TRANS-GENERATIONAL AND REPRODUCTIVE IMPACTS OF ACUTE EARLY-LIFE RADIATION ON THE HOUSE CRICKET, *ACHETA DOMESTICUS*

TRANS-GENERATIONAL AND REPRODUCTIVE IMPACTS OF ACUTE EARLY-LIFE RADIATION ON THE HOUSE CRICKET, *ACHETA DOMESTICUS*

By TAMARA M. FUCIARELLI, H.BSc

A Thesis Submitted to the School of Graduate Studies in Partial Fulfilment of the Requirements for the Degree Master of Science

McMaster University MASTER OF SCIENCE (2019) Hamilton, Ontario (Biology)

TITLE: Transgenerational and reproductive impacts of acute early-life radiation on the house cricket, *Acheta domesticus*

AUTHOR: Tamara M. Fuciarelli, H.BSc (McMaster University)

ADVISOR: Dr. C. David Rollo (McMaster University)

NUMBER OF PAGES: xv, 121

Abstract

Stress is ubiquitous for all organisms, however, impacts vary depending on type and strength of the stressor, as well as the organism's tolerance. Moreover, stress responses can be described by several models; linear, threshold, or hormetic. Generally ionizing radiation has been described by a "linear no threshold" model where all exposure has negative impacts. However, considerable research, both past and present, suggests that a "hormetic" or threshold model may better describe radiation exposure. My research focused on adult male house crickets, (*Acheta domesticus),* and the effects of early-life radiation on a variety of life history and reproductive traits, molecular biomarkers, and trans-generational impacts. Each life history and reproductive trait was also analyzed for its dose-response relationship.

Males exposed to radiation showed threshold responses in both hatching success (of 200 eggs) as well as mating success. Doses below 7Gy showed no decline in hatching success nor mating success, whereas above this threshold severe declines occurred, with 12Gy males being sterile. Sexual signals, both acoustic and chemical, were also altered by exposure. Life history traits in F0 males included a dose-dependent reduction in growth rate. Hormetic responses emerged in longevity and survivorship in 7Gy and 10Gy males and their offspring. The dose response relationship for most life-history and reproductive traits analyzed were best described by quadratic and non-linear models. Responses to radiation were also reflected by DNA methylation in F0 and F1 offspring. Generally, radiation impacts on life-history and reproductive traits were best described by non-linear modelling. Multiple aspects of sexual signalling were disrupted by radiation, including courtship signalling and pheromone signatures. Finally, radiation impacts emerged in F1 offspring that expressed significantly extended longevity and superior survivorship with no large reduction in growth rate.

Acknowledgments

There are numerous individuals I would like to thank for providing me with their time, effort, and knowledge that has helped me to successfully complete all the work I have over the past two years. Firstly, I would like to thank my supervisor, Dr. David Rollo for guiding me throughout my thesis work and always providing me with new outlooks and creative ideas to make my work unique. I would also like to thank my committee members Dr. Carmel Mothersill and Dr. Rama Singh for their guidance and ideas.

I would also like to thank the members of our lab, especially Dr. Jen Lemon, whose training and patience helped me to acquire the skills I needed to pursue new lines of research. Also, I would like to thank Akile Ozkan, Xiaobing Li, and Marina Korobeynikova for all their help in data collection as well for the help on sick days.

I would like to thank MITACS and our partner Bruce Power for providing the funding for my research to Dr. C. David Rollo (McMaster University).

A final thanks goes out to Klavdiy Popov, without you I may not of had the strength to get through the endless days and late nights. Thank you for all your help and support in and out of the lab, I really could not have done it without you.

Thesis Organization and Format

This thesis is organized in a “sandwich” thesis format, with a general introduction, three chapters, and a general conclusion. Chapter 1 and 2 cover empirical research collected throughout the two-year period, focusing on multi-generational and reproductive effects of radiation exposure. The third chapter focuses on the concept of “hormesis” as the current model for radiation exposure, and how my research applies to this paradigm. The final conclusion briefly considers each chapter, limitations, and areas for future research.

Table of Contents

Declaration of Academic Achievement

Introduction and Conclusion

(September 2017 – August 2019)

All ideas expressed are of T.M. Fuciarelli unless otherwise referenced.

Chapter 1 [Impacts of Juvenile Irradiation on Subsequent Mating and Reproduction in the cricket *Acheta domesticus*]

(September 2017 – August 2019)

Animal breeding performed by **A. Ozkan**

Irradiation conducted by **T.M Fuciarelli**

Experimental design by **C.D Rollo** and **T.M Fuciarelli**

Data Collection conducted by **T.M Fuciarelli**

Statistical analysis by **T.M Fuciarelli**

Writing and ideas by **T.M Fuciarelli** and those referenced within

Intellectual input provided by **C.D Rollo**

Chapter 2 [Impacts of Paternal Irradiation on F1 Offspring Life History Features]

(September 2017 – August 2019)

Animal breeding performed by **A. Ozkan**

Irradiation conducted by **T.M Fuciarelli**

Experimental design by **C.D Rollo** and **T.M Fuciarelli**

Data Collection conducted by **T.M Fuciarelli, A. Ozkan, M. Korobeynikova, and X. Li**

Statistical analysis by **T.M Fuciarelli**

Writing and ideas by **T.M Fuciarelli** and those referenced within

Intellectual input provided by **C.D Rollo**

Chapter 3 [Radiation impacts and the Hormetic Model]
(September 2017 – August 2019)

All ideas expressed are of T.M. Fuciarelli unless otherwise referenced.

Intellectual input provided by **C.D Rollo**

List of Figures

List of Tables

List of Supplementary Figures

List of Abbreviations

LNT: Linear-No-Threshold

ANOVA: Analysis of Variance

HSD: Honest significant difference

ROS: Radical oxygen species

NRf2: Nuclear factor erythroid 2-related factor 2

FOXO: Forehead box transcription factor

Gy: Short for the unit of radiation exposure “Gray”

AMPK: An energy sensing factor

Akt: Also called PKB, it is the final effector of Insulin and IGF-1 signalling

TOR: The “Target of Rapamycin,” a factor associated with protein synthesis, cell proliferation and growth

IIS: Insulin/insulin-like growth factor signaling pathway

CncC: Cap ‘n’ Collar isoform-C, it is the insect homolog to mammalian Nrf2

Introduction

This work examines the effects of male early life irradiation on life history and reproduction, specifically; mate choice, attractiveness, trans-generational impacts, and specific aspects of epigenetic and stress response pathways. The data collected were then analyzed to determine whether the current linear-no-threshold model adequately describes the trends being analysed. This introduction generally outlines aspects of radiation, its target and non-target affects, the radiation model, and our model organism, the cricket *Acheta domesticus*.

The stress: Ionizing radiation

All living things have an ability to respond and adapt to internal and external disturbances (Rattan, 2008). Although not easy to define, we describe stress as any aspect, whether it be chemical, physical, or biological that acts on a living system to initiate a counter physiological response (Rattan, 2008). Stressors do not always cause negative outcomes, a successful response by an organism to a stressor can improve or stabilize overall organism and cellular health (Rattan, 2008). It is only when an incomplete or failed response occurs that negative and damaging impacts emerge (Rattan, 2008).

Ionizing radiation acts as a stressor through the creation of reactive oxygen species (ROS) at levels causing damage or mortality (Einor et al. 2016). When absorbed by living cells, ionizing radiation, through the production of ROS can induce direct breakage of the bonds of important biological macromolecules, including DNA (Einor et al. 2016). ROS however, occur naturally in cells, performing important functions in cell signaling, cell division, immunity, and stress responses (Ma, 2013; Einor et al. 2016).

The level of ROS is controlled and maintained by a several cellular antioxidants (Einor et al. 2016). Damage only occurs when there is an increase in ROS's due to stress such as radiation exposure, causing an imbalance between ROS production and antioxidant mechanisms. When an imbalance occurs between ROS production and antioxidant capacity it is referred to as oxidative stress. As a result, on a cellular level, oxidative stress may manifest as damage to DNA, epigenetic changes, as well as accelerated cell aging and apoptosis. At an organism and organ level oxidative stress may manifest as reduced function, growth, fertility, and longevity (Ma, 2013; Einor et al. 2016).

Radiation impacts

Germline cells may be especially vulnerable to oxidative stress, leading to consequences in mate choice if detected by potential mates and trans-generational effects on future generations (Metcalfe & Alonso-Alvarez, 2010). Transgenerational effects through the germline can be transmitted either through maternal or paternal lines. Abundant studies have focused on maternal effects but paternal effects have been largely ignored. However, there are important portions of variance in traits explained only by paternal effects (Weigensberg et al. 1998). The impact of oxidative stress on the paternal germline, and its potential transgenerational effects on offspring also have implications for sexual selection (Velando et al. 2008).

Oxidative stress caused by radiation may both stimulate or inhibit reproduction depending on exposure, environmental conditions, and lifestyle (Metcalfe & Alonso-Alvarez, 2010). Many forms of secondary sexual traits and behavior may signal an individual's oxidative status to potential mates. Sexual selection would thus promote the evolution of signals of the intrinsic quality of males. Females may avoid males with damage if that damage correlates with

the expression of secondary sexual characteristics (Velando et al. 2008). Radiation exposure, however, may not always have negative consequences if at low doses. Many studies on invertebrates show that exposure to low doses early in life can increase resistance to later life exposure (Costantini et al. 2010; Costantini et al. 2014).

The effects of ionizing radiation on lifespans and other traits may therefore seem to be paradoxical. High levels of radiation reduce longevity and fertility, presumably due to cellular damage from oxidative stress. However, smaller doses can extend longevity of organisms spanning insects to mammals and provide resistance to future exposure (Allen & Sohal, 1982; Costantini et al. 2010; Costantini et al. 2014).

Radiation Protection

The current internationally recognized model for radiation protection for human's is the linear-no-threshold (LNT) model (Doss, 2013). This model postulates that the dose-response relationship between exposure and carcinogenic effects is linear and all doses are harmful (Averbeck, 2009; Doss, 2013). Although this model has been accepted world-wide for decades, there is considerable debate relative to its validity in modelling radiation exposure (Doss, 2013). This debate stems from extensive data indicating that there are doses that are beneficial or non-harmful across traits and species (Averbeck, 2009). A beneficial response is generally referred to as hormesis. This, and threshold response models are examined in this study and will be analyzed for a variety of traits using our model organism, the house cricket.

The model organism: *Acheta domesticus*

The house cricket, *Acheta domesticus,* was chosen as our model species. It has served to examine numerous scientific hypotheses, particularly in animal behavior (Tregenza & Wedell, 1997). House crickets are ideal for lifetime and developmental studies as they have short lifespans of approximately 120 days when raised at 30ºC, and cohorts of known age can be easily obtained from eggs (Lyn et al. 2010). Juveniles are generally similar to adults, gender can be recognized by ovipositor development in late-instar juveniles (Lyn et al. 2010), and maturation is accompanied by development of wings and genitalia. Crickets can be raised in large numbers with low maintenance and costs, and juveniles and adults have similar nutritional requirements (Lyn et al. 2010; Calabrese, 2013). This cricket model has been employed for years in Dr. Rollo's lab with respect to radiation impacts on behaviour, immune functions, growth, maturation, reproduction and aging.

Insects diversely respond to radiation and other stressors. Due to the needs of insect population control, sterilization by radiation and associated phenotypic alterations represents a large literature across diverse species. Doses between 70-100Gy are used to sterilize certain "pest" species (Bakri et al. 2005). Insects vary widely in the dose required for sterilization (ranging from approximately 5 – 300Gy) (Bakri et al. 2005) Other than abundance of knowledge on sterilization, there are relatively few studies exploring the physiological pathways by which insects respond to exposure, or potential trans-generational impacts of low to high doses on future generations.

Goals

The goal of this thesis was to determine potential impacts, whether positive or negative, of radiation exposure on our model organism, and how this pertains to the various models of radiation impacts. Specifically, my focus was on paternal aspects and their dose- response relationships for exposed males and also their offspring. This included various life history, reproductive, stress and epigenetic biomarkers relevant to sex specific differences to exposure, pathways mediating responses to radiation stress, and the potential for generational and epigenetic changes that may persist trans-generationally.

References

Allen, R., & Sohal, R. (1982). Life-lengthening effects of γ-radiation on the adult housefly, *Musca domestica*. *Mechanisms of Ageing and Development,20*(4), 369-375. doi:10.1016/0047-6374(82)90104-x

Averbeck, D. (2009). Does scientific evidence support a change fom the LNT model for low-dose radiation risk extrapolation? *Health Physics,97*(5), 493-504. doi:10.1097/hp.0b013e3181b08a20

Bakri, A., Mehta, K., & Lance, D. R. (2005). *Sterilizing insects with ionizing radiation*. *Springer*. doi:https://doi.org/10.1007/1-4020-4051-2_9

Calabrese, E. J. (2013). Low doses of radiation can enhance insect lifespans. *Biogerontology,14*(4), 365-381. doi:10.1007/s10522-013-9436-5

Costantini, D., Metcalfe, N. B., & Monaghan, P. (2010). Ecological processes in a hormetic framework. *Ecology Letters,13*(11), 1435-1447. doi:10.1111/j.1461-0248.2010.01531.x

Costantini, D., Monaghan, P., & Metcalfe, N. B. (2014). Prior hormetic priming is costly under environmental mismatch. *Biology Letters,10*(2), 20131010-20131010. doi:10.1098/rsbl.2013.1010

Doss, M. (2013). Linear no-threshold model vs. radiation hormesis. *Dose-Response,11*(4). doi:10.2203/dose-response.13-005.doss

Einor, D., Bonisoli-Alquati, A., Costantini, D., Mousseau, T., & Møller, A. (2016). Ionizing radiation, antioxidant response and oxidative damage: A meta-analysis. *Science of The Total Environment,548-549,* 463-471. doi:10.1016/j.scitotenv.2016.01.027

Lyn, J. C., Naikkhwah, W., Aksenov, V., & Rollo, C. D. (2010). Influence of two methods of dietary restriction on life history features and aging of the cricket *Acheta domesticus*. *Age,33*(4), *509-522*. doi:10.1007/s11357-010-9195-z

Ma, Q. (2013). Role of Nrf2 in oxidative stress and toxicity. *Annual Review of Pharmacology and Toxicology,53(1), 401-426.* doi:10.1146/annurev-pharmtox-011112-140320

Metcalfe, N. B., & Alonso-Alvarez, C. (2010). Oxidative stress as a life-history constraint: The role of reactive oxygen species in shaping phenotypes from conception to death. *Functional Ecology,24*(5), 984-996. doi:10.1111/j.1365-2435.2010.01750.x

Rattan, S. I. (2008). Hormesis in aging. *Ageing Research Reviews,*7(1), 63-78. doi:10.1016/j.arr.2007.03.002

Tregenza, T., & Wedell, N. (1997). Definitive evidence for cuticular pheromones in a cricket. *Animal Behaviour,54(4), 979-984.* doi:10.1006/anbe.1997.0500

Velando, A., Torres, R., & Alonso-Alvarez, C. (2008). Avoiding bad genes: Oxidatively damaged DNA in germ line and mate choice. *BioEssays,30(11-12), 1212-1219.* doi:10.1002/bies.20838

Weigensberg, I., Carriere, Y., & Roff, D. A. (1998). Effects of male genetic contribution and paternal investment to egg and hatchling size in the cricket, *Gryllus firmus*. *Journal of Evolutionary Biology,11*(2), 135-146. doi:10.1046/j.1420-9101.1998.11020135.x

CHAPTER 1

Impacts of Juvenile Irradiation on Subsequent Mating and Reproduction in the Cricket, *Acheta domesticus*

Abstract

"Choosy" females are considered able to assess potential mates for defects, damage and general fitness. Radiation induces oxidative stress that impacts diverse traits including reproductive and other life history features. Males of the cricket, *Acheta domesticus,* attract females with sexual signals that span songs, chemical pheromones, and visual/physical aspects. This study assessed the effects of radiation on male sexual signal modalities and associated female preference. Furthermore, the impacts of male irradiation as early juveniles (14 days old) on subsequent reproduction and offspring, including maternal egg production, incubation period and hatchling success were also analyzed. Results revealed a dose-dependent decline in mating success for irradiated males, potentially due to altered behaviour, song and/or chemical signalling. Impacts of radiation on reproductive output expressed a threshold, only showing declines in females paired with males exposed to > 7Gy. This study indicates that radiation significantly impacts reproductive success of this insect and highlights the mechanism as defective sexual signalling.

Introduction

For sexual species, successful mating is crucial for individual fitness (Botha et al. 2017). Sexual signalling consists of multiple modalities, each of which contributes to aspects of success (Rebar et al. 2009). Among crickets, female reproductive success translates into oviposition rates. For males, success reflects their ability to court females and sire subsequent offspring (Simmons, 1987). Male sexual signals allow females to evaluate their fitness and avoid those showing signs of damage/reduced fitness (Velando et al. 2008). For *Acheta domesticus,* male sexual signals include song (volume, quality), chemicals (cuticular pheromones), and physical features (e.g., size, damage, mating behaviour) (Tregenza & Wedell, 1997; Thomas & Simmons, 2010; Botha et al. 2017).

Visual aspects of sexual signaling may be less important as crickets are mainly nocturnal. Alternatively, chemical and acoustic aspects are known to be necessary for male success (Thomas & Simmons, 2010). Most studies of male attractiveness in *Acheta* have focused on courtship songs and body size, while cuticular pheromones have received much less attention (Tregenza & Wedell, 1997). Determining radiation impacts on male attractiveness and subsequently reproductive success is one focus of this study. With our model organism we explore the impacts of a critical stressor (radiation) on an insect (a phyla with the greatest number of species and population abundance in terrestrial ecosystems). Results may be of value in stress physiology and molecular realms, but may also extrapolate to environments contaminated with radiation associated with pollution, nuclear accidents, and war.

Accumulating evidence suggests that some environmental stressors can inflict heritable mutations and epigenetic changes in the germ line (Velando et al. 2008). Oxidative stress (a key aspect of radiation exposure) may impact reproductive fitness of males to a greater extent due to

greater vulnerability of sperm (Velando et al. 2008). Consequently, it would be advantageous for females to avoid males exposed to oxidative stress (Velando et al. 2008). Indeed, females of some species may reject male suitors that have altered sexual signalling that reflects radiation damage (chemical, visual, and acoustic calls) (Velando et al. 2008).

Radiation can cause direct damage, but ionization of water that generates reactive oxygen species in animals is considered the major impact. To determine oxidative impacts on attractiveness one must consider the sequential aspects of mating that normally progress predictably to success (Balakrishnan & Pollack, 2017). Song is a critical aspect mediating contact of the sexes, followed by stereotypic courtship (Balakrishnan & Pollack, 2017). The male continues producing the courtship song while turning away from the female to allow the female to mount him (Balakrishnan & Pollack, 2017). Mounting by the female is required for successful spermatophore transfer (Balakrishnan & Pollack, 2017). Throughout this process the females can assess cuticular pheromones, acoustic, visual, and other physical signals from the male (Balakrishnan & Pollack, 2017). Deficiencies in any of pheromones, song, or physical aspects may reduce male mating success (Balakrishnan & Pollack, 2017). Each modality of male characteristics/signalling may provide a multitude of information to respective females.

Cuticular pheromones (both lipid and hydrocarbon) are synthesized in the epidermal cells of insects (Assis et al. 2016). Hydrocarbons are the predominant component of insect cuticular pheromones and have been documented in over 100 species (Assis et al. 2016). Males and females of *Acheta domesticus* have been shown by chromatography, to produce different compositions and quantities of cuticular hydrocarbon pheromones (Warthen & Uebel). In my study male exposure to radiation was analyzed with chromatography across a range of doses

(including controls) to detect specific hydrocarbon pheromones and possible radiation alterations.

Acoustic signals are well studied aspects of male secondary sexual traits (Simmons et al. 2010). Males of many species use acoustic signals to compete for access to females, and females assess mates based on information in songs (Simmons et al. 2010). The song of a male cricket is produced by specialized structures in both of their two front wings: the file and scraper (Huber & Thorson, 1985). Each component is present on each male wing, with sound is produced by closing and opening the wings. As the wings close the file and scraper rub together causing the wings to oscillate and create sound (Huber & Thorson, 1985). Sequential closing and opening of the wings produce the song and the speed and strength of oscillations produce different song parameters (Huber & Thorson, 1985).

Male crickets use two different acoustic signals during courtship, a calling song to attract distant females and a courtship song once the female arrives (Rebar et al. 2009). The courtship song conveys species recognition and elicits female mounting in various cricket species (Rebar et al. 2009). Unlike calling songs, courtship songs are more species specific and highly variable, condition dependent, and vital in female choice (Rebar et al. 2009). Any alterations in development, thickness, or structure of male wings due to early-life irradiation of imaginal discs could alter songs, or in extreme cases, silence males altogether.

Male size is an important sexual trait in the vast majority of cricket species in which females are the choosey sex. Body size influences short-term male mating success in the field cricket, *Gryllus bimaculatus,* suggesting relevance to *Acheta domesticus* (Simmons, 1988). In *G. bimaculatus*, smaller males have limited reproductive potential. They have a higher cost for spermatophore production and longer periods between mating's (Simmons, 1988). Larger males

also have greater success attracting females. Whether this reflects stronger sexual signals or superior competition requires study (Simmons, 1988). Moreover, exceptionally small males may fail to transfer spermatophores once mounted (Simmons, 1988). Exposing juveniles to high-dose radiation may stunt growth rate and reduce maturation size of males, thus compromising reproductive success.

My study explores radiation impacts on male sexuality/success, including acoustic, chemical, and visual aspects determining attractiveness and competence of males to attract and fertilize females. I analyze variation in mating success in terms of ability to attract and mate females and subsequent reproductive output (i.e., oviposited eggs, incubation period, hatching success and total successful fecundity).

Methods

Breeding Colony

Experimental groups of *Acheta domesticus* were generated in a large colony housed in an acrylic terrarium (93 x 64.2 x 46.6 cm), insulated with 1.5 cm thick Durofoam insulation. Meshed holes in housing container roofs allowed fans to provide air circulation. The colony was maintained at 29^{o} +/- 2^{o} C on a 12 h day-12h night photoperiod. The breeding colony fed *ad libitum* with chick feed (Country Range MultiFowl Grower®, 17% protein) and *ad libitum* distilled water (soaked cellulose sponges) replaced daily. Crickets were provided with egg-carton shelters, and paper towels sprayed daily with water and replaced weekly. The colony was provided with oviposition medium (Vigoro Organic Garden Soil®) in small plastic containers. These were collected daily and incubated until hatching, providing cohorts of nymphs of known age. All experimental groups were housed and provided the same conditions as the colony.

Experimental Groups

All experimental specimens were generated from oviposition containers and collected on known hatching dates. For song analyses and mounting responses crickets were separated into 4 groups of ~70 individuals per group; Control (0 Gy), 2Gy, 6Gy, and 12Gy. For hatching and mating success, groups of individuals were separated into 6 experimental groups of approximately 200 individuals; Control (400), 2Gy, 4Gy, 7Gy, 10Gy, and 12Gy. Each experimental group was held in plastic housing containers (15cmx11.5cmx9cm) with mesh-covered cut-out lids for air circulation. Food (Country range, 17% protein Multi-Fowl Grower), distilled water (soaked cellulose sponges) and egg-carton shelters were provided *ad libitum.* All experimental groups were irradiated for a specific duration at a dose rate of 0.25Gy/min at the Taylor Radiobiology Source at McMaster University. All groups were then immediately brought to McMaster's Life Sciences Building (LSB) where they remained for life. At approximately 30-40 d of age (once gender was indicated by early ovipositor development), all females were removed from experimental groups.

Pheromone Analysis/Chemical Signals

Pheromone isolation was conducted on 6 randomly selected unmated male crickets from each experimental group. Individuals ~ a week post-maturation were removed and immobilized with CO_2. They were then weighed and then swirled in hexane for 5 min at a concentration of 5ml per 0.5g of cricket mass in a sterilized glass container. After 5 min in hexane, crickets were removed and disposed of. Glass containers with the collected hexane and pheromones were then sealed with an air tight lid and wrapped in elastic tape to ensure no outside contamination could occur. Samples were stored at 4^oC until processing.

Samples were analysed at the McMaster regional centre for mass spectrometry laboratory at A.N Bourns Sciences Bldg. Samples were first vortexed and then evaporated using nitrogen gas. Once evaporated samples were reconstituted in 40ul of pure hexane and 10ul of internal standard (Napthalene-d8), making each sample volume totalling 50ul. Samples were subsequently processed using an Agilent 5973/6890 for gas chromatography and mass spectrometry analysis. Output was then analyzed using Bruker Compass DataAnalysis 4.0. Significant peaks were first identified using the DataAnalysis program and the area/intensity under each peak was recorded. The average intensity of each peak in the control group was then compared to each irradiated group.

Song Analysis & Mating Behavior

Twenty randomly selected males from each experimental group were used for song analysis as well as collection of mating parameters. Each male was recorded in a small housing container with a shelter and sufficient food and distilled water. Once paired with a female, time to commence singing was noted and singing was recorded using a Sony Stereo Digital Voice Recorder. If and when mounting of the male was noted. If males failed to mate during a 1 h period they were considered unsuccessful. If singing did not occur within 1 h the male was excluded from analysis. Recordings were analyzed for key song quality parameters using Audacity 2.1.3 audio program. The mass of both the male and female were recorded to detect correlation to mating success.

Mating Success (Oviposition)

At midlife, approximately 56-67 days post-hatching, experimental males from each group (Control, 2Gy, 4Gy, 7Gy, 10Gy, 12Gy) were individually paired with a virgin female for 24 h. Both male and female mass was recorded. During this period each pair had access to food, distilled water and oviposition medium. Mating was considered successful if females oviposited in the medium within 24 h.

Hatching Success & Hatching Time

At midlife (~56-67 d post hatching), males from each experimental group were paired with 15-20 untreated virgin females. Both sexes were weighed. Oviposition medium was available for 72 h and replaced daily with new containers. Two hundred eggs from the first 24 h period were removed and placed into fresh medium to assess hatching time and success. Hatching and success was also recorded for the subsequent 48 h.

Statistics

For mating success, mounting success, latency to mate, latency to sing, and mass comparison all values are shown as mean values with standard error. All data were analyzed with one-way ANOVA and Tukey's HSD post-hoc multiple comparisons test. Means were accompanied with standard error. Statistics for hatching time, and hatching success remain outstanding. Pheromone composition was analyzed using the mean area under each significant peak between groups. A two-way ANOVA and Tukey's HSD post-hoc multiple comparisons test was employed. Acoustic signal structure was not statistically analyzed due to the complexity of signals. Parameters analysed in control males included inter-chirp time, duration of chirps, pulses

per chirp and amplitude/loudness?). All statistical analyses were completed using Prism Graph Pad 8.

Results

Acoustic Signals

Results for measures of male song are provided in **Table 1.0**. The range in which males were able to perform normally was designated as "typical" (see **Fig. 1.4a).** Measures could not be analyzed in 12Gy males as their songs were severely degraded **(**see **Fig. 1.4b)**. Several males from this group were unable to produce any audible sound.

Chemical Signals

The typical ion chromatogram for a control male cricket, as well as the 26 significant peaks used for analysis are indicated in **Fig. 1.1a** and **Fig. 1.1b.** The typical mass/charge diagram for each significant peak is shown in **Fig. 1.2b**. Each significant peak was shown to have a very similar "staircase" like mass/charge pattern, indicating its hydrocarbon composition**.** Results for the area/intensity under each peak indicated significant differences between controls and irradiated groups for peak 22 and 23 (**Fig. 1.3)**. For peak 22 significant differences were observed compared to control; 2Gy ($p = 0.0039$), 4Gy ($p = 0.0214$), 7Gy ($p<0.0001$), 12Gy ($p=0.0325$). For peak 23 significant differences of $p < 0.0001$ were observed in 2Gy, 4Gy, 7Gy, and 12Gy groups compared to control. These significant differences constituted to changes in signal strength for both peak 22 and peak 23.

Visual Signals

Analysis of the average mass of successful males from each group compared to the average mass of all tested males in that group showed no significant differences (**Fig. 1.5a)**. Similarly, no significant differences were found between the mass of successful versus non-successful males across groups **(Fig. 1.5b).** This indicates that male body mass was not a critical factor in female mate choice. Males however in irradiated groups showed significant declines ($p < 0.0001$) in maturation mass compared to controls in 7Gy (12%), 10Gy (21%), and 12Gy (34%) groups **(Fig. S1.2).**

Latency to Hatch & Latency to Sing

Results analyzing the time that irradiated males took to initiate courtship songs when paired with a female showed no significant differences compared to controls **(Fig. 1.6)**. Song initiation was recorded when males were observed to have wing oscillations associated with song production, if sound was not produced, which was evident in highly irradiated males this wing movement was still considered as the time to initiate singing. Latency to mate (the time for a female to mount a male after introduction), showed significant delay in the 12Gy group ($p = 0.0106$) compared to controls **(Fig. 1.7)**. No other groups showed significant differences.

Mating Success (Mounting/Oviposition)

Radiation impacts on male mating success were categorized as to whether females mounted the male or as subsequent oviposition by the female if mounting had occurred. For mounting success, significant reductions were only found in the 12Gy ($p < 0.0001$) group compared to controls **(Fig. 1.8).** Reductions in subsequent oviposition following mounting were

found for 10Gy (p = 0.01) and 12Gy (p=0.0052) exposure **(Fig. 1.9)**. A non-significant but evident reduction in oviposition following mating was observed in low and middle dose groups with reductions of 38% (2Gy), 33% (4Gy) and 47% (7Gy) compared to controls. Non-significance may be due to inadequate sample size.

Hatching Success

Hatching success was quantified as the number of eggs that hatched from a sample of 200 eggs from each group **(Fig. 1.10)**. Compared to controls with ~58% of eggs hatching, the 4Gy group showed a slight reduction to 48% and the 7Gy a slight increase at 62%. Large declines were observed for 10Gy with only 17.5% hatching and for 12Gy with 0% hatching (i.e., sterilized).

Time to Hatch

Time for 3 separate egg collections to hatch was recorded for each radiation dose **(Fig. 1.11).** Oviposition containers were provided and left in each group housing container (Control, 4Gy, 7Gy, 10Gy, and 12Gy) with approximately 20 virgin females for 3 consecutive 24 h periods. Hatching occurred 12-16 d following oviposition. There were no significant differences in hatching time for groups in which hatching occurred. In the 10Gy group one container failed to hatch, and no hatching occurred following 12Gy exposure.

Discussion

Female preference for specific male characteristics has been well studied and strongly varies among species (Ryan 1990; Prokop et al. 2012). Female responses to male *Acheta*

domesticus include visual, chemical (pheromones), and acoustic signals all of which serve to convey genetic and physical condition (Velando et al. 2008). The concept that "Choosy" females best avoid low quality males traces to Darwin (1859) and remains a guiding paradigm today (Velando et al. 2008).

I tested the effects of ionizing radiation on diverse sexual signals of male *Acheta domesticus,* and consequent impacts on female mate choice (courtship song production, pheromone quantification, and body size). I measured overall male "attractiveness" by evaluating female choice through latency to mount the male, mating success, and subsequent oviposition, incubation period and hatching success.

Wing development

The song of the male house cricket is critical to elicit females to approach, mount and transfer/accept spermatophores (Gray & Eckhardt, 2001). Males actually produce three distinct acoustic signals, each with a distinct role in successful mating (Wagner & Reiser, 1999; Gray & Eckhardt, 2001). The first, an "aggressive" call is used in male-male encounters to assert dominance and competitively obtain access to females (Gray & Eckhardt, 2001). A "calling" song attracts females from a distance, and a much quieter "courtship" song is employed during contact with females to elicit mating (Wagner & Reiser, 1999; Gray & Eckhardt, 2001). All three acoustic signals are generated by specialized wing structures (Huber & Thorson, 1985). Each wing has a "file" that contains striations that a separate wing component, the "scraper," rubs against (Huber & Thorson, 1985). This rubbing generates vibrations of the surrounding wing membrane, and via opening and closing the wings, generates the appropriate "song." See **Figure S1.0** for a visual representation of these wing components and how the song is produced.

One focus of my research was the impact of radiation on the courtship song used to elicit female mounting. Consistent with the results of previous studies, male courtship songs were recorded and were shown to have a specific pattern of intermittent chirps (opening and closing of the wings) which contain a variable number of pulses **(Fig. 1.4a)**. Control males showed similar but individual variability in acoustic signalling. Variability included average length of inter-chirp interval, average chirp length, peak amplitude, and average number of pulses per chirp **(Table 1).**

The results of courtship song recordings of males irradiated at 12Gy revealed very distorted, irregular patterns significantly distinct from any control recordings **(Fig. 1.4b).** 12Gy males lacked distinct chirps or pulses, unlike controls in which these were prominent and relatively consistent. Compared to controls these males showed significantly reduced acoustic amplitude and a significant portion of irradiated males did not produce any audible sound despite visible movement of the wings. The musculature controlling wing movement seemed to be intact in 12Gy individuals as even those with severely damaged or foreshortened wings showed typical wing movements see **Figure S1.6**. This indicates that impacts of radiation on song are specifically associated with specific damage to song production structures such as the "file and scrapper."

If the file striations or scraper structure are damaged, then rubbing the wings together may produce altered or muted sounds or even complete loss of sound production. This is consistent with the results of the recording of 12Gy males **(Fig. 1.4b)**. There is a lack of knowledge in the literature on stress impacts on the development of either the scraper or file wing components. However, there have been studies which have indicated morphological damage due to radiation in other insect species (Seth & Reynolds, 1993). A study using codling moths and Co^{60} irradiators exposed eggs to 4650 rad (~50Gy) (Proverbs & Newton, 1962).

Individuals survived and successfully developed into adults; however they were significantly malformed (Proverbs & Newton, 1962). Although doses were significantly higher than those used in this study due to species-specific radioresistance it does indicate that juvenile irradiation can alter subsequent adult wing development.

Further analysis into wing morphology of irradiated males, specifically of the scrapper and file components would be necessary to determine why irradiated males are unable to produce quality courtship songs. Currently, there is literature on the file, stridulatory component in several different species in the order Orthoptera (Chamorro-Rengifo et al. 2014). This was completed in this study using a stereomicroscope and can be easily completed in our lab using current equipment to compare control and irradiated male wings (Chamorro-Rengifo et al. 2014).

Another aspect in which malformed or dysfunctional wing components may affect male mating success is the calling song. Males produce this type of signal at a distance to facilitate female attraction and guidance (Rost & Honegger, 1987). Males, then initiate their courtship song during which females have the ability to accept or reject the suitor (Rost & Honegger, 1987; Gray & Eckhardt, 2000; Wagner & Reiser, 2000). Of females that approach males at close range, 70% or more are normally likely to mate. For this reason, male calling songs should cover a wide distance (see Gray & Eckhardt, 2001). Since irradiated males produce distorted courtship songs with reduced amplitude it is logical to assume that their calling song may be affected as well, further reducing their ability to attract and mate with females. Researchers have manually mutilated cricket wings to show that damage to the file and scrapper alter or mute sound production (Walker 1962), but there does not appear to be any studies on radiation damage. It is highly significant that radiation impacts may influence populations of insects by altering their

sexual communication systems (as species-specific acoustic signalling is a widespread and precise mechanism critically facilitating reproduction).

Chemical Distortion

Pheromones are vital to communication in the vast majority of organisms (Lockey, 1985; Johansoon & Jones, 2007). In insects, pheromones are characterized for >100 species. These are generally produced and released through the exoskeleton (Kortet & Hendrick, 2005). For reproduction, insect pheromones are critical in mate choice, species, and mate recognition, as well as mate assessment (Kortet & Hendrick, 2005; Johansoon & Jones, 2007). In my study the production and alteration of cuticular pheromones, (specifically volatile hydrocarbons), were analysed in both irradiated and control male crickets. Previous studies using cricket species have shown that volatile hydrocarbon pheromones are distinct between genders and among species (Warthen & Uebel, 1980). Results indicate a specific signature associated with males supporting a species-specific pheromone **(Fig. 1.2)**. Both irradiated and control males showed similar 26 hydrocarbon peaks in their pheromone and these were used for analysis **(Fig. 1.1).**

Although the same 26 peaks were apparent in all groups, the intensity was altered between control and irradiated males. Two of the larger peaks, 22 and 23, were significantly altered in intensity in males exposed to radiation doses spanning 2-12Gy **(Fig. 1.3)**. This could have a variety of consequences for males, including reduced mating success as these signals are very likely used by females to identify gender and/or conspecifics. Studies where female *Gryllus bimaculatus* were washed of their pheromones resulted in males ceasing all mating behavior, indicating the importance of chemical communication in crickets (Tregenza & Wedell, 1996). Future experiments to test the specific influence of pheromone alterations irrespective of other

sexual signals would be most interesting. To reduce interference from prominent acoustic signalling, wingless irradiated males could be tested for female preference against wingless controls (with value of long distance songs being dispensed with via confining male and female crickets to close proximity).

Pheromones, like other traits, may convey information about male quality to females (Kortet & Hedrick, 2005 Rantala et al. 2002). Differences in intensity seen in peak 22 and 23 may signal to females that these males are defective. The concept that females can detect damage in males and thereafter choose not to mate with them has been extensively studied (Velando et al. 2008). Recent studies showed that female rats detect and avoid males with transgenerational epigenetic defects due to fungicide exposure (Crews et al. 2007). The authors suggested that females detected this damage via male odour (Crews et al. 2007). Alterations in irradiated male crickets indeed result in reduced reproductive success as shown by mounting success and latency to mate **(Fig. 1.8 & Fig. 1.9)**. Confirmation will require identification of the hydrocarbons associated with peaks 22 and 23.

Body Size

Both acoustic and chemical signalling are important in female mate choice and sexual recognition as indicated in both the literature as well as in this study (Balakrishnan & Pollack, 1966; Wagner & Reiser, 1999; Nagamoto et al. 2005; Assis et al. 2016). Body size is another signal widely associated with female choice (Simmons, 1988). In crickets, small males may be unable to transfer their spermatophore during mating and they also require more time to produce spermatophores (Sakaluk, 1984; Simmons, 1988). Reduced body size is also correlated with lower sperm count in spermatophores (Sakaluk, 1984). My results looked specifically at female

choice to accept or reject a single male and did not test male body size as a factor in female mate choice between multiple suiters. The experiment was conducted in this way to eliminate the factor of male-male competition, and so females were given a yes/no scenario when assessing a single male.

My results indicated that there was no significant difference between the average body size of successful males compared to their unsuccessful counterparts either within their own group or all groups **(Fig. 1.5a/b).** Males that were in highly irradiated groups that had smaller body size were generally less successful in mating. However, it was not the largest of males in these groups that were successful as indicated in **Figure 1.5a/b.** This indicates that females were not judging potential suiters based solely on their body size. It is important to also note; my results indicate that male body size is not a factor for female choice when there is an absence of other competing males where male-male fighting may occur as well as for females to compare multiple potential mates. My results only indicate that irradiated males are less successful then controls as a result of the other consequences of irradiation, not body size specifically in the scenario in which a female is assessing only a single male.

Although females may not use male body size as a criterion for choice, male body size is vital in male-male competition in cricket species to obtain access to females (Kortet & Hedrick, 2005; Killian & Allen, 2008). Through male-male competition body size will directly affect male mating success as larger males will generally access more females (Kortet & Hedrick, 2005). When males encounter other males, aggressive behavior to assert dominance has been described, with behaviors including aggressive songs, antennal fencing, and tactile combat (Killian & Allen, 2008). Females may also use these competitions as a criterion for their choice as well, with studies suggesting they prefer the scent of dominant males over subordinates (Kortet &

Hedrick, 2005; Killian & Allen, 2008). To test whether this is the case male-male competitions for access to females can be conducted between control and irradiated males.

Sterilization

Sterilization of insects with radiation is a key area of research for insect control (Dyck et al. 2005; Boshra, 2007). To be effective, the dose inducing sterilization must not compromise mating to non-sterilized individuals (Dyck et al. 2005). Irradiated males have been shown to reduce egg hatching success and produce less viable offspring (Boshra, 2007). This is similar to my results in male *Acheta domesticus* irradiated at 10Gy and 12Gy **(Fig. 1.10).** Males in the 10Gy group when paired with virgin females showed a 70% decrease in hatching success of resulting eggs compared to controls. The 12Gy males when paired with virgin females showed a 100% decline in hatching success, having zero eggs successfully hatch.

Males irradiated below 10Gy, the 7Gy and 4Gy group showed a 6% increase and 17% decrease respectively in hatching success compared to controls. The large decrease in hatching success in doses 10Gy and above and the apparent lack of significant effects in doses below this suggests that sterilization may express a threshold radiation dose of 7Gy. Males in 10Gy and 12Gy groups were also less competitive than crickets exposed to lower doses, indicating that higher doses impact both competitiveness in attracting mates and fertility **(Fig. 1.8 & Fig. 1.9)**. These figures suggest that for this species of cricket (*Acheta domesticus)* a dose between 7Gy and 10Gy may be ideal for insect sterilization methods; to induce reduced male fertility while maintain competitiveness in attracting mates. As insects have been shown to vary widely in doses required for sterilization this result must be applied to our model organism only.

Incubation period of eggs for all groups with viable offspring was relatively similar, suggesting that egg development was unaffected by paternal irradiation **(Fig. 1.11).** Other studies suggest that sterilization while maintaining competitiveness occurs through spermatophore disruption. A study looking at heat stress and reproductive output found a high percentage of spermatophores from heat shocked males caused reproductive failure in females (Zizzari & Ellers, 2011). Specifically, it was suggested that spermatophore dysfunction is a result of reduced sperm transfer and quality despite having passed the spermatophore successfully (IAEA, 1968). Sperm damage within the spermatophore may be the cause of the sterility in 12Gy males as well as the significantly reduced fertility in 10Gy males. Analysis of sperm and spermatophore quality and quantity would be a key area of future research to understand radiation exposure.

Reproduction

As mentioned, females can assess various signals of male condition to select fit partners (Wagner & Reiser, 1999; Gray & Eckhardt, 2001). For example, female crickets can assess immune status of males through male courtship song parameters (Rantala et al. 2002; Rantala & Kortet, 2003). In cricket species, copulation involves females mounting males to accept spermatophores following the courtship song (Balakrishnan & Pollack, 1997). Males are therefore unable to coerce females into copulation which makes the males ability to elicit the mounting response through sexual signalling vital to mating success (Balakrishnan & Pollack, 1997). Highly irradiated males showed significantly reduced ability to elicit female mounting **(Fig. 1.7).** This was evident in both the time females took to mount as well as in overall mating success **(Fig. 1.8).** As mentioned, male mating success was dependent on acoustic signalling and

chemical pheromone production. As males produce multiple signal modalities to attract and mate females, it is unclear what information is conveyed by which signal or whether the signals interact or are additive (Wagner & Reiser, 2000). This is a key area for future research.

Delayed Effects & Genomic Instability

The described experiments analyze reproduction and mating effects of males exposed to radiation. However, research has suggested that infertility and further reproductive harm through delayed effects may extend to future generations. A study conducted on tobacco hornworms using ^{60}Co as a source found that exposed individuals not only displayed major reductions in fertility, mating behavior, and competitiveness but this also emerged in the following generations (Van Der Vloedt, & Barnor, 1984; Seth & Reynolds, 1993). They indicated that for exposed males, adverse transgenerational effects included reduced hatching success in F1 offspring (IAEA, 1968; Seth & Reynolds, 1993). In our lab work conducted on irradiated females mated with virgin unirradiated males showed significant declines in hatching success and egg size at doses 4Gy and above. In my experiment irradiated males had reduced fitness both in attracting mates and egg hatching success at slightly higher doses of 7Gy and above **(Fig. 1.8 – 1.10).** It would be an interesting avenue to continue through several generations to see if this trend continues as indicated in literature as well as to compare the relative paternal and maternal effects.

Recent studies indicate that transgenerational effects through paternal exposure can emerge as genomic instability (Barber et al. 2006; Mughal et al. 2012). In mice F1 and F2 offspring of irradiated parents had increased mutation rates and DNA damage in both germline and somatic tissues (Barber et al. 2002; Barber et al. 2006). This was suggested to reflect

genomic instability in exposed parents (Mughal et al. 2012). Genomic instability was also suggested to result from alterations in the epigenome of either exposed parent (Shen & Laird , 2013). Males with delayed damage due to radiation may produce less viable offspring either in the F1 or future generations (Metcalfe & Alonso-Alvarez, 2010). Therefore, females that can detect radiation damage via male sexual signals will in theory be able to avoid irradiated males (Velando et al. 2008; Metcalfe & Alonso-Alvarez, 2010). Since this suggests an evolved discrimination system, radiation would have to alter signals visible to this discrimination. Likely aspects might include DNA damage and consequences, elevated reactive oxygen species and damage, or altered immune biomarkers (to name a few).

Conclusion

In summary, this research provides evidence that early life radiation has later life effects on male reproductive output as well as their ability to attract mates. These effects have been shown to be linear as well as threshold for the parameters analyzed. Disturbances in both pheromone signaling as well as acoustic signalling were apparent. This may have led to the reduction in mating and hatching success in higher dosed groups, specifically in the 10Gy and 12Gy groups that were observed. Although there was not a significant decline in mating success in middle and low dose groups a small reduction was still apparent. Despite this slight decline these groups were able to produce viable offspring, showing no effects in hatching success. This overall suggests that although radiation exposed males at low-middle doses are able to reproduce viably, females are still able to detect “damage” as evidenced by slight reduction in mating success. For high doses this was most apparent, with males showing a reduction in hatching success and a significant reduction in mating success. This therefore indicates that females were

actively choosing to not avoid mating with these males. Overall my research suggests that "choosy" females can detect and avoid radiation-damaged mates, likely via radiation-associated alterations in sexual signalling.

References

A. M. V. Van Der Vloedt, & Barnor, H. (1984). Effects of ionizing radiation on tsetse biology. Their relevance to entomological monitoring during integrated control programmes using the sterile insect technique. *International Journal of Tropical Insect Science,5*(05), 431-437. doi:10.1017/s174275840000878x

Assis, B. A., Trietsch, C., & Foellmer, M. W. (2016). Male mate choice based on chemical cues in the cricket *Acheta domesticus* (Orthoptera: Gryllidae). *Ecological Entomology,42*(1), 11-17. doi:10.1111/een.12353

Balakrishnan, R., & Pollack, G. S. (1996). Recognition of courtship song in the field cricket, *Teleogryllus oceanicus*. *Animal Behaviour,51*(2), 353-366. doi:10.1006/anbe.1996.0034

Balakrishnan, R., & Pollack, G. (1997). The role of antennal sensory cues in female responses to courting males in the cricket *Teleogryllus oceanicus*. *Journal of Experimental Biology,200*, 511-522.

Barber, R., Plumb, M. A., Boulton, E., Roux, I., & Dubrova, Y. E. (2002). Elevated mutation rates in the germ line of first- and second-generation offspring of irradiated male mice. *Proceedings of the National Academy of Sciences,99*(10), 6877-6882. doi:10.1073/pnas.102015399

Barber, R. C., Hickenbotham, P., Hatch, T., Kelly, D., Topchiy, N., Almeida, G. M., . . . Dubrova, Y. E. (2006). Radiation-induced transgenerational alterations in genome stability and DNA damage. *Oncogene,25*(56), 7336-7342. doi:10.1038/sj.onc.1209723

Boshra, S. (2007). Effect of high-temperature pre-irradiation on reproduction and mating competitiveness of male *Sitotroga cerealella* (Olivier) and their F1 progeny. *Journal of Stored Products Research,43*(1), 73-78. doi:10.1016/j.jspr.2005.11.002

Botha, L. M., Jones, T. M., & Hopkins, G. R. (2017). Effects of lifetime exposure to artificial light at night on cricket (*Teleogryllus commodus*) courtship and mating behaviour. *Animal Behaviour,129*, 181-188. doi:10.1016/j.anbehav.2017.05.020

Chamorro-Rengifo, J., Braun, H., & Lopes-Andrade, C. (2014). The secret stridulatory file under the right tegmen in katydids (Orthoptera, Ensifera, Tettigonioidea). *Zootaxa,3821*(5), 590. doi:10.11646/zootaxa.3821.5.7

Crews, D., Gore, A. C., Hsu, T. S., Dangleben, N. L., Spinetta, M., Schallert, T., . . . Skinner, M. K. (2007). Transgenerational epigenetic imprints on mate preference. *Proceedings of the National Academy of Sciences,104*(14), 5942-5946. doi:10.1073/pnas.0610410104

Darwin, Charles, 1809-1882. (1859*). On the origin of species by means of natural selection, or preservation of favoured races in the struggle for life*. London :John Murray,

Dyck, V. A., Hendrichs, J., & Robinson, A. S. (2005). *Sterile insect technique: Principles and practice in area-wide integrated pest management*. Dordrecht: Springer.

Gray, D. A., & Eckhardt, G. (2001). Is cricket courtship song condition dependent? *Animal Behaviour,62*(5), 871-877. doi:10.1006/anbe.2001.1825

Huber, F., & Thorson, J. (1985). Cricket Auditory Communication. *Scientific American,253*(6), 60-68. doi:10.1038/scientificamerican1285-60

The International Atomic Energy Agency. (1968). Isotopes and radiation in entomology. In *Proceedings of a Symposium on the Use of Isotopes and Radiation in Entomology*. Vienna.

Iwasaki, M., & Katagiri, C. (2008). Cuticular lipids and odors induce sex-specific behaviors in the male cricket *Gryllus bimaculatus*. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology,149*(3), 306-313. doi:10.1016/j.cbpa.2008.01.008

Killian, K. A., & Allen, J. R. (2008). Mating resets male cricket aggression. *Journal of Insect Behavior,21*(6), 535-548. doi:10.1007/s10905-008-9148-x

Kortet, R., & Hedrick, A. (2005). The scent of dominance: Female field crickets use odour to predict the outcome of male competition. *Behavioral Ecology and Sociobiology,59*(1), 77-83. doi:10.1007/s00265-005-0011-1

Lockey, K. H. (1985). Insect cuticular lipids. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry,81*(2), 263-273. doi:10.1016/0305-0491(85)90311-6

Luke, G. A., Riches, A. C., & Bryant, P. E. (1997). Genomic instability in haematopoietic cells of F1 generation mice of irradiated male parents. *Mutagenesis,12*(3), 147-152. doi:10.1093/mutage/12.3.147

Metcalfe, N. B., & Alonso-Alvarez, C. (2010). Oxidative stress as a life-history constraint: The role of reactive oxygen species in shaping phenotypes from conception to death. *Functional Ecology,24*(5), 984-996. doi:10.1111/j.1365-2435.2010.01750.x

Mughal, S. K., Myazin, A. E., Zhavoronkov, L. P., Rubanovich, A. V., & Dubrova, Y. E. (2012). The dose and dose-rate effects of paternal irradiation on transgenerational instability in mice: A radiotherapy connection. *PLoS ONE,7*(7). doi:10.1371/journal.pone.0041300

Nagamoto, J., Aonuma, H., & Hisada, M. (2005). Discrimination of conspecific individuals via cuticular pheromones by males of the cricket *Gryllus bimaculatus*. *Zoological Science*,*22*(10), 1079-1088. doi:10.2108/zsj.22.1079

Prokop, Z. M., Michalczyk, Ł, Drobniak, S. M., Herdegen, M., & Radwan, J. (2012). Meta-analysis suggests choosy females get sexy sons more than "good genes". *Evolution,66*(9), 2665-2673. doi:10.1111/j.1558-5646.2012.01654.x

Proverbs, M. D., & Newton, J. R. (1962). Some effects of gamma radiation on the reproductive potential of the codling moth, *Carpocapsa pomonella* (L.) (Lepidoptera: Olethreutidae). *The Canadian Entomologist*,*94*(11), 1162-1170. doi:10.4039/ent941162-11

Rantala, M. J., & Kortet, R. (2003). Courtship song and immune function in the field cricket *Gryllus bimaculatus*. *Biological Journal of the Linnean Society,79*(3), 503-510. doi:10.1046/j.1095-8312.2003.00202.x

Rantala, M. J., Jokinen, I., Kortet, R., Vainikka, A., & Suhonen, J. (2002). Do pheromones reveal male immunocompetence? *Proceedings of the Royal Society of London. Series B: Biological Sciences*,*269*(1501), 1681-1685. doi:10.1098/rspb.2002.2056

Rebar, D., Bailey, N. W., & Zuk, M. (2009). Courtship songs role during female mate choice in the field cricket *Teleogryllus oceanicus*. *Behavioral Ecology,20*(6), 1307-1314. doi:10.1093/beheco/arp143

Rost, R., & Honegger, H. W. (1987). The timing of premating and mating behavior in a field population of the cricket *Gryllus campestris* L. *Behavioral Ecology and Sociobiology,21*(5), 279-289. doi:10.1007/bf00299965

Ryan, M. (1990). Signals, species, and sexual selection. *American Scientist,78*(1), 46-52.

Seth, R., & Reynolds, S. (1993). Induction of inherited sterility in the tobacco hornworm *Manduca sexta* (Lepidoptera: Sphingidae) by substerilizing doses of ionizing radiation. *Bulletin of Entomological Research,83*(2), 227-235. doi:10.1017/s0007485300034714

Shen, H., & Laird, P. (2013). Interplay between the cancer genome and epigenome. *Cell*,*153*(1), 38-55. doi:10.1016/j.cell.2013.03.008

Simmons, L. W., Tinghitella, R. M., & Zuk, M. (2010). Quantitative genetic variation in courtship song and its covariation with immune function and sperm quality in the field cricket *Teleogryllus oceanicus*. *Behavioral Ecology,21*(6), 1330-1336. doi:10.1093/beheco/arq154

Simmons, L. W. (1987). Female choice contributes to offspring fitness in the field cricket, *Gryllus bimaculatus* (De Geer). *Behavioral Ecology and Sociobiology,21*(5), 313-321. doi:10.1007/bf00299969

Simmons, L. (1988). Male size, mating potential and lifetime reproductive success in the field cricket, *Gryllus bimaculatus* (De Geer). *Animal Behaviour,36*(2), 372-379. doi:10.1016/s0003-3472(88)80008-3

Thomas, M. L., & Simmons, L. W. (2010). Cuticular hydrocarbons influence female attractiveness to males in the Australian field cricket, *Teleogryllus oceanicus. Journal of Evolutionary Biology,23*(4), 707-714. doi:10.1111/j.1420-9101.2010.01943.x

Tregenza, T., & Wedell, N. (1997). Definitive evidence for cuticular pheromones in a cricket. *Animal Behaviour,54*(4), 979-984. doi:10.1006/anbe.1997.0500

Velando, A., Torres, R., & Alonso-Alvarez, C. (2008). Avoiding bad genes: Oxidatively damaged DNA in germ line and mate choice. *BioEssays,30*(11-12), 1212-1219. doi:10.1002/bies.20838

Wagner, W. E., & Reiser, M. G. (2000). The importance of calling song and courtship song in female mate choice in the variable field cricket. *Animal Behaviour,59*(6), 1219-1226. doi:10.1006/anbe.1999.1428

Walker, T. J. (1962). Factors responsible for intraspecific variation in the calling songs of crickets. *Evolution,16*(4), 407. doi:10.2307/2406176

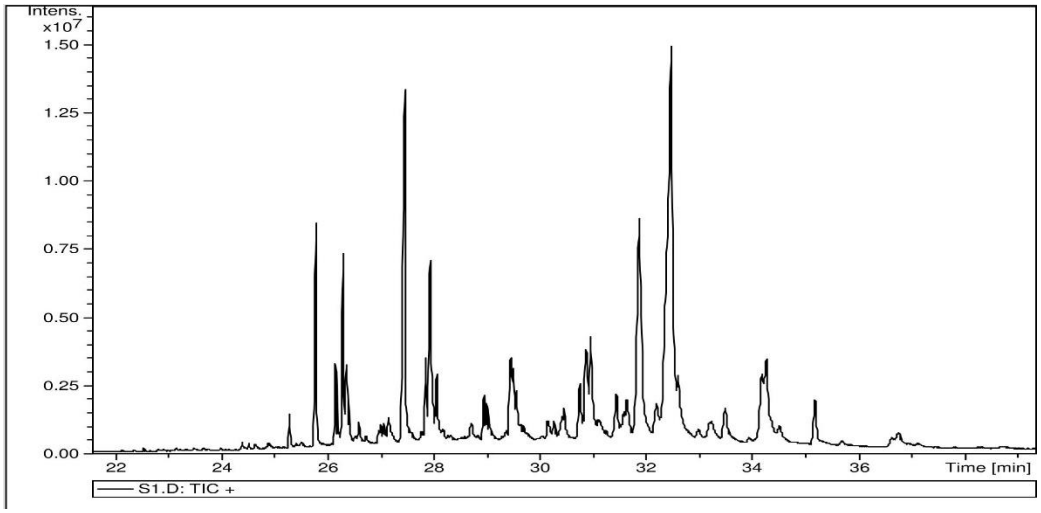
Warthen, J., & Uebel, E. (1980). Comparison of the unsaturated cuticular hydrocarbons of male and female house crickets, *Acheta domesticus* (L.) (Orthoptera: Gryllidae). *Insect Biochemistry,10*(4), 435-439. doi:10.1016/0020-1790(80)90015-3

Zizzari, Z. V., & Ellers, J. (2011). Effects of exposure to short-term heat stress on male reproductive fitness in a soil arthropod. *Journal of Insect Physiology,57*(3), 421-426. doi:10.1016/j.jinsphys.2011.01.002

Table 1: Summary Table of Parameters Collected for Control Male Courtship Songs.

Recording	Average Length of Interchirp Interval (s)	Average Chirp Length (s)	Peak Amplitude (dBFS)	Average Number of Pulses Per Chirp
1	1.13	0.2224	-1.2	2.467
2	1.1702	0.2743	-0.6	3.133
3	1.1	0.2143	-3.4	3.133
4	0.6409	0.1324	-2.2	2.867
5	0.4329	0.1667	-1.8	2.2
6	0.3857	0.1674	-2.2	2.27
7	0.4216	0.2	-7.3	3
8	0.3485	0.2407	-6.2	3
9	0.4589	0.1042	-1.3	1.933
10	0.3496	0.2305	-6.3	2.667
11	1.093	0.1853	-1	2.867
12	0.4231	0.1608	-0.9	1.8
13	1.319	0.194	-1.7	2.533
14	.4446	0.1803	-0.2	3.4
15	1.039	0.1925	-1.6	2.8
16	0.2042	0.1063	-2.7	1.933
17	0.431	0.1305	-1.4	2.133
18	0.447	0.1637	-0.8	2.067
19	0.4566	0.1561	-0.8	2.067
20	1.1689	0.157	-1.3	1.933
21	0.354	0.1489	-0.7	2.333
22	0.3085	0.152	-4	2.733

a)



b)

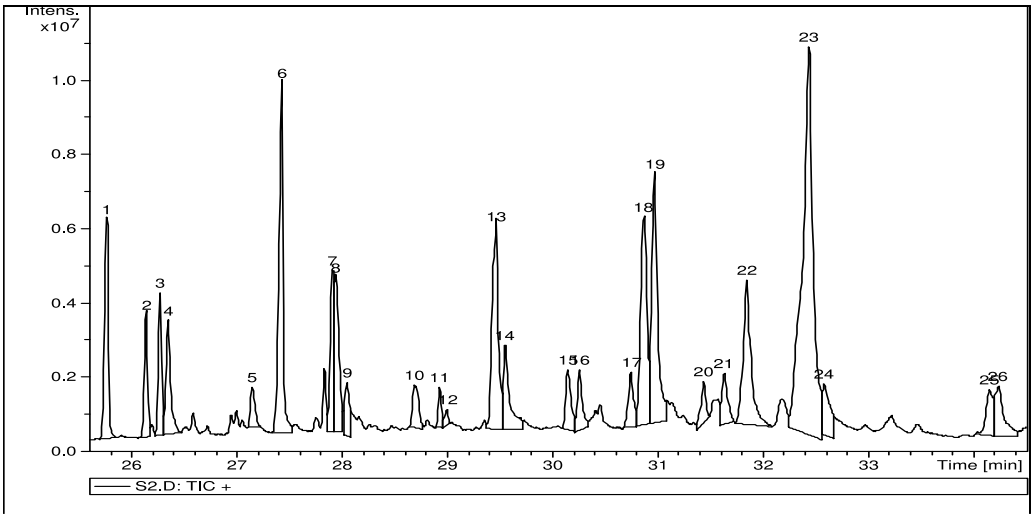
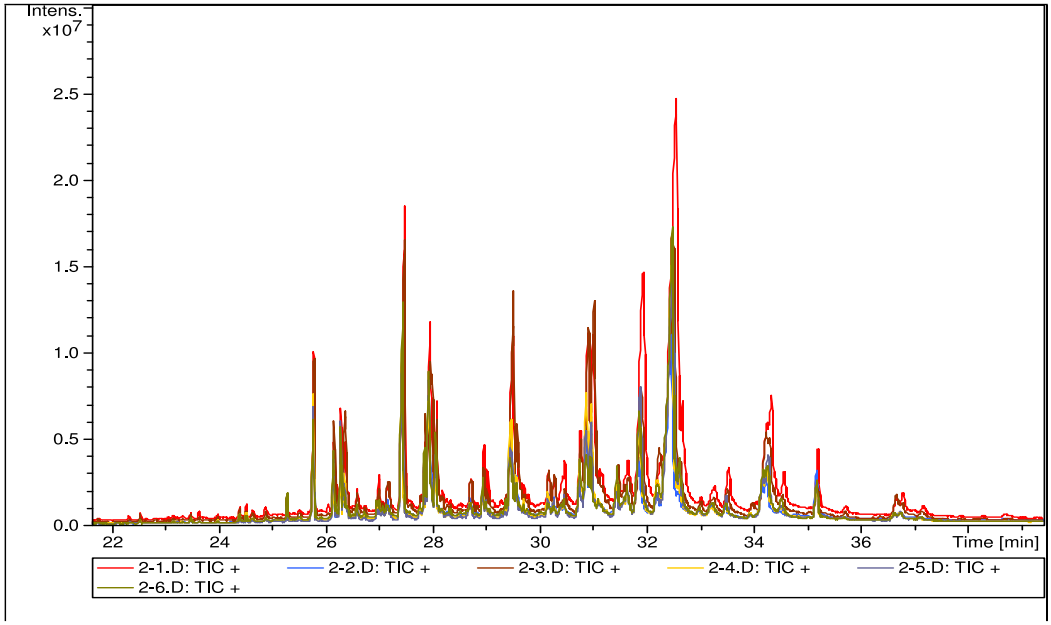


Figure 1.1: A Typical total ion chromatogram of a control male *Acheta domesticus* taken approximately 1 week post maturation. Each peak corresponds to a specific hydrocarbon compound (a). Typical total ion chromatogram of a 2Gy male cricket irradiated at 14 days of age at 0.25Gy/min. This figure shows the 26 significant peaks used for analysis (b).

a)



b)

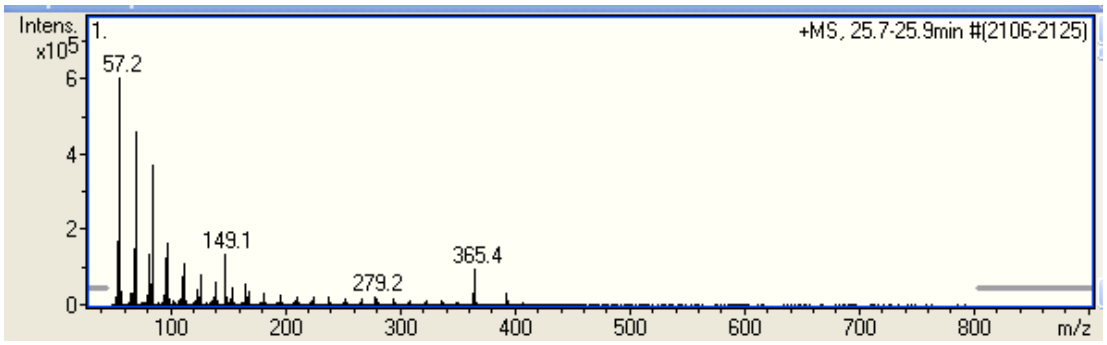


Figure 1.2: A Stacked total ion chromatogram for all 2Gy males analyzed showing the general consistency of male pheromone signature. Males were irradiated at 14 days of age at a dose rate of 0.25Gy/min with samples collected 1 week post maturation (a). A typical mass/charge of a hydrocarbon (b). Each peak mass/charge diagram was checked to insure its hydrocarbon identity.

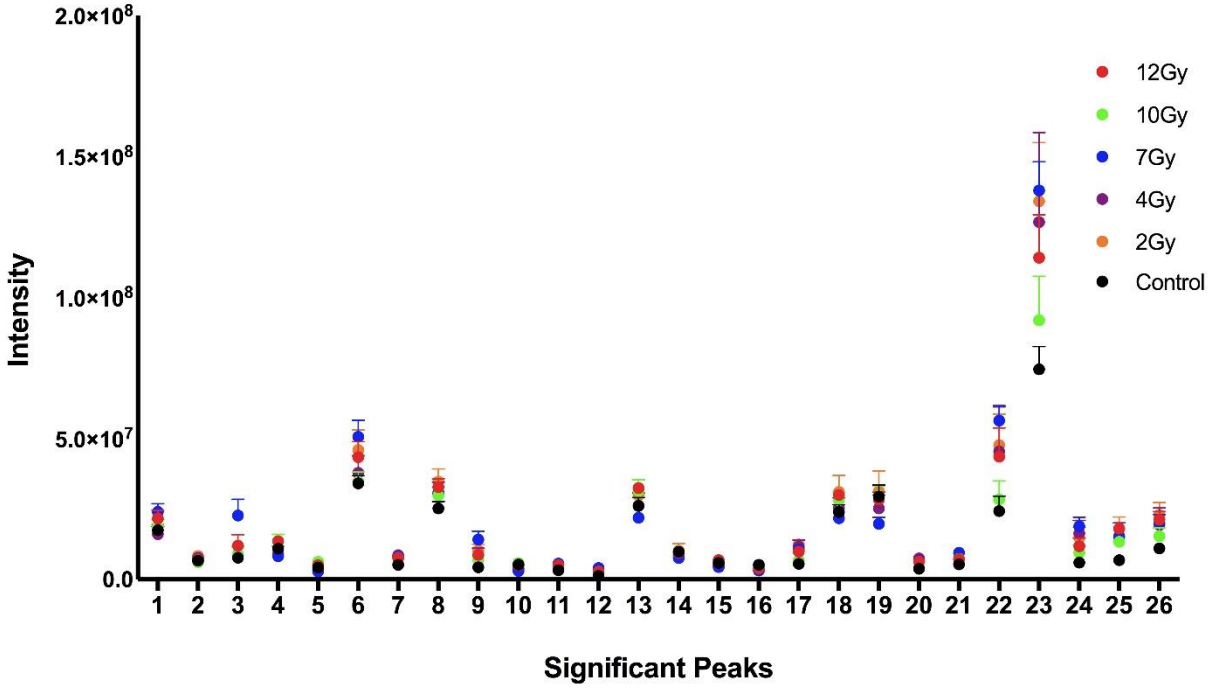
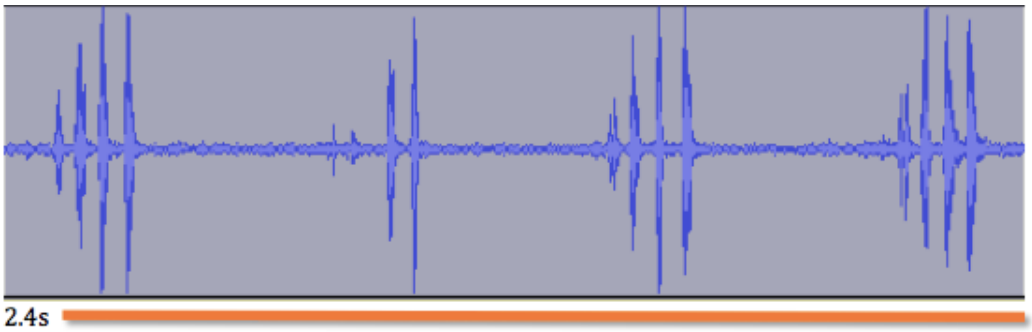


Figure 1.3: Dose-response effects of early life radiation on hydrocarbon pheromone production, specifically the effect on each of the 26 significant hydrocarbon peaks identified using gas-liquid chromatography. All individuals were irradiated at 14 days of age at 0.25G Gy/min with pheromone extraction occurring 1 week post maturation. Values are shown as the mean area under each peak for 6 individuals from each radiation group (Control, 2Gy, 4Gy, 7Gy, 10Gy, 12Gy) +/- SEM. Significant differences were identified for peak 22; 2Gy ($p= 0.0039$), 4Gy ($p = 0.0214$), 7Gy ($p <0.0001$), and 12Gy ($p=0.0325$) and 23; 2Gy,4Gy, 7Gy, and 12Gy ($p<0.0001$) compared to control values. All significant differences were analyzed compared to control values using a 2-way ANOVA followed by a Tukey's HSD test.

a)



b)

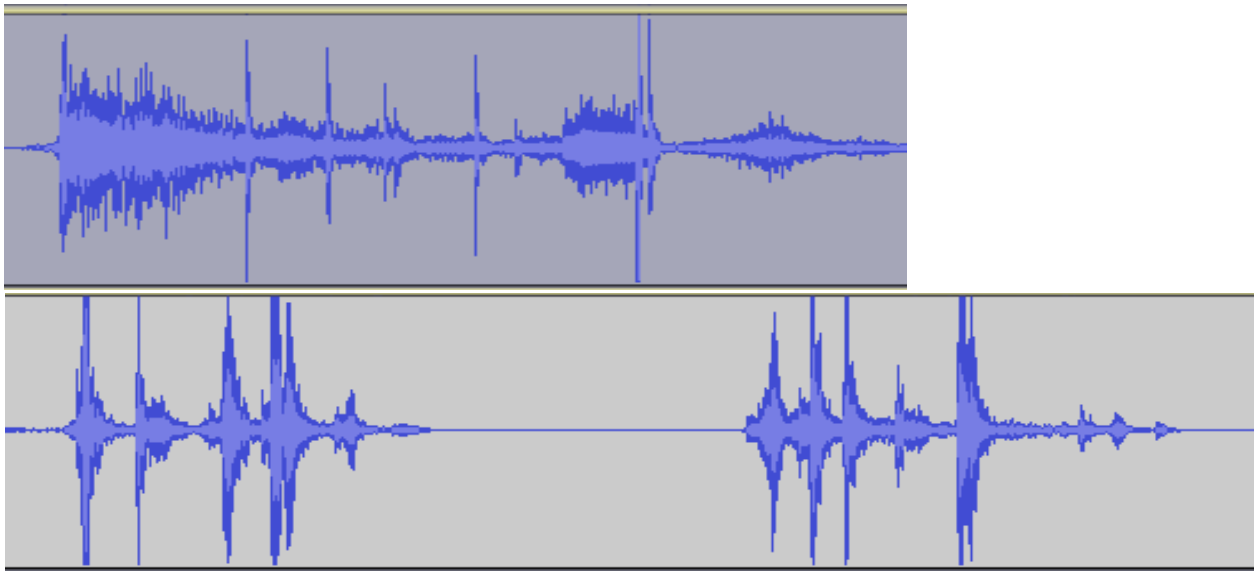
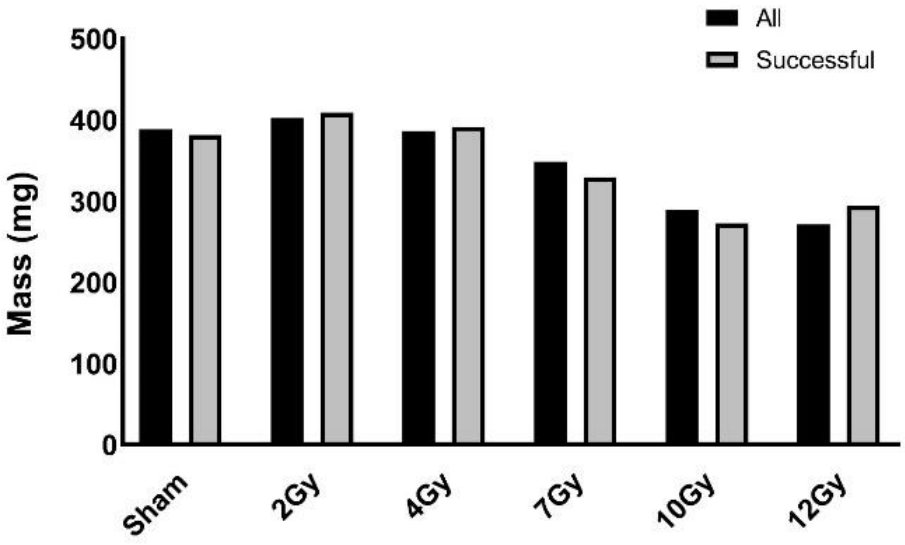


Figure 1.4: The typical audio recording of a control male *Acheta domesticus* courtship song (a) and a irradiated (12Gy) male courtship song (b). Irradiated males were exposed at 14 days of age at 0.25Gy/min. Males were recorded between 1-3 weeks post maturation using a Sony Stereo Digital Voice Recorder to record songs and Audacity 2.1.3 audio program to analyze song parameters.

a)



b)

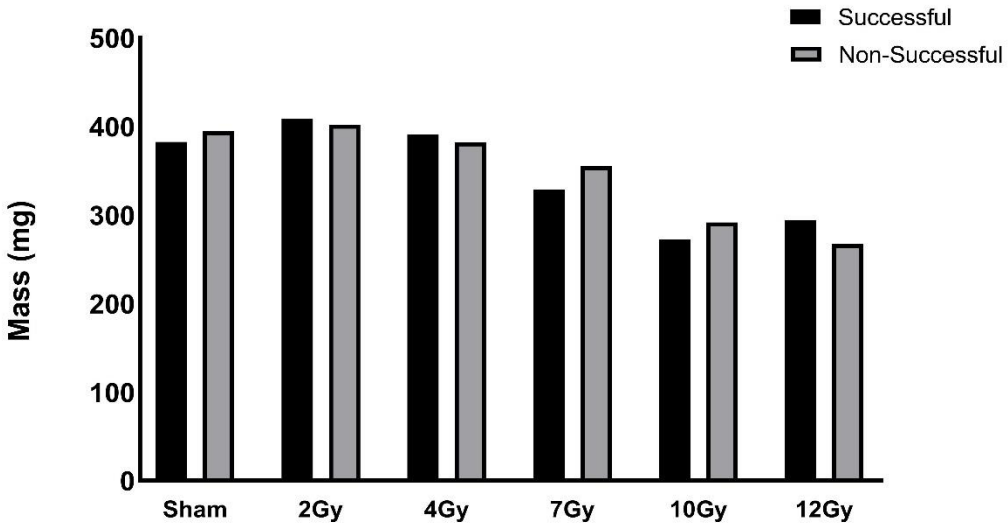


Figure 1.5: Effects of size on mating success in *Acheta domesticus* with irradiated males exposed to early life radiation. All males were exposed at 0.25Gy/min at 14 days of age and mated between 1-3 weeks post maturation with virgin females. Values are shown as the mean mass of all males tested from each group; Control (n =28), 2Gy (n =9), 4Gy (n =30), 7Gy (n=30), 10Gy (n=27), 12Gy (n=20) compared to the mean mass of all successful males for each irradiated group (a). No significant differences were observed between average mass of non-successful males versus the average mass of successful males in any groups (b). All differences were analyzes using a one-way ANOVA followed by a Tukey's HSD test for comparisons between groups.

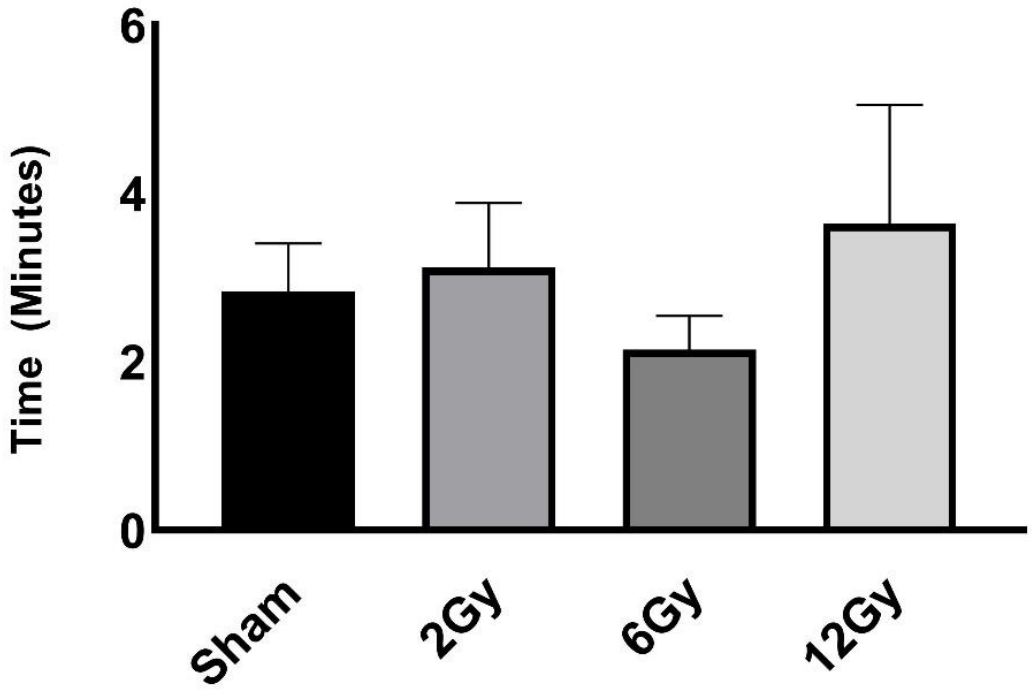


Figure 1.6: Effects of early life radiation on male *Acheta domesticus* latency to sing. Males were irradiated at 14 days of age at a dose rate of 0.25Gy/min and paired with virgin females 1-3 weeks post maturation. Time was recorded as time from when female was added to male container to when sound was produced by male wing striations. Values are the mean time for each groups Control (n= 18), 2Gy (n=17), 6Gy (n=24), 12Gy (n=14) +/- SEM. No significant differences were identified between groups. All differences were compared to control groups using a one-way ANOVA followed by a Tukey's HSD test.

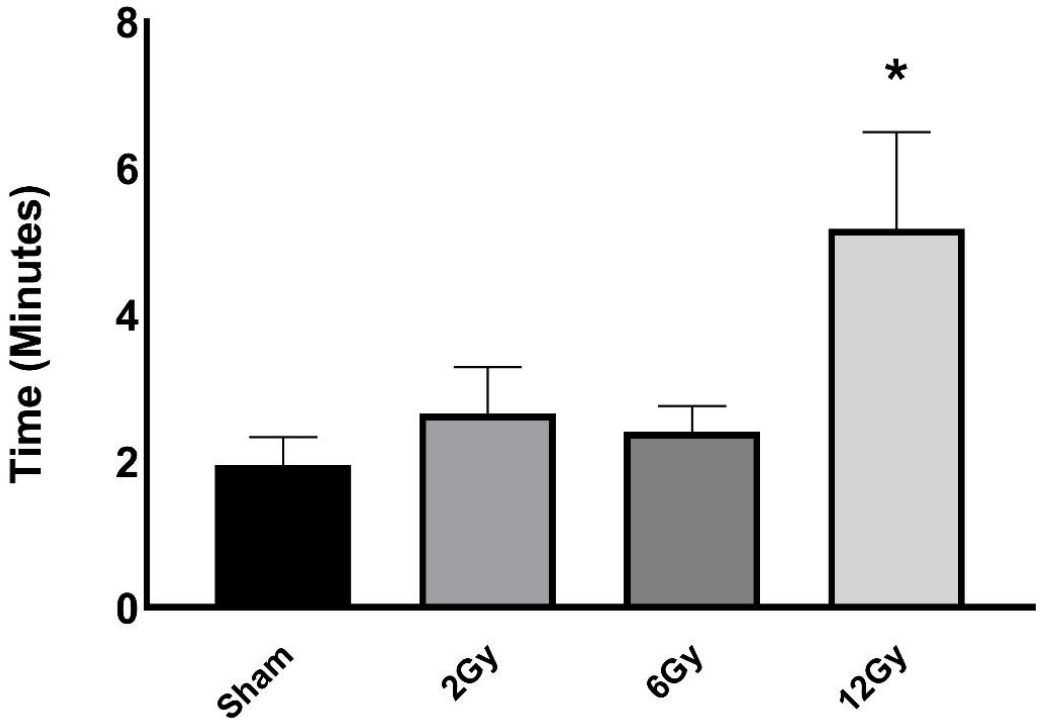


Figure1.7: Effects of early life radiation on male *Acheta domesticus* latency to mount. Males were irradiated at 14 days of age at a dose rate of 0.25Gy/min and paired with virgin females 1-3 weeks post maturation. Time was recorded as time from when the female was added to the male container to when females mounted males, indicating that mating had occurred. Values are the mean time for each group; Control (n= 18), 2Gy (n=17), 6Gy (n=24), 12Gy (n=14) +/- SEM. Significant differences were indicated in only the 12Gy group (p=0.0106) compared to controls. All differences were compared to control groups using a one-way ANOVA followed by a Tukey's HSD test.

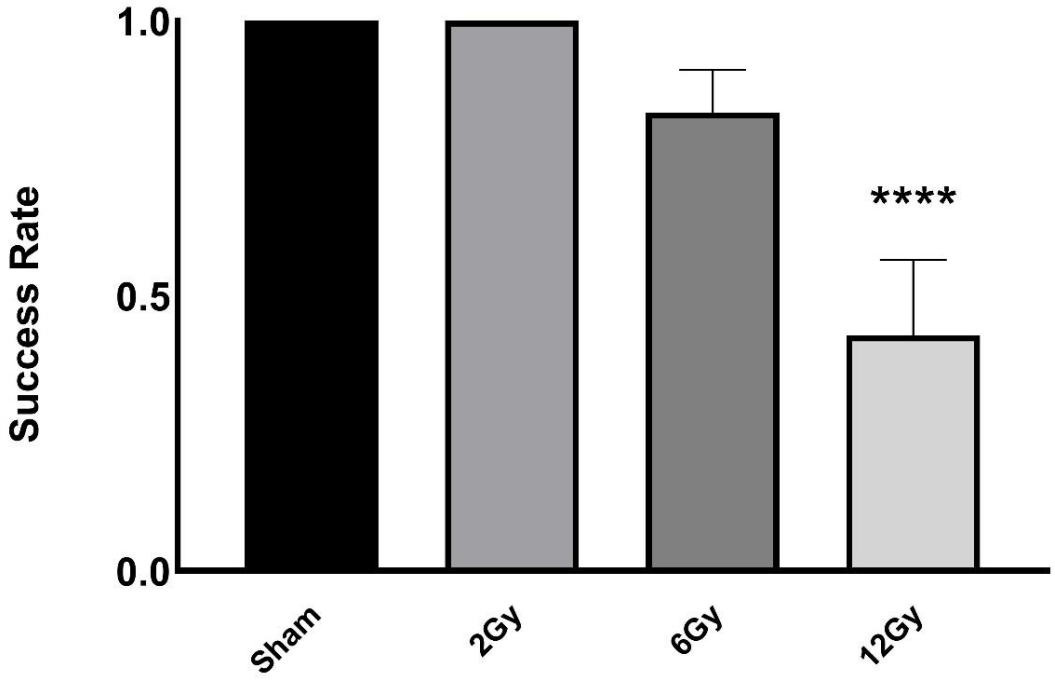


Figure 1.8: Effects of early life radiation on male *Acheta domesticus* mounting success. Males were irradiated at 14 days of age at a dose rate of 0.25Gy/min and subsequently paired with virgin females 1-3 weeks post maturation. Success was indicated as females mounting males as well as the transfer of spermatophore to the female. Values are the rate of success for each group; Control (n= 18), 2Gy (n=17), 6Gy (n=24), 12Gy (n=14) +/- SEM. Significant differences were indicated in only the 12Gy group ($p < 0.0001$) compared to controls. All differences were compared to control groups using a one-way ANOVA followed by a Tukey's HSD test.

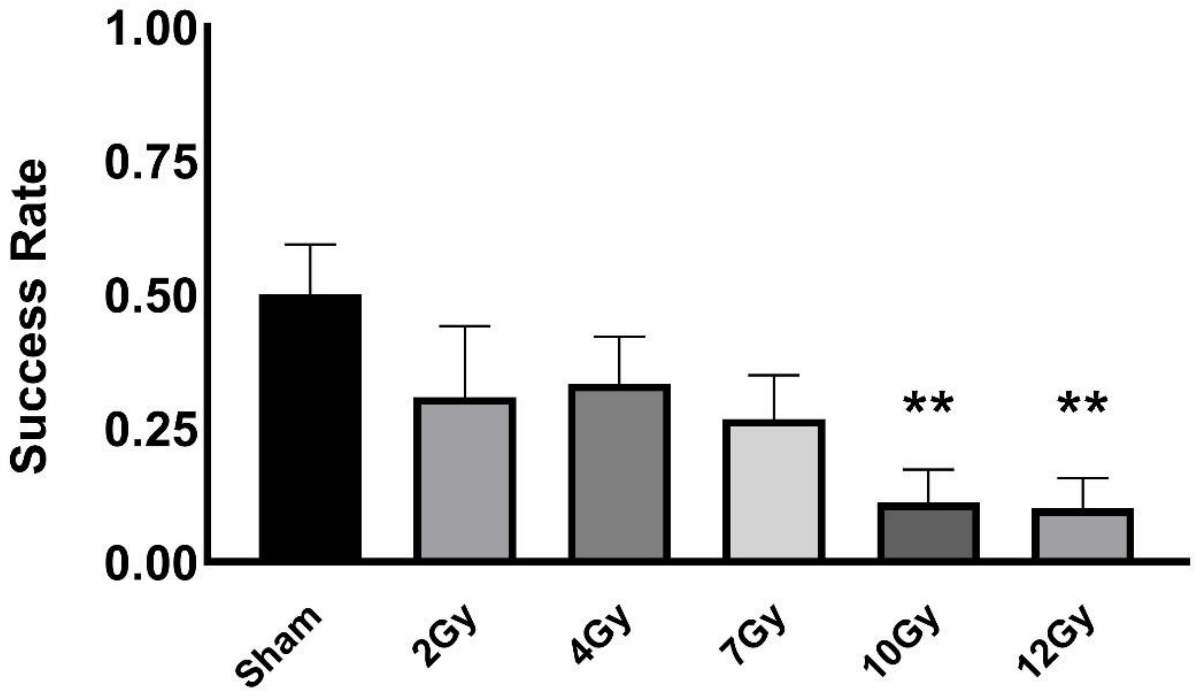


Figure 1.9: Effects of early life radiation on male *Acheta domesticus* mating with oviposition success. Males were irradiated at 14 days of age at a dose rate of 0.25Gy/min and paired with virgin females 1-3 weeks post maturation. Success was indicated as spermatophore transfer and subsequent laying of eggs into oviposition medium within the next 24 hours. Values are the rate for each groups Control (n =28), 2Gy (n =9), 4Gy (n =30), 7Gy (n=30), 10Gy (n=27), 12Gy (n=20) +/- SEM. Significant differences were indicated in only the 10Gy (p =0.0100) and 12Gy (p= 0.0052) groups compared to controls. All differences were compared to control groups using a one-way ANOVA followed by a Tukey's HSD test.

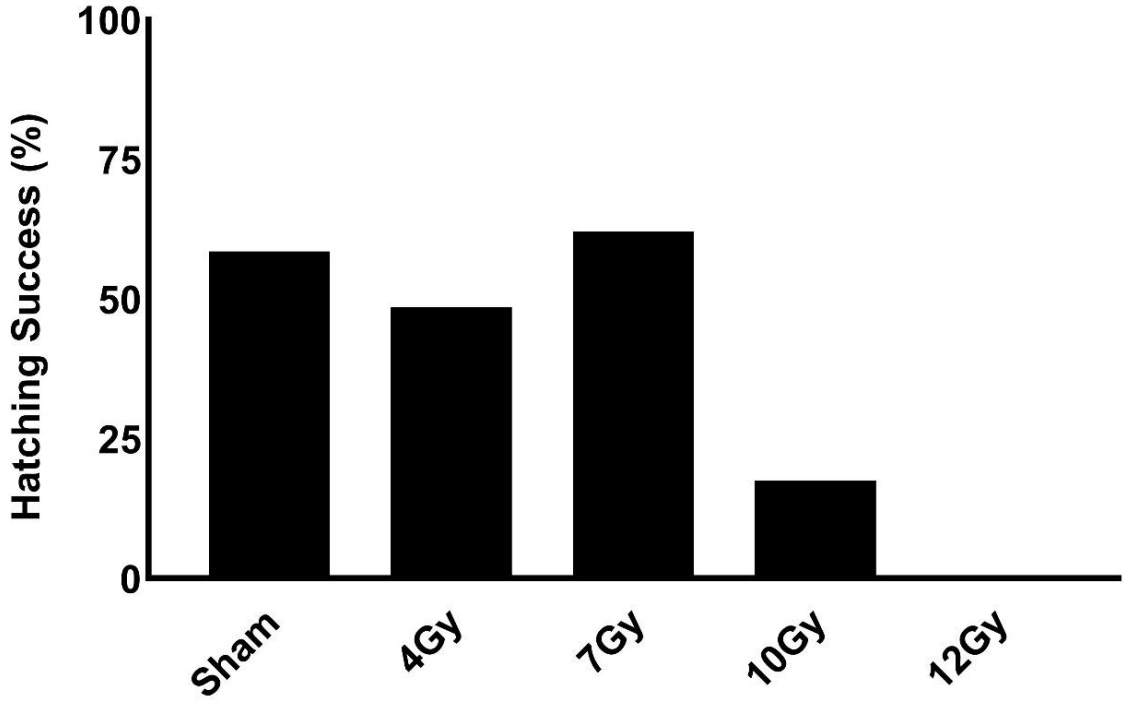


Figure 1.10: Effects of early life radiation on male *Acheta domesticus* hatching success. Males were irradiated at 14 days of age at a dose rate of 0.25Gy/min and subsequently paired with virgin females 1-3 weeks post maturation with oviposition medium being collected after a 24-hour period. Values are the percent of individuals which hatched from 200 collected eggs. Due to this being conducted a single time significant differences are not reported. The 12Gy group is reported as 0% as they failed to hatch.

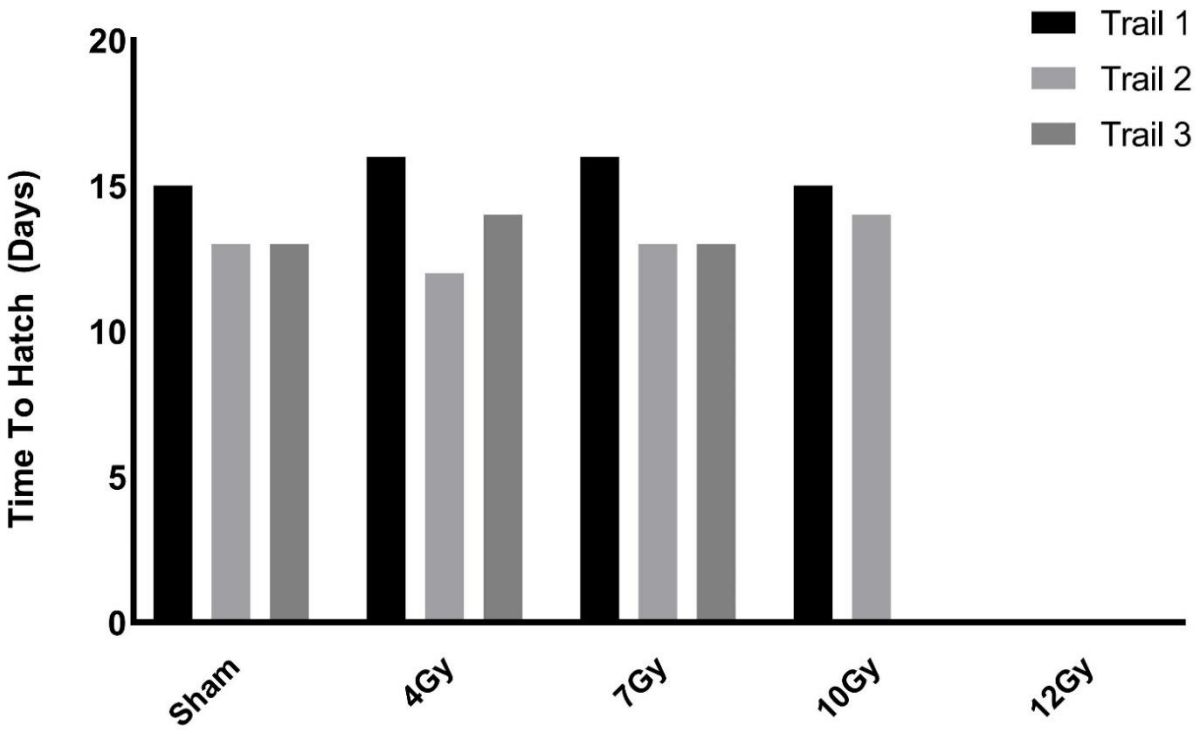


Figure 1.11: Effects of early life radiation on male *Acheta domesticus* hatching time. Males were irradiated at 14 days of age at a dose rate of 0.25Gy/min. Groups were paired with virgin females 1-3 weeks post maturation with oviposition medium being collected for 3, 24-hour periods. Values are the time in days it took from oviposition collection to hatch. Due to this being conducted for only three oviposition containers, significant differences are not reported. The 12Gy group is reported as 0% as they failed to hatch as well as one 0% marked for one 10Gy test.

CHAPTER 2

Impacts of Paternal Irradiation on F1 Offspring Life History Features

Abstract

Life generally responds and adapts to internal and external disturbances. When experiencing a disturbance an organism initiates a coordinated stress response, allowing the ability to resist or suppress the negative impacts of stress. Ionizing radiation is a stressor that impacts organisms through direct DNA damage and indirect epigenetic changes. These impacts not only effect those exposed but also offspring; if DNA damage occurs in the germline, or epigenetic changes are inherited. Generally, this has been studied through the maternal line, with paternal inheritance of stress being ignored or considered irrelevant. This study assessed life history and reproductive impacts of early acute irradiation on male *Acheta domesticus*. This was completed for both exposed males as well as their F1 offspring in order to detect potential paternal effects. Impacts on several molecular biomarkers; FOXO, Nrf2, and methylation for both F0 and F1 offspring were also investigated. Results indicated sufficient evidence to suggest paternal generational effects due to radiation exposure for several life history traits. Irradiated males as well as their offspring experiences increased survivorship and longevity at mid range doses (7Gy & 10Gy). Irradiated males experienced reduced growth rate due to their exposure however, offspring seemed to mostly avoid this outcome. Some changes to global methylation were also identified. Overall, this study indicates paternal irradiation not only impacts those exposed but can lead to impacts on offspring life history features.

Introduction

Life generally responds and adapts to internal and external disturbances (Rattan, 2008). Although difficult to define, stress is a disturbance (whether it be chemical, physical, or biological), that disrupts homeostasis, and elicits coordinated stress responses (Rattan, 2008; Mothersill & Seymour, 2013; Chaby, 2016). Stress responses have the ability to resist or suppress stressors and improve or stabilize organismal and cellular health (Rattan, 2008; Chaby, 2016). However, if stress is prolonged or sufficient to exceed tolerance, dysfunction and damage may emerge (Rattan, 2008).

Radiation as a stressor

Ionizing radiation acts as a stressor mainly through the ionization of water within cells to generate free oxygen radicals (ROS) (Einor et al. 2016). Under normal conditions ROS can serve as important intracellular signalling molecules, and levels are adaptively regulated by diverse cellular antioxidant mechanisms (D'Autreaux & Toledano, 2007). Oxidative stress occurs when ROS exceeds the tolerance of the stress response system (Einor et al. 2016). Consequently, cells may express depleted antioxidants and heat shock proteins, and widespread damage to organelles, cell membranes and DNA (Einor et al. 2016).

At the organismal level, high and/or prolonged oxidative stress can inflict damage or epigenetic changes in DNA, derive pervasive membrane damage and alter cellular constituents. Impacts can extend to alterations in regulatory systems accompanied by widespread apoptosis or accelerated cell senescence and organismal aging (Einor et al. 2016). At an organism level oxidative stress may manifest as reduced growth, infertility, and truncated longevity (Einor et al. 2016).

Diverse environmental stressors can elicit common stress resistance pathways, thus providing a "unity amid diversity" for stress research and organismal mechanisms and regulatory responses (Cypser & Johnson, 2002). Important aspects of stress responses extend to epigenetic modifications, altered cellular pathways (TOR Vs FOXO) and organismal (Growth hormone vs Hypothalamic-Adrenal Axis) regulatory watersheds. This ultimately impinges life history features (growth, maturation, body size, fertility and survivorship). Key biomarkers of stress response systems altered life-histories and epigenetic inheritance are the focus of this thesis.

Epigenetic Inheritance and Paternal Effects

Lamarck's discredited theory of evolution (~1830) suggested that acquired characteristics could be inherited. More recently, considerable literature indicates that parents exposed to environmental stressors can alter the stress response systems of their offspring (Lachmann & Jablonka, 1996; Seong et al. 2013). Inheritance based on environmental experience can only occur if maternal or paternal germline DNA were to be directly altered or undergoes "epigenetic" alterations that are heritable (Lachmann & Jablonka, 1996; Anway, 2005). Ionizing radiation, which directly and indirectly alters DNA, can result in elevated mutation rates (including the germline), as well as epigenetic alterations involving chromatin structure and methylation patterns (Baulch, 2001; Barber et al. 2002; Mousseau & Moller, 2014). Such changes can persist across multiple generations, resulting in profound effects on offspring phenotypes (Burton & Metcalfe, 2014).

Studies of epigenetic inheritance and its mechanisms have largely focused on maternal effects and assume paternal effects are absent or unimportant, particularly in species without paternal care (like crickets) (Bonduriansky & Head, 2007; Bonduriansky & Day, 2009). In

insects, maternal effects due to environmental stress have been demonstrated in over 70 species and impacts extend to mate choice, egg size, oviposition behavior, as well as epigenome modifications (Mousseau, 1998; Mousseau & Fox, 1998; Soubry, 2014).

As for paternal effects, recent studies found that they do indeed exist and are important in offspring development (even in insect species with virtually no paternal investment). Paternal effects mainly include the general vulnerability of the male germ line to both genetic and non-genetic changes compared to their egg counterparts (Nomura, 1982; Velando et al. 2008; Rodgers et al. 2013). Moreover, the male germ line has higher mutation rates tracing to poor defence mechanisms in sperm (Chong et al., 2007; Metcalfe & Alonso-Alvarez, 2010; Bonisoli-Alquati et al. 2011). Today, the main focus of stress, including radiation, are direct effects of exposure (mainly mutations) and there has been limited examination of trans-generational impacts of parental exposure (especially paternal contributions).

Epigenetics is the process of altering DNA regulation, management, or expression that can impact aspects such as cell differentiation or organismal phenotypes without altering DNA sequences (Rando, 2012; Crino & Breuner, 2015). Important aspects include DNA methylation (that alter gene expression), chromatin states (histone proteins), maternal and paternal effects, behavioral diversity, phenotypic plasticity, and gender-biased gene expression (Baulch, 2001; Anway, 2005; Bonduriansky et al. 2011). Epigenetic alterations in the germline can persist for several generations. In this way epigenetic modifications can either reduce fitness or play an adaptive role in relatively rapid adjustments to environmental and habitat changes in a lineage, perhaps acting as a short-term buffer allowing natural selection to make actual changes to the genome (Bonduriansky et al. 2011).

Studies analyzing the effects of epigenetic inheritance often focus on stressors such as toxins, pollutants, and nutrient availability (Jirtle & Skinner, 2007; Rando, 2012). Although we understand that epigenetic alterations occur and are inherited, the mechanism on how this occurs is not fully understood (Bonduriansky et al. 2011; Rodgers et al. 2013). Here the epigenetic factor examined was changes in global methylation in response to radiation (oxidative stress).

Cytosine DNA methylation (5-methylcytosine) has been demonstrated in various eukaryotic organisms (Field et al. 2004). This occurs in species to varying degrees. In insects and other invertebrates global methylation only constitutes approximately 0-3%, with methylation occurring specifically on genes (Field et al. 2004). Methylation strongly alters gene expression and regulation with consequences extending to life-histories and the expressed organismal phenotype (Velando et al. 2008; Glastad et al. 2011).

Changes to methylation patterns were long thought to be uninheritable since the vast majority of DNA methylation marks are erased after fertilization (Velando et al. 2008). However, a small subset of imprinted genes are maintained and can be transmitted to offspring by either parent (Velando et al. 2008; Johannes et al. 2009; Bale, 2014; Rodgers et al. 2015). Stress-induced alterations in methylation patterns can elicit positive adaptations to stressful environments but also have the potential to produce maladaptive methylation patterns. Such modifications can result in pathology and phenotypic aberrations that transcend levels of organization (Velando et al. 2008).

Stress Response Pathways

Analyzing changes in key components of the cellular stress response pathways and their possible inheritance is an important area relevant to environmental stress and adaptation. Nrf2, “the master antioxidant coordinator,” critically regulates stress responses, including transcription

of multiple antioxidant genes that mitigate ROS (Moller et al. 2005; Carmon-Ramirez et al. 2013; Ma, 2013; Zhang et al. 2018).

FOXO (Forkhead box O) transcription factors are a family of highly conserved regulators impacting the cell cycle, apoptosis, stress responses, and metabolism (Eijkelenboom & Burgering, 2013). In vertebrates, FOXOs are regulated by several factors through phosphorylation/dephosphorylation at specific sites which lead to either nuclear exclusion or nuclear localization/transcription (Wang et al., 2017). When FOXO is localized in the cytoplasm it is inactive and is transported to the proteasome and degraded. Low activity of FOXOs and their target genes can result in foreshortened lifespan and elevated age-related diseases, whereas nuclear localization/activation is associated with life extension (Finkel & Holbrook, 2000; Eijkelenboom & Burgering, 2013; Bednarova et al. 2015; Want et al. 2017). FOXO activation is triggered by stress and inhibition of the suppressive signalling of the insulin/IGF-1 PI3K pathway to Akt/PKB. Disinhibition of FOXO allows numerous impacts, including AMPK phosphorylation of specific sites on FOXO (that results in nuclear localization and transcription of numerous stress response genes (Davila & Aleman, 2008; Wang et al. 2017). Deactivation occurs when Akt/PKB is upregulated and phosphorylates other specific FOXO sites leading to nuclear exclusion (Wang et al. 2017).

AMP-activated protein kinase (AMPK), a master energy sensor also plays a key role in other aspects of stress response in that it inhibits the target of rapamycin (TOR) (Geer et al. 2007). The complex interplay between FOXO, Akt, AMPK, and TOR plays a key role in the meditation and regulation of stress and stress responses, as well as the resulting physiological and phenotypic alterations.

Life History Trade-offs and Adaptation

Life-history theory recognizes trade-offs among fitness components, with organisms balancing investment in reproduction (both current and future) against costs of self-maintenance and defense (Alonso-Alvarez et al. 2006; Petes et al., 2008; Hall et al. 2010). This is because reproduction is particularly expensive and stress responses may prioritize energy allocation to survival and self-maintenance (Petes et al. 2008). Management of trade-offs importantly determines fitness and consequently strongly contributes to organismal evolution (Alonso-Alvarez et al. 2006).

Growth rate is a key priority that can constrain other life-history traits. Large mature body size can mediate a variety of benefits associated with predation, mating, fecundity and intraspecific competition (Alonso-Alvarez et al. 2007; Dmitriew, 2010). Regardless, most organisms do not grow at a maximal rate due to associated oxidative stress, and because it does not leave flexibility for meeting environmental constraints and stressors (Alonso-Alvarez et al. 2007; Dmitriew, 2010; Lee et al. 2012). Trade-offs emerge among numerous life history traits (e.g., growth, life-span/survivorship, reproductive effort, dispersal, diapause, defense and immunity) (see Metcalfe & Monaghan, 2003).

Stress, and its additive effects are generally considered to be overwhelmingly negative, however there is also growing support for an adaptive role of stress in shaping individual and offspring phenotypes (Bonduriansky & Head, 2007; Constantini, 2014; Crino & Breuner, 2015). The ability of parents to pass on adaptations that improve offspring performance and stress responses are critical to fitness, survival and propagation (Bonduriansky & Head, 2007; Crino & Breuner, 2015). This may be a cost for parents, but not necessarily to offspring, who may inherit stress adaptions but not the damage associated with parental exposure. Consequently, offspring

are likely to do best in environments that are similar to those inhabited by parents (Bonduriansky & Head, 2007; Constantini, 2014; Crino & Breuner, 2015). This then suggests that habitat choice is likely a key aspect of population success.

The heritability of features beneficial to a given environment may only increase fitness if the environment is relatively stable across generations (Constantini, 2014). In the case of environmental mismatch, adults and offspring may have reduced fitness (Constantini, 2014). This may be especially true for populations that are exposed to multiple acute or relatively short-term stressors since current adaptations may be compromised as the prevailing stressor changes. One of the key aspects of this study is the effects of paternal irradiation on crickets, the life history and reproductive traits affected, as well as the transfer or heritability of parental condition to F1 offspring. Furthermore, unirradiated F1 offspring will be analyzed to see if in the absence of the same environmental stressor/condition as their parents if these inherited adaptations will be beneficial.

In this study, I analyzed the paternal effects of early life exposure to radiation. I emphasized life history traits of F0 and F1 generations (including growth, maturation, reproduction and survivorship/longevity). The overarching purpose was to detect possible trans-generational impacts mediated via the paternal line. To this end I analyzed several relevant epigenetic markers and general stress response biomarkers. The latter included global methylation, the Forkhead transcription factor (FOXO) and the specific stress mediated Nrf2 transcription factor (at several time points in F0 and F1 offspring). I assessed lifetime life history features in male house crickets (*Acheta domesticus*) irradiated as 14 d old juveniles at 6 doses; Control, 2Gy, 4Gy, 7Gy, 10Gy, and 12Gy. F1 offspring were not irradiated but all dosage groups were monitored throughout their lifetime to detect trans-generational effects.

Methods

Breeding Colony

The breeding colony which provided specimens for this chapter was described in the methods section in Chapter 1.

Experimental Groups

All experimental specimens were obtained from a single fresh oviposition container that was placed in the breeding colony at 2-3 weeks post maturation for a single day. Oviposition medium was then removed from the breeding colony after the 24 h period and subsequently kept moist for 2 weeks until hatching occurred. Within 4 days of hatching all remaining eggs and oviposition medium were removed. The experimental group was then held in plastic housing containers (15cm x 11.5cm x 9cm) with a mesh lid for air circulation. Food was the same as provided to the main colony and water was provided via soaked cellulose sponges *ad libitum.* Egg-carton shelters were also provided. 14 d after the single experimental group had hatched from oviposition medium, individuals were then separated into the 6 experimental groups used for this study; containing 200 individuals each; Control (400), 2Gy, 4Gy, 7Gy, 10Gy, and 12Gy. Groups were irradiated at 0.25Gy/min at the Taylor Radiobiology Source at McMaster University. Various exposures were obtained by duration of irradiation. All groups were then immediately brought to the laboratory where they remained in the same housing conditions as above. At approximately 30-40 d of age (when gender was apparent), all females were removed from experimental groups.

Mating

At midlife, (approximately 56-67 d post hatching), experimental males from each group were paired with 15-20 control females which were previously separated prior to maturation to ensure virginity. Groups were provided with oviposition medium for 72 h with oviposition medium being replaced daily. Following the three-day mating period females were removed and experimental males were placed in new containers to monitor survivorship. Two hundred eggs from the first oviposition container were removed and placed into fresh medium and recorded for time to hatch as well as hatching success as described in Chapter 1. Individuals hatching in this container in all groups were maintained and used in later ELISA analyses. The remaining two oviposition containers were left to hatch with hatching time recorded as described in Chapter 1. Individuals from these groups were subsequently maintained to obtain F1 life history features such as growth, development, and survivorship as described for the F0 generation.

Growth Rate and Survivorship

All individuals in experimental groups, (juveniles and adults) were weighed every second day. For juvenile groups, mass was obtained by weighing crickets in groups of 10, 5, 3, and eventually individually as groups became smaller (and more manageable) and individuals larger. Average mass was estimated by dividing the mass of each group by the group size. Mass was obtained and recorded in grams using an analytical balance. All experimental groups were checked daily for maturation, indicated by the adult molt and expression of wings. Once mature males were placed into adult housing containers according to their experimental group. Maturation mass (g) and development time (days to mature) were recorded and employed to

calculate growth rate. Mortality of juveniles and adults were recorded daily until all individuals died.

Measures of Epigenetic Alternations: Global Methylation

Measurements of global DNA methylation were completed on crickets in each radiation group F0: Control, 2Gy, 4Gy, 7Gy, 10Gy, 12Gy and F1: 4Gy, 7Gy) over 3 time points: Early Juvenile (6 hours, 1 day,3 days, and 7 days), Late Juvenile (27 days), and Adults (1 week post-maturation). At each time point crickets were flash frozen in liquid nitrogen and stored at -80^{o}C. Once all samples were collected full body samples were brought to 0^{o}C on ice, homogenized in 300ul-4ml of STM buffer (depending on cricket age/size) and centrifuged at various speeds to break and spin out all cellular components until only the clear nuclear fraction remained. Prior to analysis with assay kits, sample DNA concentration (ug/ml) was estimated to allow standardization of kit results. Global DNA methylation was measured using the MethylFlash™ Global DNA Methylation (5-mC) ELISA Kit (EPIGENTEK, Farmingdale, NY, USA, catalog # P-1030).

Measures of Stress: FOXO & Nrf2

Tests of phospho-FOXO (Ser253) and the Nrf2 transcription factor were completed on crickets in each radiation group. For FOXO this included F0: Control, 7Gy, 12Gy, F1: 4Gy, and 7Gy over 3 time points, Early Juvenile (6 hours, 1 day, and 7 days), Late Juvenile (27 days), and Adults (1 week post-maturation). For the Nrf2 assays, groups included were F0: control, 4Gy, 7Gy, 10Gy, 12Gy, F1: 4Gy, and 7Gy over 3 time points, Early Juvenile (6 hours, 1 day, and 7

days), Late Juvenile (27 days), and Adults (1-week post-maturation). At each time point crickets were flash frozen in liquid nitrogen and stored at -80ºC.

Once all samples were collected, whole body samples were brought to 0ºC on ice, then homogenized in 300ul-4ml of STM buffer (depending on cricket age/size) and centrifuged at various speeds to break and spin out all cellular components until a clear nuclear fraction as well as a cytosolic fraction was obtained. Prior to kit analyze all samples were assayed for DNA concentration (ug/ml) to standardize kit outputs. Phospho-FOXO was measured using Human and Mouse Phospho-FOXO3 (S253) ELISA (RayBiotech, Norcross, GA, USA, catalog # PEL–FOXO3-S253-1) since there is no specific kit for insects other than for *Drosophila*. This kit was specific for vertebrate FOXO3 but FOXOs are strongly conserved so we explored potential applicability. Nrf2 levels were measured using Nrf2 transcription Factor Assay Kit (Cayman Chemical, Ann Arbor, MI, USA, catalog # 600590). This kit was specific for vertebrate Nrf2 but Nrf2 is also strongly conserved so we explored potential applicability.

Statistics

Growth rate results are presented as means ± standard error. All data were analyzed with a one-way ANOVA followed by a Tukey's HSD post-hoc multiple comparisons test to differentiate among groups. Significance was set for $p < 0.05$. For survivorship curves, significant differences from controls were determined with the Gehan-Breslow-Wilcoxon test. Differences in maximal longevity of F0 and F1 groups were analyzed using a one-way ANOVA on the average age of the remaining 10% of individuals. This constituted approximately n = 12 for F0 and n = 45 for F1 groups, with the expectation of F1 10Gy group in which 10% of the group constituted only 6

individuals due to significantly reduced reproductive output. All statistical analyses were carried out with Prism Graph Pad 8.

Results

F0 Juvenile and Adult Growth Rates

Growth rates were collected for juvenile F0 irradiated males only **(Fig. 2.1).** For this cohort significant reductions, $p < 0.0001$ were observed in the 4Gy, 7Gy,10Gy, and 12Gy sub-groups with average growth rate reductions relative to controls of approximately 12%, 14%, 31%, and 47% for respective treatments. The 2Gy sub-group showed no significant reductions. Adult growth rates were also collected for F0 males **(Fig. 2.2)** with no sub-group showing any significant difference in growth rate compared to controls.

F1 Juvenile Growth Rates

Juvenile growth rates for F1 groups both female, male, and combined were collected. For **combined** F1 juvenile growth rate **(Fig. 2.3)** a significant decrease in growth rate was observed for the 4Gy, 7Gy ($p < 0.0001$) and 10Gy ($p = 0.031$) groups. This reduction constituted 17%, 8% and 8% decreases, respectively, compared to controls. For F1 **females only** juvenile growth rates, significant decreases were observed in 4Gy, 7Gy ($p < 0.0001$), and 10Gy ($p = 0.0347$) groups **(Fig. 2.4)**. This constituted approximate reductions of 16%, 7%, and 9% respectively. For F1 **Males only,** juvenile growth rates showed significant reductions in the 4Gy and 7Gy ($p<0.0001$) groups compared to controls **(Fig. 2.5),** with reductions of 16% and 11% respectively.

F0 Survivorship and Longevity

For F0 males, the Gehan-Breslow-Wilcoxon test showed significant differences in survivorship for the 7Gy ($p < 0.001$) and 10Gy ($p = 0.006$) groups compared to controls (**Fig. 2.6a)**. Increases in maximal longevity were also observed in 2Gy, 7Gy, 10Gy, and 12Gy ($p < 0.0001$) groups (based on average age of the last 10% of individuals surviving in each group versus controls) **(Fig. 2.6b)**. Increases in longevity amounted to 14% (2Gy), 14% (7Gy), 15% (10Gy) and 12% (12Gy).

F1 Survivorship and Longevity

For combined male/female F1 offspring, the Gehan-Breslow-Wilcoxon test indicated significant differences from F0 in survivorship curves for 4Gy, 7Gy, and 10Gy groups ($p < 0.0001$) when compared to controls **(Fig. 2.7a)**. Maximal longevity showed a slight decrease for the 4Gy group ($p=0.0022$), a highly significant increase for the 7Gy group ($p =0.005$), and a large increase for the 10Gy groups ($p < 0.0001$) compared to controls **(Fig. 2.7b)**. The 10Gy longevity increase constituted a remarkable 39% increase relative to controls.

Global Methylation

Significant differences in global methylation were not resolved, likely due to small sample sizes; however, general trends were discerned. In all F0 radiation groups (Control – 12Gy) there was a decrease in % methylation with age **(Fig. 2.8).** At higher doses the 7Gy ,10Gy, and 12Gy groups showed the greatest decreases in adulthood compared to controls. In F1 groups this trend was reversed, both F1 4Gy and 7Gy groups showed a much lower early

juvenile % methylation than controls and proceeded to increase to typical % methylation levels by adulthood **(Fig. 2.9)**.

FOXO

Insects have a single version of FOXO whereas vertebrates have several. FOXO activity depends on the phosphorylation of specific sites that either result in nuclear exclusion (during high nutrient availability) or nuclear localization and transcription (during stressful conditions) (Wang et al. 2017) The kit used in this experiment specifically detects whether the Ser253 site on FOXO3 (which leads to nuclear exclusion) is phosphorylated. Generally, FOXO phosphorylation sites are highly conserved but still differ slightly between humans and mice (Wang et al. 2017). Differences in FOXO3 structure between human FOXO3 and insect FOXO, dFOXO may affect antibody binding leading to reduced detection.

Preliminary testing with nuclear phosphorylated FOXO3 concentrations all showed a late juvenile increase compared to controls **(Fig. S1.7)**. This was true for both F0 (7Gy and 12Gy) and F1 (4Gy and 7Gy) groups. Early juvenile and adult levels showed no obvious increase in concentration. Cytosolic phosphorylated FOXO3 concentrations **(Fig. S1.8)** showed a similar, but less pronounced trend of late juvenile increase in concentration compared to controls. The F1 4Gy group is the exception, showing lower concentrations compared to the controls. Cytosolic changes were much less pronounced then nuclear changes.

These results, however, are inconsistent with the vertebrate role of phospho-FOXO3 (Ser253) in that it should not be present in high concentrations during stressful conditions. Furthermore, when FOXO3 is phosphorylated at this specific site it is transported to the cytoplasm, so nuclear levels would be expected to be low. My results indicated increased

phosphorylated FOXO3 in the nucleus that would suggest that FOXO was being suppressed. Regardless, the compatibility of the vertebrate FOXO3 kit for a cricket species remains an unknown aspect.

Nrf2

Significant differences were not reported due to limitations in sample size; however, general trends were observed. Nrf2 concentrations for the F0 groups (4Gy, 7Gy, and 10Gy) showed only slight differences in the late juvenile age group, with early juvenile and adult groups showing little variation **(Fig. S1.9)**. F1 groups (4Gy and 7Gy) showed a similar trend with slight increases in the late juvenile age, with adults and early juveniles showing little variation **(Fig. S1.10).** It is also likely that due to the vertebrate specific nature of this kit that detection in crickets was not possible.

NOTE:

Key molecular entities (FOXO and Nrf2) are highly conserved in insects but plate reader kits were not available for crickets. I tested mammalian kits in the hope that there would be sufficient crossover in these highly conserved molecules to provide some detection. There were some interesting trends that at least indicate that a more specific analytical approach would be worthwhile.

Discussion

Responses to stress are complex, impacting organisms at the molecular-cellular level as well as at the level of organismal life-history features and behavior. On a cellular level, stress

research often focuses on the insulin/insulin like signaling pathway (IIS) which plays a vital role in maintenance and organismal environmental stress responses (Nielson et al. 2008). This pathway is also highly conserved in its effects and factors involved, having been shown in species spanning vertebrates, invertebrates and nematodes (Tatar et al. 2003; Martins et al. 2015). In insects, the IIS pathway is complex with many key players including the insulin receptor lnR, and its targets Akt, FOXO, and TOR (Das & Dobens, 2015). This pathway is fundamental, impacting broad areas including life history traits (growth, longevity, and stress resistance) (Nielson et al. 2008).

I tested the effects of an acute environmental stressor, ionizing radiation, on the development of the House Cricket (*Acheta domesticus)*. Specifically, how this environmental stressor impacted specific aspects of the IIS pathway and its subsequent effects on life-history traits, including longevity. I also examined whether these stress response adjustments in irradiated fathers were maintained into the F1 generation.

Insulin/Insulin Like Signalling Pathway

One of the most well studied and robust pathways in stress and growth research is the insulin/insulin- like signalling pathway (IIS) (Bjedov & Partridge, 2011). This pathway plays a key role in stress responses, resistance, growth, and longevity. One of the key transcription factors in my study is FOXO (inhibits growth, cell proliferation, protein synthesis and promotes apoptosis, stress resistance, DNA repair, antioxidants and longevity). It is also a key transcriptional effector of the IIS pathway (Nielsen et al. 2008; Martins et al. 2015). In mammals, there are several versions of FOXO, invertebrates however generally have only one (e.g., dFOXO in *Drosophila*). This makes analysis and understanding of stress responses simpler

as the single dFOXO transcription factor likely covers a greater watershed of stress responses compared to vertebrates with multiple forms of FOXO (see Martins et al. 2015).

As mentioned above FOXO transcription factors are mainly regulated by phosphorylation/dephosphorylation at specific sites by effectors that respond to environmental conditions (Kramer et al. 2003; Essers et al. 2004; Nielsen et al. 2008; Wang et al. 2017; Zhang et al. 2017). If conditions are good, phosphorylation occurs by several molecules including Akt at specific sites, leading to nuclear exclusion, suppression of transcription, and transfer to the proteasome and degradation (Wang et al. 2017). Under poor conditions, Akt is downregulated and molecules such as JNK and AMPK phosphorylate FOXO at other sites leading to nuclear localization and transcription of stress response genes (Wang et al. 2017).

My results showed a large increase in deactivated phospho-FOXO3 (Ser253) in the nucleus, with a much smaller increase in the cytoplasm during only the late juvenile phase **(Fig. 2.10 & Fig. 2.11).** Phospho-FOXO3 (Ser253) in vertebrates is not present in the nucleus as this phosphorylated site cannot enter the nucleus (Wang et al. 2017). My results however suggest otherwise. There is a high likelihood that my results are contradictory to the literature on the function and regulation of FOXO due to the fact that the kit used in this experiment is specifically for vertebrate FOXO3. The FOXO family of transcription factors are highly conserved, with origin likely predating the divergence of invertebrates and vertebrates (Hosaka et al. 2004; Puig & Tjian, 2006). In vertebrates however there are several different FOXO's, each with individualized functions. Each also has a unique expression in tissues as well as distinct responses to a variety of conditions (Hosaka et al. 2004). In insects the only ortholog to mammalian FOXO is dFOXO which has been shown to regulate longevity and growth in *Drosophila* in response to nutrient availability (Junger et al. 2003; Hwangbo et al. 2004; Puig &

Tjian, 2006). dFOXO has also been shown to be most closely related to the mammalian FOXO1 which may also have resulted in our FOXO3 kit not providing accurate results (Puig & Tjian, 2006).

In regards to regulation, similar to the mammalian FOXO, dFOXO is inhibited by insulin signaling during high nutrient availability through a phosphorylation/dephosphorylation process. dFOXO in *Drosophila* has been shown to be regulated by targeting the insulin receptor gene specifically (Puig & Tjian, 2006). This allows for the insulin receptor to regulate itself based on environmental nutrient conditions. Specifically, when nutrient availability is poor, dFOXO upregulates the synthesis of insulin receptors which allows cells to become highly sensitive to changes in insulin (Puig & Tjian, 2006). Future research into insulin signaling should focus on insect dFOXO, or to explore the possibility of using a mammalian FOXO1 kit as my work indicates that the mammalian FOXO3 kit fails to produce accurate results in crickets.

FOXO plays a vital role in the IIS pathway in its effects on life history traits, however, there are other factors that should also be investigated that also play a role in longevity, survivorship, and growth that may be responsible, or partly responsible for the trends seen in crickets. Specifically, this would include AMPK, Akt, and TOR two of which direct lead to FOXO phosphorylation and regulation. Akt as mentioned above, regulates FOXO directly through the IIS signalling pathway, deactivating FOXO through phosphorylation/nuclear exclusion during high nutrient availability (Nielsen et al. 2008; Zhang et al. 2017). Growth factors and insulin (good conditions) upregulate Akt and trigger a series of cellular responses, including inhibition of FOXO and other key elements in stress response (Tothova et al. 2007; Bjedov & Partridge, 2011).

AMPK also acts on FOXO, during stressful conditions, it phosphorylates specific sites to activate and allow for the transcription of stress response genes (Wang et al. 2017). The TOR kinase, which has been shown to influence growth, lifespan, and stress resistance through its own TOR pathway is still interconnected with the IIS pathway through FOXO activation (Hay, 2011; Robida-Stubbs, 2012; Sun et al. 2017). FOXO activation negatively impacts TOR expression, which has been shown to increase lifespan during stressful conditions (Sun et al. 2017).

Target of rapamycin (TOR) is an atypical serine/threonine kinase that has been identified in a wide range of organisms (Hay, 2011; Zhai et al. 2015). It is also a major regulator of growth and metabolism in eukaryotes in relation to amino acid availability, growth factors, energy status and stress (Bjedov & Partridge, 2011). The major components of the TOR pathway are two highly conserved complex's (TORC1 and TORC2) (Bjedov & Partridge, 2011; Zhai et al. 2015). When active, TOR promotes cell growth, metabolism, and organismal homeostasis (Lee et al. 2019). As mentioned above FOXO, TOR, and AMPK are all parts of an interconnected stress response where both the IIS and TOR pathways are interacting. Under a variety of stressful conditions AMPK activates substrates in order to conserve ATP levels through inhibition of key biosynthesis enzymes (Kimura et al. 2003). When active AMPK therefore has an inhibitory effect on TOR, and consequently, growth, but has been shown to increase longevity (Sun et al. 2017).

In summary, growth inhibition due to stress may be a result of this complex stress response pathway in which Akt inhibition activates FOXO, promotes AMPK and results in TOR down-regulation, resulting in the life-extension and growth deficiencies seen in radiation exposed crickets. The mammalian FOXO3 kit used in this experiment, which was exploratory in nature was likely incompatible with the insect dFOXO. This was likely due to variation in the

structure of dFOXO which functions more closely to FOXO1 and is likely to have different phosphorylation sites then the mammalian FOXO3 to be viable. Future research however needs to be conducted on insect specific dFOXO as well as other key players to determine the overall effect of stress on longevity and growth.

Immune Response

Nuclear factor E2-related factor 2 (Nrf2) is a transcription factor in mammals that generally regulates cellular redox homeostasis and oxidative balance during periods of stress (Sykiotis & Bohmann, 2007; Surh & Na, 2008). Under normal conditions Nrf2 is predominately localized in the cytoplasm as an inactive complex with the protein Keap1. Keap1 is sensitive to changes in cellular redox potential and if oxidative stress is detected it releases Nrf2. This then allows Nrf2 to translocate into the nucleus and initiate specific gene transcription (Surh & Na, 2008). Nrf2 target genes are associated with antioxidant defense, detoxification, anti-inflammatory mediators, and proteasome function (Surh & Na, 2008). In insects, it has been shown that there is a single homolog of Nrf2 named Cap 'n' Collar isoform-C (CncC) (Sykiotis & Bohmann, 2007; Wilding, 2018). CncC, like its mammalian counterpart is regulated by the Keap1 protein (Sykiotis & Bohmann, 2007).

In *Drosophila*, this pathway is activated by oxidative stress and functions to initiate antioxidant and detoxification systems (Sykiotis & Bohmann, 2007). Interestingly, this same study found that mutants lacking Keap1, had constitutive CncC activity and life extension, likely due to increased CncC activity (Sykiotis & Bohmann, 2007). The direct association of CncC with oxidative balance makes it an ideal biomarker and regulator of cellular stress responses.

For this experiment the kit used was specific to the mammalian Nrf2, which has structural similarities to the insect CncC (Pitoniak & Bohmann, 2015). The experiment conducted was meant to be exploratory, to identify possible responses to oxidative stress in crickets, as well as if the homolog CncC in insects was closely related enough to be identified by the mammalian Nrf2 kit. This study however, showed very little variation among groups or with age in terms of Nrf2 concentrations in irradiated crickets or their offspring **(Fig. 2.12).** This is likely due to the incompatibility with the insect homolog of Nrf2 with the mammalian kit used. As described above the insect homolog of Nrf2 is the CncC transcription factor, and although it has sequences of homology to the mammalian Nrf1 and Nrf2 it is likely this was not enough to produce viable results using the mammalian kit (Pitoniak & Bohmann, 2015). For future research it is suggested to analysis the effects of specifically the insect homologue of Nrf2, CncC or its target genes.

Paternal Effects & Epigenetic Inheritance

Epigenetics, is an aspect that can allow parents to pass down genetic "knowledge" of the environment they experienced, thereby potentially improving the fitness of offspring. Epigenetics is an important phenomenon in studies of adaptation and evolution, especially in challenging and stressful environments. Research into the indirect genetic effects of stress, environmental exposure, future generations and the mechanism in which this occurs has mainly focused on maternal inheritance (Cordier, 2007; Curley et al. 2011; Soubry, 2014). Conversely, paternal effects have been largely unstudied since sperm lack much else other than the genetic material itself (Cordier, 2007; Curley et al. 2011; Soubry, 2014). However, more recent research in mammals and insects have found that paternal effects can indeed play a role in epigenetic

inheritance through mechanisms such as DNA methylation and histone modifications (Soubry, 2014). A variety of stressors have been shown to initiate epigenetic changes, including diet, oxidative stress, heat stress, and more. Studies in *Telostylinus angusticolli* showed the transfer of both maternal and paternal condition to offspring due to changes in diet (Bonduriansky & Head, 2007). My results are consistent with paternal inheritance of epigenetic modifications by environmental stress. Trans-generational effects were evident in offspring traits such as growth rate **(Fig. 2.5),** survivorship/longevity **(Fig. 2.6a/b, Fig. 2.7a/b)**, and in some stress biomarkers **(Fig. 2.10, Fig 2.11).** Interestingly, the impacts in F1 offspring tended to be generally positive, with offspring avoiding growth rate declines evident in irradiated parents while maintaining or increasing longevity and survivorship benefits. This indicates that paternal environmental stress, in this case acute radiation exposure, can alter the offspring phenotype and this appears to be beneficial.

Although there are several types of epigenetic modifications that can occur in the genome to alter gene expression, I focused on methylation since this is a known effector of epigenetic change. Methylation in most species occurs exclusively at cytosines within the sequence CpG, although in some species there are non-CpG methylation sites as well (Hendrich & Tweedie, 2003). Previously, it was thought that the zygote underwent a process of de-methylation, in which all parental imprinting was essentially erased (Curley et al. 2011). However, it has now been shown that the germline does indeed have select genes that retain at least portions of their altered methylation states that are inherited by offspring (Curley et al. 2011). Notably, these select genes appear to be sensitive to environmental stressors (Curley et al. 2011).

Methylation patterns are different between vertebrates and invertebrates, particularly in the percent and types of methylation patterns. Invertebrates typically have lower levers of

methylation, either showing none, or at most an intermediate level of global methylation (Hendrich & Tweedie, 2003). They also differ from vertebrates in that the distance between methylation sites are much farther apart, with alternating areas of methylated and unmethylated DNA (Hendrich & Tweedie, 2003). Although many insect species have not been analyzed for methylation content, *Drosophila* methylation is only present during early development and that in general this de-methylation into adulthood is associated with increases gene expression (Hendrich & Tweedie, 2003).

Results from global CpG methylation from irradiated male crickets and their offspring did show a decline from the early juvenile stage to adulthood **(Fig. 2.8).** Overall these changes constituted only a small overall decline in methylation. Despite this, all higher dosed groups, 7Gy, 10Gy, and 12Gy showed a decline from late juvenile to adulthood that exceeded that of lower dose and control groups. For offspring, F1 4Gy and 7Gy groups showed a much lower percent methylation then controls but eventually increased to near control levels throughout life **(Fig. 2.9)**. This may indicate that there might be an alternation in the development of methylation patterns due to stress, therefore altering offspring gene expression.

Another explanation for this change in pattern could be changes in the type of methylation. Recently, CpT sites were identified as the preferred recognition sequence for *Drosophila* (Hendrich & Tweedie, 2003). This may suggest that *Acheta domesticus* may also prefer these sites and have higher global methylation then previously thought. This alternative should be studied in the future to understand global methylation, and its changes due to stress by analyzing both CpG and CpT sites.

Life History Trade-Offs

Trade-offs between growth, somatic maintenance, and reproduction are an integral aspect of life history theory (Fox & Czesak, 2000; Hall et al. 2010). Using *Acheta domesticus* as a model, I found that juvenile growth in irradiated F0 males showed a dose-dependent decrease in the highest dosed groups (10Gy & 12Gy) showing a 31% and 47% reduction in growth compared to controls **(Fig 2.1).** Males receiving 4Gy and 7Gy (medium dose) only showed moderate declines of 12% and 14% respectively. This is consistent with studies that have also detected growth deficiencies among irradiated species (Engel, 1967). Furthermore, as shown in results from Chapter 1, a dose-dependent decline in reproduction was observed, with the 12Gy group being sterile, and the 10Gy group showing significant declines. Irradiation had a similarly negative effect on both growth and reproduction in *Acheta domesticus*.

Remarkably, the decline in growth and reproduction was accompanied with significant increases in survivorship and longevity for most exposed groups. Most notably, the reproductively sterile 12Gy group and dysfunctional 10Gy group both expressed significantly reduced growth but large increases in longevity (15% and 12%, respectively) **(Fig. 2.6)**. The 2Gy and 7Gy groups also showed increases in longevity with 7Gy exposure (i.e., increased mid-life survivorship with only minor declines in growth and reproduction). Only the 4Gy group showed no significant increases in longevity.

These results are generally consistent with evidence from other studies identifying trade-offs between reproduction and growth versus life extension (Metcalfe & Monaghan, 2003; Lee et al. 2012; Lyn et al. 2012; Hans et al. 2015). Unpublsihed work in our lab which focuses on this paradigm with irradiated female *Acheta domesticus* detected a trade-off between longevity and growth (Shephard, 2017). Specifically, moderate doses increased mean longevity and

survivorship but in turn reduced growth in irradiated females (Shephard, 2017). This has been suggested to reflect reduced oxidative damage associated with growth and reproduction. However, there is no consensus in the literature as to the specific mechanism mediating such trade-offs (Metcalfe & Alonso-Alvarez, 2010; Selman et al. 2012).

Interestingly, unirradiated F1 offspring showed very similar, and in some cases more exaggerated trends than their exposed parents. Both male and female offspring showed slight declines in juvenile growth rates which was irrespective of parental dose, just an "all-or-none" decline **(Fig. 2.3-2.5).** This decline although significant, did not correspond to large declines in longevity or survivorship but instead longevity and survivorship increases. Mid-high doses, 7Gy and 10Gy, like their parents showed a significant increase in mid-life survivorship, with the 10Gy group exceeding their exposed parents' survivorship and longevity by double. The F1 7Gy and 10Gy groups had longevity increases of 10% and 39% respectively **(Fig. 2.7b)**.

The 4Gy group showed very little change in survivorship and slight reduction in longevity compared to controls. These results indicate that offspring seem to inherit their parent's condition despite not being directly exposed themselves. This is consistent with the theory that the effects of stress can be inherited through genetic and epigenetic mechanisms as described above (Seong et al. 2011). The number of generations in which this effect is possible from paternal exposure is a possible area of future research.

Conclusion

In summary this research provides ample evidence for the existence of paternal transgenerational effects associated with radiation exposure. In life history parameters offspring showed increased survivorship and longevity associated with paternal exposure, indicating some

form of mechanism for the inheritance of paternal condition. Offspring were able to achieve survivorship and longevity benefits without the growth rate declines observed in exposed F0 fathers. Although FOXO and Nrf2 kits provide questionable data on stress responses this highlights an area for future work. Furthermore, methylation patterns showed some change due to radiation in parents as well as offspring, however, to make conclusions more research much be conducted to acquire statistically relevant results. Overall, my research suggests that paternal effects not only exist but are relevant in analyzing transgenerational effects of stress and that more work should focus on this avenue of research.

References

Alonso-Alvarez, C., Bertrand, S., Devevey, G., Prost, J., Faivre, B., Chastel, O., & Sorci, G. (2006). An experimental manipulation of life-history trajectories and resistance to oxidative stress. *Evolution,60*(9), 1913. doi:10.1554/05-644.1

Alonso-Alvarez, C., Bertrand, S., Faivre, B., & Sorci, G. (2007). Increased susceptibility to oxidative damage as a cost of accelerated somatic growth in zebra finches. *Functional Ecology,21*(5), 873-879. doi:10.1111/j.1365-2435.2007.01300.x

Anway, M. D. (2005). Epigenetic transgenerational actions of endocrine disruptors and male fertility. *Science,308*(5727), 1466-1469. doi:10.1126/science.1108190

Bale, T. L. (2014). Lifetime stress experience: Transgenerational epigenetics and germ cell programming. *Dialogues Clin Neurosci,*(16), 3rd ser., 297-305. Retrieved from www.ncbi.nlm.nih.gov/pmc/articles/PMC4214173/.

Barber, R., Plumb, M. A., Boulton, E., Roux, I., & Dubrova, Y. E. (2002). Elevated mutation rates in the germ line of first- and second-generation offspring of irradiated male mice. *Proceedings of the National Academy of Sciences,99*(10), 6877-6882. doi:10.1073/pnas.102015399

Baulch, J. E. (2001). Heritable effects of paternal irradiation in mice on signaling protein kinase activities in F3 offspring. *Mutagenesis,16*(1), 17-23. doi:10.1093/mutage/16.1.17

Bednářová, A., Kodrík, D., & Krishnan, N. (2015). Knockdown of adipokinetic hormone synthesis increases susceptibility to oxidative stress in *Drosophila* — A role for

dFoxO? *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology,171*, 8-14. doi:10.1016/j.cbpc.2015.03.006

Bjedov, I., & Partridge, L. (2011). A longer and healthier life with TOR down-regulation: Genetics and drugs. *Biochemical Society Transactions,39*(2), 460-465. doi:10.1042/bst0390460

Bonduriansky, R., & Head, M. (2007). Maternal and paternal condition effects on offspring phenotype in *Telostylinus angusticollis* (Diptera: Neriidae). *Journal of Evolutionary Biology,20*(6), 2379-2388. doi:10.1111/j.1420-9101.2007.01419.x

Bonduriansky, R., & Day, T. (2009). Nongenetic inheritance and its evolutionary implications. *Annual Review of Ecology, Evolution, and Systematics,40*(1), 103-125. doi:10.1146/annurev.ecolsys.39.110707.173441

Bonisoli-Alquati, A., Møller, A. P., Rudolfsen, G., Saino, N., Caprioli, M., Ostermiller, S., & Mousseau, T. A. (2011). The effects of radiation on sperm swimming behavior depend on plasma oxidative status in the barn swallow (*Hirundo rustica). Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology,159*(2), 105-112. doi:10.1016/j.cbpa.2011.01.018

Burton, T., & Metcalfe, N. B. (2014). Can environmental conditions experienced in early life influence future generations? *Proceedings of the Royal Society B: Biological Sciences,281*(1785), 20140311-20140311. doi:10.1098/rspb.2014.0311

Chaby, L. E. (2016). Why are there lasting effects from exposure to stress during development? An analysis of current models of early stress. *Physiology & Behavior,164*, 164-181. doi:10.1016/j.physbeh.2016.05.032

Chong, S., Vickaryous, N., Ashe, A., Zamudio, N., Youngson, N., Hemley, S., Whitelaw, E. (2007). Modifiers of epigenetic reprogramming show paternal effects in the mouse. *Nature Genetics,39*(5), 614-622. doi:10.1038/ng2031

Cordier, S. (2008). Evidence for a role of paternal exposures in developmental toxicity. *Basic & Clinical Pharmacology & Toxicology,102*(2), 176-181. doi:10.1111/j.1742-7843.2007.00162.x

Costantini, D. (2014). Variation in oxidative stress threats and hormesis across environments. *Oxidative Stress and Hormesis in Evolutionary Ecology and Physiology,*75-109. doi:10.1007/978-3-642-54663-1_3

Crino, O. L., & Breuner, C. W. (2015). Developmental stress: Evidence for positive phenotypic and fitness effects in birds. *Journal of Ornithology,156*(S1), 389-398. doi:10.1007/s10336-015-1236-z

Curley, J. P., Mashoodh, R., & Champagne, F. A. (2011). Epigenetics and the origins of paternal effects. *Hormones and Behavior,59*(3), 306-314. doi:10.1016/j.yhbeh.2010.06.018

Cypser, J. R., & Johnson, T. E. (2002). Multiple stressors in *Caenorhabditis elegans* induce stress hormesis and extended longevity. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences,57*(3). doi:10.1093/gerona/57.3.b109

Das, R., & Dobens, L. L. (2015). Conservation of gene and tissue networks regulating insulin signalling in flies and vertebrates. *Biochemical Society Transactions,43*(5), 1057-1062. doi:10.1042/bst20150078

Dautréaux, B., & Toledano, M. B. (2007). ROS as signalling molecules: Mechanisms that generate specificity in ROS homeostasis. *Nature Reviews Molecular Cell Biology,8*(10), 813-824. doi:10.1038/nrm2256

Dávila, D., & Torres-Aleman, I. (2008). Neuronal death by oxidative stress involves activation of FOXO3 through a two-arm pathway that activates stress kinases and attenuates insulin-like growth factor I signaling. *Molecular Biology of the Cell,19*(5), 2014-2025. doi:10.1091/mbc.e07-08-0811

Dmitriew, C. M. (2010). The evolution of growth trajectories: What limits growth rate? *Biological Reviews,86*(1), 97-116. doi:10.1111/j.1469-185x.2010.00136.x

Dubrova, Y. E., Plumb, M., Brown, J., Fennelly, J., Bois, P., Goodhead, D., & Jeffreys, A. J. (1998). Stage specificity, dose response, and doubling dose for mouse minisatellite germ-line mutation induced by acute radiation. *Proceedings of the National Academy of Sciences,95*(11), 6251-6255. doi:10.1073/pnas.95.11.6251

Eijkelenboom, A., & Burgering, B. M. (2013). FOXOs: Signalling integrators for homeostasis maintenance. *Nature Reviews Molecular Cell Biology,14*(2), 83-97. doi:10.1038/nrm3507

Einor, D., Bonisoli-Alquati, A., Costantini, D., Mousseau, T., & Møller, A. (2016). Ionizing radiation, antioxidant response and oxidative damage: A meta-analysis. *Science of The Total Environment,548-549*, 463-471. doi:10.1016/j.scitotenv.2016.01.027

Engel, D. W. (1967). Effect of singe and continuous exposures of gamma radiation on the survival and growth of the blue crab, *Callinectes sapidus*. *Radiation Research,32*(4), 685. doi:10.2307/3572280

Essers, M. A., Weijzen, S., Vries-Smits, A. M., Saarloos, I., Ruiter, N. D., Bos, J. L., & Burgering, B. M. (2004). FOXO transcription factor activation by oxidative stress mediated by the small GTPase Ral and JNK. *The EMBO Journal,23*(24), 4802-4812. doi:10.1038/sj.emboj.7600476

Field, L. M., Lyko, F., Mandrioli, M., & Prantera, G. (2004). DNA methylation in insects. *Insect Molecular Biology,13*(2), 109-115. doi:10.1111/j.0962-1075.2004.00470.x

Finkel, T., & Holbrook, N. J. (2000). Oxidants, oxidative stress and the biology of ageing. *Nature,408*(6809), 239-247. doi:10.1038/35041687

Fox, C. W., & Czesak, M. E. (2000). Evolutionary ecology of progeny size in arthropods. *Annual Review of Entomology,45*(1), 341-369. doi:10.1146/annurev.ento.45.1.341

Fuse, Y., & Kobayashi, M. (2017). Conservation of the Keap1-Nrf2 system: An evolutionary journey through stressful space and time. *Molecules,22*(3), 436. doi:10.3390/molecules22030436

Greer, E. L., Dowlatshahi, D., Banko, M. R., Villen, J., Hoang, K., Blanchard, D., Brunet, A. (2007). An AMPK-FOXO pathway mediates longevity induced by a novel method of dietary restriction in C. elegans. *Current Biology,17*(19), 1646-1656. doi:10.1016/j.cub.2007.08.047

Glastad, K. M., Hunt, B. G., Yi, S. V., & Goodisman, M. A. (2011). DNA methylation in insects: On the brink of the epigenomic era. *Insect Molecular Biology,20*(5), 553-565. doi:10.1111/j.1365-2583.2011.01092.x

Hall, M. E., Blount, J. D., Forbes, S., & Royle, N. J. (2010). Does oxidative stress mediate the trade-off between growth and self-maintenance in structured families? *Functional Ecology,24*(2), 365-373. doi:10.1111/j.1365-2435.2009.01635.x

Hans, H., Lone, A., Aksenov, V., & Rollo, C. D. (2015). Impacts of metformin and aspirin on life history features and longevity of crickets: Trade-offs versus cost-free life extension? *Age,37*(2). doi:10.1007/s11357-015-9769-x

Hay, N. (2011). Interplay between FOXO, TOR, and Akt. *Biochimica Et Biophysica Acta (BBA) - Molecular Cell Research,1813*(11), 1965-1970. doi:10.1016/j.bbamcr.2011.03.013

Hendrich, B., & Tweedie, S. (2003). The methyl-CpG binding domain and the evolving role of DNA methylation in animals. *Trends in Genetics,19*(5), 269-277. doi:10.1016/s0168-9525(03)00080-5

Hosaka, T., Biggs, W. H., Tieu, D., Boyer, A. D., Varki, N. M., Cavenee, W. K., & Arden, K. C. (2004). Disruption of forkhead transcription factor (FOXO) family members in mice reveals their functional diversification. *Proceedings of the National Academy of Sciences,101*(9), 2975-2980. doi:10.1073/pnas.0400093101

Hwangbo, D. S., Gersham, B., Tu, M., Palmer, M., & Tatar, M. (2004). *Drosophila* dFOXO controls lifespan and regulates insulin signalling in brain and fat body. *Nature,429*(6991), 562-566. doi:10.1038/nature02549

Johannes, F., Porcher, E., Teixeira, F. K., Saliba-Colombani, V., Simon, M., Agier, N., . . . Colot, V. (2009). Assessing the impact of transgenerational epigenetic variation on complex Traits. *PLoS Genetics,5*(6). doi:10.1371/journal.pgen.1000530

Jünger, M. A., Rintelen, F., Stocker, H., Wasserman, J. D., Végh, M., Radimerski, T., . . . Hafen, E. (2003). The *Drosophila* Forkhead transcription factor FOXO mediates the reduction in cell

number associated with reduced insulin signaling. *Journal of Biology,2*(3), 20. doi:10.1186/1475-4924-2-20

Kimura, N., Tokunaga, C., Dalal, S., Richardson, C., Yoshino, K., Hara, K., . . . Yonezawa, K. (2003). A possible linkage between AMP-activated protein kinase (AMPK) and mammalian target of rapamycin (mTOR) signalling pathway. *Genes to Cells,8*(1), 65-79. doi:10.1046/j.1365-2443.2003.00615.x

Kodrík, D., Bednářová, A., Zemanová, M., & Krishnan, N. (2015). Hormonal regulation of response to oxidative stress in insects—An Update. *International Journal of Molecular Sciences,16*(10), 25788-25816. doi:10.3390/ijms161025788

Kramer, J. M., Davidge, J. T., Lockyer, J. M., & Staveley, B. E. (2003). Expression of *Drosophila* FOXO regulates growth and can phenocopy starvation. *BMC Developmental Biology,3*(1), 5. doi:10.1186/1471-213x-3-5

Lachmann, M., & Jablonka, E. (1996). The inheritance of phenotypes: An adaptation to fluctuating environments. *Journal of Theoretical Biology,181*(1), 1-9. doi:10.1006/jtbi.1996.0109

Lee, J. H., Budanov, A. V., Park, E. J., Birse, R., Kim, T. E., Perkins, G. A., . . . Karin, M. (2010). Sestrin as a feedback inhibitor of TOR that prevents age-related pathologies. *Science,327*(5970), 1223-1228. doi:10.1126/science.1182228

Lee, W., Monaghan, P., & Metcalfe, N. B. (2012). Experimental demonstration of the growth rate-lifespan trade-off. *Proceedings of the Royal Society B: Biological Sciences,280*(1752), 20122370-20122370. doi:10.1098/rspb.2012.2370

Lyn, J., Aksenov, V., Leblanc, Z., & Rollo, C. D. (2012). Life history features and aging rates: insights from intra-specific patterns in the cricket *Acheta domesticus*. *Evolutionary Biology,39*(3), 371-387. doi:10.1007/s11692-012-9160-0

Martins, R., Lithgow, G. J., & Link, W. (2015). Long live FOXO: Unraveling the role of FOXO proteins in aging and longevity. *Aging Cell,15*(2), 196-207. doi:10.1111/acel.12427

Metcalfe, N. B., & Alonso-Alvarez, C. (2010). Oxidative stress as a life-history constraint: The role of reactive oxygen species in shaping phenotypes from conception to death. *Functional Ecology,24*(5), 984-996. doi:10.1111/j.1365-2435.2010.01750.x

Metcalfe, N., & Monaghan, P. (2003). Growth versus lifespan: Perspectives from evolutionary ecology. *Experimental Gerontology,38*(9), 935-940. doi:10.1016/s0531-5565(03)00159-1

Møller, A. P., Surai, P., & Mousseau, T. A. (2005). Antioxidants, radiation and mutation as revealed by sperm abnormality in barn swallows from Chernobyl. *Proceedings of the Royal Society B: Biological Sciences,272*(1560), 247-253. doi:10.1098/rspb.2004.2914

Mothersill, C., & Seymour, C. (2013). Radiation-induced bystander effects and stress-induced mutagenesis. *Stress-Induced Mutagenesis,*199-222. doi:10.1007/978-1-4614-6280-4_10

Mousseau, T. (1998). The adaptive significance of maternal effects. *Trends in Ecology & Evolution,13*(10), 403-407. doi:10.1016/s0169-5347(98)01472-4

Mousseau, T. A., & Moller, A. P. (2014). Genetic and ecological studies of animals in Chernobyl and Fukushima. *Journal of Heredity,105*(5), 704-709. doi:10.1093/jhered/esu040

Nielsen, M. D., Luo, X., Biteau, B., Syverson, K., & Jasper, H. (2008). 14-3-3ε antagonizes FoxO to control growth, apoptosis and longevity in *Drosophila*. *Aging Cell,7*(5), 688-699. doi:10.1111/j.1474-9726.2008.00420.x

Nomura, T. (1988). X-ray- and chemically induced germ-line mutation causing phenotypical anomalies in mice. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis,198*(2), 309-320. doi:10.1016/0027-5107(88)90008-5

Petes, L. E., Menge, B. A., & Harris, A. L. (2008). Intertidal mussels exhibit energetic trade-offs between reproduction and stress resistance. *Ecological Monographs,78*(3), 387-402. doi:10.1890/07-0605.1

Pitoniak, A., & Bohmann, D. (2015). Mechanisms and functions of Nrf2 signaling in *Drosophila*. *Free Radical Biology and Medicine,88*, 302-313. doi:10.1016/j.freeradbiomed.2015.06.020

Puig, O., & Tjian, R. (2006). Nutrient availability and growth: Regulation of insulin signaling by dFOXO/FOXO1. *Cell Cycle,5*(5), 503-505. doi:10.4161/cc.5.5.2501

Rando, O. J. (2012). Daddy issues: Paternal effects on phenotype. *Cell,151*(4), 702-708. doi:10.1016/j.cell.2012.10.020

Rattan, S. I. (2008). Hormesis in aging. *Ageing Research Reviews,7*(1), 63-78. doi:10.1016/j.arr.2007.03.002

Robida-Stubbs, S., Glover-Cutter, K., Lamming, D., Mizunuma, M., Narasimhan, S., Neumann-Haefelin, E., . . . Blackwell, T. (2012). TOR signaling and Rapamycin influence longevity by regulating SKN-1/Nrf and DAF-16/FoxO. *Cell Metabolism,15*(5), 713-724. doi:10.1016/j.cmet.2012.04.007

Rodgers, A. B., Morgan, C. P., Bronson, S. L., Revello, S., & Bale, T. L. (2013). Paternal stress exposure alters sperm MicroRNA content and reprograms offspring HPA stress axis regulation. *Journal of Neuroscience,33*(21), 9003-9012. doi:10.1523/jneurosci.0914-13.2013

Selman, C., Blount, J. D., Nussey, D. H., & Speakman, J. R. (2012). Oxidative damage, ageing, and life-history evolution: Where now? *Trends in Ecology & Evolution,27*(10), 570-577. doi:10.1016/j.tree.2012.06.006

Seong, K., Li, D., Shimizu, H., Nakamura, R., & Ishii, S. (2011). Inheritance of stress-induced, ATF-2-dependent epigenetic change. *Cell,145*(7), 1049-1061. doi:10.1016/j.cell.2011.05.029

Shephard, A. M. (2017). *Effects of early-life ionizing radiation exposure on the life-history of the cricket, Acheta domesticus* (Unpublished master's thesis). McMaster.

Soubry, A., Hoyo, C., Jirtle, R. L., & Murphy, S. K. (2014). A paternal environmental legacy: Evidence for epigenetic inheritance through the male germ line. *BioEssays,36*(4), 359-371. doi:10.1002/bies.201300113

Sun, X., Chen, W., & Wang, Y. (2017). DAF-16/FOXO transcription factor in aging and longevity. *Frontiers in Pharmacology,8*. doi:10.3389/fphar.2017.00548

Surh, Y., Kundu, J., & Na, H. (2008). Nrf2 as a master redox switch in turning on the cellular signaling involved in the induction of cytoprotective genes by some chemopreventive phytochemicals. *Planta Medica,74*(13), 1526-1539. doi:10.1055/s-0028-1088302

Sykiotis, G. P., & Bohmann, D. (2008). Keap1/Nrf2 signaling regulates oxidative stress tolerance and lifespan in *Drosophila*. *Developmental Cell,14*(1), 76-85. doi:10.1016/j.devcel.2007.12.002

Tatar, M., Bartke, A., & Antebi, A. (2003). The endocrine regulation of aging by insulin-like signals. *Science,299*(5611), 1346-1351. doi:10.1126/science.1081447

Tothova, Z., Kollipara, R., Huntly, B. J., Lee, B. H., Castrillon, D. H., Cullen, D. E., . . . Gilliland, D. G. (2007). FoxOs are critical mediators of hematopoietic stem cell resistance to physiologic oxidative stress. *Cell,128*(2), 325-339. doi:10.1016/j.cell.2007.01.003

Velando, A., Torres, R., & Alonso-Alvarez, C. (2008). Avoiding bad genes: Oxidatively damaged DNA in germ line and mate choice. *BioEssays,30*(11-12), 1212-1219. doi:10.1002/bies.20838

Vellai, T., Takács-Vellai, K., Sass, M., & Klionsky, D. J. (2009). The regulation of aging: Does autophagy underlie longevity? *Trends in Cell Biology,19*(10), 487-494. doi:10.1016/j.tcb.2009.07.007

Wang, X., Hu, S., & Liu, L. (2017). Phosphorylation and acetylation modifications of FOXO3a: Independently or synergistically? *Oncology Letters,13*(5), 2867-2872. doi:10.3892/ol.2017.5851

Wilding, C. S. (2018). Regulating resistance: CncC:Maf, antioxidant response elements and the overexpression of detoxification genes in insecticide resistance. *Current Opinion in Insect Science,27*, 89-96. doi:10.1016/j.cois.2018.04.006

Zhai, Y., Sun, Z., Zhang, J., Kang, K., Chen, J., & Zhang, W. (2015). Activation of the TOR signalling pathway by glutamine regulates insect fecundity. *Scientific Reports,5*(1). doi:10.1038/srep10694

Zhang, X., Wang, T., Lin, X., Denlinger, D. L., & Xu, W. (2017). Reactive oxygen species extend insect life span using components of the insulin-signaling pathway. *Proceedings of the National Academy of Sciences,114*(37). doi:10.1073/pnas.1711042114

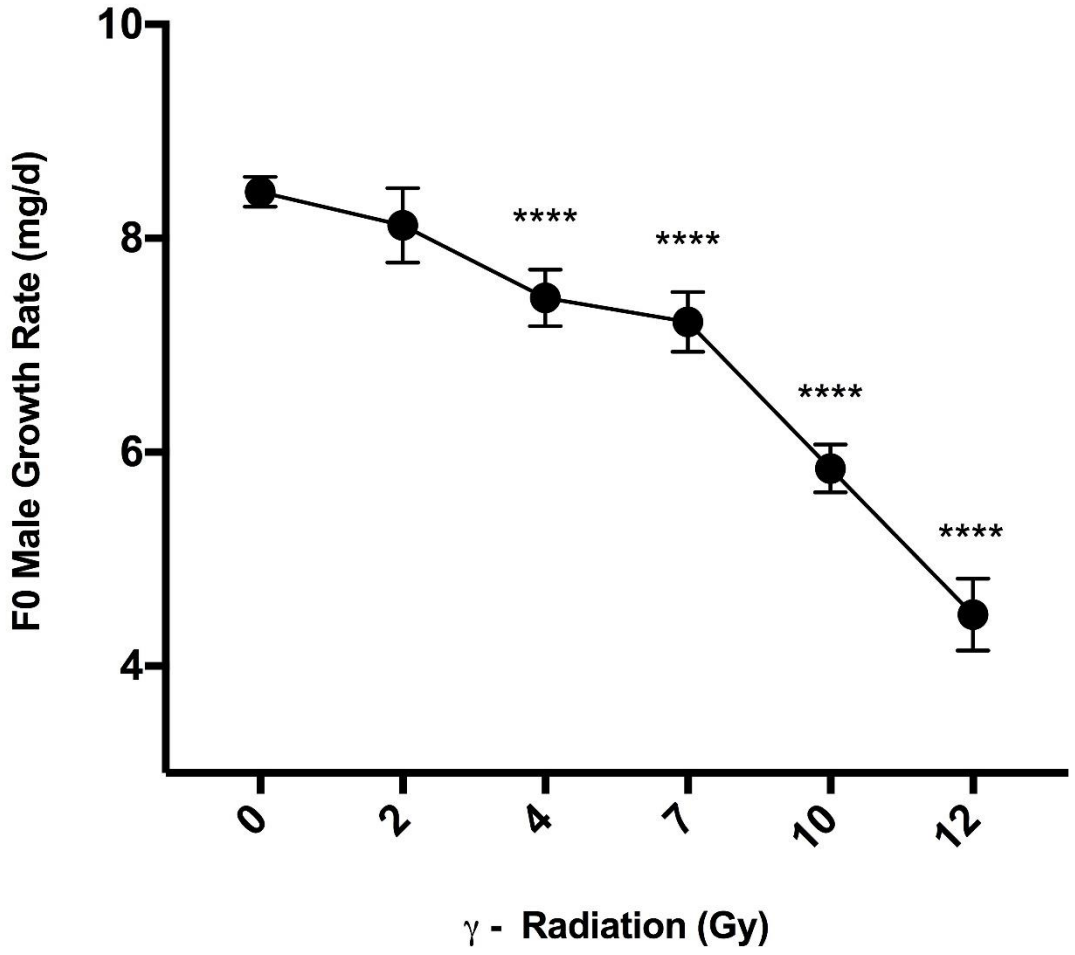


Figure 2.1: Dose-response effects of early juvenile radiation on juvenile growth rates of F0 male *Acheta domesticus*. All crickets were irradiated (dose-rate = 0.25Gy/min) at 14 days old. Growth rates were calculated by dividing the mass at maturation (mg) by the time taken to reach maturation (days) for each individual male. Groups encompassed 0 (n=52), 2Gy (n=45), 4Gy (n=55), 7Gy (n=62), 10Gy (n=63), 12Gy (n=29). Values are represented as the mean of each radiation group +/- SEM with significant values (**** for $p<0.0001$). All groups were tested for normality prior to analysis. Significant differences were identified using a one-way ANOVA followed by a Tukeys HSD test comparing all groups to the 0Gy group.

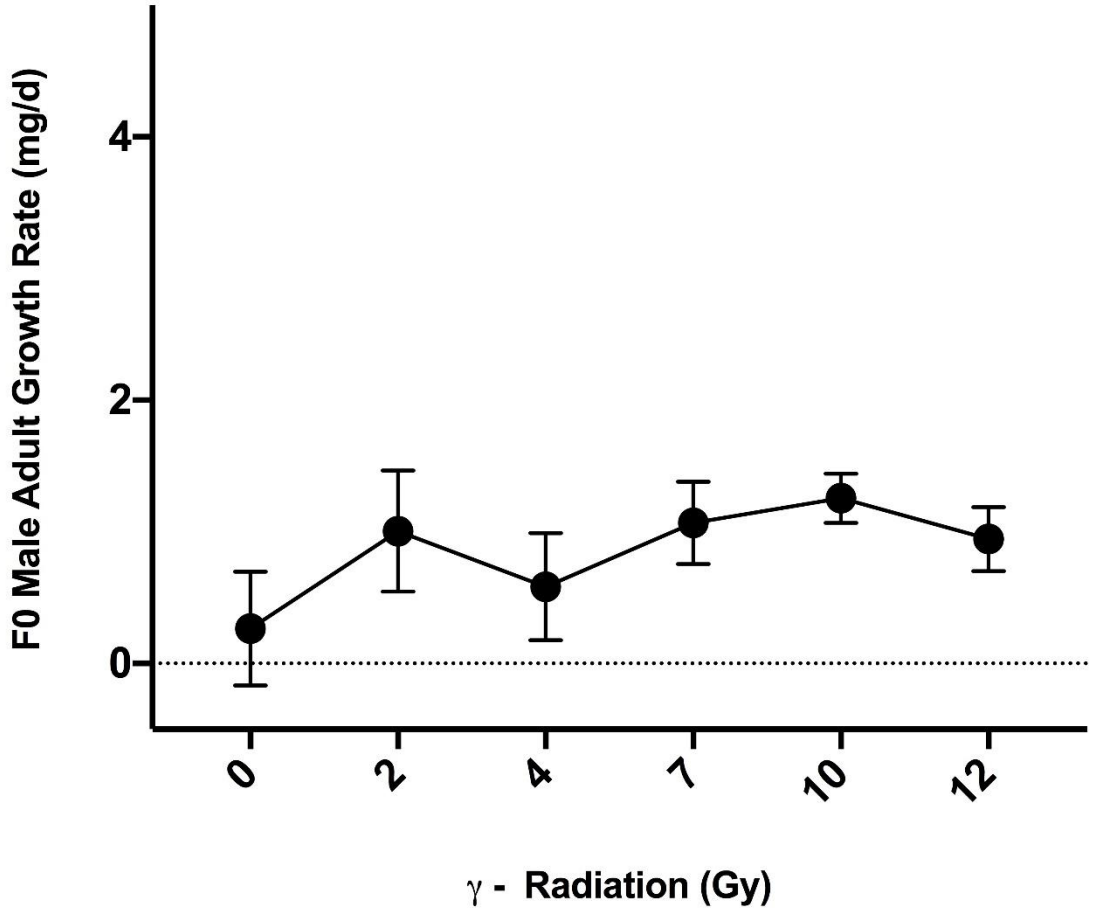


Figure 2.2: Dose-response effects of early juvenile radiation on adult growth rates of F0 male *Acheta domesticus*. All crickets were irradiated (dose-rate = 0.25Gy/min) at 14 days old. Growth rates were calculated by dividing the different between late and early adulthood (mg) by the difference in time between late and early adulthood (days) for each individual male. Groups encompassed 0 (n=14), 2Gy (n=15), 4Gy (n=17), 7Gy (n=32), 10Gy (n=39), 12Gy (n=21). Values are represented as the mean of each radiation group +/- SEM. All groups were tested for normality prior to analysis. Significant differences were identified using a one-way ANOVA followed by a Tukeys HSD test comparing all groups to the 0Gy group.

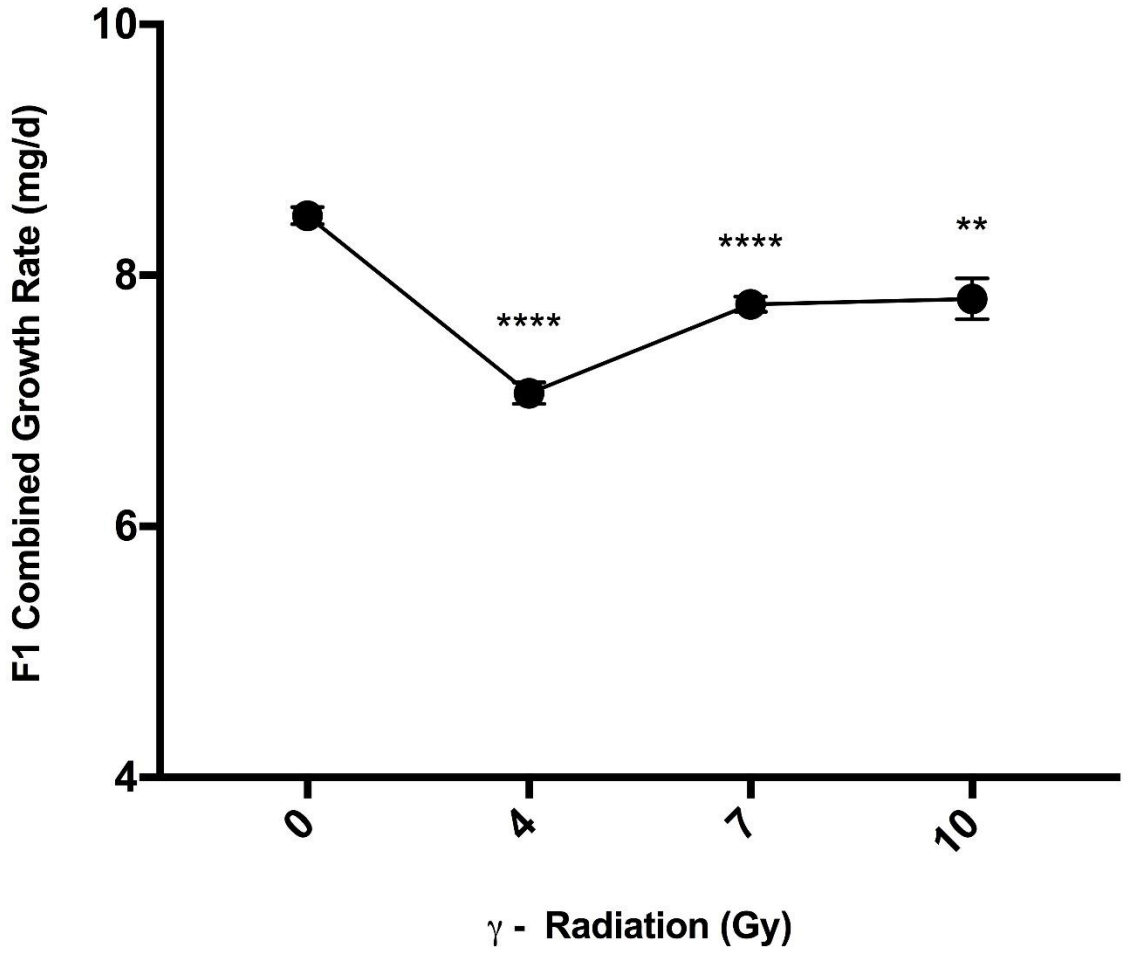


Figure 2.3: Dose-response effects of paternal early juvenile radiation on growth rates of F1 male/female combined *Acheta domesticus*. Growth rates were calculated by dividing the mass at maturation (mg) by the time taken to reach maturation (days) for each individual male. Groups encompassed 0 (n=211), 4Gy (n=113), 7Gy (n=368), 10Gy (n=36). Values are represented as the mean growth rate of each group +/- SEM with significant values (**** $p < 0.0001$, ** $p < 0.001$). All groups were tested for normality prior to analysis. Significant differences were identified using a one-way ANOVA followed by a Tukey's HSD test comparing all groups to the 0Gy group.

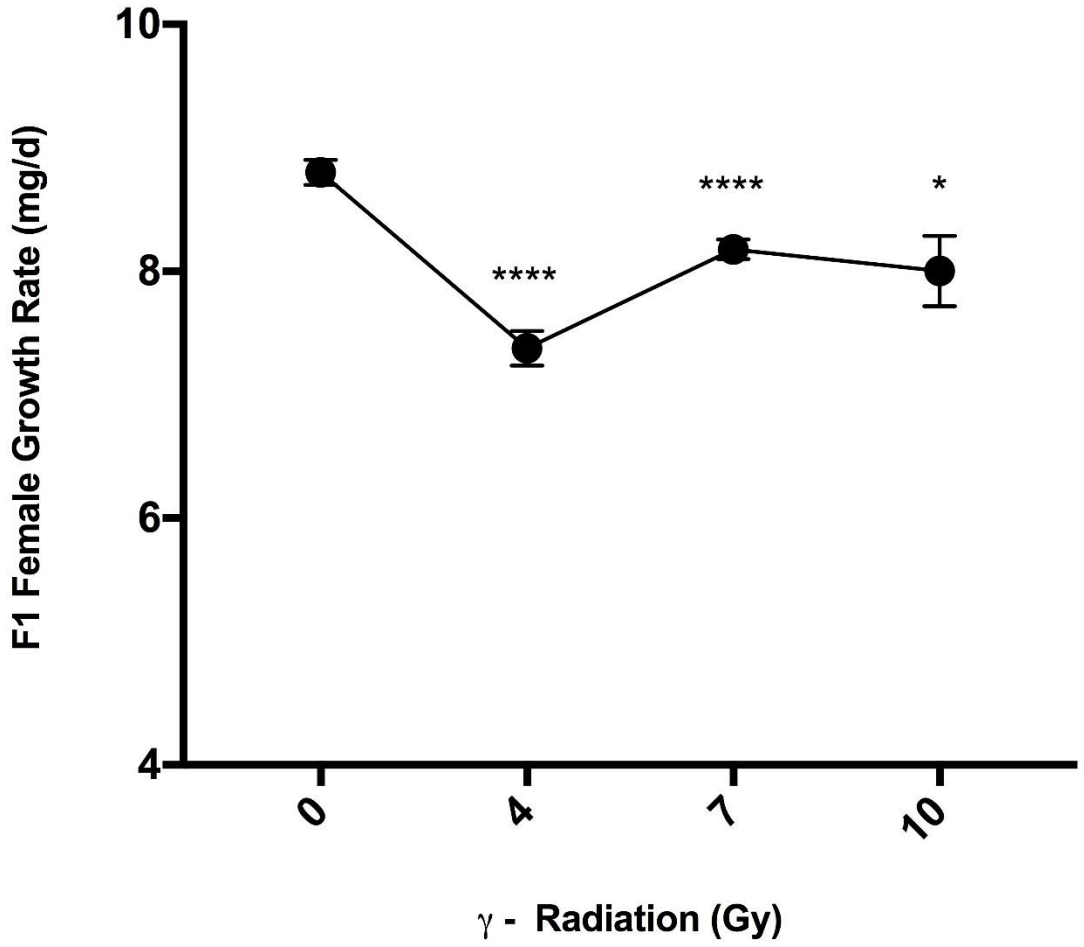


Figure 2.4: Dose-response effects of paternal early juvenile radiation on growth rates of F1 Female *Acheta domesticus*. Growth rates were calculated by dividing the mass at maturation (mg) by the time taken to reach maturation (days) for each individual male. Groups encompassed 0 (n=103), 4Gy (n=39), 7Gy (n=197), 10Gy (n=15). Values are represented as the mean growth rate of each group +/- SEM with significant values (**** $p < 0.0001$, * $p < 0.1$). All groups were tested for normality prior to analysis. Significant differences were identified using a one-way ANOVA followed by a Tukey's HSD test comparing all groups to the 0Gy group.

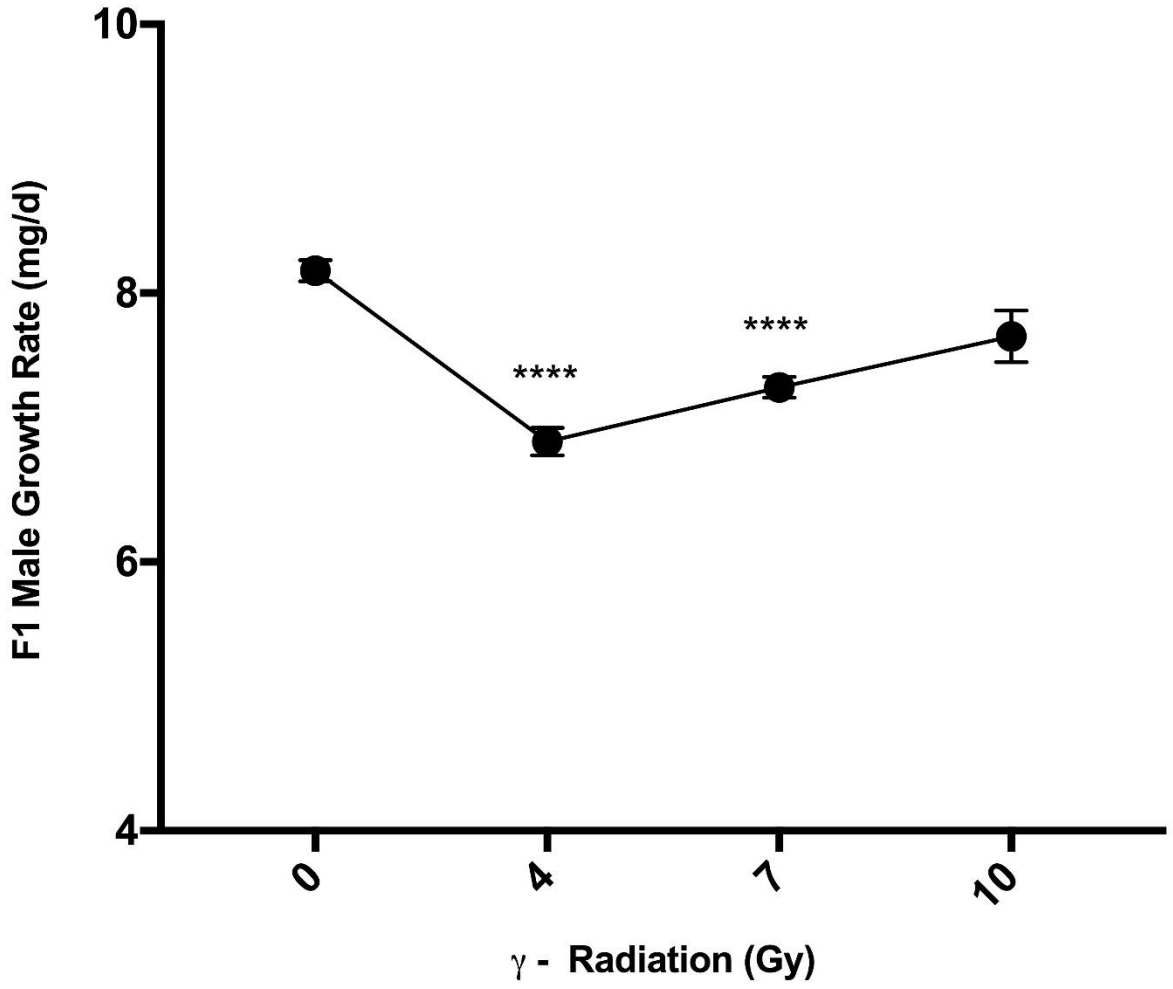
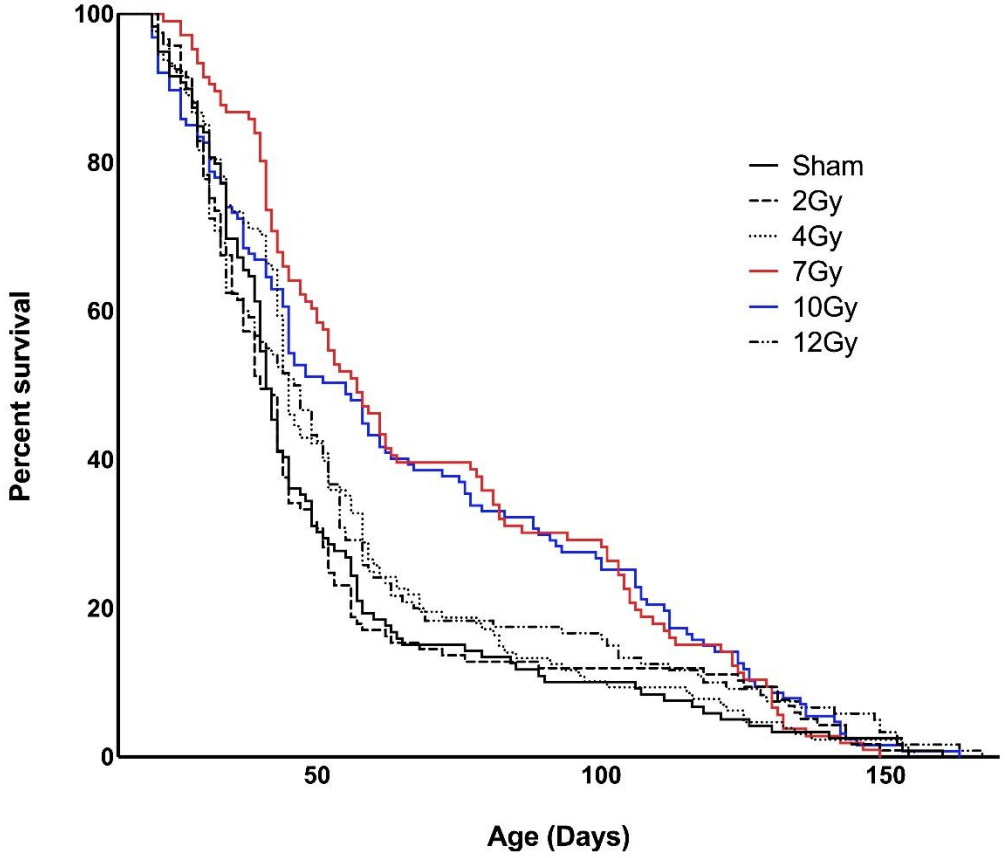


Figure 2.5: Dose-response effects of paternal early juvenile radiation on growth rates of F1 male *Acheta domesticus*. Growth rates were calculated by dividing the mass at maturation (mg) by the time taken to reach maturation (days) for each individual male. Groups encompassed 0 (n=106), 4Gy (n=72), 7Gy (n=169), 10Gy (n=19). Values are represented as the mean growth rate of each group +/- SEM with significant values (**** $p < 0.0001$). All groups were tested for normality prior to analysis. Significant differences were identified using a one-way ANOVA followed by a Tukey's HSD test comparing all groups to the 0Gy group.

a)


100
80
60
40
20
0
Percent survival
50
100
150
Age (Days)
Sham
2Gy
4Gy
7Gy
10Gy
12Gy

b)

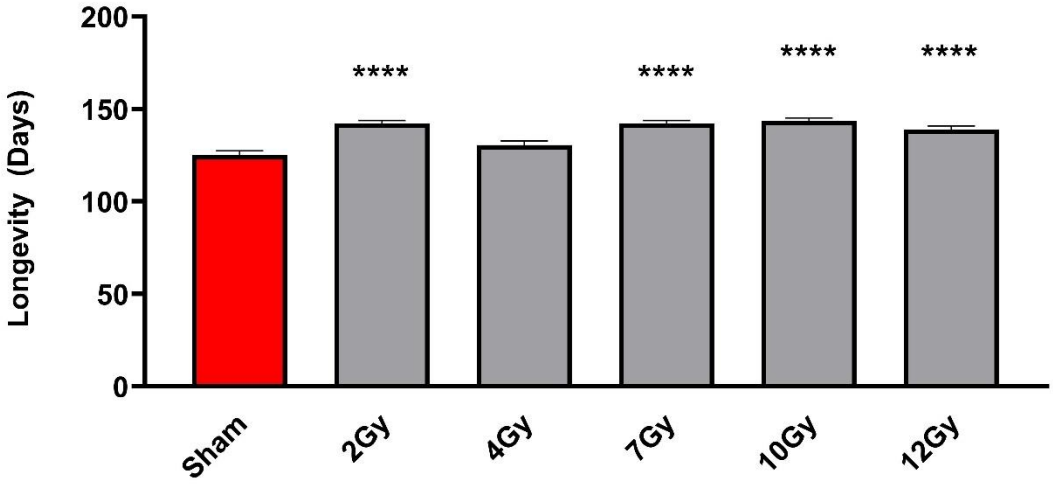
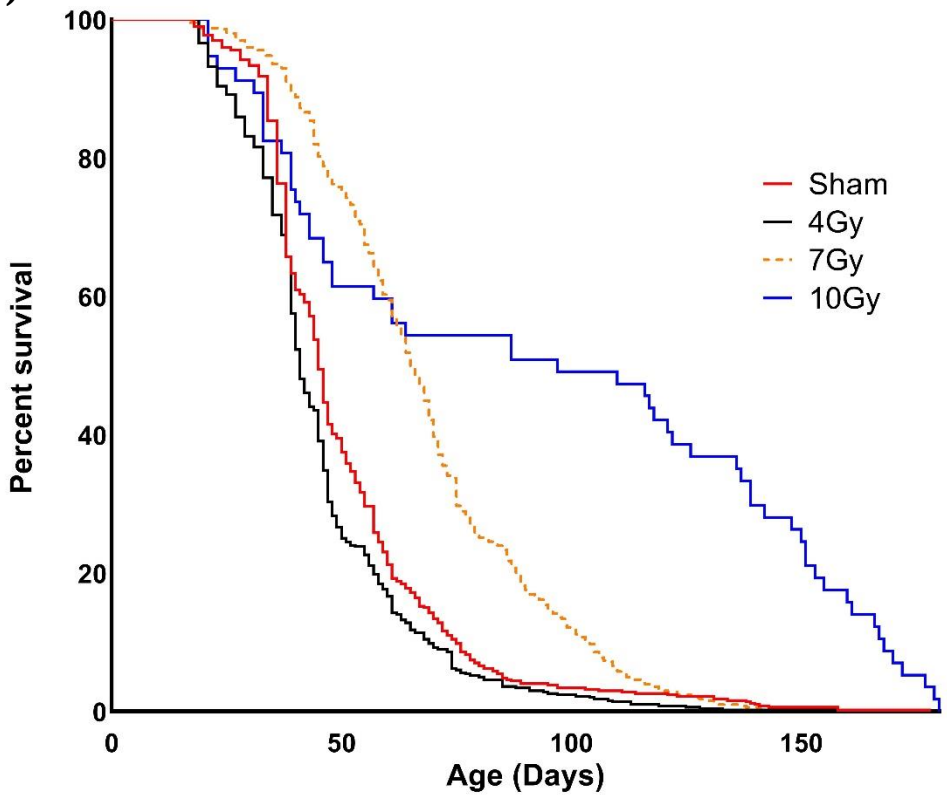

200
150
100
50
0
Longevity (Days)
****
****
****
****
Sham
2Gy
4Gy
7Gy
10Gy
12Gy

Figure 2.6: Kaplan-Meier survival curves for populations of male *Acheta domesticus* 0Gy (n=119), 2Gy (n=117), 4Gy (n = 128), 7Gy (n=106), 10Gy (n=127), 12Gy (n=120). Irradiation was conducted at 14 days of age at a dose-rate of 0.25Gy/min. A Gehan-Breslow-Wilcoxon test showed significant differences in survivorship in both 7Gy ($p < 0.0001$) and 10Gy ($p < 0.01$) as compared to the 0Gy sham group (a). ANOVA with a post-hoc Tukey's HSD test on the average age of the last 10% of surviving individuals, approximately n=12, showed a significant increase in maximal longevity for 2Gy, 7Gy, 10Gy, 12Gy ($p < 0.0001$) compared to controls. Values are represented as the mean age of each group +/- SEM (b)

a)


100
80
60
40
20
0
Percent survival
0
50
100
150
Age (Days)
Sham
4Gy
7Gy
10Gy

b)

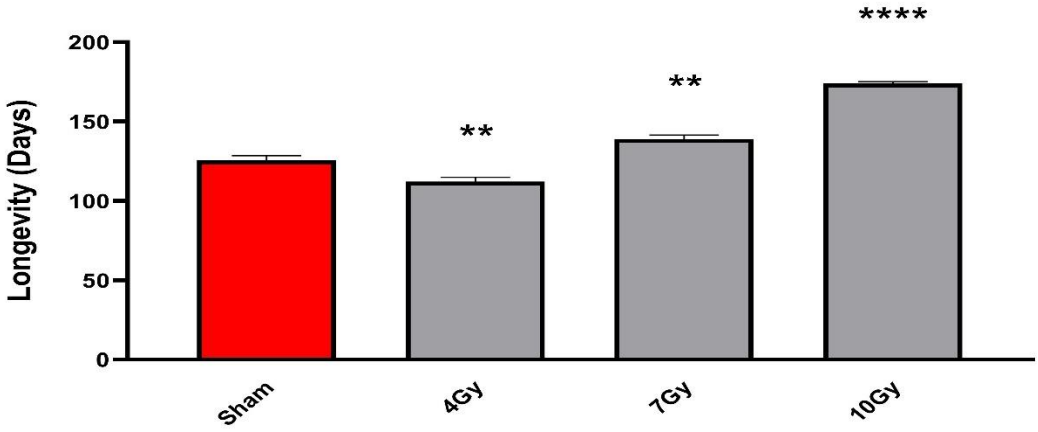

200
150
100
50
0
Longevity (Days)
**
**
****
Sham
4Gy
7Gy
10Gy

Figure 2.7: Kaplan-Meier survival curves for populations of male and female *Acheta domesticus* Sham (n=506), 4Gy (n=499), 7Gy (n=501), 10Gy (n=57). Individuals were offspring of males irradiated as described in Figure 2.6 and non-irradiated, virgin females. A Gehan-Breslow-Wilcoxon test, indicating variation in survivorship curves showed significant differences in survivorship in all groups; 4Gy ($p < 0.0001$), 7Gy ($p < 0.0001$) and 10Gy ($p < 0.0001$) as compared to the 0Gy sham group (a). ANOVA with a post-hoc Tukey's HSD test on the average age of the last 10% of surviving individuals showed alterations in maximal longevity in all groups, a slight decrease in the 4Gy ($p=0.0022$) and slight increase in 7Gy ($p = 0.0046$), while 10Gy showed significant increases ($p<0.0001$) which constitutes a 39% increase relative to the control. Values are represented as the mean age of each group +/- SEM (b).

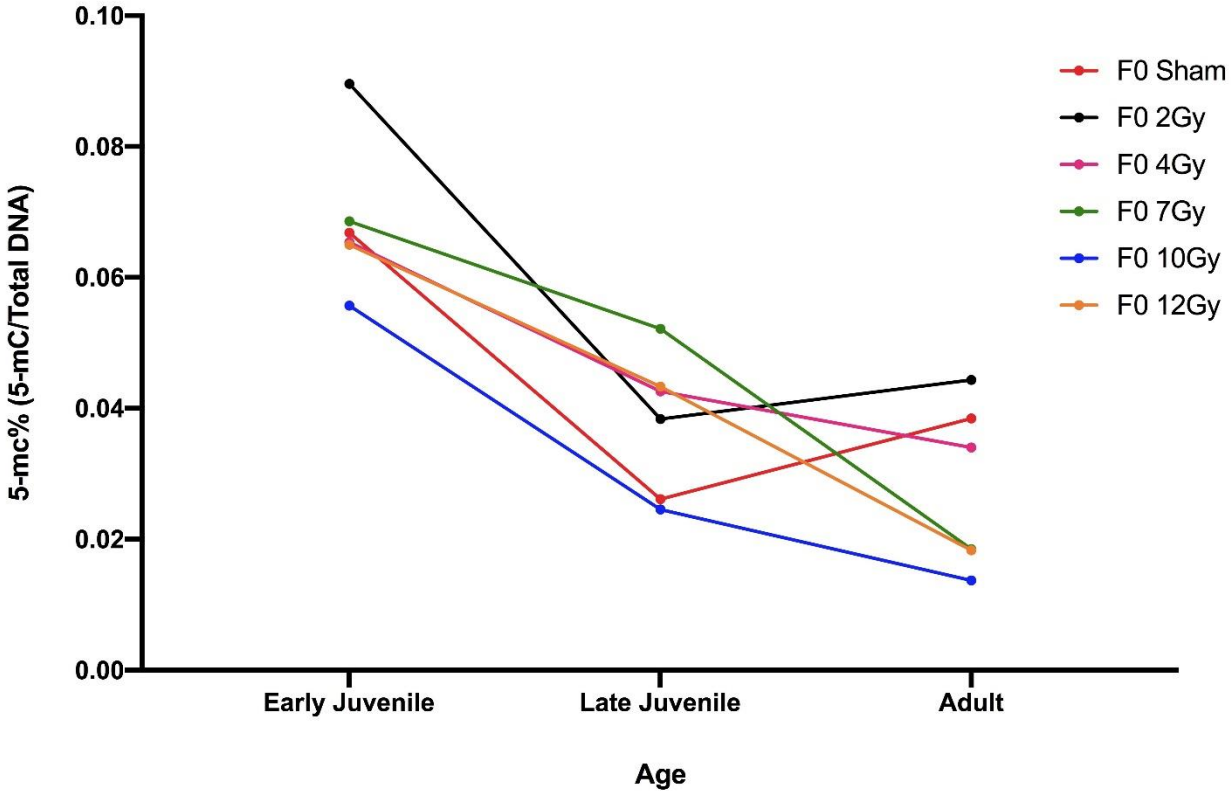


Figure 2.8: Effects of early life radiation on Global methylation (5-mC/Total DNA) from crickets exposed to early life radiation at 14 days old at various life stages. Samples were obtained from 3 time points post radiation, early juvenile (6hours, 1 Day, 3 Day, 7 Day), late juvenile (26 Days), and adult (1 week post-maturation). Values are shown as means of each age for each radiation group. Significant differences are not reported due to limited sample sizes.

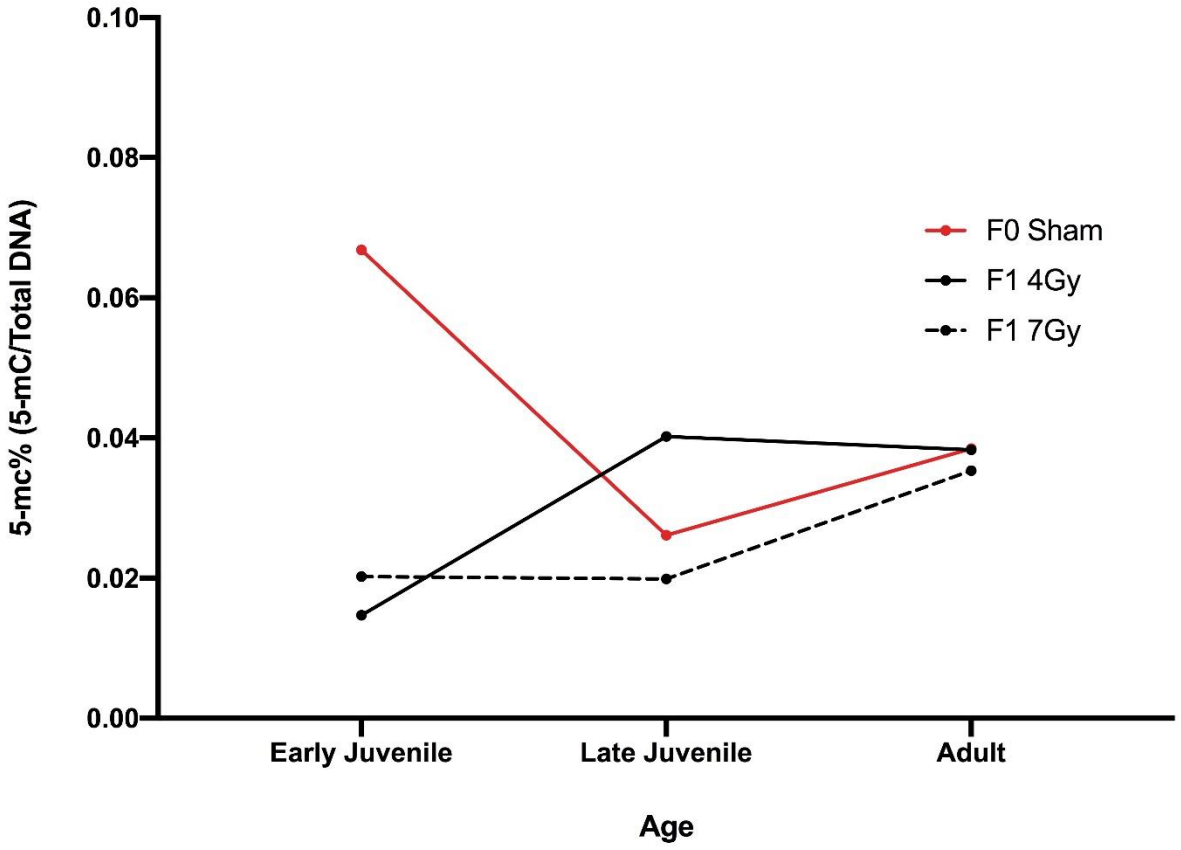


Figure 2.9: Effects of early life radiation on Global methylation (5-mC/Total DNA) from the offspring of crickets exposed to early life radiation at 14 days old. Samples were obtained from 3 time points, early juvenile (15 Days), late juvenile (30 Days), and adult (1 week post-maturation). Significant differences are not reported due to limited sample sizes.

CHAPTER 3

Radiation Impacts and the Hormetic Model

Abstract

Ionizing radiation exposure is often described by the linear-no-threshold (LNT) model. This defines a stressor as being negative or detrimental across all doses. However, there is accumulating evidence that the LNT model does not best fit the dose-response impacts of radiation. Other models, such as the threshold or hormetic model may better describe these effects since exposure may be beneficial at low doses or impacts only emerge when a threshold is reached. Using the model organism, *Acheta domesticus,* the effects of low, medium, and high doses of radiation on a variety of traits were modeled with either a linear or quadratic function. The traits analyzed included both life history and reproductive measures in irradiated males and their offspring. For almost all traits a quadratic non-linear model best described the dose-response curves (with only a single exception). Some traits also showed thresholds where impacts were not evident below medium doses. In some cases hormetic doses for specific traits were higher than expected. Overall the LNT model failed to fit the dose-response curve for life history and reproductive traits. Furthermore, various traits responded to radiation differently. More research into specific dose-responses of various traits are required to better understand the impacts of exposure, and particularly to identify hormetic ranges.

Introduction

Since the discovery of radioactivity more than a century ago, scholars have sought connections between radiation and heath (Macklis, 1990; Vaiserman, 2010). Initially, it was recognized that ionizing radiation could benefit or cure a wide range of diseases (Macklis, 1990; Prekeges, 2003; Vaiserman, 2010). One of the more striking treatments was "mild radium ingestion therapy" (Macklis, 1990; Vaiserman, 2010). This "radiation cure" was abandoned after a number of patients and scientists exposed to radiation died (Macklis, 1990; Prekeges, 2003; Vaiserman, 2010).

In the following years the "threshold model" gained recognition (Vaiserman, 2010). This suggested that harmful effects only occurred above a critical "threshold" dose (Vaiserman, 2010). Subsequently, Muller (1930) changed scientific opinion about radiation dose-response relationships by incorrectly concluding that germline mutation frequency was exactly proportional to dose. Moreover, people impacted by the WWII nuclear bombing of Japan suggested a "linear-no-threshold model (LNT)" was an accurate representation of radiation dose-response (Muller 1930; Prekeges, 2003; Vaiserman, 2010). As of today, the LNT model is the current prevailing dogma for radiation impacts and is the basis for international regulations on radiation exposure.

The LNT model issued by radiation protection agencies currently suggests that cancer risk increases linearly with radiation dose, i.e. risk increases at a similar rate across low to higher doses (Vaiserman, 2010). However, studies conducted on cancer risk for low-level exposure (e.g., X-rays, natural background) found much lower impacts than predicted by LNT model (Vaiserman, 2010). In fact, low dose exposure was associated with reduced spontaneous cancer

(i.e., a benefit) (Vaiserman, 2010). These and many other studies suggest that the LNT model does not accurately predict exposure risk whereas better representation is achieved by the "hormetic model" (that predicts that large doses are harmful but small ones are beneficial) and the "threshold model" (where low doses may be harmless) (Upton, 2001; Vaiserman, 2010).

Hormesis is the induction of beneficial effects from a range of low doses that are otherwise harmful at higher doses (Cypser & Johnson, 2002; Constantini et al. 2010; Moller et al. 2010). Studies spanning bacteria to vertebrates express hormetic dose responses for over 1,000 environmental stressors (including radiation) (Cypser & Johnson, 2002; Constantini et al, 2010; Vaiserman, 2010; Calabrese, 2013). Hormetic benefits have been detected in disease resistance, lifespan extension, and immune surveillance (Cypser & Johnson, 2002; Vaiserman, 2010; Calabrese, 2013).

Besides detection of hormesis across a diverse range of organisms (including invertebrates), there is also evidence for long-term negative impacts of radiation exposure (Bonner, 2003; Vaiserman, 2010). These are often referred to as "delayed effects" as they are sometimes not apparent in irradiated parents or in the first several generations of offspring. Delayed effects can emerge in generations produced by irradiated parents, even at doses so low that the parents seemed unaffected (Bonner, 2003; Vaiserman, 2010). A landmark study of trans-generational effects was conducted in Chernobyl bank voles that showed persistent chromosomal damage and increased embryonic lethality spanning >20 generations (Dubrova, 2003). In this study the levels of damage in offspring many generations later were comparable to individuals that received the initial exposure (Dubrova, 2003). Delayed effects of radiation exposure are a current area of research and understanding such effects at low doses is critical.

The purpose of this study was to determine the dose response relationship in male *Acheta domesticus* and their F1 male and female offspring. Variables included life history and reproductive traits, growth rate, longevity, oviposition and mounting. Each trait was analyzed to determine if the dose response relationship was best described by the LNT, hormetic, or threshold models.

Methods

Experimental groups & Data Collection

Experimental groups used for modeling analysis in this Chapter of life history and reproductive traits for both F0 irradiated males and their F1 offspring were obtained from rearing colonies as described in the Methods of Chapter 1 and 2. Methods used for the data collection of life history and reproductive traits for irradiated F0 males and F1 offspring used in this chapters modeling analysis was conducted as described in the methods portion of both Chapter 1 and Chapter 2. Data previously graphed in Chapters 1 and 2 for life history and reproductive traits were then fitted to both linear and quadratic models to compare fits.

Statistics

Dose-response relationships for both life history and reproductive traits were based on mean responses to each dose. To determine whether any traits were responding to radiation exposure in a linear or non-linear fashion, traits were fitted to both linear and quadratic functions **(Table 3.0).** A non-linear response was indicated if the fitted quadratic function best explained more variance than a linear regression (i.e., comparing the r^2 and the sum of squares for "goodness of fit). This indicates a superior "goodness of fit". If the sum of squares and the R^2 value were contradicting (i.e each indicated a different model for "best fit") the results were

listed as inconclusive. As p-values are not reported in non-linear regression models this was not including in determining which model best fit the data.

A trait was considered hormetic if first the modeling analysis indicated through R^2 and sum of squares values that the quadratic model was best describing the data and secondly, if the graph visually indicated that a low dose showed a beneficial response compared to higher doses and control groups. A threshold response for a trait was indicted if again the modeling analysis indicated through R^2 and sum of squares values that the quadratic model was best describing the data and secondly, if there were no evident negative effects until a specific dose. All statistical analyses were conducted using Prism Graphpad 8.

Results

Preferred Models

After fitting both life history and reproductive traits to both quadratic and linear models result indicated that almost all traits were best described by the quadratic function with the exception of F0 male longevity (**Table 3.0)**. Of the 8 traits listed for both F0 and F1 offspring, 5 showed R^2 values greater than 0.9. Only F0 longevity results were inconclusive with conflicting R^2 and sum of squares values. The F1 growth rate also had a weaker R^2 correlation indicating this may also be inconclusive. Overall, most traits were best described by the quadratic model (indicating that radiation impacts were non-linear).

Hormesis & Threshold responses

Hormetic responses were only evident in longevity and survivorship traits for both F0 males and F1 offspring. However, these beneficial responses were at higher doses than expected. In both F0 and F1 offspring 7Gy and 10Gy groups showed significant increases. Doses >10Gy were similar to controls (**Fig. 2.6 & Fig. 2.7)**.

Reproductive traits; mating (oviposition & mounting), latency to mate, and hatching success were all best described by the quadratic model. Each of these traits, however, also expressed a clear threshold response: i.e., no significant difference from controls until doses of 10Gy and above (**Fig. 1.7 – Fig. 1.10)**.

Response Rates

The growth rate of F0 irradiated males was best modeled by a quadratic function but neither reflected a threshold or hormetic response. F0 male growth rate visually seemed to decline in a linear manor, however the rate at which this decline occurred was not proportional to dose which is likely why the quadratic function modelled the response better. Reductions in growth were moderate as dose increased to 7Gy. After this point however, in 10Gy and 12Gy groups growth declines were much more pronounced. Specifically, between 10Gy and 12Gy groups alone, which is only a 2Gy difference growth rate declined 23% compared to the decline between controls and 7Gy groups, a 7Gy difference, which constituted only a 14.5% decline in growth rate **(Fig. 2.1).**

Discussion

Ionizing radiation, despite its currently bad reputation, is a ubiquitous element in all environments, including air, water, soil, bodies, tools and structures (Bonner, 2003; Luckey, 2006). Humans experience background radiation doses of ~2-2.5mSv/year, with some geographic regions receiving 5 times higher (Bonner, 2003). As background radiation is a normal and natural environmental feature, fitness might be expected to be highest around these background levels (Parsons, 2000). Interestingly however, organismal fitness tends to decline at doses significantly below background and increase at levels substantially above background (Parsons, 2000). The cause of this phenomenon has been suggested to be a result of adaptive responses; that if exposed to low, above background doses of radiation an organism will "adapt" and display increased defence mechanisms and therefore future radio resistance (Parsons, 2000). Whether exposure to radiation is negative at all doses, hormetic, threshold, or some other dose-response trend was the focus of this study.

The Linear-No-Threshold (LNT) Model & Hormetic Doses

Despite deficiencies, the linear-no-threshold model is the current established international standard for radiological protection (Averbeck, 2009). It is based the assumption that exposure to ionizing radiation increases carcinogenic risk linearly with dose and that all exposure is harmful (Averbeck, 2009). Current and past literature indicates that this model poorly fits the actual dose response relationship, especially at relatively low doses or dose-rates (Averbeck, 2009; Doss, 2013). Humans exposed to radiation from atomic bombs, nuclear waste, and medical therapy showed significant reductions in cancer at doses between 0.3 – 0.7Gy (Doss, 2013).

Curves fitted to life history and reproductive parameters of our crickets also found the best-fitting model was non-linear **(Table 3.0).** Reproductive traits tended to show threshold responses, where significant declines in reproduction were not apparent until mid- to high-doses. Longevity and survivorship showed hormetic doses. Growth rate in F0 males, visually looked like a linear response, however, due to dose not being directly proportional to growth rate declines this was still modelled better by a quadratic curve. Different traits therefore responded variably to doses, but none showed a linear (LNT) type dose-response. Deviation from the LNT model has been consistently shown in a vast number of species and traits at doses ranging from below 1Gy to above 10Gy depending on the organism (Allen & Sohal, 1982; Lithgow et al. 1995; Caratero et al. 1998; Cypser & Johnson, 2002; Shephard et al. 2018). The conclusion of this work not only rejects the LNT model as a blanket model for all dose response relationships, but also highlights the fact that stressors may act on organismal traits in different magnitudes. As described above, some traits showed hormetic as well as threshold responses to exposure with harmful doses varying depending on the trait. This suggests that future research should focus on analyzing dose-response relationships for a multitude of reproductive and life-history endpoints in order to understand the complex nature in which an organism response to stress.

Hormesis had been defined as a dose-response relationship described by low dose enhancement and high dose impairment (Calabrese & Baldwin, 2003). This produces a “U” or “J” shaped curve for dose-responses as found in a variety of species and toxic agents (Calabrese & Baldwin, 2003). In this study, however, hormesis was only documented for longevity and survivorship **(Fig. 3.1 & Fig. 3.2).** Other traits such as growth rate and reproductive parameters showed no hormetic responses but instead threshold or other non-linear responses. Regarding longevity and survivorship, beneficial effects were observed in mid-to-high range doses above

7Gy. Specifically, the most beneficial dose was the 7Gy group and their offspring, as they showed very little declines in reproductive parameters (as this was below the reproductive threshold) but maintained longevity and survivorship enhancements. In comparison, the 12Gy group were sterile, and 10Gy males also showed a sharp decline in reproductive output and other life history parameters. These results suggest that the hormetic, or beneficial doses in crickets exposed to radiation is around 7Gy (the mid range dose). This is slightly higher than would be predicted by the hormetic model, which describes hormetic doses as being quite low. However, one must consider what a "lose dose" is for this species, the age of exposure, and whether radiation is delivered chronically or acutely.

There are extreme differences in radio sensitivities between different kingdoms as well as between closely related species (Sparrow & Miksche, 1961). Insect species are generally more radiation resistant than mammals, with studies showing insect cells to be between 3-104 times more resistant then mammalian cells (LaChance & Graham, 1984). Therefore, when analyzing the effects of radiation on insects it is important to understand that doses that may be lethal or harmful to mammals may not elicit similar responses in insects. A dose of 7Gy may indeed be a low dose for our cricket species but lethal in some mammalian species.

A study conducted on adult *Acheta domesticus* in 1971 showed that life expectancy in females was increased at doses between 500 to 2000 R which translates to about 5 – 17Gy, and that life expectancy reductions were not seen until significantly higher doses (between 4000 – 10000R) (Hunter & Krithayakiern, 1971). In this same study reproductive parameters were much more impacted by these doses than were life history parameters; with reproductive traits (fecundity, fertility, and oviposition period) decreasing significantly at doses as low as 1000R while life history parameters (life expectancy) only showing declines at doses of 4000R (Hunter

& Krithayakiern, 1971). In comparison, hormetic doses for mammals range between 0.1 – 100mGy with beneficial endpoints in immune function, fertility, and longevity (Luckey, 2006; Scott et al. 2007). These studies, along with my results, indicate that the doses that significantly reduce fitness are much higher than would be expected for mammals and consequently, hormetic doses may also be shifted higher than the micro doses reported to achieve hormesis in mammals.

Another important aspect in radiation responses is chronic versus acute doses as well as variable dose rates. Acute and chronic exposure, depending on the dose, causes differential impacts on an organism (ICRP, 2007; Mughal et al. 2012). In humans, chronic exposures of 0.3 – 0.5Gy/year can impact the immune, haemopoietic, and neural systems, but levels below 0.1Gy have no apparent effect (ICRP, 2007). If this 0.3 – 0.5Gy dose was applied acutely, it would cause acute radiation syndrome with symptoms of nausea, vomiting, bone marrow loss, and potentially death (CDC, 2018). Despite the wealth of knowledge on the effects of acute versus chronic exposure in humans, there is a paucity of data on other organisms (ICRP, 2008). In non-human research there is wide variation in species, dose rates, exposure periods, types of source, and age of exposure that limits out ability to extrapolate or generalize (ICRP, 2008). Furthermore, acute exposures are most prevalent in literature which may not be as relevant when extrapolating to exposure in the natural environmental with chronic background emissions (ICRP, 2008). In this study, doses were administered acutely at a dose rate of 0.25Gy/min, whether or not the effects observed would be different if exposed chronically is an area for future research.

In summary the effects of exposure on different traits and species has been shown time and time again to be more complex than the current LNT model. Countless studies including this

one indicate that more research needs to be completed to understand how and why radiation effects traits differently, why some dose ranges are hormetic, and how this varies among species.

Adaptative Responses vs. Delayed Effects

Past radiation exposure experiments for the most part focus on targeted effects of radiation such as direct DNA damage and direct mutations (Kadhim et al. 2004). Currently however, the focus of radiation exposure has shifted to non-targeted effects; bystander effects, genomic instability and adaptive responses (Zhou et al. 2003; Kadhim et al. 2004). Genomic instability is the destabilization of offspring genomes of parents who were exposed to a mutagen (Chaudhry et al. 2012; Mughal et al. 2012; Gomes & Dubrova, 2015). This destabilization results in the accumulation of mutations and the subsequent health effects that can occur in F1 offspring that continue to persist to future generations (Kadhim et al. 2004; Gomes & Dubrova, 2015). Interestingly, some studies have indicated that only paternal exposure elicits the genomic instability and its consequences seen in exposed parents (Gomes & Dubrova, 2015).

Genomic instability has been suggested to be a result of epigenetic modifications; altered methylation processes, acetylation, phosphorylation and deficiencies in cellular repair mechanisms (although exact mechanisms remain unknown) (Kadhim et al. 2004; Seong et al. 2011). Bystander effects are a phenomenon where radiation-damaged cells can elicit damage in non-irradiated cells (Mothersill & Seymour, 2001; Zhou et al. 2003). Specifically, non-irradiated cells have been shown to display induced mutagenesis despite absence of ROS (Zhou et al. 2003). The bystander effect has also been detected in a variety of cell lines given both densely and sparsely ionizing radiation exposures (Mothersill & Seymour, 2001). Current research has indicated that this may be a result of gap-junction mediated cell-cell communication or other

transmissible factors, but the exact mechanism is currently under study (Mothersill & Seymour, 2001; Zhou et al. 2003). Both bystander effects and genomic instability are negative effects caused by seemingly harmless low-dose exposure as well as higher doses (Kadhim et al. 2004). Currently, our knowledge on these two subjects are limited and are a current area of research.

Although the bystander effect and genomic instability are two highly negative outcomes of low dose radiation exposure there is a conflicting, positive outcome called adaptive response (Kadhim et al. 2004). This is the process in which resistance to a harmful dose of radiation is achieved through an earlier, less harmful dose (Kadhim et al. 2004). Adaptive response is mediated by the synthesis of proteins, and through the up and down regulation of genes involved in stress pathways (Kadhim et al. 2004). A study using 13 different human cell lines demonstrated a weak inverse relationship between adaptive response and bystander effects (Mothersill et al. 2002). Similarly, a study examining the same relationship showed that adaptive response to low doses ionizing radiation protected against the bystander effect (Sawant et al. 2001). The relationship between the bystander effect, adaptive response, which are short term effects and transgenerational genomic instability which can impact future generations is an area for future research in the area of the non-targeted effects of radiation.

Differential Responses: Gender & Traits

Cricket responses to radiation exposure vary based on the trait being analyzed, with stimulatory doses being evident for some traits, absent, or negative for others (Calabrese & Blain, 2005). This may also depend on the gender of the individual as well, with some studies indicating that females and males respond differentially to exposure for some traits. There have been studies conducted in our lab that found that reproductive parameters including hatching

success, egg size, early-adult rate of reproduction and lifetime fecundity showed hormetic responses in irradiated females between 0 - 2Gy (Shephard et al. 2018). Unpublished data from our laboratory also showed a sharp, near-zero decline in oviposition rate in females irradiated at 5- 7Gy. In my first two chapters reproductive decline did not occur in irradiated males until doses of 10Gy and above, in parameters such as hatching success and mating success **(Fig. 1.9 & Fig. 1.10).** This indicates that between sexes the male house cricket is more radio-resistant than females for reproductive traits, as they can still reproduce and produce viable offspring at doses much higher than in females.

Another aspect of differential responses is the effect of exposure on different traits. Reproductive parameters in crickets seem to be most sensitive to radiation exposure, with sterilization in males occurring at 12Gy and near sterilization at 10Gy **(Fig. 1.10).** Other traits, such as longevity are increased at these same seemingly negative doses, with males irradiated at 10Gy and 12Gy having significant longevity increases. In general, reduced reproduction or sterility is associated with life extension across many phyla.

Growth rate, another life history trait showed declines at doses above 4Gy **(Fig. 2.1)**. Survivorship on the other hand showed mid-life increases at 7Gy and 10Gy. As with reproduction, growth (another aspect of production) is also strongly linked to longevity. These results suggest that radiation impacts different life history and reproductive parameters differently, and impacts on one aspect can cascade to other aspects. Future research in analyzing effects of radiation on different life history and reproductive parameters should look at a variety of traits before making general conclusions about radio-resistance and impacts as what may be beneficial or detrimental to one trait may not be true for others.

Conclusion

In summary this research provides further evidence that the LNT model for radiation exposure fails to accurately describe the effects of exposure on a variety of life history and reproductive traits in male *Acheta domesticus*. Radiation exposure has been shown, in this and other studies, to impact male and female crickets differently, and within each gender to effect life history and reproductive traits differently. Beneficial and detrimental doses in each parameter analyzed were different, indicating that there is not a dose that all traits respond to the same. Furthermore, all traits analyzed in this study with male house crickets failed to be predicted by a linear model. Finally, hormetic doses were only observed in males for survivorship and longevity parameters and were at doses significantly higher than what would be expected by the hormetic model. Other parameters did not show any beneficial effects of radiation, however, hormetic doses may be occurring at doses below 2Gy which was the lowest dose used in this study. Overall this affirms the simplicity of the LNT model and its failure to be a general rule for radiation exposure. Future research should continue to look at a variety of traits and their dose response curves, gender and species differences, as well as doses below 2Gy.

References

Allen, R., & Sohal, R. (1982). Life-lengthening effects of γ-radiation on the adult housefly, Musca domestica. *Mechanisms of Ageing and Development,20*(4), 369-375. doi:10.1016/0047-6374(82)90104-x

Averbeck, D. (2009). Does scientific evidence support a change from the lnt model for low-dose radiation risk extrapolation? *Health Physics,97*(5), 493-504. doi:10.1097/hp.0b013e3181b08a20

Barber, R., Plumb, M. A., Boulton, E., Roux, I., & Dubrova, Y. E. (2002). Elevated mutation rates in the germ line of first- and second-generation offspring of irradiated male

mice. *Proceedings of the National Academy of Sciences,99*(10), 6877-6882. doi:10.1073/pnas.102015399

Barber, R. C., Hickenbotham, P., Hatch, T., Kelly, D., Topchiy, N., Almeida, G. M., . . . Dubrova, Y. E. (2006). Radiation-induced transgenerational alterations in genome stability and DNA damage. *Oncogene,25*(56), 7336-7342. doi:10.1038/sj.onc.1209723

Bonner, W. M. (2003). Low-dose radiation: Thresholds, bystander effects, and adaptive responses. *Proceedings of the National Academy of Sciences,100*(9), 4973-4975. doi:10.1073/pnas.1031538100

Calabrese, E. J., & Baldwin, L. A. (2003). HORMESIS: The dose-response revolution. *Annual Review of Pharmacology and Toxicology,43*(1), 175-197. doi:10.1146/annurev.pharmtox.43.100901.14022

Calabrese, E., & Blain, R. (2005). The occurrence of hormetic dose responses in the toxicological literature, the hormesis database: An overview. *Toxicology and Applied Pharmacology,202*(3), 289-301. doi:10.1016/j.taap.2004.06.023

Calabrese, E. J. (2013). Low doses of radiation can enhance insect lifespans. *Biogerontology,14*(4), 365-381. doi:10.1007/s10522-013-9436-5

Caratero, A., Courtade, M., Bonnet, L., Planel, H., & Caratero, C. (1998). Effect of a continuous gamma irradiation at a very low dose on the life span of mice. *Gerontology,44*(5), 272-276. doi:10.1159/000022024

CDC Radiation Emergencies | Acute Radiation Syndrome: A Fact Sheet for Physicians. (n.d.). Retrieved from https://www.cdc.gov/nceh/radiation/emergencies/arsphysicianfactsheet.htm

Chaudhry, M. A., Omaruddin, R. A., Kreger, B., Toledo, S. M., & Azzam, E. I. (2012). Micro RNA responses to chronic or acute exposures to low dose ionizing radiation. *Molecular Biology Reports,39*(7), 7549-7558. doi:10.1007/s11033-012-1589-9

Costantini, D., Metcalfe, N. B., & Monaghan, P. (2010). Ecological processes in a hormetic framework. *Ecology Letters,13*(11), 1435-1447. doi:10.1111/j.1461-0248.2010.01531.x

Cypser, J. R., & Johnson, T. E. (2002). Multiple stressors in *Caenorhabditis elegans* induce stress hormesis and extended longevity. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences,57*(3). doi:10.1093/gerona/57.3.b109

Doss, M. (2013). Linear no-threshold model vs. radiation hormesis. *Dose-Response,11*(4). doi:10.2203/dose-response.13-005.doss

Dubrova, Y. E. (2003). Radiation-induced transgenerational instability. *Oncogene,22*(45), 7087-7093. doi:10.1038/sj.onc.1206993

Gomes, A. M., Barber, R. C., & Dubrova, Y. E. (2015). Paternal irradiation perturbs the expression of circadian genes in offspring. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis,775*, 33-37. doi:10.1016/j.mrfmmm.2015.03.007

Hunter, P. E., & Krithayakiern, V. (1971). Effect of gamma radiation upon life expectancy and reproduction in the house cricket, *Acheta domesticus* (Orthoptera: Gryllidae)1. *Annals of the Entomological Society of America,64*(1), 119-123. doi:10.1093/aesa/64.1.119

ICRP. (2008). ICRP Publication 108: Environmental protection: the concept and use of reference animals and plants. *Ann. Icrp*, 38(1), doi:10.1088/0952-4746/32/1/b01

Kadhim, M. A., Moore, S. R., & Goodwin, E. H. (2004). Interrelationships amongst radiation-induced genomic instability, bystander effects, and the adaptive response. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis,568*(1), 21-32. doi:10.1016/j.mrfmmm.2004.06.043

Lachance, L. E., & Graham, C. K. (1984). Insect radiosensitivity: Dose curves and dose-fractionation studies of dominant lethal mutations in the mature sperm of 4 insect species. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis,127*(1), 49-59. doi:10.1016/0027-5107(84)90139-8

Lithgow, G. J., White, T. M., Melov, S., & Johnson, T. E. (1995). Thermotolerance and extended life-span conferred by single-gene mutations and induced by thermal stress. *Proceedings of the National Academy of Sciences,92*(16), 7540-7544. doi:10.1073/pnas.92.16.7540

Luckey, T. D. (2006). Radiation hormesis: The good, the bad, and the ugly. *Dose-Response,4*(3). doi:10.2203/dose-response.06-102.luckey

Macklis, R. M. (1990). Radithor and the era of mild radium therapy. *JAMA: The Journal of the American Medical Association,264*(5), 614. doi:10.1001/jama.1990.03450050072031

Metcalfe, N. B., & Alonso-Alvarez, C. (2010). Oxidative stress as a life-history constraint: The role of reactive oxygen species in shaping phenotypes from conception to death. Functional Ecology,24(5), 984-996. doi:10.1111/j.1365-2435.2010.01750.x

Møller, A. P., & Mousseau, T. A. (2012). The effects of natural variation in background radioactivity on humans, animals and other organisms. *Biological Reviews,88*(1), 226-254. doi:10.1111/j.1469-185x.2012.00249.x

Mothersill, C., & Seymour, C. (2001). Radiation-induced bystander effects: Past history and future directions. *Radiation Research,155*(6), 759-767. doi:10.1667/0033-7587(2001)155[0759:ribeph]2.0.co;2

Mothersill, C., Seymour, C. B., & Joiner, M. C. (2002). Relationship between radiation-induced low-dose hypersensitivity and the bystander effect. *Radiation Research,157*(5), 526-532. doi:10.1667/0033-7587(2002)157[0526:rbrild]2.0.co;2

Mughal, S. K., Myazin, A. E., Zhavoronkov, L. P., Rubanovich, A. V., & Dubrova, Y. E. (2012). The dose and dose-rate effects of paternal irradiation on transgenerational instability in mice: A radiotherapy connection. PLoS ONE,7(7). doi:10.1371/journal.pone.0041300

Muller, H. J. (1930). Radiation and genetics. *The American Naturalist,64*(692), 220-251. doi:10.1086/280313

Parsons, P. A. (2000). Hormesis: An adaptive expectation with emphasis on ionizing radiation. *Journal of Applied Toxicology,20*(2), 103-112. doi:10.1002/(sici)1099-1263(200003/04)20:23.0.co;2-o

Prekeges, J. L. (2003). Radiation hormesis, or, could all that radiation be good for us? *J Nucl Med Technol,31*, 11-17.

Sawant, S. G., Randers-Pehrson, G., Geard, C. R., And, D. J., & Hall, E. J. (2001). The bystander effect in radiation oncogenesis: transformation in C3H 10T½ cells in vitro can be initiated in the unirradiated neighbors of irradiated cells. *Radiation Research,155*(3), 397-401. doi:10.1667/0033-7587(2001)155[0397:tbeiro]2.0.co;2

Scott, B. R., Haque, M., & Palma, J. D. (2007). Biological basis for radiation hormesis in mammalian cellular communities. *International Journal of Low Radiation,4*(1), 1. doi:10.1504/ijlr.2007.014485

Shephard, A. M., Aksenov, V., Tran, J., Nelson, C. J., Boreham, D. R., & Rollo, C. D. (2018). Hormetic effects of early juvenile radiation exposure on adult reproduction and offspring performance in the cricket (*Acheta domesticus*). *Dose-Response,16*(3), 155932581879749. doi:10.1177/1559325818797499

Upton, A. C. (2001). Radiation hormesis: Data and interpretations. *Critical Reviews in Toxicology,31*(4-5), 681-695. doi:10.1080/20014091111956

Vaiserman, A. M. (2010). Radiation hormesis: Historical perspective and implications for low-dose cancer risk assessment. *Dose-Response,8*(2). doi:10.2203/dose-response.09-037.vaiserman

Velando, A., Torres, R., & Alonso-Alvarez, C. (2008). Avoiding bad genes: Oxidatively damaged DNA in germ line and mate choice. BioEssays,30(11-12), 1212-1219. doi:10.1002/bies.20838

Zhou, H., Randers-Pehrson, G., Geard, C. R., Brenner, D. J., Hall, E. J., & Hei, T. K. (2003). Interaction between radiation-induced adaptive response and bystander mutagenesis in mammalian cells. *Radiation Research,160*(5), 512-516. doi:10.1667/rr3083

Table 3.0: Preferred Linear or Quadratic Model Life History and Reproductive Traits

Trait	Generation	Equation	R^2	Sum of Squares	Preferred Model
Longevity	F0	$y = -0.1826x^2 + 3.202x + 127.8$	0.5139	137.3	Inconclusive
		$y = 0.9923x + 131.2$	0.3794	175.3	
	F1	$y = 1.211x^2 - 7.017x + 124$	0.9835	34.95	Quadratic
		$y = 4.940x + 111.8$	0.6307	782.3	
Growth Rate	F0	$y = -0.02268x^2 - 0.03382x + 8.304$	0.9765	0.2635	Quadratic
		$y = -0.3083x + 8.722$	0.9241	0.8496	
	F1	$y = 0.03362x^2 - 0.3799x + 8.387$	0.7004	0.3001	Quadratic
		$y = -0.04779x + 8.031$	0.1249	0.8765	
Mating (Ovipostion)	F0	$y = 0.0007308x^2 - 0.03932x + 0.4610$	0.9039	0.01081	Quadratic
		$y = -0.03047 + 0.4476$	0.8985	0.01142	
Mating (Mounting)	F0	$y = -0.003472x^2 - 0.006945x\ 1.010$	0.9976	0.0005197	Quadratic
		$y = -0.4960x + 1.063$	0.9476	0.01143	
Latency to Mate	F0	$y = 0.02751x^2 - 0.08914x + 2.2202$	0.9363	0.4002	Quadratic
		$y = 0.2488x + 1.795$	0.8274	1.085	
Hatching Success	F0	$y = -0.7235x^2 + 3.999x + 55.62$	0.8765	366.5	Quadratic
		$y = 68.27x - 4.692$	0.06774	960.6	

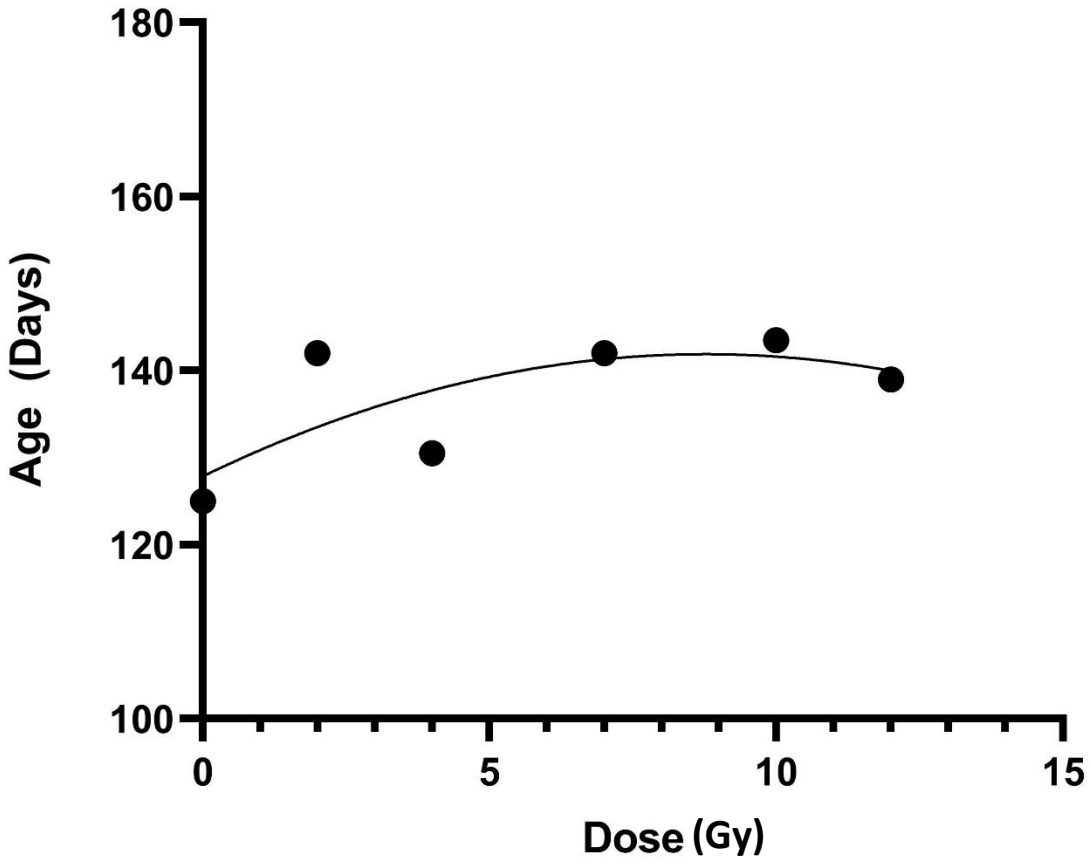


Figure 3.1: Dose Response curve for the longevity of irradiated male *Acheta domesticus.* All males were irradiated at 14 days of age at a dose rate of 0.25Gy/min. Significant differences between doses can be found in Figure 2.6b. Fitted to the graph is the quadratic function $y = -0.1826x^2 + 3.202x + 127.8$, R^2 value of 0.5139. This graph shows increased longevity in all doses compared to the control.

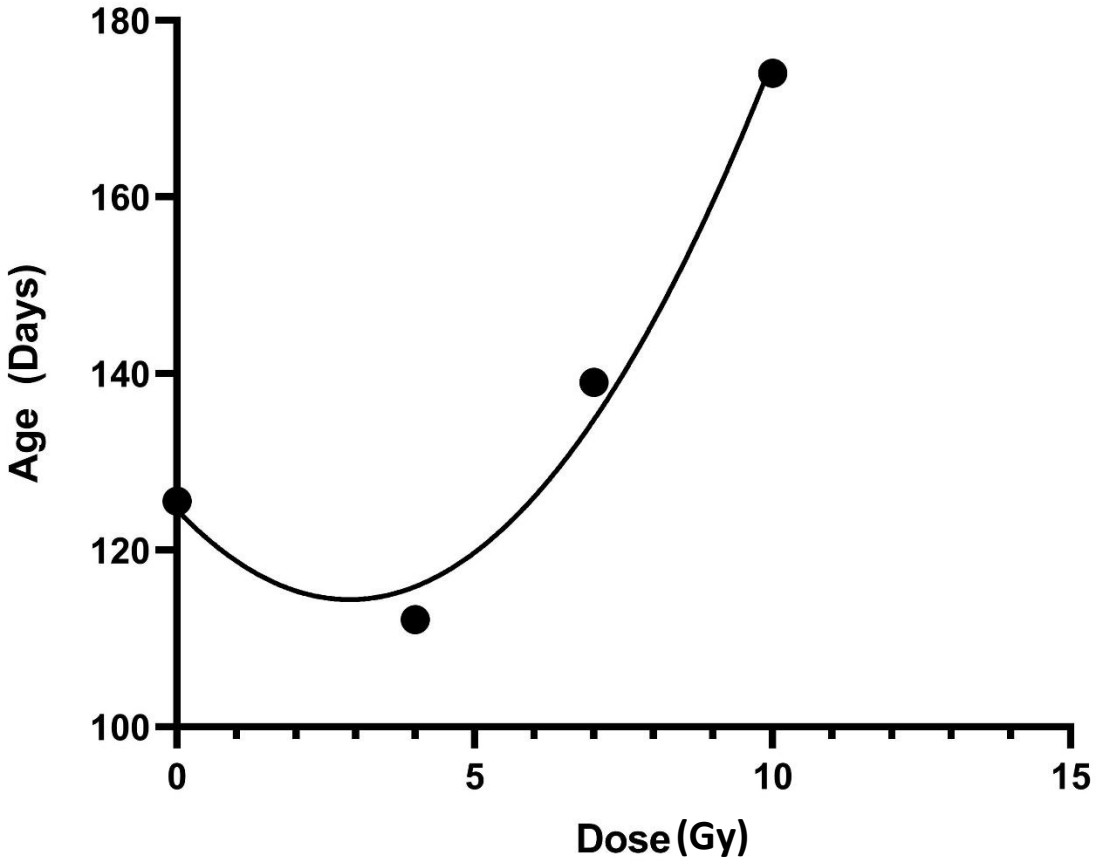


Figure 3.2: Dose response curve for longevity of F1 offspring of males exposed to radiation as early juveniles. Significant differences between doses can be found in Figure 2.7b. Fitted to the graph is the quadratic function $y = 1.211x^2 - 7.017x + 124$, R^2 value of 0.9835. This graph shows a slight decrease in longevity at 4Gy but then a very large significant increase in the highest doses tested compared to controls. P value

Conclusions

Summary, Limitations, and Future Directions

Summary

In general, my research furthers the understanding of radiation effects for the insect species *Acheta domesticus.* In my first chapter, I was able to determine disturbances in male mating ability through both their sexual signals and reproductive success. I was also able to determine the dose in which sterility occurs though male exposure. In my second chapter, I was able to conclusively show generational effects of paternal exposure in the F1 generation in several life history traits including growth, longevity, and survivorship. Furthermore, I was able to detect occurrences of gene regulation alterations and epigenetic changes in both exposed males and their offspring. These biomarkers however require further research and analysis to make definitive conclusion. In my final chapter I discussed hormesis and other dose-response models and how it pertains to the research conducted in this thesis. I was able to show that no trait was described best by the LNT model. I also discussed how radiation effects sex and traits differently. Finally, I showed how longevity showed beneficial responses to radiation which occurred in both those exposed and their offspring. Overall this thesis furthers our understanding of dose-response, generational effects, as well as attractiveness and mate choice.

Limitations

One of the main limitations of each of the chapters in this thesis is that the data used was collected in a laboratory setting. Natural environments are not static, nor are they free from a variety of stressors. Organisms experience variations in a number of other variables throughout

their lifetime which may be described as stress. Therefore, it is unlikely in natural environments that a species will experience a singular stress, immune from other potential stressors such as temperature and food availability. Another major limitation is the density in which cricket species typically live in their natural environments. Studies conducted analyzing the effects of population density on crickets determined changes in development and growth in densely populated groups versus low density groups (Iba et al., 1995). In lab conditions, crickets are held in relatively dense groups compared to that of their natural environment which may affect the results observed. Finally, the last major limitation is dose-rate. During the experiments dose is delivered acutely at a consistent dose-rate. Although this is necessary for consistency in determining the amount of stress received it is unlikely in natural environments where exposure would occur. Doses in natural environments are likely to be chronic not acute. These limitations are the detriments of experimenting in a laboratory setting but also necessary.

Future Directions

Chapter 1

Future research in this area should consider specific components of sexual signals, (i.e., hydrocarbon alterations and development of specific wing components that are being altered or damaged. Furthermore, male-male interactions which may play an indirect role in female choice would be of great interest. Finally, a deeper look into male sterilization, specifically the components of the spermatophore that are rendering males sterile could give more insight into the sterile male technique and its method of sterilization. My research generally suggests that insects, specifically crickets, have intricate communication and reproduction that can be easily disrupted by early life stressors such as radiation. A closer look into these areas could benefit our

understanding of how species generally respond to environmental stressors, and especially radiation exposure that can have sudden and persistent ecosystem impacts in this age of nuclear energy and weaponization.

Chapter 2

Future work on generational effects should consider extending this experiment to future generations to determine whether the responses seen in survivorship and longevity remain, eventually level off, or drop back to control levels. Also, using more generations may shed light into the potential for the delayed effects of exposure that are not apparent in just one generation. Specifically, genomic instability and the consequences of this would be an important avenue for future research. Further analysis into the stress response pathways and its components including FOXO3, Nfkb, TOR, AMPK, and Akt to name a few would be ideal for understand specifically how radiation is effecting the organism on a cellular level. Furthermore epigenetic markers such as the CpT sites instead of the common CpG should be analyzed for this species. Future research into these areas will prove to further our understanding of stress-response, its mechanisms, and how stress shapes the life history of those exposed as well as future generations.

Chapter 3

Future directions in the area of radiation dose-response modeling should focus on determining specific effects of exposure on specific traits rather then a general one model fits all approach. This would allow for a better understanding of beneficial and harmful doses as it pertains to specific reproductive and life history traits. As well, more data should be collected on sex and species-specific responses to radiation as the results of this study as well as other data collected in our lab suggests that males and females have variable resistances to radiation.

Supplementary Figures

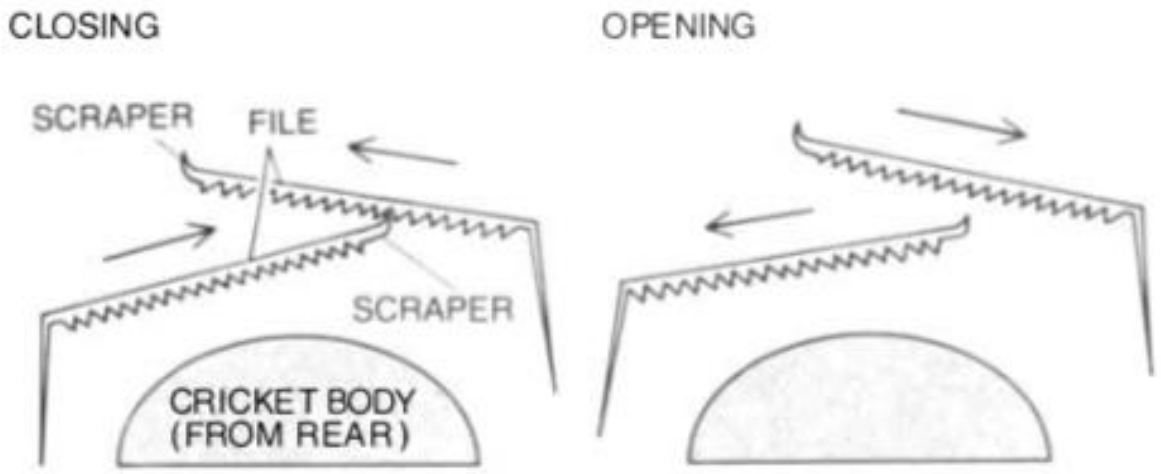


Figure S1.1: A stylized diagram of the components and movements of a male cricket used in order to produce courtship songs. (Franz & Thorson, 1983). The image shows the scraper and file which are the components producing sound. This occurs with the scraper rubbing against the striations on the file creating oscillations and sound as the wings open and close.

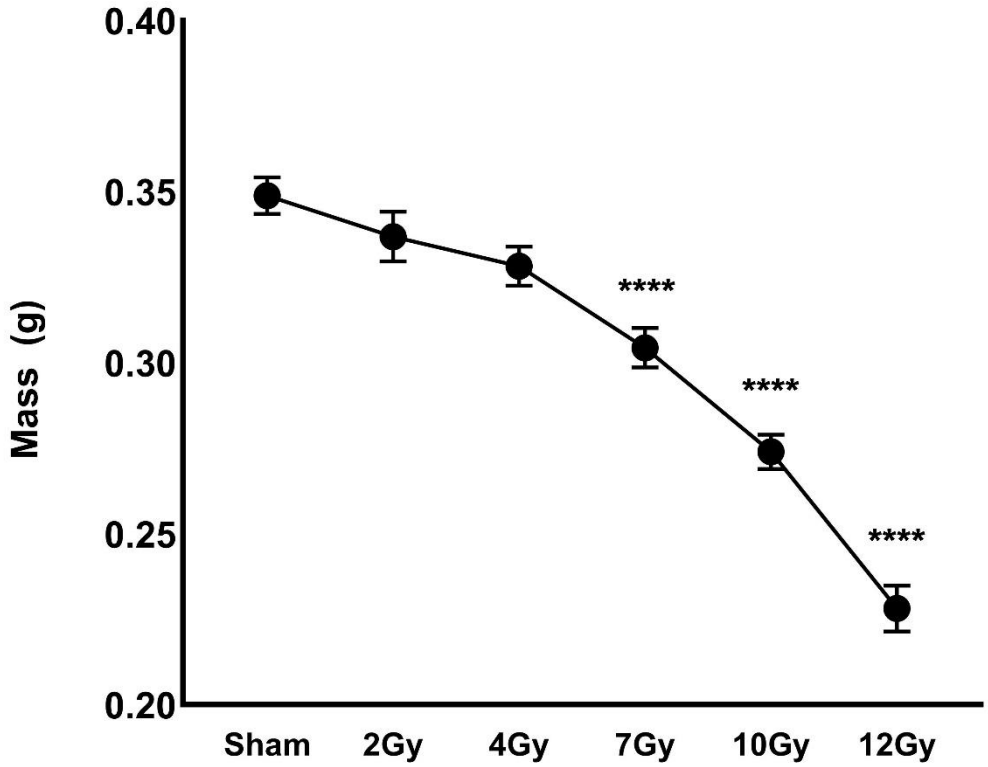


Figure S1.2: Dose-response effects of early juvenile radiation on maturation mass of F0 male *Acheta domesticus*. All crickets were irradiated (dose-rate = 0.25Gy/min) at 14 days old. Groups encompassed 0 (n=52), 2Gy (n=45), 4Gy (n=55), 7Gy (n=62), 10Gy (n=63), 12Gy (n=29). Values are represented as the mean of each radiation group +/- SEM with significant values (**** for $p<0.0001$). All groups were tested for normality prior to analysis. Significant differences were identified using a one-way ANOVA followed by a Tukeys HSD test comparing all groups to the 0Gy group.

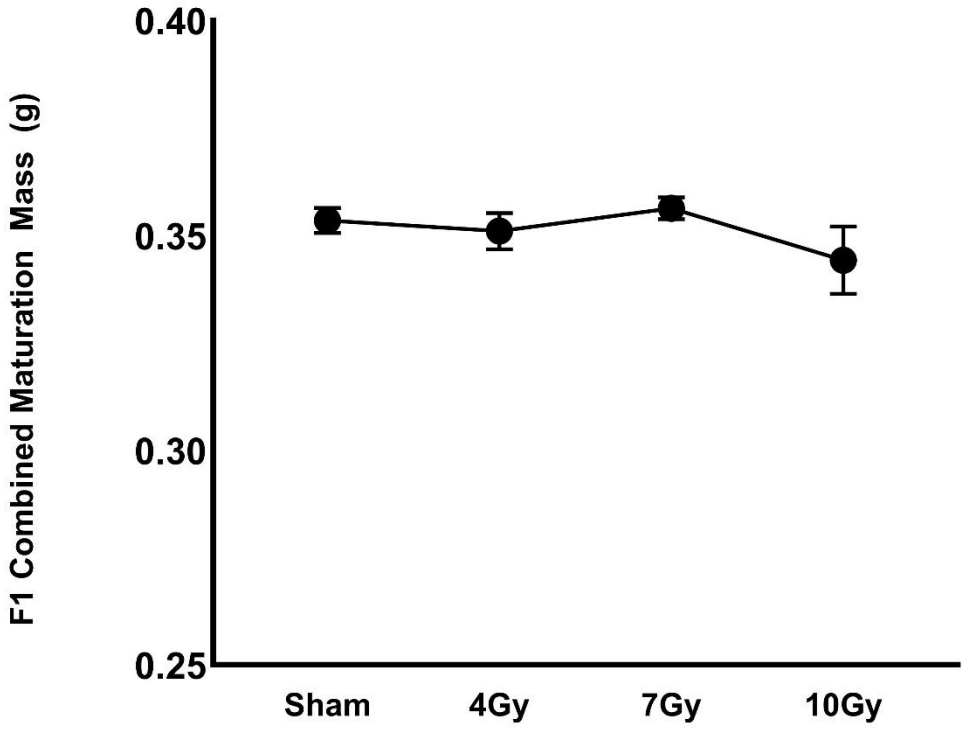


Figure S1.3: Dose-response effects of paternal early juvenile radiation on maturation mass of F1 male/female combined *Acheta domesticus*. Groups encompassed 0 (n=211), 4Gy (n=113), 7Gy (n=368), 10Gy (n=36). Values are represented as the mean maturation mass of each group +/- SEM. Groups were tested for normality prior to analysis. Significant differences were identified using a one-way ANOVA followed by a Tukey's HSD test comparing all groups to the 0Gy group. No significant differences were observed.

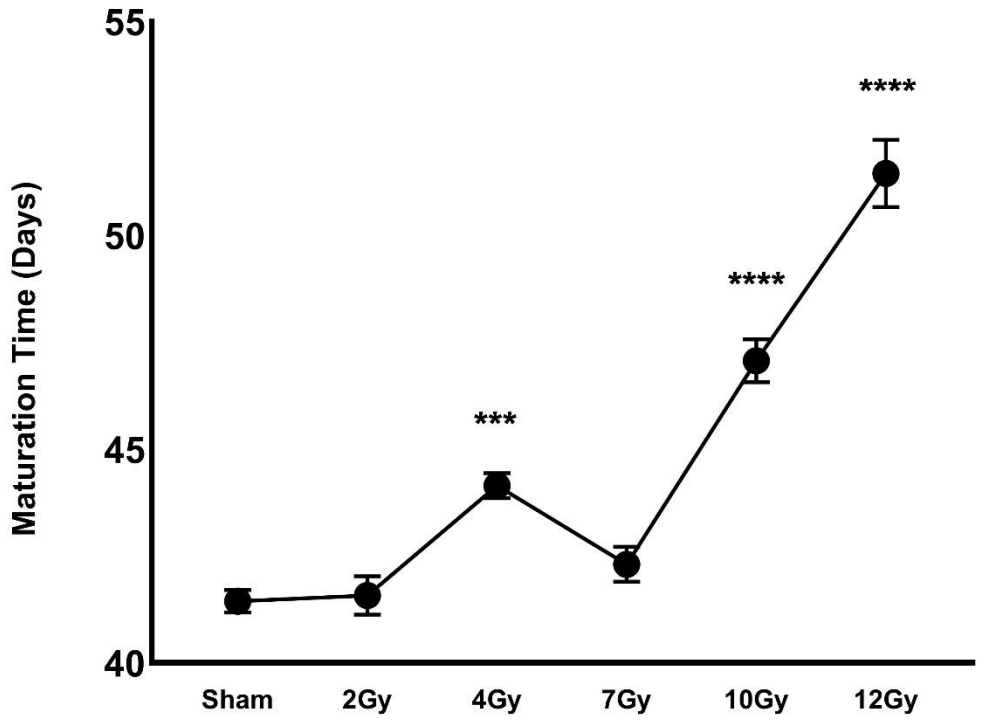


Figure S1.4: Dose-response effects of early juvenile radiation on maturation time of F0 male *Acheta domesticus*. All crickets were irradiated (dose-rate = 0.25Gy/min) at 14 days old. Groups encompassed 0 (n=52), 2Gy (n=45), 4Gy (n=55), 7Gy (n=62), 10Gy (n=63), 12Gy (n=29). Values are represented as the mean of each radiation group +/- SEM with significant values **** for $p<0.0001$ and *** $p = 0.0002$). All groups were tested for normality prior to analysis. Significant differences were identified using a one-way ANOVA followed by a Tukeys HSD test comparing all groups to the 0Gy group.

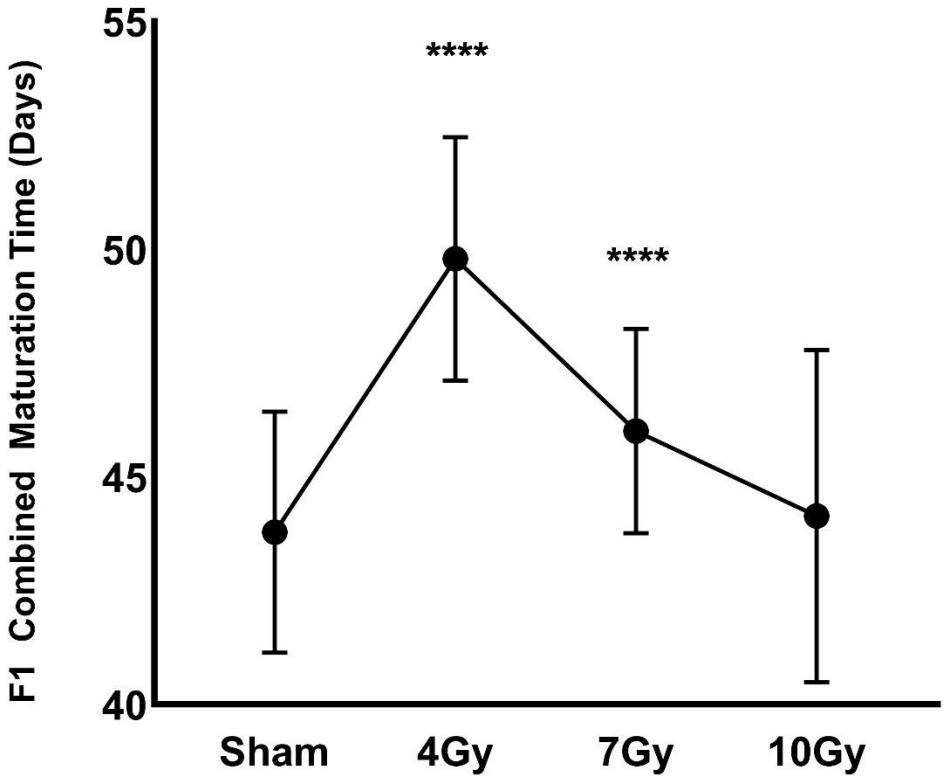


Figure S1.5: Dose-response effects of paternal early juvenile radiation on maturation time of F1 male/female combined *Acheta domesticus*. Groups encompassed 0 (n=211), 4Gy (n=113), 7Gy (n=368), 10Gy (n=36). Values are represented as the mean maturation time of each group +/- SEM with significant values (**** $p < 0.0001$). All groups were tested for normality prior to analysis. Significant differences were identified using a one-way ANOVA followed by a Tukey's HSD test comparing all groups to the 0Gy group.

a) b)

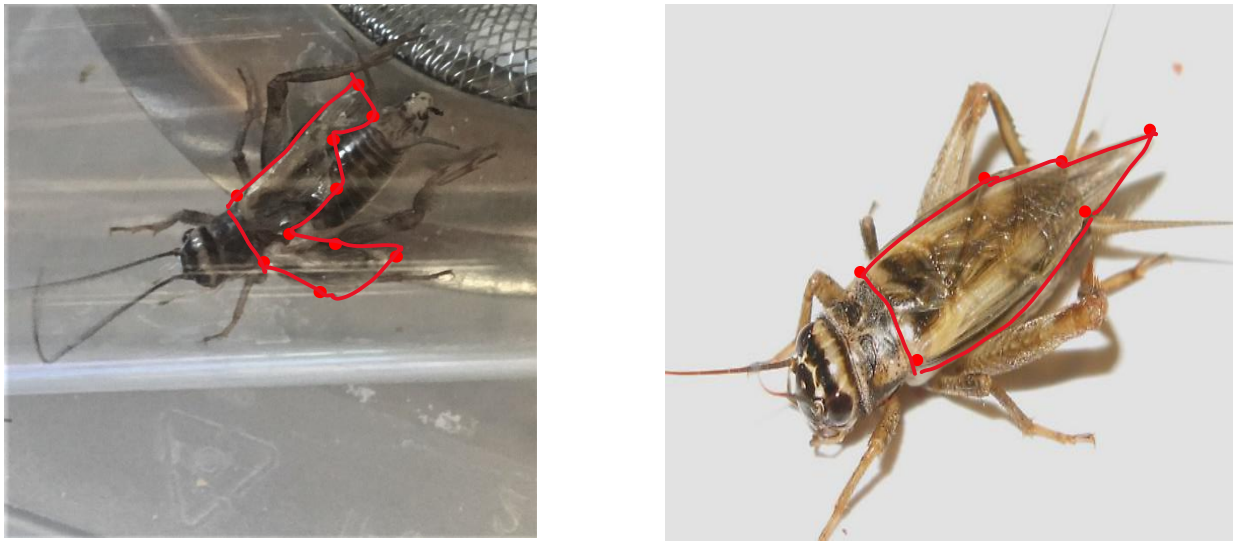


Figure S1.6: An example of a 12Gy cricket with malformed wings (a) and a control male cricket (b). The 12Gy male has shortened and broken wings, as indicated by its exposed body which is usually covered by wings (a). Highlighted in red is the general shape of the wings in both the 12Gy male and control. The malformation in the 12Gy male is obvious by the apparent separation of the two wing components compared to the control.

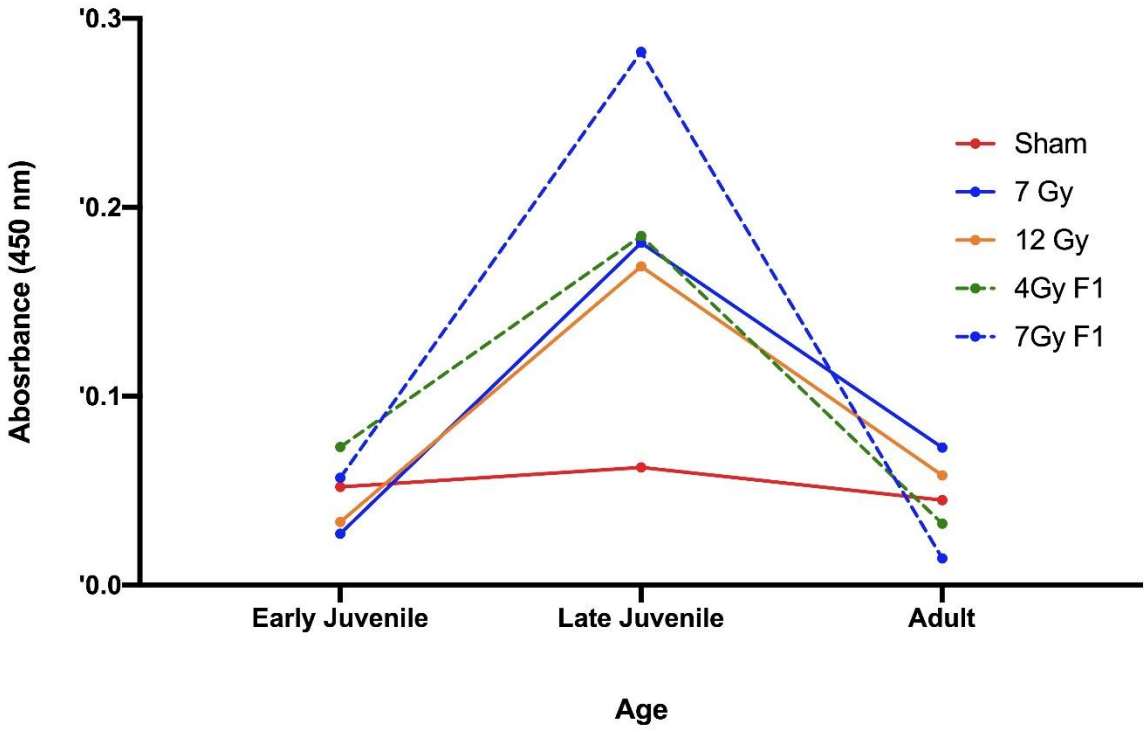


Figure S1.7: Effects of early life radiation on nuclear Phospho-FOXO3 (Ser253) from of crickets exposed to early life radiation at 14 days old and their offspring. F0 samples were obtained from radiation groups from 3 time points post radiation, early juvenile (6 hours, 1 Day, and 7 Days), late juvenile (26/27 Days), and adult (1 week post-maturation). F1 samples were taken at 3 time points as well, early juvenile (15 days), late juvenile (30 days), and adults (1 week post maturation). Values are shown as means of each age for each radiation group. Significant differences are not reported due to limited sample sizes.

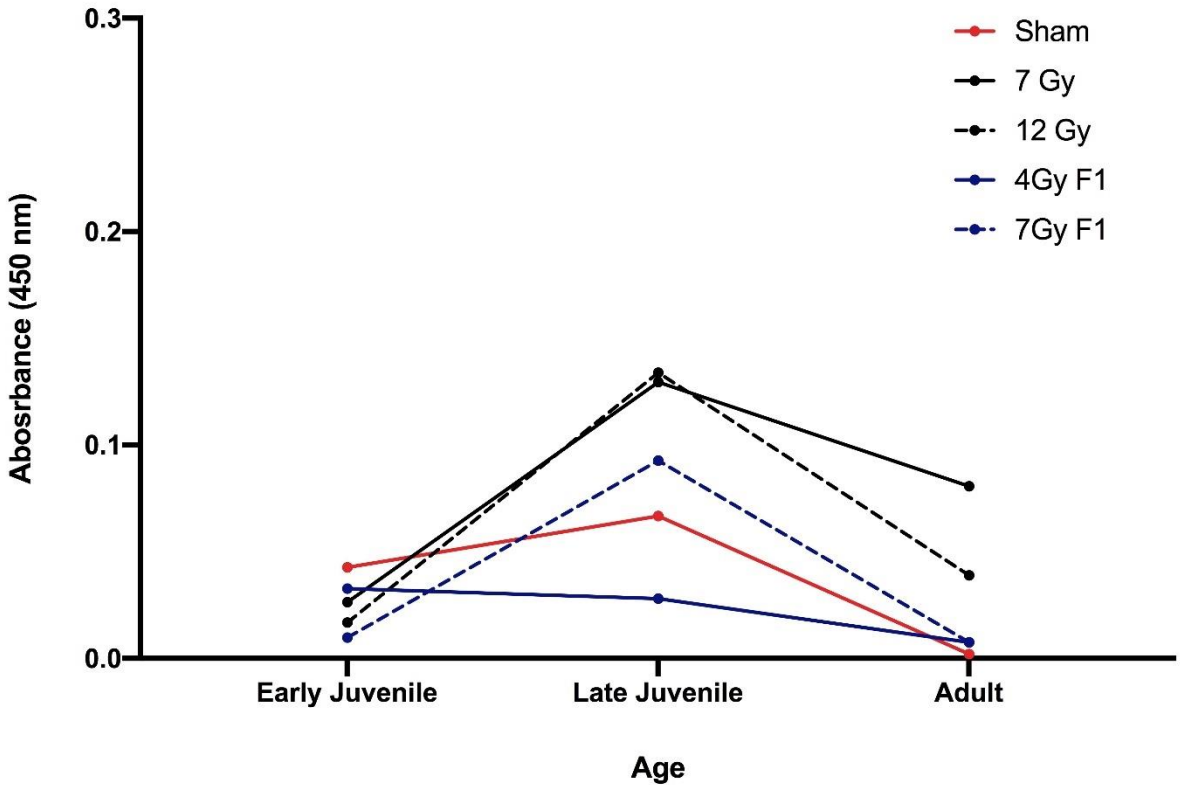


Figure S1.8: Effects of early life radiation on Cytosolic Phospho-FOXO3 (Ser253) from of crickets exposed to early life radiation at 14 days old and their offspring. F0 samples were obtained from radiation groups from 3 time points post radiation, early juvenile (6 hours, 1 Day, and 7 Days), late juvenile (26/27 Days), and adult (1 week post-maturation). F1 samples were taken at 3 time points as well, early juvenile (15 days), late juvenile (30 days), and adults (1 week post maturation). Values are shown as means of each age for each radiation group. Significant differences are not reported due to limited sample sizes.

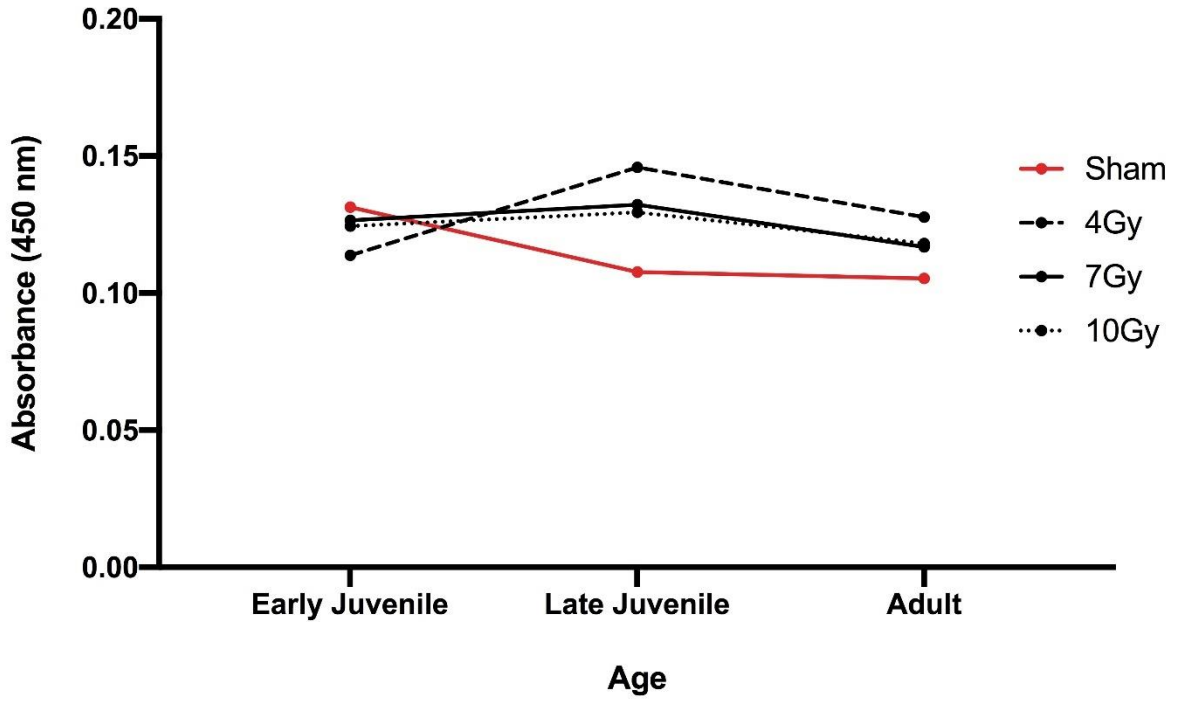


Figure S1.9: Effects of early life radiation on Nrf2 concentrations of crickets exposed to early life radiation at 14 days old. Samples were obtained from radiation groups from 3 time points post radiation, early juvenile (6hours, 1Day, and 7Days), late juvenile (26/27 Days), and adult (1 week post-maturation). Values are shown as means of each age for each radiation group. Significant differences are not reported due to limited sample sizes.

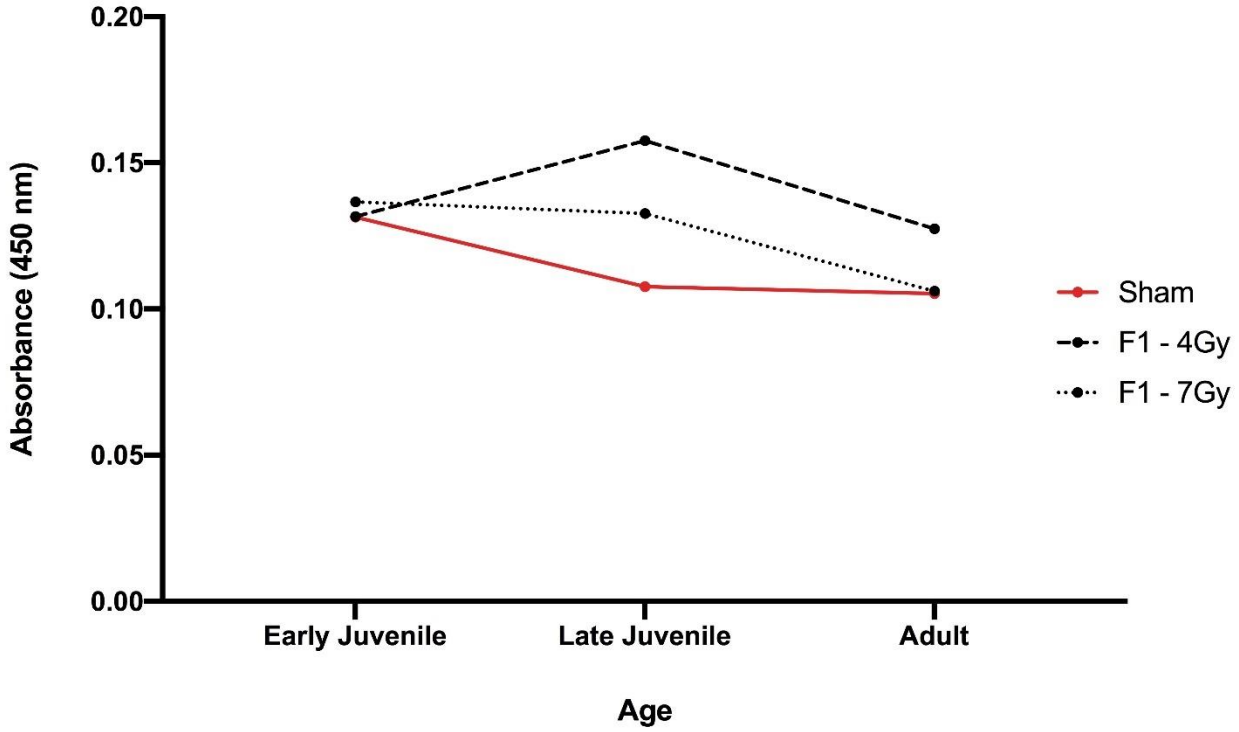


Figure S1.10: Effects of early life radiation on Nrf2 concentrations of the offspring of male crickets exposed to early life radiation at 14 days old. F1 samples were taken at 3 time points as well, early juvenile (15 days), late juvenile (30 days), and adults (1 week post maturation). Values are shown as means of each age for each radiation group. Significant differences are not reported due to limited sample sizes.