix Figure S2; $p<0.001$). Except for two colonies at 50 Gy, none of the queens irradiated at doses between 50 and 250 Gy were able to produce eggs that reached the pupal stage.

The completion of metamorphosis, resulting in the emergence of worker ants, was observed in the control group starting from day 49, with no significant difference between the tested conditions at this point (Fig. 4 and Appendix Figure S2; $p>0.05$). A similar production of workers was observed in replicates exposed to radiation levels between 0 and 25 Gy from day 56 until the end of the experiment, although there seemed to be a trend towards reduced worker production in samples exposed to 25 Gy of radiation (Appendix Figures S3, S4 and S5). For 50 Gy-exposed colonies, only two colonies gave birth

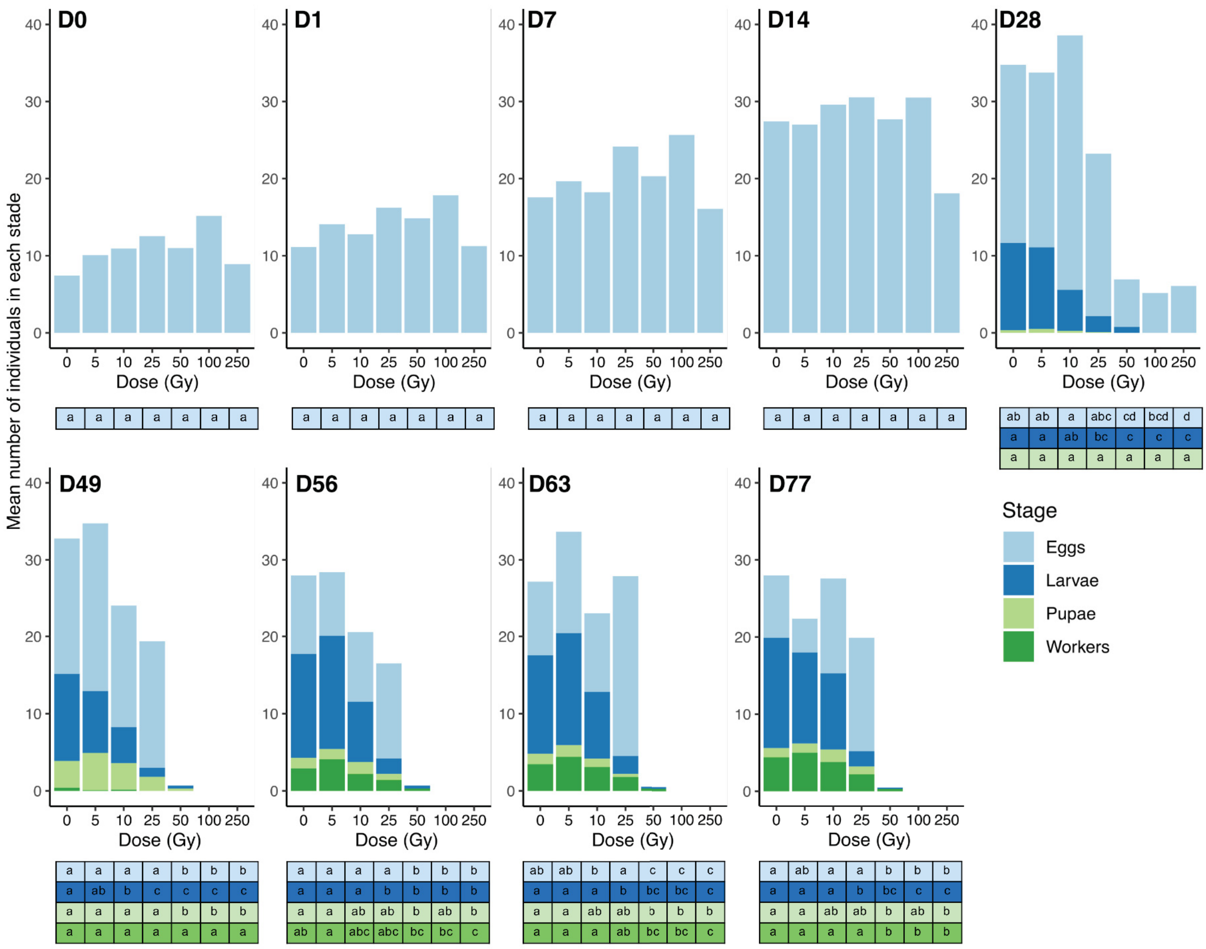


Figure 4 – Effects of X-ray radiation doses on reproductive capacity and developmental progression in *Lasius niger* queens. Each barplot represents the mean number of individuals of each stage for each irradiation dose: 0 Gy (n=12), 5 Gy (n=12), 10 Gy (n=13), 25 Gy (n=13), 50 Gy (n=13), 100 Gy (n=6) and 250 Gy (n=12). Statistical comparisons between the doses are shown with letters, no statistical difference was observed between conditions sharing the same letter. For all comparisons, the letter "a" was assigned to the higher value.

to one and three worker(s) by the end of the experiment. No worker was observed in replicates, which had been exposed 0 to 25 Gy.

To summarize, doses of 5 and 10 Gy exerted no discernible influence on offspring production. Exposure to 25 Gy significantly impacted larval production from day 28 onwards but had no impact on egg, pupae and worker production until the end of the experiment. Finally, an exposure to 50 Gy or greater showed a pronounced effect on offspring production across all stages, from day 28 until the conclusion of the experiment.

## Discussion

In this study, we present the first comprehensive investigation into the response of the common black ant *Lasius niger* to increasing levels of X-ray exposure over a period of 11 weeks. Our study provides results for two distinct aspects of radiation resistance in the field of radiation biology: the ability to survive acute irradiation events, and the ability to produce viable and fertile offspring after the irradiation.

### No impact of a 250 Gy exposure on *L. niger* queens' survival after 77 days

The first aspect of radioresistance is the short-term individual survival to acute irradiation, with the critical factor being the duration from radiation exposure to the organism's death. Survival times for mammals exposed to lethal doses of radiation depend on the amount of radiation administered to the animal. Mammals exposed to 1000 Gy perish within minutes, while exposure to 10 to 100 Gy results in survival ranging from 3 to 5 days, and exposure to 2 to 10 Gy can lead to survival times extending between 30 to 60 days (Coggle 1983 in Von Zallinger & Tempel 1998). When evaluating radioresistance, arthropods generally exhibit greater resilience compared to many higher vertebrates, although they are not as robust as entities such as bacteria. Mature insects display significant resistance to irradiation, largely attributable to the fact that their bodies primarily consist of specialized cells that have ceased to divide. These specialized cells inherently possess greater resilience to the detrimental effects of irradiation than cells that are either in a state of division or have yet to specialize (Dyar's rule, see Bakri *et al.* 2005).

In this study, we found no significant difference in terms of survival rate between unirradiated ants and acutely exposed animals up to 11 weeks post-irradiation. However, we cannot exclude the possibility that differences may be observed with extended durations of exposure, as *Lasius niger* queens can live for almost 30 years (Kramer *et al.* 2016). Alternatively, future research may explore radiation resistance for higher doses of X-ray exposure (e.g., $>1000$ Gy) to determine whether exposed queens experience a measurable reductions in survival within the 11 weeks experimental window, allowing us to observe radiation effects on lifespan more quickly, without needing to account for their potential 30-year longevity. The impact of radiation on living organisms can furthermore be influenced by numerous technical factors, including dose rate and aspects such as heat stress induced by irradiation facilities or thermal stress caused by cooling systems. These factors complicate the interpretation of results, and it remains unclear whether the high survival rates observed in this study are due to *Lasius niger*'s inherent radioresistance or to these technical aspects. For example, while our experiment showed high survival rates across all radiation doses after 11 weeks, similar studies on other ant species under comparable conditions have reported reduced lifespans (Follet & Taniguchi 2007; Calcaterra *et al.* 2012; Coulin *et al.* 2014; Follet *et al.* 2016), raising the question of whether technical factors or species-specific resistance primarily explains our findings. Further investigations are needed to disentangle these variables.

**50 Gy exposure prevents survival of *L. niger* progeny after 49 days**

The second aspect of radioresistance is the long-term species survival to acute irradiation, which means the survival of the progeny. From an evolutionary perspective, this latter definition holds greater significance, as an organism's survival following radiation exposure is futile if it cannot produce viable and fertile offspring, ultimately leading to a dead end ("delayed killing" described by Enzmann & Haskins 1938).

Insects have a high radiation resistance compared to vertebrates, but this radioresistance considerably varies among this class, independently of insect evolution and phylogeny (Paithankar *et al.* 2022). The International Database on Insect Disinfestation And Sterilization (IDIDAS: http://www-ididas.iaea.org/ididas/, see Bakri *et al.* 2005) provides a comprehensive range of radiation doses required to achieve sterility in insects and related arthropods, with variations observed both between and within different orders. The mean sterilization doses span from 130 to 400 Gy for Lepidoptera (based on 71 species in Bakri *et al.* 2005), 30 to 280 Gy for Acari (24 spp.), 40 to 200 Gy for Coleoptera (78 spp.), 10 to 180 Gy for Hemiptera (26 spp.), 20 to 160 Gy for Diptera (85 spp.), 20 to 150 Gy for Araneae (2 spp.), 5 to 140 Gy for Blattodea (5 spp.), 100 Gy for Thysanoptera (10 spp.). Sterilization doses as low as 4 Gy were reported in *Schistocerca gregaria* (Forskål, 1775) (Orthoptera, Acrididae) (Dushimirimana *et al.* 2012) and 5 Gy in *Blaberus craniifer* (Burmeister, 1838) (Blattodea, Blaberidae) (Gecheva & Apostolova 1986). IDIDAS currently (May 2023) reports sterilization doses spanning from 50 and 140 Gy for Hymenoptera (7 spp.) and between 70 and 125 Gy for Formicidae (4 spp.).

In this study, we observed that the reproduction of early fecundated *Lasius niger* queens was not significantly impacted until they were exposed to 25 Gy of X-ray radiation. Although a few workers were found after exposure to a dose of 50 Gy, it seems that this dosage was the minimum required to sterilize *Lasius niger* queens. This suggests that radiosterilization of *Lasius niger* occurs within a narrow range of X-ray doses between 25 and 50 Gy. In comparison to other studies focusing on different ant species, *Lasius niger* queens appear to be less radiotolerant than previously investigated species, whose sterilization doses ranged from 70 to 125 Gy (Follett & Taniguchi 2007; Calcaterra *et al.* 2012; Coulin *et al.* 2014; Follett *et al.* 2016). However, comparing our results directly with those of previous research remains a challenging task. While previous studies utilized Cobalt-60 (Co-60) emitting 1.17 MeV gamma rays, our experimental design employed an X-ray spectrum ranging from 225 to 20 keV. Examining electron stopping power (i.e., the rate at which an electron loses its kinetic energy as it traverses matter) in water (a biological tissue analog) reveals that at Co-60's energy, the photoelectric effect generates electrons with a stopping power of about 1.85 MeV $cm^2/g$ (Berger 1995). However, for the lower energies in our X-ray spectrum, stopping power significantly increases, ranging from 2.65 to 22.56 MeV $cm^2/g$ (up to 12 times higher). This suggests our X-ray irradiation may induce more complex and potentially harder-to-repair lesions in *L. niger* queens compared to Co-60. This difference in lesion complexity could explain the observed lower sterilization threshold in our study. To validate this hypothesis, it would be intriguing to expose other species to our X-ray design and compare the results.

Currently, there is no definitive explanation for the observed radioresistance differences among Formicidae species. Several factors may contribute to radioresistance, such as DNA repair pathways, antioxidant defenses, and the activation of stress response mechanisms (Krisko & Radman 2010; Daly 2012; Krisko *et al.* 2012; Ryabova *et al.* 2017; Lim *et al.* 2019; Fortunato *et al.* 2021; Reindl *et al.* 2023). Further research is needed to elucidate the specific contributions of these factors and how they interact to influence radioresistance. One possible contributing factor could be the presence of varying centromere structures, such as holocentric chromosomes (Zedek & Bureš 2019). Holocentric chromosomes have multiple centromeres distributed along their length, which may provide certain advantages in maintaining genome integrity after exposure to radiation. Indeed, a study analyzing the effects of ionizing radiation on over 250 species of arthropods showed that holocentric species were more radiation resistant than monocentric ones (Zedek & Bureš 2019). Further studies combining radioresistance data and genomic structure of ants may contribute to the testing of this hypothesis and the diversity of radiation resistance reported among Formicidae species. Finally, genomic and transcriptomic studies may also help identify potential genetic or molecular factors that could be involved in the radioresistance of ants. Investigating these factors could provide valuable insights into the mechanisms underlying the varying degrees of radioresistance in different ant species.

This research indirectly addresses broader questions about survival strategies of various species in extreme environmental conditions, such as those potentially resulting from a nuclear event. Our data confirm that young queens of *Lasius niger*, as well as those of other ant species, are capable of reproducing following acute exposures of 25 Gy. Given their subterranean lifestyle, it is highly likely that queen ants, as the colony founders, would be protected from the effects of radiation or be less affected in many cases, especially when distant from the epicenter of a nuclear blast. This protection may also extend to their survival during the fallout period, where the residual radioactive particles that settle on the ground could have less impact on their subterranean habitats. As has been demonstrated at sites with radioactive sources, ant populations can persist over long periods (Perrault & Castet 1988). However, ants do not appear to be more resistant than other insect species, and humans are particularly sensitive to the effects of ionizing radiation (less than 10 Gy; e.g., Mole 1984). By contrast, only a handful of animals can reproduce following exposure to high doses (> 500 Gy) of ionizing radiation, such as certain tardigrades and bdelloid rotifers (Gladyshev & Meselson 2008; Charlotta Nilsson *et al.* 2010; Beltran-Pardo *et al.* 2015; Hespeels *et al.* 2023). This leads to an important consideration regarding the impact of radiation dose rates on biological outcomes. Specifically, very low dose rates or chronic exposure may allow for the repair of sublethal damage in germ cells before the occurrence of subsequent damage. On the contrary, acute irradiation doses do not ensure the repair of this type of damage during exposure and are consequently more deleterious. This observation is crucial in understanding the differential impacts of radiation on biological systems and raises questions about the thresholds of repair and damage in various species, including ants.

## Conclusion

In conclusion, this study not only enhances our understanding of ant radioresistance inhabiting many geolocations around the world but also offers valuable insights into the popular culture fascination with ants' presumed high radiation resistance. As observed in other Formicidae species, *Lasius niger* queens appear to survive up to doses of 250 Gy for at least 11 weeks. Whether their hypothetical premature death compared to unirradiated individuals occurs later remains to be determined. However, we observed the sterilization of most queens at doses of 50 Gy, which seems to be lower than those previously reported for other ant species. By investigating the radioresistance of *Lasius niger*, our research helps to shed light on the reality of ant resilience in the face of extreme radiation exposure and provides a more accurate representation of their capacity to withstand such conditions.

## Data availability

The full data set is available on request to the corresponding authors.

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# Appendix

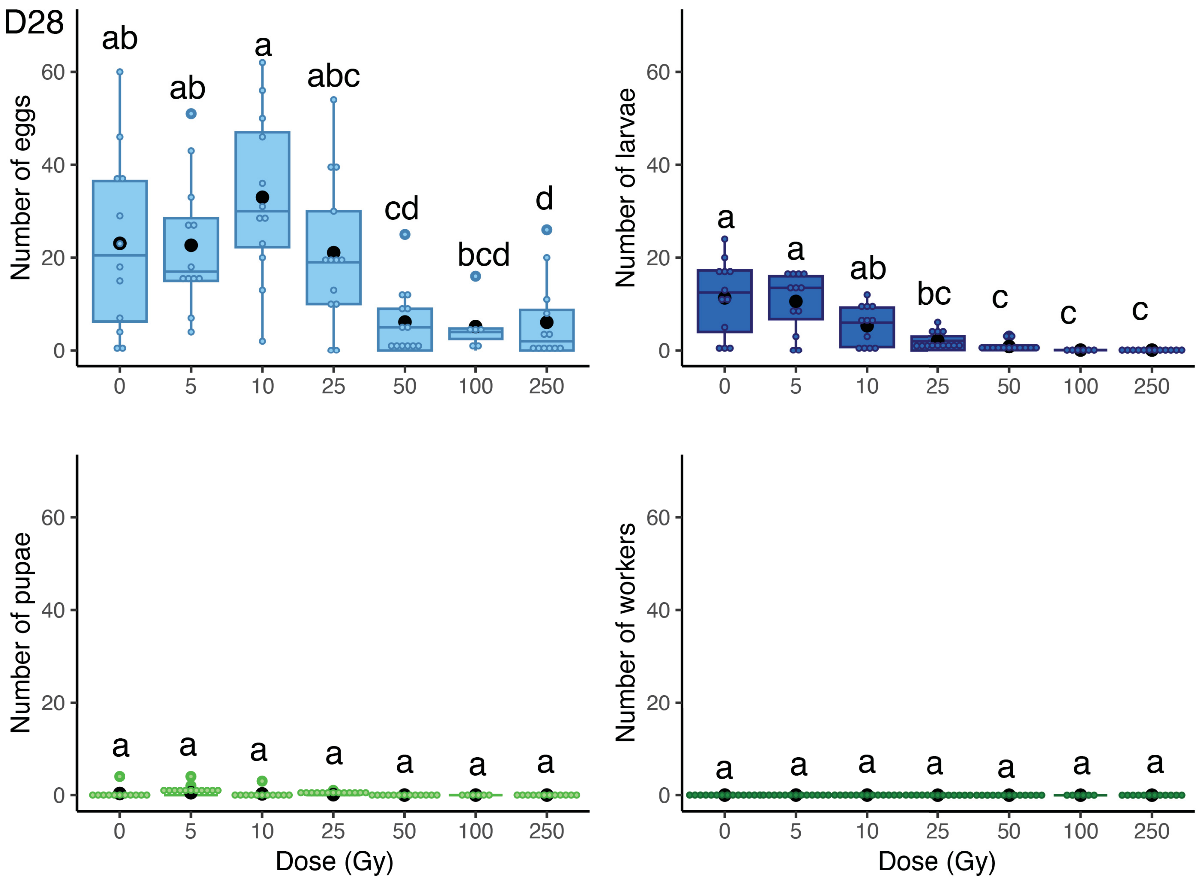


Figure S1 – Evolution of colony foundations at day 28, for tested radiation doses ranging from 0 to 250 Gy. The color and letter codes used for egg (top left), larva (top right), pupa (bottom left) and worker (bottom right) productions refer to Figure 3.

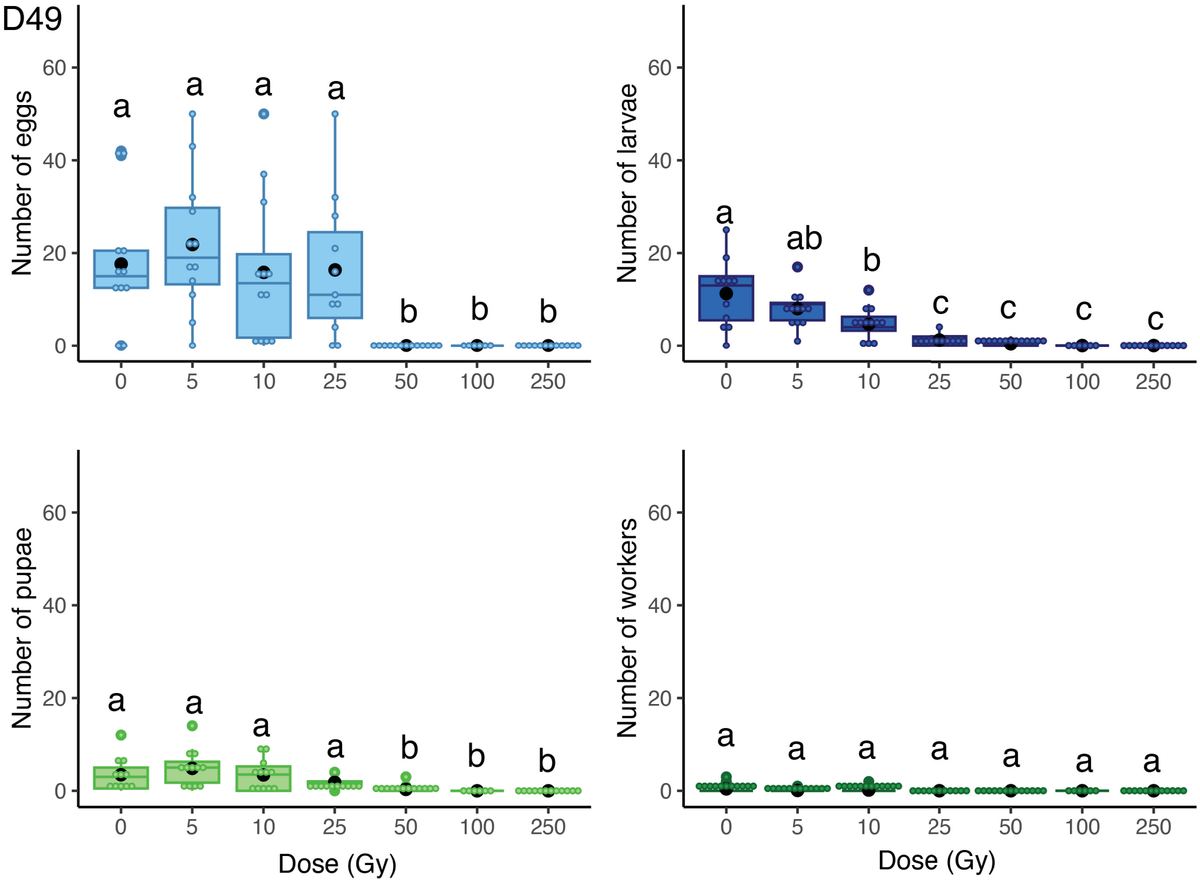


Figure S2 – Evolution of colony foundations at day 49, for tested radiation doses ranging from 0 to 250 Gy. The color and letter codes used for egg (top left), larva (top right), pupa (bottom left) and worker (bottom right) productions refer to Figure 3.

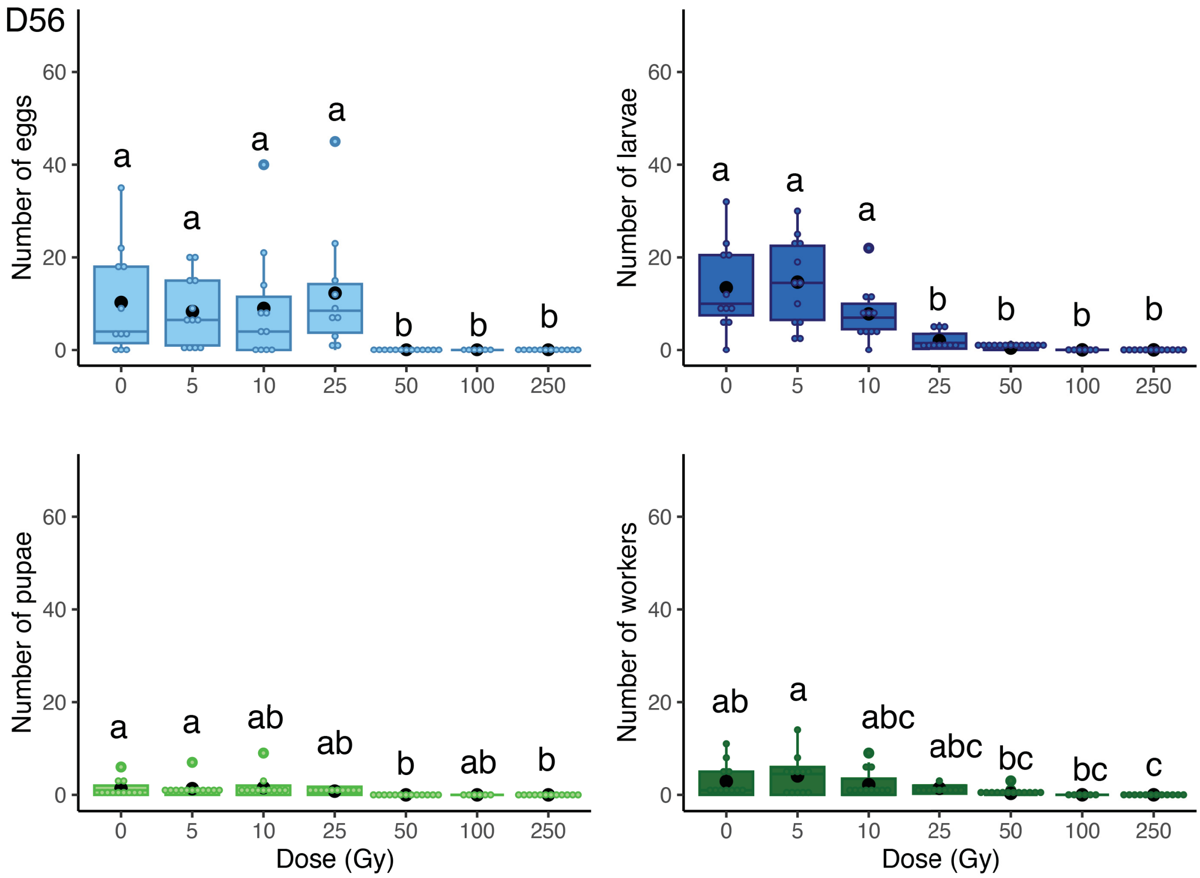


Figure S3 – Evolution of colony foundations at day 56, for tested radiation doses ranging from 0 to 250 Gy. The color and letter codes used for egg (top left), larva (top right), pupa (bottom left) and worker (bottom right) productions refer to Figure 3.

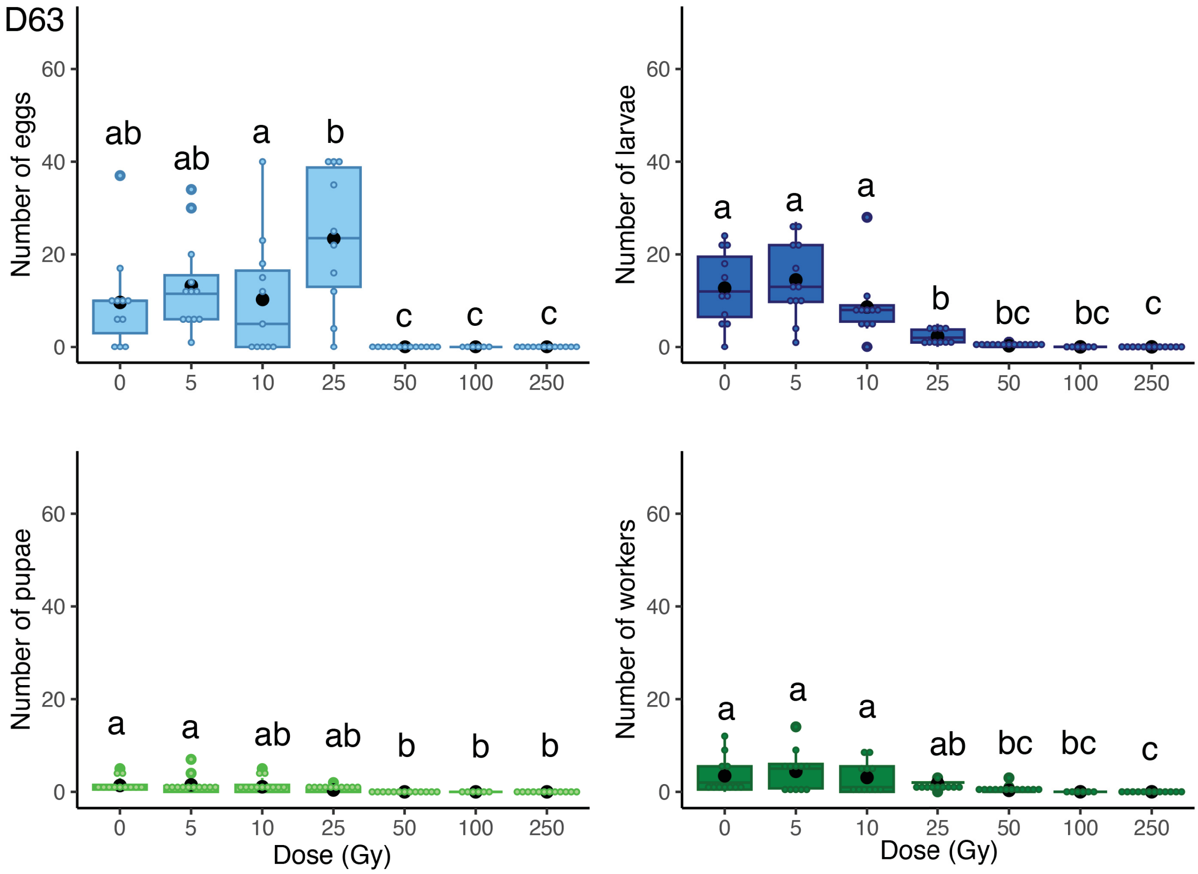


Figure S4 – Evolution of colony foundations at day 63, for tested radiation doses ranging from 0 to 250 Gy. The color and letter codes used for egg (top left), larva (top right), pupa (bottom left) and worker (bottom right) productions refer to Figure 3.

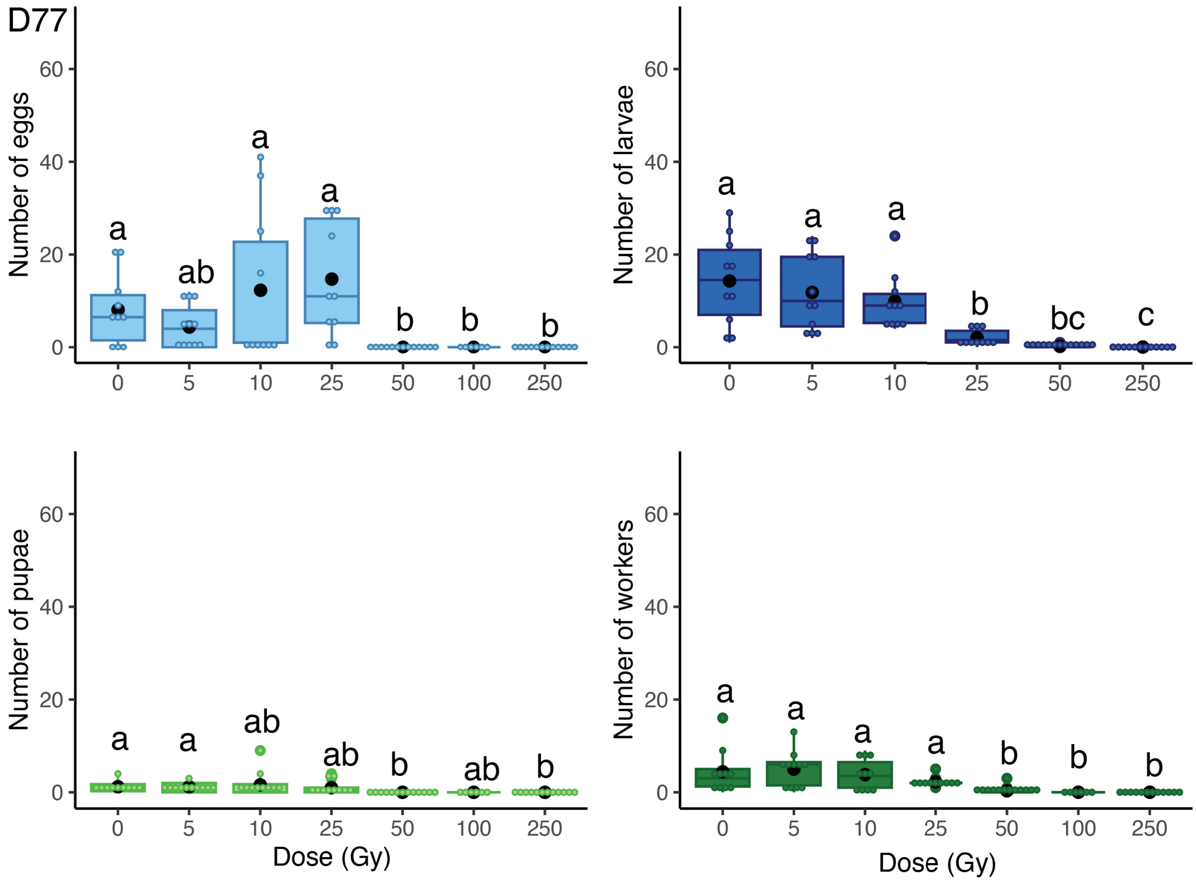


Figure S5 – Evolution of colony foundations at day 77, for tested radiation doses ranging from 0 to 250 Gy. The color and letter codes used for egg (top left), larva (top right), pupa (bottom left) and worker (bottom right) productions refer to Figure 3.