1 **Chernobyl-level radiation exposure damages bumblebee reproduction: a laboratory**

2 **experiment**

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7 The consequences for wildlife of living in radiologically contaminated environments are

8 uncertain. Previous laboratory studies suggest insects are relatively radiation-resistant;

9 however, some field studies from the Chernobyl Exclusion Zone report severe adverse effects

10 at substantially lower radiation dose rates than expected. Here we present the first laboratory

11 investigation to study how environmentally-relevant radiation exposure affects bumblebee life-

12 history, assessing the shape of the relationship between radiation exposure and fitness-loss.

13 Dose rates comparable to the Chernobyl Exclusion Zone (50-400 μGy h^{-1}) impaired

14 bumblebee reproduction and delayed colony growth but did not affect colony weight or

15 longevity. Our best-fitting model for the effect of radiation dose rate on colony queen

16 production had a strongly non-linear concave relationship: exposure to only 100 μGy h^{-1}

17 impaired reproduction by 30-45%, while further dose rate increases caused more modest

18 additional reproductive impairment. Our data indicate that the practice of estimating effects of

19 environmentally-relevant low dose rate exposure by extrapolating from high dose rates may

20 have considerably underestimated the effects of radiation. If our data can be generalised, they

21 suggest insects suffer significant negative consequences at dose rates previously thought

22 safe; we therefore advocate relevant revisions to the international framework for radiological

23 protection of the environment.

24

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

26 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.
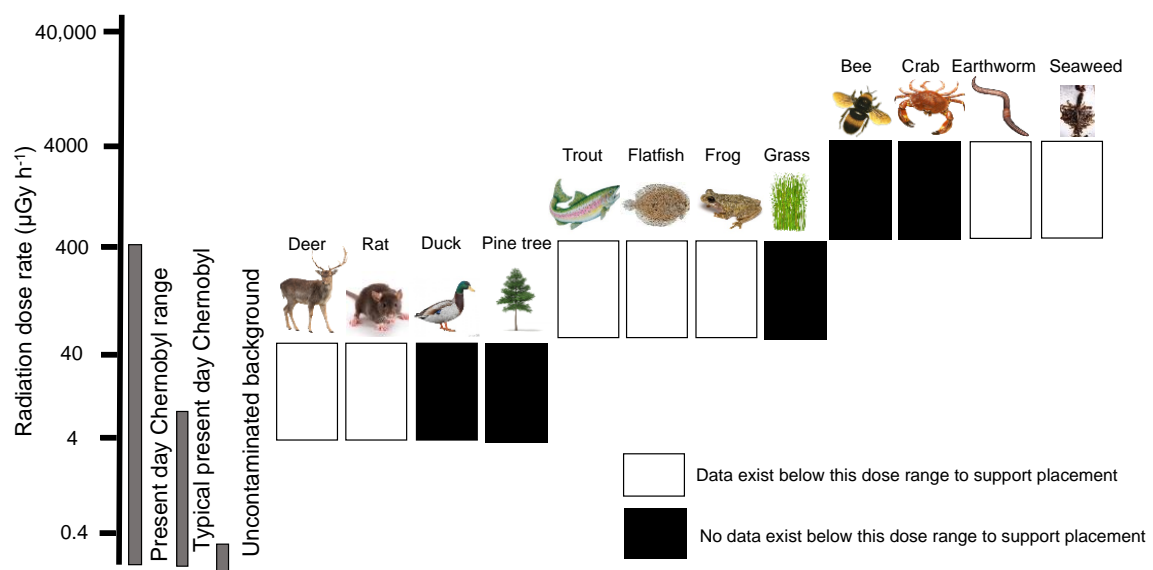
Figure 1.

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

71 demonstrated for context (Chernobyl dose rates are now chronic and low, approximately 1%

72 of those at the time of the accident in 1986) [26].

73

74 The International Commission on Radiological Protection (ICRP) provides global

75 recommendations for the protection of both humans and wildlife. For wildlife, Reference

76 Animals and Plants (RAPs) are used; each RAP has a designated dose rate band (a DCRL:

77 Derived Consideration Reference Level) within which deleterious effects are predicted to start

78 occurring (figure 1: [23]). The insect RAP is a "eusocial bee". The framework suggests insects

79 are less radiosensitive than other taxa (figure 1), predicting negative effects on bees only at

80 the highest radiation levels considered (400 – 4000 µGy h^{-1}). However, no experimental

81 studies exist on bees within or below this dose rate band and there are relatively limited data

82 for other invertebrates to justify this conclusion [23]. Our work provides the first experimental

83 test to examine whether current assumptions about bee radiosensitivity are correct. We

84 studied bumblebees, not only because of there is a key knowledge gap associated with

85 radiological protection but also because bumblebees provide essential pollination services

86 that underpin ecosystem function [36].

87 To our knowledge, we have conducted the first experiment to bridge the gap between

88 laboratory and field radiosensitivity studies in insects. We used an experimental radiation

89 exposure facility to address three questions: (i) does chronic exposure to radiation dose rates

90 found in the Chernobyl Exclusion Zone affect bumblebees; (ii) what is the shape of the

91 relationship between dose rate and fitness effects; and (iii) is there a clear lower threshold

92 below which bees are not affected? Under controlled conditions bumblebee colonies were

93 exposed to a radiation gradient for a substantial proportion of colony lifespan, then after this

94 laboratory exposure period bumblebees foraged naturally outside. We assessed bumblebee

95 colony fitness by measuring key life-history traits: reproductive success, colony growth, as

96 well as the longevity of colonies, workers and queens. The production of new queens by

97 bumblebee colonies is the most important metric of reproductive success because queens are

98 the only individuals to overwinter and form new colonies the following year [37]. Our results

99 demonstrate that radiation exposure causes reproductive impairment and that dose rates in
100 the range currently found within the Chernobyl Exclusion Zone contribute disproportionately
101 to this damage.

102 **2. Materials and Methods**

103 (a) Experimental Design

104 Fifty-nine commercial *Bombus terrestris audax* colonies (comprising a queen, workers and
105 brood) were purchased from Biobest®. For four weeks colonies were positioned in a climate-
106 controlled (25°C and a 12 hr, L:D light cycle (07h – 19h)) radiation facility containing a gamma-
107 emitting caesium-137 source and a control area shielded from radiation. We chose a gamma
108 emitting source because estimates have shown that 95% of the dose rate for wild bumblebees
109 in the Chernobyl Exclusion Zone is due to external gamma radiation [38]. Bumblebee colonies
110 were housed in a standard box (25cm (l) x 30cm (w) 20cm (h)) for the duration of the
111 experiment and rotated 180° every two days to reduce within-colony radiation exposure
112 heterogeneity. Each colony in the radiation treatment group received a unique dose rate (20 -
113 3000 µGy h^{-1}) to encompass both the dose rate range measurable in the Chernobyl Exclusion
114 Zone and the range of the bee DCRL (figure 1). We achieved the radiation gradient by varying
115 colony distance from the caesium-137 source within the radiation facility and verified dose
116 rates post-experiment using dosimeters at each colony's position. Monitoring, maintenance
117 and bee-marking were conducted in a neighbouring unirradiated area under red light.

118 Colonies were weighed before the experiment and were distributed across the
119 radiation exposure gradient randomly, except for colony start weight, which we systematically
120 ensured was not associated with radiation dose rate ($F_{1, 57} = 0.07$, p = 0.791). Anti-wax moth
121 concentrate (Certan/B401® from Dragonfli, 1 in 20 in water) was applied to colony containers
122 at weeks two and five of the experiment to protect against wax moth infestation (which was
123 subsequently not observed). Colonies received *ad libitum* dried pollen and artificial nectar
124 (Biogluc®); Biogluc reservoir weight was measured weekly during the laboratory exposure

125 phase to assess food intake. In exposure week three, before colonies were moved outdoors,

126 a subset of 30 workers from 29 of the colonies were marked with individual coloured number

127 tags attached to the thorax to enable estimation of worker longevity [37].

128 After four weeks, radiation exposure stopped; colonies were moved from the radiation

129 facility and randomly assigned outdoor locations in the University of Stirling campus gardens

130 (56.1454° N, 3.9206° W) which has background radiation levels (0.11 $\pm$ 0.01 µGy h^{-1}).

131 Colonies were sheltered from the weather in plastic boxes and were situated close to

132 ornamental gardens, deciduous woodland and mixed farmland. Natural foraging was

133 encouraged by reducing the pollen supply two days before outdoor placement and preventing

134 access to Biogluc® from the day colonies were placed outdoors. Total colony weight was

135 measured weekly throughout both radiation exposure and field phases until colony death.

136 Once outside, colonies were checked post-sunset every 1-2 days to determine colony and

137 queen death dates. Tagged workers were recorded as alive, dead or missing. Worker

138 longevity was expressed as the number of days between the tagging date and the day of death

139 or absence. No absent bumblebees subsequently returned to the colony. Queen longevity was

140 defined as the time in days from the start of the experiment to the date at which the queen

141 was observed dead. Colonies were considered dead once the queen had died and fewer than

142 five workers remained; colonies were then euthanised at -80°C for 45 minutes. Bumblebees

143 construct wax cells in which pupae develop; males and workers develop in similar-sized cells,

144 whereas queen cells are much larger [36]. Posthumous colony dissection allowed colony

145 counts of the combined worker and male population, as well as queen production [39]. Pupal

146 cell counts included emerged and non-emerged cells: 100% of the queen cells and 93% ($\pm$

147 3% SE) of worker/male cells had emerged.

148

149 (b) Statistical Analysis

150 Analyses were conducted in R (version 3.5.1 [40]). Maximal models were established and

151 each predictor's inclusion was evaluated using an F or chi-squared test to compare the

152 goodness of fit between models; non-significant predictor variables were removed (in addition

153 we calculated Akaike Information Criterion (AICc) for model terms on removal). For most

154 analyses the unit of replication was the colony; however for cases involving repeated

155 measures or where parameters were assessed for individual bees, we used mixed effects

156 models with colony as a random effect to account for non-independence. Model diagnostics

157 were checked to validate fit to assumptions. Means are stated in the text ± standard error.

158 We constructed negative binomial generalised linear models to investigate

159 associations between colony queen production and dose rate using "glm.nb" from the MASS

160 package [41]. The maximal model's predictor variables were: dose rate, colony start weight,

161 whether workers from the colony were tagged, and a colony start weight by dose rate

162 interaction. We tested for a non-linear relationship between queen production and dose rate:

163 first in separate negative binomial generalised linear models using either a square-root or a

164 natural log transformation of dose rate (ln(dose rate + 1)), then using a saturating exponential

165 function in Nonlinear least squares (nls) (f(dose rate)=k1*(1-exp[-k2*dose rate]). We

166 compared these models using AICc and the plotted residuals to evaluate model fit. Factors

167 influencing the combined count of worker and male pupal cells were assessed using the same

168 model structure and dose rate transformations.

169 Peak colony weight occurred only at either week four or five; therefore, to assess

170 factors influencing this timing we used binomial generalised linear models with predictors for

171 dose rate, colony start weight, and a colony start weight by dose rate interaction. We tested

172 for non-linear effects of radiation using the same transformations as we did for queen
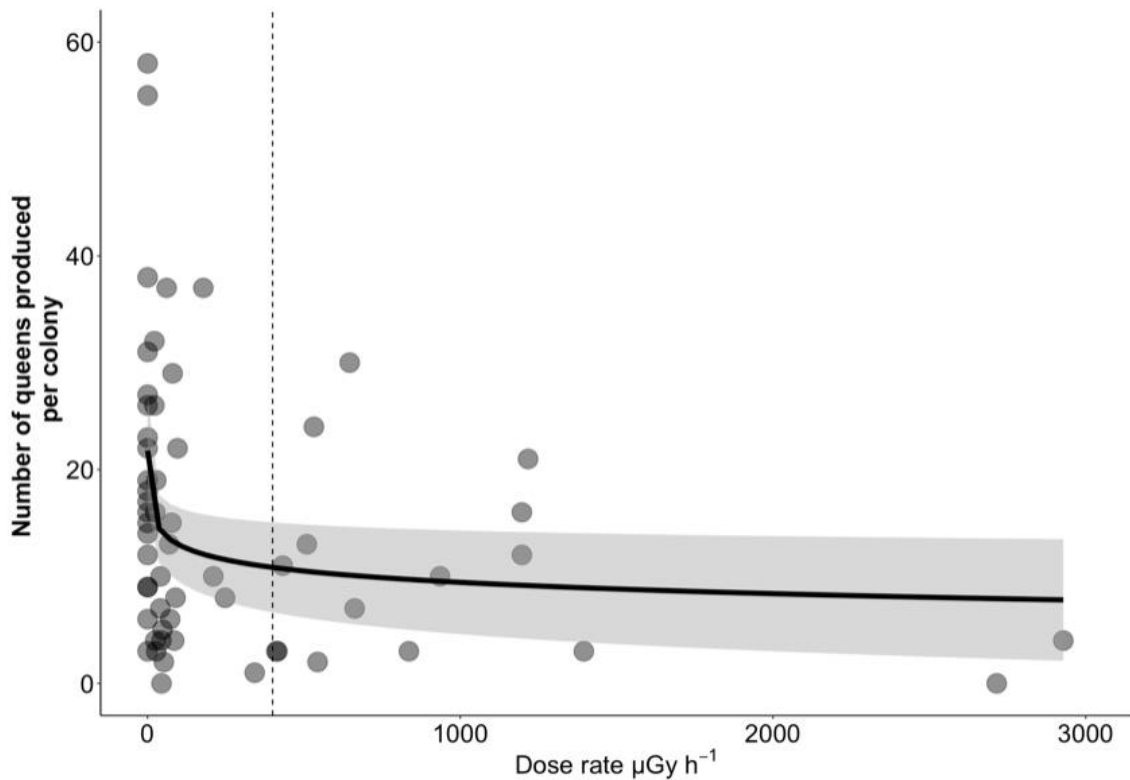
173 production.

174 Separate Cox's proportional hazards models ("coxph" from the package survival) [42]

175 were used to analyse factors influencing colony and queen longevity: dose rate, colony start

176 weight and tagged status were predictor variables. Drivers of worker longevity variation were

177 assessed using mixed-effects Cox models ("coxme" [42]): models included dose rate, colony

178 start weight and worker status (observed dead or absent from colony) as fixed effects, and

179 colony identity as a random effect.

180   The magnitude of colony weight change was assesed in two time periods: the

181   exposure phase (weeks 1 – 4: increasing weight) and the field release phase (week five

182   onwards: declining weight). Linear mixed-effects models were fitted using "lmer" from the lme4

183   package [43] with colony identity as a random effect to account for repeated measures. Fixed

184   covariates were dose rate, colony start weight, experiment week and all two-way interactions.

185   We included interaction terms to test whether the effect of dose rate on colony weight was

186   influenced by either colony start weight or week number. The same model structure was used

187   to investigate variation in Biogluc® consumption.

188   **3. Results**

189   (a) Effect of radiation exposure on queen production

190   Queen production per colony ranged from zero to 58 (mean = 16.1 ± 1.67 SE). Bumblebee

191   colonies exposed to radiation produced fewer queens (mean = 12.0 ± 3.50 SE, n = 39) than

192   unexposed colonies (mean = 21.1 ± 3.37 SE, n = 20). Initially, we fitted a linear relationship

193   between dose rate and queen production, demonstrating a strongly significant negative effect

194   of radiation on reproduction ($F_{1, 57} = 7.31$, p = 0.007, figure S1, table S1). We tested for effects

195   of colony start weight, tagging status of workers and a start weight by dose rate interaction,

196   which were not significant (table S1).

197

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

213   model predicted a 46% reduction in colony queen production at only 100 µGy h^{-1}; at 400 µGy

214   h^{-1} reproduction decreased by 51% and then decreased only modestly further (by 59%) in

215   colonies exposed to 3000 µGy h^{-1}. In contrast, the linear model (figure S1) predicted that

216   colony queen production declined by 6% at 100 µGy h^{-1}, 28% at 400 µGy h^{-1} and 82% in

217   colonies exposed to 3000 µGy h^{-1}. The results from the square-root transformed model were

218   intermediate between these two. Within our dataset, two colonies from the control area

219   produced very high numbers of queens and might be considered outliers (30% more queens

220   than other colonies). The relationship between dose rate and queen production remained

221   significant when these two data points were removed from all the above models (log dose

222   rate: ($F_{1, 55}$ = 4.54, p = 0.033, table S3); square root dose rate: ($F_{1, 55}$ = 5.28, p = 0.022); and

223   the linear dose rate model ($F_{1, 55}$ = 5.76, p = 0.016, table S1).

224   Our data provide compelling evidence for substantial fitness consequences to

225   bumblebees across the dose rate range we assessed (figure 2). Next, we investigated the

226   strength of support for negative effects on queen production at lower dose rates relevant to

227   the Chernobyl Exclusion Zone. We progressively truncated the dataset to exclude dose rates

228   above 2000, 1000, 500, 100 and 50 µGy h^{-1} and fitted the same models as the original

229   analysis. This process inevitably reduced our sample size and statistical power.

230   The negative effect of radiation remained significant in all these analyses as low as 50

231   µGy h^{-1} when using a log-transformation of dose rate to generate a non-linear fit (table S3).

232   Whereas the linear-effect model using the untransformed dose rate predictor was significant

233   for some but not all these truncated data sets (table S1). The linear model provided

234   additional evidence that the effect of radiation on queen production was curved. If the effect

235   had been linear with no threshold over the dose rate range we studied, we would have

236   expected the parameter estimate for the impact of radiation per unit exposure to be broadly

237   similar over the full range. Instead, the parameter estimate dramatically increased as we

238   restricted our analysis to progressively lower dose rates (table S1), supporting the non-linear

239   curve that our logarithmic transform model previously revealed.

240

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

242   As a second metric of reproduction, the combined number of worker and male cells produced

243   by each colony was counted (worker and male cells are indistinguishable). We tested for a

244   correlation between queen production and worker-male production and detected a weak

245   negative relaitonship (Pearson r = -0.29, n = 59, P = 0.021) suggesting that, whilst these two

246   reproduction metrics are not fully independent of one another, they may trade-off. Mean

247   worker-male cells per colony was 263.1 (± 8.00 SE). Worker-male production reduced

248   significantly in response to increasing radiation (linear dose rate predictor: $F_{1,\ 55}$ = 4.37, p =

249   0.041, table S4).
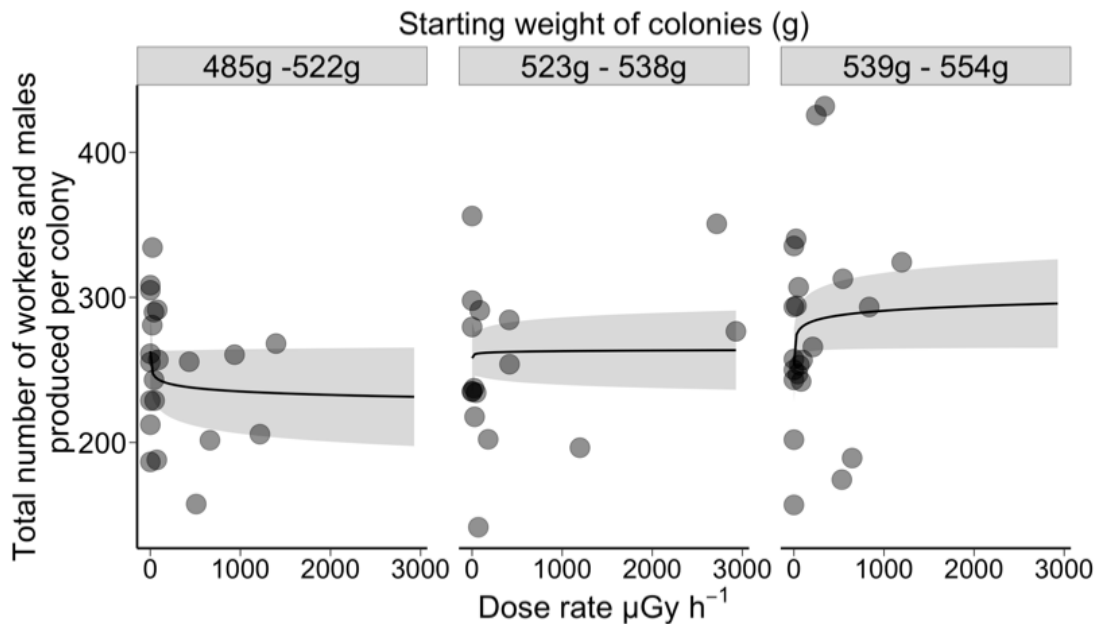
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251   **Figure 3.**

252   The effect of radiation on bumblebee colony ability to produce males and workers, for colonies

253   of different starting weight. Colonies were put into three start weight groups (low, mid and

254   high) of approximately equal sample size (n = 20, 20, 19 respectively) to maintain equivalent

255   statistical power for each. The points and fitted lines are derived from a negative binomial

256   model containing a log-transformed dose rate predictor and its interaction with colony starting

257   weight (specified as a three-level factor). Whilst the negative effect of radiation was individually

258   significant for the lighter colonies, the positive slopes for mid-weight and the heaviest colonies

259   were not significant (see text). The shaded area around the fitted lines represents the 95%

260   confidence interval. Y-axis is offset from zero to show control colony data effectively.

261

262   We compared the fit of models containing non-linear transformations of the dose rate

263   predictor (log transformation AICc = 651.4; square-root transformation AICc = 652.5) with the

264   untransformed linear variable (AICc = 653.0). The strongest support was for a curvilinear log-

265   transformation of dose rate ($F_{1, 55}$ = 6.99, p = 0.010, table S5), although AICc separation

266   between models was only 1.6 points.

267   The negative effect of radiation on colony worker-male production varied according to

268   colony start weight (log-transformed dose by start weight interaction: $F_{1, 55}$ = 7.10, p = 0.010,

269   figure 3, table S5). We investigated this interaction by dividing the dataset into three colony

270   weight classes (table S5): the negative effect of log-transformed dose rate on worker-male

271   production in the smaller colonies was marginally significant ($F_{1, 18}$ = 3.31, p = 0.088) but the

272   positive effect was not significant in mid-weight ($F_{1, 18}$ = 0.26, p = 0.611) or heavier colonies

273   ($F_{1, 17}$ = 2.18, p = 0.157). We repeated these analyses for the linear model: whilst the interaction

274   between dose rate and colony start weight was significant, the individual tests of the effect of

275   dose rate in each of the three weight subgroups were not (table S4). Effect of radiation on

276   colony growth timing.

277

278   (c) Effect of radiation on colony growth timing

279   Bumblebee colonies typically undergo a growth phase as workers are produced, then peak in

280   weight as colonies generate reproductives towards the end of the colony cycle [36,44]. All

281   colonies reached peak weight either in week four or five of the experiment (colonies were

282   placed outside to forage at the beginning of week five, which curtailed further growth).

283   Increasing radiation dose rate was significantly associated with an increased probability of

284   delay in reaching peak weight ($\chi^2_{1, 57}$ = 11.08, p = 0.0008, figure 4, table S6). Our model

285 predicted that 50% of control colonies reached peak weight at the later date, whereas for those

286 exposed to 400 µGy h⁻¹ this percentage increased to 81%. The binomial response in this

287 analysis and the logit transformation underlying the model make it difficult to assess the extent

288 of non-linearity in the effect of radiation on peak weight timing. Nevertheless, we tested for

289 curvature in this relationship using log-transformed (AICc = 75.1) and square-root transformed

290 (AICc = 71.5) dose rate, but a linear fit using the untransformed dose rate predictor was

291 superior (AICc = 69.9).
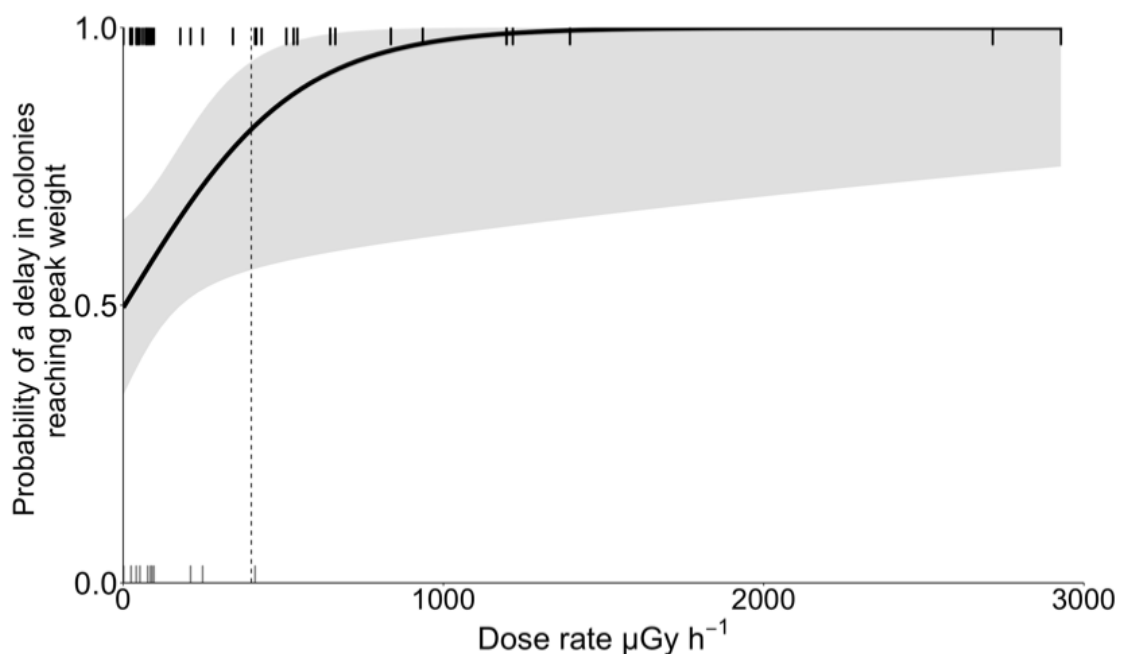
292

293 **Figure 4.**

294 The effect of radiation on the number of experimental weeks it took bumblebee colonies to

295 reach peak weight (week four or five). The fitted line is the predicted relationship from a

296 binomial generalised linear model; the shaded area shows the 95% confidence intervals. The

297 dotted line indicates the upper limit of dose rates measured in the Chernobyl Exclusion Zone.

298 Tick marks at the top and bottom of the figure show raw data points.

299

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

301 At week three, a subset of 30 workers from half the colonies (n = 29 colonies, 870 bees) were

302 marked with number tags to assess individual worker longevity after field release. Of these

303 workers, 76% disappeared from the colony within 48 hours of release and were not sighted

304 again. The remaining marked workers either died in the nest (n = 47) or were subsequently

305 recorded absent (n = 166). Cox survival analysis on these remaining workers detected a

306 negative but non-significant effect of radiation dose rate on worker longevity (χ^2_1 = 1.12, p =

307 0.261, table S7). There was no significant longevity difference between bumblebees that died

308 in the nest or were recorded absent (χ^2_1= 2.47, p = 0.115, table S7). Colony longevity was not

309 significantly affected by dose rate (table S8), nor was queen longevity (queen death date was

310 successfully recorded in 20 (33%) of the colonies**)** (table S9). Colonies from which workers

311 were tagged lived longer than untagged colonies (table S8); these tagged colonies were

312 distributed evenly across the radiation gradient ($F_{1,57}$ = 0.07, p = 0.791). Tagging workers did

313 not affect colony queen production ($F_{1,56}$ = 1.87, p = 0.171), nor influence production of

314 workers and males ($F_{1,54}$ = 0.08, p = 0.767).

315   All colonies gained weight throughout the four-week laboratory radiation exposure phase

316 and lost weight during the outdoor period (due to loss of individuals and resources) until colony

317 death. However, there was no relationship between radiation dose rate and colony weight

318 change during either of these phases (tables S10 and S11). Similarly, artificial nectar

319 (Biogluc®) consumption increased over time during the radiation exposure phase but was not

320 influenced by dose rate (table S12).

321 **4. Discussion**

322 Here we experimentally demonstrate that bumblebees are negatively affected by exposure to

323 ionising radiation at dose rates significantly lower than previously thought likely to impact

324 insects. We assessed the impact of radiation exposure on eight fitness-related traits: colony

325 queen production, male-worker production, colony growth timing, colony weight, colony nectar

326   consumption, worker lifespan, queen lifespan and colony lifespan. We found negative effects

327   of radiation exposure on the first three of these; radiation dose rates equivalent to the more

328   contaminated areas of the Chernobyl Exclusion Zone impaired both reproduction metrics and

329   delayed colony growth.

330   Several of our analyses suggested that the effect of increasing radiation dose rate on

331   bumblebee reproductive fitness was non-linear. Contrary to established hypotheses that lower

332   dose rates of radiation have little effect on insects, our best-fitting models indicated that a rapid

333   decline in bumblebee reproduction occurred over the range 0-100 μGy h^{-1} and that further

334   increases in radiation dose rate had relatively smaller additional effects. Our evidence for non-

335   linear effects is from two sources. Firstly, for our analyses of colony queen production, the

336   best fitting model contained a log transformation of dose rate, although AICc improvement

337   relative to a linear predictor was only modest. Secondly, when using an untransformed dose

338   rate predictor in models that assumed a linear effect of radiation, as we progressively restricted

339   our analysis to lower and lower dose rates the relationship between dose rate and reproductive

340   impairment became considerably steeper. We also tried analyses using other non-linear

341   modelling techniques, but for these the fit was generally poor. Radiation protection for wildlife

342   frequently relies on knowledge of the shape of the relationship between radiation exposure

343   and damage caused; this is particularly important to enable extrapolations to low dose

344   exposures where biological effects may be challenging to detect. Our study suggests that

345   linear extrapolations to low dose rates may substantially underestimate radiation effects, at

346   least for bumblebees.

347   Our data unambiguously demonstrate a negative effect of radiation on bumblebee

348   colony queen production when considering the full dose rate range in our experiment (0-3000

349   μGy h^{-1}). Determining a conclusive lower threshold at which these effects start occurring is

350   difficult because as we restricted our analysis to progressively lower dose rates our sample

351   size declined (for example, we only had seven radiation-exposed colonies below 50 μGy h^{-1}).

352   However, our analyses certainly provide no evidence that this deleterious effect of radiation

353   exposure disappeared in the 50-100 µGy h^{-1} range: tests in this range assuming a non-linear

354   dose rate effect were significant.

355   Our study makes a major step forwards to resolve the ongoing controversy of whether

356   radiation dose rates at contaminated sites such as the Chernobyl Exclusion Zone are

357   damaging to invertebrates. Our best-fitting model, with log-transformation of the dose rate

358   predictor, indicated that exposure to 100 µGy h^{-1} decreased bumblebee colony queen

359   production by 46%. Queens are demographically limiting, so this decrease in queen

360   production has the potential to substantially impact bumblebee populations [45].

361   Approximately comparable to our findings, pesticide exposure has been reported to reduce

362   bumblebee colony founding success by 26% and was predicted to dramatically increase the

363   likelihood of local population extinction [46]. Impaired pollination services due to radiation-

364   induced reductions in bumblebee population size could drive negative consequences for the

365   whole ecosystem [47]. Furthermore, we suggest that our laboratory estimates of the negative

366   consequences of radiation exposure may be conservative compared with radiation effects on

367   wild populations that are exposed to multiple other stressors.

368   Radiation exposure also reduced the combined total of males and workers that

369   colonies produced. As for queen production, our analyses suggested that this effect of

370   radiation was non-linear and that the negative effects started to plateau above approximately

371   100 µGy h^{-1}. However, this reduction in worker-male production occurred only in smaller

372   colonies; heavier colonies appeared to be protected from adverse radiation effects. We

373   speculate that either larger colonies could buffer against radiation stress, or that in colonies

374   which started the experiment smaller, a greater proportion of the future worker-male

375   population might have been at a particularly radiosensitive pre-adult stage. Reduced male

376   output directly impairs colony reproductive success [48] and a smaller worker cohort reduces

377   the colony's capacity to support new queens [49].

378   Radiation also delayed the time that colonies took to attain their peak weight. Control

379   colonies had a 50% probability of experiencing a one-week delay in reaching their peak

380   weight; whereas exposure to 400 µGy h^{-1} increased this probability of reproductive delay to

381   81%. The timing of peak weight can be used as a proxy to indicate the time reproductives are

382   produced [50]. Reproductive delay is a common stress-response in bumblebees [51,52].

383   Colonies which generate reproductives earlier have greater reproductive success as they can

384   produce more queens and maximise mating opportunities for males [53,54]. Our colonies were

385   released to forage in the field after one month of irradiation; many workers disappeared from

386   the colonies at this point and colony weight gain stopped. Therefore, our experimental design

387   artificially curtailed colony growth, which may mean that our estimate of the growth delay

388   caused by radiation is an underestimate.

389   Previous laboratory studies, typically using acute exposures, have suggested that

390   invertebrates are relatively resistant to ionising radiation [55]. The consequences of acute

391   high-dose radiation exposure to animals have been well-studied [22,56,57]; acute dose rates

392   are thought to overwhelm repair mechanisms and result in significant damage [58]. In

393   comparison, we have limited knowledge as to the effects of chronic low-dose radiation

394   exposure on wildlife [59]. Our colonies chronically-exposed to 100 μGy h^{-1} for four weeks

395   received an accumulated dose of 0.07 Gy, while the most highly exposed colonies received 2

396   Gy over this time period. Our effects are comparable to previous studies that delivered similar

397   total doses acutely: exposure to 1-2 Gy is reported to cause 50% mortality in sub-adult

398   Hymenoptera [60], and exposure to 60 Gy reduced the number of eggs laid by queen ants by

399   90% [61]. However, we acknowledge that there is minor uncertainty as to the dose each

400   individual bee received because bees were free to move around inside their colony box,

401   furthermore bees developed and eclosed at different times during the experiment.

402   Our data show chronic low-dose exposure is more harmful than previously thought.

403   We propose two hypotheses which could explain these effects at chronic low dose rates. (I)

404   Radiation-induced oxidative damage might directly harm cells with negative consequences for

405   reproductive capacity. However, we note that dose rates of 400 μGy h^{-1} and below are

406   predicted to create too few reactive oxygen species to cause significant damage from oxidative

407   stress [19]. Alternatively, (II) radiation effects might activate bumblebee stress responses,

408   which could drive costly tradeoffs with reproduction. Defence and repair mechanisms, such

409   as mounting an immune response, can indeed impair fitness in bumblebees [62,63].

410   Our findings have significant implications for the international environmental protection

411   framework used by the ICRP in which radiation dose rate bands (DCRLs) for different taxa

412   predict the exposure at which negative effects are likely to occur [23]. We provide the first data

413   to evaluate the DCRL for bees, which ICRP use as a reference to represent all insects. We

414   demonstrate a substantial reduction in bumblebee queen production (approximately -50%) at

415   the lowest end of the current dose rate band (400 μGy h^{-1}). We recommend to the ICRP that

416   the dose rate band should be lowered to 40-400 μGy h^{-1}, placing insects in the same

417   radiosensitivity category as some vertebrates (Figure 1). However, we have very few data for

418   dose rates below 40 μGy h^{-1} (only 3 exposed colonies), preventing us from making any strong

419   conclusions below this, which would require us to extrapolate our findings to unstudied dose

420   rates.

421   Our findings demonstrate that the most contaminated areas of the Chernobyl Exclusion

422   Zone could cause substantial damage to bumblebees. Nevertheless, our analyses suggest it

423   would be unlikely that bumblebees would experience severe adverse effects at the very low,

424   near-background Chernobyl dose rates (eg 0.01 μGy h^{-1}) reported by some authors [15,16].

425   **5. Conclusion**

426   Our findings help resolve an ongoing controversy in radioecology and call for changes in

427   radiological protection practice. We provide the first experimental evidence of significant

428   detrimental effects on insect reproductive success at environmentally-relevant dose rates

429   previously not predicted to affect insects. Furthermore, our results suggest that the impact of

430   radiation is non-linear, such that bumblebee fitness-loss increases rapidly at lower dose rate

431   ranges. We suggest that radiation exposure could have significant implications for wild insect

432   populations in the more highly contaminated areas of post-disaster sites such as the

433   Chernobyl Exclusion Zone.

434

Data accessibility

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.

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	ΔAICc
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
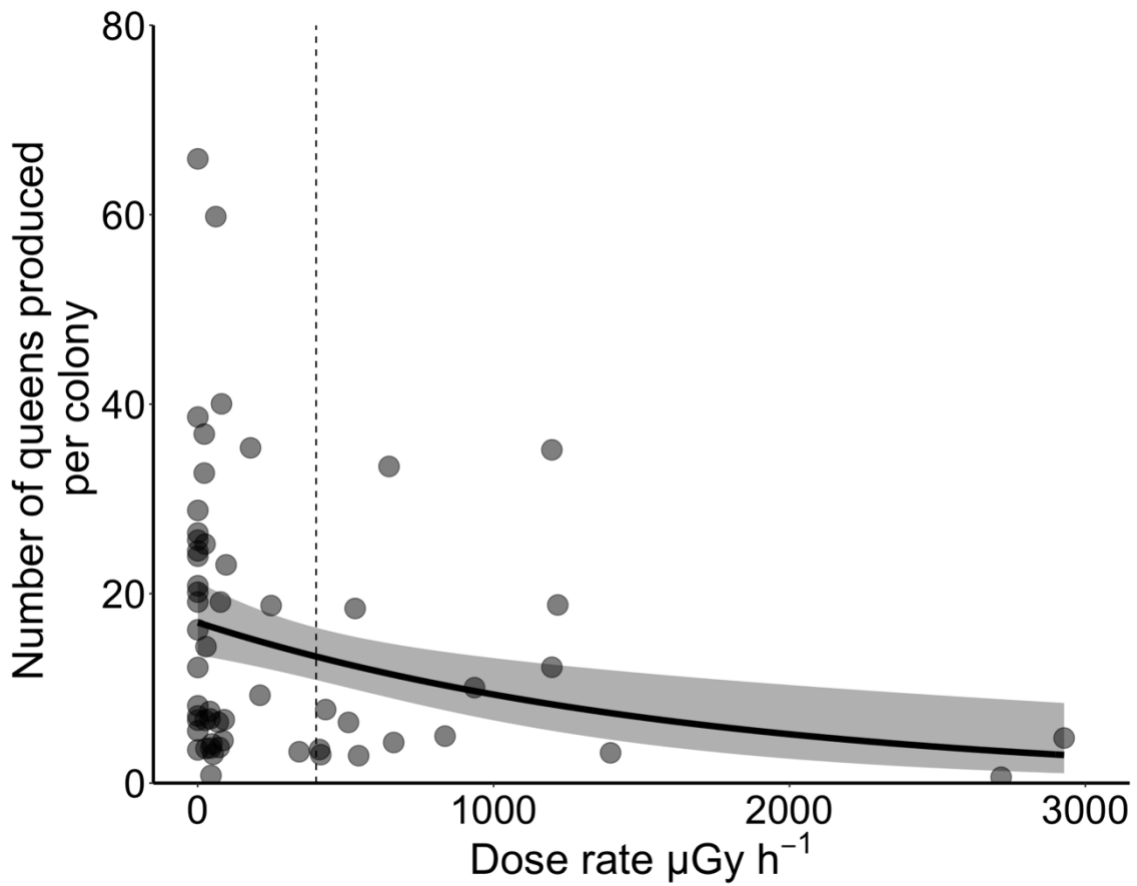
Figure S1.

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	χ^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	χ^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 ($\mu Gy\ h^{-1}$)	*-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	χ^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 ($\mu Gy\ h^{-1}$)	*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	χ^2	P value	ΔAICc
Colony start weight (g)	*0.0180*	*0.0100*	*2.92*	*0.0876*	*+0.7*
Dose rate ($\mu Gy\ h^{-1}$)	*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	χ^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	χ^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	χ^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 ($\mu Gy\ h^{-1}$)	*-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*