# Chernobyl-level radiation exposure damages bumblebee reproduction: a laboratory experiment

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The consequences for wildlife of living in radiologically contaminated environments are uncertain. Previous laboratory studies suggest insects are relatively radiation-resistant; however, some field studies from the Chernobyl Exclusion Zone report severe adverse effects at substantially lower radiation dose rates than expected. Here we present the first laboratory investigation to study how environmentally-relevant radiation exposure affects bumblebee life-history, assessing the shape of the relationship between radiation exposure and fitness-loss. Dose rates comparable to the Chernobyl Exclusion Zone (50-400 µGy $h^{-1}$) impaired bumblebee reproduction and delayed colony growth but did not affect colony weight or longevity. Our best-fitting model for the effect of radiation dose rate on colony queen production had a strongly non-linear concave relationship: exposure to only 100 µGy $h^{-1}$ impaired reproduction by 30-45%, while further dose rate increases caused more modest additional reproductive impairment. Our data indicate that the practice of estimating effects of environmentally-relevant low dose rate exposure by extrapolating from high dose rates may have considerably underestimated the effects of radiation. If our data can be generalised, they suggest insects suffer significant negative consequences at dose rates previously thought safe; we therefore advocate relevant revisions to the international framework for radiological protection of the environment.

Keywords: ionising radiation; environmental protection; life history, insect, environmental contamination

## 1. Introduction

Ionising radiation damages biological molecules and cells [1,2], and at high dose rates can cause death or significant fitness loss to organisms [3,4]. Most ionising radiation in the environment comes from natural sources. However, some geographic regions have elevated radionuclide levels due to anthropogenic activities, resulting in considerable radiation exposure to wildlife [5,6]. There remains no consensus whether chronic low-dose exposure is detrimental to wildlife because field studies are frequently contradictory [7–10]. Some evidence suggests these levels of radiation do not affect wildlife [11,12]. Whereas several studies in the Chernobyl Exclusion Zone report significant adverse effects on wildlife at exceptionally low radiation levels, equivalent to background dose rates in uncontaminated environments (e.g. 0.1 µGy $h^{-1}$) [13–16]. However, these findings have been challenged as unreliable by some authors; therefore considerable uncertainty remains as to the effects of chronic low-dose radiation on wildlife [10,17–20]

Laboratory-based experiments investigating radiation effects on insects typically use acute radiation exposure (high dose rates delivered over a short time) and assess crude metrics such as death or sterility [21,22]. Such laboratory studies are often used to extrapolate to the likely effects of chronic low-dose rates in contaminated natural environments such as the Chernobyl Exclusion Zone, yet they may have limited ecological relevance [23,24]. Unambiguous determination of the effects of chronic low-dose radiation exposure for wildlife is imperative to predict radiation impacts on ecosystem function in contaminated environments [25], advise in the case of future radiation accidents [26], adhere to ethical obligations associated with environmental protection [27], and to test whether current regulations are fit for purpose [28].

The immediate effects of substantial exposure to high dose rate radiation are relatively well known, both for humans [2] and animals [29]. The impacts of sporadic or continuous exposure to low dose rates are harder to estimate with certainty, in part due to the stochastic nature of some radiation damage, but also due to incomplete understanding of the links between molecular damage and subsequent morbidity [30] and of the effectiveness of repair processes [31]. Radiation biologists have attempted to estimate the effects of low dose exposure using data from higher dose scenarios, often using linear relationships such as the controversial Linear No Threshold Model [32,33]. In other contexts non-linear relationships between dose and damage have been proposed; these usually assume that damage escalates with increasing dose, resulting in a low dose region with relatively little biological effect that is perceived as ‘low risk’. Nevertheless, empirical tests of the shape of the relationship between radiation dose and subsequent fitness remain relatively rare for animals [34,35]. Knowledge of this relationship is, however, crucial for estimating the ecological impacts of environmental radiological contamination and for radioprotection policy.

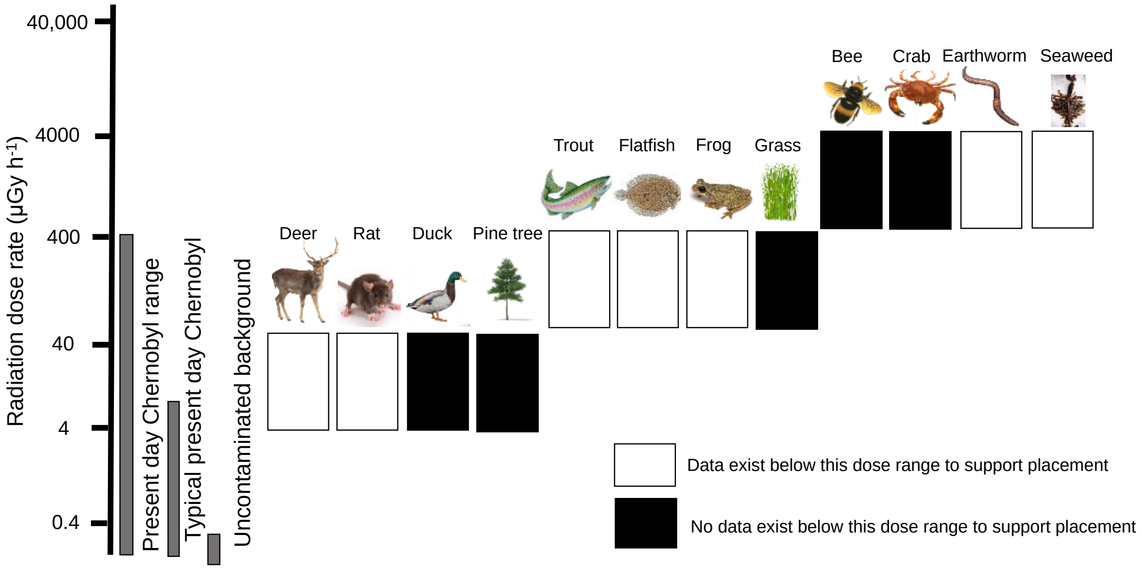

**Figure 1.**

Predicted radio-sensitivity of each Reference Animal and Plant (RAP) based on the Derived Consideration Reference Levels (DCRLs) established by the International Commission on Radiological Protection (ICRP) [23]. DCRLs predict the dose rate range within which radiation is likely to start having deleterious effects (mortality, morbidity or reproduction) on an individual organism [23]. Estimated dose rates for the present-day Chernobyl Exclusion Zone are demonstrated for context (Chernobyl dose rates are now chronic and low, approximately 1% of those at the time of the accident in 1986) [26].

The International Commission on Radiological Protection (ICRP) provides global recommendations for the protection of both humans and wildlife. For wildlife, Reference Animals and Plants (RAPs) are used; each RAP has a designated dose rate band (a DCRL: Derived Consideration Reference Level) within which deleterious effects are predicted to start occurring (figure 1: [23]). The insect RAP is a “eusocial bee”. The framework suggests insects are less radiosensitive than other taxa (figure 1), predicting negative effects on bees only at the highest radiation levels considered (400 – 4000 µGy $h^{-1}$). However, no experimental studies exist on bees within or below this dose rate band and there are relatively limited data for other invertebrates to justify this conclusion [23]. Our work provides the first experimental test to examine whether current assumptions about bee radiosensitivity are correct. We studied bumblebees, not only because of there is a key knowledge gap associated with radiological protection but also because bumblebees provide essential pollination services that underpin ecosystem function [36].

To our knowledge, we have conducted the first experiment to bridge the gap between laboratory and field radiosensitivity studies in insects. We used an experimental radiation exposure facility to address three questions: (i) does chronic exposure to radiation dose rates found in the Chernobyl Exclusion Zone affect bumblebees; (ii) what is the shape of the relationship between dose rate and fitness effects; and (iii) is there a clear lower threshold below which bees are not affected? Under controlled conditions bumblebee colonies were exposed to a radiation gradient for a substantial proportion of colony lifespan, then after this laboratory exposure period bumblebees foraged naturally outside. We assessed bumblebee colony fitness by measuring key life-history traits: reproductive success, colony growth, as well as the longevity of colonies, workers and queens. The production of new queens by bumblebee colonies is the most important metric of reproductive success because queens are the only individuals to overwinter and form new colonies the following year [37]. Our results demonstrate that radiation exposure causes reproductive impairment and that dose rates in the range currently found within the Chernobyl Exclusion Zone contribute disproportionately to this damage.

## 2. Materials and Methods

### (a) Experimental Design

Fifty-nine commercial *Bombus terrestris audax* colonies (comprising a queen, workers and brood) were purchased from Biobest®. For four weeks colonies were positioned in a climate-controlled (25°C and a 12 hr, L:D light cycle (07h – 19h)) radiation facility containing a gamma-emitting caesium-137 source and a control area shielded from radiation. We chose a gamma emitting source because estimates have shown that 95% of the dose rate for wild bumblebees in the Chernobyl Exclusion Zone is due to external gamma radiation [38]. Bumblebee colonies were housed in a standard box (25cm (l) x 30cm (w) 20cm (h)) for the duration of the experiment and rotated 180° every two days to reduce within-colony radiation exposure heterogeneity. Each colony in the radiation treatment group received a unique dose rate (20 - 3000 $\mu Gy\ h^{-1}$) to encompass both the dose rate range measurable in the Chernobyl Exclusion Zone and the range of the bee DCRL (figure 1). We achieved the radiation gradient by varying colony distance from the caesium-137 source within the radiation facility and verified dose rates post-experiment using dosimeters at each colony's position. Monitoring, maintenance and bee-marking were conducted in a neighbouring unirradiated area under red light.

Colonies were weighed before the experiment and were distributed across the radiation exposure gradient randomly, except for colony start weight, which we systematically ensured was not associated with radiation dose rate ($F_{1,\,57} = 0.07$, $p = 0.791$). Anti-wax moth concentrate (Certan/B401® from Dragonfli, 1 in 20 in water) was applied to colony containers at weeks two and five of the experiment to protect against wax moth infestation (which was subsequently not observed). Colonies received *ad libitum* dried pollen and artificial nectar (Biogluc®); Biogluc reservoir weight was measured weekly during the laboratory exposure phase to assess food intake. In exposure week three, before colonies were moved outdoors, a subset of 30 workers from 29 of the colonies were marked with individual coloured number tags attached to the thorax to enable estimation of worker longevity [37].

After four weeks, radiation exposure stopped; colonies were moved from the radiation facility and randomly assigned outdoor locations in the University of Stirling campus gardens (56.1454° N, 3.9206° W) which has background radiation levels (0.11 ± 0.01 µGy $h^{-1}$). Colonies were sheltered from the weather in plastic boxes and were situated close to ornamental gardens, deciduous woodland and mixed farmland. Natural foraging was encouraged by reducing the pollen supply two days before outdoor placement and preventing access to Biogluc® from the day colonies were placed outdoors. Total colony weight was measured weekly throughout both radiation exposure and field phases until colony death.

Once outside, colonies were checked post-sunset every 1-2 days to determine colony and queen death dates. Tagged workers were recorded as alive, dead or missing. Worker longevity was expressed as the number of days between the tagging date and the day of death or absence. No absent bumblebees subsequently returned to the colony. Queen longevity was defined as the time in days from the start of the experiment to the date at which the queen was observed dead. Colonies were considered dead once the queen had died and fewer than five workers remained; colonies were then euthanised at -80°C for 45 minutes. Bumblebees construct wax cells in which pupae develop; males and workers develop in similar-sized cells, whereas queen cells are much larger [36]. Posthumous colony dissection allowed colony counts of the combined worker and male population, as well as queen production [39]. Pupal cell counts included emerged and non-emerged cells: 100% of the queen cells and 93% (± 3% SE) of worker/male cells had emerged.

### (b) Statistical Analysis

Analyses were conducted in R (version 3.5.1 [40]). Maximal models were established and each predictor’s inclusion was evaluated using an F or chi-squared test to compare the goodness of fit between models; non-significant predictor variables were removed (in addition we calculated Akaike Information Criterion (AICc) for model terms on removal). For most analyses the unit of replication was the colony; however for cases involving repeated measures or where parameters were assessed for individual bees, we used mixed effects models with colony as a random effect to account for non-independence. Model diagnostics were checked to validate fit to assumptions. Means are stated in the text ± standard error.

We constructed negative binomial generalised linear models to investigate associations between colony queen production and dose rate using “glm.nb” from the MASS package [41]. The maximal model’s predictor variables were: dose rate, colony start weight, whether workers from the colony were tagged, and a colony start weight by dose rate interaction. We tested for a non-linear relationship between queen production and dose rate: first in separate negative binomial generalised linear models using either a square-root or a natural log transformation of dose rate (ln(dose rate + 1)), then using a saturating exponential function in Nonlinear least squares (nls) (f(dose rate)=k1*(1-exp[-k2*dose rate]). We compared these models using AICc and the plotted residuals to evaluate model fit. Factors influencing the combined count of worker and male pupal cells were assessed using the same model structure and dose rate transformations.

Peak colony weight occurred only at either week four or five; therefore, to assess factors influencing this timing we used binomial generalised linear models with predictors for dose rate, colony start weight, and a colony start weight by dose rate interaction. We tested for non-linear effects of radiation using the same transformations as we did for queen production.

Separate Cox’s proportional hazards models (“coxph” from the package survival) [42] were used to analyse factors influencing colony and queen longevity: dose rate, colony start weight and tagged status were predictor variables. Drivers of worker longevity variation were assessed using mixed-effects Cox models (“coxme” [42]): models included dose rate, colony start weight and worker status (observed dead or absent from colony) as fixed effects, and colony identity as a random effect.

The magnitude of colony weight change was assesed in two time periods: the exposure phase (weeks 1 – 4: increasing weight) and the field release phase (week five onwards: declining weight). Linear mixed-effects models were fitted using “lmer” from the lme4 package [43] with colony identity as a random effect to account for repeated measures. Fixed covariates were dose rate, colony start weight, experiment week and all two-way interactions. We included interaction terms to test whether the effect of dose rate on colony weight was influenced by either colony start weight or week number. The same model structure was used to investigate variation in Biogluc® consumption.

## 3. Results

### (a) Effect of radiation exposure on queen production

Queen production per colony ranged from zero to 58 (mean = 16.1 ± 1.67 SE). Bumblebee colonies exposed to radiation produced fewer queens (mean = 12.0 ± 3.50 SE, n = 39) than unexposed colonies (mean = 21.1 ± 3.37 SE, n = 20). Initially, we fitted a linear relationship between dose rate and queen production, demonstrating a strongly significant negative effect of radiation on reproduction ($F_{1, 57} = 7.31$, $p = 0.007$, figure S1, table S1). We tested for effects of colony start weight, tagging status of workers and a start weight by dose rate interaction, which were not significant (table S1).

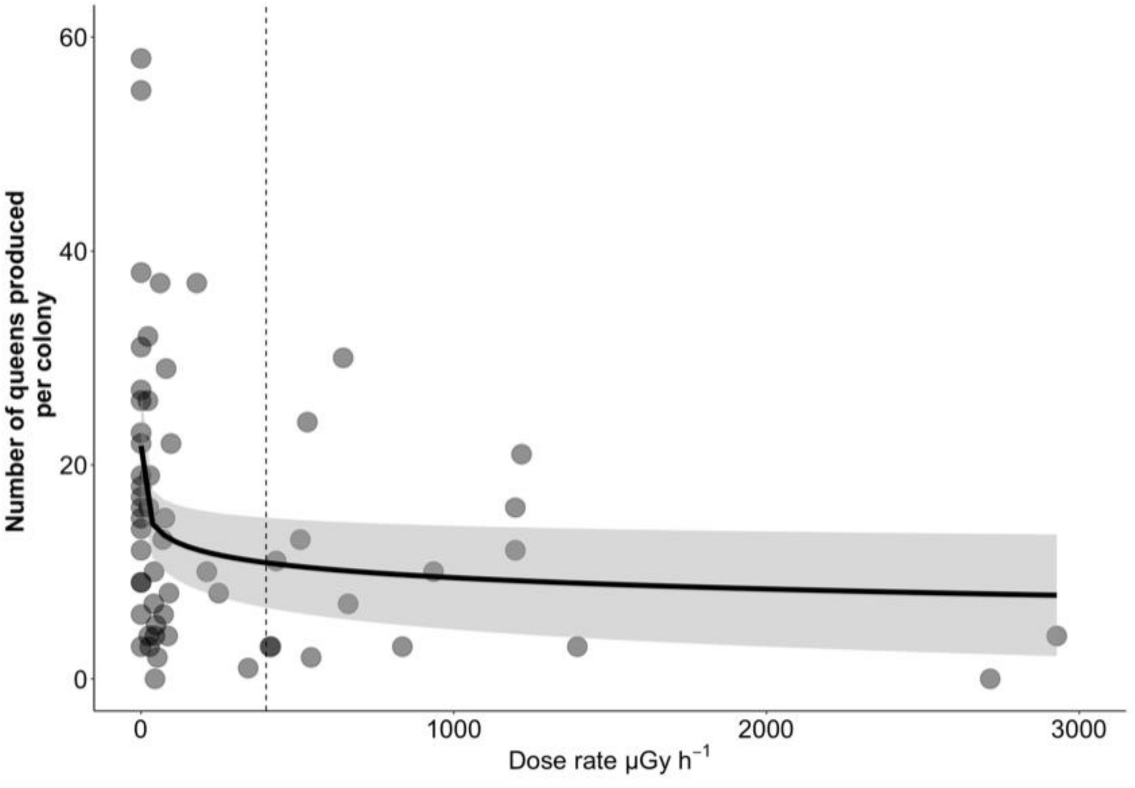

**Figure 2.**

The impact of radiation on bumblebee colony queen production (n = 59). Plotted points are partial residuals from a negative binomial model with a log-transformed dose rate predictor. The fitted line with 95% confidence intervals is derived from the model. The vertical dotted line indicates the upper limit of dose rates know to occur in the Chernobyl Exclusion Zone [26]. Y-axis is offset from zero to show control colony data effectively.

Next, we tested whether the effect of radiation dose rate was non-linear using the same model structure and compared the fit of models with different curvilinear transformations of dose rate. A log-transformed dose rate predictor produced the best fitting model (AICc = 437.4, table S2), compared to a square-root transformation (AICc = 437.9, table S2), the original linear fit (AICc = 438.2, table S2) and the saturating expotential function (AICc = 469.5, table S2). While the log, square root and linear models all fell within 2 AICc points of each other, the best-fitting log-transformed model suggested substantial nonlinearity in the effect of elevating radiation dose rate ($F_{1, 57}$ = 8.85, p = 0.004, figure 2, table S3). The log-transformed model predicted a 46% reduction in colony queen production at only 100 µGy h$^{-1}$; at 400 µGy h$^{-1}$ reproduction decreased by 51% and then decreased only modestly further (by 59%) in colonies exposed to 3000 µGy h$^{-1}$. In contrast, the linear model (figure S1) predicted that colony queen production declined by 6% at 100 µGy h$^{-1}$, 28% at 400 µGy h$^{-1}$ and 82% in colonies exposed to 3000 µGy h$^{-1}$. The results from the square-root transformed model were intermediate between these two. Within our dataset, two colonies from the control area produced very high numbers of queens and might be considered outliers (30% more queens than other colonies). The relationship between dose rate and queen production remained significant when these two data points were removed from all the above models (log dose rate: ($F_{1, 55}$ = 4.54, p = 0.033, table S3); square root dose rate: ($F_{1, 55}$ = 5.28, p = 0.022); and the linear dose rate model ($F_{1, 55}$ = 5.76, p = 0.016, table S1).

Our data provide compelling evidence for substantial fitness consequences to bumblebees across the dose rate range we assessed (figure 2). Next, we investigated the strength of support for negative effects on queen production at lower dose rates relevant to the Chernobyl Exclusion Zone. We progressively truncated the dataset to exclude dose rates above 2000, 1000, 500, 100 and 50 µGy h$^{-1}$ and fitted the same models as the original analysis. This process inevitably reduced our sample size and statistical power.

The negative effect of radiation remained significant in all these analyses as low as 50 µGy h$^{-1}$ when using a log-transformation of dose rate to generate a non-linear fit (table S3). Whereas the linear-effect model using the untransformed dose rate predictor was significant for some but not all these truncated data sets (table S1). The linear model provided additional evidence that the effect of radiation on queen production was curved. If the effect had been linear with no threshold over the dose rate range we studied, we would have expected the parameter estimate for the impact of radiation per unit exposure to be broadly similar over the full range. Instead, the parameter estimate dramatically increased as we restricted our analysis to progressively lower dose rates (table S1), supporting the non-linear curve that our logarithmic transform model previously revealed.

### (b) Effect of radiation exposure on male and worker output

As a second metric of reproduction, the combined number of worker and male cells produced by each colony was counted (worker and male cells are indistinguishable). We tested for a correlation between queen production and worker-male production and detected a weak negative relaitonship (Pearson r = -0.29, n = 59, P = 0.021) suggesting that, whilst these two reproduction metrics are not fully independent of one another, they may trade-off. Mean worker-male cells per colony was 263.1 (± 8.00 SE). Worker-male production reduced significantly in response to increasing radiation (linear dose rate predictor: $F_{1,\ 55} = 4.37$, p = 0.041, table S4).

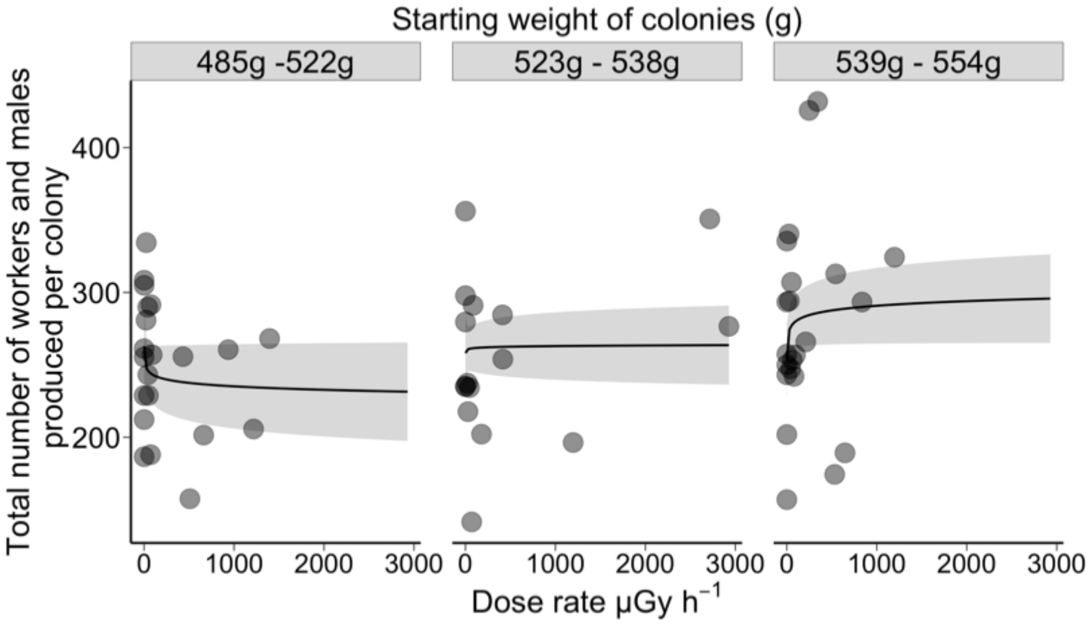

**Figure 3.**

The effect of radiation on bumblebee colony ability to produce males and workers, for colonies of different starting weight. Colonies were put into three start weight groups (low, mid and high) of approximately equal sample size (n = 20, 20, 19 respectively) to maintain equivalent statistical power for each. The points and fitted lines are derived from a negative binomial model containing a log-transformed dose rate predictor and its interaction with colony starting weight (specified as a three-level factor). Whilst the negative effect of radiation was individually significant for the lighter colonies, the positive slopes for mid-weight and the heaviest colonies were not significant (see text). The shaded area around the fitted lines represents the 95% confidence interval. Y-axis is offset from zero to show control colony data effectively.

We compared the fit of models containing non-linear transformations of the dose rate predictor (log transformation AICc = 651.4; square-root transformation AICc = 652.5) with the untransformed linear variable (AICc = 653.0). The strongest support was for a curvilinear log-transformation of dose rate ($F_{1,\ 55}$ = 6.99, p = 0.010, table S5), although AICc separation between models was only 1.6 points.

The negative effect of radiation on colony worker-male production varied according to colony start weight (log-transformed dose by start weight interaction: $F_{1,\ 55}$ = 7.10, p = 0.010, figure 3, table S5). We investigated this interaction by dividing the dataset into three colony weight classes (table S5): the negative effect of log-transformed dose rate on worker-male production in the smaller colonies was marginally significant ($F_{1,\ 18}$ = 3.31, p = 0.088) but the positive effect was not significant in mid-weight ($F_{1,\ 18}$ = 0.26, p = 0.611) or heavier colonies ($F_{1,\ 17}$ = 2.18, p = 0.157). We repeated these analyses for the linear model: whilst the interaction between dose rate and colony start weight was significant, the individual tests of the effect of dose rate in each of the three weight subgroups were not (table S4). Effect of radiation on colony growth timing.

### (c) Effect of radiation on colony growth timing

Bumblebee colonies typically undergo a growth phase as workers are produced, then peak in weight as colonies generate reproductives towards the end of the colony cycle [36,44]. All colonies reached peak weight either in week four or five of the experiment (colonies were placed outside to forage at the beginning of week five, which curtailed further growth). Increasing radiation dose rate was significantly associated with an increased probability of delay in reaching peak weight ($\chi^2_{1,\ 57}$ = 11.08, p = 0.0008, figure 4, table S6). Our model predicted that 50% of control colonies reached peak weight at the later date, whereas for those exposed to 400 µGy $h^{-1}$ this percentage increased to 81%. The binomial response in this analysis and the logit transformation underlying the model make it difficult to assess the extent of non-linearity in the effect of radiation on peak weight timing. Nevertheless, we tested for curvature in this relationship using log-transformed (AICc = 75.1) and square-root transformed (AICc = 71.5) dose rate, but a linear fit using the untransformed dose rate predictor was superior (AICc = 69.9).

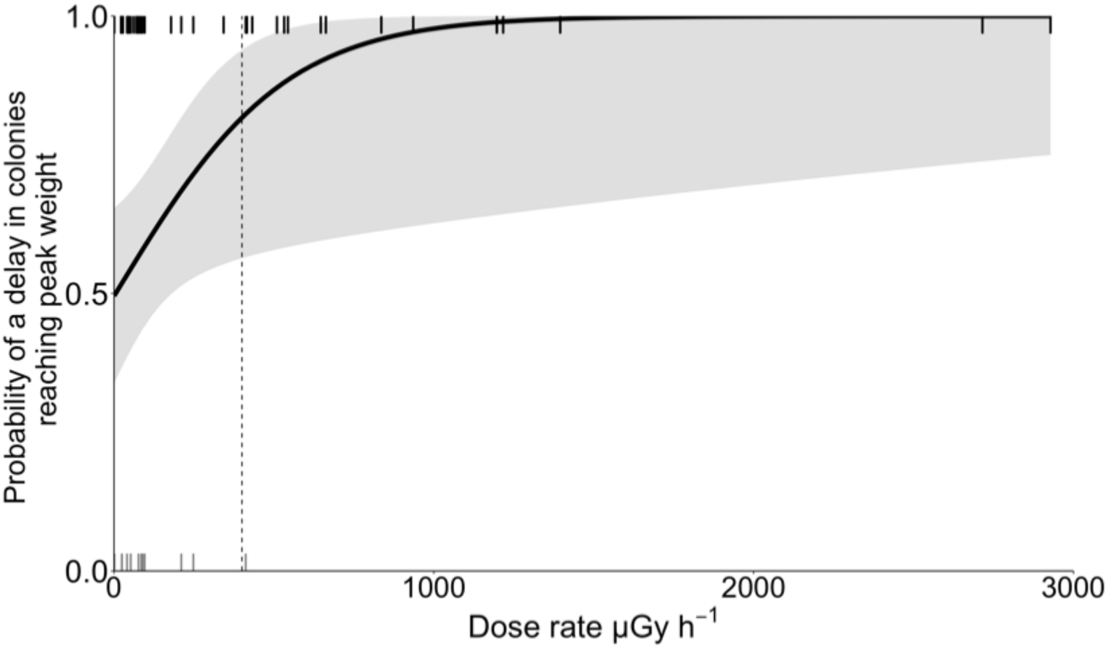

**Figure 4.**

The effect of radiation on the number of experimental weeks it took bumblebee colonies to reach peak weight (week four or five). The fitted line is the predicted relationship from a binomial generalised linear model; the shaded area shows the 95% confidence intervals. The dotted line indicates the upper limit of dose rates measured in the Chernobyl Exclusion Zone. Tick marks at the top and bottom of the figure show raw data points.

### (d) Effect of radiation on longevity and colony weight change

At week three, a subset of 30 workers from half the colonies (n = 29 colonies, 870 bees) were marked with number tags to assess individual worker longevity after field release. Of these workers, 76% disappeared from the colony within 48 hours of release and were not sighted again. The remaining marked workers either died in the nest (n = 47) or were subsequently recorded absent (n = 166). Cox survival analysis on these remaining workers detected a negative but non-significant effect of radiation dose rate on worker longevity ($\chi^2_1 = 1.12$, p = 0.261, table S7). There was no significant longevity difference between bumblebees that died in the nest or were recorded absent ($\chi^2_1= 2.47$, p = 0.115, table S7). Colony longevity was not significantly affected by dose rate (table S8), nor was queen longevity (queen death date was successfully recorded in 20 (33%) of the colonies**)** (table S9). Colonies from which workers were tagged lived longer than untagged colonies (table S8); these tagged colonies were distributed evenly across the radiation gradient ($F_{1, 57} = 0.07$, p = 0.791). Tagging workers did not affect colony queen production ($F_{1, 56} = 1.87$, p = 0.171), nor influence production of workers and males ($F_{1, 54} = 0.08$, p = 0.767).

All colonies gained weight throughout the four-week laboratory radiation exposure phase and lost weight during the outdoor period (due to loss of individuals and resources) until colony death. However, there was no relationship between radiation dose rate and colony weight change during either of these phases (tables S10 and S11). Similarly, artificial nectar (Biogluc®) consumption increased over time during the radiation exposure phase but was not influenced by dose rate (table S12).

## 4. Discussion

Here we experimentally demonstrate that bumblebees are negatively affected by exposure to ionising radiation at dose rates significantly lower than previously thought likely to impact insects. We assessed the impact of radiation exposure on eight fitness-related traits: colony queen production, male-worker production, colony growth timing, colony weight, colony nectar consumption, worker lifespan, queen lifespan and colony lifespan. We found negative effects of radiation exposure on the first three of these; radiation dose rates equivalent to the more contaminated areas of the Chernobyl Exclusion Zone impaired both reproduction metrics and delayed colony growth.

Several of our analyses suggested that the effect of increasing radiation dose rate on bumblebee reproductive fitness was non-linear. Contrary to established hypotheses that lower dose rates of radiation have little effect on insects, our best-fitting models indicated that a rapid decline in bumblebee reproduction occurred over the range 0-100 µGy h$^{-1}$ and that further increases in radiation dose rate had relatively smaller additional effects. Our evidence for non-linear effects is from two sources. Firstly, for our analyses of colony queen production, the best fitting model contained a log transformation of dose rate, although AICc improvement relative to a linear predictor was only modest. Secondly, when using an untransformed dose rate predictor in models that assumed a linear effect of radiation, as we progressively restricted our analysis to lower and lower dose rates the relationship between dose rate and reproductive impairment became considerably steeper. We also tried analyses using other non-linear modelling techniques, but for these the fit was generally poor. Radiation protection for wildlife frequently relies on knowledge of the shape of the relationship between radiation exposure and damage caused; this is particularly important to enable extrapolations to low dose exposures where biological effects may be challenging to detect. Our study suggests that linear extrapolations to low dose rates may substantially underestimate radiation effects, at least for bumblebees.

Our data unambiguously demonstrate a negative effect of radiation on bumblebee colony queen production when considering the full dose rate range in our experiment (0-3000 µGy h$^{-1}$). Determining a conclusive lower threshold at which these effects start occurring is difficult because as we restricted our analysis to progressively lower dose rates our sample size declined (for example, we only had seven radiation-exposed colonies below 50 µGy h$^{-1}$). However, our analyses certainly provide no evidence that this deleterious effect of radiation exposure disappeared in the 50-100 μGy $h^{-1}$ range: tests in this range assuming a non-linear dose rate effect were significant.

Our study makes a major step forwards to resolve the ongoing controversy of whether radiation dose rates at contaminated sites such as the Chernobyl Exclusion Zone are damaging to invertebrates. Our best-fitting model, with log-transformation of the dose rate predictor, indicated that exposure to 100 μGy $h^{-1}$ decreased bumblebee colony queen production by 46%. Queens are demographically limiting, so this decrease in queen production has the potential to substantially impact bumblebee populations [45]. Approximately comparable to our findings, pesticide exposure has been reported to reduce bumblebee colony founding success by 26% and was predicted to dramatically increase the likelihood of local population extinction [46]. Impaired pollination services due to radiation-induced reductions in bumblebee population size could drive negative consequences for the whole ecosystem [47]. Furthermore, we suggest that our laboratory estimates of the negative consequences of radiation exposure may be conservative compared with radiation effects on wild populations that are exposed to multiple other stressors.

Radiation exposure also reduced the combined total of males and workers that colonies produced. As for queen production, our analyses suggested that this effect of radiation was non-linear and that the negative effects started to plateau above approximately 100 μGy $h^{-1}$. However, this reduction in worker-male production occurred only in smaller colonies; heavier colonies appeared to be protected from adverse radiation effects. We speculate that either larger colonies could buffer against radiation stress, or that in colonies which started the experiment smaller, a greater proportion of the future worker-male population might have been at a particularly radiosensitive pre-adult stage. Reduced male output directly impairs colony reproductive success [48] and a smaller worker cohort reduces the colony's capacity to support new queens [49].

Radiation also delayed the time that colonies took to attain their peak weight. Control colonies had a 50% probability of experiencing a one-week delay in reaching their peak weight; whereas exposure to 400 μGy $h^{-1}$ increased this probability of reproductive delay to

81%. The timing of peak weight can be used as a proxy to indicate the time reproductives are produced [50]. Reproductive delay is a common stress-response in bumblebees [51,52]. Colonies which generate reproductives earlier have greater reproductive success as they can produce more queens and maximise mating opportunities for males [53,54]. Our colonies were released to forage in the field after one month of irradiation; many workers disappeared from the colonies at this point and colony weight gain stopped. Therefore, our experimental design artificially curtailed colony growth, which may mean that our estimate of the growth delay caused by radiation is an underestimate.

Previous laboratory studies, typically using acute exposures, have suggested that invertebrates are relatively resistant to ionising radiation [55]. The consequences of acute high-dose radiation exposure to animals have been well-studied [22,56,57]; acute dose rates are thought to overwhelm repair mechanisms and result in significant damage [58]. In comparison, we have limited knowledge as to the effects of chronic low-dose radiation exposure on wildlife [59]. Our colonies chronically-exposed to 100 µGy $h^{-1}$ for four weeks received an accumulated dose of 0.07 Gy, while the most highly exposed colonies received 2 Gy over this time period. Our effects are comparable to previous studies that delivered similar total doses acutely: exposure to 1-2 Gy is reported to cause 50% mortality in sub-adult Hymenoptera [60], and exposure to 60 Gy reduced the number of eggs laid by queen ants by 90% [61]. However, we acknowledge that there is minor uncertainty as to the dose each individual bee received because bees were free to move around inside their colony box, furthermore bees developed and eclosed at different times during the experiment.

Our data show chronic low-dose exposure is more harmful than previously thought. We propose two hypotheses which could explain these effects at chronic low dose rates. (I) Radiation-induced oxidative damage might directly harm cells with negative consequences for reproductive capacity. However, we note that dose rates of 400 µGy $h^{-1}$ and below are predicted to create too few reactive oxygen species to cause significant damage from oxidative stress [19]. Alternatively, (II) radiation effects might activate bumblebee stress responses, which could drive costly tradeoffs with reproduction. Defence and repair mechanisms, such as mounting an immune response, can indeed impair fitness in bumblebees [62,63].

Our findings have significant implications for the international environmental protection framework used by the ICRP in which radiation dose rate bands (DCRLs) for different taxa predict the exposure at which negative effects are likely to occur [23]. We provide the first data to evaluate the DCRL for bees, which ICRP use as a reference to represent all insects. We demonstrate a substantial reduction in bumblebee queen production (approximately -50%) at the lowest end of the current dose rate band (400 µGy $h^{-1}$). We recommend to the ICRP that the dose rate band should be lowered to 40-400 µGy $h^{-1}$, placing insects in the same radiosensitivity category as some vertebrates (Figure 1). However, we have very few data for dose rates below 40 µGy $h^{-1}$ (only 3 exposed colonies), preventing us from making any strong conclusions below this, which would require us to extrapolate our findings to unstudied dose rates.

Our findings demonstrate that the most contaminated areas of the Chernobyl Exclusion Zone could cause substantial damage to bumblebees. Nevertheless, our analyses suggest it would be unlikely that bumblebees would experience severe adverse effects at the very low, near-background Chernobyl dose rates (eg 0.01 µGy $h^{-1}$) reported by some authors [15,16].

## 5. Conclusion

Our findings help resolve an ongoing controversy in radioecology and call for changes in radiological protection practice. We provide the first experimental evidence of significant detrimental effects on insect reproductive success at environmentally-relevant dose rates previously not predicted to affect insects. Furthermore, our results suggest that the impact of radiation is non-linear, such that bumblebee fitness-loss increases rapidly at lower dose rate ranges. We suggest that radiation exposure could have significant implications for wild insect populations in the more highly contaminated areas of post-disaster sites such as the Chernobyl Exclusion Zone.

## Data accessibility

Data are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.j3tx95xb5

## Authors' Contributions

K.R., P.R.W., D.C. and M.C.T. designed the study. K.R. collected the data. Data were analysed by K.R. and M.C.T. The manuscript was written by K.R. and edited by P.R.W., D.C., M.T.

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## Competing interests

We declare we have no competing interests.

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## Supplementary Materials

**Table S1.**

Parameter estimates from a negative binomial generalised linear model testing the effect of radiation dose rate on colony queen production in which the dose rate predictor was untransformed. The estimated reduction in queen production at 100 µGy h$^{-1}$ was calculated using the predict function. The slope estimates and intercepts have not been back-transformed from the log-link underlying the model. At the top of the table are the parameters from the full model, including the values for non-significant terms (indicated in italics) in reverse order of the removal sequence during model simplification. In the second section of the table we progressively restrict the analysis to data from smaller and smaller dose rates to investigate whether there is a lower dose rate threshold below which the negative effect of dose rate on queen production becomes undetectable. The final section of the table repeats these analyses after removal of data from two colonies which produced especially large numbers of queens and which might be considered outliers. The AICc for the best model was 438.2

| **Data set** | **N** | **Estimated % reduction at 100 µGy h$^{-1}$** | **Intercept** | **Estimate** | **Standard error** | **F** | **P value** | **$\Delta$AICc** |
|---|---|---|---|---|---|---|---|---|
| **Full data set** | | | | | | | | |
| Dose rate (µGy h$^{-1}$) | 59 | 6 | 2.86 | -0.0006 | 0.0002 | 7.31 | 0.0068 | |
| *Tagging status of workers* | | | | *-4.5632* | *3.3191* | *1.94* | *0.1695* | *+0.8* |
| *Colony start weight (g)* | | | | *0.0091* | *0.0801* | *0.02* | *0.8651* | *+1.3* |
| *Colony start weight by dose rate interaction* | | | | *-0.0011* | *0.0001* | *0.02* | *0.8653* | *+1.2* |
| **Testing for a threshold dose rate for the effect of radiation** | | | | | | | | |
| Dose rate < 2000 µGy h$^{-1}$ | 57 | 5 | 2.84 | -0.0005 | 0.0003 | 2.36 | 0.1243 | |
| Dose rate < 1000 µGy h$^{-1}$ | 53 | 11 | 2.88 | -0.0009 | 0.0005 | 3.30 | 0.0690 | |
| Dose rate < 400 µGy h$^{-1}$ | 46 | 26 | 2.96 | -0.0030 | 0.0010 | 6.55 | 0.0104 | |
| Dose rate < 100 µGy h$^{-1}$ | 39 | 48 | 3.01 | -0.0064 | 0.0040 | 2.74 | 0.0974 | |
| Dose rate < 50 µGy h$^{-1}$ | 30 | 99 | 3.16 | -0.0283 | 0.0077 | 9.95 | 0.0020 | |
| **Testing for a theshold dose rate for the effect of radiation with the two highest queen producing colonies removed (<50 queens)** | | | | | | | | |
| All dose rates | 57 | 3 | 2.75 | -0.0005 | 0.0002 | 5.76 | 0.0163 | |
| Dose rate < 2000 µGy h$^{-1}$ | 55 | 5 | 2.72 | -0.0003 | 0.0003 | 1.23 | 0.2668 | |
| Dose rate < 1000 µGy h$^{-1}$ | 51 | 8 | 2.87 | -0.0005 | 0.0005 | 1.96 | 0.1607 | |
| Dose rate < 400 µGy h$^{-1}$ | 44 | 22 | 2.82 | -0.0025 | 0.0010 | 4.78 | 0.0286 | |
| Dose rate < 100 µGy h$^{-1}$ | 37 | 33 | 2.82 | -0.0379 | 0.0038 | 1.03 | 0.3078 | |
| Dose rate < 50 µGy h$^{-1}$ | 28 | 89 | 2.97 | -0.0225 | 0.0073 | 7.08 | 0.0077 | |

**Table S2.**

The fit for the three alternative negative binomial models exploring curvature in the effect of dose rate on colony queen production. Models were negative binomial generalised linear models with different non-linear transformations of dose rate except for the saturating exponential function which was fitted using non-linear least squares (nls). For the negative binomial models (log, square root and no transformation) the parameter estimates are on a transformed log scale. For the nls model (saturating exponential function) the parameter estimates are on the original scale.

| **Dose rate predictor** | **AICc** | **Intercept** | **Estimate** | **SE** | **P value** |
|---|---|---|---|---|---|
| Log-transformed | 437.4 | 2.98 | -0.0991 | 0.0328 | 0.0023 |
| Square root transformed | 437.9 | 2.97 | -0.0255 | 0.0083 | 0.0022 |
| No transformation | 438.2 | 2.86 | -0.0006 | 0.0002 | 0.0021 |
| **Parameters from nls** | **AICc** | **K1** | **K2** | **SE** | **P value** |
| Saturating exponential function | 469.5 | 17.71 | -0.0006 | 0.0004 | 0.1241 |

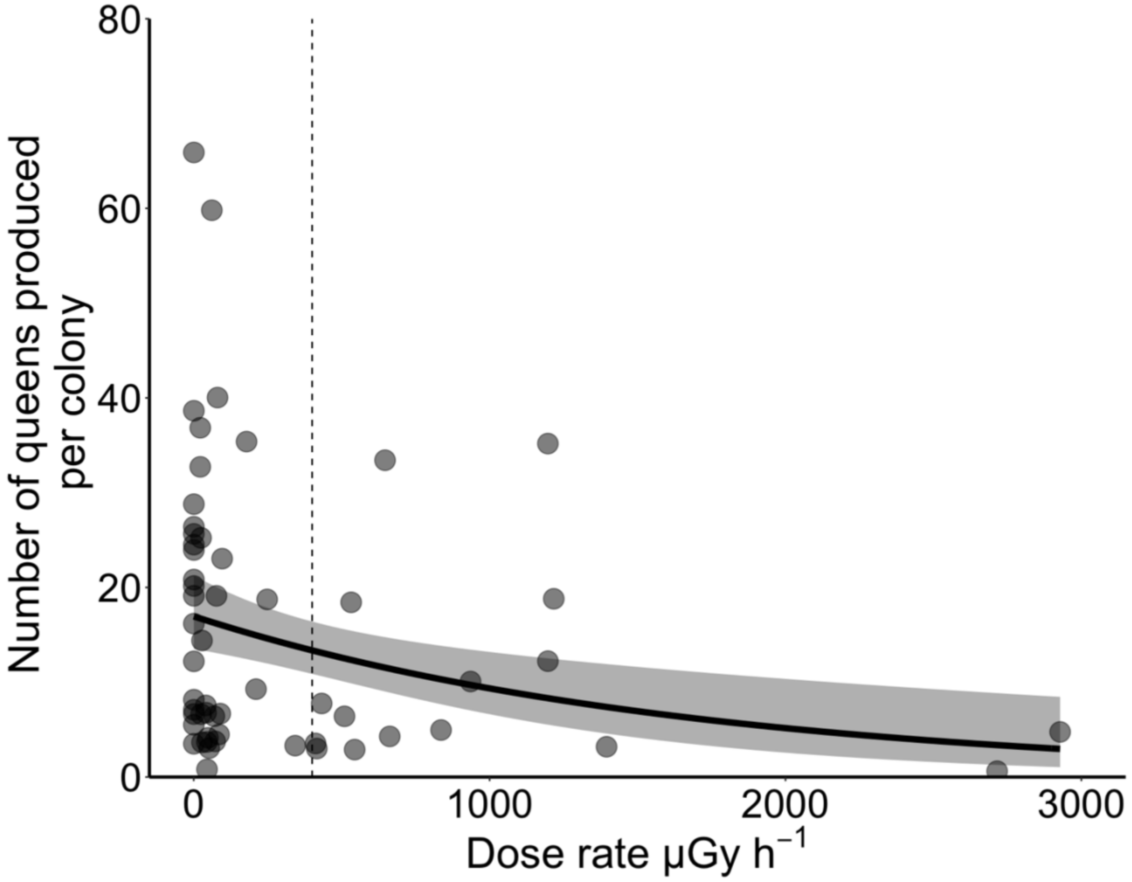

**Figure S1.**

The impact of radiation on bumblebee colony queen production (n = 59) assuming a linear effect of dose rate. Plotted points are partial residuals from a negative binomial model with an untransformed dose rate predictor. The fitted line with 95% confidence intervals is derived from the model. The vertical dotted line indicates the upper limit of dose rates know to occur in the Chernobyl Exclusion Zone [26]. Y-axis is offset from zero to effectively show control colony data.

**Table S3.**

Parameter estimates from a negative binomial generalised linear model testing the effect of radiation dose rate on colony queen production in which the dose rate predictor was log transformed. The estimated reduction in queen production at 100 µGy h$^{-1}$ was calculated using the predict function. The slope estimates and intercepts have not been back-transformed from the log-link underlying the model. At the top of the table are the parameters from the full model, including the values for non-significant terms (indicated in italics) in reverse order of the removal sequence during model simplification. In the second section of the table we progressively restrict the analysis to data from smaller and smaller dose rates to investigate whether there is a lower dose rate threshold below which the negative effect of dose rate on queen production becomes undetectable. The final section of the table repeats these analyses after removal of data from two colonies which produced especially large numbers of queens and which might be considered outliers. The AICc for the best model was 437.4

| **Data set** | **N** | **Estimated % reduction at 100 µGy h$^{-1}$** | **Intercept** | **Estimate** | **SE** | **F** | **P value** | **ΔAICc** |
|---|---|---|---|---|---|---|---|---|
| **Full data set** | | | | | | | | |
| Dose rate (µGy h$^{-1}$) | 59 | 46 | 2.98 | -0.0991 | 0.0328 | 8.85 | 0.0035 | |
| *Tagging status of workers* | | | | *-0.3061* | *0.2130* | *1.87* | *0.1711* | *+2.1* |
| *Colony start weight (g)* | | | | *0.0008* | *0.0002* | *0.03* | *0.8472* | *+2.5* |
| *Colony start weight by dose rate interaction* | | | | *-1.61 x 10$^{-6}$* | *1.08 x 10$^{-5}$* | *0.02* | *0.8723* | *+1.5* |
| **Testing for a threshold dose rate for the effect of radiation** | | | | | | | | |
| Dose rate < 2000 µGy h$^{-1}$ | 57 | 40 | 3.08 | -0.1066 | 0.0416 | 6.48 | 0.0108 | |
| Dose rate < 1000 µGy h$^{-1}$ | 53 | 43 | 3.10 | -0.1201 | 0.0461 | 6.53 | 0.0105 | |
| Dose rate < 400 µGy h$^{-1}$ | 46 | 48 | 3.14 | -0.1493 | 0.5458 | 6.76 | 0.0092 | |
| Dose rate < 100 µGy h$^{-1}$ | 39 | 45 | 3.12 | -0.1413 | 0.0662 | 4.31 | 0.0377 | |
| Dose rate < 50 µGy h$^{-1}$ | 30 | 60 | 3.18 | -0.2357 | 0.0859 | 6.05 | 0.0130 | |
| **Testing for a theshold dose rate for the effect of radiation with the two highest queen producing colonies removed (<50 queens)** | | | | | | | | |
| All dose rates | 57 | 34 | 2.93 | -0.0905 | 0.0411 | 4.54 | 0.0330 | |
| < 2000 µGy h$^{-1}$ | 55 | 29 | 2.89 | -0.0717 | 0.0416 | 2.96 | 0.0852 | |
| < 1000 µGy h$^{-1}$ | 51 | 32 | 2.91 | -0.0821 | 0.0461 | 3.09 | 0.0786 | |
| < 400 µGy h$^{-1}$ | 44 | 36 | 2.94 | -0.1044 | 0.0544 | 3.40 | 0.0651 | |
| < 100 µGy h$^{-1}$ | 37 | 31 | 2.90 | -0.0872 | 0.0649 | 1.77 | 0.1826 | |
| < 50 µGy h$^{-1}$ | 28 | 48 | 2.96 | -0.0814 | 0.0814 | 3.76 | 0.0523 | |

**Table S4.**

Parameter estimates from models which investigated how the combined number of workers and males produced by bumblebee colonies was influenced by radiation dose rate (untransformed) and colony start weight. All predictors from the global model are presented at the top of the table, including values for a non-significant term (indicated in italics) at the point of removal during model simplification. The lower section of the table presents tests of the effect of the dose rate predictor in colonies of three different starting weight categories (small, medium and large) to examine the nature of the start weight by dose rate interaction. The AICc for the best model was 653.0

| **Predictors** | **Estimate** | **SE** | **F** | **P value** | **ΔAICc** |
|---|---|---|---|---|---|
| Full dataset (n= 59) | | | | | |
| Colony start weight (g) | 0.3241 | 0.3726 | 0.75 | 0.3884 | |
| Dose rate (µGy $h^{-1}$) | -0.8642 | 0.4132 | 4.37 | 0.0410 | |
| Colony start weight by dose rate interaction | -0.0021 | 0.0007 | 4.50 | 0.0383 | |
| *Tagged status of workers* | *6.4632* | *15.5514* | *0.17* | *0.6794* | *+2.3* |
| **Colonies with a start weight less than 527g (n = 20)** | | | | | |
| Dose rate (µGy $h^{-1}$) | -0.0581 | 0.0331 | 3.11 | 0.9770 | |
| **Colonies with a start weight between 527 and 548g (n = 20)** | | | | | |
| Dose rate (µGy $h^{-1}$) | 0.0162 | 0.0153 | 1.16 | 0.2924 | |
| **Colonies with a start weight more than 548g (n = 19)** | | | | | |
| Dose rate (µGy $h^{-1}$) | 0.0670 | 0.0445 | 2.28 | 0.1477 | |

**Table S5.**

Parameter estimates from models which investigated how the combined number of workers and males produced by bumblebee colonies was influenced by radiation dose rate (log-transformed) and colony start weight. All predictors from the global model are presented at the top of the table, including values for a non-significant term at the point of removal during model simplification (indicated in italics). The lower section of the table presents tests of the effect of the dose rate predictor in colonies of three different starting weight categories (small, medium and large) to examine the nature of the start weight by dose rate interaction. The AICc for the best model was 651.4

| **Predictors** | **Estimate** | **SE** | **F** | **P value** | **ΔAICc** |
|---|---|---|---|---|---|
| Full dataset (n= 59) | | | | | |
| Colony start weight (g) | 0.0271 | 0.4131 | 0.06 | 0.8061 | |
| Dose rate (µGy $h^{-1}$) | -132.50 | 50.3210 | 6.99 | 0.0100 | |
| Colony start weight by dose rate interaction | 0.2473 | 0.0933 | 7.10 | 0.0102 | |
| *Tagged status of workers* | *4.5984* | *15.4142* | *0.08* | *0.7667* | +5.8 |
| **Colonies with a start weight less than 527g (n = 20)** | | | | | |
| Dose rate (µGy $h^{-1}$) | -6.992 | 3.782 | 3.31 | 0.0881 | |
| **Colonies with a start weight between 527 and 548g (n = 20)** | | | | | |
| Dose rate (µGy $h^{-1}$) | 2.044 | 3.960 | 0.26 | 0.6114 | |
| **Colonies with a start weight more than 548g (n = 19)** | | | | | |
| Dose rate (µGy $h^{-1}$) | 7.113 | 4.812 | 2.18 | 0.1566 | |

**Table S6.**

Parameter estimates from binomial models which investigated if radiation dose rate influenced whether colonies reached peak mass in week four or week five of the experiment. All predictors from the global model are presented; values for non-significant terms (indicated in italics) are given at the point they were removed during model simplification in reverse order of the removal sequence. The binary response variable (peaking in either week four and five) was fitted with a logit link. n = 59 colonies. The AICc for the best model was 69.9.

| **Predictors** | **Estimate** | **SE** | **$\chi^2$** | **P value** | **ΔAICc** |
|---|---|---|---|---|---|
| Dose rate (µGy $h^{-1}$) | 0.0038 | 0.0018 | 11.08 | 0.0008 | |
| *Colony start weight (g)* | *0.0079* | *0.0013* | *0.23* | *0.6306* | *+2.0* |
| *Colony start weight by dose rate interaction* | *-0.00002* | *0.00005* | *-0.14* | *0.7045* | *+5.2* |

**Table S7.**

Parameter estimates from Cox proportional hazards mixed effects models which investigated if worker longevity was influenced either by radiation dose rate or the manner of a worker's death (worker confirmed dead by identification of a body in the colony, or recorded missing from the colony); a random effect for colony was included. All predictors from the global model are presented (which were all not significant). Predictors are listed in reverse order of the model simplification sequence; parameters were calculated at the point of removal from the model. n = 213 bees. The AICc for the 'intercept only' best model was 366.2.

| **Predictors** | **Estimate** | **SE** | **$\chi^2$** | **P value** | **ΔAICc** |
|---|---|---|---|---|---|
| *Worker confirmed dead or recorded absent from the colony* | *0.2962* | *0.1855* | *2.47* | *0.1154* | *-1.4* |
| *Dose rate (µGy $h^{-1}$)* | *-0.0007* | *0.0006* | *1.12* | *0.2612* | *-1.5* |
| *Colony start weight (g)* | *0.0097* | *0.0054* | *1.11* | *0.2921* | *-1.7* |

**Table S8.**

Parameter estimates from Cox proportional hazards models which investigated if colony longevity was influenced by radiation dose rate. All predictors from the global model are presented including the values for non-significant terms (indicated in italics). Non-significant predictors are listed in reverse order of the model simplification sequence; parameters were calculated at the point of removal from the model. n = 59 colonies. The AICc for the best model was 369.4.

| **Predictors** | **Estimate** | **SE** | **$\chi^2$** | **P value** | **ΔAICc** |
|---|---|---|---|---|---|
| Tagged status of workers | 0.6210 | 0.2700 | 6.37 | 0.0201 | |
| *Colony start weight (g)* | *-0.0049* | *0.0055* | *0.78* | *0.3774* | *-1.2* |
| *Dose rate (µGy $h^{-1}$)* | *0.0002* | *0.0003* | *0.41* | *0.5182* | *-1.4* |

**Table S9.**

Parameter estimates from Cox proportional hazards models which investigated if queen longevity was influenced by radiation dose rate. All predictors from the global model are presented (which were all not significant). Predictors are listed in reverse order of the model simplification sequence; parameters were calculated at the point of removal from the model. n = 20 queens. The AICc score for the best model was 84.7.

| **Predictors** | **Estimate** | **SE** | **$\chi^2$** | **P value** | **ΔAICc** |
|---|---|---|---|---|---|
| *Colony start weight (g)* | *0.0180* | *0.0100* | *2.92* | *0.0876* | +0.7 |
| *Dose rate (µGy $h^{-1}$)* | *0.0009* | *0.0006* | *1.80* | *0.1797* | +2.1 |
| *Tagged status of workers* | *0.0547* | *0.4808* | *0.01* | *0.9092* | +2.5 |

**Table S10.**

Parameter estimates from linear mixed effects models which investigated whether radiation dose rate influenced colony weight during weeks 1-4 during the laboratory exposure stage of the experiment. All predictors from the global model are presented; values for non-significant terms (indicated in italics) are given at the point they were removed during model simplification in reverse order of the removal sequence. n = 59 colonies. The AICc score for the best model was 1707.2.

| **Predictors** | **Estimate** | **SE** | **$\chi^2$** | **P value** | **ΔAICc** |
|---|---|---|---|---|---|
| Colony start weight (g) | 1.1510 | 0.2350 | 43.7 | <0.0001 | |
| Experiment week number | 65.8532 | 2.0001 | 278.0 | <0.0001 | |
| *Dose rate (µGy $h^{-1}$)* | *0.0023* | *0.0072* | *0.14* | *0.7031* | *+9.9* |
| *Dose rate by experiment week interaction* | *0.0004* | *0.0003* | *2.05* | *0.1521* | *+19.4* |
| *Start weight by experiment week interaction* | *0.0097* | *0.0082* | *1.49* | *0.2211* | *+13.5* |
| *Colony start weight by dose rate interaction* | *- 0.0023* | *0.0044* | *0.30* | *0.5818* | *+25.2* |

**Table S11.**

Parameter estimates from linear mixed effects models which investigated whether radiation dose rate influenced colony weight during weeks 5-8 during the field natural foraging stage of the experiment. All predictors from the global model presented; values for non-significant terms (indicated in italics) are given at the point they were removed during model simplification in reverse order of the removal sequence. n = 59 colonies. The AICc for the best model was 1298.4.

| **Predictors** | **Estimate** | **SE** | **$\chi^2$** | **P value** | **ΔAICc** |
|---|---|---|---|---|---|
| Colony start weight (g) | 0.6110 | 0.2891 | 37.6 | <0.0001 | |
| Experiment week number | -27.4411 | 3.4640 | 4.50 | 0.0338 | |
| *Dose rate (µGy $h^{-1}$)* | *-0.0075* | *0.0121* | *0.42* | *0.5187* | *+3.4* |
| *Colony start weight by experiment week interaction* | *0.0124* | *0.0151* | *0.68* | *0.4073* | *+3.3* |
| *Colony start weight by dose rate interaction* | *0.0001* | *0.0007* | *0.48* | *0.8260* | *+18.2* |
| *Dose rate by experiment week interaction* | *0.0037* | *0.0073* | *0.16* | *0.6883* | *+12.4* |

**Table S12.**

Parameter estimates from linear mixed effects models which investigated whether radiation dose rate influenced colony food consumption (artificial nectar) during the laboratory exposure stage of the experiment. All predictors from the global model presented; values for non-significant terms (indicated in italics) are given at the point they were removed during model simplification in reverse order of the removal sequence. n = 59 colonies. The AICc for the best model was 3299.7.

| **Predictors** | **Estimate** | **SE** | **$\chi^2$** | **P value** | **ΔAICc** |
|---|---|---|---|---|---|
| Colony start weight (g) | 0.4811 | 0.4020 | 1.46 | 0.2260 | |
| Experiment week number | 209.2021 | 48.385 | 18.20 | <0.0001 | |
| Colony start weight by experiment week interaction | -0.8025 | 0.0890 | 68.62 | <0.0001 | |
| *Dose rate (µGy $h^{-1}$)* | *-0.0045* | *0.0204* | *0.12* | *0.7342* | *+8.7* |
| *Dose rate by experiment week interaction* | *-1.5625* | *2.2478* | *0.54* | *0.4596* | *+19.5* |
| *Colony start weight by dose rate interaction* | *-0.1175* | *0.4517* | *0.07* | *0.7857* | *+34.0* |