Réponses évolutives de populations de nématodes (Caenorhabditis elegans) exposées à des rayonnements ionisants

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Affidavit

Je soussigné, Loïc Quevarec, déclare par la présente que le travail présenté dans ce manuscrit est mon propre travail, réalisé sous la direction scientifique de Christelle Adam-Guillermin (directrice de thèse) et Jean-Marc Bonzom (co-directeurs de thèse) dans le respect des principes d'honnêteté, d'intégrité et de responsabilité inhérents à la mission de recherche. Les travaux de recherche et la rédaction de ce manuscrit ont été réalisés dans le respect à la fois de la charte nationale de déontologie des métiers de la recherche et de la charte d'Aix-Marseille Université relative à la lutte contre le plagiat.

Ce travail n'a pas été précédemment soumis en France ou à l'étranger dans une version identique ou similaire à un organisme examinateur.

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Liste de publications et participation aux conférences

1) Liste des publications (acceptées, en préparation…) dans le cadre de cette thèse :

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Résumé

Les pressions anthropiques sur les écosystèmes, comme les pollutions par des substances radioactives, peuvent mettre à mal la survie et le maintien des populations. Face aux stresseurs, les individus peuvent répondre rapidement par plasticité phénotypique et à plus long terme, les populations peuvent s'adapter. Toutefois, ces réponses s'accompagnent de coûts en ressource et peuvent induire une perte de *fitness* dans d'autres environnements. Mais si ces réponses sont insuffisantes, les populations peuvent décliner, voire disparaître. La prise en compte de ces mécanismes est importante pour améliorer l'évaluation des risques écologiques d'une contamination de l'environnement et en particulier pour estimer les conséquences des stresseurs, telle que la pollution radioactive, sur l'évolution des populations et leur maintien à long terme.

Dans ce contexte, l'objectif de ce projet est d'étudier expérimentalement l'évolution de populations de *Caenorhabditis elegans* exposées à des rayonnements ionisants gamma (Cs-137) et d'évaluer les coûts associés. Pour cela, des populations de *C. elegans* ont été exposées à trois débits de dose (0 - 1,4 et 50 $mGy.h^{-1}$) pendant 20 générations. Plusieurs traits d'histoire de vie ont été mesurés : la croissance des populations, la fréquence des mâles, le succès d'éclosion, la fécondité individuelle et à l'échelle de la population. Les modifications des traits observés ont été étudiées à l'aide de transplantations réciproques et de modèles mathématiques pour établir les causes sous-jacentes de ces changements. Enfin, les populations qui ont évolué dans les différentes conditions d'irradiation ont été exposées à un second stresseur, la bactérie pathogène *Serratia marcescens*, afin d'estimer les coûts adaptatifs.

A 50 $mGy.h^{-1}$ nous avons observé une diminution globale de la croissance des populations, de la reproduction, de la survie des embryons et de la *fitness*, mais une augmentation de la fréquence des mâles par rapport à la condition contrôle. A 1,4 $mGy.h^{-1}$, nous avons mesuré une diminution de la fécondité à l'échelle de la population et de la *fitness* et une augmentation de la croissance des populations et de la fréquence des mâles par rapport à la condition contrôle. De plus, nos résultats montrent qu'une pression de sélection s'est exercée sur les populations exposées aux rayonnements ionisants ce qui a conduit à une adaptation de ces populations. Les résultats suggèrent une tendance évolutive vers une amélioration de la survie des embryons et une histoire de vie plus lente en réponse à une faible irradiation. Au fort débit de dose, une amélioration de la *fitness* a été observée en quelques générations. De plus, l'irradiation favorise la reproduction par croisement (*outcrossing*). Ces changements se sont accompagnés de coûts. La diminution du succès d'éclosion et de la taille de ponte tardive des populations irradiées remises dans un environnement non irradié démontre la présence de coûts adaptatifs aux rayonnements gamma. D'autre part, l'évolution des populations sous rayonnements ionisants a induit un coût sur la capacité de survivre face à une bactérie pathogène.

Ces résultats montrent qu'une pression anthropique comme les rayonnements ionisants peut modifier la trajectoire évolutive d'une population, questionnant sur la survie à long terme de ces organismes et soulignant la nécessité d'une telle démarche dans le cadre d'une évaluation du risque écologique.

Mots clés : rayonnements ionisants, évolution expérimentale, adaptation locale, coût adaptatif, *Caenorhabditis elegans*

Abstract

Anthropogenic pressures on ecosystems, such as pollution by radioactive substances, can affect the survival and maintenance of populations. Faced with stressors, individuals can respond rapidly through phenotypic plasticity and in the longer term, populations can adapt. However, these responses are accompanied by resource costs and can lead to a loss of fitness in other environments. Nevertheless, if these responses are insufficient, populations may decline or even disappear. Considering these mechanisms is important to improve the ecological risk assessment of environmental contamination, and in particular to estimate the consequences of stressors, such as radioactive pollution, on the evolution of populations and their long-term maintenance.

In this context, the aim of this project is to experimentally study the evolution of *Caenorhabditis elegans* populations exposed to gamma ionizing radiation (Cs-137) and to evaluate the associated costs. For this purpose, *C. elegans* populations were exposed to three levels of ionizing radiation (0 - 1.4 and 50 $mGy.h^{-1}$) for 20 generations. Several life history traits were measured: population growth rate, male frequency, hatching success, individual and realized fecundity. The observed trait changes were studied using reciprocal transplants and mathematical models to establish the underlying causes of these changes. Finally, populations that evolved under the different radiation conditions were exposed to a second stressor, the pathogenic bacterium *Serratia marcescens*, to estimate adaptive costs.

We observed for high dose rates a decrease in population growth rate, reproduction, embryo survival and fitness, and an increase in male frequency compared to the control condition. We observed for low dose rates a decrease in realized fecundity and fitness, and an increase in population growth rate and male frequency compared to the control condition. Furthermore, results showed that selection pressure was exerted on populations exposed to ionizing radiation leading to adaptation. Results suggested an evolutionary trend towards an improvement of embryo survival and a slower life history in response to low dose rates. We observed that fitness increased within a few generations for high dose rates. In addition, irradiation promotes reproduction by outcrossing. These changes were accompanied by costs. The lower hatching success and late brood size of the irradiated populations back to the control environment reveal some costs of adaptation to gamma radiation. In addition, the evolution of populations under ionizing radiation induced a cost on survival to a pathogenic bacterium.

Our results show that an anthropogenic pressure such as ionizing radiation can modify the evolutionary trajectory of a population, questioning the long-term survival of these organisms and highlight the need for such an approach in the context of an ecological risk assessment.

Key words: ionizing radiation, experimental evolution, local adaptation, adaptive cost, *Caenorhabditis elegans*

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Glossaire

Adaptation locale *(Local adaptation).* Processus par lequel des variations présentes dans une population sont sélectionnées du fait de leurs caractères avantageux dans les conditions environnementales locales (l'habitat). Ce processus induit une amélioration de la valeur sélective (*fitness)* de cette population dans son habitat local par rapport à d'autres populations provenant d'autres habitats (Kawecki et Ebert, 2004).

Biodiversité *(Biodiversity)*. Désigne la totalité des gènes (= diversité génétique), des espèces (= diversité spécifique), des écosystèmes (= diversité des écosystèmes : forêt, lac, prairie, marin côtier…), ainsi que toutes les interactions au sein et entre ces niveaux d'organisation (= diversité fonctionnelle).

Compromis *(trade-off).* Situation où l'allocation de ressources (nourriture, temps ou encore l'espace : Garland, 2014) dans un trait améliorant la *fitness*, induit un coût représenté par une diminution d'allocation de ressources pour un autre trait. Généralement, on utilise le terme *trade-off* pour décrire invariablement la relation fonctionnelle reliant deux traits et la corrélation statistique négative observée entre eux (Roff et Fairbairn, 2007).

Conflit sexuel *(Sexual conflict).* Cas où les individus de différents sexes d'une même espèce ont des intérêts évolutifs opposés par rapport à un caractère qu'ils partagent.

Coût adaptatif *(Adaptation cost).* Réduction de la valeur sélective (*fitness*) d'un individu, et plus précisément d'un génotype, dans des environnements autres que celui pour lequel il est adapté.

Croisement *(Outcrossing)*. La fusion de gamètes provenant d'individus distincts.

Evolution expérimentale *(Experimental evolution)*. L'évolution expérimentale consiste à exposer des populations à des environnements contrôlés (en laboratoire ou en milieu sauvage) pendant plusieurs générations pour étudier les changements dans le temps et en particulier les processus évolutifs et leur issue (Bataillon *et al.*, 2010).

Expérience de transplantation réciproque *(Reciprocal transplant experiment).* Expérience consistant à déplacer des individus d'une population de leur environnement d'origine vers l'environnement d'une autre population, et *vice-versa*. Cette approche permet en particulier de mettre en évidence l'adaptation d'une population à son environnement.

Fécondité réalisée (ou écologique) *(Realized (or ecological) fecundity).* La fécondité réalisée est le nombre observé de descendants produits dans une population dans des conditions environnementales réelles. Elle s'oppose à la fécondité maximale (ou physiologique) qui est le nombre maximal théorique de descendants produits dans une population en l'absence de contraintes écologiques (Tarsi et Tuff, 2012).

Gray (Gy). Unité de mesure exprimant la dose de rayonnements ionisants absorbée par un organisme et plus précisément l'énergie absorbée par unité de masse. Ainsi, un Gray représente un joule absorbé dans un kilogramme de matière (1 $J.kg^{-1}$). Bien souvent les Grays sont exprimés par unité de temps ($Gy.h^{-1}$ ou $Gy.j^{-1}$ ou $Gy.an^{-1}$), on parle alors de débit de dose.

Plasticité phénotypique *(Phenotypic plasticity).* La propriété d'un génotype donné à produire des phénotypes différents en réponse à des conditions environnementales distinctes (Pigliucci, 2001). On peut différencier deux grandes catégories de plasticité phénotypique : la plasticité développementale (effets transgénérationnels) et la plasticité réversible (acclimatation) (Beaman *et al.*, 2016 ; Gutschick et BassiriRad, 2003).

Population efficace *(Effective population).* Le nombre d'individus qui participent effectivement à la production de la génération suivante (Sbordoni *et al.*, 2012).

Rayonnement ionisant *(Ionizing radiation).* Un rayonnement possédant suffisamment d'énergie pour modifier la charge électrique des atomes qu'il traverse, on parle d'ionisation. On retrouve plusieurs types de rayonnements ionisants, principalement alpha, bêta, gamma et X.

Stresseur (ou facteur de stress) *(Stressor (or stress factor))*. Facteur biotique ou abiotique qui entraîne une réduction de la *fitness* moyenne de la population (Theodosiou *et al.*, 2019).

Système immunitaire *(Immune system)*. Correspond à l'ensemble des mécanismes biologiques de défense de l'organisme (barrières physiques, cellules spécialisées, molécules…). Généralement, on différencie deux sous-systèmes : le système immunitaire inné et le système

immunitaire acquis (ou adaptatif). Le système immunitaire permet de détecter et de répondre à une grande variété de pathogènes. Le déclenchement du système immunitaire est appellé la réponse immunitaire *(Immune response)*. Dans le chapitre 4 nous utilisons le terme ***Host defense*** comme un synonyme de système immunitaire (Delves et Roitt, 2000 ; Levinson *et al.*, 2018).

Traits d'histoire de vie *(life history traits)*. Tous les traits qui contribuent directement à la production d'une descendance et à la survie, par exemple l'âge de la première reproduction, la fréquence de la reproduction, le nombre d'œufs et/ou de descendants produits par événement reproductif, l'investissement parental dans chaque descendant, le sex-ratio, la croissance ou encore la durée de vie (Reznick *et al.*, 2000).

Valeur sélective (*Fitness)*. Le concept de valeur sélective (le mot *fitness* est le terme le plus souvent employé en français) a pour but de décrire la capacité d'un individu, et plus largement d'un génotype, à se reproduire. *In fine*, la mesure de la *fitness* permet de quantifier la sélection naturelle (Lenormand *et al.*, 2016 ; Nowak, 2019). Généralement, on détermine la *fitness* d'un génotype par le nombre total de descendants, estimé à l'aide du produit de la survie et de la fertilité de l'individu.

Zone d'exclusion *(Exclusion zones)*. Zone géographique où la contamination radioactive a conduit à la réglementation des activités humaines, en particulier une évacuation des populations humaines présentent dans cette zone et une restriction de son accès.

Introduction générale

La biodiversité est présente à plusieurs échelles d'organisation du vivant, comprenant la diversité génétique au sein d'une espèce (diversité intraspécifique), la diversité des espèces ou des taxons (diversité spécifique ou taxonomique), et la diversité des écosystèmes (diversité écosystémique) (Hamilton, 2005 ; Jabiol, 2010). Les écosystèmes ont été définis à l'origine comme « l'unité de base de la nature » par Tansley, avant que cette définition soit ensuite largement débattue par la communauté scientifique (Blew, 1996 ; Tansley, 1935). Aujourd'hui, dans le domaine de l'écologie, on peut définir un écosystème comme l'ensemble des êtres vivants (biocénose), des caractéristiques de l'environnement (biotope) et de toutes les interactions présentes dans un espace donné. Les écosystèmes et la diversité d'espèces qui les composent sont étroitement liés. Schwartz *et al.* (2000) ont montré par exemple que le fonctionnement d'un écosystème était positivement corrélé à sa diversité d'espèces. Elle va largement influencer le fonctionnement de l'écosystème, notamment sa productivité, sa biomasse, sa résistance aux invasions biologiques ou encore sa stabilité (Loreau et de Mazancourt, 2013 ; Loreau, 1998 ; Tilman *et al.*, 2014). Ainsi, lorsque la diversité d'espèces est modifiée ou s'érode, par exemple en lien avec les activités anthropiques, on peut s'attendre à un impact fort sur les écosystèmes, pouvant provoquer leur dérèglement ou leur disparition. La Plateforme intergouvernementale scientifique et politique sur la biodiversité et les services écosystémiques (IPBES) estimait en 2019 que les trois quarts de l'environnement terrestre et environ deux tiers du milieu marin ont été significativement modifiés par l'activité anthropique (IPBES, 2019). De son côté, le Fonds mondial pour la nature (WWF) indiquait dans un rapport en 2020 que les populations animales à travers le monde avaient décliné de 68 % entre 1970 et 2016 (WWF, 2020). Cinq grandes causes ont été identifiées pour expliquer ce phénomène : la perte et la dégradation des habitats (sols, forêts, rivières…), la surexploitation des ressources (pêche, exploitation du bois…), le changement climatique, les espèces invasives et les pollutions (physique et chimique). Ainsi, au cours de l'Anthropocène, les activités humaines ont provoqué une diversification et une accélération des changements environnementaux, mettant en péril la biodiversité.

Face à ces contraintes, les organismes peuvent répondre de différentes manières. Si les organismes sont suffisamment mobiles, ils peuvent migrer vers d'autres environnements moins stressants, mais qui peuvent être potentiellement différents de leur milieu d'origine. Si les

organismes restent en contact avec le stresseur, ils peuvent parfois répondre rapidement par des mécanismes de plasticité phénotypique. Ces modifications sont généralement réversibles et non héritables. A plus long terme, il est également possible d'observer une adaptation locale des populations à la suite de la sélection naturelle d'individus possédant une meilleure *fitness* (valeur sélective) face à ce stresseur (Boyd *et al.,* 2016). Cette gamme de réponses ne garantit pourtant pas la croissance et la survie des populations exposées aux stresseurs. En effet, si les réponses sont insuffisantes, les populations peuvent progressivement décliner et risquent l'extinction (Boyd *et al.*, 2016 ; Otto, 2018). Dans le cas d'espèces sessiles ou peu mobiles, la migration vers un environnement moins contraignant est compromise. De plus, les processus de plasticité phénotypique et d'adaptation peuvent être insuffisants, ne permettant pas d'améliorer la *fitness* des organismes ou pouvant provoquer une augmentation de la vulnérabilité des organismes. En outre, les mécanismes sous-jacents à l'amélioration de la *fitness* sont souvent accompagnés de coûts (Reznick *et al.*, 2000 ; Roff et Fairbairn, 2007). Il est possible de distinguer deux coûts en ce qui concerne les réponses plastiques : les coûts liés à l'expression d'un phénotype sous-optimal dans un environnement donné (c'est-à-dire les coûts de l'inadéquation phénotype-environnement) et les coûts liés à la plasticité elle-même (coût de maintenance, de production et génétique) (DeWitt *et al.,* 1998 ; Van Kleunen et Fischer, 2005 ; Auld *et al.,* 2010). L'augmentation du coût en ressources pour répondre à une contrainte peut entraîner des conséquences sur d'autres traits d'histoire de vie. En effet, l'allocation de ressource dans un trait pour l'améliorer peut entraîner un coût sur un autre trait et *vice versa*, créant des compromis (*trade-off*) (Reznick *et al.,* 2000 ; Roff et Fairbairn, 2007 ; Garland, 2014). Ces processus contraignent les trajectoires évolutives d'une population et donc peuvent les affecter à long terme (Roff et Fairbairn, 2007 ; Walsh et Blows, 2009). L'adaptation locale des organismes à un environnement donné présente également un coût se traduisant notamment par des compromis évolutifs (*evolutionary trade-off*). L'adaptation des organismes à un facteur de stress les rend potentiellement moins bien adaptés à d'autres environnements ou tout changement de leur environnement (Bergelson et Purrington, 1996 ; Coustau *et al.*, 2000).

Tous ces mécanismes évolutifs peuvent moduler la réponse dans le temps des organismes soumis à un stresseur, que ce soit en améliorant leur survie au fil du temps (réponse plastique, adaptation), ou au contraire en provoquant une détérioration de la survie ou de la reproduction des individus au sein d'une population. Cette problématique est primordiale dans le domaine de l'écotoxicologie, qui étudie tout particulièrement le devenir et les impacts des polluants sur les écosystèmes. Pour une meilleure évaluation des risques écologiques d'une contamination de l'environnement, il est important de s'intéresser aux mécanismes évolutifs et de comprendre

les conséquences des changements évolutifs sur le maintien à long terme des populations (Dutilleul *et al.*, 2013). La question évolutive est de plus en plus prise en compte en écotoxicologie, mais reste assez peu étudiée dans le domaine de la radioécologie, qui s'intéresse entre autres à l'impact sur les organismes des substances radioactives dans l'environnement. La majorité des travaux dans ce domaine se concentrent sur les effets directs des rayonnements sur les organismes, mais beaucoup moins sur les effets à l'échelle des populations pendant plusieurs générations (Bréchignac *et al.*, 2016 ; Guirandy *et al.*, 2022).

Dans ce contexte, l'objectif principal de ce projet de doctorat est d'étudier les effets multigénérationnels des rayonnements ionisants sur plusieurs traits d'histoire de vie de populations de *Caenorhabditis elegans* et en particulier sur leur l'évolution.
L'émission de rayonnements ionisants dans l'environnement a particulièrement augmenté depuis la fin de la Seconde Guerre mondiale, en relation avec les activités humaines (essais atmosphériques d'armes nucléaires, cycle du combustible nucléaire, accidents nucléaires majeurs…) (Rhodes *et al.*, 2020), et ils affectent parfois fortement les écosystèmes. Par exemple, les accidents majeurs des centrales nucléaires de Tchernobyl (Ukraine) ou de Fukushima (Japon) ont libéré respectivement $1{,}4\times10^{19}$ et $5{,}2\times10^{17}$ Bq de radionucléides dans l'environnement (IAEA, 2006 ; Okano *et al.*, 2016).
Les rayonnements ionisants peuvent engendrer des effets délétères sur les organismes, pouvant notamment affecter la survie, la reproduction ou le développement (par exemple Real *et al.*, 2004 ; Dallas *et al.*, 2012). Par ailleurs, plusieurs travaux ont démontré que ces effets délétères pouvaient se transmettre génétiquement ou non sur plusieurs générations (Hertel-Aas *et al.*, 2007 ; Buisset-Goussen *et al.*, 2014 ; Sarapultseva et Dubrova, 2016 ; Smith *et al.*, 2016 ; Guédon *et al.*, 2021).

Dans ce manuscrit, une première partie intitulée « état des connaissances » permettra d'éclairer le lecteur sur l'effet des rayonnements ionisants sur les traits d'histoire de vie étudiés dans ce projet et leurs conséquences à l'échelle des populations. Ensuite, nous décrirons brièvement les réponses que peuvent mettre en place les organismes face aux stresseurs. En tant qu'animal modèle utilisé dans ce travail, nous décrirons brièvement la biologie de cet organisme ainsi que la méthodologie appliquée pour étudier l'impact des rayonnements ionisants sur l'évolution des populations. Par la suite, les résultats et les discussions des travaux de recherche de cette thèse seront présentés sous forme de publications organisées en 4 chapitres.

Dans un premier temps, sous la forme d'un article de revue de littérature, nous montrons que l'approche évolutive est peu étudiée en radioécologie, alors qu'elle peut aider à caractériser et comprendre les effets des rayonnements ionisants sur les organismes (chapitre 1). Dans cet article, nous nous sommes focalisés sur : (i) les propriétés mutagènes des rayonnements ionisants et leur perturbation des processus évolutifs, (ii) les expositions à différentes échelles de temps, conduisant à une interaction entre l'évolution passée et contemporaine et (iii) la nécessité d'appréhender les spécificités des zones d'exclusion et comment l'approche évolutive pourrait concilier les résultats obtenus *in natura* et en laboratoire.

Ensuite, nous nous sommes intéressés aux changements démographiques de populations de *C. elegans* exposées pendant plusieurs générations à des rayonnements ionisants (chapitre 2). Pour comprendre les variations démographiques, la reproduction et la survie des individus ont été suivies en parallèle. La mesure du taux de croissance des populations est fondamentale en écotoxicologie (Sibly *et al.*, 2002), elle permet d'intégrer les interactions entre les traits d'histoire de vie et elle fournit une mesure plus pertinente de l'impact écologique d'un stresseur que des mesures au niveau individuel (Forbes et Calow, 1999). Ainsi, nous avons étudié pendant plusieurs générations l'évolution de populations irradiées et étudié les variations des traits mesurés.

Un focus a ensuite été réalisé sur les changements de stratégie de reproduction induits par les rayonnements ionisants (chapitre 3). En effet, il a déjà été démontré que les rayonnements ionisants peuvent induire des effets délétères sur la reproduction de *C. elegans* (Dubois *et al.*, 2018 ; Lecomte-Pradines *et al.*, 2017 ; Maremonti *et al.*, 2019), ce qui pourrait avoir des conséquences sur la stratégie de reproduction et le sex-ratio des populations irradiées, et *de facto* influencer la sélection sexuelle et l'évolution de la population (Janicke et Morrow, 2018). Nous avons tout particulièrement quantifié l'effet des rayonnements ionisants sur l'évolution du sex-ratio des populations et déterminé les causes des changements observés.

Finalement, nous avons étudié les coûts de l'adaptation (c'est-à-dire les compromis évolutifs ; Kawecki et Ebert, 2004) aux rayonnements ionisants en mesurant la survie de populations de *C. elegans* irradiées pendant plusieurs générations puis exposées à une bactérie pathogène, *Serratia marcescens* (chapitre 4). Le système immunitaire d'un organisme et notamment sa réponse immunitaire peut être affectée par les rayonnements ionisants et est fortement sujette à des *trade-offs*, car son maintien est coûteux en énergie mais essentiel à la survie de l'organisme (Lochmiller et Deerenberg, 2000 ; Schwenke *et al.*, 2016).

Etat des connaissances

1. Impact des rayonnements ionisants

Dans cette partie nous abordons seulement les effets des rayonnements ionisants sur certains traits d'histoire de vie en lien avec notre projet de recherche. Nous nous concentrerons sur l'impact des rayonnements ionisants sur la reproduction, la survie et la défense immunitaire des organismes. Ensuite, nous abordons les conséquences de ces changements à l'échelle des populations et de leur démographie.

1.1. Impact sur les traits d'histoire de vie des organismes

1.1.1. Impact sur la reproduction

Les rayonnements ionisants peuvent affecter la reproduction de nombreuses espèces (Dallas *et al.*, 2012 ; Real *et al.*, 2004). La diminution de la reproduction des organismes peut être liée à de multiples causes à différentes échelles, que ce soit par exemple des modifications comportementales, des traits sexuels secondaires, du système reproducteur, de l'environnement hormonal, des gamètes ou du développement embryonnaire. Dans le cas des rayonnements ionisants, des travaux ont décrit certaines de ces causes, résumées ici de manière non exhaustive.

Les rayonnements ionisants peuvent altérer l'accès à la reproduction des organismes. Fuciarelli et Rollo (2021) se sont par exemple intéressés à l'impact des rayonnements ionisants sur le chant des grillons domestiques mâles (*Acheta domesticus*). Les auteurs montrent que l'exposition aux rayonnements (à partir de 7 Gy) modifie leur chant, réduisant ainsi les capacités des mâles à s'accoupler avec succès. Les rayonnements ionisants peuvent également retarder la reproduction des organismes en provoquant des retards ou des anomalies de développement et de croissance. Par exemple, Alonzo *et al.* (2008) ont montré qu'une irradiation alpha à un débit de dose de 15 $mGy.h^{-1}$ entraînait un retard de reproduction de 3 jours chez des daphnies (*Daphnia magna*). Le début de la reproduction étant directement conditionné par la taille des daphnies, les auteurs suggèrent que le retard de reproduction est lié à un retard de croissance au stade juvénile. Les rayonnements ionisants sont également connus pour provoquer des retards de développement chez l'humain (Elgazzar et Kazem, 2015) ou chez le moustique (*Aedes Aegypti*) après une exposition de 5 Gy (Asman et Rai, 1972). Les

auteurs montrent que l'exposition aux rayonnements ionisants des œufs de *Aedes Aegypti* provoquait ensuite un retard de développement des larves qui en sont issues.

Les rayonnements ionisants affectent en particulier les gonades et les gamètes, et provoquent des dommages chromosomiques ce qui peut réduire la fécondité des organismes (Cosgrove et Blaylock, 1971 ; Perlowagora-Szumlewicz, 1964 ; Rackham et Woodhead, 1984). Hyodo-Taguchi et Egami (1976) ont mis en évidence un impact des rayonnements ionisants sur la gamétogenèse du poisson médaka (*Oryzias latipes*). Les résultats montrent une réduction du nombre de spermatogonies cinq jours après une irradiation aiguë de 1 Gy (rayon X). Chez le poisson zèbre (*Danio rerio)*, Hurem *et al.* (2018) ont montré qu'une irradiation gamma pouvait provoquer une grave perturbation de la maturation des ovocytes à 8,7 $mGy.h^{-1}$ et une absence de développement des organes reproducteurs à 53 $mGy.h^{-1}$. Chez les humains des résultats ont montré une surmortalité par apoptose des cellules germinales fœtales à partir d'une dose de 0,1 Gy (Guerquin *et al.*, 2009 ; Lambrot *et al.*, 2007). Au contraire, d'autres travaux ont montré une accélération de la spermatogenèse du médaka (*Oryzias latipes*) après une irradiation de 4,75 Gy, mais au prix d'une augmentation du nombre d'anomalies morphologiques des spermatozoïdes et d'une surmortalité des spermatozoïdes anormaux (Kuwahara *et al.*, 2003). Des anomalies gamétiques aux niveaux de la région de la tête et du flagelle des spermatozoïdes ont également été rapportées chez un coléoptère (*Blaps sulcata*) à 3 $\mu Gy.h^{-1}$ (Kheirallah *et al.*, 2017). *In natura*, des impacts similaires sur la reproduction ont été identifiés à des débits de dose relativement faibles. Par exemple, Lerebours *et al.* (2018) ont observé une augmentation de la proportion d'ovocytes immatures chez des perches (*Perca fluviatilis*) collectées dans le lac Glubokoye, se situant proche de la centrale nucléaire accidentée de Tchernobyl (Ukraine). Le débit de dose moyen dans le lac a été estimé à 15,7 $\mu Gy.h^{-1}$.

Les effets délétères des rayonnements ionisants sur les gonades et les gamètes peuvent provoquer une baisse du nombre de descendants. Des travaux ont par exemple montré une diminution de la taille de ponte chez des invertébrés comme la daphnie (*Daphnia magna*) après une exposition à 0,38 $mGy.h^{-1}$, le bourdon terrestre (*Bombus terrestris audax*) à 100 $\mu Gy.h^{-1}$ ou encore chez un planorbe (*Biomphalaria glabrata*) après une irradiation de 40 Gy (Gilbin *et al.*, 2008 ; Perlowagora-Szumlewicz, 1964 ; Raines *et al.*, 2020). D'autres travaux ont montré un impact sur la fécondité de plusieurs vertébrés lié à une diminution du nombre de descendants, par exemple chez le guppy (*Poecilia Reticulata*) à 1,7 $mGy.h^{-1}$, le poisson zèbre (*Danio rerio*) à 53 $mGy.h^{-1}$ ou chez le médaka (*Oryzias latipes*) à 362 $mGy.h^{-1}$ (Hurem *et al.*, 2018 ; Hyodo-Taguchi et Etoh, 1983 ; Woodhead, 1977). A des débits de dose généralement élevés, on peut

observer une stérilisation temporaire ou complète des organismes. Woodhead (1977) a montré que dès 12,7 mGy.h^{-1} chez le guppy (*Poecilia reticulata*) une stérilité fonctionnelle permanente provoquée par des modifications de fonctions hypophysaires. La stérilisation des organismes par les rayonnements ionisants est notamment utilisée pour contrôler les populations d'organismes considérés comme nuisibles dans le domaine de la santé ou de l'agronomie (Bakri *et al.*, 2005).

La viabilité des embryons et des stades précoces de vie est également un point essentiel pour expliquer une diminution de la fécondité chez les organismes irradiés. Des travaux ont montrés une diminution de la survie des larves de plusieurs espèces de vers exposés à une irradiation chronique à 0,19, 3,2 et 4 mGy.h^{-1} chez respectivement *Neanthes arenaceodentata*, *Ophryotrocha diadema* et *Eisenia fetida* (Harrison et Anderson, 1994 ; Hertel-Aas *et al.*, 2007 ; Knowles et Greenwood, 1994) ou chez le moustique *Aedes aegypti* après une irradiation de 3 Gy (Shetty *et al.*, 2016). Dans les zones radiocontaminées à la suite de l'accident nucléaire de Kyshtym (Russie) en 1957, Fesenko (2019) indiquait que chez la grenouille des champs (*Rana arvalis*), les œufs étaient de plus petite taille et les embryons possédaient une mortalité accrue par rapport aux grenouilles provenant des zones non radiocontaminées. Des travaux ont suggéré que la réduction de la capacité d'éclosion et de la survie des embryons pouvaient être induit par une diminution du succès de fécondation, à des mutations létales dans les cellules germinales ou des dommages pendant l'embryogenèse (Hertel-Aas *et al.*, 2007 ; Perlowagora-Szumlewicz, 1964).

Chez *C. elegans*, l'impact des rayonnements ionisants sur la reproduction a également été étudié. Il a été montré qu'une irradiation gamma externe induisait un retard de croissance et de la reproduction à un débit de dose de 26,8 mGy.h^{-1} (Lecomte-Pradines *et al.*, 2017). Par une approche de modélisation utilisant le DEB-tox (*Dynamic Energy Budget theory in toxicology*), les auteurs ont montré que ces retards pouvaient être liés à une augmentation des coûts de croissance et de maturation. Sur la taille de ponte elle-même, des travaux ont montré une diminution chez la souche N2 d'environ 25 %, 35 %, 43 % et 61 % à des débits de dose de rayonnements gamma respectivement de 42,7 mGy.h^{-1}, 50 mGy.h^{-1}, 108 mGy.h^{-1} et 228 mGy.h^{-1} (Buisset-Goussen *et al.*, 2014 ; Dufourcq-Sekatcheff *et al.*, 2021 ; Maremonti *et al.*, 2019). Une autre étude a montré une diminution de la taille de ponte de 23 % à 50 mGy h^{-1} pour la génération parentale (F0), puis une diminution jusqu'à 40 % pour les générations suivante (entre F1 et F3), indiquant de probables effets transgénérationnels, et en particulier épigénétiques (Guédon *et al.*, 2021). Plusieurs travaux ont expliqué cette diminution de la taille de ponte chez

C. elegans par une diminution du nombre de cellules germinales par apoptose radio-induite, une diminution de la production de spermatides et une diminution de la quantité et de la qualité des spermatozoïdes après une irradiation (Guédon *et al.*, 2021 ; Maremonti *et al.*, 2019). De plus, des résultats ont montré des dommages à l'ADN des noyaux germinaux mitotiques causés par une irradiation gamma aiguë (30 Gy) (Park *et al.*, 2016) et des dommages à l'ADN embryonnaire (Maremonti *et al.*, 2019). Dubois *et al.* (2019) ont montré que la radioactivité modulait l'expression des protéines impliquées dans la reproduction. Les auteurs indiquent que ces changements pouvaient être un marqueur sensible et prédictif pour identifier un impact des rayonnements ionisants sur un organisme.

En ce qui concerne la mortalité des embryons chez *C. elegans*, après une irradiation aiguë de 50 Gy au stade L4/jeune adulte chez la souche N2, Dubois *et al.* (2018) ont mis en évidence une diminution du succès d'éclosion d'environ 20 % dans la descendance. Dans cette même étude, aucune diminution du succès d'éclosion n'a été observée avec une irradiation chronique de la génération parentale entre le stade embryon et L4/jeune adulte à 50 $mGy.h^{-1}$ (soit 3,3 Gy cumulé). Dubois *et al.* (2018) ont montré que la diminution du succès d'éclosion était corrélée à la carbonylation des protéines, suggérant que la diminution du succès d'éclosion pourrait être expliquée par des phénomènes d'apoptose.

1.1.2. Impact sur la mortalité des organismes adultes

La plupart des études sur les effets des rayonnements ionisants sur la mortalité des organismes ont été réalisées dans le cadre de recherches médicales ou écotoxicologiques. La durée de l'exposition, la dose, le stade de vie de l'organisme, la radiosensibilité des espèces peuvent, entre autres, largement influer sur le taux de mortalité. Par exemple, Tester *et al.* (1968) ont montré une variation du simple au double de la dose létale 50 % (DL_{50}) pour trois espèces de canard appartenant au même genre (4,25 Gy pour *Anas crecca,* 6,27 Gy pour *Anas discors* et 7,84 Gy pour *Anas clypeata*). La DL_{50} correspond à la dose déduite statistiquement, censée provoquer la mort de 50 % des individus d'une population animale donnée dans des conditions d'expérimentation précises (Trevan et Dale, 1927). Sur des groupes phylogénétiques plus éloignés, la variation peut être encore plus importante. A titre d'exemple, les DL_{50} des invertébrés aquatiques ont été estimées entre 2,1 et 2780 Gy, chez des poissons elles sont comprises entre 7 à 60 Gy et chez des mammifères entre 1 et 15 Gy (Copplestone *et al.*, 2001 ; Dallas *et al.*, 2012). Concernant *C. elegans*, des travaux ont montré un taux de mortalité d'environ 55 % pour la souche N2 à une dose de 60 Gy (Clejan *et al.*, 2006). Pour des espèces possédant des mues, comme les arthropodes, il est possible d'observer une augmentation de la mortalité au cours du cycle de vie. Par exemple, Shetty *et al.* (2016) ont observé une

augmentation de la mortalité entre le stade pupe et l'émergence des adultes dès une irradiation de 3 Gy chez *Aedes aegypti*.

Les causes de la mortalité diffèrent suivant les espèces. Chez les oiseaux, la mortalité est liée à d'importantes hémorragies intestinales généralisées, puis à un effondrement circulatoire et une insuffisance rénale (Tester *et al.*, 1968). Chez les mammifères, la mortalité des individus est d'abord provoquée par une atteinte de la moelle osseuse et une altération du tissu hématopoïétique, puis d'une atteinte gastro-intestinale et du système nerveux central (Badr et Badr, 1971).

1.1.3. Facteur de morbidité : impact sur le système immunitaire de l'organisme

La morbidité peut être définie comme tout impact préjudiciable sur les processus biologiques qui entraîne une diminution de la valeur adaptative d'un organisme, à court ou à long terme (Dallas *et al.*, 2012). Il existe un grand nombre de traits affectant la morbidité. Dans ce paragraphe nous avons décidé de nous focaliser que sur le système immunitaire de l'organisme, qui peut être particulièrement affectée par les rayonnements ionisants.

Les rayonnements ionisants peuvent avoir des impacts négatifs sur les défenses immunitaires des organismes, jusqu'à provoquer une immunosuppression aux plus fortes doses (Cui *et al.*, 2017). Parallèlement, ils peuvent également affecter indirectement la réponse immunitaire *via* le stress oxydant qu'ils occasionnent (Mittal *et al.*, 2013). Chez les humains, des travaux ont montré des effets délétères des rayonnements ionisants sur l'immunité innée et acquise à différentes échelles : moléculaire (expression des gènes, anticorps, antigènes…), cellulaire (leucocytes, lymphocytes…) ou tissulaire (rate, thymus, moelle osseuse…) (Anderson et Warner, 1976 ; Lumniczky *et al.*, 2021 ; Manda *et al.*, 2012). Plusieurs pathologies ont été associées aux effets des rayonnements ionisants et du stress oxydant qui en découle (cancers, diabètes, maladies neurodégénératives, maladies cardiovasculaires, maladies circulatoires…) (Little Mark P. *et al.*, 2012 ; Shah *et al.*, 2012 ; Sumner, 2007 ; Toyokuni, 1999). En ce qui concerne les espèces sauvages, quelques travaux menés dans des zones radiocontaminées proches de la centrale accidentée de Tchernobyl ont identifié un impact délétère des rayonnements ionisants sur la réponse immunitaire de l'hirondelle rustique (*Hirundo rustica*) avec un niveau de radiation ambiant compris entre 2,6 et 4,4 µGy ou chez le campagnol roussâtre (*Myodes glareolus*) avec un débit de dose ambiant compris entre 15 et 18 $\mu Gy.h^{-1}$ (Camplani *et al.*, 1999 ; Kesäniemi *et al.*, 2019). Expérimentalement, un impact délétère des rayonnements ionisants sur la réponse immunitaire a été identifié chez la truite arc-en-ciel (*Oncorhynchus mykiss*) à un débit de dose de 4,66 $mGy.h^{-1}$ ou la pyrale à houppes blanches

(*Orgyia leucostigma*) après une irradiation à 300 Gy (Knowles, 1992 ; Rossmoore et Hoffman, 1971).

Au contraire, d'autres résultats montrent que les rayonnements ionisants peuvent stimuler la réponse immunitaire (Shibamoto et Nakamura, 2018). Par exemple, une irradiation chronique de 0,95 $mGy.h^{-1}$ réduit significativement l'apparition de tumeurs cancéreuses chez la souris grise (*Mus musculus*) (Sakai *et al.*, 2003). Les auteurs suggèrent que ce résultat peut être expliqué par une stimulation des fonctions immunitaires par les rayonnements ionisants. Une autre étude a montré qu'une irradiation à 0,2 Gy chez la drosophile (*Drosophila melanogaster*) peut améliorer la résistance aux infections bactériennes (Seong *et al.*, 2012). Les espèces réactives de l'oxygène jouent également un rôle important dans le système immunitaire. Par exemple, en cas d'infection bactérienne, ils favorisent l'activité des macrophages ou de l'inflammasome (Chen *et al.*, 2018 ; West *et al.*, 2011).

Chez *C. elegans*, à notre connaissance très peu d'études se sont intéressées à l'effet des rayonnements ionisants sur ses défenses immunitaires. Après une irradiation gamma de *C. elegans* a 50 Gy, Liu *et al.* (2022) ont mis en évidence une diminution de la survie des nématodes infectés par la bactérie *Pseudomonas aeruginosa*. Au contraire, Kimura *et al.* (2012) ont observé une amélioration de la survie des nématodes face au pathogène *P. aeruginosa* après qu'ils ont été irradiés trois fois à une dose de 100 Gy avec des rayons X. Les auteurs ont montré que les rayonnements ionisants activent des gènes de la réponse immunitaire innée.

Globalement, de nombreuses questions demeurent quant à l'impact des rayonnements ionisants sur le système immunitaire et en particulier dans le cas des faibles doses (Lumniczky *et al.*, 2018, 2021). Le Comité scientifique des Nations unies pour l'étude des effets des rayonnements ionisants (United Nations Scientific Committee on the Effects of Atomic Radiation, UNSCEAR) considère les rayonnements ionisants comme un agent modulateur de la réponse immunitaire (Annexe D, UNSCEAR, 2008). L'UNSCEAR ajoute dans ce rapport que les rayonnements ionisants influencent la réponse immunitaire de nombreuses façons et parfois de manière opposée suivant de nombreux paramètres, par exemple la dose, le débit de dose, l'âge, le patrimoine génétique, l'état de santé, les comorbidités ou les co-stresseurs environnementaux.

1.2. Conséquences populationnelles et démographiques

L'impact des rayonnements ionisants à l'échelle individuelle peut avoir des conséquences directes au niveau de la population par le biais d'une diminution de la reproduction ou de la survie, comme décrit ci-dessus. Des effets sur des paramètres démographiques, qui jouent un

rôle important dans le maintien d'une population, tels que la croissance, la densité ou le sex-ratio d'une population, pourraient également être observés (Le Galliard *et al.*, 2005 ; Sibly *et al.*, 2002).

Quelques études se sont intéressées à l'effectif de populations sauvages par des approches de comptage dans des zones contaminées par des radionucléides. Par exemple, après la catastrophe de Kychtym en 1957 dans le complexe nucléaire de Maïak (Russie), une diminution des populations a été observée dans les années qui ont suivi l'accident chez des invertébrés, des petits mammifères ou encore chez des espèces d'oiseaux (Fesenko, 2019). Des diminutions similaires ont été observées chez différentes espèces dans la zone d'exclusion de Tchernobyl (oiseaux, abeilles, papillons, sauterelles, libellules, araignées, mammifères) ou à Fukushima (oiseaux, papillons et cigales) (Mousseau et Møller, 2014). Il est intéressant de noter que les effectifs d'araignées avaient au contraire augmentés l'année qui a suivie l'accident de Fukushima (Mousseau et Møller, 2014). Les auteurs ont suggéré qu'une diminution de la pression de prédation, par exemple par les oiseaux, pourrait expliquer ce résultat. Une autre approche pour évaluer la taille d'une population efficace est d'étudier la diversité génétique des individus qui la compose. Par exemple, Car *et al.* (2021) ont montré à travers l'étude de la diversité génétique et par des réseaux d'haplotypes mitochondriaux, que des populations de rainettes (*Hyla orientalis*) de la zone d'exclusion de Tchernobyl possédaient une petite taille efficace environ 30 ans après l'accident nucléaire.

Quelques travaux en laboratoire se sont également intéressés à l'impact des rayonnements ionisants sur les populations. Par exemple, Marshall (1966) a exposé une population de daphnies (*Daphnia pulex*) à différents niveaux de rayonnement gamma pendant 55 semaines. Les auteurs ont alors observé une extinction des populations exposées à un débit de dose compris entre 158 et 187 $mGy.h^{-1}$. A des débits de dose plus faibles, compris entre 31 et 158 $mGy.h^{-1}$, la densité de population était en quasi-équilibre, mais la densité moyenne était d'autant plus faible que le débit de dose était important. D'autres travaux ont conclu qu'une irradiation (rayonnement alpha) pouvait avoir des conséquences négatives importantes sur la dynamique des populations, après avoir montré que ce stresseur provoquait chez *Daphnia magna* un retard de développement et de reproduction (Alonzo *et al.*, 2008).

L'étude de l'impact des rayonnements ionisants sur les populations s'appuie également sur les outils de modélisation. Par exemple, un modèle mathématique développé chez la souris grise (*Mus musculus*) permet d'estimer l'impact d'une exposition chronique ou aiguë sur des

populations à partir de paramètres spécifiques de l'organisme (taux de croissance de la population, reproduction et capacité de réparation) et de l'irradiation (débit de dose, quantité de dommages induits par l'irradiation, dommages létaux irréversibles, dommages sur le système de réparation) (Kryshev et Sazykina, 2015). Les résultats de ce modèle ont montré que la survie d'une population de souris soumise à une exposition chronique était bien meilleure que celle de populations soumises à la même dose mais sur un très court terme (exposition aiguë). Par ailleurs, Alonzo *et al.* (2016) ont modélisé les réponses de populations à un rayonnement gamma externe chronique chez 12 espèces (*Neanthes arenaceodentata, Ophryotrocha diadema, Physa heterostropha, Eisenia fetida, Lumbricus terrestris, Porcellio scaber, Oryzias latipes, Poecilia reticulate, Oncorhynchus tschawytscha, Mus musculus, Canis familiaris* et *Capra hircus*). Les auteurs ont mis en évidence que les réponses des populations différaient selon le paramètre individuel affecté (survie des juvéniles ou des adultes, retard de maturité ou réduction de la fécondité), le paramètre de population considérée (en particulier le taux de reproduction) et l'histoire de vie des espèces étudiées. Ces travaux prédisent un risque de légère baisse de la dynamique des populations à partir respectivement d'un débit de dose chronique de 2 $\mu Gy.h^{-1}$ et 970 $\mu Gy.h^{-1}$ chez des mammifères et des invertébrés du sol.

Les rayonnements ionisants peuvent également affecter des paramètres démographiques, entraînant des conséquences sur les populations. Par exemple, l'impact peut être différent selon le stade de vie et le sexe des organismes (Dallas *et al.*, 2012 ; Møller *et al.*, 2012). Ces effets peuvent modifier la structure d'âge d'une population, affectant largement sa croissance et sa taille (Hoy *et al.*, 2020). De plus, les effets négatifs des rayonnements ionisants sur la reproduction et la survie des organismes peut modifier la densité de population, qui joue un rôle important sur la croissance d'une population (Tanner, 1966). Une petite taille de population efficace peut engendrer une dépression de consanguinité et de la dérive génétique, pouvant diminuer la *fitness* des individus d'une population (Hedrick et Kalinowski, 2000). Dans le contexte des rayonnements ionisants, à notre connaissance une seule étude s'est intéressée à la dérive génétique de population de daphnies (*Daphnia pulex*) provenant de la zone d'exclusion de Tchernobyl (Goodman *et al.*, 2022). Les auteurs ont observé une faible structuration génétique des populations, indiquant une faible dérive génétique des populations (Goodman *et al.*, 2022). D'autres travaux montrent que les rayonnements ionisants peuvent provoquer une modification du sex-ratio, notamment chez *Aedes aegypti* à partir d'une irradiation de 35 Gy en augmentant la différenciation des embryons en mâle (Shetty *et al.*, 2016). Un changement de sex-ratio a également été observé chez l'hirondelle rustique (*Hirundo rustica*) dans des zones contaminées de la région de Tchernobyl, en lien avec un plus fort taux de mortalité des femelles

(Møller *et al.*, 2012). Les auteurs indiquent qu'un biais du sex-ratio peut augmenter le risque d'extinction locale, comme le démontre Le Galliard *et al.* (2005) chez des populations de lézard vivipare (*Lacerta vivipara*) avec à l'origine un léger excès de mâle.

Malgré l'importance de l'étude des effets à l'échelle d'une population, la majorité des travaux en radioécologie se concentre sur les effets individuels et sub-individuels. Pourtant, du point de vue de la protection de l'environnement, les principales échelles d'intérêt sont les populations ou les écosystèmes (Dallas *et al.*, 2012). Geras'kin (2016) ajoute que la compréhension des effets des rayonnements ionisants à l'échelle de la population permettrait de mieux identifier les populations touchées et de prévoir les conséquences écologiques de l'exposition aux rayonnements.

2. Les réponses face à un stresseur environnemental

Les organismes sont capables de répondre aux modifications de leur environnement suivant différentes stratégies afin de minimiser ou de se soustraire aux effets d'un stresseur. Ces réponses biologiques sont extrêmement variées dans le règne du vivant, mais on peut toutefois dégager un schéma global de réponses : les organismes peuvent fuir par migration ou rester exposés au stresseur, dans ce cas les organismes peuvent survivre grâce à des mécanismes de plasticité phénotypique et à plus long terme d'adaptation (Figure 1). Si la réponse des organismes est insuffisante, la population risque l'extinction (Boyd *et al.*, 2016). Il est important de noter que ces réponses sont liées et interagissent entre elles. Dans cette section nous nous intéresserons tout particulièrement à la plasticité phénotypique, l'adaptation locale et les coûts qui peuvent en découler.

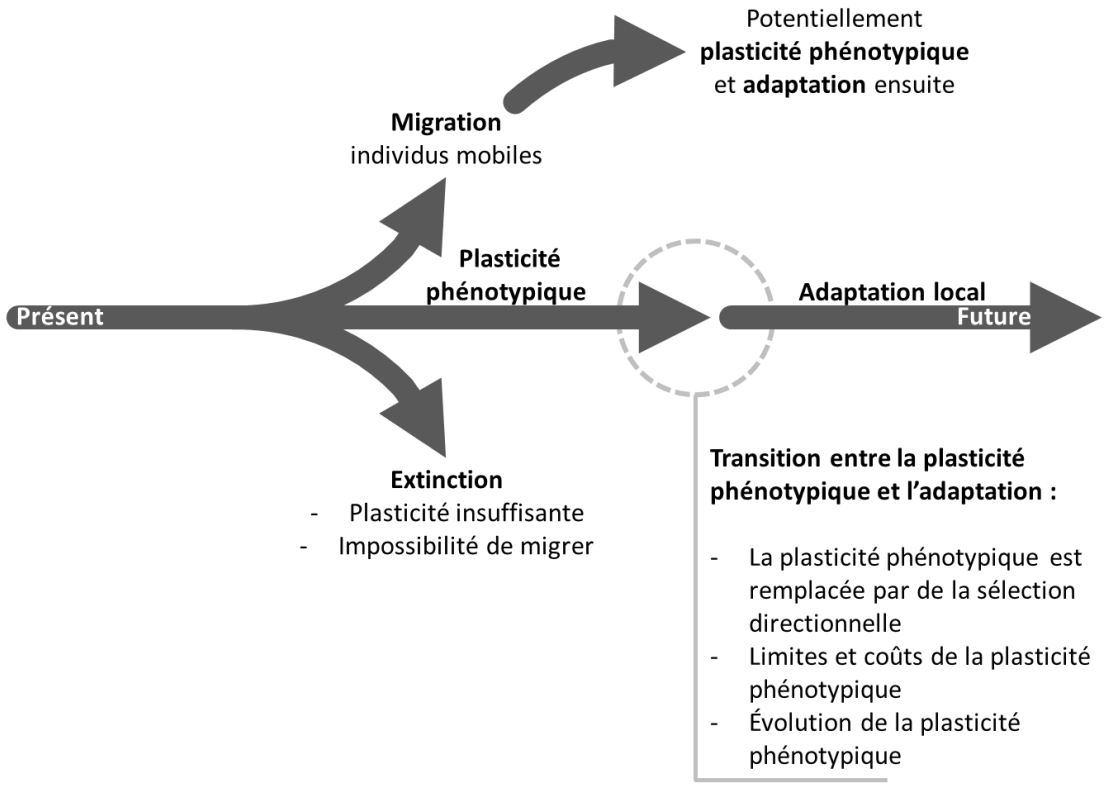

Figure 1 : schéma conceptuel résumant quatre réponses globales d'un organisme face à des conditions environnementales changeantes. A court terme, dans certaine situation, il est possible d'observer de la migration, de la plasticité phénotypique ou l'extinction des populations. A plus long terme (quelques générations) des phénomènes d'adaptation locale peuvent être mesurés. A la suite de la migration des populations, la plasticité phénotypique et l'adaptation peuvent être nécessaires pour que les populations se maintiennent dans le nouvel environnement. Le cercle en pointillé représente la transition entre les mécanismes de plasticité phénotypique et l'adaptation. Les mécanismes de cette transition ne sont pas encore totalement connus et des incertitudes demeurent (Ghalambor *et al.*, 2015). Boyd *et al.* (2016) proposent trois alternatives permettant d'expliquer cette transition, représentées ici sous le cercle en pointillé. Schéma modifié à partir de Boyd *et al.* (2016).

2.1. La plasticité phénotypique

La plasticité phénotypique peut se définir comme *« the property of a given genotype to produce different phenotypes in response to distinct environmental conditions »* (Pigliucci, 2001). Elle peut concerner tout type de trait, qu'il soit morphologique, comportemental, physiologique ou bien métabolique et permet de répondre de manière relativement rapide à un changement de l'environnement (Lafuente et Beldade, 2019). Les mécanismes de plasticité phénotypique sont généralement considérés comme non héritables, la modification ne sera pas transmise aux générations suivantes. Dans les faits, la plasticité phénotypique regroupe plusieurs types de réponses s'appuyant sur des mécanismes différents. On peut différencier

deux grandes catégories de plasticité phénotypique : la plasticité développementale et la plasticité réversible (acclimatation) (Beaman *et al.*, 2016 ; Gutschick et BassiriRad, 2003).

La plasticité développementale est induite par l'environnement gamétique parental et embryonnaire, impliquant bien souvent les effets parentaux et les transmissions épigénétiques comme la méthylation de l'ADN, c'est-à-dire des effets transgénérationnels (Kappeler et Meaney, 2010 ; Uller, 2008). Ainsi, les indices environnementaux externes vont influencer le développement de l'organisme entraînant des changements de phénotypes pendant le reste de sa vie et bien souvent irréversibles (Lafuente et Beldade, 2019 ; West-Eberhard, 2003). Par exemple chez la punaise du chou arlequin (*Murgantia histrionica*), les individus exposés à un environnement plus froid pendant leur développement présentent un corps plus petit et plus sombre que ceux élevés dans un environnement plus chaud (Sibilia *et al.*, 2018). Plus généralement, chez de nombreux Sauropsides, l'environnement thermique pendant l'incubation peut influencer les traits morphologiques, physiologiques ou comportementaux des individus (While *et al.*, 2018). La plasticité développementale joue un rôle important pour améliorer la *fitness* des individus, notamment si les indices environnementaux sont fiables et prévisibles, par exemple dans le cas de variations saisonnières comme la photopériode, l'abondance des ressources ou la présence de prédateurs (Bateson *et al.*, 2004 ; Kasumovic *et al.*, 2009). Au contraire si les effets des conditions passées ne correspondent pas aux conditions actuelles, la plasticité développementale peut avoir un effet négatif sur la *fitness* (Bateson *et al.*, 2004). D'autres recherches suggèrent que la plasticité développementale agit comme un tampon contre les perturbations non seulement environnementales mais aussi génétiques (Moczek, 2015).

La seconde catégorie de plasticité phénotypique est la plasticité réversible ou acclimatation, qui est une réponse temporaire à un changement de l'environnement et a lieu chez les organismes juvéniles ou adultes. Ainsi, contrairement à la plasticité développementale, le trait retrouve généralement sa valeur initiale une fois l'environnement revenu à son état initial. Cette caractéristique permet aux organismes d'ajuster leur phénotype dans le temps en fonction des changements environnementaux, même non prévisibles (Beaman *et al.*, 2016 ; Canale et Henry, 2010). Cette plasticité réversible permet d'améliorer la *fitness* des individus face à de nouvelles contraintes de l'environnement. La plasticité réversible concerne de très nombreux traits et peut être observée chez probablement tous les organismes (Gabriel *et al.*, 2005). Pour ne donner que quelques exemples marquants, nous pouvons citer des changements de couleurs de nombreux organismes (par exemple chez des mammifères, des crustacés, les caméléons ou encore les céphalopodes) en réponse à des changements biotiques ou abiotiques de leur environnement,

des modifications comportementales en présence de prédateurs ou encore une diminution de la consommation en O_2 chez des organismes soumis à des conditions hypoxiques (Storz *et al.*, 2010).

Beaman *et al.* (2016) défendent l'idée que ces deux catégories de plasticité phénotypique sont mécaniquement liées et se modulent réciproquement. Les auteurs indiquent que l'étude de ces interactions pourrait permettre de mieux prévoir la réaction des organismes face aux changements futurs.

La plasticité phénotypique est une réponse relativement immédiate à un changement d'environnement, mais elle joue un rôle essentiel à plus long terme, puisqu'elle participe à moduler l'évolution des espèces à long terme. La plasticité développementale peut être adaptative et son évolution peut être façonnée par la sélection naturelle. A plus long terme, elle peut faciliter l'évolution de nouveaux caractères (Moczek *et al.*, 2011). D'autres recherches suggèrent que la plasticité phénotypique de manière générale pourrait faciliter l'adaptation ou promouvoir la diversification des organismes (Gibert, 2020 ; Kelly *et al.*, 2011 ; Lafuente et Beldade, 2019). Enfin, pour faire face à un nouveau stresseur, la plasticité phénotypique et particulièrement sa magnitude, peut être sélectionnée (Kelly *et al.*, 2011 ; Lande, 2009 ; Visser, 2008).

2.2. Adaptation locale

Dans leur environnement naturel, les populations sont souvent confrontées de manière chronique à des stresseurs environnementaux. En quelques générations, dans certains cas, les populations peuvent répondre à ces stresseurs par adaptation locale. Cette réponse repose sur la sélection naturelle de variations qui sont avantageuses dans ces conditions, en améliorant globalement la survie et/ou le succès reproducteur des individus, c'est-à-dire la *fitness* (Kawecki et Ebert, 2004). La sélection naturelle agit sur la fréquence génétique des variations entre les générations. Les variations génétiques sélectionnées sont préexistantes dans la population, ainsi la diversité génétique peut être importante pour l'adaptabilité d'une population (Booy *et al.*, 2000). Ce processus permet théoriquement aux populations d'être adaptées de manière optimale aux conditions locales de l'environnement après plusieurs générations. Il sous-tend que les populations soient devenues potentiellement mal adaptées à d'autres conditions environnementales (Kawecki et Ebert, 2004). La mesure de la *fitness* est un bon moyen de caractériser l'adaptation relative d'une population à son environnement. Un individu adapté à son environnement aura potentiellement une plus grande descendance et une meilleure survie que des individus de la même espèce moins bien adaptés. Pour illustrer ces propos avec

des exemples historiques, on peut citer la sélection d'un phénotype sombre (*carbonaria*) chez la phalène du bouleau (*Biston betularia*) dans les environnements industriels noircis par les suies, conférant à ces papillons un meilleur camouflage et une meilleure survie face aux prédateurs par rapport au phénotype clair (*typica*) beaucoup plus visible (Bishop, 1972 ; Kettlewell, 1958). Un autre exemple est celui de l'adaptation de la forme du bec en fonction du régime alimentaire des espèces de pinsons de Darwin (*Geospizinae*) aux Galápagos (Boag Peter T. et Grant Peter R., 1981 ; Darwin, 1964 ; Price *et al.*, 1984). L'adaptation est un processus important à la survie et la reproduction des organismes à long terme face aux nombreuses variations de leur environnement.

Cependant, l'adaptation locale d'une population n'est pas toujours détectable (Gandon et Michalakis, 2002 ; Hereford, 2009) et peut même être considérée comme absente (Houwenhuyse *et al.*, 2021). Hereford (2009) explique que *« Studies that do not demonstrate local adaptation should not be looked on as failed experiments but as demonstrations that populations may be prevented from reaching adaptive optima »*. Plusieurs mécanismes empêchant l'adaptation ont déjà été identifiés, tels qu'un flux génétique restreint, une sélection trop faible ou trop forte dans un environnement temporellement variable (Comeron *et al.*, 2008 ; Gandon et Michalakis, 2002 ; Kawecki et Ebert, 2004 ; Reeve et Sherman, 1993). Par ailleurs, l'adaptation locale peut être plus difficile à établir en présence de compromis (*Trade-off*) (Buchanan *et al.*, 2018 ; Marshall et Sinclair, 2010). L'identification et l'intégration de ces mécanismes sont essentielles pour caractériser les réponses à long terme des populations aux stresseurs environnementaux.

2.3. Coûts et compromis (*trade-off*)

Une amélioration de la *fitness* se traduit globalement par une augmentation de l'allocation de ressources dans les traits qui contribuent à cette amélioration. L'augmentation de l'allocation de ressources représente un coût pour l'organisme (Reznick *et al.*, 2000). La quantité de ressources (énergie, espace, temps…) acquise par un individu étant limitée (Garland, 2014), ces coûts entrainent une diminution de l'investissement dans d'autres traits ; on parle alors de compromis (*trade-off*) (Boggs, 1992 ; Reznick *et al.*, 2000 ; Roff et Fairbairn, 2007 ; Steiner et Pfeiffer, 2007). Généralement, le terme *trade-off* est employé pour décrire invariablement la relation fonctionnelle reliant deux traits et la corrélation statistique négative observée entre eux (Roff et Fairbairn, 2007). Il est intéressant de noter que Stearns (1989) distinguait les *trade-off*s intra-individuels (entre deux traits d'un individu) et les *trade-off*s intergénérationnels (entre un trait de la génération parentale et un trait de la descendance), qui s'apparentent à des effets parentaux. Les *trade-offs* ont fait l'objet de plusieurs modélisations, afin d'en étudier le fonctionnement et de comprendre les mécanismes sous-jacents. Par exemple, le « modèle en

Y » de De Jong et Van Noordwijk (1992), largement utilisé, intègre à la fois les traits affectant l'acquisition des ressources et son allocation. Il décrit deux stratégies d'allocation alternatives, soit en priorisant la survie (viabilité), soit la reproduction (fécondité). Stearns (1989) identifie trois approches pour mesurer et analyser les *trade-offs* : une approche génotypique (étude des corrélations génétiques entre plusieurs traits), phénotypique (mesures de traits avec une relation négative directement liée à la reproduction ou à la survie) et dite de « structure intermédiaire » (liant les deux approches précédentes). Stearns définit cette dernière approche comme les mécanismes physiologiques et de développement sous contrôle endocrinologique qui aboutissent à la répartition des ressources entre les fonctions de reproduction, de croissance, d'entretien, de stockage et de survie. Chez *C. elegans*, un exemple de *trade-off* a été particulièrement étudié entre la longévité et la reproduction des hermaphrodites. Certains individus porteurs de mutation sur le gène *daf-2* (voie de signalisation de l'insuline) possèdent une fertilité beaucoup plus faible que des individus *wild type* (souche N2), mais ils possèdent une longévité bien supérieure (Jenkins *et al.*, 2004). Les auteurs précisent que les mutants possèdent toutefois une *fitness* plus faible que les individus N2 et démontrent qu'en seulement quatre générations la population de mutants s'éteint.

Dans le cadre de l'adaptation, il est possible de considérer la question des *trade-offs* à plus long terme (Bennett et Lenski, 2007). Lorsqu'une population s'adapte à un nouvel environnement, cela implique la sélection de traits qui améliore la *fitness* de cette population. Cependant, l'adaptation peut s'accompagner de la « détérioration » d'autres traits, potentiellement moins ou peu importants dans ce nouvel environnement. Dans ce cas de figure on parle également de *trade-off* (ou *evolutionary trade-off*), mais où le déclin de certains caractères au cours de l'adaptation est considéré comme un coût (ou contrainte) adaptatif (Bennett et Lenski, 2007). Ce coût peut être particulièrement visible lorsqu'une population adaptée à un environnement donné est placée dans un environnement différent ; il est alors possible d'observer une perte de *fitness*, indiquant une moins bonne adaptation (Bergelson et Purrington, 1996 ; Coustau *et al.*, 2000). Ainsi, des organismes adaptés à un environnement donné seront potentiellement moins performants dans d'autres environnements. Cette vision du coût associé à l'adaptation était déjà défendue par Darwin (1964) dans « *the origin of species by means of natural selection* » en affirmant : « *Natural selection is continually trying to economise in every part of the organisation. If under changed conditions of life a structure, before useful, becomes less useful, its diminution will be favoured, for it will profit the individual not to have its nutriment wasted in building up a useless structure* ». Depuis, ce paradigme s'est largement ancré en biologie évolutive, notamment pour étudier l'histoire de vie

ou l'évolution morphologique et physiologique des organismes (Bennett et Lenski, 2007). Une des approches les plus utilisées pour démontrer l'existence d'un *trade-off* est la réalisation d'expériences de sélection expérimentale, dans lesquelles sont étudiées un trait sélectionné, tout en mesurant des traits corrélés. Novak *et al.* (2006) ont par exemple étudié le *trade-off* entre le taux de croissance et le rendement de populations d'*Escherichia coli* (quantité de bactéries atteinte pendant la phase stationnaire de croissance pour une quantité de ressource donnée) après que celles-ci a évolué pendant 20 000 générations sous un régime de sélection constant (quantité de glucose limitante). Les auteurs ont alors observé un *trade-off* entre les deux traits, suggérant que les populations avaient évolué de différente manière dans cet environnement. Certaines populations possédaient une croissance rapide au détriment de son rendement, et inversement pour d'autres.

L'existence des *trade-offs* et leur rôle prépondérant dans le façonnement des trajectoires évolutives des organismes ne font plus aucun doute. Pourtant, on comprend encore relativement mal l'évolution et le fonctionnent de ces *trade-offs*, que ce soit théoriquement ou empiriquement (Roff et Fairbairn, 2007 ; Walsh et Blows, 2009).

3. L'évolution expérimentale : une méthode pour étudier les processus évolutifs

Dans cette section nous expliquerons l'intérêt de cette approche et du modèle biologique utilisé dans ce cadre pour répondre aux objectifs de ce projet de thèse.

L'évolution expérimentale consiste à exposer des populations à des environnements contrôlés pendant plusieurs générations pour étudier les changements dans le temps, et en particulier les processus évolutifs et leur issue (Bataillon *et al.*, 2010). Cette approche permet d'étudier de nombreux aspects de l'évolution, en s'appuyant sur un large spectre de complexité d'expérience : du plus simple en étudiant une population et un caractère, pour étudier par exemple les bases théoriques de la génétique des populations, à l'étude de plusieurs caractères sur un organisme, les interactions entre les organismes ou les théories touchant l'évolution et l'écologie des organismes et des communautés (Bataillon *et al.*, 2010). La complexité peut largement être modulée par la durée de l'expérience, allant de quelques générations à de très nombreuses, comme le projet *E. coli long-term evolution experiment* (LTEE) initié en laboratoire il y a plus de 30 ans par Richard Lenski de l'Université d'État du Michigan et comptabilisant plus de 70000 générations (Grant *et al.*, 2021 ; Lenski *et al.*, 1991).

D'un point de vue mécanistique, l'évolution expérimentale est très utile pour étudier les forces évolutives (mutation, sélection, dérive génétique et migration) de manière indépendante les unes des autres, et permet *de facto* de démontrer leur effet indépendant sur des populations (Bataillon *et al.*, 2010 ; Kawecki *et al.*, 2012). Elle est également utilisée pour tester le potentiel d'évolution (évolvabilité) d'un caractère et de son impact sur d'autres caractères, permettant d'étudier comment des paramètres peuvent imposer des contraintes sur l'évolution des organismes (Bataillon *et al.*, 2010 ; Colegrave et Collins, 2008). De plus, l'évolution expérimentale permet d'étudier les trajectoires évolutives de populations (Teotónio *et al.*, 2017). Pour cela on peut répéter une expérience plusieurs fois indépendamment pour comparer les différentes trajectoires, permettant d'analyser si l'évolution « prend les mêmes chemins » entre les différents réplicats. D'un point de vue pratique, l'évolution expérimentale est un outil indispensable pour appréhender les réponses des organismes face à des stresseurs et de comprendre la dynamique d'adaptation des populations (Kawecki *et al.*, 2012). Cette approche permet par exemple d'étudier l'apparition d'adaptation suivant des gradients d'exposition à une pollution, en analysant la *fitness* relative entre plusieurs populations indépendantes. A l'inverse, on peut comparer la *fitness* de plusieurs populations génétiquement différentes (souche, lignée…) face à une même pollution. Ensuite, il est théoriquement possible de caractériser cette adaptation et les variants génétiques qui en sont à l'origine, en s'appuyant notamment sur des

expériences de transplantation réciproque, de jardin commun et par des investigations à l'échelle génétique (Evans *et al.*, 2021 ; Kawecki et Ebert, 2004). Les travaux de Van Doorslaer *et al.* (2007) sont un bon exemple de cette approche, en étudiant l'adaptation dans un contexte de réchauffement climatique. Les auteurs ont cultivé des populations de zooplancton (*Simocephalus vetulus*) dans trois milieux différents de température (température ambiante ou 2 scénarios de réchauffement climatique) pendant un an, puis les ont soumis à différentes températures. Ils montrent une meilleure survie pour les populations provenant du scénario de réchauffement le plus important. L'évolution expérimentale permet également de mettre en évidence les coûts à l'adaptation (Dutilleul *et al.*, 2017 ; Jasmin et Kassen, 2007). Pour cela, on s'appuie généralement sur des expériences de transplantation réciproque, où l'on mesure la perte de *fitness* de population d'individus associée au changement d'environnement, par rapport à leur environnement d'origine.

La réussite d'une expérience d'évolution expérimentale est particulièrement dépendante de l'organisme modèle choisi et de ses qualités pratiques en fonction de la question biologique étudiée (Kawecki *et al.*, 2012). En général, les organismes choisis sont des micro-organismes unicellulaires comme la bactérie *Escherichia coli* (Grant *et al.*, 2021 ; Lenski *et al.*, 1991) ou la levure *Saccharomyces cerevisiae* (Dunham Maitreya J. *et al.*, 2002), mais on retrouve également des métazoaires comme la mouche du vinaigre (*Drosophila melanogaster*) (Tobler *et al.*, 2014), la daphnie (*Daphnia magna*) (Ebert, 2008) ou encore les nématodes (*Caenorhabditis elegans)* (Dutilleul *et al.*, 2014 ; Teotónio *et al.*, 2017). Ces organismes possèdent des avantages pour les expériences d'évolution expérimentale. Par exemple, ils sont faciles à propager et à compter, ils possèdent une reproduction rapide, il est possible d'avoir de grande population dans un volume restreint, ils sont biologiquement bien décrits et certains d'entre eux, comme les nématodes ou les bactéries, peuvent être stockés à -80°C afin de figer les processus évolutifs. Dans le cadre de l'évolution expérimentale, il est possible de s'intéresser à de nombreux paramètres, par exemple des traits d'histoire de vie, de virulence/infectivité, de coopération ou de biodiversité (Bataillon *et al.*, 2010). C'est pourquoi, il est primordial de prendre en compte l'adéquation entre l'organisme modèle et les traits d'intérêts pour étudier l'évolution de la population.

L'évolution expérimentale est une approche indispensable, car elle rend compte d'une complexité du vivant et de mécanisme qu'il est impossible de percevoir par une observation « instantanée ». La prise en compte dans les expériences d'évolution expérimentale des changements dans le temps a permis de réaliser des avancées fondamentales dans la

connaissance du vivant, dont on mesure l'importance par l'aphorisme de Theodosius Dobzhansky *« Nothing in biology makes sense except in the light of Evolution »*.

4. *Caenorhabditis elegans*

Ici nous abordons succinctement la biologie de *C. elegans* (cycle de vie, reproduction…) et présentons les avantages de ce modèle animal pour mener à bien des études de biologie évolutive expérimentale.

4.1. Ecologie générale et cycle de vie

Caenorhabditis elegans est un petit ver nématode d'environ un millimètre de la famille des Rhabditidae. Cet organisme est un métazoaire transparent et non parasitaire. Depuis sa découverte en 1900 dans des échantillons d'humus d'Algérie (Maupas, 1900), il a été retrouvé à de nombreux endroits comme par exemple en Amérique du nord et du sud, en Europe, en Afrique, en Australie ou à Hawaï (Frézal et Félix, 2015). Ils sont principalement présents dans des zones humides et tempérées, dans du matériel végétal en décomposition riche en bactérie (fruits pourris, composte), ce qui représente une abondante source de nourriture pour *C. elegans* (Blaxter et Denver, 2012 ; Frézal et Félix, 2015).

Le cycle de vie de *C. elegans* se compose de quatre stades larvaires - L1, L2, L3, L4 - qui sont suivis par l'âge adulte (Corsi *et al.*, (2015) ; Figure 2). Mais dans certaines conditions environnementales stressantes (manque de nourriture, température excessive…) les individus au stade L1 peuvent alors passer en stade *dauer*. Ce stade particulier leur permet de vivre plusieurs mois dans des conditions environnementales difficiles et de se disperser pour en particulier trouver de nouvelles sources de nourriture. Cette dispersion peut être facilitée par un comportement phorétique des nématodes (phoronte) avec d'autres espèces hôtes (Kiontke et Sudhaus, 2006). Dès lors qu'une nouvelle source de nourriture est découverte, les individus en

dauer reprennent leur cycle en L4 et les populations croissent à nouveau et colonisent la source de nourriture.

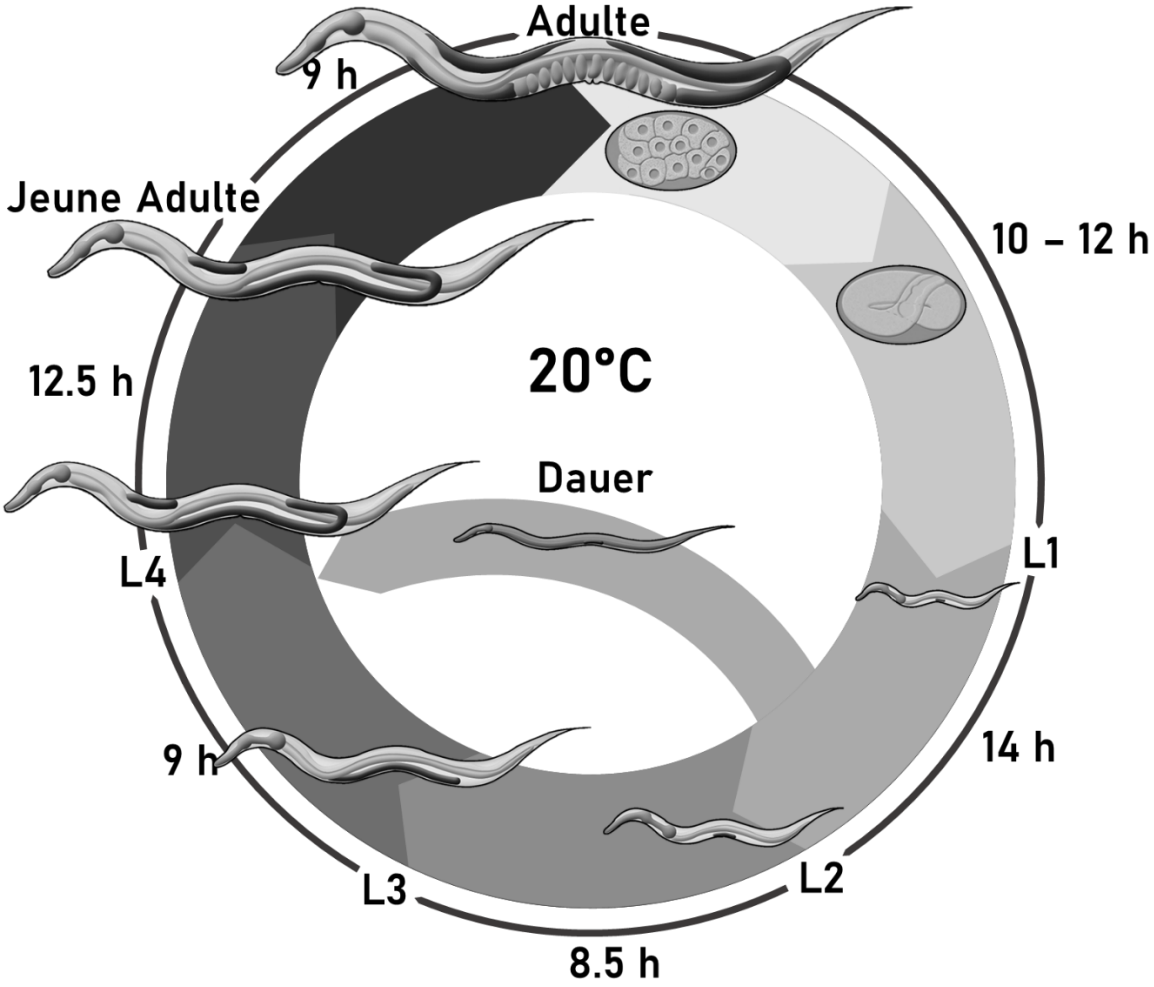

Figure 2 : cycle de vie simplifié de *C. elegans* à 20°C, le cycle débute avec la ponte de l'œuf (embryon) et le développement *ex utero*. Le schéma est modifié à partir du WormAtlas : https://www.wormatlas.org/hermaphrodite/introduction/mainframe.htm.

4.2. Reproduction

Le genre *Caenorhabditis* est particulièrement intéressant pour étudier l'évolution des interactions sexuelles, les conflits sexuels, l'outcrossing ou le sex-ratio, car il est possible d'observer une grande variabilité des systèmes d'accouplement et de la fréquence des mâles suivant les espèces et les populations (Palopoli *et al.*, 2015).

L'information génétique est supportée par 5 paires de chromosomes autosomes et une paire de chromosomes sexuels. Le sexe de l'animal est déterminé génétiquement par le nombre de chromosomes X. Les mâles n'ont qu'un seul chromosome X (XØ), les hermaphrodites en possèdent une paire (XX) (Zarkower, 2006). Les hermaphrodites se reproduisent principalement par autofécondation et leur quantité limitée de spermatozoïdes limite la

production de leur progéniture à environ 300 individus, qui seront tous hermaphrodites (Barr *et al.*, 2018 ; Chasnov, 2013 ; Cutter *et al.*, 2019). Une fois les spermatozoïdes épuisés, les hermaphrodites semblent libérer du « lait vitellin » (libre et dans les ovocytes) par la vulve provenant de la dégradation de leur propre corps. Ce processus participerait à améliorer la croissance et la reproduction de la progéniture (Kern *et al.*, 2021). Les hermaphrodites peuvent également s'accoupler avec des mâles et l'accouplement avec plusieurs mâles peut produire jusqu'à environ 1000 descendants, car les spermatozoïdes provenant des mâles sont produits continuellement et en grand nombre (Chasnov, 2013 ; Cutter *et al.*, 2019 ; Singson, 2001). Les ovocytes fécondés par un mâle produisent une descendance avec 50 % de mâles et 50 % d'hermaphrodites.

C. elegans est un organisme androdioïque, c'est-à-dire que les populations sont composées principalement d'individus hermaphrodites et quelques rares mâles (Figure 3 ; Herman, 2005). La fréquence des mâles varie entre 0 et 22 % suivant les populations sauvages (Anderson *et al.*, 2010). La rareté relative des mâles suggère un avantage sélectif de l'autofécondation par rapport à l'*outcrossing* (Cutter *et al.*, 2019 ; Stewart et Phillips, 2002) et une différence de stratégies reproductives entre les sexes, conduisant potentiellement à des conflits sexuels (Chapman, 2006). En effet, il semble que l'accouplement avec des mâles puisse entraîner un coût, par exemple une mortalité accrue et une diminution du succès reproducteur des hermaphrodites qui s'accouplent avec des mâles de manière répétée (Cutter *et al.*, 2019). Cependant, la fréquence des mâles peut augmenter fortement en cas de stress sévère (Lopes *et al.*, 2008 ; Lynch *et al.*, 2018 ; Morran *et al.*, 2009a ; Rose et Baillie, 1979). Cette observation suggère qu'il existe soit une diminution du coût de l'*outcrossing* pour les hermaphrodites, soit un avantage pour les mâles dans des conditions de stress. Bien que l'avantage de la reproduction biparentale soit encore un sujet de débat en biologie évolutive, il a été suggéré que chez *C. elegans*, l'*outcrossing* améliore la sélection naturelle, permettant une adaptation plus rapide et une purge plus efficace des mutations délétères (Cutter *et al.*, 2019).

La différenciation en mâle chez *C. elegans* peut également être liée à une anomalie de non disjonction du chromosome X pendant la méiose (Broverman et Meneely, 1994). Cette anomalie conduit les hermaphrodites à produire un gamète XX et un gamète Ø, qui produisent respectivement un hermaphrodite et un mâle. La fréquence de la non disjonction est d'environ 0,001- 0,002 chez la souche de laboratoire N2 (Corsi *et al.*, 2015 ; Stewart et Phillips, 2002), mais peut augmenter dans des conditions de stress, par exemple avec une température élevée

(Ayyadevara *et al.*, 2014 ; Cutter *et al.*, 2003) ou une exposition à des rayonnements ionisants (Kim, 1985).

Figure 3 : photographie d'un individu adulte mâle (A) et hermaphrodite (B) de nématode *C. elegans* (taille : environ 1 mm). Les mâles sont reconnaissables à leur l'extrémité caudale formant une queue arrondie indiquée par une flèche noire sur la photographie (crédit photo : Loic Quevarec/IRSN).

4.3. Modèle biologique

C. elegans est un organisme qui possède de nombreux atouts pour réaliser des recherches en biologie. Depuis les travaux fondateurs de Sydney Brenner dans les années 1960 sur cet organisme (Brenner, 1974), un grand nombre d'avancées et de connaissances scientifiques ont été acquises grâce à ce modèle animal (Blaxter et Denver, 2012). Cette dynamique toujours d'actualité autour du modèle *C. elegans* s'explique notamment par la facilité de son élevage et de sa multiplication, de sa petite taille, de son cycle de vie court, sa transparence ou encore le fait qu'il soit possible de le conserver vivant à -80 °C ou dans de l'azote liquide (Corsi *et al.*, 2015). Cette particularité permet de maintenir aisément à long terme des populations (souches, mutants, lignées transgéniques…) sans difficultés d'entretiens et sans changement génétique au fil du temps. Ce modèle biologique possède également l'atout d'être très bien décrit à l'échelle génétique, cellulaire, métabolique ou comportementale (Corsi *et al.*, 2015 ; Couillault et Kurz, 2010 ; Lemieux et Ashrafi, 2015 ; Schafer, 2005 ; Sulston *et al.*, 1983). En outre, *C. elegans* est le premier animal à avoir été séquencé entièrement (*C. elegans* Sequencing Consortium*, 1998), ouvrant les portes à de nouvelles recherches et découvertes. Encore aujourd'hui les

informations génétiques sont régulièrement mises à jour et enrichies (Grove *et al.*, 2018) ce qui en fait l'un des organismes modèles les mieux décrit et compris à cette échelle.

Nombre de ses avantages l'on propulsé comme modèle de choix dans le domaine de la biologie évolutive. Son cycle de vie extrêmement court en fait un des organismes métazoaires les plus utilisés pour réaliser des expériences multigénérationnelles et ainsi étudier, par exemple, les trajectoires évolutives de populations exposées à un stresseur, les mécanismes génétiques ou les stratégies de reproduction (Dutilleul *et al.*, 2017 ; Lopes *et al.*, 2008 ; Morran *et al.*, 2009b ; Noble *et al.*, 2017 ; Rose et Baillie, 1979 ; Yue *et al.*, 2021). Certaines souches ont été développées dans le but d'étudier des mécanismes évolutifs chez *C. elegans*. Par exemple la population A6140, créée à partir d'un mélange de 16 isolats (souches ou populations) (Noble *et al.*, 2017 ; Teotonio *et al.*, 2012), possède la particularité d'avoir une grande diversité génétique et environ 20 % de mâles, permettant de favoriser le brassage génétique, les mécanismes de sélection et d'adaptation. Cette population a initialement permis d'étudier l'évolution de l'*outcrossing* chez *C. elegans* (Teotonio *et al.*, 2012). Les auteurs ont notamment montré que la présence des mâles permettait de faciliter l'adaptation à de nouveaux environnements et que l'*outcrossing* pouvait être adaptatif. Plus récemment, cette population a permis de cartographier et de décrire l'architecture génétique de la fertilité des nématodes (https://lukemn.github.io/cemee/ ; Noble *et al.*, 2017) ou d'étudier l'évolution de population de *C. elegans* exposés à des stresseurs comme le sel (NaCl) ou l'uranium (Dutilleul *et al.*, 2013, 2014, 2015, 2017). Ces derniers auteurs ont étudié les mécanismes d'acclimatation, d'adaptation ou les coûts génétiques à travers des expériences multigénérationnelles et des transplantations réciproques.

Tel que décrit ci-dessus, par ses caractéristiques et les travaux antérieurs déjà réalisés en biologie évolutive, c'est cette population A6140 qui a été utilisée dans ce travail de recherche de doctorat.

Objectifs spécifiques et hypothèses

Cette thèse est organisée en 4 chapitres. Chaque chapitre comporte une publication.

Tout d'abord, à travers une revue de littérature, notre objectif était de montrer toute la pertinence d'étudier les effets des rayonnements ionisants sur les organismes sous l'angle de l'approche évolutive (chapitre 1).

Par la suite, nous avons étudié les effets chroniques des rayonnements ionisants sur la démographie de populations de *C. elegans* et sur plusieurs traits d'histoire de vie (chapitre 2) et en particulier sur la stratégie de reproduction (chapitre 3).

Enfin, dans le dernier chapitre, nous avons abordé la question des coûts adaptatifs et des compromis (*trade-off*) associés (chapitre 4).

Dans cette section nous présentons les objectifs spécifiques de chaque chapitre et les hypothèses associées. Ensuite, les résultats et les discussions des travaux de recherche pour chacun de ces objectifs sont présentés sous forme de publications rédigées en anglais.

Chapitre 1 - Dans quelle mesure l’approche évolutive peut contribuer à améliorer nos connaissances sur les effets des rayonnements ionisants sur les organismes ?

Au travers de cette revue de littérature, trois points en particulier ont été abordés : (i) les propriétés mutagènes des rayonnements ionisants et les conséquences de ces perturbations sur les processus évolutifs, (ii) les expositions à différentes échelles de temps, conduisant à une interaction entre l'évolution passée et contemporaine, (iii) la nécessité d'appréhender les spécificités des zones d'exclusion radio-contaminées (c’est-à-dire des zones où les populations humaines sont quasiment absentes) et comment l'évolution pourrait concilier les résultats obtenus dans ces zones et en laboratoire.

Dans ce travail, nous tentons de démontrer que l'évolution peut contribuer à fournir une vision intégrative des conséquences écologiques des rayonnements ionisants. Cette approche de biologie évolutive peut contribuer à expliquer les différences de sensibilité aux rayonnements ionisants entre les espèces, à améliorer notre estimation des impacts des rayonnements ionisants sur les populations et à aider à identifier les caractéristiques environnementales ayant un impact sur les organismes (par exemple, interaction avec d'autres polluants, migration des populations, changements environnementaux anthropiques).

Chapitre 2 - Quels sont les effets d'une irradiation chronique sur l'évolution et la démographie de populations de *C. elegans* ?

Comme vu en introduction, les rayonnements ionisants peuvent induire des impacts délétères sur la reproduction et le développement précoce de *C. elegans*. Ces deux paramètres jouent un rôle prépondérant sur la croissance d'une population.

L'objectif de cette étude était (i) de caractériser les changements au sein d'une population de *C. elegans* exposée aux rayonnements gamma et (ii) d'étudier les mécanismes sous-jacents, en déterminant si les variations observées étaient liées à de la plasticité phénotypique ou à des processus adaptatifs.

Nous avons émis l'hypothèse que les rayonnements ionisants réduisaient directement le taux de croissance de la population et ralentissaient le cycle de vie, notamment en diminuant la reproduction des individus et en affectant le développement et la survie des individus à des stades de vie précoces.

Nous avons étudié le taux de croissance et les traits d'histoire de vie d'une population de *C. elegans* exposée à des rayonnements ionisants gamma pendant 60 jours, soit 20 générations. Des expériences de transplantation réciproque ont été menées pour identifier si les variations observées étaient liées à de la plasticité phénotypique ou à de l'adaptation génétique.

Chapitre 3 - Les rayonnements ionisants peuvent-ils être à l'origine d'un changement de la stratégie de reproduction ?

Les rayonnements ionisants peuvent conduire à des effets délétères sur la reproduction de *C. elegans* et en particulier sur les gamètes. Ces effets pourraient avoir des conséquences sur la stratégie de reproduction et le sex-ratio des populations irradiées, influençant la sélection sexuelle et l'évolution de la population.

L'objectif de cette étude était (i) de tester si l'exposition aux rayonnements gamma modifiait le sex-ratio de populations de *C. elegans* et (ii) d'étudier les mécanismes évolutifs qui sous-tendent ces changements.

Nous avons émis l'hypothèse que les rayonnements ionisants modifient le sex-ratio par une diminution du succès de fertilisation des hermaphrodites par rapport à la fertilisation des ovocytes par les mâles, entrainant une augmentation de la fréquence des mâles. Dans un environnement stressant, l'*outcrossing* pouvant être avantageux, nous avons émis l'hypothèse que cette stratégie serait sélectionnée dans les conditions irradiées, entrainant un maintien de la forte fréquence des mâles même après la fin de l'irradiation.

Pour tester ces hypothèses nous avons étudié le sex-ratio de populations de *C. elegans* exposées à des rayonnements ionisants pendant 20 générations. Nous avons également réalisé des expériences de transplantation réciproque pour évaluer si les changements observés étaient de nature génétique (adaptation). Enfin, nous avons appliqué un modèle mathématique pour examiner les mécanismes évolutifs qui pouvaient être responsables des changements de sex-ratio.

Chapitre 4 - Est-ce que l'évolution à long terme dans un environnement irradié a pu être à l'origine d'un coût adaptatif sur le système immunitaire de *C. elegans* ?

Les rayonnements ionisants peuvent affecter l'efficacité du système immunitaire des organismes contre des pathogènes, que ce soit en le stimulant à des débits de dose relativement faible, ou au contraire en le diminuant à des débits de dose plus élevée. De plus, la réponse immunitaire est fortement sujette aux *trade-offs*, notamment parce que son maintien est particulièrement coûteux en ressources, mais essentiel à la survie de l'organisme.

L'objectif de cette étude était donc (1) de déterminer si les populations qui ont évolué dans des environnements irradiés possédaient une réponse immunitaire altérée face à une bactérie pathogène et (2) de mettre en évidence l'apparition ou non d'un *trade-off* évolutif, entre l'efficacité du système immunitaire contre un pathogène et la *fitness*, chez les populations irradiées.

Nous avons émis deux hypothèses : (i) les populations irradiées possèdent un système immunitaire moins efficace face à un pathogène, révélant un coût adaptatif aux rayonnements ionisants ; (ii) l'amélioration de l'efficacité d'adaptation des populations aux rayonnements ionisants conduirait à un *trade-off* évolutif avec l'efficacité du système immunitaire de *C. elegans.*

Pour atteindre cet objectif, après avoir été irradiées pendant 17 générations, les populations de nématodes ont été exposées pendant deux jours à une bactérie pathogène, *Serratia marcescens*. Le taux de survie des nématodes a alors été estimé, nous renseignant ainsi sur l'efficacité du système immunitaire face au pathogène.

Chapitre 1 - Evolutionary approach for pollution study: the case of ionizing radiation

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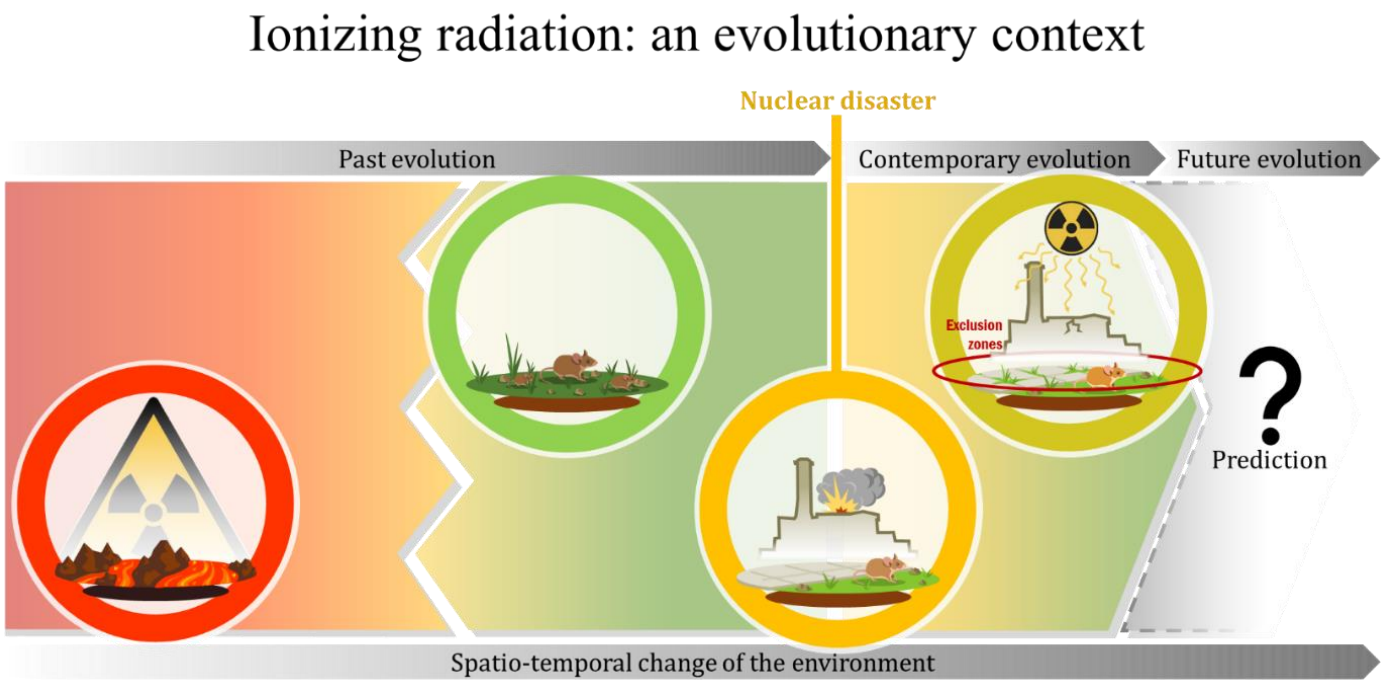
Key words - Evolution, ecotoxicology, evolutionary processes, radioecology, Ionizing radiation, Exclusion zones

Abstract

Estimating the consequences of environmental changes, specifically in a global change context, is essential for environmental protection or conservation issues. In the case of pollutants, the interest in using an evolutionary approach to investigate their consequences has been emphasized since the 2000s, but these studies remain rare compared to the characterization of direct effects on individual features. In radioecology, this lack of study with an evolutionary approach is particularly true, and that is why we focused on this study case to develop our ideas. In this review, we aim to show why and how evolutionary approach should be better used to characterize the effects of ionizing radiation on organisms. We suggest that evolution may help to provide an integrative view on the biological consequences of ionizing radiation. We focused on three topics: (i) the mutagenic properties of ionizing radiation and its disruption of evolutionary processes, (ii) exposures at different time scales, leading to an interaction between past and contemporary evolution, and (iii) the necessity to apprehend the specificities of exclusion zones and how evolution could conciliate the results obtained in the field and in the laboratory. This approach can contribute to explain species differences in the sensitivity to ionizing radiation, to improve our estimation of the impacts of ionizing radiation on populations, and to help identify the environmental features impacting organisms (e.g., interaction with other pollutants, migration of populations, anthropogenic environmental changes). This approach would benefit from being integrated to the ecological risk assessment process.

Graphical abstract



1. Introduction

In ecotoxicology, the awareness of evolution is particularly marginal with still few studies investigating changes in the intensity of evolutionary processes faced with pollutants compared to mechanistic studies investigating effects at the individual scale (Coutellec and Barata, 2013; Straub et al., 2020). In addition to physiological or biochemical measurements, efforts since the 2000s have focused on the development of new molecular biological biomarkers, in particular using omics tools (Forbes et al., 2006; Zhang et al., 2018). The interest for molecular biology has allowed for example to explain genetically the sensitivity of organisms to pollutants (Jo et al., 2009) or to study their mode of action (Larras et al., 2020; Peng et al., 2016). Concurrently, the integration of evolutionary approach to investigate the effects of pollutants on the environment has been developed with the emergence of studies in "evolutionary ecotoxicology" (see for example Bickham et al., 2000). This approach allowed to make assumptions about the intensity of evolutionary processes, notably supported by the development of molecular biology (Belfiore and Anderson, 1998).

With the emergence of evolutionary ecotoxicology studies, using evolutionary approach to investigate the effects of anthropogenic ionizing radiation became quickly apparent (see Matson et al., 2000; Theodorakis and Shugart, 1997; Theodorakis et al., 2001). Because of its mutagenic properties, the focus on ionizing radiation permitted to determine the persistence of these genetic modifications through generations and to compare them with the impact of other evolutionary processes and demographic patterns, such as gene flow (see Theodorakis, 2001). Thus, several studies analysed the evolutionary consequences of ionizing radiation following nuclear disasters, on genotypes (for example Baker et al., 2017; Dubrova et al., 1996; Fuller et al., 2019; Meeks et al., 2009) and phenotypes (Ellegren et al., 1997; Ruiz-González et al., 2016). These studies allowed both to study effects at the population level, which integrates individuals effects (Auerbach, 1984; Bréchignac et al., 2016), and to involve the temporal dimension, and take into account the dynamics at work. However, studies investigating evolution and its processes remain still relatively scarce in radioecology (see Box 1 for the case of field studies of ionizing radiation effects).

Nuclear energy is currently democratized and the use of radionuclides diversified, leading to several cases of anthropogenic exposure of the environment to ionizing radiation (powerplant accidents, atomic weapon tests, etc.) (Rhodes et al., 2020). The most obvious and dramatic examples of an anthropogenic exposure of the environment to ionizing radiation are nuclear disasters, such as the Kyshtym (29 September 1957), Chernobyl (26 April 1986), or Fukushima accident (11 March 2011). In these disaster areas, organisms can be exposed to relatively high dose rates for several decades. Radioactive contamination has led to regulation of human

activities in these areas, called exclusion zones, populations were evacuated and access was restricted. There is still scientific debate on the observed ionizing radiation effects on the environment after a nuclear disaster for low-doses (inferior to 100 µGy.h^{-1}) (Beresford et al., 2020).The scientific debate also concerns the linearity of the dose-response relationship for low-doses and the existence of a threshold under which no effect is expected (Tubiana et al., 2006). There are thus major scientific but also societal challenges related to the study of the effects of ionizing radiation on the environment (Centemeri et al., 2021).

In this review, our aim is to show why and how evolutionary questioning is indispensable to shed new light on radioecology and notably wildlife in exclusion zones. Indeed, ionizing radiation can disrupt evolution by their effect on inheritance and populations (Part 2), and consequently have an impact on organisms at ecological and geological time scales (Part 3). In addition, evolution is necessary to apprehend the specificities of exclusion zones, and make the link between laboratory and field studies (Part 4).

2. Ionizing radiation promotes the apparition of genotypic and phenotypic changes, and can modify evolutionary history

2.1. Impact on genetic and non-genetic inheritance

Ionizing radiation is mutagen and induces genetic alteration (Breimer, 1988) through direct effects on the DNA or through indirect oxidative damages (Einor et al., 2016). This alteration occurs at the chromosomes level, with chromosomal aberrations (Bender et al., 1974; Sudprasert et al., 2006), and at the DNA molecule level, with single or several base lesions (e.g., single or double strand breaks, base oxidation: Frankenberg-Schwager, 1990; Sage and Shikazono, 2017). It results in mutations such as substitutions, deletions/insertions, copy-number variants (Adewoye et al., 2015; Han and Yu, 2010). Ionizing radiation has a significant impact on spermatozoa, related to a negative regulation of DNA repair and to the loss of apoptotic capacities during spermatogenesis (Lewis and Aitken, 2005). Because of an important cellular activity (replication for example), reproduction and the first steps of development can be highly vulnerable to mutations. Specifically, deleterious mutations could impair it, leading sometimes to gamete loss or the death of the embryo.

Increased germline mutation rate can increase the number of mutations transmitted to the offspring. For example, germline mutation rate has increased in humans following nuclear weapon tests or nuclear disasters (Dubrova, 2003, 2006; Dubrova et al., 1996, 2002; Costa et al., 2018). The same increased mutation rate occurred in barn swallows (*Hirundo rustica*) (Ellegren et al., 1997) and in plants: scots pine (*Pinus sylvestris)* and wheat (*Triticum aestivum*) (Geras'kin and Volkova, 2014; Kovalchuk et al., 2003). Not all heritable mutations are conserved at the population scale, but some mutations may confer a higher individual fitness and increase in frequency in the population, as discussed for *Drosophia* exposed to X-rays (Nöthel, 1983). The majority of *de novo* mutations is either neutral for fitness or deleterious (Eyre-Walker and Keightley, 2007). If the short-term impact on individuals is limited, an accumulation of slightly deleterious mutations could have harmful long-term population effects (with interaction between genes called "synergistic epistasis" for example: Domínguez-García et al., 2019) especially in small populations which could experience a "mutational meltdown", leading to extinction (Loria et al., 2019; Lynch et al., 1995).

Mutations are thus interesting as a "potential" for evolution, creating diversity, but may represent a load for small populations (Lynch and Gabriel, 1990). Nevertheless, while adaptive evolution has often been interpreted as solely driven by selection, mutations can also be seen for themselves as an "orienting factor", conditioning the adaptive response (Yampolsky and Stoltzfus, 2001). In particular, specific types of mutation (i.e., mutation spectrum) can mediate a specific adaptive response (Cano Alejandro V. et al., 2022). For example, in the case of

environmental stressors, some results indicate that the types of mutation generated are stressor-specific and can lead to different evolutionary responses (Agashe, 2017). Mutations generated after an exposure to ionizing radiation can be specific to oxidative stress, as observed on crustaceans exposed to a relatively high background radiation which showed a higher proportion of *C:G→A:T* mutation type in more radioacontaminated areas (Saclier et al., 2020). Ionizing radiation could thus be an interesting case study to test the potential consequences of such mutational signature on evolutionary processes. Another transmitted variation increasingly studied in radioecology is the non-genetic transmission of information through generations (see Box 1). This idea of non-genetic transmission can be related to the concept of "non-targeted effects", defined as the effects that do not require direct exposure by irradiation, especially in the case of low-doses (Desouky et al., 2015). While non-targeted effects describe effects on cells, tissues or organs non-directely exposed to ionizing radiation, they also describe effects on organisms non-exposed to ionizing radiation but from which parents were exposed, and not mediated by DNA directely exposed to ionizing radiation, which refers to a non-genetic transmission of information. For example, an increased tendancy for mutations (i.e., “genetic instability”, Langie et al., 2015) can be transmitted after an exposure to ionizing radiation, leading to a higher rate of mutations in offspring (Dubrova, 2006) rather than the transmission of germline mutations. Because of the epigenetic nature (in a conservative sense, see Heard and Martienssen, 2014) of genomic instability, it can be seen as a non-genetic transmission of information.

Until today, genetic instability has been an important subject of investigations in radioecology (Dubrova, 2003; Yeager et al., 2021). These "epigenetic" mechanisms differ from the dose-effect relationship (Morgan and Sowa, 2009), and can also be studied as part of variation impacting evolution. As proposed by supporters of an "extended evolutionary synthesis" (Danchin et al., 2011; Laland et al., 2015), this non-genetic inheritance could be compared to the genetic inheritance with some specificities (Danchin et al., 2019). Several studies have observed radiation-induced transgenerational modifications (parental effects, epigenetic modification or genetic instability, Dubrova and Sarapultseva 2020) for example in *Daphnia magna* (Parisot et al., 2015; Sarapultseva and Dubrova, 2016; Trijau et al., 2018), *Danio rerio* (Guirandy et al., 2022), *Oncorhynchus mykiss* (Smith et al., 2016), *Caenorhabditis elegans* (Buisset-Goussen et al., 2014; Guédon et al., 2021) or *Eisenia fetida* (Hertel-Aas et al., 2007). Although some results suggested the same kind of transgenerational modification in Chernobyl or Fukushima, on butterflies (*Zizeeria maha*), rodents (*Myodes glareolus*), plants (*Arabidopsis thaliana*), birds (*Hirundo rustica*) or humans (*Homo sapiens*) (see for example Aghajanyan et al., 2011; Hancock et al., 2019; Omar-Nazir et al., 2018; Shuryak and Brenner,

2021), the proximate mechanism remains to be fully investigated. Nevertheless, the existence of several epigenetic changes may indicate that the same kind of phenomenon should appear in the field. For example, a modification of genome-wide DNA methylation has been observed at Chernobyl for Brassicaceae (Horemans et al., 2018), and Scots pine trees (Volkova et al., 2018), and at Fukushima on tree frogs (Gombeau et al., 2020). These changes are observed at a single time, but recent results suggest a possible transmission over generations (Horemans et al., 2019; Laanen et al., 2021). Non-genetic inheritance could be a particularly important mechanism to generate novelty, following a nuclear disaster (for example Kuijper and Hoyle, 2015; Lind and Spagopoulou, 2018).

2.2. Other consequences at the population level

Ionizing radiation is not solely a factor of novelty due to its mutagenic effect. Oxidative stress caused by ionizing radiation can cause stress to individuals (oxidative damage to DNA, lipids, proteins, and many metabolites: Bonisoli-Alquati et al., 2010; Dauer et al., 2010; Einor et al., 2016; Galván et al., 2014) that can modify individual fitness. Thus, individual scale changes can be reflected at the population scale (i) by the apparition of population effects not equal to the sum of individual effects (see for an application on ionizing radiation Monte, 2009) and (ii) by population changes over generations through the modification of the intensity of evolutionary processes. Evolutionary processes are all the mechanisms by which inherited variations are modified in a population (inducing its evolution). By studying these evolutionary processes - especially through genetic variation - we can estimate the long-term consequences of individual ionizing radiation effects. This approach was conducted in evolutionary ecotoxicology by Bickham (2011) who proposed the four cornerstones of genetic response of populations to a contaminant exposure: (1) genetic variability caused by increased mutation rates; (2) neutral modifications of genetic diversity; (3) genetic variability caused by gene flow induced by dispersal; and (4) selection through the survival of the fittest. These processes can be distinguished but they also interact. For example, neutral genetic drift can interact with

mutations by favouring the fixation of new genetic variation. Mutations can also generate new variation on which selection can act (Figure 4).

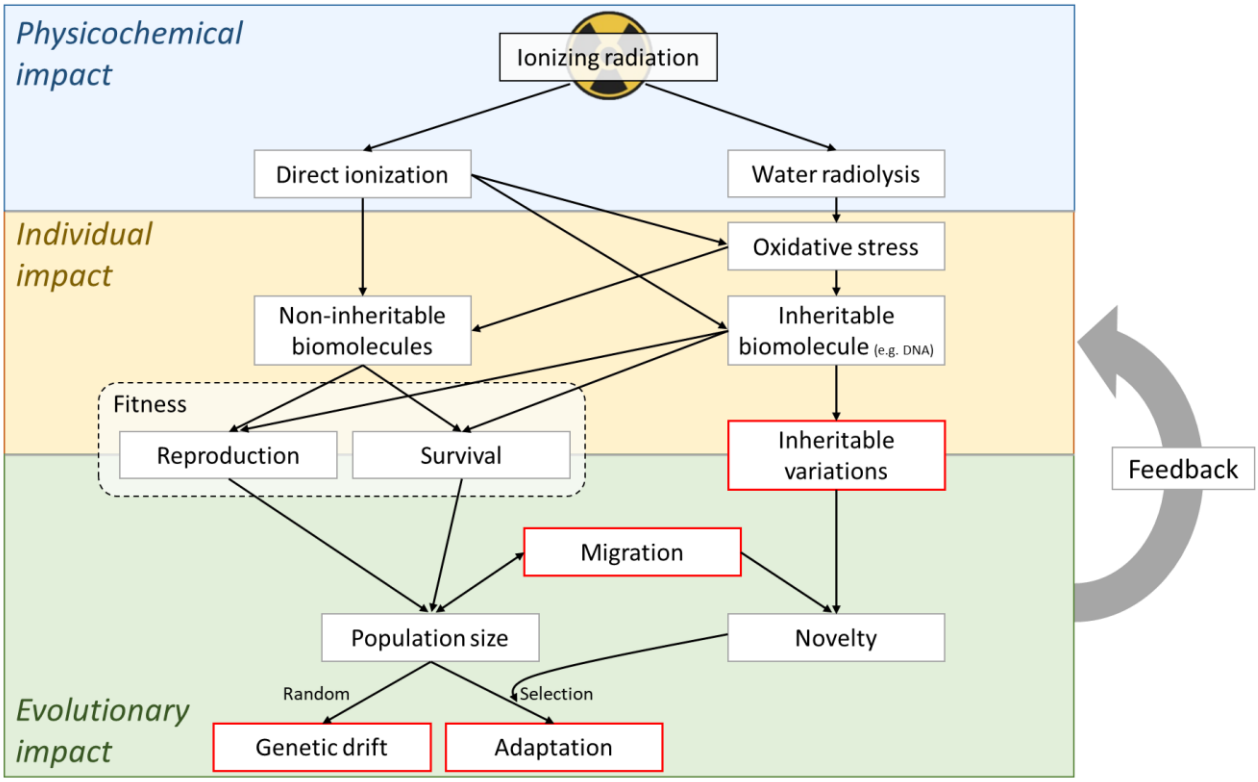


Figure 4: conceptual schema which presents the impact of ionizing radiation on the evolutionary processes (framed in red). Effects of ionizing radiation starts from physicochemical impacts (area in blue), which operates in two ways: it is generally considered that ionizing radiation affects living organisms by direct ionization of biomolecules (proteins, lipids, carbohydrates and nucleic acids) or indirectly by water radiolysis. Physicochemical impacts lead to (I) individual changes (area in yellow). Direct or indirect ionization can be at the origin of an oxidative stress, which impacts biomolecules, whether they are transmitted between generations (e.g., DNA) or not. The modification of biomolecules can lead to an alteration of individual traits, and consequently challenges the differential reproduction and survival of organisms (i.e., evolutionary fitness). Conjointly, ionizing radiation can cause new inheritable variations (e.g., mutations). Introducing new variations and altering the fitness of organisms have an (II) evolutionary impact at the population level (area in green). Fitness change and migrations of individuals (i.e., emigration and immigration) alter population characteristics (size, dynamics, etc.). Depending on the effective population size, genetic drift resulting from the random fixation of variations can be modulated. But the fixation of variations can also be the result of selective process, which can lead to adaptation. Adaptation may be enhanced by the contribution of novel variations emerging from immigration and radiation-induced heritable variations. It is important to note that evolutionary impacts can modulate individual effects (Feedback - grey arrow): for example, population adaptation to ionizing radiation may modify the impact of this stressor (sensitivity), improving evolutionary fitness, which can then impact the intensity of evolutionary processes.

Genetic drift, which is the neutral fixation of genetic variations, is an evolutionary process dependent on population sizes. In a population with a small effective population size (i.e., the

individuals involved in reproduction: Nei and Tajima, 1981), stochastic effects can decrease of genetic diversity. In evolutionary ecotoxicology, because of a potential effect of pollutants on demography and population sizes, this phenomenon has been called “genetic erosion” (van Straalen and Timmermans, 2002) and may lead to a decreased evolutionary potential (Frankham et al., 1999). For example, a reduced genetic diversity attributed to genetic drift was observed on marsh frogs living in a contaminated industrial place of Azerbaijan (Matson Cole W. et al., 2006). In the case of ionizing radiation, a study of tree frogs living in the Chernobyl region showed an increased mitochondrial diversity in the most contaminated area (Car et al., 2021). This diversity is attributed to mutations, but genetic drift is necessary to explain the fixation of these mutations over generations (Whitlock and Bürger, 2004). Other studies of populations subject to radioactive contamination did not highlight any increase in genetic drift (e.g., earthworms and aquatic crustaceans in Chernobyl : Fuller et al., 2019; Newbold et al., 2019) ; *Trifolium pratense* in Komi Republic (Rybak et al., 2018). Du Boulay (1977) proposed that genetic drift could purge chromosomal aberrations induced by irradiation. Anecdotally mechanisms can probably allow the emergence of advantageous variations (reverse or back mutation and compensatory mutations) in a context of genetic drift (Whitlock and Bürger, 2004).

When population size is reduced in a contaminated place, migration processes can buffer the impact of genetic drift by providing new variations in the area. With low population size in a “poor-quality” habitat (sink), the competition for resources may be less important by a decrease in reproduction or survival, making that habitat more attractive and promote the migration of individuals from good quality habitats with a demographic surplus (source). This phenomenon is called “source-sink” dynamics and may affect the viability of sink populations (Dias, 1996; Pulliam, 1988). Reduced population size observed for some organisms in the most polluted places (Horiguchi et al., 2016; Mappes et al., 2019; Møller and Mousseau, 2007, 2013) makes them particularly favourable to such dynamics. Frequent migrations toward the Chernobyl most contaminated area has been tested with stable isotopes for barn swallows (*Hirundo rustica*), suggesting a demographic sink (Møller et al., 2006). A population genetic approach also emphasizes the existence of a population sink on kangaroo rats (*Dipodomys merriami*) in the Nevada test Site, where nuclear weapons were tested between 1951 and 1963 (Theodorakis et al., 2001). Migration processes can thus modify the genetic diversity of organisms and, beside source-sink dynamics, be important evolutionary processes. According to Baker et al. (2017) a higher mutation rate explained the higher mitochondrial diversity of bank voles (*Myodes glareolus*) in the most contaminated area of the Chernobyl region. But migration can also generate this diversity (Kesäniemi et al., 2018; Matson et al., 2000), and explain bank vole

population dynamics (Vives i Batlle et al., 2020). While migrations can have beneficial effects by increasing the diversity and thus the evolutionary potential of contaminated populations, deleterious effect can emerge from outbreeding depression and lead to the extinction of the population independently of the low-quality of the habitat (Vuillaume et al., 2015). Outbreeding depression is still poorly studied in ecotoxicological context, or mainly in the laboratory for the selection of strains for effective chemical testing (Brown et al., 2009), but it is a serious consideration in conservation biology management (Edmands, 2007).

Radiocontaminated places like the Chernobyl or Fukushima regions are thus of interest to study the fundamental question: how can wild populations cope with a suddent environmental change in their environment? Currently, there is still too few studies to provide definitive conclusions. Specifically, compared to mutations or selection, genetic drift and gene flow have been relatively understudied in the case of ionizing radiations (see Box 1). While a lot of research is centred on the selection/adaptation process, many studies fail to evaluate adaptation process by not using suitable methodology (i.e., common garden, reciprocal transplant, genetic approaches) (Møller and Mousseau, 2016).

3. Multiple temporalities in the exposure to ionizing radiation

3.1. From acquired individual responses to heritable population modifications

Organisms may be able to cope with rapid environmental change by plastically changing hormone concentrations, development, etc. (Taborsky et al., 2021). If this change persists, organisms displaying better ability to survive and reproduce will have more descendants, i.e., a better fitness. And if this ability is heritable, it can be selected over generations, and lead to evolution. While character states selected, called adaptations, are phenotypically comparable to plastic responses, these adaptations are transmitted over generations. Acquired individual plastic modifications may also sometimes be transmitted across generations (see the idea of non-genetic transmission discussed part 2.1), but they are generally shakier than selected modifications (Danchin et al., 2019). Identifying the mechanisms at the origin of responses to ionizing radiation may help understand the potential long-term consequences of these modifications (inertia, genetic erosion, genetic load, lost of evolutionary potential) (Chevin et al., 2010).

There are several ways to identify, or demonstrate, case of selection or even adaptation (see Lambert et al., 2021). First, as mentioned in the section 2.2, genetic diversity may help to identify populations or even loci submitted to selection (Bickham, 2011), and with next-generation sequencing, gene-environment and gene-phenotype association approaches can also be conducted to identify loci displaying signatures of selection (Oziolor et al., 2017, 2019). For example, directed evolution experiments in the laboratory with *Escherichia coli* have created strains that are ultra-resistant to ionizing radiation. With next-generation sequencing, the authors demonstrated that resistance is mainly linked to only three variants involved in DNA repair (Byrne et al., 2014). Second, by testing the inertia of phenotypic modification, it may be possible to identify cases of selection and adaptation (Kawecki and Ebert, 2004). ‘Common garden experiments’ consist in rearing in the same conditions and during several generations organisms from differentially contaminated places, to test whether the phenotypic differences between these populations are caused by phenotypic plasticity or genetic differentiation. There are several examples at Chernobyl for grasshoppers (*Chorthippus albomarginatus*) (Bonisoli-Alquati et al., 2017) or plants (Boratyński et al., 2016; Pernis et al., 2020) or on the laboratory for bacteria exposed to ^{60}Co irradiation for hundreds of generations (Harris et al., 2009). Third, the gold standard is "reciprocal transplant". In these experiments, there is not just one rearing place, but organisms from contaminated and non-contaminated are both reared for ideally at least four generations in contaminated and non-contaminated places, to ensure that the differences between populations reflected genetic differentiation (Badyaev and Uller, 2009; Kawecki et al., 2012; Dutilleul et al., 2014). Local adaptation occurs if the selected traits

improve fitness in the contaminated environment. For the Chernobyl region, two studies conducted reciprocal transplant experiments to test for the adaptation to ionizing radiation of organisms exposed to ionizing radiation for several generations: Ruiz-González et al. (2016) identified bacterial communities from intermediate-exposed site in Chernobyl which survived and reproduced better under exposure to ionizing radiation, even than higher contaminated places in Chernobyl. In contrast Arnaise et al. (2020) showed no evidence of adaptive response to selection to ionizing radiation for anther-smut fungus (*Microbotryum lychnidis-dioicae*). Laboratory studies on nematodes (*Caenorhabditis elegans*) have also characterized selection processes under exposure to radionuclides with reciprocal transplant experiments (Dutilleul et al., 2017; Quevarec et al., 2022).

Background ionizing radiation is consistent over time at an ecological time scale, even in the highest naturally radioactive places like Ramsar (Iran) (Ghiassi-nejad et al., 2002) or Kerala (India) (Derin et al., 2012). On the contrary, after anthropogenic radioactive contamination, the exposure of organisms to ionizing radiation may be acute before a fast decrease and a decreasing plateau at a lower dose rate (see Caplin and Willey, 2018 for an example of the evolution of activity after the Chernobyl accident). These temporal radioactive dynamics may modulate the response of organisms, depending on their past exposure and the exposure of their ancestors, and thus complicate the study of the dose-effect relationship. The difficulty in interpreting the observed changes could be amplified by another aspect of radiation, ionizing radiation can accelerate the rate of adaptation (see the hypothesis made by Grodzinsky, 2016). For example, are trait differences between organisms exposed to current low dose rates and "control populations" caused by radiation they are currently experiencing or the consequence of past modifications, transmitted since the first high doses? At Chernobyl for example, some authors argued that current doses are too low to induce observed damages (Smith et al., 2012), and oxidative stress (Baker and Chesser, 2000; Deryabina et al., 2015).

To study the temporality of modifications, some studies compare doses and effects at different times, to determine if the effects are caused by current or historical/ancient doses. For example Omar-Nazir et al. (2018) studied the link between mutation rate estimated on birds more than 20 years after the Chernobyl accident to a reconstruction of dose absorbed by birds the first six months after the accident. Authors suggested that the acute dose initially received by the birds still had effects on mutation rate several generations later. The first doses after the Chernobyl accident were also estimated on *Drosophila* and bank voles and associated with the effects observed during the first ten years. These studies suggested transgenerational effects related to the first doses received following the Chernobyl accident (Hancock et al., 2019, 2020). At the Fukushima prefecture, the same kind of approach was undertaken on pale blue

grass butterfly (Hancock et al., 2019). The authors indicated the existence of genetic instability induced by radiation, but they do not question the evolutionary component of changes.

3.2. Implications of the long-term and the historical evolutionary dimension

From the origin of life, background radiation exposure significantly decreased (Caplin and Willey, 2018). For example, radiation dose rate from internal Potassium 40 has decreased by a factor of eight since 4 Ga ago (Karam and Leslie, 1999). Thus, Todd (1994) has proposed that selection for radiation resistance occurred early in the evolution of life, and played a role in the establishment of DNA repair mechanisms. According to this hypothesis, Wassmann et al. (2010) showed the possibility to select resistance to radiation in conditions of UV exposure mimicking the Archaean period, when the earth did not have a significant ozone layer. They studied the evolution of *Bacillus subtilis* during 700 generations and showed that the populations developed resistance to UV and ionizing radiation by adaptive mechanisms. This resistance also seemed to extend to hydrogen peroxide, increased salinity and desiccation (Wassmann et al., 2010). Similarly, the extreme resistance and radiotropism of certain species of melanized fungi (Dadachova and Casadevall, 2008; Robertson et al., 2012) would go back to the beginning of the Cretaceous. Indeed, a large quantity of highly melanized fungal spores may have been found in deposits from the early Cretaceous, while this period is characterized by the extinction of many animal and plant species. The authors explain that this period coincides with Earth's crossing the 'magnetic zero', resulting in the loss of its 'shield' against cosmic radiation (Dadachova and Casadevall, 2008). Because the intensity of exposure to cosmic radiation has largely decreased since then, this raises the question if this stressor is still sufficient to maintain a selective pressure on resistance mechanisms, and especially repair systems.

Other theories have proposed explanations for the emergence of resistance mechanisms to ionizing radiation. Resistance to radiation may arise from an exaptation (Gould and Vrba, 1982). Exaptation must be understood as an adaptive trait for a function, for which they had not been initially selected. Theoritically, this process may provide resistance to ionizing radiation without the resistance trait being specifically selected for that stressor. For Shuryak, (2020) resistance to ionizing radiation may result from selection for resistance to stressors, such as oxidative stress, desiccation, or salinity. For example, non-extremophile species can tolerate radioactive contamination (Shuryak, 2020). Resistance of rotifers to ionizing radiation seems to come from an adaptation to desiccation (Gladyshev and Meselson, 2008). Radiation-resistant biofilm communities at Chernobyl are adaptated to UV and desiccation (Ragon et al., 2011). Resistance to ionizing radiation in the amoeba, *D. discoideum*, may come from its resistance to

genotoxic effects from the organisms it feeds on (Todd, 1994). More broadly, every preexisting trait can influence sensitivity to ionizing radiation. For example, in plant taxa, reproduction strategy, root system, or life-span modulate the exposure to radionuclides and determine the fitness in contaminated areas (Shuryak, 2020).

Resistance traits to ionizing radiation seem to have appeared in different forms and at multiple times, and is therefore not specific to some phylogenetic groups (Shuryak, 2020). But, the lack of radioecological knowledge of the majority of living species (Sazykina, 2018) dramatically limits the characterization of these traits through the tree of life. However, it is known that some taxonomic groups are resistant to ionizing radiation, indicating a probable appearance of this trait upstream of the group. In a context of acute radiation, Harrison and Anderson (1996) underlines the existence of a great disparity of radiosensitivity between taxonomic groups. For example, Gymnosperms are radiosensitive species, whereas fungi, bryophytes or pteridophytes seem more resistant to ionizing radiation (Real et al., 2004). The genus *Betula* is known to have greater tolerance to ionizing radiation than gymnosperms (Shuryak, 2020). Within metazoans, homeothermic species, and notably mammals, are more sensitive to this stress than invertebrates such as rotifers, Porifera and Hydrozoa (Harrison and Anderson, 1996; Gladyshev and Meselson, 2008; Sazykina, 2018). Among microorganisms, the paraphyletic group of bacteria and protozoa, can show very high resistance to ionizing radiation (Harrison and Anderson, 1996). Rely on phylogeny to study species sensitivity to ionizing radiation should thus be better developed to improve predictions (Box 3).

4. From exclusion zones to experimental evolution

Following major nuclear accidents, humans have establish exclusion zones to reduce their activities in the most contaminated places (Alexis-Martin and Davies, 2017; Hamada et al., 2012; Smith and Beresford, 2005). The effect of ionizing radiation on wildlife in these places is subjected to active debate (Beaugelin-Seiller and Garnier-Laplace, 2020; Beaugelin-Seiller et al., 2020a; Smith, 2020), with important societal consequences (Fassert and Hasegawa, 2019; Oughton, 2011; Stawkowski, 2016) and high visibility in the media (Beresford et al., 2020). This debate poses the question of the status of wildlife in contaminated areas and their capability to persist and adapt under ionizing radiation. In practice, this debate concerns for example the dose threshold at which no effect on organisms is expected or the difference between effects in the field and under laboratory conditions. In this section, we would like to introduce the idea that this current debate is mostly based on snapshots of the situation, neglecting eco-evolutionary dynamics. As human activities modify the environment and have evolutionary consequences on populations, radioecology may benefit by better considering them in exclusion zones. Taking an evolutionary perspective, a better dialogue may also take place between researchers who conduct field or laboratory experiments.

4.1. Debate on wildlife status in exclusion zones

Human exclusion from the most contaminated places is frequent after major nuclear accidents, for sanitary reasons (Bondarkov et al., 2011). After the Kyshtym disaster in 1957, the Eastern Ural State Radioactive Reserve was created restricting the acces by humans (Barescut et al., 2009; Pozolotina V N and V, 1991). The Chernobyl Exclusion Zone was set up in 1986 in Polesia, around the powerplant. A "difficult to return zone" has been defined in the most contaminated places around the Fukushima Daiichi power plant (Harada et al., 2014).

Exclusion zones are directly characterized by an increase in ionizing radiation, and indirectly by the drastic decrease in human activities during several years or decades. Human activities cause effects on wildlife which can counteract or exacerbate the effects of ionizing radiation, and it is thus necessary to consider the interaction between these two stressors. For example, human activities were included in a study of the abundance of several vertebrate species in the Fukushima prefecture (Lyons et al., 2020). We can describe exclusion zones as a biogeographical island (MacArthur and Wilson, 1963; Wilson and MacArthur, 1967), in discontinuity with surrounding areas and with two specific characteristics: nuclear pollution and wilderness. These two characteristics have each a specific dynamic. Both are expected to disappear in the future (radioactive decay and human resettlement) but in the first years the radioactive decay is far faster than the increase in human activities. By studying the relative

level of these two characteristics, their temporal dynamics, and the modifications they induce on organisms, it could be possible to characterize the specific effect of ionizing radiation, considering in the same time the consequences of human activities (Figure 5). We may get inspired from the study of the interaction between two other kinds of abiotic (urban) island (heat and pollution, see Li et al., 2018; Ulpiani, 2021).

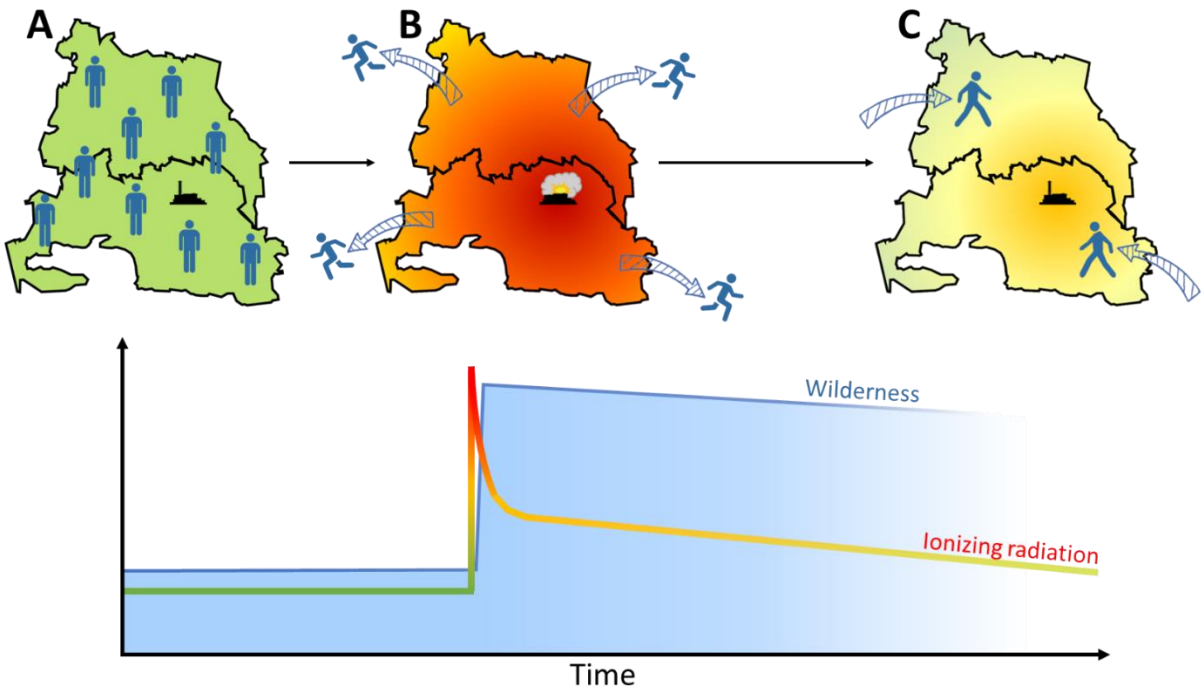


Figure 5: Temporal change in nuclear pollution (Ionizing radiation emitted by radionuclides remaining in the environment), and human activity (wilderness generated by the exclusion zone) in a theoretical area (based on the CEZ) following a nuclear accident. (A) before the accident, human activity is high (wilderness low) and there is no significant ionizing radiation level. (B) The nuclear accident occurs, quickly causing a gradient in ionizing radiation culminating near the nuclear power plant (orange to red area). This event induces a rapid and massive evacuation of the area by humans and the implementation of legislation to drastically restrict access to this area, causing a quick increase in wilderness. (C) After several months or years depending on the contamination, the radioactive decay causes a fast decrease in ionizing radiation up to an almost constant level (i.e., exponential decay), but still relatively important (orange to yellow area) and human activities are slowly recovering in the area (slow decrease of wilderness).

These two characteristics are differently perceived by organisms: ionizing radiation is imperceptible for the overwhelming majority of organisms (with very few known exceptions, such as mushrooms which move towards ionizing radiation sources; Steinhauser, 2015; Dadachova et al., 2007; Zhdanova et al., 2004), contrary to the decrease in human activities. Land cover changes were monitored in the Chernobyl region (Gemitzi, 2020) and the Fukushima prefecture (Sekizawa et al., 2015). These environmental modifications attract some species like mammals (Lyons et al., 2020; Webster et al., 2016). As discussed before (part 2.2), radioactive environment may induce a source-sink dynamic, which could be reinforced by low human activities. Thus, exclusion zone can be perceived as attractive for some species (cues), although being of poor-quality (outcomes), and thus represent an "ecological trap" (Battin, 2004). However, if the benefits of the human departure are higher than the ionizing radiation costs, stay in exclusion zone is beneficial for organisms and thus this area can become 'source' habitat. In order to display this potential trap in exclusion zones, it is necessary to make longitudinal studies of populations (i.e., evolution) by considering fitness measures, genetic diversity, landscape genetics, etc. (Robertson and Blumstein, 2019). Beyond the specific case of source-sink dynamic, the characteristics of exclusion zones may influence otherwise population dynamic. For example, as it is already well described in urban ecology, the modification of interaction with humans may influence more broadly gene flow between wildlife populations (for example Miles et al., 2019; Zuñiga-Palacios et al., 2021). This is the case, even if this modification is a decrease in human activities as observed in exclusion zones. For example, Matsushima et al. (2021) showed in the Fukushima prefecture a reduced suitable habitat for a frog species dependant on rice paddies since human departure and agricultural decrease.

In addition to the focus on wildlife in exclusion zones, study surrounding populations could help us to clarify the potential effects of ionizing radiation. Indeed, populations in exclusion zones share a common evolutionary history with surrounding populations which is at the origin of inter-individual variations at different levels (e.g., phenotypic, genotypic, demographic, etc.). Clarify these 'natural geographic variations' can help to decipher the changes caused by ionizing radiation. This biogeographical perspective has been conducted on bank voles in Ukraine compared to CEZ populations (Meeks et al., 2009) and tree frogs in Europe compared to CEZ populations (Car et al., 2021). Currently, studies on the long-term effects of ionizing radiation in exclusion zones rarely include the evolutionary history of populations. Studies mentioned describing the debate only focus on a snapshot of abundance or interspecific diversity (e.g. Deryabina et al., 2015; Møller and Mousseau, 2013), but they do not provide any

information on their long term dynamic. Thus, they only give partial information on organisms, notably on individual health status, survival, reproduction and migration.

4.2. Field vs laboratory debate: an evolutionary contribution?

The part of the debate on wildlife in exclusion zones which rely on the differences of effects of ionizing radiation between field and laboratory studies can be clarified in the light of evolution. Meta-analysis highlighted that organisms are more sensitive to ionizing radiation in the field than in controlled laboratory experiments (Garnier-Laplace et al., 2013; Beaugelin-Seiller et al., 2020). While Smith (2020) identified methodological issues, such as the non consideration of studies showing no effect and a flawed analysis of metadata, however, these differences of sensibility remain plausible hypothesis even if not yet properly tested (Beresford et al., 2016). Garnier-Laplace et al. (2013) suggested that these differences can be explained by ecological mechanisms or different exposure to ionizing radiation. Studies on other stressors have also identified differences in organism sensitivity between laboratory and field studies, due to the organism variability, their ecology and the tools used for their study (tests and data analysis) (Chapman, 2006; Schmitt-Jansen et al., 2008). Here, we put forward an evolutionary approach to contribute to clarifying the causes of these differences of sensibility between field and laboratory studies.

In the field, populations exposed to a given dose rate have generally been exposed during several generations to this stressor. This chronic exposure impacted the evolutionary trajectory of populations, which may have modified the response to ionizing radiation compared with ancestral population ‘naive’ to ionizing radiation. An effect displayed at a given dose rate and time can thus be the result of several generations irradiated. In laboratory conditions, organisms are usually not exposed to irradiation until experiments. Thus, at the same dose rate than in the field, responses are expected to be different due to an evolutionary trajectory not impacted by ionizing radiation. However, it is possible to simulate the exposure of several generation through experimental evolution (Lampe et al., 2017).

In addition to the difference in past exposure between laboratory and field populations, the organisms used in the experiments will largely influence their sensitivity to ionizing radiation, especially considering their past evolutionary history. For example, the evolvability (i.e., the capability to adapt, by providing variation, shaping the effect of variation, and shaping the process of selection (Riederer et al., 2022)) of a diverse population will potentially be much better than a population with little diversity (Watson, 2021). The choice of a laboratory model and how to breed this model could also drastically modify the evolutionary response to a stressor (Cutter et al., 2019). For example, the *Caenorhabditis elegans* N2 strain reference,

probably the most widely used strain of *C. elegans* (Zhao et al., 2018), is rather unrepresentative of *C. elegans* as a whole. For example, males of the N2 strain are particularly inefficient for reproduction, influencing largely sex-ratio of the population (Anderson et al., 2010). Yet, by using another strain with higher male frequency and genetic diversity, Quevarec et al. (2022) showed that ionizing radiation alter the reproductive strategy of a population, by outcrossing selection in a few generations. This result would probably have been more difficult to observe with the N2 strain and without multigenerational experiments.

Although evolution may explain a part of the apparent differences of organism sensitivity between laboratory and field conditions, these two ways of experiment are not supposed to give the same type of information, and this initial debate could be artificial and based on a mutual incomprehension of both approaches. Field studies show the real consequences of exposure to ionizing radiation, and hypothesis have to be done for example on past evolutionary processes. The processes inferred can then help to design laboratory studies, in which mechanisms can be demonstrated. Experimental evolution can thus fullfill the role of dialogue between field and laboratory experiments on the question of evolution. Some of laboratory studies have already investigated the effect of ionizing radiation on evolutionary mechanisms in bacteria (Harris et al., 2009; Lampe et al., 2019) or *Caenorhabditis elegans* (Quevarec et al., 2022).

5. Conclusion

Human activity has largely contributed to the increase of radionuclide emissions in the environment, leading in several places to a chronic exposure (e.g., up to several decades) of organisms never experienced since several million years. It appeared essential to consider the long-term impact of ionizing radiation on these organisms, to preserve the integrity of ecosystems. In this review, we showed that an evolutionary context may help to give an integrative view on the biological responses to ionizing radiation. This “top-down” approach opens notably the gate to including spatial and temporal dimensions. Considering the temporal dimension, the evolutionary history helps to better understand the sensitivity or robustness of different organisms to this environmental stress. Additionally, the study of the current evolution of a population contributes to estimate the impact of environmental stressors on the following generations and their fate. Study of evolution is also interesting to be better anchored in the spatial dimension, by helping to consider other environmental characteristics (e.g., interaction with other pollutants, migration of populations, or more broadly the whole set of anthropic environmental changes).

In this review, we emphasize that ionizing radiation is intimately linked to evolutionary change, whether from a biological/process (i.e., the induction of inheritable biological changes and by its potential impact on fitness) or historical/pattern point of view (i.e., the current situation of exclusion zones or the history of irradiation on earth). Radioecology is therefore an invaluable study case for applying the concepts and methods of evolutionary biology in the study of a pollutant (as it has been and is done marginally). But the interest of an evolutionary perspective is much broader than radioecology, and the points highlighted in this review are relevant for research on pollutants and are already used depending on the field. Finally, this approach is relevant to identify endpoints measurement for ecological risk assessment (Straub et al., 2020).

6. Acknowledgements

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Box 1: How central is evolution in radioecology field studies?

To analyze the consideration and treatment of evolution in radioecology, we conducted a systematic Web of Science survey in the subset of field radioecology studies. Details about the query and the 98 analyzed studies is available in the Supplementary Information section. To represent these studies and their treatment of evolution, a Principal Component Analysis was performed on nine evolution-related characteristics extracted from these studies (Figure 6).

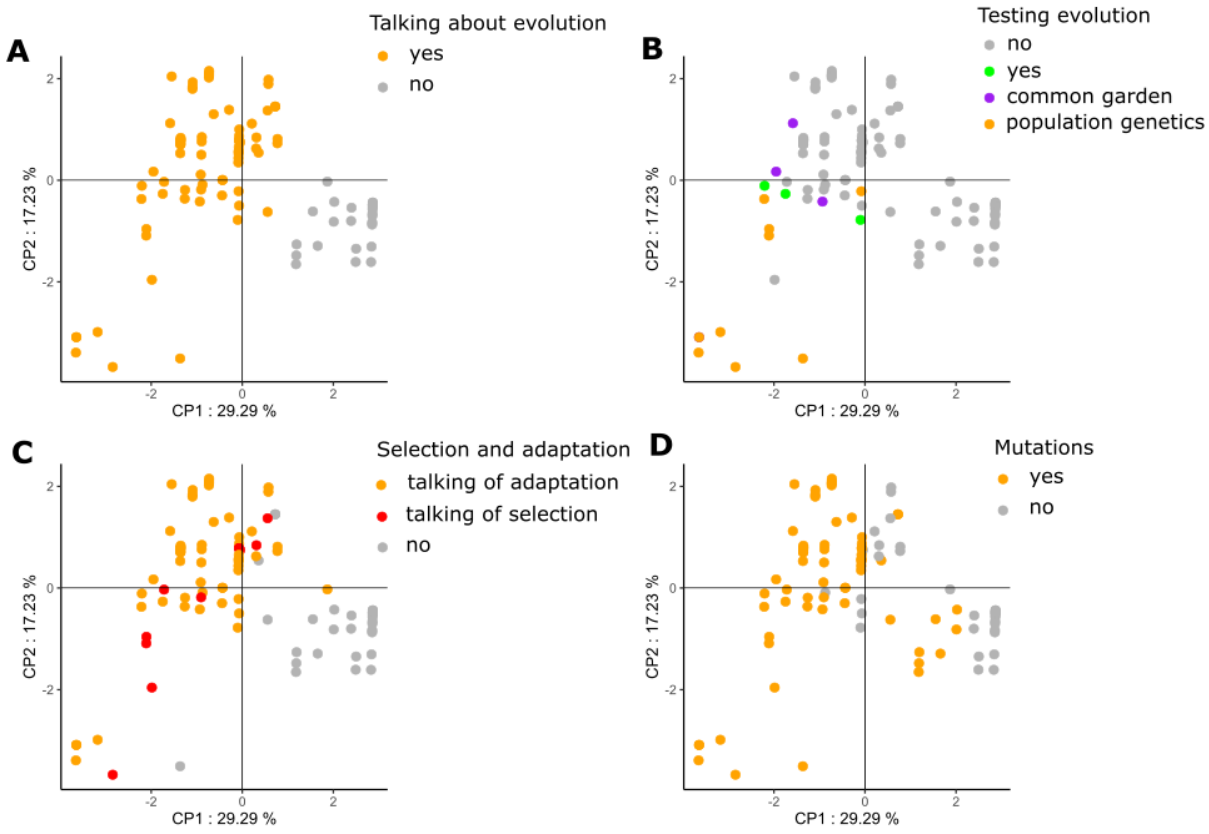

Figure 6: Representation of a subsample of radioecology field studies from a PCA conducted on nine evolution-related characteristics. Four of these characteristics are represented because of their particularly interesting distribution: (A) evolution talk, (B) evolution study, (C) adaptation talk and (D) mutation talk.

For all these studies, a vast majority talk about evolution (71), indicating the current and past interest on these topics. A very low number of papers (8) study evolution compared to papers only talking about evolution (about 11%, Figure 6A, B). As observed in a large number of papers, evolution is often only mentioned as context or hypothesis for perspective, but evolution is still poorly studied for itself, thus hypothesis (often adaptation, see Møller and Mousseau, 2016) remain to be tested. Many studies talk about adaptation and/or selection (67) and mutations (65), two major evolutionary processes. But contrary to the use of adaptation and/or selection which is linked to the fact of talking about evolution (only five differences, chisq:

p=8e-16, Figure 6C), there are more studies talking about mutations which do not talk about evolution (7) and studies talking about evolution which do not talk about mutations (13, total chisq: p=2e-6, Figure 6D), as for migrations not linked to talking about evolution (chisq: p=0.6). Thus, mutations and migrations are sometimes taken as an individual effect, impacting only the individual exposed, and not as an evolutionary process, creating genetic diversity for example. Few other studies deal with evolution, or studies testing evolution over years (Wilcox: p=0.93), but more studies talking about non-genetic transgenerational modifications (Wilcox: p=0.016, Figure 7B), also linked to the fact of talking about evolution (Wilcox: p=0.007, Figure 7A). This can indicate a shift in the view of evolution applied to ionizing radiation, closer to the ideas of an inclusive evolutionary synthesis (Danchin et al., 2019; Laland et al., 2015).

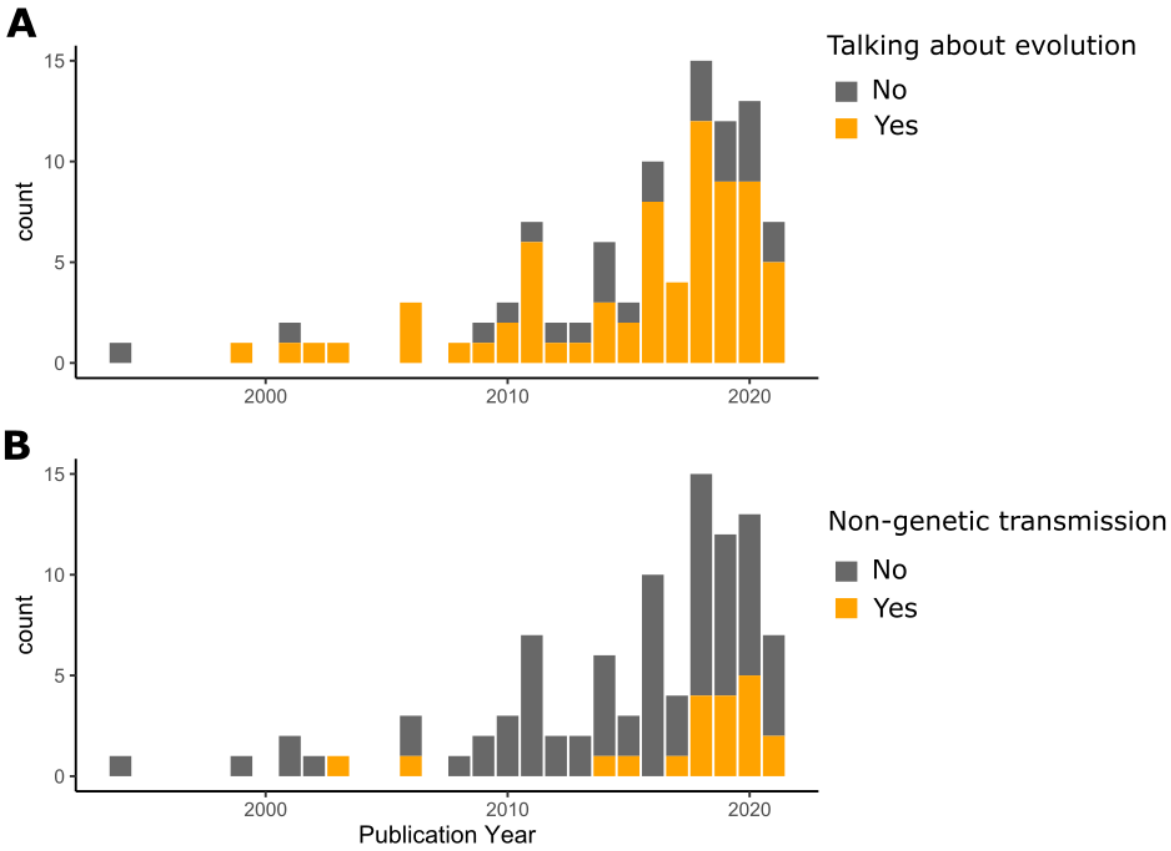

Figure 7: Temporal changes of the topics covered by a subsample of radioecology field studies. A. the fact of talking about evolution. B. the fact of talking about a transmission over generations not only from genetic material.

Box 2: Semantical point

From ‘radio-adaptation’ to ‘adaptation’

In radioecology, the word ‘radio-adaptation’ is defined as an individual response after a first exposure to ionizing radiation (Rodgers and Holmes, 2008; Rodgers et al., 2001; Wickliffe et al., 2003). For example, Rodger and Holmes (2008) highlighted a reduction of micronuclei - a biomarker of DNA double strand breaks - for mice exposed to an acute dose of 1.5 Gy when mice were exposed first to 10 cGy priming doses. Thus, in radioecology, ‘adaptation’ word is far from its classical meaning in evolutionary biology. That is why, even if these studies use ‘adaptation’ word, they focused on a plastic, individual response and did not studied ‘adaptation’ as an evolutionary process.

Hormesis, the incomplete intrepretation

In (eco)toxicology, "hormetic" effect of a pollutant, like ionizing radiation is defined as a biphasic dose-response relationship, usually considered ‘positive’ for the organism at low doses and harmful at high doses (Mattson, 2008). This concept appears difficult to involve in an evolutionary point of view. Indeed, in evolutionary biology, it is difficult to describe an effect as ‘positive’ *per se*, because a response is part of a trade-off. Because resources are limited, a positive effect on a trait may be costly and may be antagonist of another trait (Reznick et al., 2000). In the case of ionizing radiation, evolutionary trade-offs (i.e., the trade-off on how some traits are selected at the expense of other traits) were tested in Chernobyl on life-history traits in wild carrots (*Daucus carota*) (Boratyński et al., 2016), between sperm length and integrity on barn swallows (Hermosell et al., 2013) or between developmental rate and resistance to DNA damage in grasshoppers (*Chorthippus albomarginatus*) (Bonisoli-Alquati et al., 2017).

Exclusion zones as “natural laboratories”

Human exclusion zones are frequently compared to "natural laboratories", and are considered as privileged places to study the long-term effects of ionizing radiation on wildlife (see for example Goodman et al., 2019; Jernfors et al., 2021; Mousseau and Møller, 2020; Orizaola, 2020). This term appears confusing because it refers to the idea of a controlled experiment in natural environment. This type of controlled experiment is what Diamond (1983) defined by the term ‘field experiment’ in which only the parameter of interest (here “ionizing radiation”) is modified and other avariables controlled. But all studies in exclusion zones are closer to ‘natural experiment’ as they do not control the ionizing radiation dose, but only compare the effect of different doses by the site selection. In our opinion, the use of “natural

laboratories" and "field experiments" is more consistent with the study of Amiro and Sheppard (1994) or that of Woodwell (1962) in which an irradiator has been set in natural area to observe the potential ionizing radiation effects.

Box 3: Using evolution to assess ecological radiological risk

Evolutionary tools and concepts are not solely interesting for scientific purposes in radioecology, but also to enrich the ecological risk assessment, which is the practice seeking describing, predicting, and controlling risks to ecosystems from human activities (Suter II, 2016). The implementation of evolution in ecological risk assessment is still scarce in the case of pollutants like pesticides (Straub et al., 2020; Topping et al., 2020), metals (Hoeks et al., 2020), and more broadly chemicals (Rodríguez-Romero et al., 2021; Saaristo et al., 2018). In the case of ionizing radiation, evolution is also poorly considered, and from the topics described in this review we identified four points on which evolution can inform ecological radiological risk assessment:

(i) Considering the evolutionary pattern: phylogenetically-based species sensitivity

Differences in species sensitivity to ionizing radiation is a central element to predict risks associated with ionizing radiation. As detailed in part 3.2, the specific differences in ionizing radiation sensitivity can be studied through the lens of evolutionary history, and this may help to improve the integration of specific sensitivity in risk assessment. Current kinship relationships (phylogeny) may reflect the sensitivity of organisms to different environmental stressors, and phylogeny can be used to predict sensitivity and risks associated. The idea behind this "phylogenetically-based species sensitivity", is that more species are related, more chance they have homologous characters states, from which depend on their sensitivity to ionizing radiation. To our knowledge, this approach has never been conducted for ionizing radiation – there is still a lot of incertainties on species sensitivity to ionizing radiation (Bréchignac et al., 2016) – but some models tried to predict sensitivity of species to chemical pollutants (see for example Guénard et al., 2011; Malaj et al., 2016) or tested the influence of relationship among species on several pollutants (Brady et al., 2017; Mikryukov et al., 2020; Raimondo et al., 2010), and show that whithout testing the sensitivity of each species, phylogeny may help its prediction.

(ii) Considering the evolutionary processes

a. Exposure: the complexity to estimate representative dose at the population level

International organizations in ecological radiological risk assessement advocate assessing risks at the ecosystem and population levels (IAEA, 2014; ICRP, 2007). But the doses on which risks are assessed are mostly individual dose rates at a certain time point (see for example the ERICA tool which is mostly used to estimate an individual dose rate (Brown et al., 2008)), and

are not reflecting the multiplicity of exposures at ecosystem and population levels. Some authors argued the necessity to integrate “historical doses”, from which ancestors of current individuals were exposed (notably in the first times after nuclear accidents) when dose rates were more elevated (Mothersill et al., 2018) (see part 2.1). In this debate including evolutionary processes bring us to consider doses in the first times after accidents, doses cumulated through the life of individuals (as discussed in Stark et al., 2017) and the accumulation of doses since the beginning of the exposure, over generations. Indeed, changes in exposed populations can accumulate over generations. For example, if mutations are induced by ionizing radiation, these mutations can accumulate over generations and lead to profound changes which cannot be only associated with late doses (see this point discussed in Car et al., 2021).

b. Effects

i. Evolution in action: the fitness endpoint

By investigating the evolutionary processes occurring in populations exposed to ionizing radiation, it is possible to assess the ability of current and future populations to persist in the environment, and assess their evolutionary potential. In particular, the concept of fitness, still little used in radioecology, could be interesting to avoid measures centred on mortality, and neglecting sublethal effects (Straub et al., 2020). The definition of fitness would also benefit from clarification. Indeed, in studies interested in the effects of ionizing radiation, fitness is sometimes used to qualify mortality (Nohara et al., 2014), reproduction (as in Beresford et al., 2020 about Fuller et al., 2018; Lerebours et al., 2018), or individual health status (notably in medicine: Piscione et al., 2017; Putora et al., 2019, and agronomy: Cai et al., 2018; Yang et al., 2017). To better determine the consequences at population level, because of a possible trade-off between mortality and reproduction, it is essential to define fitness as a product of survival and reproduction measures. In evolutionary biology, the concept of fitness led to major debates in its precise definition (in Dawkins, 1982, chapter "An agony in five fits"; in Grene, 1986, chapter "Adaptation") but this practical definition (a combination of survival and reproduction) can be implemented in ecological radiological risk assessment. In the 80s, (Auerbach, 1984) already preconized the use of fitness to study the effect of ionizing radiation on populations. Its use is still scarce, but some studies showed the interest of this estimate, for example on nematodes *Caenorabditis elegans* (Dutilleul et al., 2017) showed with fitness (computed as the product of fertility by survival rate or fertility multiplied by survival frequency) the existence of costs of adaptation to ionizing radiation, which may affect the persistence of populations. Goodman et al. (2019) calculated also fitness, as the rate of population increase, including

measures of the proportion of surviving females and the number of offspring produced, and showed no relation of fitness with exposure to ionizing radiation.

ii. What are the past and current evolutionary processes? Intra and interspecific variation endpoint

Another way to monitor past and current evolutionary processes, and predict evolutionary potential, is to be attentive to intraspecific variations (Des Roches et al., 2021). Several tools like measures of genetic diversity can report how different populations from the same species evolve depending on environmental changes. These approaches are increasingly studied, for example in the Chernobyl exclusion zone (Baker et al., 2017; Car et al., 2021; Fuller et al., 2019), and may be integrated into risk assessment in the future. The study of interspecific diversity indices like phylogenetic diversity, defined as "the sum of the branch lengths of the minimum spanning path joining a set of taxa on a tree" (Véron et al., 2019), seems also particularly interesting for estimating *a posteriori* effects of contamination on communities.

Chapitre 2 - Ionizing radiation affects the demography and the evolution of *Caenorhabditis elegans* populations

Loïc Quevarec, Denis Réale, Elizabeth Dufourcq-Sekatcheff, Olivier Armant, Christelle Adam-Guillermin, Jean-Marc Bonzom

Key words - Experimental evolution, population growth rate, stressful environment, life history traits, local adaptation, environmental safety

Abstract

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

1. Introduction

Since the end of World War II, some military or civil activities have increased the release of radionuclides in various ecosystems (Rhodes et al., 2020). For instance, the accidents at the Chernobyl (Ukraine) or Fukushima (Japan) nuclear power plants have released respectively 1.4×10^{19} and 5.2×10^{17} Bq of radionuclides into the environment (IAEA, 2006; Okano et al., 2016). Ionizing radiation can affect survival, reproduction and development of plants, mammals, fish, or invertebrates (Dallas et al., 2012; Real et al., 2004). Furthermore, its deleterious effects can last over several generations (i.e., *Eisenia fetida*: Hertel-Aas et al., 2007; *Daphnia magna*: Sarapultseva and Dubrova, 2016; *Oncorhynchus mykiss*: Smith et al., 2016; *Caenorhabditis elegans*: Buisset-Goussen et al., 2014; Guédon et al., 2021). However, studies testing multigenerational effects of exposure to ionizing radiation on population growth are lacking.

Regulatory ecological risk assessment of pollutants (chemical, ionizing radiation, etc.) have important limitations. It is largely based on single-species toxicity tests performed in the laboratory under standardized conditions, and mostly over a short period of time, over one or two generations (Kapustka, 2008). As a result, such tests may strongly underestimate the level of risk for natural populations. For example, the effects of pollutants may persist across several generations, by inducing genetic change or non-genetic inheritance (Danchin et al., 2011). They, thus, can have evolutionary consequences on populations, which can change our diagnostics of ecological risk assessment (Coutellec and Barata, 2011; Straub et al., 2020). In the presence of a pollutant, life history traits can show either phenotypical plastic, genetic or epigenetic changes in response to these new environmental conditions (Hoffmann and Willi, 2008). If adaptive, this change in life history traits can in turn improve the fitness of the population and lead to higher population growth rate and resilience (De Leániz et al., 2007; Dutilleul et al., 2017).

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

evolutionary dynamics of a population affected by pollution, and therefore improve the assessment of ecological risks caused by pollutants.

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

Here, we studied the population growth rate and associated change in life history traits of a *C. elegans* population that we chronically exposed to ionizing radiation (gamma irradiation) for 60 days, or around 20 generations, under experimental conditions. The aim of this study was (1) to characterize *C. elegans* population change exposed to gamma radiation, and (2) to investigate the underlying mechanisms, by determining whether the observed variations are related to phenotypic plasticity or adaptive processes. We used the nematode *C. elegans* (Nematoda, Rhabditidae) an androdiecious organism because of its short life cycle (reaches sexual maturity within three days at 20° C), its short lifespan (21 days at 20° C), and its high fecundity (300 offspring in selfed hermaphrodites, up to 1000 when crossed with males). *C. elegans* is a powerful model for evolutionary experiments (Braendle *et al.*, 2007; Goussen *et al.*, 2013; Gray & Cutter, 2014; Dutilleul *et al.*, 2017). It was previously found that ionizing radiation reduces reproduction and somatic growth in *C. elegans* (Buisset-Goussen et al., 2014; Dufourcq-Sekatcheff et al., 2021; Guédon et al., 2021; Lecomte-Pradines et al., 2017; Maremonti et al., 2019), we hypothesized that ionizing radiation reduces population growth rate and slows life history.

2. Material and methods

2.1. Test organism and population maintenance

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

2.2. Irradiation conditions

The external gamma radiation exposure was conducted at the Mini Irradiator for Radio Ecology ^{137}Cs irradiation facilities, at the *"Institut de Radioprotection et de Sûreté Nucléaire"* (MIRE, IRSN, Cadarache, France). We used the same irradiation facilities, and the same protocol as previously described by Buisset-Goussen *et al.* (2014). The populations of *C. elegans* were exposed to gamma radiation at 20 °C and 80% relative humidity. We measured dose rates with radiophoto luminescent (RPL) micro-dosimeters twice during the experiment. As explained in Quevarec *et al.* (2022), the Petri dishes were placed vertically in the irradiator to homogenize the dose received over the entire dish. Placing the plates at different distances from the source and separated by shields (Petri dish filled with lead filings) allowed us to obtain the required dose rates.

2.3. Multigenerational experiment

We used three dose rate gamma radiation treatments: control (0.0 $mGy.h^{-1}$), low irradiation (1.4 $mGy.h^{-1}$), and high irradiation (50.0 $mGy.h^{-1}$). Low irradiation treatment had an environmental reality. In the Chernobyl Exclusion Zone, terrestrial wildlife could be exposed

to dose rates up to ~10 mGy.h^{-1} (Garnier-Laplace et al., 2013). High irradiation treatment was selected because studies have shown an impact on *C. elegans* reproduction at a similar dose rate (Buisset-Goussen et al., 2014; Dubois et al., 2018; Dufourcq-Sekatcheff et al., 2021; Guédon et al., 2021; Maremonti et al., 2019). For each treatment, we created five independent replicates taken from the stock population and maintained for over 60 days, with a transfer to new Petri dishes once every three days. Although three days correspond to about one generation in natural conditions, gamma irradiation has been shown to delay growth and reproduction at high dose rates (Lecomte-Pradines et al., 2017), and we cannot guarantee that every three-day transfer corresponds to a generation. However, we assume that 60 days would corresponds to about 20 generations. We thus describe the dynamics of changes during the experiment as a function of the number of days, based on the number of three-day transfers.

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

$$R = \frac{lnN_t - lnN_0}{t}$$

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

was transferred to a new well in the plate. After 2 days (144 h after egg collection), we counted late brood size. We calculated the total brood size as the sum of early and late brood size.

2.4. Reciprocal-transplant experiments

We performed a reciprocal-transplant experiments between the control and both the low and the high irradiation treatment to test for the hypothesis of irradiation-induced adaptive changes in the treated populations (Figure 8). We used hatching success and brood size as indexes of fitness at the population level.

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

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

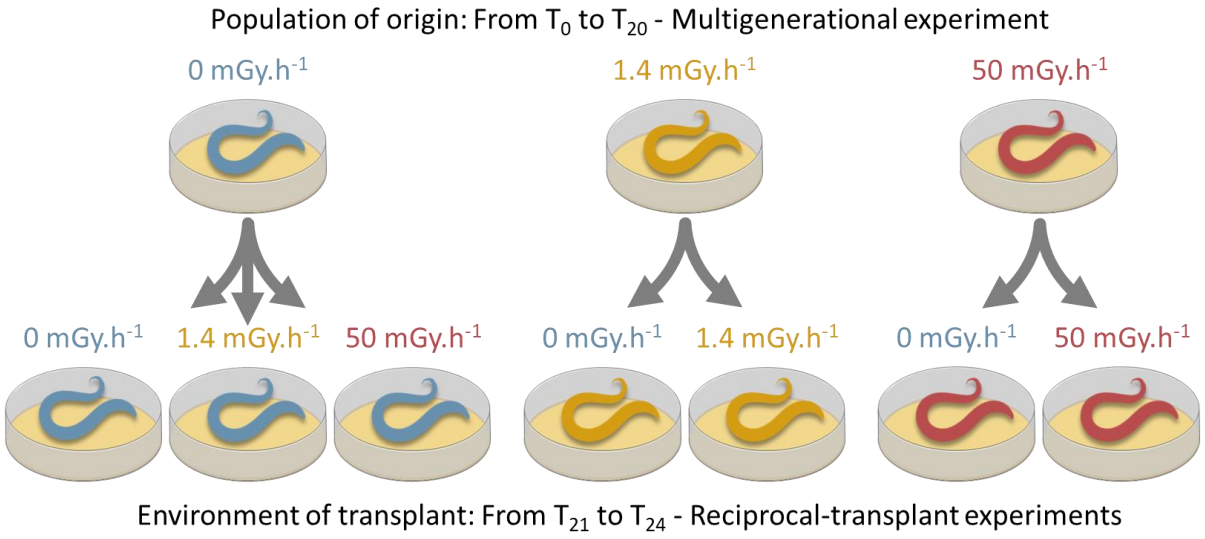

Figure 8: Schematic overview of the reciprocal-transplant experiment design for *C. elegans* populations in three gamma radiation treatments. After 20 transfers in the initial environment

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

2.5. Statistical analysis

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

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

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

Because we used GAMMs and GLMMs with different link functions, we provide the estimated parameters in the text after back transforming the coefficient using the inverse log

function or inverse logit function (untransformed coefficient are shown in the Tables). The log-transformed raw data of realized fecundity are also back transformed in the text.

3. Results

3.1. Multigenerational experiment

Control, low and high irradiation treatments showed population growth rates from larval to adult stage equal to 1.51, 1.53 and 1.48, respectively (Table 1). Control populations showed a population growth rate significantly lower than the low irradiation treatment, and significantly higher than the high irradiation treatment (Table 1a; Figure 9a). In the control populations, population growth rate decreased slightly, but significantly, between transfers 1 and 20. Population growth rate fluctuated significantly without increasing or decreasing in both irradiation treatments, and its frequency increased with the treatment dose (Table 1b; Figure 9a, Figure B.1).

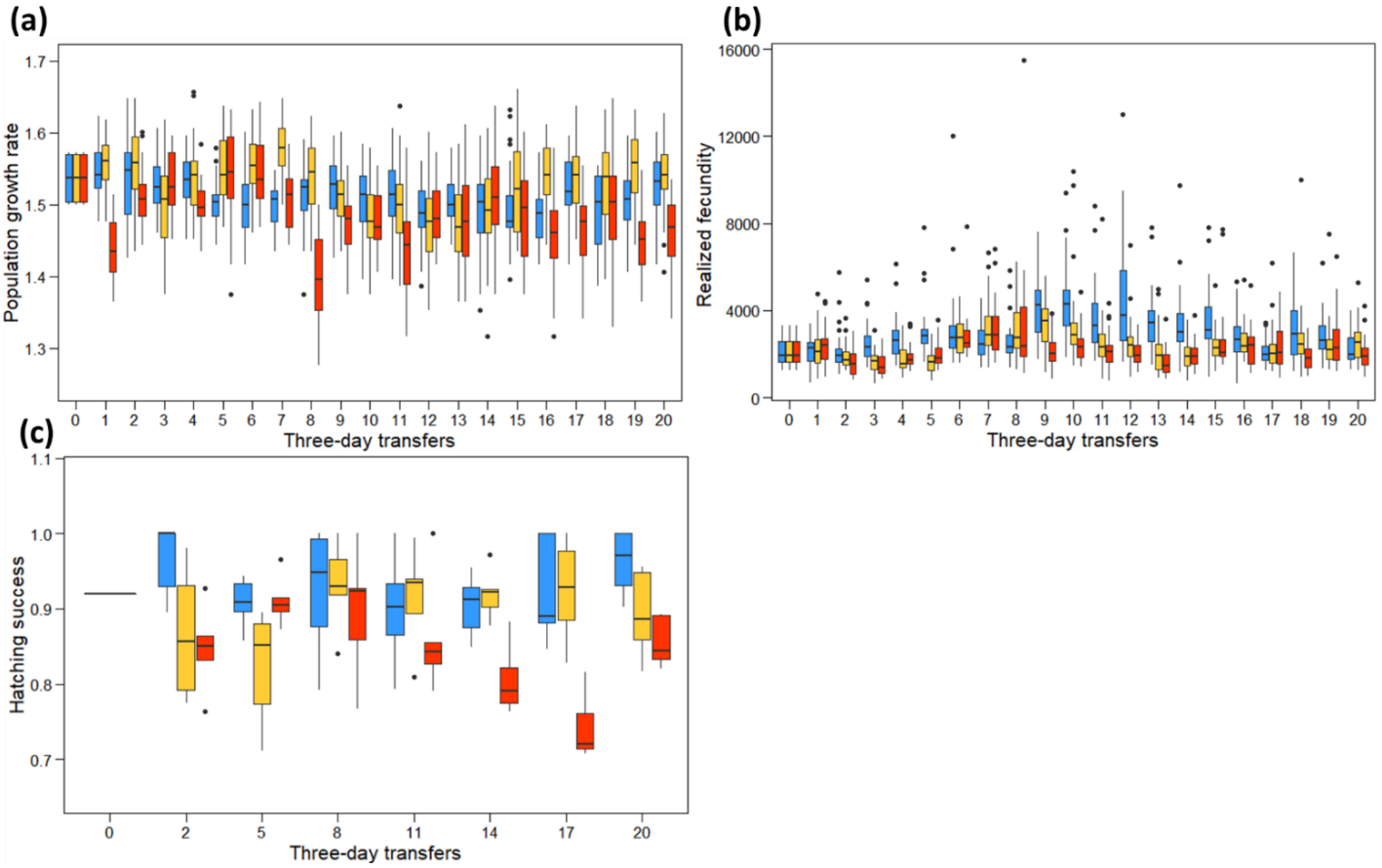

Figure 9: Boxplot of **(a)** population growth rate and **(b)** realized fecundity over time during 20 three-day transfers and **(c)** hatching success over time (i.e., three-day transfers: 0, 2, 5, 8, 11, 14, 17 and 20) for *C. elegans* populations living in different gamma radiation environments. Blue: control; yellow: low radiation (1.4 $mGy.h^{-1}$); red: high radiation (50.0 $mGy.h^{-1}$).

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

a)	Estimate	Std. Error	t	P	
(Intercept)	0.413	0.003	123.822	2.00 e-16	***
Gamma Low	0.010	0.005	2.086	0.037	*
Gamma High	-0.020	0.005	-4.182	3.02 e-05	***

Approximate significance of smooth terms

b)	edf	Ref.df	F	P	
s (Time): Control	1.980	1.980	6.788	0.003	**
s (Time): Gamma Low	6.565	6.565	9.238	2.00 e-16	***
s (Time): Gamma High	11.474	11.474	6.275	2.00 e-16	***

Control, low and high treatment populations showed realized fecundity equal to 2779, 2243 and 2053 eggs per 1000 individuals, respectively. Realized fecundity was significantly higher in the control than in the low and high irradiation treatments (Table 2a; Figure 9b). Realized fecundity in the control populations increased slightly from transfers 1 to 10, and decreased slightly, but significantly from transfers 10 to 20. Realized fecundity fluctuated significantly without increasing or decreasing in both irradiated treatments (Table 2b; Figure 9b, Figure B.2).

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

a)	Estimate	Std. Error	T	P	
(Intercept)	7.935	0.028	287.832	2.00 e-16	***
Gamma Low	-0.214	0.039	-5.477	4.94 e-08	***
Gamma High	-0.304	0.039	-7.784	1.18 e-14	***

Approximate significance of smooth terms

b)	Edf	Ref.df	F	P	
s (Time): Control	6.208	6.208	14.226	2.00 e-16	***
s (Time): Gamma Low	8.184	8.184	11.330	2.00 e-16	***
s (Time): Gamma High	9.210	9.210	6.309	2.00 e-16	***

The hatching success rate was estimated at 0.92, 0.90 and 0.85 for control, low and high irradiation treatments, respectively (Table 3). Control populations showed a significantly higher proportion of hatched eggs than high irradiation populations but did not differ

significantly from low irradiation populations (Table 3a; Figure 9c). Hatching success in high treatment fluctuated across time (Table 3b; Figure 9c, Figure B.3).

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

a)	Estimate	Std. Error	t	P	
(Intercept)	2.433	0.170	14.340	2.00 e-16	***
Gamma Low	-0.194	0.232	-0.837	0.405	*
Gamma High	-0.674	0.226	-2.991	0.004	**

Approximate significance of smooth terms

b)	Edf	Ref.df	F	P	
s (Time): Control	1.852	1.852	1.549	0.167	
s (Time): Gamma Low	1.000	1.000	2.737	0.101	
s (Time): Gamma High	2.783	2.783	4.111	0.0102	*

Mean early brood size was at 252, 253 and 231 eggs per individual for control, low and high irradiation treatments, respectively (Figure 10a). High irradiation treatment showed a significantly smaller proportion of early brood size (Kruskal-Wallis chi-squared = 10.651, df = 2, p = 0.00487) than the control (p = 0.012, Holm adjustment method) and low irradiation treatment (p = 0.012, Holm adjustment method). In contrast, no significant difference was observed for low irradiation treatment compared to control treatment (p = 0.867, Holm adjustment method). The mean late brood size was at 18, 23 and 26 for control, low and high irradiation treatments, respectively in the multigenerational experiment (Figure 10b). No significant difference was observed between treatments (Kruskal-Wallis chi-squared = 0.0442, df = 2, p = 0.978). The mean total brood size was at 268, 276 and 252 for control, low and high irradiation treatments, respectively in the multigenerational experiment (Figure 10c). High irradiation treatment showed a significantly smaller proportion of total brood size (Kruskal-Wallis chi-squared = 7.799, df = 2, p = 0.0203) than the control (p = 0.044, Holm adjustment method) and low irradiation treatment (p = 0.044, Holm adjustment method). In contrast, no significant difference was observed for low irradiation treatment compared to control treatment (p = 0.824, Holm adjustment method). Finally, results showed a negative correlation between early and late brood size in individual level for high irradiation treatment only (Spearman's rank correlation: S = 349, p = 0.0077, ρ = -0.243). No significant correlation was observed for

low irradiation treatment (Spearman's rank correlation: S = 243, p = 0.870, ρ = 0.015) and control treatment (Spearman's rank correlation: S = 362, p = 0.124, ρ = -0.139).

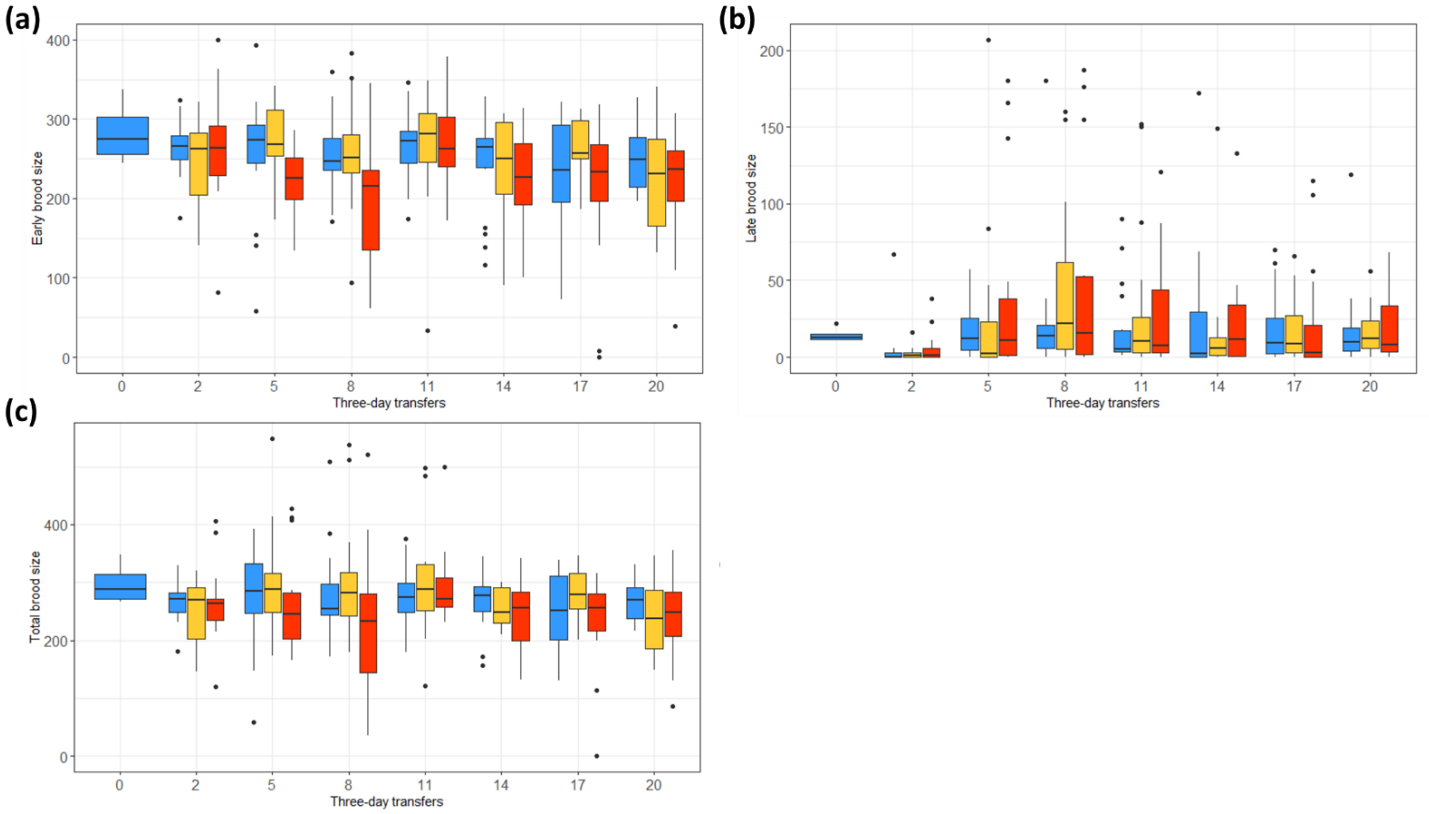

Figure 10: Boxplot of **(a)** early, **(b)** late and **(c)** total brood size over time (i.e., three-day transfers: 0, 2, 5, 8, 11, 14, 17 and 20) for *C. elegans* populations living in different gamma radiation environments. Blue: control; yellow: low radiation (1.4 $mGy.h^{-1}$); red: high radiation (50.0 $mGy.h^{-1}$).

3.2. Reciprocal transplant experiments

In the experiment comparing control and low irradiation treatment, we found a significant interaction between population of origin and environment of transplant on hatching success (Table 4a; Figure 11a). Hatching success was maximized when populations have evolved in the same environment of origin and transplant. The transplanted populations in the control treatment had a better hatching success than in low irradiation treatment. In the control to high irradiation treatment transplant, we did not find any significant results between the population of origin and the environment of the transplant on hatching success (Table 4a; Figure 11b).

The transplant between control and low irradiation treatment showed a significant interaction between population of origin and environment of transplant for late brood size (Table 4c; Figure 11e). Late brood size decreased from the control to the low irradiation environment, whereas the control population did not change its late brood size after being transplanted into a low

irradiation treatment (Table 4c; Figure 11e). In the control to high irradiation treatment transplant on late brood size and for the transplants on early and total brood size, we did not find any significant result between the population of origin and the environment of the transplant (Table 4; Figure 11).

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

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

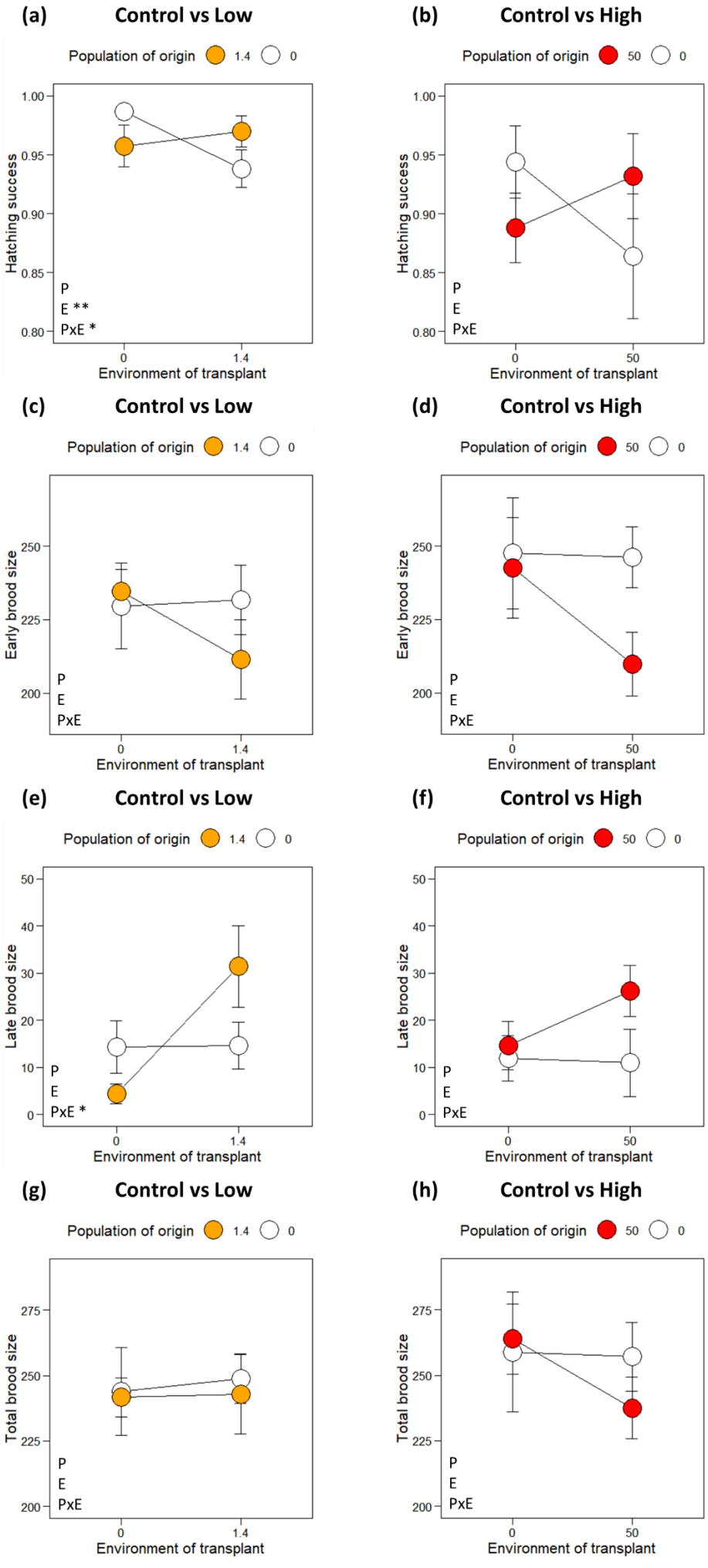
(a) Control vs Low
Population of origin 1.4 0
Hatching success
1.00
0.95
0.90
0.85
0.80
P
E **
PxE *
0
1.4
Environment of transplant
(b) Control vs High
Population of origin 50 0
Hatching success
1.00
0.95
0.90
0.85
0.80
P
E
PxE
0
50
Environment of transplant
(c) Control vs Low
Population of origin 1.4 0
Early brood size
250
225
200
P
E
PxE
0
1.4
Environment of transplant
(d) Control vs High
Population of origin 50 0
Early brood size
250
225
200
P
E
PxE
0
50
Environment of transplant
(e) Control vs Low
Population of origin 1.4 0
Late brood size
50
40
30
20
10
0
P
E
PxE *
0
1.4
Environment of transplant
(f) Control vs High
Population of origin 50 0
Late brood size
50
40
30
20
10
0
P
E
PxE
0
50
Environment of transplant
(g) Control vs Low
Population of origin 1.4 0
Total brood size
275
250
225
200
P
E
PxE
0
1.4
Environment of transplant
(h) Control vs High
Population of origin 50 0
Total brood size
275
250
225
200
P
E
PxE
0
50
Environment of transplant

Figure 11: Hatching success **(a, b)**, early **(c, d)**, late **(e, f)** and total brood size **(g, h)** of *C. elegans* populations after four transfers of reciprocal transplant between control and **(a, c, e, g)** low (1.4 $mGy.h^{-1}$) or **(b, d, f, h)** high (50.0 $mGy.h^{-1}$) irradiation treatments. Dots represent the mean of life history traits ± S.E for each new treatment. The color of dot represents the populations' treatment during the multigenerational experiment. White: control; yellow: low radiation (1.4 $mGy.h^{-1}$); red: high radiation (50.0 $mGy.h^{-1}$). The significance of each main effect [population of origin (P), environment of transplant (E) and their interaction (P × E)] is indicated in the bottom left of each graph. *, P <0.05; **, P <0.01; ***, P <0.001

4. Discussion

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

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

4.1. Multigenerational experiment: characterizing demographic change

Previous reviews have found a negative impact of ionizing radiation on reproduction and survival on many species (plants, mammals, fish, or invertebrates) (Dallas et al., 2012; Real et al., 2004). These traits can strongly affect the demography of the population (Moyson et al., 2019). In our case, the lower population growth rate of the high irradiation treatment reflects a parallel decrease in realized fecundity, brood size and hatching success. In short-lived species like *C. elegans*, fecundity can impact population growth to a higher extent than survival (Sibly et al., 2002). In the low irradiation treatment, the significant decrease in realized fecundity and the absence of a significant increase for brood size and hatching success cannot explain the increased population growth rate. This decrease in fecundity could be caused by other unmeasured parameters, changing the ratio between the number of eggs and the number of

individuals. First, low ionizing radiation could increase developmental speed during the 20 transfers, as has been shown in *C. elegans* exposed to manganese (Lin et al., 2006) or with an increase in temperature (Byerly et al., 1976). Second, higher adult survival or longer lifespan could also decrease the ratio between the number of eggs and the number of individuals. Indeed, Johnson and Hartman (1988) suggested that ionizing radiation could increase *C. elegans* lifespan at acute doses between 100 Gy and 300 Gy. Moreover, some stressors such as a dietary restriction (Kaeberlein et al., 2006) or chemicals (thioallyl compounds: Ogawa et al., 2016; mianserin: Petrascheck et al., 2009) can increase the lifespan in *C. elegans*. More generally, other unmeasured demographic traits in this study may impact population growth rate (Sibly et al., 2002), and could explain our results. For example, age structure (Hoy et al., 2020), population density (Tanner, 1966) or sex ratio (Le Galliard et al., 2005; Lenz et al., 2007) could change population growth rate.

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

Our results show that at the population level the two irradiated conditions decreased fecundity, and the decrease intensified with dose rate. Other studies on the *C. elegans* N2 strain have found a decreased fecundity of 25%, 35%, 43% and 61% in populations chronically exposed to 42.7 $mGy.h^{-1}$, 50.0 $mGy.h^{-1}$, 108.0 $mGy.h^{-1}$ and 228.0 $mGy.h^{-1}$, respectively (Buisset-Goussen et al., 2014; Dufourcq-Sekatcheff et al., 2021; Maremonti et al., 2019). Such decrease in fertility may be caused by germ cell apoptosis and reduced spermatids production (Guédon et al., 2021; Maremonti et al., 2019). Interestingly, we found a decrease in fecundity at much lower dose rate than Buisset-Goussen et al. (2014), Maremonti et al. (2019) or Dufourcq-Sekatcheff et al. (2021). First, The A6140 population used in this study may be less resistant to ionizing radiation than the N2 strain. *C. elegans* strains differ in their radioresistance (Clejan et al., 2006). Second, the multigenerational irradiation exposure could amplify deleterious reproductive effects, through for example transgenerational effects, as shown in

several studies on invertebrates (*Ophryotrocha diadema:* Knowles and Greenwood, 1994; *Daphnia magna:* Alonzo et al., 2008; *C. elegans*: Buisset-Goussen et al., 2014). As for population growth rate, under irradiated conditions the realized fecundity fluctuated significantly over time, suggesting transgenerational effect of the *C. elegans* reproduction (Yue et al., 2021). Moreover, this result seems logical since the calculation of both indexes is based on the same data set.

Offspring survival is essential in the maintenance and growth of a population over the long term. We, therefore, measured hatching success during the experiment in parallel with population growth rate. Our results showed a lower hatching success in the high irradiation treatment compared to the control treatment. A decrease in the hatching success of about 20% has already been observed with an acute gamma irradiation of 50 Gy of the L4/young adult stage with the N2 strain (Dubois et al., 2018). However, this study showed no decrease in hatching success with a chronic irradiation of 50.0 $mGy.h^{-1}$ between the egg and L4/young adult stages (Dubois et al., 2018). Other studies have shown a decrease in larval survival at much lower chronic dose rates (gamma radiation) in *Neanthes arenaceodentata* (0.19 $mGy.h^{-1}$; Harrison and Anderson, 1994), *Ophryotrocha diadema* (3.2 $mGy.h^{-1}$; Knowles and Greenwood, 1994) and *Eisenia fetida* (4.0 $mGy.h^{-1}$; Hertel-Aas et al., 2007). In our study, the significant decrease in hatching success appeared after several transfers of irradiation (Figure 9c), which may explain this difference in results. Moreover, for this study we used the A6140 population (Noble et al., 2017; Teotonio et al., 2012) and not the N2 strain, so we can expect a slightly different response. Dubois et al. (2018) showed that the decrease in hatching success was correlated with protein carbonylation and suggests that the decrease in hatching success could be possibly explained by an apoptosis phenomenon. Other work has shown that many genes can cause embryogenic mortality when inactivated in *C. elegans* (Maeda et al., 2001). An accumulation of mutations caused by chronic exposure to ionizing radiation may decrease hatching success.

We found a decrease in early and total brood size for high irradiation treatment compared to control treatment. Hermaphrodites having grown and laid eggs outside the irradiator, these results showed that early irradiation at the embryonic stage can have an impact on reproduction once adult. The observed decrease in hermaphrodite fecundity was consistent with several studies showing the deleterious impact of ionizing radiation on *C. elegans* reproduction, by decreasing the number of germ cells by radiation-induced apoptosis, and by decreasing the quantity and quality of gametes (Buisset-Goussen et al., 2014; Dufourcq-Sekatcheff et al.,

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

4.2. Reciprocal-transplant experiments: study of evolutionary changes

After the multigenerational experiment, reciprocal-transplant experiments were performed to study variations in life history traits and analyze the mechanisms underlying these changes. Our reciprocal-transplant experiments showed some adaptive responses for hatching success and late brood size in populations subjected to irradiation. Results suggested an evolutionary trend towards an improvement of embryo survival and a slower life history in response to low irradiation. The similar but nonsignificant trends observed for hatching success and late brood size for high radiation suggest adaptive process in the same direction but slowed at high doses rate. These changes could provide better resistance and fitness against ionizing radiation. Indeed, organisms with a slower life strategy invest more in self-maintenance than in reproduction, leading to increased stress tolerance, better immune response or lower rates of aging (Tökölyi et al., 2016). In *C. elegans*, studies have shown an extend lifespan and delay in reproductive timing with improved resistance to different stressors (heat, oxygen, and juglone: Cypser and Johnson, 2002; quercetin, caffeic and rosmarinic acid: Pietsch et al., 2011; arsenite: Schmeisser et al., 2013; mitochondrial stress: Maglioni et al., 2014). For high irradiation treatment, populations did not appear to adapt as successfully as populations subjected to low irradiation. In these stressful conditions, local adaptation could be limited by trade-offs among fitness-related traits (Colautti and Lau, 2015; Kraemer and Boynton, 2017), as previously

suggested between early and late brood size. In addition, regarding the lack of significant results in reciprocal-transplant experiment for hatching success at high dose rates could be related to the large amplitude of the standard error, indicating great variability in the response between biological replicates (Figure 11b). Radiation being strongly mutagenic (Breimer, 1988), we can expect a random accumulation of mutations in the irradiated populations, resulting in a divergence of phenotypic responses between independent populations.

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

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

5. Conclusion

As far as we know, this study presents one of the first characterization of the effects of chronic exposure to ionizing radiation over so many generations. Our results showed a higher *C. elegans* population growth rate for low irradiation treatment and a lower for high irradiation treatment compared to the control. The results of life history traits show a decrease of realized fecundity for both irradiation treatment. Moreover, we observed a decrease of individual fecundity and hatching success for high irradiation treatment. Finally, results suggest an adaptive response of populations that live in low irradiation conditions, showing an

improvement of embryo survival and a slower life strategy. These evolutionary changes were with some costs of adaptation.

Our study demonstrates the importance of studying the effects of a stressor, such as ionizing radiation, at the population level to assess ecological risks. Our approach at the population level allowed us to highlight effects that we could not have highlighted if we had studied the effects of ionizing radiation only at the individual level. For example, we could show a decrease in population fecundity at the lowest dose rate, while no significant variation in brood size was observed at the individual level at this dose rate. Furthermore, the fluctuation of the traits and the adaptive response observed in this work showed the importance of studying long-term effects over several generations. This work has brought a new argument to show the importance of considering demographic and evolutionary changes in ecotoxicology, and for Ecological Risk Assessment.

6. Acknowledgements

We are grateful to Henrique Teotónio and his team for their invaluable help and for providing the A6140 nematode population. We especially thank Carolyn Hall for linguistic corrections. Thanks to Sandrine Frelon, André Gilles, Morgan Dutilleul and Patrick Laloi for ideas, valuable discussion and comments during this project. This study has been funded by the French Institute of Radiological Protection and Nuclear Safety (IRSN).

Chapitre 3 - Male frequency in *Caenorhabditis elegans* increases in response to chronic irradiation

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Key words - Experimental evolution, sex ratio, stressful environment, selection, *Caenorhabditis elegans*, sexual conflict

Abstract

Outcrossing can be advantageous in a changing environment because it promotes the purge of deleterious mutations and increases the genetic diversity within a population, which may improve population persistence and evolutionary potential. Some species may therefore switch their reproductive mode from inbreeding to outcrossing when under environmental stress. This switch may have consequences on the demographic dynamics and evolutionary trajectory of populations. For example, it may directly influence the sex ratio of a population. However, much remains to be discovered about the mechanisms and evolutionary implications of sex ratio changes in a population in response to environmental stress. Populations of the androdioecious nematode *Caenorhabditis elegans*, are composed of selfing hermaphrodites and rare males. Here we investigate the changes in sex ratio of *C. elegans* populations exposed to radioactive pollution for 60 days or around 20 generations. We experimentally exposed populations to three levels of ionizing radiation (i.e. 0 $mGy.h^{-1}$, 1.4 $mGy.h^{-1}$, and 50 $mGy.h^{-1}$). We then performed reciprocal transplant experiments to evaluate genetic divergence between populations submitted to different treatments. Finally, we used a mathematical model to examine the evolutionary mechanisms that could be responsible for the change in sex ratio. Our results showed an increase in male frequency in irradiated populations, and this effect increased with the dose rate. The model showed that an increase in male fertilization success or a decrease in hermaphrodite self-fertilization could explain this increase in the frequency of males. Moreover, males persisted in populations after transplant back into the control conditions. These results suggested selection favoring outcrossing under irradiation conditions. This study shows that ionizing radiation can sustainably alter the reproductive strategy of a population,

likely impacting its long-term evolutionary history. This study highlights the need to evaluate the impact of pollutants on the reproductive strategies of populations when assessing the ecological risks.

1. Introduction

Outcrossing, the fusion of gametes from distinct individuals, plays an important role in evolution (Otto & Lenormand, 2002), is widespread in multicellular organisms, and confers many advantages. For example, the recombination caused by genetic crossbreeding generates offspring that are genetically different from their parents, and both theoretical and empirical studies have shown that the resulting increase in genetic diversity improves population persistence and evolutionary potential in response to novel environmental stresses or conditions (Colegrave, 2002; Morran *et al.*, 2009a; Seudre *et al.*, 2018). Genetic recombination also facilitates the elimination of deleterious mutations (Crow, 1994) and spreads beneficial mutations (Otto, 2009). In contrast, asexual or obligatory self-fertilizing species accumulate deleterious mutations through the Muller ratchet mechanism (Muller, 1932; Felsenstein, 1974; Morran *et al.*, 2009a) and homozygosity (Glémin & Galtier, 2012). These processes lead to the expression of recessive mutations and inbreeding depression and may lower the evolutionary potential of the population (Archetti, 2004). Although the subject is still strongly debated, asexual or obligatory self-fertilizing species are expected to have higher extinction rates than outcrossing species (Goldberg *et al.*, 2010; Ho & Agrawal, 2017; Glémin *et al.*, 2019). However, these modes of reproduction can provide fitness gains. For example they can maintain beneficial gene combinations that would be easily broken by outcrossing (i.e. outbreeding depression) (Dolgin *et al.*, 2007) and improve the chance of reproduction at low population density (Morran *et al.*, 2009b; Teotónio *et al.*, 2012).

Outcrossing may therefore be particularly effective in a changing environment (Colegrave, 2002; Morran *et al.*, 2009a). This hypothesis is supported by the finding that environmental stresses promote sexual reproduction in some organisms, such as daphnia or nematodes that usually breed asexually (Gemmill *et al.*, 1997; Dacks & Roger, 1999; Alonzo *et al.*, 2008; Camp *et al.*, 2019). Outcrossing, however, also leads to some significant costs (Otto, 2009; Lehtonen *et al.*, 2012), such as reduced growth rate (Smith & Maynard-Smith, 1978; Gibson *et al.*, 2017), increased time and energy spent finding mates, increased disease transmission, and a higher vulnerability to predators during mating (Otto & Lenormand, 2002; Grosmaire, 2018). Whether a population uses asexual reproduction to avoid these costs or sexual reproduction to benefit from outcrossing will therefore depend on many factors.

Outcrossing requires the presence of both male and female reproductive functions. The sex ratio influences sexual selection and is an important parameter in the evolution of a species (Janicke & Morrow, 2018). It also plays an essential role in the demography, mating system, and genetics of a population, and can influence its evolutionary trajectory (Hartl *et al.*, 1997; Freedberg & Taylor, 2007; Rood & Freedberg, 2016; Sowersby *et al.*, 2020). According to

Fisher's principle (Fisher, 1930), frequency-dependent selection maintains a balanced sex ratio in most populations. However, sex ratio varies widely between gonochoric taxa, and sex can be determined genetically or plastically in response to environmental signals (West & Sheldon, 2002; Székely *et al.*, 2014). Under stressful conditions, selection may favor one sex if that sex provides a greater fitness than the other. For example, maternal stress alters the relative cost of producing each sex (Geffroy & Douhard, 2019), hence production of the lowest-cost sex could confer a selective advantage (Myers, 1978). For example, Agaonid fig wasps, *Ceratosolen galili*, adjust their sex ratio in response to the number of foundresses laying eggs in a fig, which presumably functions to limit local mate competition in the offspring (Greeff *et al.*, 2020). Thus, the sex ratio of a population may change under new selection pressures (Sowersby *et al.*, 2020), and can in turn affect the evolution of genetic sex-determining mechanisms (Mank *et al.*, 2006). However, much remains to be discovered about the mechanisms and evolutionary implications of sex ratio changes in a population in response to environmental stress (Sowersby *et al.*, 2020).

At certain doses, ionizing radiation is a powerful environmental stressor, and its emission in the environment has increased with human activity since the end of World War II (Rhodes *et al.*, 2020). For instance, the accidents at the Chernobyl and Fukushima nuclear power plants have released 1.4×10^{19} Bq (IAEA, 2006) and 5.2×10^{17} Bq (Okano *et al.*, 2016) of radionuclides into the environment, respectively. Following these accidents, many surrounding ecosystems have been contaminated, with negative effects of radio-contamination on wildlife (Møller & Mousseau, 2006; Geras'kin *et al.*, 2008; Aliyu *et al.*, 2015; Cannon & Kiang, 2020). Ionizing radiation can have detrimental effects on the reproduction of mammals and fish, such as delayed reproduction or a decrease in the weight and survival of embryos (Real *et al.*, 2004) and has also been found to have deleterious effects on reproduction in many invertebrate species (Dallas *et al.*, 2012; e.g. *Caenorhabditis elegans* Buisset-Goussen *et al.*, 2014; Lecomte-Pradines *et al.*, 2017; Dubois *et al.*, 2018; Maremonti *et al.*, 2019). Irradiation may also affect the sex ratio of populations, though more work is needed in this area. In humans, the effect of ionizing radiation on the sex ratio has been studied extensively, without consistent evidence of a significant impact (Terrell *et al.*, 2011). In contrast, a change in the sex ratio induced by internal alpha irradiation, caused mainly by an accumulation of ^{241}Am in the tissues, has been observed in *Daphnia magna* exposed over three generations, with nonreproducing males appearing at the F2 generation and accounting for 31% of the total daphnids (Alonzo *et al.*, 2008). In the Chernobyl Exclusion Zone, female barn swallows (*Hirundo rustica*) showed higher mortality than males, and this supposedly affected the sex ratio of the population (Møller

et *al.* 2012). To the best of our knowledge, the effects of external exposure of ionizing radiation on sex ratio dynamics have never been studied.

In this paper, we studied the sex ratio of a population of *C. elegans* experimentally exposed to gamma radiation for 60 days or around 20 generations. The aim of this study was (1) to test whether exposure to gamma radiation changes the sex ratio of *C. elegans* populations, and (2) to investigate the mechanisms underlying these changes. The metazoan *C. elegans* (Nematoda, Rhabditidae) is an androdioecious organism. *C. elegans*, and more broadly the genus *Caenorhabditis*, is particularly useful for studying the evolution of sexual interactions and sexual conflict (Palopoli *et al.*, 2015). It is also ideal for investigating evolutionary questions about outcrossing and sex ratio, because populations are mostly composed of hermaphrodites XX with a few rare males XØ (Herman, 2005). Hermaphrodites reproduce mainly by self-fertilization, and their limited sperm quantity restrains their offspring production to about 300 individuals, all of which will be hermaphrodites (Chasnov, 2013; Barr *et al.*, 2018; Cutter *et al.*, 2019). However, hermaphrodites can also mate with males, and mating with several males can produce up to about 1000 offspring, because male sperm is produced continually and in large numbers (Singson, 2001; Chasnov, 2013; Cutter *et al.*, 2019). In addition, sperm competition induced by the presence of males could increase hermaphrodite sperm counts (Cutter, 2004). Oocytes sired by a male produce offspring that are 50% male and 50% hermaphrodite.

Male *C. elegans* are rare in the wild, between 0 and 22% of the population, and their frequency always remains lower than that of hermaphrodites (Anderson *et al.*, 2010). The relative rarity of males suggests a selective advantage of self-fertilization over outcrossing (Stewart & Phillips, 2002; Cutter *et al.*, 2019), and this difference in reproductive strategies between the sexes can lead to fitness conflicts (Chapman, 2006). Indeed, it appears that mating with males can incur a cost, for example increased mortality and decreased reproductive success of hermaphrodites who mate with males repeatedly (Cutter *et al.*, 2019). However, male frequency strongly increases under severe stress (Rose & Baillie, 1979; Lopes *et al.*, 2008; Morran *et al.*, 2009a; Lynch *et al.*, 2018). This observation suggests that there is either a decrease in the cost of outcrossing for hermaphrodites or an advantage to males under stressful conditions. Although the benefit of two-parent reproduction is still a subject of debate in evolutionary biology, it has been suggested that in *C. elegans* outcrossing enhances natural selection, allowing for faster adaptation and more effective purging of deleterious mutations (Cutter *et al.*, 2019). Given these benefits to outcrossing in a stressful environment, we hypothesized that selection for outcrossing would occur in the irradiated environments, resulting in an increase in the number of males. Mechanistically, since ionizing radiation causes

a decrease in spermatid production (Maremonti *et al.*, 2019), that affects the sperm limited hermaphrodites more strongly than males, we hypothesized that ionizing radiation would modify the sex ratio through a decrease in mating success of hermaphrodites who self-fertilize compared to males, and the consequential increase in production of male offspring. Oxidative stress and DNA damage induced by ionizing radiation decrease sperm quality and quantity by altering meiosis, decreasing sperm activation and viability and inducing germ-cell apoptosis (Maremonti *et al.*, 2019; Guédon *et al.*, 2021).

2. Material and methods

2.1. Test organism and population maintenance

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

2.2. Irradiation conditions

The external gamma radiation exposure was conducted at the Mini Irradiator for Radio Ecology ^{137}Cs irradiation facilities, at the Institut de Radioprotection et de Sûreté Nucléaire (MIRE, IRSN, Cadarache, France; Figure 12). We used the same irradiation facilities, and the same protocol as previously described by Buisset-Goussen *et al.* (2014), but with the following differences. The irradiators were placed in incubators with controlled temperature at 20 °C and 80% relative humidity. The populations of *C. elegans* were exposed to three dose rate gamma radiation treatments (5 replicates per treatment): 0 (control treatment), 1.4 (low irradiation treatment), and 50 (high irradiation treatment) mGy.h^{-1}. Low irradiation treatment had an environmental reality, Garnier-Laplace *et al.*, (2013) indicated that terrestrial wildlife could be exposed to dose rates up to ~10 mGy.h^{-1} in the Chernobyl Exclusion Zone. High irradiation treatment was chosen because several studies have shown an impact on reproduction in *C. elegans* at a similar dose rate (Buisset-Goussen *et al.*, 2014; Dubois *et al.*, 2018; Maremonti *et al.*, 2019; Dufourcq-Sekatcheff *et al.*, 2021; Guédon *et al.*, 2021). The dose rates were measured with radiophoto luminescent (RPL) micro-dosimeters twice during the experiment. As explained in Buisset-Goussen *et al.*, (2014), the Petri dishes were placed vertically in the irradiator to homogenize the dose received over the entire dish. To obtain the required dose

rates, the plates were placed at different distances from the source and separated by shields (Petri dish filled with lead filings). For technical reasons, we placed the petri dishes of the control condition in an identical incubator, with for only difference the absence of irradiation system in the incubator.

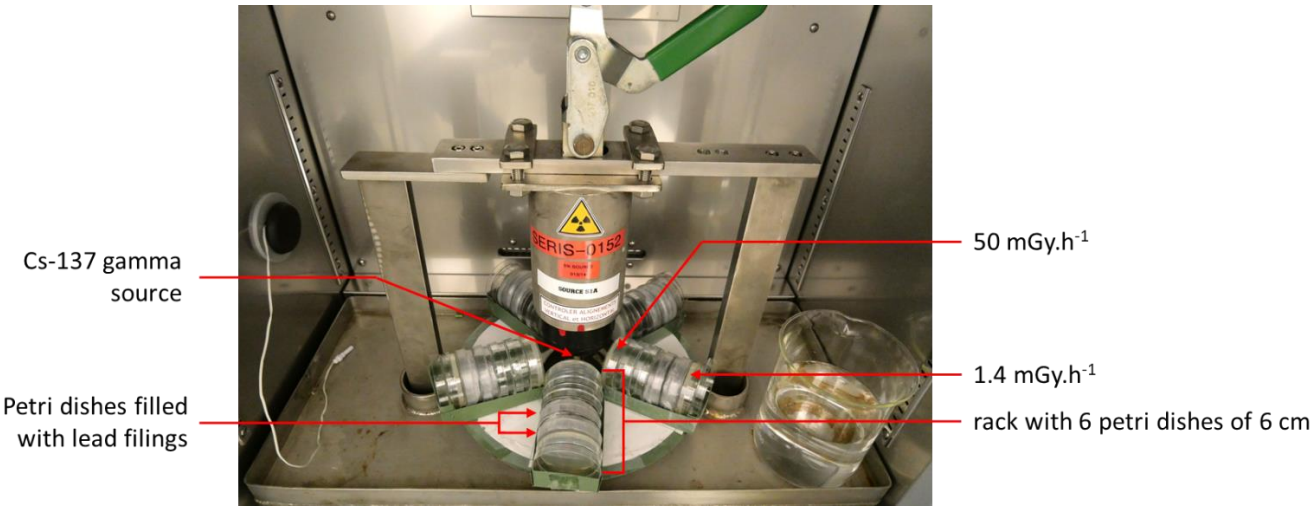

Figure 12: Irradiation system inside the incubator (credit: L. Quevarec/IRSN)

2.3. Multigenerational experiment

A multigenerational experiment was performed over 60 days (about 20 generations) with the same conditions for all populations except for the irradiation treatment (Figure 13). Dutilleul *et al.* (2014) found that this duration was long enough to detect plastic or evolutionary changes in *C. elegans*. We transferred samples of populations into new Petri dishes every three days. Although three days grossly correspond to a generation in natural conditions, gamma radiation delays growth and reproduction at high dose rates (Lecomte-Pradines *et al.*, 2017), and we cannot guarantee that every transfer corresponds to a generation. We thus describe the dynamics of changes during the experiment as a function of the number three-day transfers.

We estimated the frequency of males relative to hermaphrodites at transfer 0, 2, 5, 8, 11, 14, 17 and 20. For each estimation, we transferred 100 eggs per replicate into new Petri dishes (3 cm) containing the same medium. Forty-eight hours after the transfer, we counted males and hermaphrodites (L4 and young adult) with a stereomicroscope (Olympus SZX12, 1.6 × 90 magnification).

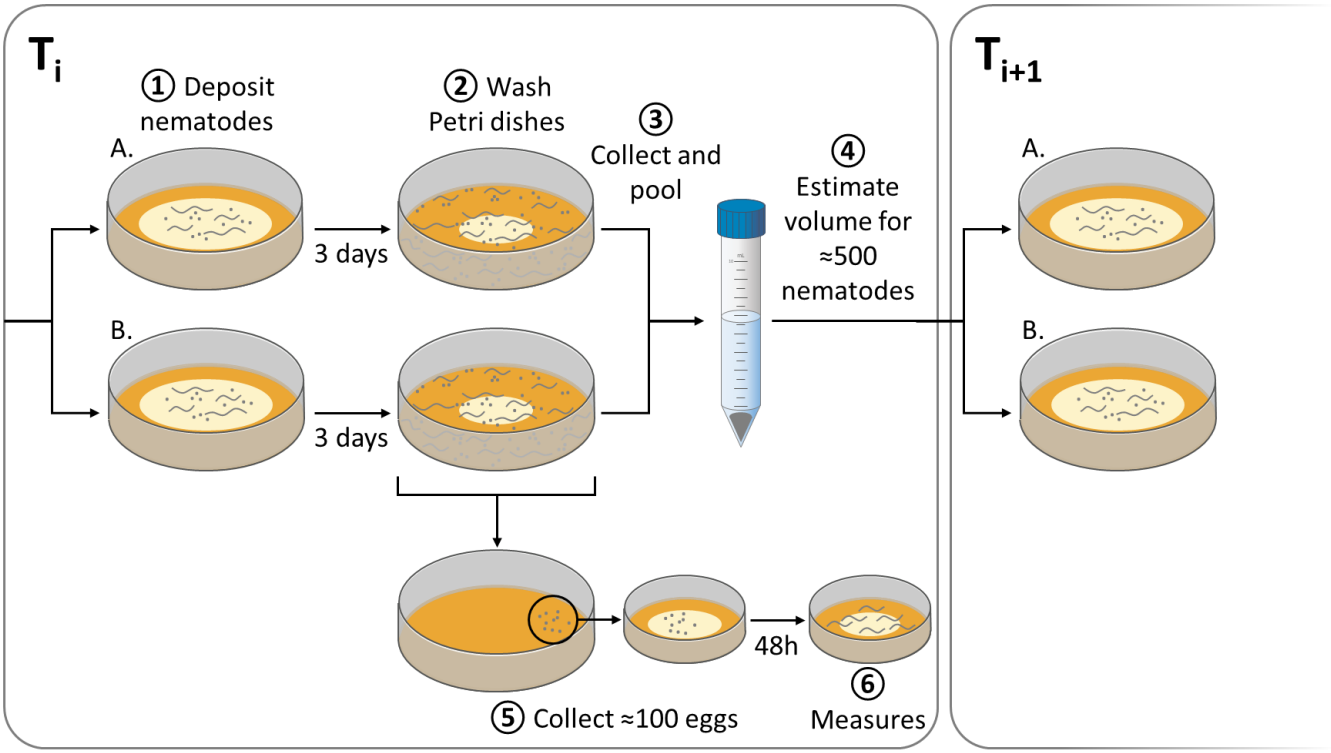

Figure 13: Schematic overview of the multigenerational experiment protocol for *C. elegans* populations under different gamma radiation treatments. For each treatment (0, 1.4 and 50 $mGy.h^{-1}$), five replicates composed of two Petri dishes (which we will call A and B) were made. **1.** These plates were seeded with about 500 individuals of all stages (egg to adult). At the time of transfer (three days), the two plates (A and B) were **2.** washed with M9 solution and **3.** pooled in 15 mL tube. **4.** A volume equivalent to ~500 nematodes was then used to seed two Petri dishes for the next transfer. This allowed us to have a population of about 1000 individuals per replicate at the beginning of each transfer, which were then spread over two Petri dishes. **5.** Simultaneously, ~100 eggs were collected and used to **6.** estimate the sex ratio for each replicate 48h later.

2.4. Reciprocal-transplant experiments

To test whether sex ratio changes were maintained if the stressor was removed, we performed a reciprocal transplant experiment between the control and both the low and the high irradiation treatment. For this experiment, we referred to the population that had evolved either in a controlled environment or in the two irradiated treatments as the "population of origin", and the environment they had evolved in as the "environment of origin". We referred to the novel environment the populations were transferred to as the "environment of transplant" (Figure 14). At transfer 20, we collected 500 individuals from each population of origin and placed them either in the same environment (environment of origin) or in one of the two environments of transplant. We created five replicates for each condition of reciprocal

transplant, resulting in 40 replicates in total. We then maintained these populations in the same conditions for 12 days, or four transfers.

At the end of the fourth transfer of reciprocal transplants, we estimated the frequency of males relative to hermaphrodites. We measured the sex ratio after four transfers to ensure that the differences between populations were not due to parental effects (Badyaev & Uller, 2009; Kawecki *et al.*, 2012; Dutilleul *et al.*, 2014).

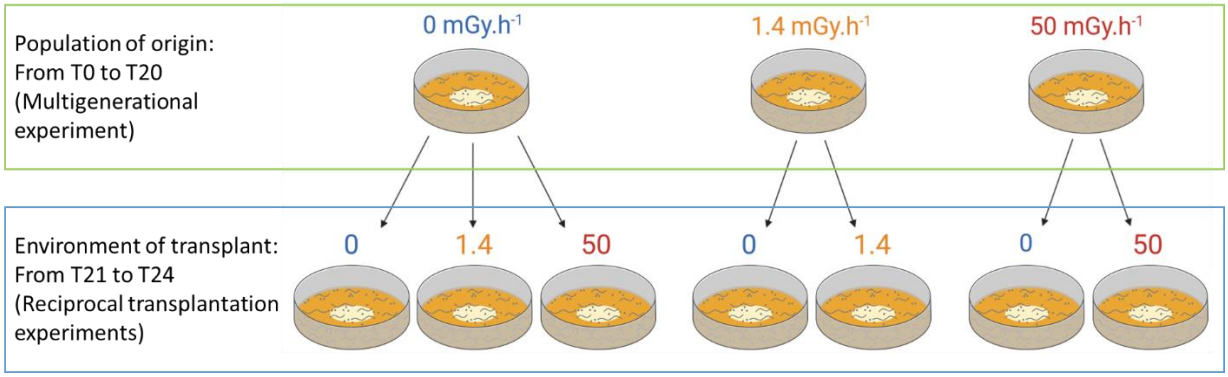

Figure 14: Schematic overview of the reciprocal-transplant experiment design for *C. elegans* populations in three gamma radiation treatments. After 20 transfers in the initial environment (Population of origin) (0, 1.4 and 50 mGy.h^{-1}) during the multigenerational experiment, populations were placed in a second environment (environment of transplant) for four transfers. This partial reciprocal transplant was performed as shown here. The measurements of sex ratio were made after 4 transfers to ensure that the differences between populations was due to genetic differentiation and not parental effects.

2.5. Stewart and Phillips model (SP model)

To investigate which factors could lead to change in the sex ratio during the experiment, we modified Stewart & Phillips (2002) model of evolution of androdioecy in *C. elegans*, following the equation:

$$m' = (1-\sigma)(1+2u)\alpha m/2T + \beta(1-\delta)(1-\alpha m)u/T,$$

and:

$$T = \alpha m[1-\sigma(1/2+u)] + \beta(1-\delta)(1-\sigma u)(1-\alpha m),$$

where α is the fertilization success of males, β is the proportion of eggs not fertilized by males that are self-fertilized, u is the rate of nondisjunction of the X chromosome, δ the degree of inbreeding depression in self-fertilized offspring, σ the relative viability difference between

males and hermaphrodites, *m* the male frequency in the current generation and *m'* the male frequency in the next generation. Following Kim's results (1985), we predicted that ionizing radiation increases the rate of nondisjunction of the X chromosome (*u*), which should increase the bias in sex-ratio in favor of males. Additionally, we predicted that the deleterious impact of radiation on spermatid production in hermaphrodites (Maremonti *et al.*, 2019), decreases the proportion of self-fertilized eggs β, and increases the fertilization success of males α, which continuously produce sperm in large numbers (Cutter *et al.*, 2019). This also should increase the proportion of males in the population.

Male fertilization success (α) covers many traits, such as the ability to find hermaphrodites or to mate with them. In the same way, the proportion of eggs not fertilized by male that are self-fertilized (β) depends on trait such as the production of hermaphrodite sperm, the proportion of viable sperm, or the ability for male sperm to outcompete hermaphrodite sperm. Our goal, however, was not to analyse the exact role of these traits in these two variables, but rather to test whether the variables in Stewart & Phillips' model (2002) could explain the temporal change in sex-ratio.

The model analyses the effect of generations, and we thus use the term generation to reflect the effect of time on sex ratio. Note that it differs from the experiment where we used the term transfer instead of generation, as explained above. However, it is important to remember that the dynamics of sex ratio in both the experiment and the model depend on across generation change. To model changes in male frequency over multiple generations, we created iterations where at each generation we replaced the male frequency by the frequency of the previous generation. The model was also modified to incorporate changes in the α and β over generations. We empirically determined the baseline values of α and β by identifying the value at which the frequency of males was stable at 0.25, the level observed in the control condition. Thus, compared to the initial model, $\alpha = 0.65$ and $\beta = 0.15$. The *u* of the A6140 was fixed at 0.005 (Teotónio *et al.*, 2012). Inbreeding depression is considered negligible in *C. elegans* (Stewart & Phillips, 2002). However, Teotónio et al. (2012) considered that inbreeding depression was present in diversified populations of 1000 individuals, but that it played a minor role in the male maintenance relative to adaptation. In our case, we had 500 individuals in each Petri dish (1000 per replicate population); we estimated that inbreeding depression had a weak contribution to male maintenance. Furthermore, with our experimental approach we could not quantify inbreeding depression. We thus chose to focus on the parameters described above. δ was set to

0. In addition, σ was also set to 0 because at the highest dose rate (i.e. 50 $mGy.h^{-1}$) used in this study, no mortality was expected (Clejan *et al.*, 2006).

2.6. Statistical analysis

We used two statistical models to analyse the change in the sex ratio over the 20 transfers. First, we used a Generalized Linear Mixed Model (GLMM) with R software (Team, 2013) and the Ade4 package (Dray & Dufour, 2007; Bougeard & Dray, 2018) to examine the strong increase in male frequency observed over the first 2 transfers. In this model, sex ratio corresponded to the frequency of males (i.e. number of males divided by the number of total individuals) and had a binomial distribution and a logit link function. No overdispersion of data was observed. The sex ratio was analysed as a function of transfer (0 and 2), treatment (i.e., control, low and high irradiation) and their interaction, with replicate ID as a random effect. Second, we used a Generalized Additive Mixed Model (GAMM) with the Mgcv package in R (Wood *et al.*, 2016) to examine the changes in sex ratio between transfer 2 and 20, because visual inspection of the data showed no simple relationship. The sex ratio was analysed as a function of transfer (2, 5, 8, 11, 14, 17 and 20), treatment (i.e., control, low and high irradiation) and their interaction as fixed effects, and replicate ID as a random effect. The smoothing was performed on the variable transfer in function of treatment. A quasi-binomial distribution and a logit link function were used and no overdispersion of data was observed. We analysed the sex ratio in the reciprocal-transplant experiment, using a GLMM, with the environment of origin, environment of transplant, and the interaction between environment of origin and environment of transplant as fixed effects. A binomial distribution and a logit link function were used and no overdispersion of data was observed. Replicate ID was included as a random effect.

3. Results

3.1. Multigenerational experiment

Between transfers 0 and 2, the significant transfer by treatment interaction, but non-significant main terms, revealed an increase in male frequency in both irradiation conditions (1.4 and 50 mGy.h^{-1}) but no change in the control treatment (Table 5; Figure 15).

Between transfers 2 and 20, male frequency was estimated at 0.25, 0.37 and 0.43 for control, low and high irradiation treatments, respectively (Table 6; estimations shown have been transformed with the inverse-logit function). Male frequency was higher in both the low and high irradiation treatments than in the control treatment (Table 6a; Figure 15). Male frequency decreased significantly across transfers in the low irradiation treatment and increased significantly in the control and high irradiation treatments (Table 6b; Figure 15, Figure C.1).

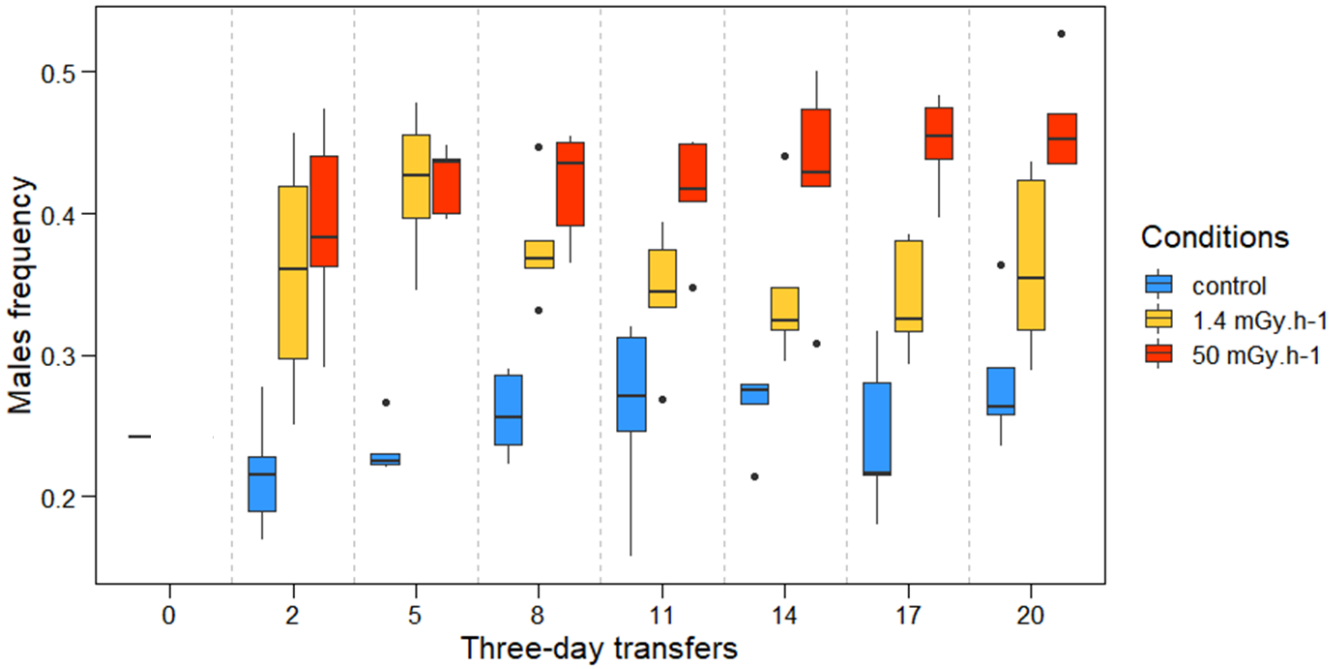

Figure 15: Boxplot of male frequency over time (i.e., three-day transfers: 0, 2, 5, 8, 11, 14, 17 and 20) for *C. elegans* populations living in different gamma radiation environments. Blue: control; yellow: low radiation (1.4 mGy.h^{-1}); red: high radiation (50 mGy.h^{-1}).

Table 5: Effect of time (i.e. three-day transfer) and gamma irradiation condition (0, 1.4 and 50 mGy.h^{-1}) on male frequency in *C. elegans*, between transfer 0 and 2. *, P <0.05; **, P <0.01; ***, P <0.001

	Estimate	Std. Error	z value	Pr(>|z|)	
(Intercept)	-1.169	2.387e-01	-4.895	9.83e-07	***
Time	-5.983e-02	1.332e-01	-0.449	0.653	
Gamma Low	6.297e-16	3.376e-01	0.000	1.000	
Gamma High	1.479e-15	3.376e-01	0.000	1.000	
Time:Gamma Low	4.014e-01	1.881e-01	2.134	0.0328	*
Time:Gamma High	3.850e-01	1.888e-01	2.039	0.0414	*

Table 6: Effects of **(a)** irradiation condition (0.0, 1.4 and 50 mGy.h^{-1}) and **(b)** Time (EDF: Effective degrees of freedom) on *C. elegans* male frequency, between transfer 2 and 20. *, P <0.05; **, P <0.01; ***, P <0.001

a)	Estimate	Std. Error	t value	Pr(>|t|)	
(Intercept)	-1.077	0. 03994	-26.97	<2e-16	***
Gamma Low	0.541	0. 05403	10.01	<2e-16	***
Gamma High	0.794	0. 05504	14.43	<2e-16	***

Approximate significance of smooth terms

b)	edf	Ref.df	F	p-value	
s(Time):Control	1	1	4.973	0.0280	*
s(Time):Gamma Low	1	1	5.162	0.0252	*
s(Time):Gamma High	1	1	5.571	0.0202	*

3.2. Reciprocal-transplant experiments

For transplants between the control and the low irradiation treatment there was no significant interaction between the effects of the population of origin and the environment of transplant on male frequency (Table 7, Figure 16a). However, control populations transplanted into a low irradiation environment had significantly more males than low irradiation populations transplanted into a control environment (Table 7; Figure 16a).

Transplants between the control and the high irradiation treatment, in contrast, showed a significant interaction between the effects of the population of origin and the environment of transplant on male frequency (Table 7; Figure 16b). Male frequency increased in the control populations that were transplanted into the high irradiation treatment but did not change in the high irradiation populations that were transplanted into the control treatment.

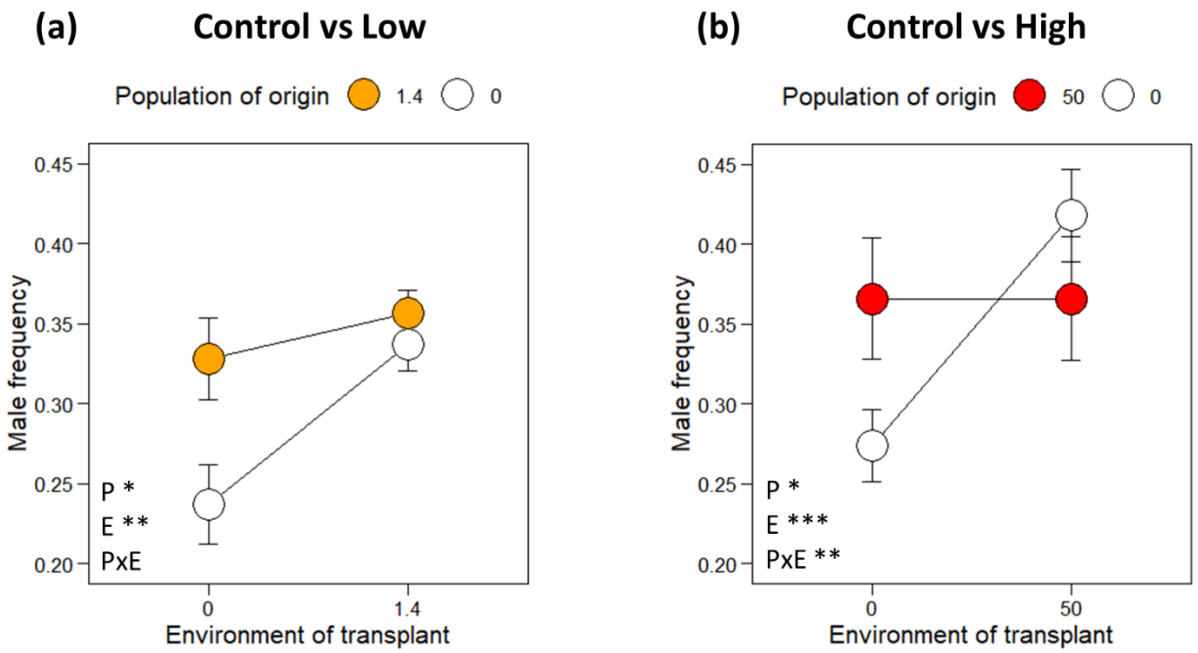

Figure 16: Sex ratio of *C. elegans* populations after four transfers of reciprocal transplant between control and both **(a)** low (a: 1.4 mGy.h^{-1}) and **(b)** high (b: 50 mGy.h^{-1}) radiation treatments. Dots represent the mean male frequency ± S.E for each new treatment. The color of dot represents the populations' treatment during the multigenerational experiment. White: control; yellow: low radiation (1.4 mGy.h^{-1}); red: high radiation (50 mGy.h^{-1}). The significance of each main effect [population of origin (P), environment of transplant (E) and their interaction (P × E)] is indicated at the bottom left of each graph. *, P <0.05; **, P <0.01; ***, P <0.001

Table 7: Results of generalized linear mixed models (GLMM) for male frequency in two reciprocal transplants between the environment of origin and the environment of transplant (between control and 1.4 mGy.h^{-1} and between control and 50 mGy.h^{-1}). *, P <0.05; **, P <0.01; ***, P <0.001

Fixed effect	LR Chisq	Df	P	
Control *versus* 1.4 mGy.h^{-1}				
population of origin	6.474	1	0.0109	*
environment of transplant	7.484	1	0.0062	**
population of origin: environment of transplant	2.601	1	0.1067	
Control *versus* 50 mGy.h^{-1}				
population of origin	3.879	1	0.0489	*
environment of transplant	15.02	1	0.0001	***
population of origin: environment of transplant	6.782	1	0.0092	**

3.3. SP model

The models showed that male frequency rapidly increased with male fertilization success (α: 0.50-0.80) and with a decrease in the proportion of eggs not fertilized by males that were self-

fertilized (β: 0.20-0.10; Figure 17A-C). Temporally stable values of α and β (Figure 17A-C) led to a plateau for male frequency after two to five generations. Increasing α or decreasing β across generations caused changes in sex ratio in the population like what we observed empirically (Figure 17D-F; Figure 15). The slight decrease in male frequency observed in the low irradiation treatment (Figure 15) was like the decrease in sex ratio caused by gradually decreasing α from 0.65 to 0.50 or increasing β between 0.15 and 0.20 (Figure 17D). The slow increase in male frequency observed in the high irradiation treatment (Figure 15) was like the results of the model when α was gradually increased from 0.65 to 0.80 or β was decreased from 0.15 to 0.10 (Figure 17F).

We then used the SP model to examine the effects of α and β in the reciprocal transplant experiment (Figure 18). Holding the values for male fertilization success (α) and the proportion of eggs not fertilized by males that are self-fertilized (β) at those observed in the initial population (i.e. α = 0.52; β = 0.15; Figure 18A, solid line; or α = 0.65; β = 0.20; Figure 18B, solid line), produced a rapid decrease in male frequency from 0.46 to 0.27 in four generations. However, an increase in α (Figure 18A) or a decrease in β (Figure 18B) dampened this effect, and better reproduced what was observed in the reciprocal transplant experiment. For example, for α = 0.80 and β = 0.15 (Figure 18A, long dash line), male frequency at generation 4 was 0.38. Similarly, with α = 0.65 and β = 0.10 (Figure 18B, long dash line), male frequency at generation 4 was 0.39.

Finally, we used the SP model to examine the effects of the rate of nondisjunction of the X chromosome (u) on male frequency (Figure 19). The models showed that male frequency rapidly decreased and then reaches a plateau after ten generations for all values of u. The level of the plateau increased with u. For example, male frequency at generation 20 was 0.16 for u = 0.005 and 0.18 for u = 0.010 (Figure 19, solid line and dot dash line).

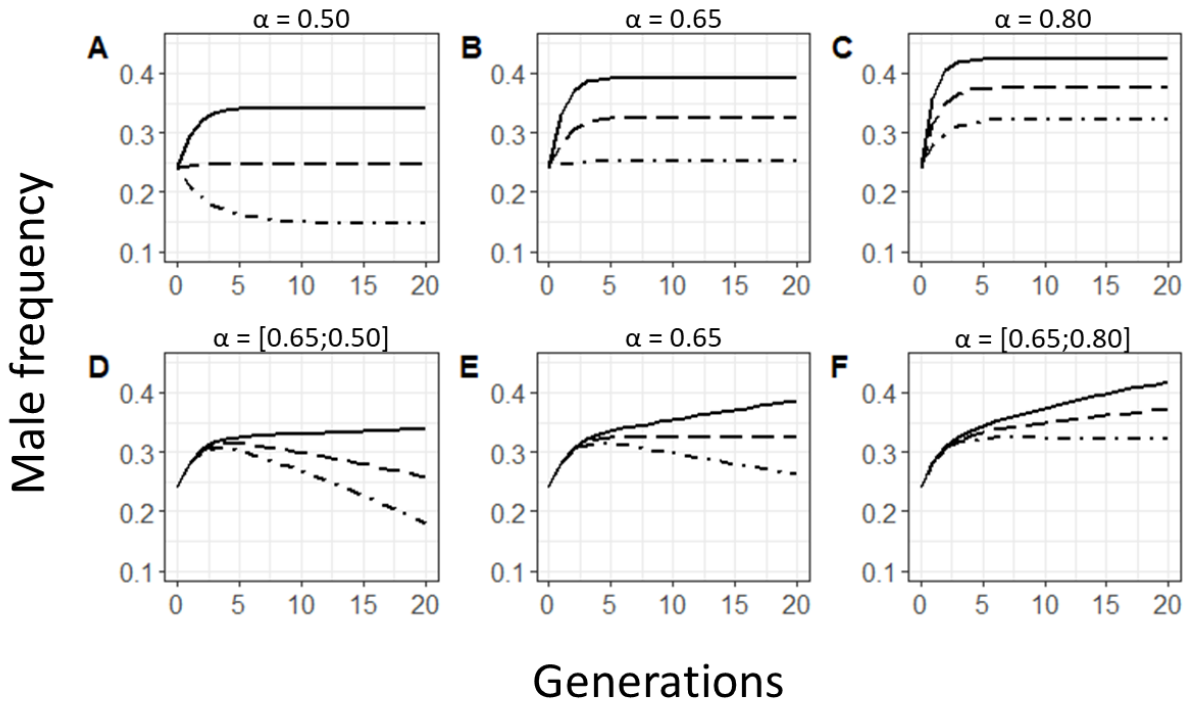

Figure 17: Model results showing changes in *C. elegans* male frequency over 20 generations as a function of male fertilization success (α) and the proportion of eggs not fertilized by males that are self-fertilized (β). This model is adapted from Stewart and Phillips, 2002. In A, B, and C, α was set to 0.50, 0.65, and 0.80, respectively. The three lines correspond to three values of β: 0.10 (solid line), 0.15 (long dash line) and 0.20 (dot dash line). In D, E and F, β decreased from 0.15 to 0.10 (by -0.0025 per generation; solid line), β = 0.15 (long dash line), and β increases from 0.15 to 0.20 (by 0.0025 per generation) (dot dash line). In D) α decreased from 0.65 to 0.50 (by -0.0075 per generation), in E) α = 0.65 and in F) α increases from 0.65 to 0.80 (by 0.0075 per generation).

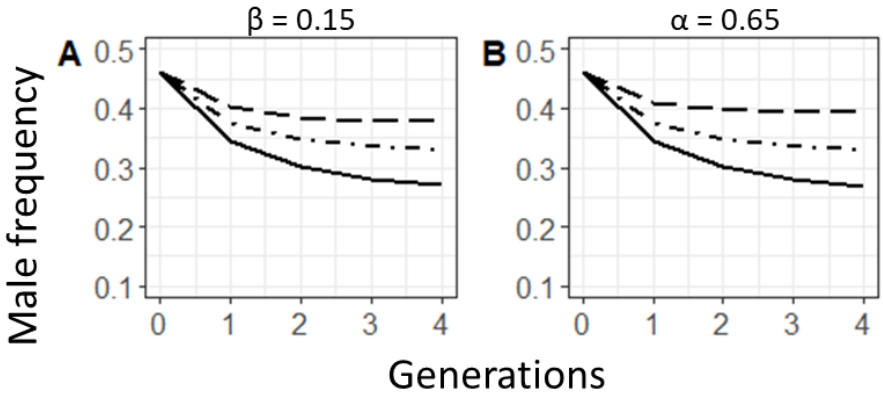

Figure 18: Model results showing the effects of *C. elegans* male fertilization success (α) and the proportion of eggs not fertilized by males that are self-fertilized (β) on male frequency over four generations. This model is adapted from Stewart and Phillips, 2002. **A.** β = 0.15 and α = 0.52 (solid line), 0.65 (dot dash line), or 0.80 (long dash). **B.** α = 0.65 and β = 0.20 (solid line), 0.15 (dot dash line), or 0.10 (long dash).

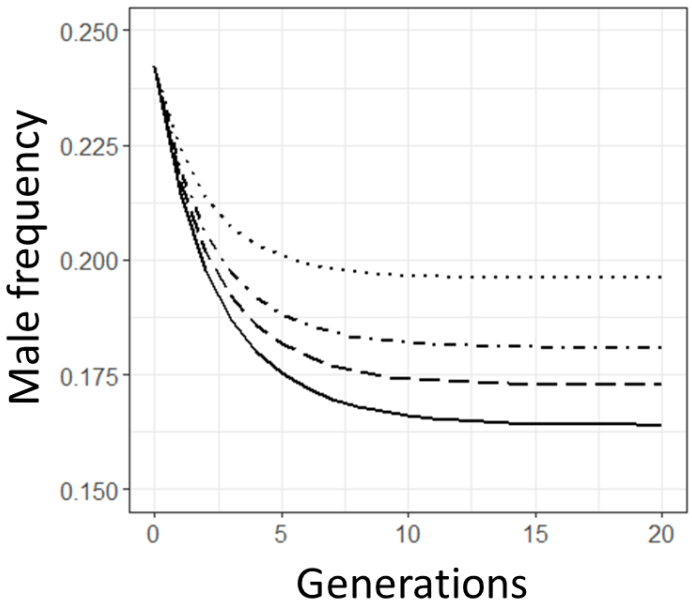

Figure 19: Models showing changes in male frequency over 20 generations in response to changes in the rate of nondisjunction of the X chromosome (u). This model is adapted from Stewart and Phillips, 2002. The four lines correspond to four values of u: 0.005 (solid line), 0.0075 (long dash line), 0.010 (dot dash line) and 0.015 (dot line).

4. Discussion

Our results show that ionizing radiation increased male frequency in *C. elegans* populations (Tables 1 and 2; Figure 15). Before irradiation, males made up slightly less than 25% of the populations. In the two irradiation-treatment groups, the proportion of males increased by more than 10% after only two three-day transfers, and the increase was stronger under high than under low irradiation treatments. Over the subsequent 18 three-days transfers male frequency increased slowly in the high irradiation treatment and decreased slowly in the low irradiation treatment, to reach 43% and 37% overall, respectively. In contrast, male frequency did not change significantly in the control treatment (Figure 15). The reciprocal-transplant experiment showed that switching back to a non-irradiated environment for four transfers did not reduce the high male frequency of the originally highly-irradiated populations. Based on the SP model, these results may be explained by an increase in male fertilization success or a decrease in the proportion of self-fertilized eggs under high gamma radiation conditions that is either irreversible or slow to reverse. These empirical and modelling results suggest that irradiation generates conditions that favor outcrossing in *C. elegans*, by providing a male fitness advantage over hermaphrodites.

4.1. Increased male frequency in response to radiation

Knowing that ionizing radiation has a deleterious impact on the reproduction of *C. elegans* (Buisset-Goussen *et al.*, 2014; Lecomte-Pradines *et al.*, 2017; Maremonti *et al.*, 2019), we hypothesized that irradiation could directly influence the sex ratio. As expected, male frequency increased in the presence of gamma radiation, and this effect was amplified under the higher dose rate (Table 5; Figure 15, Figure 16). This effect is similar to that of other stressors, such as pesticide, starvation, or temperature, on male frequency in *C. elegans* (Rose & Baillie, 1979; Lopes *et al.*, 2008; Morran *et al.*, 2009a). Additionaly, experimental tests investigating the influence of mutations on the evolution of outcrossing have shown an increase in male frequency in populations with a high mutation rate, induced by a genetic polymorphism or by the mutagen ethyl methanesulfonate (Cutter, 2005; Morran *et al.*, 2009b). Male frequency also increases in response to stress in other species (e.g. *Pristionchus pacificus*, another Rhabditida, androdioecious species, under temperature stress Morgan *et al.*, 2017, and *Daphnia magna* exposed to internal alpha irradiation, Alonzo *et al.*, 2008). It thus seems that several environmental stressors lead to the same demographic changes in androdioecious species.

In *C. elegans*, increasing male frequency under ionizing radiation could have two, non-exclusive, proximal causes: (1) an increase in outcrossing rate and (2) an increase in

nondisjunction abnormality rate of the X chromosome. Results from our modified SP model support the first explanation (Figure 17). As previously described, self-fertilization produces about 300 hermaphroditic offspring, and is restricted by the quantity of sperm produced by hermaphrodites (Chasnov, 2013; Barr *et al.*, 2018; Cutter *et al.*, 2019). In contrast, outcrossing with several males can produce up to about 1000 offspring, with 50% of the offspring being male and 50% hermaphrodite (Singson, 2001; Chasnov, 2013; Cutter *et al.*, 2019). In this configuration, hermaphroditic sperm could be the limiting gamete, and any further decrease in production could impact self-fertilized offspring production. In fact, a decrease of about 30% in spermatid number and about 25% in self-fertilized brood size has been observed at dose rate of about 40 mGy h^{-1} (Buisset-Goussen *et al.*, 2014; Maremonti *et al.*, 2019). Ionizing radiation can alter meiosis, decrease sperm activation and viability, and induce germ-cell apoptosis. (Maremonti *et al.*, 2019; Guédon *et al.*, 2021). Under these conditions, if outcrossing persists, the decreased sperm quantity in hermaphrodites will create a fitness advantage for males, causing their frequency to increase. The model shows that increasing male frequency, in turn, increases the prevalence of outcrossing and positive feed-back effects on male frequency. Outcrossing can also be favoured if male *C. elegans* mate preferentially with older or sperm-depleted hermaphrodites (Morsci *et al.*, 2011; Barr *et al.*, 2018). In fact, contact between mature hermaphroditic sperm and oocytes inhibits the production of sexual pheromones and limits attractiveness to males (Morsci *et al.*, 2011; Leighton *et al.*, 2014; Barr *et al.*, 2018). Once their sperm is depleted, hermaphrodites produce more pheromone, stop avoiding males, and expel less seminal mass, all of which facilitates outcrossing (Chasnov, 2013). Since ionizing radiation reduces the number of their spermatids (Maremonti *et al.*, 2019), it may cause hermaphrodites to become receptive to males earlier in their life.

The number of male *C. elegans* in the population may also increase because of nondisjunction abnormality of the X chromosome during meiosis (Broverman & Meneely, 1994). Usually, this step leads to hermaphrodites producing one XX and one Ø gamete, which produce a functional hermaphrodite and a male respectively. The frequency of nondisjunction is modulated by genetic variation (Hodgkin et al., 1979; Mains et al., 1990; Teotónio et al., 2006), and increases under stressful conditions (e.g. high temperature in mice, Golbus, 1983, and *C. elegans*, Cutter *et al.*, 2003, Ayyadevara *et al.*, 2014, or gamma-type radiation stress in *C. elegans*, Kim, 1985), and should therefore boost the production of males in irradiated populations (Rose & Baillie, 1979; Kim, 1985). Kim (1985) showed that an irradiation of 10 Gy doubled the nondisjunction of the X chromosome in *C. elegans*. However, the results of our SP model (Figure 19) suggest that the effect of the nondisjunction of the X chromosome on the

frequency of males is rather small in our system. Doubling the frequency of the nondisjunction of the X chromosome increased the frequency of males by only 2%. It thus seems that non-disjunction is not the main factor explaining the change in sex-ratio in favour of males.

4.2. Evolutionary responses to ionizing radiation

After the second transfer of the multigenerational experiment, male frequency increased slowly in the high irradiation treatment and decreased slowly in the low irradiation treatment. Using the modified SP model, we could reproduce similar trends by incorporating a gradual increase in male fertilization success or a gradual decrease in the proportion of self-fertilization across transfers for the high irradiation treatment, and conversely for the low irradiation treatment. These gradual changes probably reflect population evolution under stressful conditions and trait selection modifying male frequency. Additionally, populations transplanted from the high irradiation treatment to the control treatment maintained a high male frequency (Table 7; Figure 16). Results from the SP model suggest that this stability was caused by an inertia in the level of outcrossing frequency in the population (Figure 18). These results suggest that even once the stressor stopped, males continued to have a fitness advantage over hermaphrodites for several generations. Such an advantage suggests that the selective pressures under high irradiation conditions may have caused genetic changes in both hermaphrodites and males that favored outcrossing over self-fertilization. In agreement with SP model, the slow decrease in male frequency under low irradiation treatment (Table 6; Figure 15, Figure C.1) reflected a decrease of male fertilization success or an increase in the proportion of self-fertilized eggs after many generations. These results could indicate that by adapting to ionizing radiation, outcrossing became less advantageous.

Alternatively, our results could reflect epigenetic effects on hermaphrodite reproduction or demographic inertia; the high proportion of males in the population leading to an unbalance in the reproductive success of both males and hermaphrodites, even generations after the stressor is removed. The high density of males could promote cross-fertilization, maintaining a high male frequency (Cutter *et al.*, 2003; Wegewitz *et al.*, 2008). However, since the stressor is no longer present, self-fertilization reproduction should become advantageous over outcrossing, leading to a progressive return to the original male frequency (Cutter *et al.*, 2003; Wegewitz *et al.*, 2008). In our reciprocal transplantation, this could explain the slight decrease observed in the frequency of males for the low irradiation treatment (Figure 16a). Given the relatively high male frequency in the high irradiation treatment before the reciprocal transplant experiment, it may have increased the inertia and lead to an even slower decrease in male frequency (Figure 16b). However, our modelling of reciprocal transplants (Figure 18) predicts a more

rapid decrease in male frequency in the absence of population evolution than what we see in our experiment. This suggests that the inertia hypothesis alone cannot explain our results.

An increase in male frequency was also observed in *C. elegans* populations that evolved a resistance to Levamisole, an anti-parasitic pesticide (Lopes *et al.*, 2008). The authors argued that outcrossing was advantageous under these conditions, and that the increase in male frequency was "an expression of the evolution of pesticide resistance" (Lopes *et al.*, 2008). Other studies have indicated that male reproductive efficiency and the availability of hermaphrodites for outcrossing had a genetic basis (Wegewitz *et al.*, 2008; Gimond *et al.*, 2019). More generally, it has been strongly suggested that outcrossing is beneficial for fitness under stressful conditions, and facilitates adaptation to stress (Morran *et al.*, 2009a; Plesnar-Bielak *et al.*, 2017). Our results, however, suggest that a shift in the relative fitness of male and hermaphrodite reproductive strategies can explain the increase in male frequency, which in turn increases the amount of outcrossing. Rather than being the direct adaptive response of the population to novel stressors, outcrossing would be the by-product of stress-induced selection pressures on males and hermaphrodites and potential sexual conflict between them. This explanation has the advantage of not implying problematic group-selection mechanisms (Grafen 1984). The changes in male frequency observed in our experiments could also help us to understand the large disparity in male frequency among wild populations (Sivasundar & Hey, 2005; Wegewitz *et al.*, 2008; Anderson *et al.*, 2010). The relative proportion of males and hermaphrodites may have evolved under different stress regimes, with populations showing the highest rate of stressful events showing higher male frequencies than populations living under less stressful conditions.

5. Conclusion

Our results showed that ionizing radiation increased male frequency in a *C. elegans* population. This effect was amplified by increasing dose rate. The results of our mathematical model suggest that the increase in frequency of males could be explained by an increase in fertilization success of males or a decrease in self-fertilization. Moreover, after radiation was removed, males persisted at high proportions, as predicted by the mathematical model if an increase in the fertilization success of males or a decrease in the prevalence of self-fertilization has taken place. This suggests that outcrossing in androdioecious populations may represent a demographic or evolutionary response to stress, such as exposure to ionizing radiation. These results show that ionizing radiation can cause prolonged changes in the reproductive strategy of a population, likely impacting its long-term evolutionary history.

Our results highlight the importance of studying the evolutionary responses to pollution and the mechanisms involved for a robust assessment of the ecological risks of pollutants for populations. We strongly encourage the consideration of evolutionary parameters in the processes of ecological risk assessment of pollutants and more broadly of all anthropogenic stressors (climate change, etc.).

6. Acknowledgements

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Chapitre 4 - Host defense alteration in *Caenorhabditis elegans* after evolution under ionizing radiation

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Key words - Experimental evolution, evolutionary cost, fitness, host defense, Caenorhabditis elegans.

Abstract

Adaptation to a stressor can lead to costs on other traits. These costs play an unavoidable role on fitness and influence the evolutionary trajectory of a population. Host defense seems highly subject to these costs, possibly because its maintenance is energetically costly but essential to the survival. When assessing the ecological risk related to pollution, it is therefore relevant to consider these costs to evaluate the evolutionary consequences of stressors on populations. However, to the best of our knowledge, the effects of evolution in gamma-irradiated environment on host defense have never been studied. Using an experimental evolution approach, we analyzed fitness across 20 transfers (about 20 generations) in *Caenorhabditis elegans* populations exposed to 0, 1.4, and 50.0 $mGy.h^{-1}$ ionizing radiation. Then, populations from transfer 17 were exposed to the bacterial parasite *Serratia marcescens* and their survival estimated to study host defense. We found a lower fitness in both irradiated treatments compared to the control ones, but fitness increased over time in the 50.0 $mGy.h^{-1}$. Survival rate to *S. marcescens* was lower in both 1.4 and 50.0 $mGy.h^{-1}$ compared to the control. However, we did not show a trade-off relation between population fitness at the end of multigenerational experiment and host defense. These results suggested that population have adapted to ionizing radiation, but at the same time host defense has decreased. However, there does not seem to be an evolutionary trade-off between adaptive efficiency to irradiation and host defense.

1. Introduction

Wildlife is currently confronted with multiple environmental stressors such as pollution, starvation or pathogens. Within a few generations, the populations can respond to these stressors by adapting through the selection of traits that are advantageous under these new conditions (Kawecki and Ebert, 2004). Potentially, this process allows populations to be adapted to local conditions, but poorly adapted to other conditions, i.e. adaptive cost (Bennett and Lenski, 2007; Dutilleul et al., 2017; Kawecki and Ebert, 2004). Indeed, selection and adaptation can be associated with a decrease in genetic diversity, which may decrease the chances of adapting to new stressors (Jansen et al., 2011; Salice et al., 2010). Additionally, adaptation to new stressors may induce an evolutionary trade-off. This process describes situations where allocating more resources to a biological function reduces the amount of resources provided to another function, or where genes involved in increasing the value of a fitness-related traits are also involved in reducing the value of another fitness-related trait (Garland, 2014; Reznick et al., 2000; Roff and Fairbairn, 2007). Adaptive cost and evolutionary trade-offs can constrain the evolutionary trajectories of a population (Roff and Fairbairn, 2007; Walsh and Blows, 2009). For example, Dutilleul et al. (2017) have shown that *Caenorhabditis elegans* populations evolving for 22 generations adapted to their salt or uranium environment. In addition, populations evolving in a salt environment had lower fitness in a uranium environment, indicating an adaptive cost. In contrast, fitness in the salt environment was similar between uranium and salt exposed populations, indicating no adaptive cost for uranium-adapted populations. The identification and integration of these mechanisms are essential to characterize the long-term responses of populations to environmental stressors. However, the evolution and functioning of these trade-offs and associated costs is still relatively poorly understood, both theoretically and empirically (Roff et Fairbairn, 2007; Walsh et Blows, 2009).

The host defense, the protection of an organism against infections, is a trait relevant to study adaptive costs and trade-offs. Studies have observed evolutionary trade-offs between host defense and life history traits in bacteria (*Pseudomonas syringae*: Koskella et al., 2012), plant (*Arabidopsis thaliana*: Tian et al., 2003), and animals (*Biomphalaria glabrata*: Langand et al., 1998, *Drosophila melanogaster*: Kraaijeveld and Godfray, 1997; Vijendravarma et al., 2009, *Gallus domesticus:* Verhulst S. et al., 1999). The presence of these trade-offs may be related to the fact that host defense is both costly and essential to the survival of the organism (Lochmiller and Deerenberg, 2000; Schwenke et al., 2016). Organisms continually interact with parasites that can impose detrimental effects to them. These interactions favor selection and evolution of high diversity of defense mechanisms to increased host defense to maintain their fitness under infection (Penley et al., 2018). However, host defense is resource expensive with costs of

upregulating the immune system upon parasitic infection (i.e., inducible costs) and costs of maintaining immunological machinery even lacking parasitism (i.e., constitutive costs) (Antonovics and Thrall, 1994; Sandland and Minchella, 2003a). However, the mechanisms inducing these costs associated with host defense are poorly understood. For example, several studies did not observe significant costs of host defense on life history traits when populations were exposed to parasites (*Caenorhabditis elegans:* Penley et al., 2018, *Drosophila melanogaster*: Faria et al., 2015; Gupta et al., 2016, *Poecilia reticulata*: Dargent et al., 2013, *Trichoplusia ni*: Milks et al., 2002). The absence of detection of host defense costs can be related to a lack of genetic variation on traits which a trade-off can be manifested (Penley et al., 2018). Alleles that confer greater defense could be strongly selected for in previous exposure and could lead to selective sweeps. In this case, the lack of diversity within the host population could prevent the observation of a trade-off (Penley et al., 2018). Also, environmental variables could influence the detection of defense costs in host-parasite system (Sandland and Minchella, 2003a). For example, Sandland and Minchella (2003b) have shown a trade-off between immune responses and life history traits in *Lymnaea elodes* population exposed to the parasite *Echinostoma revolutum*. The detection of this trade-off depended of nutrient availability. Studies investigating the costs associated with the host defense rarely incorporate other variations in environmental conditions (Sandland and Minchella, 2003a), which may expose the existence of trade-offs.

Ionizing radiation and the oxidative stress it induces can have negative impacts on the host defense, up to causing immunosuppression at the highest doses (Cui et al., 2017; Mittal et al., 2013). These negative impacts are observed on innate and acquired immunity at molecular (gene expression, antibodies, antigens, cytokines…), cellular (leucocytes, T cells…) and tissue level (spleen, thymus, marrow…) (Anderson et Warner, 1976; Lumniczky et al., 2021; Manda et al., 2012). On wildlife, rare studies in the Chernobyl exclusion zone (Ukraine) have shown a decrease of immune response in *Hirundo rustica* populations and identified evidence of oxidative stress and immunosuppression in *Myodes glareolus* populations (Camplani et al., 1999; Kesäniemi et al., 2019). Also, a previous irradiation can alter host defense to parasites. Liu *et al.* (2022) have shown, for example, a decrease of *C. elegans* survival to *Pseudomonas aeruginosa* after gamma irradiation at 50 Gy. In contrast, studies have shown that ionizing radiation can also stimulate host defense and immune response (Shibamoto and Nakamura, 2018). For example, Seong et al. (2012) have shown an increased survival rate of *Drosophila melanogaster* to *Staphylococcus aureus* or *Pseudomonas aeruginosa* after being irradiated (gamma rays) at 0.2 Gy. Similarly, Kimura et al. (2012) observed an activation of innate immunity genes and an increased survival of *C. elegans* to *Pseudomonas aeruginosa* after being

irradiated (X-rays) at 100 Gy. Host defense plays an unavoidable role in survival and fitness, influencing evolutionary trajectory of populations. In a context of ecological risk assessment of radioactive pollution, it is therefore relevant to study the evolutionary responses of the host defense to this environmental stressor. However, to the best of our knowledge, the effects of evolution in gamma-irradiated environment, particularly adaptive cost on host defense have never been studied.

In this study, we investigated the potential impact on host defense of *C. elegans* populations after multigenerational exposure to ionizing radiation. In previous studies, we showed that long-term irradiation can modify life history traits in *C. elegans* (e.g. sex ratio, population growth rate, hatching success or fecundity) and alter evolutionary trajectories through selection and adaptation mechanisms (Quevarec et al., 2022, Quevarec et al., *in prep*).

Here, we tested whether these evolutionary changes have induced host defense costs. For this purpose, we infected with the bacteria *Serratia marcescens,* both *C. elegans* treatments chronically exposed to ionizing radiation during about 17 generations and treatments that stayed in a non-irradiated environment. The survival rate of the nematodes in each population allowed us to estimate the efficiency of their host defense. We also hypothesized that adaptive evolution to irradiation should trade-off with immune defense. We, thus, compared the host defense (survival) to infection by *S. marcescens* between control and irradiated populations, and tested for the presence of a negative correlation between fitness of irradiated populations and survival in bacteria-infected populations.

2. Material and methods

2.1. Test organism and population maintenance

We cultured population of *C. elegans* A6140 on 6 cm Petri dishes at 20 °C and 80% relative humidity to have a generation time of approximately 3 days (Byerly et al., 1976; Noble et al., 2017; Teotonio et al., 2012). Petri dishes were filled with 12 mL of nematode growth medium (NGM) and seeded with *Escherichia coli* bacteria (OP50 strain) ad libitum (Quevarec et al., 2022). Before exposure to ionizing radiation, the stock population was maintained for at least 25 three-day transfers (around 25 generations). Every three days, we washed nematodes off the Petri dishes with an M9 solution. We then estimated the number of individuals and we transferred 1000 nematodes at all developmental stages into two new dishes to ensure they were fed *ad libitum*.

2.2. Irradiation conditions

The external gamma radiation exposure was conducted at the Mini Irradiator for Radio Ecology ^{137}Cs irradiation facilities, at the *"Institut de Radioprotection et de Sûreté Nucléaire"* (MIRE, IRSN, Cadarache, France). We used the same irradiation facilities and the same protocol as previously described by Quevarec et al. (2022). The Petri dishes were placed vertically in the irradiator to homogenize the dose received over the entire dish. Placing the plates at different distances from the source and separated by shields (Petri dish filled with lead filings) allowed us to obtain the required dose rates.

2.3. Multigenerational experiment: fitness index estimate

We used three dose rate gamma radiation treatments: control (0.0 $mGy.h^{-1}$), low irradiation (1.4 $mGy.h^{-1}$), and high irradiation (50.0 $mGy.h^{-1}$). For each treatment, we created five independent replicates taken from the stock population and maintained for over 60 days, with a transfer to new Petri dishes once every three days. At the beginning of each transfer, each replicate contained initially 1000 worms equally distributed into two Petri dishes (initial density of 500 worms/plate) (Quevarec et al., 2022).

We estimated fitness (i.e., realized fecundity x survival rate) at transfer 0, 2, 5, 8, 11, 14, 17 and 20. Realized fecundity corresponded to the number of eggs / 1000 hatched individuals / unit time (i.e., from larval to adult stage; population estimated after 3 days of growth), based on the definition of Tarsi and Tuff (2012) and described in Quevarec et al. (*in prep*). Survival

rate corresponded to the hatching success at transfer 0, 2, 5, 8, 11, 14, 17 and 20, as described in Quevarec et al. (*in prep*).

2.4. Estimation of the immune response — survival assays

At transfer 17, populations from each replicate and each treatment were cultured in control condition for about 10 generations and were sent to Morran's laboratory at Emory University (Atlanta, USA) to study host defense.

To estimate the effects of irradiation on *C. elegans* host immune response, we assessed the 48-hour survival of the experimental populations and of the ancestral population. We used the bacterial parasite, *Serratia marcescens* strain SM2170, to infect *C. elegans* on Serratia Selection Plates (SSPs) (Morran et al., 2009b). SSPs are 10 cm Petri dishes containing NGM-Lite agar (US Biological, Swampscott, MA). One side of the SSP was seeded with 30 μL of an overnight culture of SM2170. The opposite side of the SSP was seeded with 50 μL of an overnight culture of OP50 *Escherichia coli* to serve as a relatively benign food source for nematodes surviving parasite exposure. The plates were incubated at 28°C for 24 hours. Then, 20 μL of ampicillin (200 μg/mL) was applied to the middle of the plate in an area without SM2170 or OP50, as a means to stop the spread of SM2170 into OP50. Finally, approximately 200 (ranging from 154 to 336) L4 and young adult nematodes were transferred onto the SM2170 side of the plate and maintained on the SSP for 48 hours at 20°C. After 48 hours, we counted the number of live worms on each SSP and determined the 48-hour survival rate for each technical replicate (Penley et al., 2017). Survival rate was measured as the number of living worms divided by the total number of worms plated.

We assayed 49 SSPs (4 SSPs replicates for the ancestral population + 3 SSPs replicates x 15 replicates of experimental populations).

2.5. Evolutionary trade-off

At transfer 17, we estimated a standardized fitness of each population from the fitness index described above. We calculated standardized fitness *x* as:

$$x = \frac{X - \mu}{\sigma}$$

Where X is the value of fitness index to standardize, μ is the mean fitness index of the treatment (control, low or high irradiation) and σ is the standard deviation of fitness index to a treatment. This calculation allows us to compare the fitness of populations independently of the variation induced by the treatment (i.e., confounding factors). We studied the correlation

between standardized fitness of irradiated populations and survival in bacteria-infected populations.

2.6. Statistical analysis

Before the analysis, we log-transformed data of fitness index. We used a Mixed Generalized Additive Model (GAMM) with R software (Team, 2013) and the Mgcv package (Wood et al., 2016) to analyze fitness index with Gaussian distribution. No overdispersion of the data was observed. We analyzed fitness index as a function of number of transfers (a continuous variable previously standardized), irradiation treatment (i.e., control, low and high irradiation) and their interaction as fixed effects. ID of the replicate and measurement group ID were added as random effects. The smoothing was performed on the variable transfer in function of treatment.

We analyzed survival rate and the correlation between the survival rate of infected worms and the standardized fitness at transfer 17 with quasi-binomial (logit link function) distribution for both, using a Generalized Linear Mixed Model (GLMM) (Ade4 package ; Bougeard and Dray, 2018; Dray and Dufour, 2007 and glmmTMB package ; Brooks et al., 2017). No overdispersion of the data was observed. Survival rate was analyzed as a function of irradiation treatment (i.e., ancestor, control, low and high irradiation) as fixed effects, and the ID of the replicate as a random effect. For correlation between the survival rate and the standardized fitness, survival rate was analyzed as a function of irradiation treatment (i.e., control, low and high irradiation), standardized fitness, and their interaction as fixed effects and replicate ID as a random effect.

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

3. Results

3.1. Multigenerational experiment

Control, low and high irradiation treatments showed a fitness index equal to 2323, 1902 and 1667, respectively (Table 8a; estimations shown have been transformed with the inverse-log function). Fitness index was significantly higher in the control than in the low and high irradiation treatments (Table 8; Figure 20). In the control populations, fitness index varied significantly across transfers increasing slightly from transfers 1 to 10 and decreasing slightly from transfers 10 to 20 (Figure 20 , Figure D.1). Fitness index increased significantly until transfer 8 then stabilized in high irradiation treatments (Table 8b; Figure 20, Figure D.1). In the low irradiation treatments, fitness index did not vary significantly across transfers (Table 8b; Figure 20, Figure D.1).

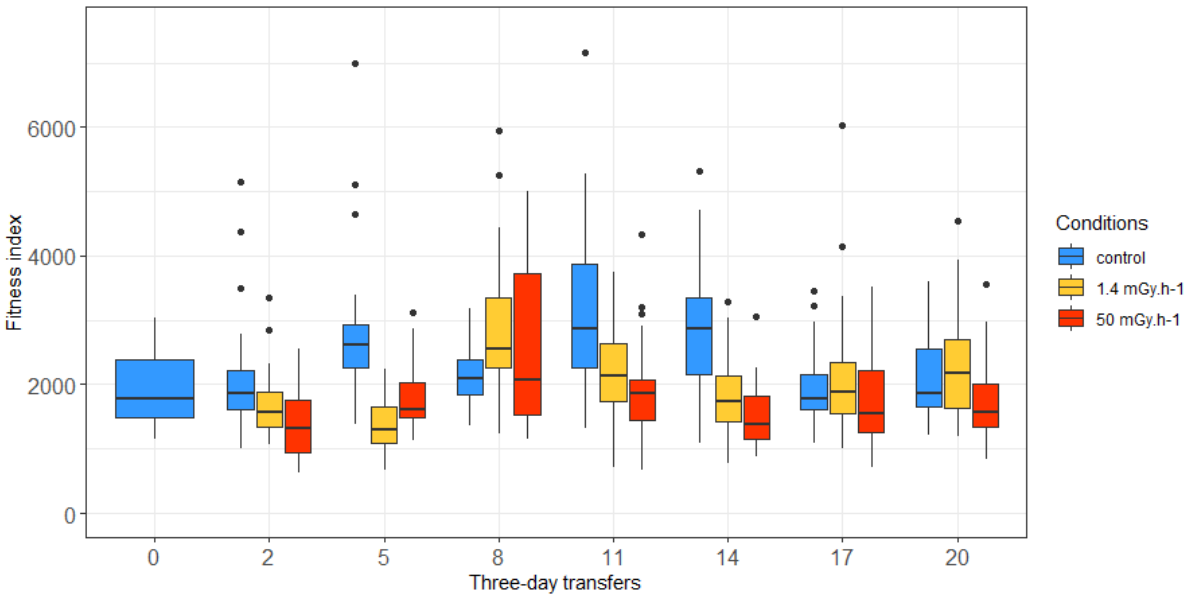

Figure 20: Boxplot of fitness index over time (i.e., three-day transfers: 0, 2, 5, 8, 11, 14, 17 and 20) for *C. elegans* populations living in different gamma radiation environments. Blue: control; yellow: low radiation (1.4 $mGy.h^{-1}$); red: high radiation (50.0 $mGy.h^{-1}$).

Table 8: Effects of **(a)** gamma irradiation condition (0.0, 1.4 and 50.0 mGy.h^{-1}) and **(b)** Time (EDF: Effective degrees of freedom) on *C. elegans* population fitness index during the 20 transfers of a multigenerational experiment. *, P <0.05; **, P <0.01; ***, P <0.001

a)	Estimate	Std. Error	t value	Pr(>|t|)	
(Intercept)	7.750	0.042	185.35	<2.00 e-16	***
Gamma Low	-0.200	0.059	-3.39	0.0007	***
Gamma High	-0.331	0.059	-5.66	2.36 e-08	***

Approximate significance of smooth terms

b)	edf	Ref.df	F	p-value	
s(Time):Control	2.409	2.409	3.678	0.017	*
s(Time):Gamma Low	1.000	1.000	3.689	0.055	
s(Time):Gamma High	3.470	3.470	4.223	0.003	**

3.2. Estimation of the immune response — survival assays

Survival rate was estimated at 0.81 for the ancestor population, 0.81 for the control, and 0.64 and 0.59 for the low and high irradiation treatments, respectively (Table 9). The irradiated populations showed a significantly lower proportion of live individuals than the control populations. This corresponded to 17% decline in survival in the low and a 22% decline in survival in the high irradiation treatment compared to the control populations (Figure 21). The ancestral population did not differ significantly from the control populations (Table 9; Figure 21).

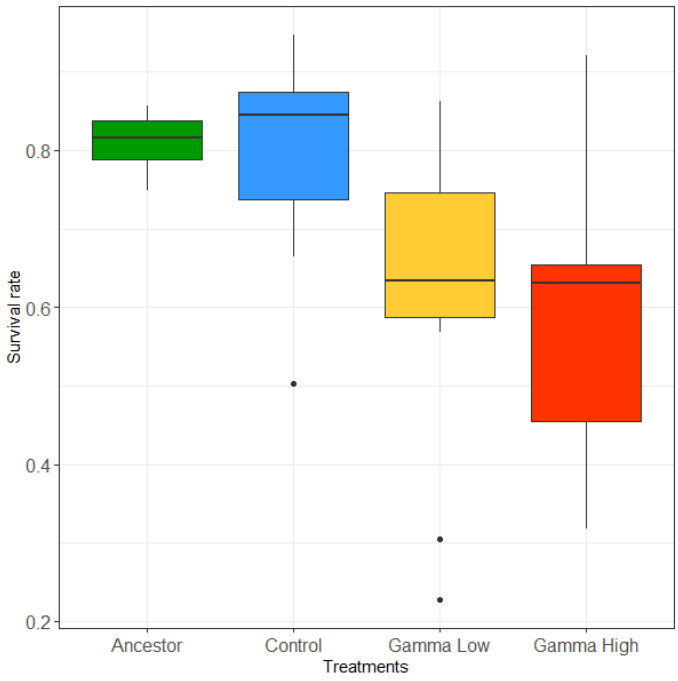

Figure 21: Boxplot of survival rate after two days of exposure to *S. marcescens* for *C. elegans* from the ancestral population (green; at transfer 0) or from gamma irradiated populations at transfer 17 (Blue: control; yellow, low radiation, 1.4 mGy.h^{-1}; red, high radiation, 50.0 mGy.h^{-1}).

Table 9: Effect of original environment on survival rate after two days of exposure to *S. marcescens* for *C. elegans* populations from transfer 0 (ancestor) or from transfer 17 that evolved in different gamma radiation environments (0.0, 1.4 and 50.0 mGy.h^{-1}). *, P <0.05; **, P <0.01; ***, P <0.001

	Value	Std. Error	DF	t-value	p-value	
(Intercept)	1.453	0.269	33	5.402	0.000	***
Gamma Low	-0.857	0.376	12	-2.283	0.042	*
Gamma High	-1.071	0.369	12	-2.903	0.013	*
Ancestor	-0.005	0.620	12	-0.008	0.994	

3.3. Evolutionary trade-off

Survival rate of infected worms showed a non-significant increase with standardized fitness at transfer 17 in the control populations (inverse-logit transformed slope coefficient = 0.74; Table 10). Survival rate of infected worms increased with standardized fitness at transfer 17 in the low and high irradiation treatment, although this increase was not significantly different from zero (0.90 and 0.89, respectively; Figure 22). Survival rate was estimated at 0.81 for the control, and 0.65 and 0.59 for the low and high irradiation treatments, respectively (Table 10).

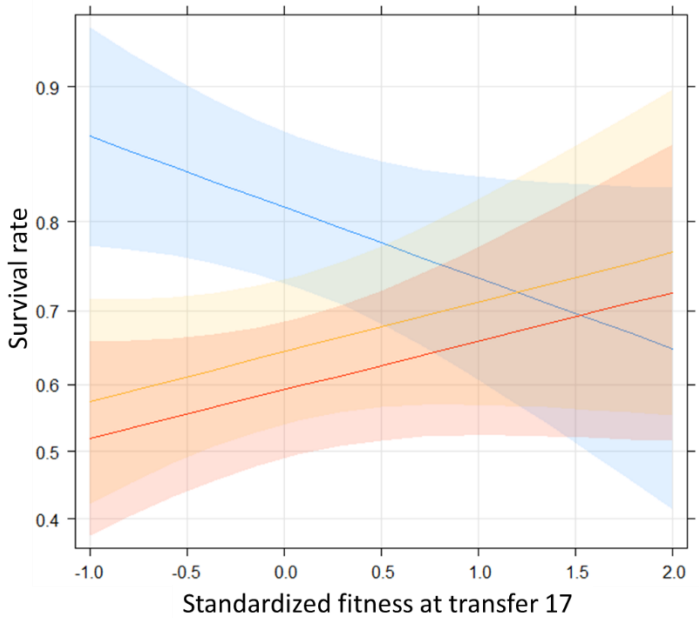

Figure 22: This graphical representation is estimated by GLMM. Shaded areas represent 95% confidence intervals. Lines showing the relationship between survival rate after two days of exposure to *S. marcescens* and standardized fitness for *C. elegans* populations at transfer 17 that evolved in different gamma radiation environments. Blue: control; yellow: low radiation (1.4 mGy.h^{-1}); red: high radiation (50.0 mGy.h^{-1}).

Table 10: Effect of gamma irradiation condition (0, 1.4 and 50 $mGy.h^{-1}$) on standardized fitness and survival rate after two days of exposure to *S. marcescens* for *C. elegans* populations from transfer 17. *, P <0.05; **, P <0.01; ***, P <0.001

	Value	Std. Error	DF	t-value	p-value	
(Intercept)	1.472	0.240	30	6.131	0.000	***
Gamma Low	-0.871	0.332	9	-2.621	0.028	*
Gamma High	-1.100	0.324	9	-3.393	0.008	**
Fitness	-0.426	0.236	9	-1.805	0.105	
Gamma Low:Fitness	0.726	0.332	9	2.188	0.057	
Gamma High:Fitness	0.719	0.319	9	2.257	0.050	

4. Discussion

We showed that ionizing radiation decreased the fitness of *C. elegans* populations (Table 8; Figure 20). Fitness decreased by 18% and 28% for low and high irradiation treatments compared to the control treatment, respectively. When exposed to the bacterial parasite *S. marcescens* populations that have evolved in an irradiated environment showed a decline in survival compared to the control populations. These results validate our hypothesis that adaptation to ionizing radiation was associated with a cost on the effectiveness of host defense. However, we did not observe any significant correlation between fitness of irradiated populations at the end of the multigenerational experiment (i.e. adaptation efficiency to irradiation) and host defense for the three treatments (Figure 22). These results indicate that there was no visible cost to being better adapted to irradiation on the defense host.

4.1. Decreased fitness in response to ionizing radiation

In multigenerational experiment, our results showed a lower fitness in two irradiated conditions compared to the control conditions, and this effect increased with the dose rate. To our knowledge, studies describing the effects of ionizing radiation on fitness are scarce (Goodman et al., 2019). However, some studies have shown a decrease in reproduction following irradiation in many species (Dallas et al., 2012; Real et al., 2004), specifically in *C. elegans* from 42.7 $mGy.h^{-1}$ (Buisset-Goussen et al., 2014; Dufourcq-Sekatcheff et al., 2021; Guédon et al., 2021; Maremonti et al., 2019), and in a multigenerational experiment from 1.4 $mGy.h^{-1}$ with the *C. elegans* A6140 population (Quevarec et al., *in prep*). Also, studies have shown a decrease in survival following irradiation in *C. elegans*. Clejan et al. (2006) showed a decrease of about 55% in the survival rate of *C. elegans* (N2 strain) exposed to an acute ionizing radiation dose of 60 Gy. We demonstrated a 7% decrease on hatching success (i.e., survival of larvae) in populations of *C. elegans* A6140 exposed for 20 generations to a dose rate of 50 $mGy.h^{-1}$ (Quevarec et al., *in prep*). These results corroborate that a dose rate of 50 $mGy.h^{-1}$ (i.e., high irradiation treatment) has a negative impact on the population fitness of *C. elegans*. For low irradiation treatment (i.e., 1.4 $mGy.h^{-1}$), the decrease in fitness in *C. elegans* is consistent with our previous studies, where we observed a decrease in realized fecundity for 20 generations to a dose rate of 1.4 $mGy.h^{-1}$ (Quevarec et al., *in prep).* In contrast, no effect was observed in *C. elegans* (N2 strain) on hatching success between 6.6 and 45 $mGy.h^{-1}$ for shorter experiments (three generations: Buisset-Goussen et al., 2014; one generation: Dubois et al., 2018), and on the number of larvae per hermaphrodite (i.e., reproduction) at 28 $mGy.h^{-1}$ (three generations: Buisset-Goussen et al., 2014). However, other studies have shown a decrease of survival of larvae at much lower chronic dose rates (gamma radiation) in other worms: 0.19

mGy.h^{-1}, 3.2 mGy.h^{-1} and 4 mGy.h^{-1} in *Neanthes arenaceodentata* (Polychaeta), *Ophryotrocha diadema* (Polychaeta) and *Eisenia fetida* (Oligochaeta), respectively (Harrison and Anderson, 1994; Hertel-Aas et al., 2007; Knowles and Greenwood, 1994).

Our results also showed that fitness increased until transfer 8 then stabilized in high irradiation treatments. Results and the shape of the curve suggested a local adaptation of populations to ionizing radiation, as described by Silander et al. (2007). Authors showed that fitness of populations adapting to a constant environment reaches a plateau due to a change in the ratio of beneficial to deleterious mutations. Transgenerational effects could also explain fitness changes in high irradiation treatments. For example, Yue et al. (2021) has shown oscillatory changes in *C. elegans* reproduction exposed for 11 generations to 1-ethyl-3-methylimidazolium bromide-related transgenerational effect. For low irradiation treatments, the similar but nonsignificant increase observed for fitness suggest process in the same direction but slowed at low doses rate. These results contrast with our previous studies, where we observed an adaptive response on embryo survival and a slower life history of populations that live in low irradiation conditions (Quevarec et al., *in prep*). According to the intensity of ionizing radiation, these opposing conclusions suggested that evolutionary response (i.e., transgenerational effects or adaptation) could take place on different traits. Indeed, it is known that the response of organisms may be different according to the ionizing radiation dose (Elgazzar and Kazem, 2015; Liu et al., 2019; Tubiana et al., 2006).

4.2. Evolution of the host defense

The defense of the organism is essential for its survival and can be estimated by exposing individuals to a pathogen (Penley et al., 2017). We showed that *C. elegans* populations that evolved in an irradiated environment survived less well to *S. marcescens* than control populations (Figure 21). We observed a decrease in survival rates for both irradiated conditions. These results suggested that long-term exposure to ionizing radiation decreased the effectiveness of the population defense. It was likely that these changes had a genetic cause, since populations from the different irradiation treatments (0.0, 1.4 and 50.0 mGy.h^{-1}) were placed in same environmental conditions without irradiation (i.e., common garden) for 10 generations before the beginning of exposure to the pathogen. Indeed, it is known that there is considerable variation in *C. elegans* responses to pathogenic bacteria, notably linked to genetic variability (Meisel and Kim, 2014; Penley et al., 2018; Schulenburg and Ewbank, 2004). However, we cannot totally exclude that at least part of the changes were related to long-term transgenerational epigenetic effects (Klosin et al., 2017). This suggests that adaptation to ionizing radiation was associated with an evolutionary cost on the effectiveness of host defense.

Several studies have also shown deleterious effects of ionizing radiation on defense of the organism in one-generation experiment. Liu et al. (2022) found a decrease in survival of *C. elegans* infected with *Pseudomonas aeruginosa* when irradiated at a dose of 50 Gy with gamma radiation. Rossmoore and Hoffman (1971) showed increased mortality of *O. leucostigma* larvae exposed to the pathogen *Bacillus thuringiensis* ten days after a gamma irradiation acute dose of 300 Gy. More specifically, studies showed deleterious effects of ionizing radiation on immune response in *Oncorhynchus mykiss* at 4.66 $mGy.h^{-1}$ on humoral immune response (Knowles, 1992), in *Myodes glareolus* between 15 $\mu Gy.h^{-1}$ and 18 $\mu Gy.h^{-1}$ on pathways associated with immunity (e.g., impaired antigen processing and activation of leucocytes involved in inflammatory responses) (Kesäniemi et al., 2019) or in *Hirundo rustica* between 2.6 µGy et 4.4 µGy on lymphocyte, immunoglobulin and spleen (Camplani et al., 1999). Interestingly, control populations had a similar survival rates than the ancestral population, suggesting that without the stressor, the immune response remained stable over time.

4.3. Absence of evolutionary trade-off between adaptive efficiency to irradiation and host defense

It is known that the host defense and reproduction of the organism require a significant resource investment (Naim et al., 2020), and that frequently a trade-off in resource allocation exists between these two functions (see introduction). Thus, it is particularly relevant to study whether an evolutionary trade-off exists between fitness and the immune response. However, our results did not show a negative correlation between fitness of irradiated populations at transfer 17 and survival in bacteria-infected populations. These results showed that the populations that were best adapted to ionizing radiation were not less resistant to *S. marcescens*. This suggests that the efficiency of adaptation to ionizing radiation was not associated with an evolutionary cost on host defense. Similarly, Penley et al. (2018) have shown with a multigenerational experiment in *C. elegans* populations in the presence of *S. marcescens* that elevated levels of host defense are not associated with a fitness cost. This absence of cost associated with host defense after experimental evolution with parasites has been observed in *Drosophila melanogaster* (Faria et al., 2015; Gupta et al., 2016), *Poecilia reticulata* (Dargent et al., 2013) or *Trichoplusia ni* (Milks et al., 2002). It was interesting to note the presence of a trend toward improved host defense with increasing fitness for the low and high irradiation treatment. These trends suggested that *C. elegans* populations better adapted to ionizing radiation also seemed to survive better to *S. marcescens.*

Alternatively, our approach may have some limitations, which might explain their failure to support the trade-off hypothesis. The experimental evolution approach does not always allow

detecting and evaluating the efficiently the evolution of resistance and associated costs (Kawecki et al., 2012; Penley et al., 2018). For example, laboratory experimental evolution limit the number of selective pressures acting on the population, which could increase the likelihood of evolving resistance without associated costs (Penley et al., 2018). Outside the experimental conditions, evolutionary mechanisms can make it difficult to detect trade-offs. For example, Novak et al. (2006) explained that the absence of a trade-off could be caused by the selection for other traits might indirectly affect the traits of interest. In our case, for example, the selection of a trait involved in improving fitness to ionizing radiation could also improve host defense (i.e., exaptation: Gould and Vrba, 1982). Additionally, Novak et al. (2006) suggested a further explanation for the absence of a trade-off is that if the population was not well adapted to the selective environment, it might have been able to improve one trait without degrading the second trait. Thus, it is conceivable that the populations had not evolved long enough in an irradiated environment to observe a trade-off between the studied traits.

5. Conclusion

Our results showed that the fitness of irradiated *C. elegans* populations increased over time, but remains lower overall than fitness of control populations. The evolution in irradiate environment has resulted in greater susceptibility to the pathogen *S. marcescens*. This effect was amplified by increasing the dose rate. These results suggested the presence of an evolutionary trade-off between adaptation to ionizing radiation and *C. elegans* defense. However, we did not show any trade-off between fitness at the end of multigenerational experiment and *C. elegans* survival to *S. marcescens*, suggesting that a better adaptive efficiency to ionizing radiation was not related to evolutionary costs on host defense. Organisms are often exposed to different stressors in time and space. Understanding these stressors on the evolutionary trajectories of populations and the associated costs is an important challenge. In an ecological risk assessment process, it is therefore important to ask whether a population can adapt to one or more stressors, and at what cost? Our study emphasizes this last point, since we have shown that even if a population seems to adapt to a stressor (here ionizing radiation), this had an evolutionary cost on host defense.

6. Acknowledgements

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Discussion générale et perspectives

1. Limites de l'approche méthodologique

A la suite de ce travail, avec le recul, il est maintenant possible d'émettre quelques critiques vis-à-vis des choix méthodologiques et expérimentaux.

1.1. Choix du modèle : entre référence et exotisme

Nous avons choisi une population de *C. elegans* particulière (A6140) qui possédait plusieurs caractéristiques intéressantes pour étudier les réponses évolutives. Cette population possédait une grande diversité génétique issue du croisement de 16 souches ou populations de *C. elegans*, fournissant ainsi un support idéal à la sélection génétique (Teotonio *et al.*, 2012). En comparaison, la souche N2 - largement utilisée dans de nombreux laboratoires de recherche - possède une diversité génétique très faible ce qui aurait été problématique pour ce type d'étude. De plus, la population A6140 possède une forte fréquence de mâles (Teotonio *et al.*, 2012), indiquant la présence de croisements entre individus et donc d'un brassage génétique plus important que des souches plus pauvres en mâles comme c'est le cas avec la souche N2.

Toutefois, la très grande majorité des travaux expérimentaux, et en particulier pour étudier les effets des radiations sur *C. elegans*, sont réalisés avec la souche N2 (Zhao *et al.*, 2018). Dans ce travail, nous avons utilisé une autre population, A6140. Nous devons donc rester prudents quand nous discutons nos résultats avec d'autres résultats obtenus sur la souche N2. Il est possible que la population A6140 réponde différemment aux rayonnements ionisants que la souche N2. En effet, Clejan *et al.* (2006) ont montré par exemple des taux de survie différents selon les souches de *C. elegans* lorsqu'elles étaient exposées à des rayonnements ionisants.

La souche N2 est adaptée aux conditions de laboratoire ce qui conduit à des changements génétiques et comportementaux particuliers (Anderson *et al.*, 2010; Cutter *et al.*, 2019; Zhao *et al.*, 2018). A la différence de notre population A6140, les mâles de la souche N2 sont très peu efficaces pour se reproduire (Anderson *et al.*, 2010) ; ce qui explique leur très faible fréquence dans les populations de N2 (0,1 %). La sélection relâchée dans les laboratoires, par rapport à l'environnement naturel, a probablement rendu les croisements des mâles N2 obsolètes (Anderson *et al.*, 2010). Cette différence démographique par rapport à notre population entraine probablement des conséquences à toutes les échelles, modifiant largement la réponse aux

rayonnements ionisants de N2 par rapport à la population A6140 utilisée dans ce travail de doctorat.

1.2. Synchronisation ou “mix-stage” (chevauchement des générations)

L’objectif majeur de ce projet était de caractériser au mieux les effets des rayonnements ionisants sur des populations. Pour atteindre cet objectif, nous avons voulu minimiser les biais expérimentaux associés à la manipulation des populations de nématodes et les facteurs de stress autres que les rayonnements ionisants. Nous avons donc limité au maximum la manipulation des populations, en choisissant par exemple de ne pas synchroniser les populations, à la différence de beaucoup d’autres études.

Au sein d’une population de *C. elegans* constituée d’individus à différents stades de développement (embryon, larve L1, L2…), la synchronisation consiste à sélectionner des individus au même stade de développement. Lors d’expériences multigénérationnelles, cette pratique permet de s’assurer d’avoir des générations discrètes. La synchronisation chez *C. elegans* repose sur des techniques de *bleaching* et de *starvation*. Ces techniques consistent respectivement à tuer l’ensemble des nématodes à l’exception des embryons (œufs) avec une solution d’hypochlorite de sodium et à bloquer le développement des individus au stade L1 en laissant les populations une nuit sans nourriture (Porta-de-la-Riva *et al.*, 2012). Nous n’avons pas souhaité utiliser cette approche, car ces techniques représentent des facteurs de stress qui pourraient s’avérer aussi ou voire plus importants que les rayonnements ionisants eux-mêmes. Ensuite, ces techniques nécessitent du temps pour être menées à bien, notamment la *starvation* qui implique de laisser les nématodes une nuit dans un incubateur en milieu liquide. Dans nos conditions, cela aurait provoqué une absence d’irradiation pendant près d’une journée entre chaque génération. Nous ne pouvions pas exclure qu’une irradiation discontinue ait un effet sur les réponses évolutives des populations. A titre indicatif, Park *et al.* (2016) ont mis en évidence chez *C. elegans* une réparation des cassures de l’ADN seulement six heures après une irradiation gamma aiguë de 30 Gy.

Outre le fait que le *bleaching* pourrait être un facteur de stress important, cette étape pourrait être une contrainte majeure à la reproduction des mâles chez cette espèce. En effet, le *bleaching* consiste à tuer tous les nématodes sauf les œufs 3 jours après la ponte. Ainsi, si l'on considère que les mâles se reproduisent plutôt avec des hermaphrodites âgés ou ceux ayant épuisé leur propres spermatozoïdes (Barr *et al.*, 2018; Morsci *et al.*, 2011), le *bleaching* pourrait tuer les

mâles avant même qu'ils aient eu accès à la reproduction. Dans notre cas, le chevauchement causé par l'absence de *bleaching* peut permettre aux mâles de se reproduire.

Tous ces éléments nous ont conduits à écarter ces méthodes et à privilégier la non synchronisation des populations. Bien entendu notre choix expérimental n'est pas sans conséquences. En effet, avec une population de *C. elegans* synchronisée, la taille efficace de la population est très proche de la taille estimée, ce qui permet de mesurer plus précisément la fécondité et la *fitness* d'une population. Par ailleurs, avec une population synchronisée, nous aurions pu estimer plus précisément la taille des populations dans les gouttes de 5 µl car nous n'aurions eu que des individus de grandes tailles. Au contraire les populations non synchronisées se caractérisent par un chevauchement des générations (*mix-stage*), complexifiant la manipulation des nématodes ou l'estimation de certaines mesures (fécondité à l'échelle de la population et la croissance de la population). Par exemple, lorsqu'on observe sous loupe binoculaire les populations de *C. elegans*, il y a des œufs non fécondés très ressemblant à ceux fécondés. Il est alors aisé de se tromper entre les deux. Il est probable qu'avec des populations synchronisées, le lien entre la fécondité et la croissance des populations aurait été beaucoup plus clair. D'un point de vue évolutif, le *mix-stage* permet une reproduction intergénérationnelle, là où la synchronisation ne le permet pas, affectant dans les deux cas l'évolution des populations et probablement l'adaptation locale.

Bien entendu, cela représente un compromis expérimental, la *starvation* aurait pu être adaptée *via* l'utilisation d'un irradiateur plus volumineux pour éviter la discontinuité de l'irradiation. De plus, d'autres techniques de synchronisation moins stressantes existent, par exemple à travers l'utilisation de gradients de sucrose ou la collecte d'œufs (Dufourcq-Sekatcheff *et al.*, 2021; Dutilleul *et al.*, 2017b), mais ces techniques sont moins adaptées lorsque l'on manipule des populations de grande taille. En définitif, nous avons choisi de privilégier des expériences avec plusieurs conditions d'irradiations et plus de réplicats biologiques, plutôt que de se concentrer sur une synchronisation parfaite.

1.3. Problèmes expérimentaux

Le comptage des nématodes dans les gouttes s'est avéré difficile en raison de la présence d'embryons non fécondés ressemblant aux embryons fécondés et la persistance d'amas issus du tapis bactérien (non éliminés malgré les lavages).

D’autres problèmes ont eu lieu pendant les mesures de taille de ponte : des hermaphrodites réussissaient à migrer sous la gélose, rendant le comptage des embryons et le repiquage beaucoup plus complexe. Plusieurs mesures de ponte ont d’ailleurs été écartées à cause de cela. Il est possible que ce comportement soit directement lié au choix des plaques de culture (12 puits) pour cette mesure. Il semblerait que l’évaporation soit plus importante dans ces plaques que dans les boîtes de Pétri conventionnelles de 3 cm de diamètre, causant une rétractation de la gélose plus prononcée et favorisant la migration des nématodes sous la gélose.

Pour les expériences de transplantation réciproque, des problèmes ont été à l’origine d’une réussite partielle de certaines expériences et de la non-utilisation de plusieurs résultats (Figure 24). Lors de ces expériences, la charge de travail nous a contraint à manipuler à deux personnes, ce qui est toujours une source de biais potentiel. De plus, la place limitée dans l’irradiateur MIRE (*Mini Irradiator for Radio Ecology*) et le manque de temps, ne nous a pas permis de réaliser des transplantations réciproques entre les conditions 1,4 et 50 $mGy.h^{-1}$. Pourtant cette expérience aurait pu nous apporter des informations très intéressantes sur les différences de réponses suivant l’intensité de l’irradiation, notamment pour déterminer si l’adaptation locale à un débit de dose est également vraie à l’autre débit de dose ou si des mécanismes différents ont été sélectionnés suivant le débit de dose.
La place limitée dans l’irradiateur MIRE nous a obligé à réaliser les transplantations au plus faible débit de dose dans un second temps, après avoir congelé les populations. Cette étape a eu des conséquences directes sur certaines populations, comme par exemple une mortalité accrue des individus et une forme de synchronisation des populations, puisque ce sont principalement les stades L1 et L2 qui survivent à la congélation. Couplés aux effets expérimentateurs, les impacts étaient si importants que beaucoup des résultats obtenus n’ont pas été retenus. Une seconde expérience a été réalisée pour tenter de corriger ces problèmes. Malgré cela, des biais persistaient dans l’estimation de l’effectif des populations, ce qui nous a conduit à écarter les mesures de croissance des populations et de fécondité à l’échelle de la population (*realized fecundity*) au moment de l’analyse des données de transplantations réciproques.

2. Expérience multigénérationnelle

Les résultats de l'expérience multigénérationnelle montrent qu'en augmentant le débit de dose, il y a une amplification de l'impact des rayonnements ionisants sur les organismes (Figure 23). Ainsi, à 50 $mGy.h^{-1}$ nous observons systématiquement des effets plus importants sur les différents traits mesurés qu'à 1,4 $mGy.h^{-1}$. Au plus fort débit de dose nous avons observé une diminution globale de la croissance des populations, de la reproduction (fécondité individuelle et à l'échelle de la population), de la survie des embryons et de la *fitness*. A l'inverse, nous avons observé une augmentation de la fréquence des mâles à ce débit de dose. Pour le plus faible débit de dose, la fécondité individuelle et la survie des embryons ne montraient aucune différence significative avec les populations contrôles. En revanche, nous avons mesuré une diminution de la fécondité à l'échelle de la population et de la *fitness*, une augmentation de la croissance des populations et de la fréquence des mâles par rapport à la condition contrôle.

Si nous regardons les résultats en détail, nous observons une baisse de fécondité beaucoup plus faible à l'échelle individuelle qu'à l'échelle de la population, alors que les deux mesures sont étroitement liées : la première influençant largement la seconde. Cette différence présente dans les deux débits de dose semble cohérente avec le fait que les mesures individuelles ont été réalisées sur des hermaphrodites qui ont grandi hors irradiateur. Il est probable que dans ces conditions les effets des rayonnements ionisants soient moins importants que pour les mesures populationnelles où les individus restent dans les irradiateurs. De plus, les deux mesures sont sur des échelles biologiques différentes, l'échelle populationnelle est modulée par un plus grand nombre de facteurs, comme la structure d'âge, la densité de la population ou le sex-ratio. Certains facteurs non mesurés pendant ces travaux peuvent probablement accentuer la baisse de la fécondité à l'échelle de la population. Dans notre cas, le sex-ratio peut affecter la fécondité à l'échelle de la population, mais pas à l'échelle individuelle, puisqu'elles ont été réalisées sans fécondation des mâles. Nous nous attendions à une amélioration de la fécondité à l'échelle de la population avec l'augmentation de la fréquence des mâles. Pourtant, pour les deux conditions irradiées nous mesurons une baisse de la fécondité à l'échelle de la population malgré l'augmentation de la fréquence des mâles. Une des explications serait que l'apport en spermatozoïdes des mâles soit insuffisant pour compenser la perte de fécondité liée à l'irradiation. Toutefois, autant pour le fort débit de dose cela semble vraisemblable, puisqu'une diminution de la fécondité individuelle a été mesurée, autant pour le plus faible débit de dose cela semble peu probable, car aucune diminution de la fécondité individuelle n'a été observée. A ce jour, nous n'avons pas d'explication claire à proposer concernant ces résultats.

Concernant la croissance des populations plusieurs interrogations sont posées. Pour le faible débit de dose nous avons mesuré une augmentation de la croissance des populations par rapport aux populations contrôles, ce qui semble cohérent avec l'augmentation de la fréquence des mâles, mais pas avec la diminution de la fécondité à l'échelle de la population. Pour le plus fort débit de dose, la diminution de la croissance, bien que faible, est cohérente avec la diminution de la survie des juvéniles (succès d'éclosion), la diminution de la fécondité individuelle et à l'échelle de la population. Plusieurs inconnues semblent graviter autour des résultats de la fécondité à l'échelle de la population et de la croissance des populations et tout particulièrement pour le faible débit de dose. Le fait que ces deux traits soient à l'échelle de la population et qu'ils intègrent les variations de nombreux traits non mesurés, pourrait expliquer ce manque de cohérence dans les résultats. Il est possible que plusieurs autres paramètres, non mesurés dans ce travail, puissent également influencer la croissance des populations et expliquer respectivement l'augmentation et la faible baisse de croissance par rapport aux populations contrôles pour le faible et fort débit de dose. Par exemple, ces résultats pourraient être expliqués par une meilleure survie des individus après leur éclosion, une plus grande espérance de vie ou un changement de densité de population, lequel est souvent corrélé négativement avec la croissance des populations (Sibly *et al.*, 2002).

Enfin, nous avons observé une diminution globale de la *fitness* des populations irradiées par rapport aux populations contrôles. Ce résultat est cohérent avec les résultats des traits dont cet indice de *fitness* est issu.

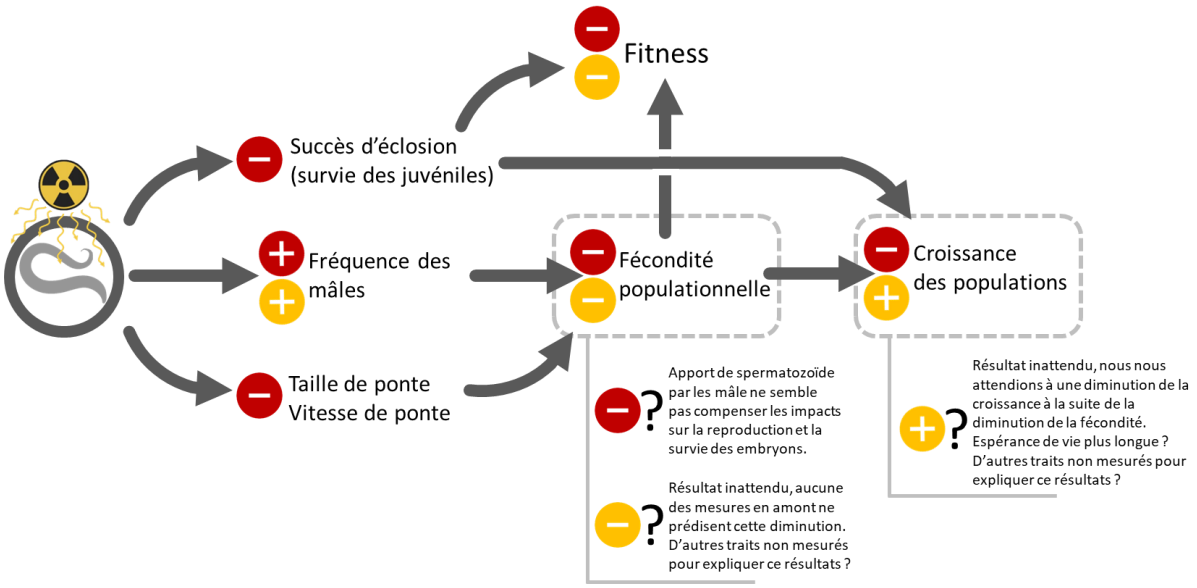


Figure 23 : représentation schématique récapitulant les principaux résultats obtenus durant l'expérience multigénérationnelle. Les « + » et les « - » indiquent respectivement une augmentation ou une diminution significative du trait en question des populations irradiées par rapport aux populations contrôles. Les résultats pour les traitements 1,4 et 50 $mGy.h^{-1}$ sont représentés respectivement en orange et en rouge.

3. Changements évolutifs des populations

Au fil des générations, les populations exposées aux rayonnements ionisants ont été sélectionnées et se sont adaptées à ce nouvel environnement (Figure 24).

Au travers des expériences de transplantation réciproque, les résultats ont montré que le succès d'éclosion et la taille de ponte tardive étaient plus élevés dans les populations après 20 transferts dans des conditions de faible irradiation que dans les populations nouvellement placées dans l'environnement irradié (Figure 24). Ces résultats suggèrent une tendance évolutive vers une amélioration de la survie des embryons et une histoire de vie plus lente en réponse à une faible irradiation. Des tendances similaires mais non significatives observées pour le fort débit de dose pour les mêmes traits suggèrent que le processus adaptatif a été ralenti.

Par ailleurs, l'augmentation de la fréquence des mâles dans les deux conditions irradiées est liée à des mécanismes de sélection de l'*outcrossing*, connus pour améliorer la *fitness* dans des conditions de stress et faciliter l'adaptation aux stresseurs. Effectivement, l'augmentation de la *fitness* observée pour le fort débit de dose, bien qu'en absolu inférieure à celle des populations en condition contrôle, suggère une adaptation des populations aux rayonnements ionisants.

L'ensemble de ces résultats révèle que ces changements évolutifs étaient associés à des coûts. La diminution du succès d'éclosion et de la taille de ponte tardive des populations irradiées puis placées dans un environnement contrôle révèlent la présence de coûts adaptatifs aux rayonnements ionisants et de possibles *trade-offs* avec d'autres traits. De plus, exposées à une bactérie pathogène, *Serratia marcescens,* les populations irradiées avaient un plus faible taux de survie que les populations non irradiées. Ce résultat indique la présence d'un coût adaptatif en lien avec l'efficacité du système immunitaire de *C. elegans*.

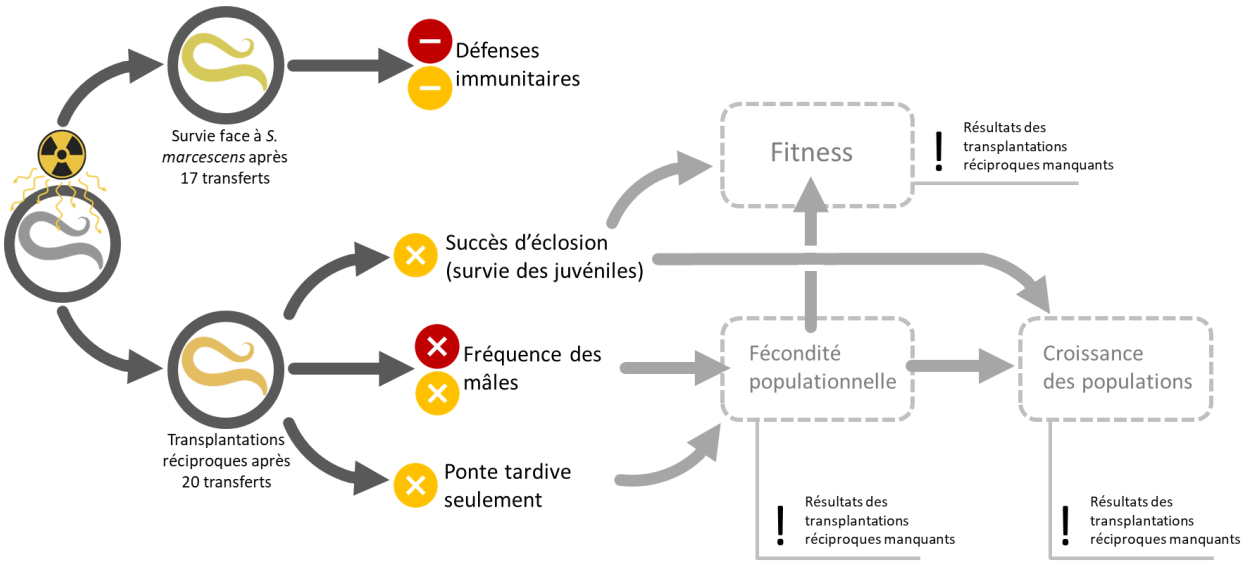


Figure 24 : représentation schématique récapitulant les principaux résultats obtenus durant les expériences de transplantations réciproques et de mesures du coût adaptatif (défenses immunitaires). Les « X » et les « - » indiquent respectivement une interaction significative et une diminution significative du trait par rapport aux résultats des populations contrôle. Les résultats pour les traitements 1,4 et 50 $mGy.h^{-1}$ sont représentés respectivement en orange et en rouge. Les traits grisés indiquent que ces résultats sont manquants, car non exploitables à l'issue des transplantations réciproques.

4. Perspectives

A la suite de ce travail de doctorat, nous présentons ici de nouvelles pistes de recherche qui nous semblent pertinentes.

4.1. S'approcher d'un réalisme environnemental en réduisant le débit de dose

Avant ce travail de doctorat, à notre connaissance, il n'existait pas de travaux qui aient mis en évidence les effets des rayonnements ionisants sur *C. elegans* à des débits de dose proches de 1,4 $mGy.h^{-1}$. Nous avons démontré à ce débit de dose des impacts à la fois sur le sex-ratio, la fécondité, la croissance des populations, la *fitness* et la réponse immunitaire de *C. elegans*. Maintenant, il nous semblerait intéressant de poursuivre ce travail sur *C. elegans* à des débits de dose plus faibles (inférieur à 200 $\mu Gy.h^{-1}$ par exemple) pour un plus grand réalisme écologique. Par exemple, Bonzom *et al.* (2016) ont estimé dans la zone d'exclusion de Tchernobyl (Ukraine) 25 ans après la catastrophe nucléaire, qu'en moyenne les microorganismes, nématodes, vers de terre et isopodes du sol étaient exposés à des débits de dose compris entre 0,3 et 150 $\mu Gy\ h^{-1}$ sur les 17 sites étudiés. Dans le cadre d'une évaluation des risques écologiques d'une contamination radioactive de l'environnement suite à un accident nucléaire majeur, comme celui de Tchernobyl, il serait donc maintenant pertinent de poursuivre ce travail de thèse à des débits de doses (tels qu'estimés par Bonzom *et al.* 2016) auxquels la faune du sol peut être exposé plusieurs dizaine d'année après l'accident. Cette démarche a déjà été appliquée, comme dans le travail de Raines *et al.* (2020), où les auteurs ont montré en laboratoire une altération de la reproduction chez le bourdon terrestre (*Bombus terrestris*) et un retard de croissance des colonies à des débits de dose comparables à ceux mesurés dans la zone d'exclusion de Tchernobyl (50-400 $\mu Gy\ h^{-1}$). Ces auteurs montrent également que la courbe dose-réponse n'est pas linéaire, les effets aux faibles débits de dose sont plus importants que ceux prédits par les modèles linéaires. Il est à noter qu'à des débits de dose encore plus faibles de 26 $nGy\ h^{-1}$ (ce qui correspond au rayonnement de fond terrestre), Lampe *et al.* (2017, 2019) n'ont pas trouvé d'effet sur la trajectoire évolutive de populations d'*E. coli* pendant 500 générations.

D'autre part, dans certains cas, les effets des rayonnements ionisants semblent suivre une relation de type hormétique, c'est-à-dire que l'on observe un effet opposé suivant que la dose reçue soit faible ou forte (Mattson, 2008). A des doses relativement faibles, inférieures à 200 mGy chez l'humain, des effets bénéfiques ont été observés, comme par exemple une augmentation de l'immunité, du niveau de « substances radioprotectrices » (enzymes antioxydantes, protéines de stress…) et de la capacité de réparation de l'ADN (Feinendegen,

2005; Shibamoto et Nakamura, 2018). S'intéresser à de faibles débits de dose chez *C. elegans* permettrait d'étudier plus en détail ces phénomènes, et en particulier mettre en évidence des coûts potentiels (c'est-à-dire la présence de *trade-offs*).

4.2. Place des mâles dans les populations

L'un des résultats les plus inattendus de ce projet était l'augmentation rapide de la fréquence des mâles dans les populations à la suite de l'exposition aux rayonnements ionisants. Cette réponse reste à ce jour encore assez peu documentée, à l'exception de quelques travaux réalisés chez *C. elegans* avec d'autres types de stresseurs : pesticide, diminution des ressources alimentaires, augmentation de la température (Lopes *et al.*, 2008 ; Morran *et al.*, 2009b ; Rose et Baillie, 1979). Une des anecdotes particulièrement intéressantes à ce sujet est que plusieurs personnes (com. pers.) travaillant sur *C. elegans* ont observé l'apparition de mâles chez la souche N2 après des *bleaching*, mais à notre connaissance ces observations n'ont jamais été publiées. Le *bleaching* pourrait donc représenter un stress pour les *C. elegans*. Il serait intéressant de faire la lumière sur ce point et de mieux quantifier le rôle du *bleaching* dans l'apparition de mâles.

Le changement de stratégie de reproduction en parallèle de l'augmentation de la fréquence des mâles semble favoriser le brassage génétique et l'adaptation locale (Teotonio *et al.*, 2012). Qu'en est-il chez des populations sauvages *in natura* ? Sur le terrain, une grande variabilité de la fréquence des mâles dans le temps et entre différentes populations de *C. elegans* (entre 0,01 et 22 %) a déjà été observée (Anderson *et al.*, 2010; Barrière et Félix, 2007). La présence des mâles chez *C. elegans* semble favorisée dans des conditions environnementales stressantes (Anderson *et al.*, 2010). Nos résultats montrent qu'il en va de même avec les rayonnements ionisants. Il serait intéressant d'étudier le sex-ratio de populations sauvages de *C. elegans* habitant des zones radio-contaminées, comme par exemple les zones proches des centrales nucléaires accidentées de Tchernobyl ou de Fukushima (IAEA, 2006; Okano *et al.*, 2016). Cette approche pourrait permettre de caractériser l'état de stress de ces populations et servir de bio-indicateur. Par exemple une forte fréquence des mâles pourrait refléter la présence d'une altération de la reproduction ou d'anomalies chromosomiques liées aux rayonnements ionisants. Au contraire, une faible fréquence des mâles pourrait indiquer une absence de stress et une adaptation des populations à leur environnement.

Les mâles jouent également un rôle dans le succès reproducteur des hermaphrodites en augmentant la taille de ponte (Chasnov, 2013; Cutter *et al.*, 2019; Singson, 2001). Caractériser

précisément l'impact de la fertilisation des mâles sur la fécondité des hermaphrodites apporterait des informations essentielles pour expliquer les dynamiques de populations. Examiner ce point nous aurait permis de mieux comprendre l'augmentation de la croissance des populations exposées au faible débit de dose, et inversement pour le fort débit de dose.

4.3. Mécanismes de défense : causes proximales

Après avoir été irradiées pendant plusieurs générations, les populations de *C. elegans* exposées à une bactérie pathogène avaient un moins bon taux de survie que les populations non irradiées. Ce résultat suggère un coût adaptatif. Comment expliquer cette sensibilité accrue à ce pathogène ? Quels sont les mécanismes sous-jacents ? Plusieurs pistes pourraient être explorées, comme des changements de la réponse immunitaire constitutive (barrière cuticulaire, pharynx broyeur qui dégrade les bactéries ingérées…), inductible (peptides antimicrobiens et protéines de défense) ou du comportement de fuite du nématode (Engelmann et Pujol, 2010; Yan *et al.*, 2020).

4.4. Mieux décrire l'évolution des populations soumises aux rayonnements ionisants

Ces travaux de thèse ont permis de mieux cerner certains aspects de l'impact des rayonnements ionisants sur l'évolution de population de *C. elegans*. Toutefois, des expériences supplémentaires auraient permis d'approfondir ces connaissances.

Dans un premier temps, et comme décrit précédemment, il serait important de réaliser de nouvelles expériences de transplantation réciproque entre toutes les conditions pour étudier les réponses adaptatives concernant la croissance, la fécondité et la *fitness* des populations. Des transplantations réciproques pourraient également être réalisées tout au long de l'expérience multigénérationnelle, par exemple toutes les 3 générations, pour ainsi mieux étudier les changements évolutifs temporels des populations. Cette approche nous permettrait en particulier de mieux expliquer (i) l'évolution des traits mesurés, (ii) les oscillations observées sur la croissance et la fécondité des populations et (iii) la baisse du succès d'éclosion observée à partir du huitième transfert au plus fort débit de dose.

Enfin, il aurait également été pertinent d'étudier l'évolution des populations par des approches de génomiques. Cette approche moléculaire permettrait de comparer les distances génétiques entre les différentes populations et d'établir si les populations se sont différenciées en fonction des traitements et au fil des générations. Il serait également possible d'étudier dans les populations la fréquence de variants présents sur des gènes associés à des fonctions

biologiques et de potentiellement déterminer s'ils sont sélectionnés ou non. Il est théoriquement possible d'établir quels gènes ou régions génomiques sont impliqués dans la réponse aux rayonnements ionisants.

4.5. Portée de ce travail de thèse

Pour clore ce chapitre, comme tout travail de recherche, il reste encore de nombreuses questions sans réponses et de nombreux axes de recherche à explorer. Néanmoins, ce doctorat a permis de mettre en avant la nécessité d'intégrer l'approche évolutive dans le cadre de l'évaluation des risques écotoxicologiques, que ce soit en lien avec une contamination radioactive ou tous autres types de stresseurs (polluants chimiques ou physiques, dérèglements climatiques…). L'évaluation des risques écotoxicologiques réglementaire pour les polluants repose en grande partie sur les tests de toxicité mono-spécifiques réalisés en laboratoire dans des conditions standardisées, et dans de nombreux cas sur une seule génération. Toutefois, les effets d'un stresseur peuvent apparaitre après plusieurs générations, en particulier dans le cas d'une exposition chronique des organismes à des concentrations sublétales, affectant ainsi les trajectoires évolutives des populations (Straub *et al.,* 2020). Sans prendre en considération les effets d'un stresseur dans le temps, il est difficile d'estimer rigoureusement son impact sur une population et de correctement déterminer des seuils de protection. Ce travail de doctorat montre à quel point il est important et pertinent d'intégrer une approche de biologie évolutive dans l'évaluation des risques écotoxicologiques.

Appendices

1. Appendice A : Informations supplémentaires du chapitre 1

Box 1: How central is evolution in radioecology field studies? - Methodology for the Web of Science query:

In 06-2021, we conducted a Web of Science "Topic" query for all years (1900-today) with (ionizing OR ionising) AND radiation AND (effect OR impact) AND (Chernobyl OR Fukushima OR Kyshtym) (649 results) and another query with (Chernobyl OR Fukushima OR Kyshtym) AND wildlife (127 results). Only research papers on the effects of ionizing radiation were manually selected based on the abstract (98 results). For these 98 papers, full text was examined to check if they (i) talk about evolution, (ii) study evolution (we qualified papers "studying evolution" if they examine transmitted variations (e.g., reciprocal transplant, and common garden centred on adaptation, or population genetics)), (iii) use the word evolution, (iv) talk about adaptation and/or selection, (v) talk about migration and/or gene flow, (vi) talk about mutations, (vii) talk about genetic drift, (viii) use the word fitness and (ix) talk about non-genetic transgenerational effects.

2. Appendice B : Figures supplémentaires du chapitre 2

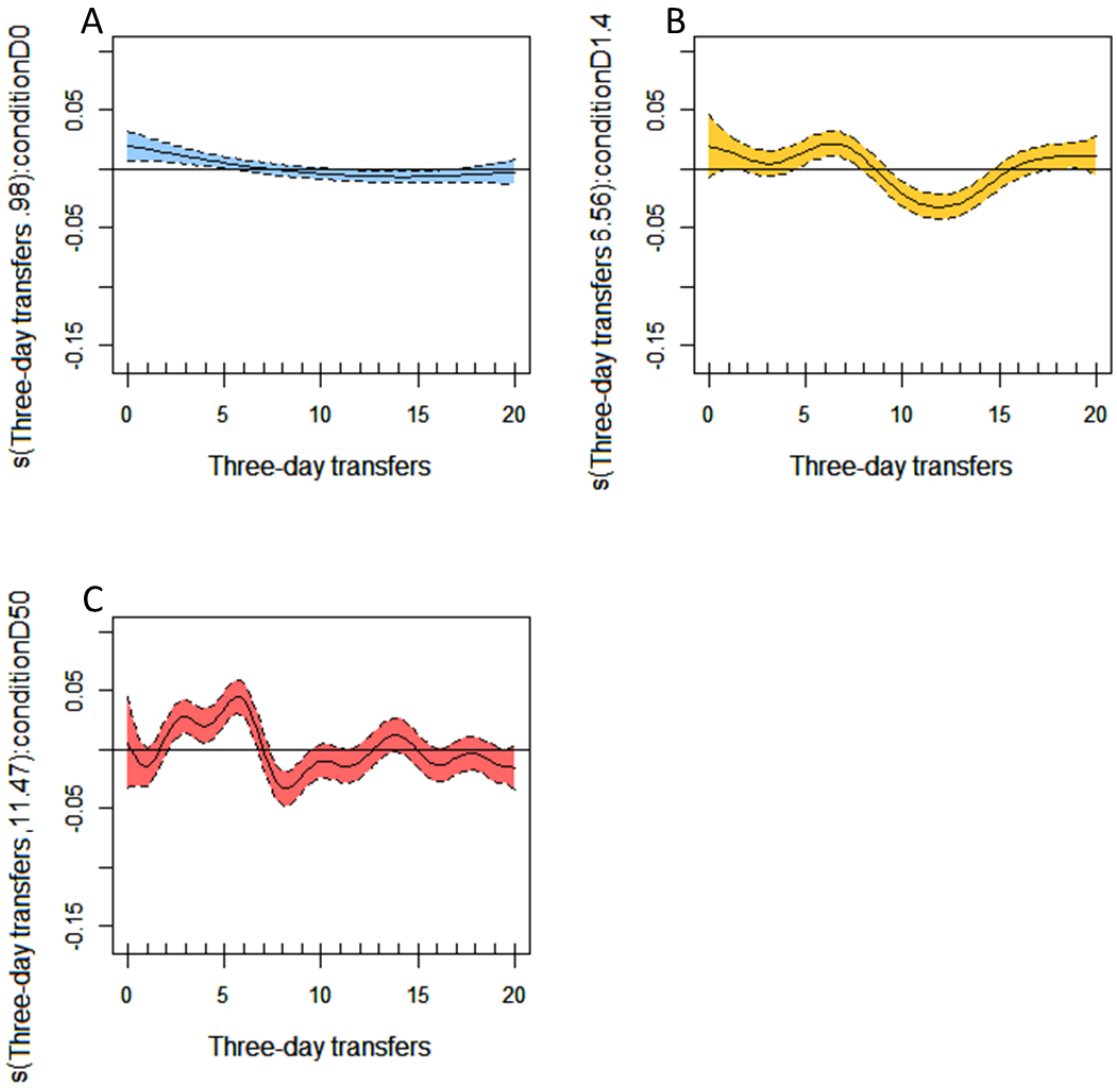


Figure B.1: This graphical representation is estimated by generalized additive mixed model (GAMM). Plots showing the partial effects of population growth rate on generation for **A.** Control, **B.** 1.4 mGy.h^{-1} and **C.** 50.0 mGy.h^{-1}. Shaded areas and dashed lines represent 95% confidence intervals. Partial response curves showing the relationship of the partial residuals of the response variable on the linear predictor scale and the relevant explanatory variables of the best approximate model. Plots were centred to have a mean value of zero along the y-axis, and the trends rather than the values of the plots were used to describe the responses to the smoothed explanatory variables.

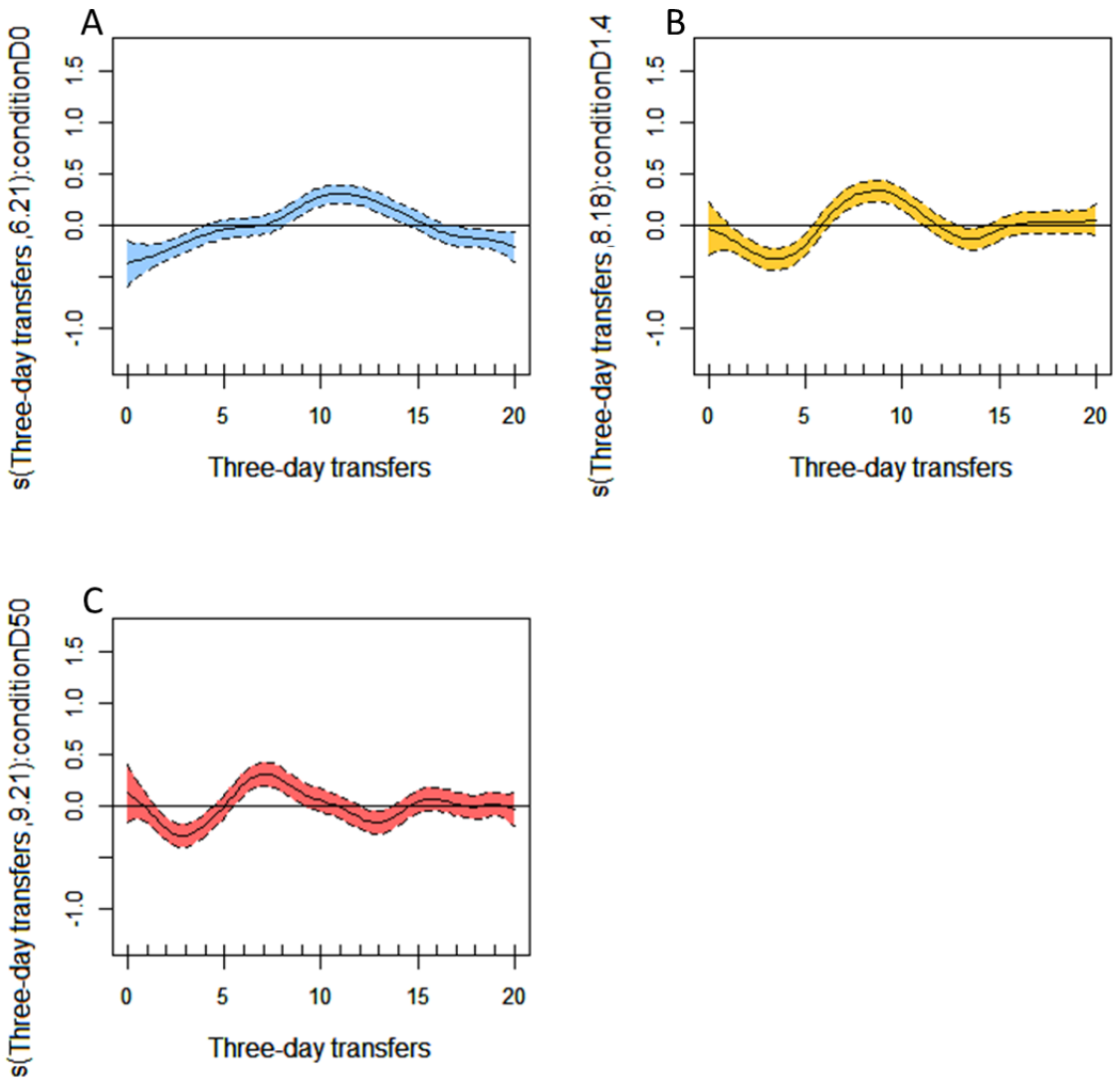


Figure B.2: This graphical representation is estimated by generalized additive mixed model (GAMM). Plots showing the partial effects of realized fecundity on generation for **A.** Control, **B.** 1.4 mGy.h^{-1} and **C.** 50.0 mGy.h^{-1}. Shaded areas and dashed lines represent 95% confidence intervals. Partial response curves showing the relationship of the partial residuals of the response variable on the linear predictor scale and the relevant explanatory variables of the best approximate model. Plots were centred to have a mean value of zero along the y-axis, and the trends rather than the values of the plots were used to describe the responses to the smoothed explanatory variables.

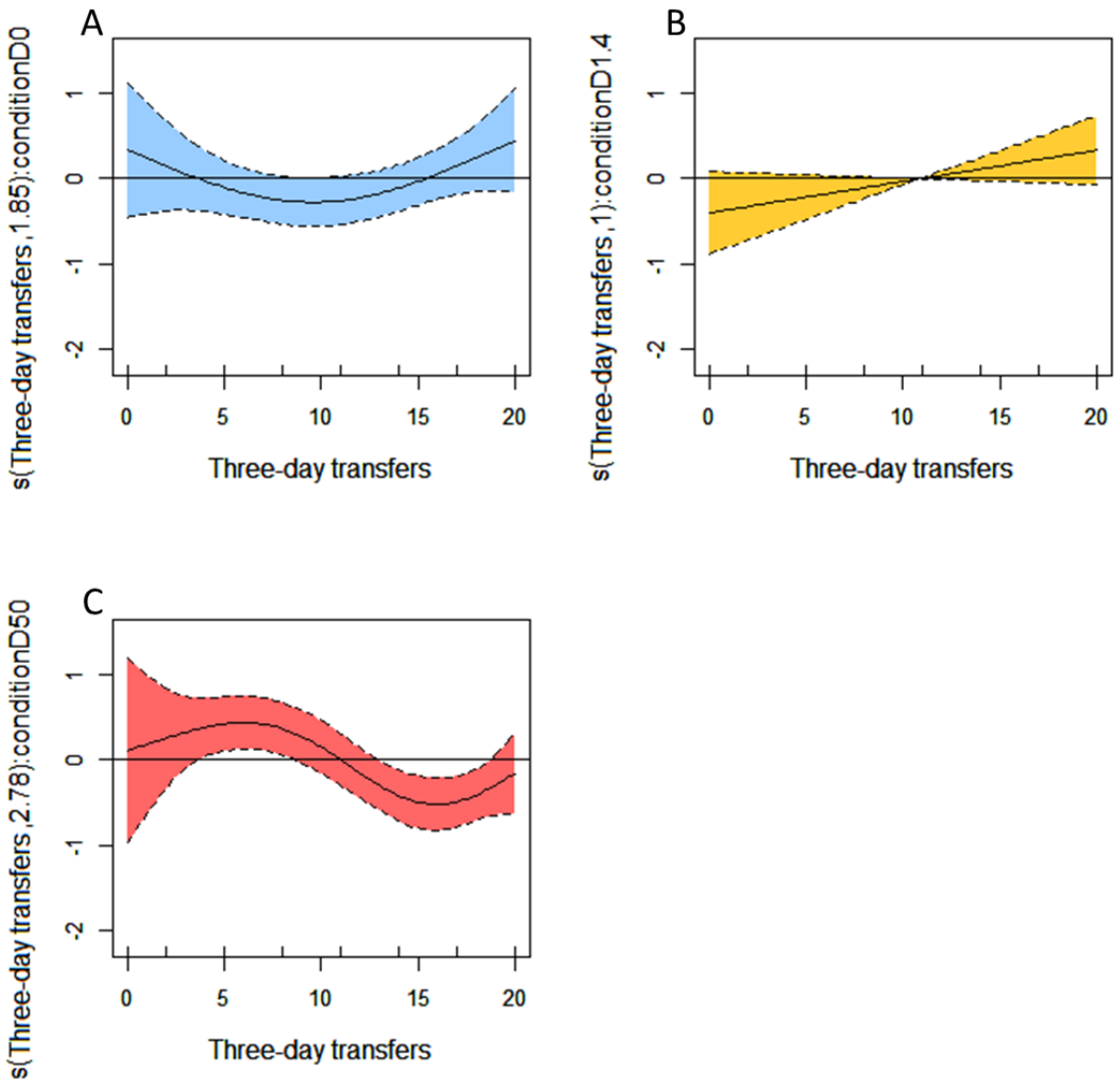


Figure B.3: This graphical representation is estimated by generalized additive mixed model (GAMM). Plots showing the partial effects of hatching success on generation for **A.** Control, **B.** 1.4 mGy.h^{-1} and **C.** 50.0 mGy.h^{-1}. Shaded areas and dashed lines represent 95% confidence intervals. Partial response curves showing the relationship of the partial residuals of the response variable on the linear predictor scale and the relevant explanatory variables of the best approximate model. Plots were centred to have a mean value of zero along the y-axis, and the trends rather than the values of the plots were used to describe the responses to the smoothed explanatory variables.

3. Appendice C : Figures supplémentaires du chapitre 3

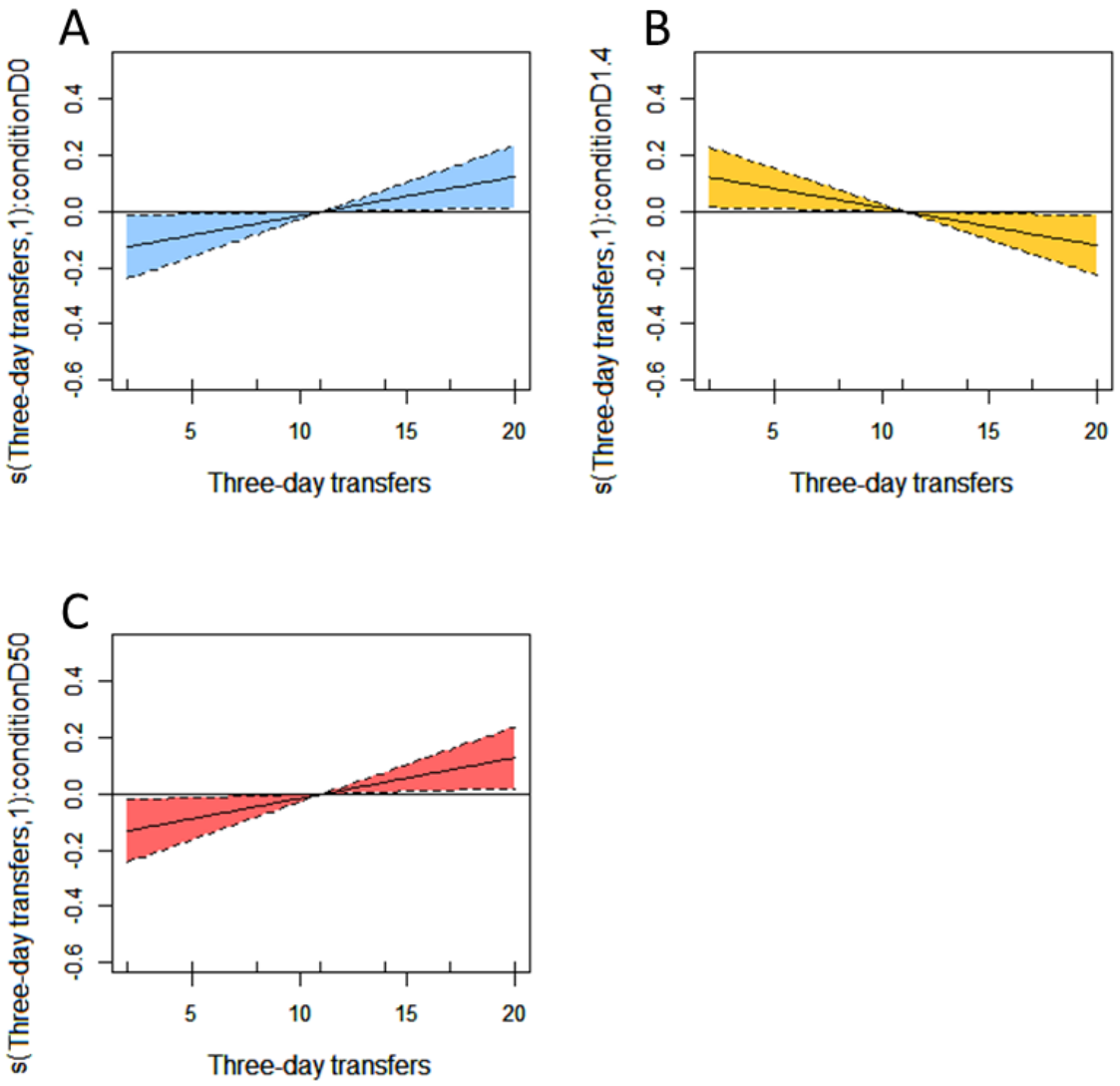


Figure C.1: Partial response curves showing the relationship of the partial residuals of the response variable on the linear predictor scale and the relevant explanatory variables of the best approximate model. Plots were centred to have a mean value of zero along the y-axis, and the trends were used, rather than the actual values of the plots, to describe the responses to the smoothed explanatory variables. The graphical representations were estimated by GAMM. Plots show the partial effects of male frequency on time (three-day transfers) for **A.** Control **B.** 1.4 mGy.h^{-1} and **C.** 50 mGy.h^{-1}. Shaded areas and dashed lines represent 95% confidence intervals.

4. Appendice D : Figures supplémentaires du chapitre 4

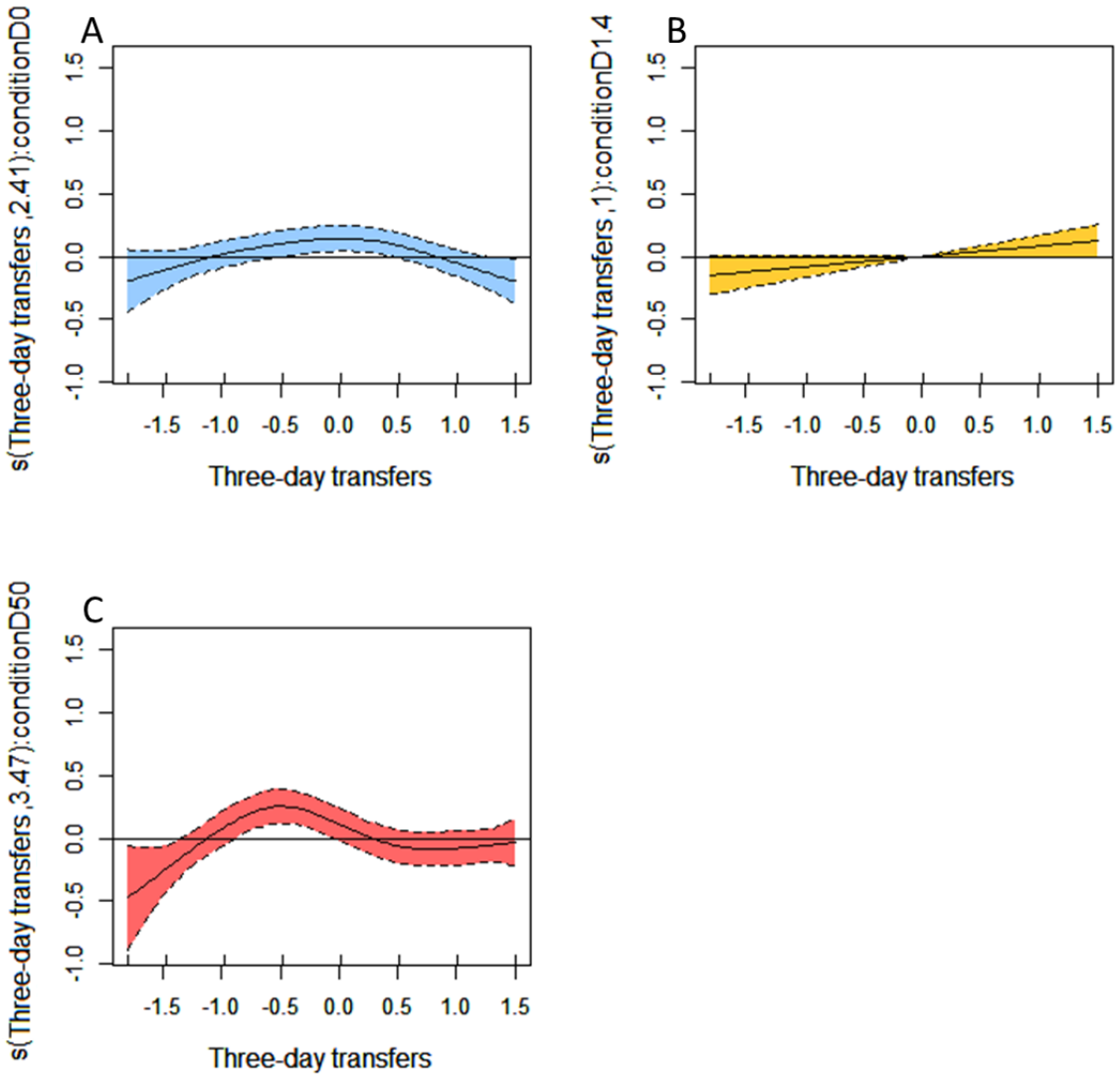


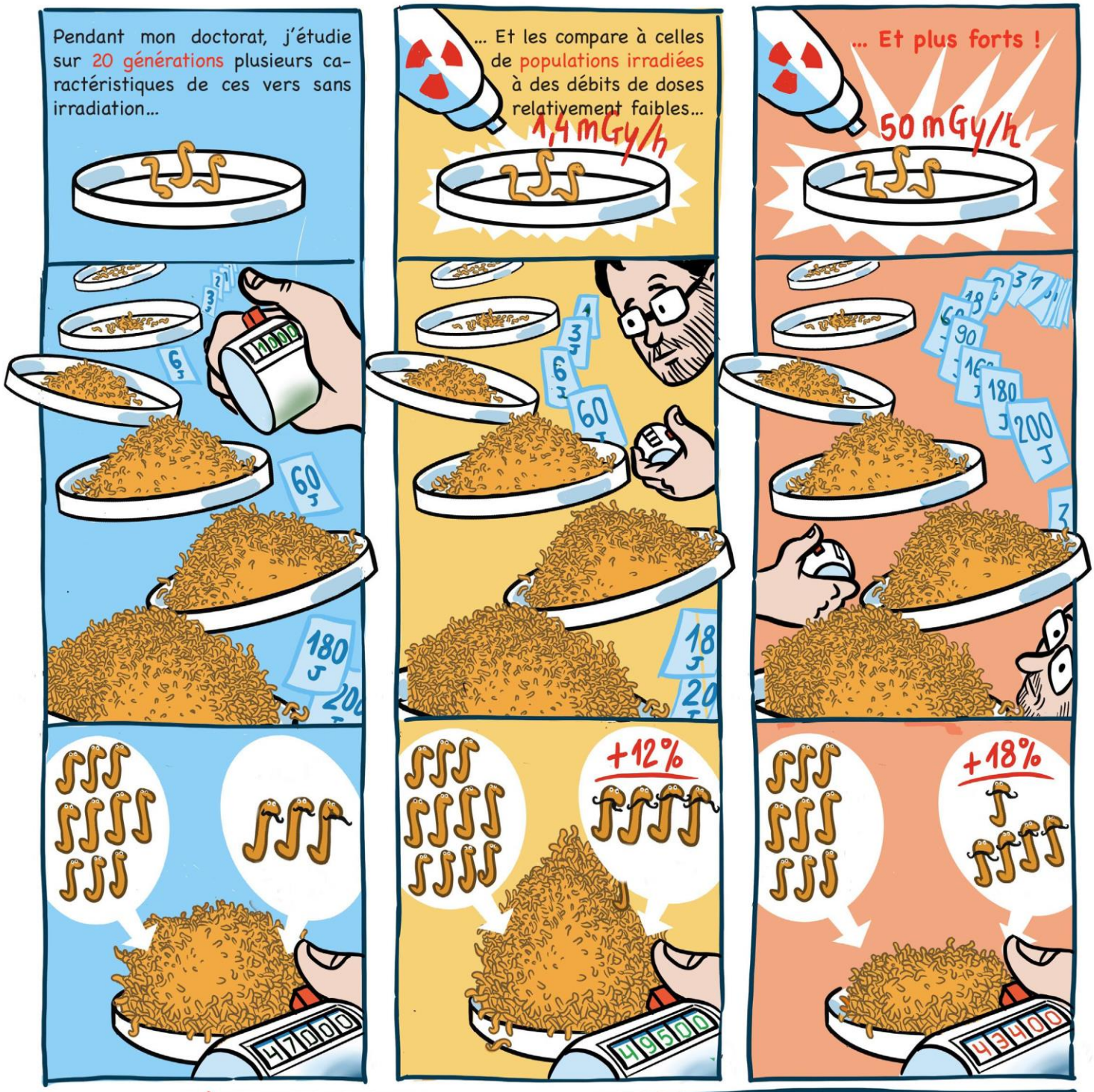
Figure D.1: This graphical representation is estimated by GAMM. Plots showing the partial effects of fitness index on generation for **A.** Control **B.** 1.4 $mGy.h^{-1}$ and **C.** 50.0 $mGy.h^{-1}$. Shaded areas and dashed lines represent 95% confidence intervals. Partial response curves showing the relationship of the partial residuals of the response variable on the linear predictor scale and the relevant explanatory variables of the best approximate model. Plots were centred to have a mean value of zero along the y-axis, and the trends rather than the values of the plots were used to describe the responses to the smoothed explanatory variables.

5. Appendice E : illustrations de la thèse dans le cadre de l'IRSN

Figure E.1 : *comic strip* représentant l'évolution du sex-ratio chez une population de *C. elegans* sous irradiation gamma pendant plusieurs générations. Offert par l'IRSN et réalisé par Olivier Legan pour avoir participer au concours "3 minutes pour une thèse" de l'IRSN.

L'IMPACT DE LA RADIOACTIVITÉ SUR LES POPULATIONS ANIMALES: ON MÈNE L'ENQUÊTE!

La pollution est un phénomène majeur qui perturbe très significativement la biodiversité. Face à la pollution comment les êtres vivants peuvent-ils résister, s'adapter ? A quel prix ? Des expériences réalisées au laboratoire sur *Caenorhabditis elegans*, des vers d'1 mm de long, peuvent nous aider à répondre à ces questions.



En étudiant les œufs pondus, l'évolution du nombre de vers et les mâles dans les populations, j'ai observé des choses très intéressantes chez les groupes irradiés ! Par exemple:

... Sous faible irradiation, l'effectif de la population augmente globalement et au contraire, ...

... Sous forte irradiation, il diminue avec la fécondité (-8% d'œufs pondus et -7% d'éclosions). Autre résultat inattendu : le nombre de mâles a fortement augmenté au fil des générations chez les vers irradiés.

Sous l'effet des rayonnements ionisants, les populations sont donc impactées : plus de mâles, baisse de la fécondité à forte dose, etc.

Maintenant, si je transplante ces populations de vers irradiées dans un environnement sans irradiation, vont-elles retrouver leurs caractéristiques du départ ?

J'ai enfilé de nouveau ma bouse blanche pour réaliser ces expériences de transplantation. Mes premiers résultats montrent que, même sans irradiation, les populations qui avaient été irradiées continuent à compter beaucoup plus de mâles, par exemple.

Donc, pendant 20 générations sous irradiation la population a évolué, s'est adaptée : cette population de vers continue à « fabriquer » plus de mâles, même sans irradiation !

Que s'est-il passé ?

C'est l'enquête

que je mène en ce moment.

Et si...

... la réponse se trouvait au niveau du génome ?

J'ai donc commencé à regarder du côté du matériel génétique, en séquençant l'ADN de toutes ces populations de vers (irradiées et non irradiées). Est-ce que génétiquement les populations irradiées sont différentes des populations non irradiées ? **L'enquête est en cours.**

Rendez-vous à ma soutenance de thèse !

RÉPUBLIQUE FRANÇAISE
Liberté Égalité Fraternité

IRSN

Loïc Quevarec est le lauréat du concours **« 3 minutes pour une thèse »** de l'IRSN en 2020. Sa thèse qui traite de la « Compréhension des mécanismes impliqués dans la réponse évolutive de populations de nématodes (C. elegans) exposées à des rayonnments ionisants » se poursuit jusque fin 2021.

Dessins: Olivier Legan

Figure E.2 : bande dessinée représentant les travaux de thèse. Offert par l'IRSN et réalisé par Olivier Legan pour avoir gagné le concours "3 minutes pour une thèse" de l'IRSN.

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