CONSEQUENCES OF STRESS ON DEVELOPMENT

THE IMPACTS OF IONIZING RADIATION ON INVERTEBRATE GROWTH, DEVELOPMENT, AND REPRODUCTION.

By TAMARA FUCIARELLI, MSc.

A Thesis Submitted to the School of Graduate Studies in Partial Fulfilment of the Requirements for the Degree Doctor of Philosophy

McMaster University © Copyright: Tamara Fuciarelli, July 2023

McMaster University DOCTOR OF PHILOSOPHY (2023)

1280 Main Street West, Hamilton, Ontario, L8S 4K1

TITLE: The impacts of ionizing radiation on invertebrate growth, development, and reproduction

AUTHOR: Tamara Fuciarelli, MSc. (McMaster University)

SUPERVISOR: Dr. C. David Rollo

NUMBER OF PAGES: xix, 211

ABSTRACT

Ionizing radiation (IR) as an environmental stressor has become a topic of great interest due to both nuclear disasters and other contamination sites. Although IR impacts on human and other mammalian species are well studied, much less is understood about impacts to invertebrate species. Here, I investigate the impacts of IR exposure on the House Cricket *Acheta domesticus,* with a focus on sexual signalling, generational impacts, and bystander effects. Initial experiments were conducted to determine the optimal living conditions for this species (< 0.93 cricket/cm^2) which were used in subsequent experiments. Mating is multifaceted in this species, males court females using chemical pheromone production and acoustic signaling. Males were shown to have IR induced changes to wing morphology/acoustic signaling and chemical pheromone production, ultimately resulting in reduced mating success. In females, similar alterations were observed due to IR exposure. Females were more susceptible to pheromone alternations, however males, likely due to the complexity of wing structures were shown to be more susceptible to wing alterations than females. Further experimentation aimed to examine the possibility of paternal and/or maternal inheritance of stress on various life-history features of F1 and F2 offspring. Results here were varied, with offspring for the most part recovering from F0 exposure. Finally, research was conducted to observe potential bystander effects; the impacts on life-history of exposure to irradiated individuals on non-irradiated individuals. Here, results indicated that bystander individuals can adjust their life history features in response to exposure to irradiated individuals. However, this work is preliminary as whole-body bystander effect research in insects is lacking. The results here indicate that although generally more radioresistant than mammals, insects can be profoundly affected by IR exposure. It is therefore prudent that further research in invertebrates, expanding on endpoints and species be conducted.

ACKNOWLEDGMENTS

There are so many people that I would like to thank for supporting me through my Ph.D. First, I would like to thank my committee, Dr. Faure, Dr. Mothersill, and Dr. Stone, as well as my supervisor Dr. C David Rollo. I could not have asked for better mentors. Committee meetings were always filled with great ideas and pathways to further my research and graduate experience. I also could not have appreciated more the freedom I was given to pursue not only the research questions I was interested in but also to pursue other interests throughout my time at McMaster. I hope our paths will cross in the future and thank you all again for your time and mentorship. I also wanted to give a special thanks to Susan Marsh-Rollo, you have supported me every step of the way through great advice and mentorship, its greatly appreciated.

I also wanted to thank my parents, for continuously supporting me through my 10+ years of post secondary education. I couldn't have done it without you, between consistently giving me rides to school, to editing my manuscripts and assignments, to listening to me contemplate why I'm still in school. You have always been in my corner, and I attribute all my current success to your support and guidance through the years. Love you both. I also wanted to thank Klavdiy, you are my rock, I don't know if I could have made it through all the bumps and turns without your love and support. Thank you for always being there and being whatever I needed.

Finally, I wanted to thank all the volunteers, thesis students, and lab techs that have been apart of our lab throughout my graduate studies. There have been many whose great work have contributed to the publications within this thesis. A big thank you to Xiaobing, thank you for all your help in experiment design, undergraduate projects, writing and editing my work, and covering for me when I'm away. It was greatly appreciated.

TABLE OF CONTENTS

List of Figures and Tables ……………………………………………………………………viii

List of Abbreviations ………………………………………………………………...…xiii

Declaration of Academic Achievement……………………………………………………....xiv

Preface…………………………………………………………………………………..……xix

Introduction……………………………………………………………………………………..2

References (for introduction)…………………………………………………………………10

Chapter 1: ***Radiation exposure causes developmental alterations in size and shape of wings and structures associated with song production in male crickets (Acheta domesticus)***
1.1 Introduction……………………………………………………………………………..13
1.2 Methods……………………………………………………………………………..……..16
1.3 Results……………………………………………………………………………………..20
1.4 Discussion…………………………………………………………………..………..21
1.5 Acknowledgements……………………………………………………………………..24
1.6 Figures and Tables…………………………………………………….………………..24
1.7 References ……………………………………………………………………………...30

Chapter 2: ***Impacts of ionizing radiation on the cuticular hydrocarbon profile and mating success of male house crickets (Acheta domesticus).***
2.1 Introduction……………………………………………………...…….…………..33
2.2 Methods……………………………………………………..…………………..35
2.3 Results……………………………………………………………………………………..38
2.4 Discussion…………………………………………………………………..………..39
2.5 Acknowledgements……………………………………………………………………..42
2.6 Figures and Tables…………………………………………………….…………..42
2.7 References ……………………………………………………………………………...48

Chapter 3: ***Ionizing radiation alters male Acheta domesticus courtship songs that are critical for mating success***
3.1 Introduction……………………………………………………………………………..51
3.2 Methods…………………………………………………………………………..……..55
3.3 Results……………………………………………………………………………………..60
3.4 Discussion…………………………………………………………………..………..62
3.5 Acknowledgements……………………………………………………….……………66
3.6 Figures and Tables…………………………………………………….………………..67
3.7 References ……………………………………………………………………………...71

Chapter 4: ***Differential impacts of ionizing radiation on a sexually dimorphic trait in male and female Acheta domesticus.***
4.1 Introduction……………………………………………………………………………..75

4.2 Methods……………………………………………………………………..…..………..78
4.3 Results…………………………………………………………………………………..83
4.4 Discussion……………………………………………………………………….…..86
4.5 Acknowledgements……………………………………………………………………..91
4.6 Figures and Tables…………………………………………………….……….92
4.7 References ……………………………………………………………………………...99

Chapter 5: ***Cuticular hydrocarbons of female house crickets (Acheta domesticus): Identification, sexual dimorphism, and stress induced impacts***
5.1 Introduction……………………………………………………………………..…..103
5.2 Methods……………………………………………………………………...…..106
5.3 Results……………………………………………………………………………..…..109
5.4 Discussion……………………………………………………………………………..110
5.5 Acknowledgements………………………………………………...…………………114
5.6 Figures and Tables…………………………………………………..……………………114
5.7 References ………………………………………………………...…………………..119

Chapter 6: ***Trans-generational paternal and maternal effects of ionizing radiation exposure on life-history features of Acheta domesticus***
6.1 Introduction………………………………………………………………..………..122
6.2 Methods…………………………………………………………………………………..128
6.3 Results…………………………………………………………………………….…..134
6.4 Discussion……………………………………………………………………………..137
6.5 Acknowledgements……………………………………………………………………143
6.6 Figures and Tables…………………………………………………………...…..144
6.7 References ……………………………………………………………….………...157
6.8 Appendix………………………………………………………………….………..163

Chapter 7: ***Radiation induced bystander effects on the life-history features of the House Cricket Acheta domesticus***
7.1 Introduction…………………………………………………………..…………..164
7.2 Methods……………………………………………………………………...……..168
7.3 Results…………………………………………………………………...…….…..171
7.4 Discussion……………………………………………………………………………..172
7.5 Acknowledgements…………………………………………………..…...……..……177
7.6 Figures and Tables……………………………………………………………….....178
7.7 References ……………………………………………………………………..……...182
7.8 Appendix………………………………………………………………………………185

Chapter 8: ***The effects of rearing density on growth, survival, and starvation resistance of the house cricket Acheta domesticus***
8.1 Introduction……………………………………………………………………..…..186
8.2 Methods………………………………………………………………………………..190
8.3 Results……………………………………………………………..…………..194
8.4 Discussion……………………………………………………………………………..195
8.5 Acknowledgements……………………………………………………………………200

8.6 Figures and Tables…………………………………………………………………….....200
8.7 References ………………………………………………………………………..…...206

Conclusions ……………………………………………………………………………...….210

LIST OF TABLES AND FIGURES

TABLES

Chapter 1

Table 1: A procustes ANOVA for centroid size (µm) and shape (Procrustes distance) on *Acheta domesticus* wing analysis.

Table 2: Procrustes ANOVA for fluctuating asymmetry in *Acheta domesticus* wings exposed to 0 (control), 4.6, 16.2, or 23.2 Gy radiation.

Chapter 2

Table 1: Two-way ANOVA results representing the relative impact of error, dose, and peak on *Acheta domesticus* cuticular hydrocarbon analysis.

Table 2: Summary of GC results for cuticular hydrocarbon peaks. Concentration for Control males, calculated as area under each peak, with % change from control (4.6-27.8Gy).

Table 3: Summary of GC/MS results for male cuticle profile with likely compound candidates using a pooled sample including individuals from 0–27.8Gy.

Chapter 4

Table 1: Procrustes ANOVA on shape for fluctuating asymmetry in male and female *Acheta domesticus* wings exposed to 0 (control), 0.58Gy, 2.3Gy, 4.6Gy, 16.2Gy, 23.2Gy, and 27.8Gy ionizing radiation.

Table 2: A procustes ANOVA for centroid size (µm) and shape (Procrustes distance) on *Acheta domesticus* wing analysis.

Table 3: Two-way ANOVA results representing the relative impact of error, dose, and sex on *Acheta domesticus* wing fluctuating asymmetry.

Chapter 5

Table 1: Two-way ANOVA results representing the relative impact of dose, peak, and interaction effects on female *Acheta domesticus* cuticular profile.

Table 2: Summary of GC results for female cuticular profile (0-27.8Gy). Significant differences from control are shown with % change from the control.
Table 3: Summary of GC/MS results for the female cuticle profile with likely compound candidates.

Chapter 6
Table 1: Summary of survival and growth data of F0 control and irradiated *Acheta domesticus,* F1 and F2 male and female offspring.

Chapter 7
Table 1: Summary of life-history (growth, longevity, survival) for *Acheta domesticus* controls, irradiated (0.58Gy, 2.3Gy, 16.2Gy), and bystander groups.

Chapter 8
Table 1: Summary of growth parameters of each *Acheta domesticus* rearing density group.
Table 2: Multiple linear regression analysis with AICc comparison was used to determine the most correct model for predicting growth rate based on sex and density group.
Table 3: Multiple linear regression analysis with AICc comparison was used to determine the most correct model for predicting survival from sex and density group.

FIGURES

Introduction
Figure 1: Image of a typical male and female of our model organism the House Cricket *Acheta domesticus.*

Chapter 1
Figure 1: Position of 11 landmarks (red dots) superimposed on a photograph of a control *Acheta domesticus* wing.
Figure 2: Principal component (PC) analysis of *Acheta domesticus* wing shape.

Figure 3: Canonical variate (CV) analysis of *Acheta domesticus* wing shape between control and radiation groups.
Figure 4: Centroid size (µm) of control and irradiated *Acheta domesticus* male wings.

Chapter 2
Figure 1: A typical total ion chromatogram of a control male *Acheta domesticus* extracted using a hexane solvent at 1 week post maturation (~50 days).
Figure 2: Dose-response effects of early life radiation on cuticular hydrocarbons, specifically the effect on each of the 26 significant hydrocarbon peaks identified using gas-liquid chromatography.
Figure 3: Effects of early life radiation on male *Acheta domesticus* mating success.

Chapter 3
Figure 1: The courtship song of a control male house cricket, *Acheta domesticus*, displayed using Raven Pro software.
Figure 2: . Impacts to courtship song parameters of high-frequency chirps of male *Acheta domesticus* exposed to ionizing radiation (0–27.8 Gy).
Figure 3: Impacts to courtship song parameters of low-frequency trills of male *Acheta domesticus* exposed to ionizing radiation (0–27.8 Gy).
Figure 4: Impacts of radiation (0–27.8 Gy) on male *Acheta domesticus* mating parameters.

Chapter 4
Figure 1: : Position of landmarks (white dots) superimposed on a photograph of a control male (a) and female (b) *Acheta domesticus* wing.
Figure 2: Principal component (PC) analysis of male and female *Acheta domesticus* wing shape. Scatterplots for both male (a) and female (b) wing shape.
Figure 3: Canonical variate (CV) analysis of wing shape variation between control and radiation groups in male (a) and female (b) *Acheta domesticus*.
Figure 4: Centroid size of control and irradiated male (a) and female (b) *Acheta domesticus* wings.

Figure 5: Fluctuating asymmetry for male and female *Acheta domesticus* exposed to IR 0-27.8Gy at 14 days of age.

Chapter 5

Figure 1: A typical total ion chromatogram of a control female *Acheta domesticus* extracted using a hexane solvent at a 5ml:0.5g hexane to cricket ratio.

Figure 2: Dose-response effects of early life radiation on the cuticular profile on the identified 20 peaks for female *Acheta domesticus*.

Chapter 6

Figure 1: Kaplan-Meier survival curves for the F0 population of control and irradiated (0.58 – 27.8Gy) male and female *Acheta domesticus.*

Figure 2: Impacts of ionizing radiation exposure (0-27.8Gy) on the growth rate of male and female *Acheta domesticus.*

Figure 3: Impacts of ionizing radiation exposure (0.58 – 27.8Gy) on male and female *Acheta domesticus* longevity.

Figure 4: Kaplan-Meier survival curves for the F1 population of offspring from paternal (a) and maternal (b) control and irradiated (0.58 – 27.8Gy) lines of *Acheta domesticus.*

Figure 5: Growth rates (mg/day) of *Acheta domesticus* for both paternally (a) and maternally (b) derived F1 male and female offspring.

Figure 6: Impacts of ionizing radiation exposure (0.58 – 27.8Gy) on F1 male and female *Acheta domesticus* derived from paternal (a) and maternal (b) F0 parental lines.

Figure 7: Kaplan-Meier survival curves for the F2 populations of offspring from paternal (a) and maternal (b) F1 groups of *Acheta domesticus.*

Figure 8: Growth rates (mg/day) of *Acheta domesticus* for both paternally (a) and maternally (b) derived F2 male and female offspring.

Figure 9: Longevity (days) of *Acheta domesticus* for both paternally (a) and maternally (b) derived F2 male and female offspring

A1: Visual representation of the methodology applied to generate the paternal and maternal F1 and F2 generations of *Acheta domesticus*.

Chapter 7

Figure 1: Impacts of ionizing radiation and RIBE on the growth rate of male and female *Acheta domesticus.*

Figure 2: Impacts of ionizing radiation and RIBE on male and female *Acheta domesticus* survivorship.

Figure 3: Impacts of ionizing radiation and RIBE on *Acheta domesticus* longevity.

A1: Diagram of experimental setup for each RIBE trial. Three

Chapter 8

Figure 1: The proportion of *Acheta domesticus* reaching maturation in each rearing density (0.09, 0.19, 0.47, and 0.93 cricket/cm^2).

Figure 2: Density-dependent effects of various rearing densities on the growth rate of male and female *Acheta domesticus*

Figure 3: Mass of adult *Acheta domesticus* used for starvation treatment from each density group.

Figure 4: Starvation resistance of adult *Acheta domesticus* reared at various densities.

LIST OF ABBREVIATIONS

IR: Ionizing Radiation

SIT: Sterile Insect Technique

ROS: Reactive Oxygen Species

NTE: Non-Targeted Effects

LNT: Linear-no-Threshold

TH: Threshold

RIBE: Radiation Induced Bystander Effect

Gy: Short for the unit of radiation exposure "Gray"

ANOVA: Analysis of Variance

DECLARATION OF ACADEMIC ACHIEVEMENT

INTRODUCTION and CONCLUSION: Unless otherwise referenced all ideas expressed are of **Tamara Fuciarelli**[1].

CHAPTER 1: *[Radiation exposure causes developmental alterations in size and shape of wings and structures associated with song production in male crickets (Acheta domesticus)]*

Breeding and husbandry of crickets were preformed by **Tamara Fuciarelli**[1]**,** as well as **Xiaobing Li**[1]**,** and **Fergus O'Conner**[1]. Experimental design and methodology were completed by **Tamara Fuciarelli** with the guidance of **Dr. Ian Dworkin**[1], and **Dr. C David Rollo**[1]. Irradiation of experimental groups was completed by **Tamara Fuciarelli.** Data collection, including wing dissections, microscopy, and landmarking was conducted by **Tamara Fuciarelli.** Data analysis and interpretation using MorphoJ was conducted by **Tamara Fuciarelli** with the guidance of **Dr. Ian Dworkin.** The manuscript and publication process were completed by **Tamara Fuciarelli** with the aid of **Dr. C David Rollo.**

CHAPTER 2: [*Impacts of ionizing radiation on the cuticular hydrocarbon profile and mating success of male house crickets (Acheta domesticus).*]

Breeding and husbandry of crickets were preformed by **Tamara Fuciarelli,** as well as **Xiaobing Li,** and **Fergus O'Conner**. Experimental design and methodology were completed by **Tamara Fuciarelli** with the guidance of **Dr. C David Rollo, Dr. Kirk Green**[2] and **Dr. Fan Fei**[2].

[1] Department of Biology, McMaster University, 1280 Main Street West, Hamilton, ON, Canada, L8S 4L8.
[2] Department of Chemistry, McMaster University, 1280 Main Street West, Hamilton, ON, Canada, L8S 4M1.

Irradiation of experimental groups was completed by **Tamara Fuciarelli.** Hydrocarbon extraction and sample preparation were conducted by **Tamara Fuciarelli.** Hydrocarbon samples were analyzed with the assistance of the McMaster Regional Center for Mass spectrometry. Data interpretation was conducted by **Tamara Fuciarelli** with the guidance of **Dr. Kirk Green** and **Dr. Fan Fei**. The manuscript and publication process were completed by **Tamara Fuciarelli** with the aid of **Dr. C David Rollo.**

CHAPTER 3: [*Ionizing radiation alters male Acheta domesticus courtship songs that are critical for mating success*]

Breeding and husbandry of crickets were preformed by **Tamara Fuciarelli,** as well as **Xiaobing Li,** and **Fergus O'Conner**. Experimental design and methodology were completed by **Tamara Fuciarelli** with the guidance of both **Dr. C David Rollo** and **Dr. Paul Faure**[3]. Irradiation of experimental groups was completed by **Tamara Fuciarelli.** Acoustic recordings, data analysis, and interpretation was conducted by **Tamara Fuciarelli** with the guidance of **Dr. Paul Faure** and **Dr. C David Rollo.** Mating trials and analysis concerning all reproductive endpoints were conduced by **Tamara Fuciarelli.** The manuscript writing and publication process were completed by **Tamara Fuciarelli** with the aid of **Dr. C David Rollo.**

CHAPTER 4: [*Differential impacts of ionizing radiation on a sexually dimorphic trait in male and female Acheta domesticus.*]

Breeding and husbandry of crickets were preformed by **Tamara Fuciarelli,** as well as **Xiaobing Li,** and **Jeffrey Xu**[4]. Experimental design and methodology were completed by **Tamara**

[3] Department of Psychology, Neuroscience and Behaviour, McMaster University, Hamilton, ON L8S 4K1, Canada
[4] Department of Biology, McMaster University, 1280 Main Street West, Hamilton, ON, Canada, L8S 4L8.

Fuciarelli with the guidance of **Dr. C David Rollo.** Irradiation of experimental groups was completed by **Tamara Fuciarelli.** Wing dissections for both male and female crickets were conducted by **Tamara Fuciarelli** and **Selvi Patel**[1]. Wing preparation and microscopy was conducted by **Selvi Patel**. Subsequent landmarking on MorphoJ, statistical analysis, and subsequent data interpretation was done by **Tamara Fuciarelli** and **Selvi Patel**. The manuscript writing and publication process were completed by **Tamara Fuciarelli** with the aid of **Dr. C David Rollo.**

CHAPTER 5: [*Cuticular hydrocarbons of female house crickets (Acheta domesticus): Identification, sexual dimorphism, and stress induced impacts*]

Breeding and husbandry of crickets were preformed by **Tamara Fuciarelli,** as well as **Xiaobing Li,** and **Jeffrey Xu**. Experimental design and methodology were done by **Tamara Fuciarelli** with the guidance of **Dr. C David Rollo,** and **Dr. Kirk Green**. Irradiation of experimental groups was completed by **Tamara Fuciarelli.** Hydrocarbon extraction and sample preparation were completed by **Selvi Patel.** Collected hydrocarbon samples were analyzed with the assistance of the McMaster Regional Center for Mass spectrometry. Data analysis interpretation was conducted by **Tamara Fuciarelli** with the guidance of **Dr. Kirk Green**. The manuscript and publication process were completed by **Tamara Fuciarelli** with the aid of **Dr. C David Rollo.**

CHAPTER 6: [*Trans-generational paternal and maternal effects of ionizing radiation exposure on life-history features of Acheta domesticus*]

Breeding and husbandry of crickets were preformed by **Tamara Fuciarelli,** as well as **Xiaobing Li,** and **Jeffrey Xu**. Experimental design and methodology were done by **Tamara Fuciarelli**

with the guidance of **Dr. C David Rollo.** Irradiation of experimental groups was completed by **Tamara Fuciarelli.** The generation of F1 and F2 maternal and paternal lines were produced by **Tamara Fuciarelli.** Data collection and analysis including the monitoring of growth, survival, and longevity endpoints were conducted by **Tamara Fuciarelli** with the help of **Xiaobing Li.** Writing of experimental results into manuscript form and editing was done by **Tamara Fuciarelli** with the help of **Dr. C David Rollo.**

CHAPTER 7: [*Radiation induced bystander effects to life-history features in the House Cricket Acheta domesticus*]

Breeding and husbandry of crickets were preformed by **Tamara Fuciarelli,** as well as **Xiaobing Li,** and **Jeffrey Xu**. Experimental design and methodology were completed by **Tamara Fuciarelli** with the guidance of **Dr. C David Rollo** and **Dr. Mothersill**[5]. Irradiation of experimental groups was completed by **Tamara Fuciarelli.** Data collection including the daily monitoring of mortality and growth was completed by **Tamara Fuciarelli.** Analysis of all results and statistical analysis was completed by **Tamara Fuciarelli.** Writing of experimental results into manuscript form and editing was done by **Tamara Fuciarelli** with the help of **Dr. C David Rollo.**

CHAPTER 8: [*The effects of rearing density on growth, survival, and starvation resistance of the house cricket Acheta domesticus*]

Breeding and husbandry of crickets were preformed by **Tamara Fuciarelli,** as well as **Xiaobing Li,** and **Jeffrey Xu**. Experimental design and methodology were done by **Tamara Fuciarelli**

[5] Department of Medical Physics and Applied Radiation Science Unit at McMaster University, Nuclear Research Building, 1280 Main Street West, Hamilton, Ontario, Canada, L8S4KI.

with the guidance of **Dr. C David Rollo.** Data collection including the separation of groups into various densities and monitoring them for daily growth and mortality parameters was completed by **Siyumi Mahavidanage**[6] with the help of **Tamara Fuciarelli.** Data analysis including statistical analysis and graphing was completed by **Siyumi Mahavidanage** with the help of **Tamara Fuciarelli.** The manuscript and publication process were completed by **Tamara Fuciarelli** with the aid of **Dr. C David Rollo.**

[6] Department of Biology, McMaster University, 1280 Main Street West, Hamilton, ON, Canada, L8S 4L8.

PREFACE

Dear reader,

This thesis is prepared in a “sandwich” format with all chapters being written in journal article format. Each chapter is written as a stand-alone piece with its own introduction assuming no prior knowledge of other chapters. There is a brief introduction and conclusion which summarizes the subsequent chapters and their relevance.

All figures, references, and appendices for each chapter are present at the end of each chapter to allow for easier access for the reader. Chapters 1-4, and 8 are represented *verbatim* from published articles. Copyright information is presented on the first page of each of these chapters. All citations and page formatting were homogenized to fit the overall presentation of this thesis.

Chapters are structured as either published papers or prepared manuscripts and therefore there is an in-depth introduction presented at the beginning of each chapter. Therefore, to minimize repetition, a general introduction and conclusion are included at the beginning and end of this thesis.

INTRODUCTION

When compared to vertebrates, invertebrates have been largely understudied in the field of radiobiology. As well, what has been researched focuses mainly on sterilization and eradication methods on a few "pest" species through the sterile insect technique (SIT). This work therefore aims to both expand on the impacts of exposure on a less studied species as well as elucidate the effects on more subtle endpoints that may be relevant to organisms living within environmental contamination sites as well as potential unintended consequences of the SIT. Here I examine the effects of early life irradiation on sexual signaling, mating, trans-generational impacts, and radiation induced bystander effects. There is also a stand-alone chapter investigating optimal densities for rearing our model *Acheta domesticus*. Each individual chapter has its own introduction covering relevant background information. This introduction will therefore give a general background on ionization radiation as a stressor, the current theories and models of exposure, as well as the generally accepted consequences of exposure and current competing theories. I will also outline our model, current gaps in research, and how my current work aims to expand on our current understanding of IR in invertebrate species.

Ionizing Radiation Models and Theories

The historically accepted theory which has dominated from the early 1950's to 1990's that is associated with IR damage and organismal response is known as target theory (Mothersill and Seymour, 2014). Target theory posits that IR damages cells when ionizing particles hit a defined target within the cell (Mothersill and Seymour, 2014). Within this framework damage can occur either directly; through the direct interaction between ionizing particles and

macromolecules but also indirectly; through the interaction between ionizing particles and water molecules, which then proceed to interact with and damage macromolecules (Desouky et al., 2015). As the cell encompasses more then 70% water, it is this indirect damage which likely predominate under target theory (Desouky et al., 2015).

Indirect damage occurs due to the highly reactive molecules which are created when these ionizing particles react with water. These molecules are classified as reactive oxygen species (ROS) and include superoxide, hydrogen peroxide, hydroxyl radicals and several other highly reactive molecules. Under normal conditions these ROS's are actually produced naturally, typically by three cellular compartments: mitochondria, endoplasmic reticulum, and the cytosol (Szumiel, 2015). As well ROS's act as important cellular molecules involved in cell signalling, cell division, immunity, and stress response (Riley, 1993; Einor et al., 2016). These molecules are tightly regulated by the cell, through both compartmentation, antioxidant defences, and enzyme systems (Riley, 1993). However, when exposure to ROS exceeds cellular tolerance, oxidative stress is induced, and damage can occur (Szumiel, 2015). Certain extraneous exposures including from chemicals, the environment, pathogens, and ionizing radiation exposure can increase levels of ROS's to these harmful levels (Szumiel, 2015).

Non targeted Effects and Radiation Induced Bystander Effects (RIBE)

The target theory of radiation exposure has dominated for many years as a relatively simple view of cellular responses to IR. However, this view has been increasingly challenged by data supporting a non-targeted effects (NTE) of IR exposure (Szumiel, 2015). NTE describes the phenomena where irradiated cells can convey damage to other, nonirradiated cells (Morgan, 2003; Desouky et al., 2015). NTE include phenomena such as the radiation induced bystander

effect (RIBE), genomic instability, and adaptive responses (Szumiel, 2015). A NTE of particular interest which will be discussed through this thesis is the radiation induced bystander effect. RIBE describes the signal mediated transfer of biological effects from an irradiated cell to a non-irradiated cell (Mothersill & Seymour, 2001; Aberbeck, 2009). Biological effects due to RIBE have been described largely in cellular models but recent studies have also indicated that RIBE can occur on and organismal level and has been shown in both mice and fish (Surinov et al., 2004; Mothersill et al., 2006). The mechanism for RIBE however is not fully elucidated but likely involves a combination of soluble factors, gap junction communication, and physical signalling (Azzam et al., 2001; Aberbeck, 2009; Desouky et al., 2015; Li et al., 2023). Although target and non-target theory seem contradictory, it is likely that NTE's will dominate at low doses where targeted effects are small, and that at high doses targeted effects will dominate where NTE's are inhibited or prevented (Mothersill and Seymour, 2014).

Ionizing Radiation Exposure and General Impacts

Ionizing radiation research has become an area of general importance for research due to its use in nuclear power generation and pest control as well as the accidental release of radionuclides during environmental disasters i.e., Chernobyl (1986) and Fukushima (2011) (Imanaka et al., 2015; Dyck et al., 2021). Given its importance, research has been conducted on IR impacts on a variety of species, although this has mostly focused on vertebrates. The generally accepted impacts of higher dose IR exposure include on a cellular level, chromosomal aberrations, mutations, cell death, DNA damage, and cancer (Little, 2003; Desouky et al., 2015). On an organismal level, high dose IR exposure has been shown to have severe negative impacts to survival, growth and reproduction (Sarapultseva and Dubrova, 2016; Fuciarelli & Rollo,

2020). As well, behavioural, morphological, and developmental impacts have also been described in several species (Mousseau and Moller, 2014). On an ecosystem level, impacts to species abundance and diversity within contaminated environments has also been observed (Mousseau and Moller, 2014). However, the strength of response and the dose required to achieve it is highly species specific, as well as dose-rate, age, and sex specific (ICRP, 2008). It is therefore highly prudent to have a diversity of data on both the species used as well as other parameters to obtain a full understanding of IR impacts. Endpoints of particular interest which are investigated throughout this thesis include paternal and maternal generational effects as well as endpoints associated with sexual signaling and mating.

Radiation Modeling and Hormesis

Modeling radiation exposure risk can be described under three models: the linear-no-threshold (LNT), threshold (TH), and hormetic model. Which of the three is the correct model is a hotly debated topic in radiobiology. Although most pharmaceuticals and chemicals are regulated using a TH dose-response model; where dose above a certain threshold will produce a biological response, the predominate model for radiation exposure is the LNT model (Vaiserman, 2010; Calabrese, 2012; Doss, 2013). The LNT model postulates that the relationship between carcinogenic effects and dose is linear, that as dose increases effects increases (Doss, 2013). This model therefore predicts that all dose is harmful. However, current research has argued that a LNT model does not accurately represent the observed data, specially in the low dose range. Instead, researchers have suggested a biphasic model or hormetic model, where the response at low doses is opposite to that at higher doses (Calabrese, 2012). The hormetic model is described by the beneficial or stimulatory response at low dose exposure while at high dose exposure

increasing detrimental effects are expected (Mothersill and Seymour, 2014). Hormetic responses have been shown to occur in studies spanning bacteria to vertebrates for over 1000 environmental stressors (Constantini et al, 2010; Vaiserman, 2010; Calabrese, 2013). Potential hormetic responses were investigated throughout the experiments presented here.

Invertebrates and Ionizing Radiation

Despite invertebrates encompassing 90% of animal life on Earth, IR impacts on invertebrate species are poorly characterised (ICRP, 2008; Dallas et al., 2012). Insects are a group of particular interest as they fulfill important roles in supporting ecosystem functioning i.e., as food sources for other species, pollinators, and as decomposers (Didham et al., 1996). However, IR research in insects has largely focused on sterilization endpoints associated with the SIT (Bakri et al., 2005; Beasley et al., 2012). SIT focused research however encompasses only species that are considered "pests" to humans i.e., those that are agricultural pests or disease vectors. This limits our knowledge of IR impacts in insects to just a few species. As well, the endpoints categorized often focus on solely male sterilization and other endpoints associated with population control. This lack of data is clearly displayed in the ICRP (2008) report in which gaps in knowledge along several non-reproductive endpoints are evident. This report also highlights the need for species and endpoint specific data. For example, the LD_{50} for adult insects ranges from 20 – 3000Gy (ICRP, 2008). This is an enormous amount of species variation of radiation tolerance. As well, in some species low doses can cause damage at more vulnerable stages in the insect life cycle. This sensitivity is clearly apparent in ecological studies in areas surrounding Chernobyl and Fukushima where increased morphological abnormalities,

generational impacts, and reduced abundance and diversity has been described (Moller and Mousseau, 2009; Hiyama et al., 2012).

This work

It is therefore the aim of this work to highlight various IR impacts in a non-pest species, the house cricket (*Acheta domesticus).* I aim to expand on both the potential consequences to individuals living within and around contamination zones as well as the potential unintended consequences of individuals released through the SIT. My work here focuses on answering three main questions:

1. What are the impacts of IR exposure to cricket sexual signaling and does this subsequently impact mating success?
2. Are there generational implications to life-history features due to IR exposure and does this differ based on maternal or paternal exposure?
3. Is RIBE present on the life-history features of non-irradiated crickets exposed to irradiated conspecifics.

Dose

It is important to note that the doses utilized throughout this thesis range from 0.58Gy – 27.8Gy. Although these are doses that can be used in the SIT, they are quite high to what is normally found in areas surrounding radiation contamination zones. Chronic low dose exposures, as found in these areas, are very difficult to replicate in the lab so acute doses are most often used. However, the high doses used here may act to simulate the damage incurred by individuals receiving these low but chronic exposures in contamination areas over many generations. It is

important though that future studies, possibly in the field, aim to better understanding of the potential differences and comparisons between chronic and acute exposures as currently there is a lack of research in this area.

Model

The model used throughout this thesis is the House Cricket, *Acheta domesticus* **(Figure 1)**. This species is a well used model in our lab as well as serving as a model for various other work related to animal behavior (Tregenza and Wedell, 1997). This model is also ideal for the research aims of this thesis due to their ideal life-history features and life cycle. *Acheta* have a mean lifespan of around 120 days when reared at 30°C (Lyn et al., 2010). Juveniles undergo a known number of molts in which the adult molt is easily identifiable as the molt in which wings develop. As well, males and females are easily identifiable in early juvenile phases as females develop an ovipositor early in development (Lyn et al., 2010). This allows for straightforward measurements of various life history features including survival, longevity, growth, and reproduction. Sex specific data can also easily be collected to elucidate sex specific impacts of IR exposure. *Acheta domesticus* are also quite easy to rear, juveniles and adults have similar nutritional requirements, and they can be raised in large numbers, and require low maintenance (Lyn et al. 2010; Calabrese, 2013). The utilization of *Acheta domesticus* as a model organism, has also been employed for years in Dr. Rollo's lab with respect to radiation impacts on behaviour, immune functions, growth, maturation, reproduction and aging.

Figure 1: Male (a) and female (b) House Crickets (*Acheta domesticus*).

REFERENCES

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.

Azzam, E. I., De Toledo, S. M., & Little, J. B. (2001). Direct evidence for the participation of gap junction-mediated intercellular communication in the transmission of damage signals from α-particle irradiated to nonirradiated cells. *Proceedings of the National Academy of Sciences*, *98*(2), 473–478.

Bakri, A., Mehta, K., & Lance, D. R. (2005). Sterilizing insects with ionizing radiation. *Sterile Insect Technique: Principles and Practice in Area-Wide Integrated Pest Management*, 233–268.

Beasley, D. E., Bonisoli-Alquati, A., Welch, S. M., Møller, A. P., & Mousseau, T. A. (2012). Effects of parental radiation exposure on developmental instability in grasshoppers. *Journal of Evolutionary Biology*, *25*(6), 1149–1162.

Calabrese, E. J. (2012). Hormesis: Improving predictions in the low-dose zone. *Molecular, Clinical and Environmental Toxicology: Volume 3: Environmental Toxicology*, 551–564.

Calabrese, E. J. (2013). Low doses of radiation can enhance insect lifespans. *Biogerontology*, *14*(4), 365–381.

Costantini, D., Metcalfe, N. B., & Monaghan, P. (2010). Ecological processes in a hormetic framework. *Ecology Letters*, *13*(11), 1435–1447.

Dallas, L. J., Keith-Roach, M., Lyons, B. P., & Jha, A. N. (2012). Assessing the impact of ionizing radiation on aquatic invertebrates: A critical review. *Radiation Research*, *177*(5), 693–716.

Desouky, O., Ding, N., & Zhou, G. (2015). Targeted and non-targeted effects of ionizing radiation. *Journal of Radiation Research and Applied Sciences*, *8*(2), 247–254.

Didham, R. K., Ghazoul, J., Stork, N. E., & Davis, A. J. (1996). Insects in fragmented forests: A functional approach. *Trends in Ecology & Evolution*, *11*(6), 255–260.

Doss, M. (2013). Linear no-threshold model vs. Radiation hormesis. *Dose-Response*, *11*(4), dose-response.

Dyck, V. A., Hendrichs, J., & Robinson, A. S. (2021). *Sterile insect technique: Principles and practice in area-wide integrated pest management*. Taylor & Francis.

Einor, D., Bonisoli-Alquati, A., Costantini, D., Mousseau, T. A., & Møller, A. P. (2016). Ionizing radiation, antioxidant response and oxidative damage: A meta-analysis. *Science of the Total Environment*, *548*, 463–471.

Fuciarelli, T. M., & Rollo, C. D. (2020). Trans-generational impacts of paternal irradiation in a cricket: Damage, life-history features and hormesis in F1 offspring. *Dose-Response*, *18*(4), 1559325820983214.

Hiyama, A., Nohara, C., Kinjo, S., Taira, W., Gima, S., Tanahara, A., & Otaki, J. M. (2012). The biological impacts of the Fukushima nuclear accident on the pale grass blue butterfly. *Scientific Reports*, *2*(1), 1–11.

ICRP, 2008. Environmental Protection - the Concept and Use of Reference Animals and Plants. ICRP Publication 108. Ann. ICRP 38 (4-6).

Imanaka, T., Hayashi, G., & Endo, S. (2015). Comparison of the accident process, radioactivity release and ground contamination between Chernobyl and Fukushima-1. *Journal of Radiation Research*, *56*(suppl_1), i56–i61.

Li, X., Seymour, C. B., Mothersill, C., & Rollo, C. D. (2023). Investigation of presence and impact of radiation-induced bystander effect in *Acheta domesticus*. *International Journal of Radiation Biology*, 1–12.

Little, J. B. (2003). Genomic instability and bystander effects: A historical perspective. *Oncogene*, *22*(45), 6978–6987.

Lyn, J. C., Naikkhwah, W., Aksenov, V., & Rollo, C. D. (2011). Influence of two methods of dietary restriction on life history features and aging of the cricket *Acheta domesticus*. *Age*, *33*, 509–522.

Møller, A. P., & Mousseau, T. A. (2009). Reduced abundance of insects and spiders linked to radiation at Chernobyl 20 years after the accident. *Biology Letters*, *5*(3), 356–359.

Morgan, W. F. (2003). Non-targeted and delayed effects of exposure to ionizing radiation: II. Radiation-induced genomic instability and bystander effects in vivo, clastogenic factors and transgenerational effects. *Radiation Research*, *159*(5), 581–596.

Mothersill, C., & Seymour, C. (2001). Radiation-induced bystander effects: Past history and future directions. *Radiation Research*, *155*(6), 759–767.

Mothersill, C., Bucking, C., Smith, R. W., Agnihotri, N., O'Neill, A., Kilemade, M., & Seymour, C. B. (2006). Communication of radiation-induced stress or bystander signals between fish in vivo. *Environmental Science & Technology*, *40*(21), 6859–6864.

Mothersill, C., & Seymour, C. (2014). Implications for human and environmental health of low doses of ionising radiation. *Journal of Environmental Radioactivity*, *133*, 5–9.

Mousseau, T. A., & Møller, A. P. (2014). Genetic and ecological studies of animals in Chernobyl and Fukushima. *Journal of Heredity*, *105*(5), 704–709.

Riley, P. A. (1994). Free radicals in biology: Oxidative stress and the effects of ionizing radiation. *International Journal of Radiation Biology*, *65*(1), 27–33.

Sarapultseva, E. I., & Dubrova, Y. E. (2016). The long-term effects of acute exposure to ionising radiation on survival and fertility in *Daphnia magna*. *Environmental Research*, *150*, 138–143.

Surinov, B. P., Isaeva, V. G., & Dukhova, N. N. (2004). Postirradiation volatile secretions of mice: Syngeneic and allogeneic immune and behavioral effects. *Bulletin of Experimental Biology and Medicine*, *138*(10), 384–386.

Szumiel, I. (2015). Ionizing radiation-induced oxidative stress, epigenetic changes and genomic instability: The pivotal role of mitochondria. *International Journal of Radiation Biology*, *91*(1), 1–12.

Tregenza, T. O. M., & Wedell, N. (1997). Definitive evidence for cuticular pheromones in a cricket. *Animal Behaviour*, *54*(4), 979–984.

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

CHAPTER 1

TITLE

Radiation exposure causes developmental alterations in size and shape of wings and structures associated with song production in male crickets (*Acheta domesticus)*

AUTHORS

Tamara M. Fuciarelli* & C. David Rollo

Department of Biology, McMaster University, 1280 Main Street West, Hamilton ON, L8S 4L8, Canada Accepted: 5 September 2020. *Corresponding author: Email fuciartm@mcmaster.ca

Published online: 21 November 2020
Entomologia Experimentalis et Applicata 2020©

CITATION

Fuciarelli, T. M., & Rollo, C. D. (2021). Radiation exposure causes developmental alterations in size and shape of wings and structures associated with song production in male crickets (*Acheta domesticus*). *Entomologia Experimentalis et Applicata*, *169*(2), 227–234.
https://doi.org/10.1111/eea.12999

1.1 INTRODUCTION

Ionizing radiation has become a focus of environmental contamination, due to expanding nuclear power generation and the Chernobyl (1986, Ukraine) and Fukushima (2011, Japan) disasters (Imanaka et al., 2015). These disasters mediated large-scale release of persistent radioactive material, particularly ^{137}Cs (Kashparov et al., 2003; Hiyama et al., 2012). Regular release of radionuclides (tritium and gamma emitters) due to 'normal nuclear operating

procedures' also contribute to environmental contaminates (Dallas et al., 2012). The global risk from accidents and standard operating procedures among 440 active reactors highlights the need for understanding both short- and long-term impacts of radiation in a diversity of species (World Nuclear Association, 2020).

Critical impacts of radiation include impacts on gonads, heritable mutations, and developmental abnormalities (ICRP, 2008). Mutagenic and epigenetic effects can also have transgenerational consequences, providing a source for heritable phenotypic variation (Beasley et al., 2012). Compared to vertebrates, data on invertebrates have been sparse and poorly characterized, even though invertebrates represent ~90% of animal life on earth (ICRP, 2008; Møller & Mousseau, 2009; Dallas et al., 2012). Most reproductive knowledge of radiation impacts on invertebrates is associated with pest control using the sterile insect technique (SIT) (Bakri et al., 2005; Klassen & Curtis, 2005; Beasley et al., 2012). Doses utilized in the SIT range greatly depend on the species of interest, but can range from 4 to 400 Gy (Dyck et al., 2005). LD_{50} (i.e., lethal dose at which 50% of test animals die) values for adult insects range amazingly from 20 to 3000 Gy, again dependent on species (ICRP, 2008). Although doses required for sterility and mortality in adult insects seem quite high, lower doses can still cause profound impacts to species. A study analyzing the pale grass blue butterfly, *Pseudozizeeria maha* (Kollar), in areas surrounding Fukushima found morphological abnormalities, and generational life-history impacts at doses between 0.055 and 0.125 Gy (Hiyama et al., 2012). Generally, data are highly focused on dose-responses achieving sterility without mortality, leaving a relative paucity of studies examining other impacts such as behavior and sexual signaling, especially at lower doses (Dyck et al., 2005). Radiation has immense potential to disrupt diverse aspects specific to sexual signaling, behavior, development, morphology, and reproductive success.

As in all sexually reproducing species, sexual signaling is vital for reproductive success and involves multiple modalities that each contribute to success (Rebar et al., 2009). In *Acheta domesticus* (L.) (Orthoptera: Gryllidae), females are the choosy sex. Male sexual behavior is complex, but species-specific acoustic signaling is crucial (Simmons et al., 2010). Signaling utilizes specialized structures to modulate aspects of acoustics including the volume, acoustic pulse rate, chirp length, and amplitude (Rebar et al., 2009; Ingleby, 2015). Production of this specialized calling is dependant on five key structures on the male forewings: chord, plectrum, mirror, file, and harp (Montealegre-Z et al., 2011) (Figure 1). The harp, mirror, and chord are the components that resonate and radiate the sound produced by the plectrum and stridulatory file (Montealegre-Z et al., 2011). Together, each wing component is vital for the oscillations generated by a male cricket, and production of an acoustic signal conveying species recognition and, likely, aspects of male fitness (Huber & Thorson, 1985; Rost & Honegger, 1987; Wagner & Reiser, 2000; Rebar et al., 2009; Montealegre-Z et al., 2011). Indeed, several studies in crickets and other song-producing species have confirmed the importance of 'correct' song production for mating success, as well as the importance of the structure and size of wing components on song production (Balakrishnan & Pollack, 1996; Simmons & Ritchie, 1996; Gray & Eckhardt, 2001; Bennet-Clark, 2003).

Here, we analyze morphological changes in wings associated with song production in male *A. domesticus* arising from early-life irradiation across a dose range of 0–23.2 Gy. We applied morphometric techniques to examine the specific acoustic structures of male wings and whether radiation impacted both the shape and size of these components, both of which would impact song production. Fluctuating asymmetry, defined as changes to bilateral traits due to the

developmental environment, was also analyzed as a biomarker of radiation stress (Beasley et al., 2013).

1.2 METHODS

Breeding colony: *Acheta domesticus* were generated in a large breeding colony housed in an acrylic terrarium (93 × 64.2 × 46.6 cm), insulated with 1.5-cm-thick Durofoam insulation. Fans provided air circulation. The colony was maintained at 29 ± 2 °C and L12:D12 photoperiod. Food consisted of *ad libitum* chick feed (17% protein, Country Range MultiFowl Grower Quick Feeds Feed Mill, Copetown, Canada) and *ad libitum* distilled water (soaked cellulose sponges) replaced daily. Crickets were provided with egg-carton shelters, and paper towels sprayed daily with distilled water and replaced weekly. The colony was provided with oviposition medium (Vigoro Organic Garden Soil, Swiss Farms Products, Marysville, OH, USA) in small plastic containers (7 × 7 × 7 cm). The oviposition containers were collected after 24 h and incubated at 29 ± 2 °C until hatching, providing cohorts of nymphs of known age.

Experimental groups: After individuals hatched from the oviposition containers (after 14 days), approximately 1000 nymphs were separated after 24 h of hatching, to again ensure same-aged individuals were used. Experimental individuals were housed in the same conditions as the breeding colony. At 14 days of age (fourth instar), approximately 600 individuals (150 per group) were randomly selected, separated, and irradiated for specific durations using a ^{137}Cs source at a dose rate of 0.58 Gy per min totaling 0, 4.6, 16.2, or 23.2 Gy at the Taylor Radiobiology Source at McMaster University (Hamilton, ON, Canada). All individuals from

each experimental group were irradiated during a single exposure; 150 individuals were chosen to ensure enough individuals survived to maturity for later analysis. All groups were then immediately brought to McMaster's Life Sciences Building where they were maintained for life. Adult males are known to fight other males for access to females, often causing bodily damage. At approximately 30 days of age, females were removed from all experimental groups; prior to this, males and females are not distinguishable – it was not until females develop an ovipositor, that females were removed. All experimental individuals used for morphometric analysis were males.

Specimen preparation and data collection: Left and right forewings from each experimental group (0–23.2 Gy) were detached at their base from anesthetized males using fine forceps. Wing removal occurred at 50–60 days of age. This time frame reflects the longer maturation time for higher dose groups. Males were approximately 2 weeks post-maturation when wings were collected. Each wing pair was then mounted between two microscope slides that were then permanently attached for later analysis. A photograph was taken of each wing pair at 0.75× magnification using a Nikon 16.25-megapixel camera mounted on a SMZ18 stereoscope (Nikon, Melville, NY, USA). Photos were digitized with tpsUtil32 (v.1.78) and tspDIG2 (v.2.31; both available at http://life.bio.sunysb.edu/morph/, courtesy Dr. FJ Rohlf). Wing samples were collected from all individuals that had survived within each group: control (n = 18), 4.6 Gy (n = 20), 16.2 Gy (n = 16), and 23.2 Gy (n = 17), totaling 71 crickets. Sample size for both left and right wings, as well as a replicate image for both, included control (n = 72), 4.6 Gy (n = 80), 16.2 Gy (n = 64), and 23.2 Gy (n = 68), totaling 284 images. Nine images were then identified as outliers and removed (1× control, 1× 16.2 Gy, 7× 23.2 Gy). Using previously published data by

Klingenberg et al. (2010), 11 'landmarks' were demarked on each wing. Landmarks chosen are those depicting areas of song production, encompassing areas of harp (landmark 2, 3, 5, 6, and 9), chord (5 and 7), mirror (landmark 6, 8, 9, 10, and 11), plectrum (landmark 1 and 4), and file (landmark 2 and 3). Landmarks are illustrated in Figure 1 from a control male. Repeatability tests were performed by photographing and landmarking each pair of wings twice, with a 2-week interval (see below).

Centroid size: As a measure of wing size, centroid size (CS) was calculated as the square root of the sum of squared distances from the assigned landmarks to their centroid (the average x and y coordinates of all landmarks) (Bookstein, 1991; Jirakanjanakit et al., 2007). Centroid size is used as a universal measure of size in geometric morphometrics (Klingenberg, 2016). Average centroid size of all individual wings in each experimental group (0–23.2 Gy) was analyzed using a one-way ANOVA followed by a Tukey honestly significant difference (HSD) post-hoc test to determine significant differences between means. Replicates were averaged in order to get a more accurate measure of each wings centroid size.

Measurement error: Image acquisition and subsequent landmarking are two of the main sources of measurement error when performing geometric morphometrics. To minimize this form of error we applied a procedure adapted from Alibert et al. (2001), as follows. After each wing was initially photographed and digitized, 2 weeks later they were repositioned, re-photographed, and re-digitized by the same researcher. This procedure facilitates the reduction of measurement error as well as image acquisition. The statistical significance of measurement error was also tested using a Procrustes ANOVA (Table 1; Klingenberg et al., 2002). The

Procrustes ANOVA is a common method for quantifying relative amounts of variation at different levels (Klingenberg & McIntyre, 1998; Klingenberg et al., 2002). All analyses were performed in MorphoJ v.1.07a (Klingenberg, 2011).

Variation in wing shape: All landmark coordinates were superimposed with a general Procrustes fit. The Procrustes fit superimposes/projects each appointed landmark for each image into tangent space. Each set of landmarks for each image is then scaled (to control for size), aligned, and rotated. This procedure simultaneously removes the influence of orientation, location, and size from each wing image (Klingenberg & McIntyre, 1998). It is also during this procedure that centroid size as described above was obtained. Prior to analysis images of opposite wings were flipped using tspDig so each wing image had the same orientation for landmarking. Variation in wing shape was then analyzed using both a principal components analysis (PCA) and a canonical variates analysis (CVA). Both describe variation in shape; however, PCA looks at major sources of variation across the entire dataset, unconnected to experimental groups whereas CVA identifies the shape variation that is associated with each experimental group and its significance (Blankers et al., 2017). Both analyses allow us to determine in what regions of the wing variation is occurring (Blankers et al., 2017). CVA was reported as P-value after 10 000 permutations. This describes whether the variance between groups is larger than could be produced by random permutation. To determine levels of fluctuating asymmetry for both control males as well as for each radiation dose a Procrustes ANOVA was completed separately for each experimental group. All shape analyses were performed in MorphoJ.

1.3 RESULTS

Variation in the dataset: Principal components analysis indicated that the first four principal components contributed to approximately 74% of total wing variation across groups (PC1, 42.6%; PC2, 13.8%; PC3, 11.5%; PC4, 5.9%). 90% Confidence ellipses indicated a distinct separation between each radiation group in comparison to controls, which is indicative of radiation-induced variation (Figure 2). Wireframe images indicated that most variation was occurring in the mirror region of the wing with most distortion occurring on landmarks 6, 8, 9, 10, and 11.

Comparison between experimental groups: CVA results indicated that three canonical variates represent 100% of variation between experimental groups, with the first two representing almost 90% of variation (CV1, 69.9%; CV2, 20%) (Figure 3). P-values from Procrustes distances among groups using 10 000 permutations showed significant differences in all radiation groups, compared to the control: 23.2 Gy ($P<0.0001$), 16.2 Gy ($P<0.0001$), and 4.6 Gy ($P = 0.0001$). Wireframe images indicated that most variation was occurring in the mirror and chord region of the wing with most distortion occurring on landmarks 4, 6, 7, 8, 9, 10, and 11.

Fluctuating asymmetry: A Procrustes ANOVA on all samples within each group indicated significant levels of fluctuating asymmetry (individual*side interaction) for control, 4.6, 16.2, and 23.2 Gy groups $P<0.0001$ (Table 2). Mean square values for fluctuating asymmetry increased with dose. The mean squares for fluctuating asymmetry exceeded

measurement error by more then fourfold in each experimental group which ensured the levels of fluctuating asymmetry were not compromised by measurement error.

Body size: One-way ANOVA indicated significant differences between groups ($F_{3,132}$ = 17.42, P<0.0001). Body size was reduced for the 16.2 Gy (Tukey's post-hoc HSD test: P = 0.040) and 23.2 Gy (P = 0.002) groups, compared to controls (Figure 4). This constituted a centroid size reduction from 1.10 mm (control) to 1.07 mm (16.2 Gy) and 1.05 mm (23.2 Gy). No significant change in wing centroid size was seen in the 4.6 Gy wings compared to the controls. A linear regression indicated a negative correlation between centroid size and radiation dose: y = −5.826 (± 0.963 SE) x + 1114 (± 5.647 SE) (R^2 = 0.215; $F_{1,134}$ = 36.63, P<0.0001).

Measurement error: The relative impact of measurement error was determined to be negligible compared to the variation between experimental groups. A Procrustes ANOVA on both centroid size and shape illustrated that measurement error was 159.8× (centroid size) and 3.4× (shape) times lower than the variation associated with experimental group (Table 1). It was concluded that measurement error did not significantly bias results.

1.4 DISCUSSION

Male crickets, like many other insects, use species and sex-specific signaling systems for mating and reproduction (Alexander, 1967; Hedwig, 2016). Acoustic signaling in particular is used by males of many species in the Orthoptera order for mate attraction and courtship (Alexander, 1967; Hedwig, 2016). These signals have been shown to be species specific, with

females only responding to signals from males of their own species, with the correct song parameters, that is, frequency and pulse rate (Walker, 1957; Otte, 1992; Hedwig, 2016). Many studies have also shown female preferences for specific features of male songs (Thomson et al., 2014).

The variety of acoustic properties is related to various morphological structures associated with song production (Otte, 1992; Montealegre-Z et al., 2011). Males produce these acoustic signals through the closing of the forewings in which the plectrum rubs against the opposing wings' file, producing sound. The sound is then amplified and radiated mainly by the harp and mirror (Montealegre-Z et al., 2011; Thomson et al., 2014). The results of the PCA, which indicates where variation is occurring across the entire dataset, indicated that 56% of the wing variation was associated mainly with the mirror region of the wing. The CVA, which determines differences in wing shape among experimental groups, also indicated that most variation was associated with the mirror region. When comparing radiation groups to the control, Procrustes distances indicated that radiation has significantly impacted wing shape in males, and that this change is mostly associated with the mirror structure.

These results are significant as the literature shows extreme specificity of these song structures, including the mirror, in producing highly specialized acoustic signaling. Although not explicitly demonstrated in this study other work has indicated that both the size and shape of these wing structures alters the songs of males. A study using seven species of crickets indicated that removal or alteration of the mirror region of forewings completely diminishes the auditory signal (Keuper et al., 1988). It was determined that the mirror functions as an amplifier for the sound produced by the file and plectrum, and that its shape is vital in that function (Keuper et al., 1988). The shape and size of the harp, another sound resonator, has also been shown to

impact the sound produced by males (Moradian & Walker, 2008). The literature seems to suggest that the alterations seen in the irradiated male wings will likely cause auditory change in male songs.

The mean squares of the Procrustes ANOVA also indicated increasing levels of fluctuating asymmetry in shape as dose increased. This is significant as increased fluctuating asymmetry has been identified as a reliable biomarker of environmental stress in insects (Beasley et al., 2013). Fluctuating asymmetry has also been indicated as a general indicator of individual quality (Lajus et al., 2015). However, these results are contrary to recent literature on radiation impacts on fluctuating asymmetry in grasshoppers in areas surrounding Chernobyl (Beasley et al., 2012). Beasley et al. (2012) indicated no radiation-induced differences in levels of fluctuating asymmetry in the Chernobyl grasshoppers exposed to several chronic low doses (0.03–50.06 μSv h^{-1}). However, this could be due to the vastly higher doses assessed in this study as well as differences between chronic vs. acute exposure (Beasley et al., 2012).

The size of sound-generating structures also has significant acoustical impacts on song production (Dambach & Gras, 1994). Our results indicated a significant reduction in centroid size (a proxy for wing size) for males irradiated at 16.2 and 23.2 Gy. The linear regression analysis suggests a significant dose-response relationship between dose and centroid size. This indicates a reduction in area for the song-producing regions of the forewing. Reduced wing size has been shown to impact the amount of muscle power available for stridulating as well as cause disruptions in sound emission due to altered membrane radius and wavelength interactions (Dambach & Gras, 1994). It is therefore likely that radiation-induced impacts on shape and size of song-producing regions will alter the male acoustic signaling. This may have a profound impact on male mating success and attractiveness. Unpublished and ongoing work in our

laboratory has suggested that indeed irradiated males have significantly reduced mating success with normal females. Whether this is due to altered signaling or other radiation-induced effects is currently being investigated.

1.5 ACKNOWLEDGMENTS

This work was supported by the Mitacs Elevate Grant (IT05851), and the NSERC Discovery Grant (RGPIN-05693-2015).

1.6 FIGURES AND TABLES

Table 1. Procrustes ANOVA values for centroid size (µm) and shape (Procrustes distance) representing the relative impact of measurement error and experimental group on *Acheta domesticus* wing analysis.

Data set	Effect	SS	MS	d.f.	F	P
Centroid size	Experimental group	13429494	4476498	3	159.83	<0.0001
	Error	20212106	150836	134		
Shape	Experimental group	0.07171732	0.0013280985	54	3.37	<0.0001
	Error	0.05275714	0.0000218728	2412		

Table 2. Procrustes ANOVA for fluctuating asymmetry in *Acheta domesticus* wings exposed to 0 (control), 4.6, 16.2, or 23.2 Gy radiation, based on shape analysis of the left and right wings, and a replicate image from each experimental group. Each wing had 11 landmarks. The 'individual*side' term provides an estimate for fluctuating asymmetry.

Treatment	Effect	SS	MS	d.f.	F	P
Control	Individual	0.04877170	0.0001593847	306	2.73	<0.0001
	Side	0.00511391	0.0002841060	18	4.86	<0.0001
	Individual*side	0.01787462	0.0000584138	306	4.39	<0.0001
	Error	0.00861967	0.0000133020	648		
4.6 Gy	Individual	0.08657076	0.0002531309	342	2.33	<0.0001
	Side	0.00834945	0.0004638583	18	4.27	<0.0001
	Individual*side	0.03715122	0.0001086293	342	8.33	<0.0001
	Error	0.00938810	0.0000130390	720		
16.2 Gy	Individual	0.14030899	0.0005996111	234	2.18	<0.0001
	Side	0.01413711	0.0007853951	18	2.86	0.0001
	Individual*side	0.06436464	0.0002750626	234	11.26	<0.0001
	Error	0.01230748	0.0000244196	504		
23.2 Gy	Individual	0.17105187	0.0006787773	252	1.03	0.41
	Side	0.01599661	0.0008887008	18	1.35	0.16
	Individual*side	0.16604519	0.0006589095	252	15.85	<0.0001
	Error	0.02244188	0.0000415590	540		

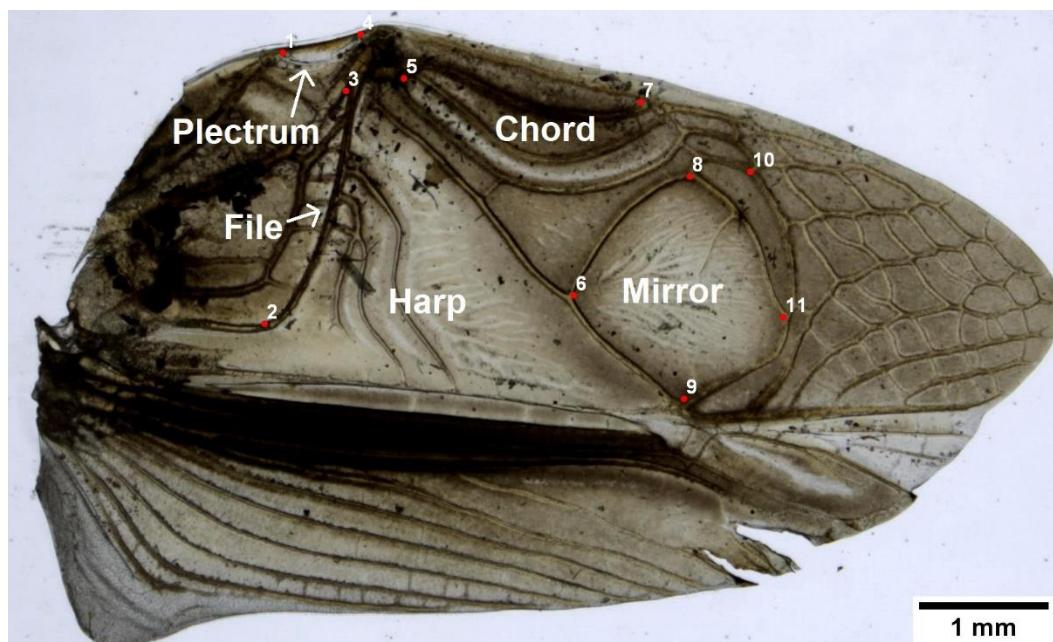


Figure 1. Position of 11 landmarks (red dots) superimposed on a photograph of a control *Acheta domesticus* wing. All photographed wings were landmarked using the same 11 landmarks which were present in all wings analyzed. Opposing wings were digitally flipped to provide the same orientation for all analyzed wings. The scale bar measures 1 mm.

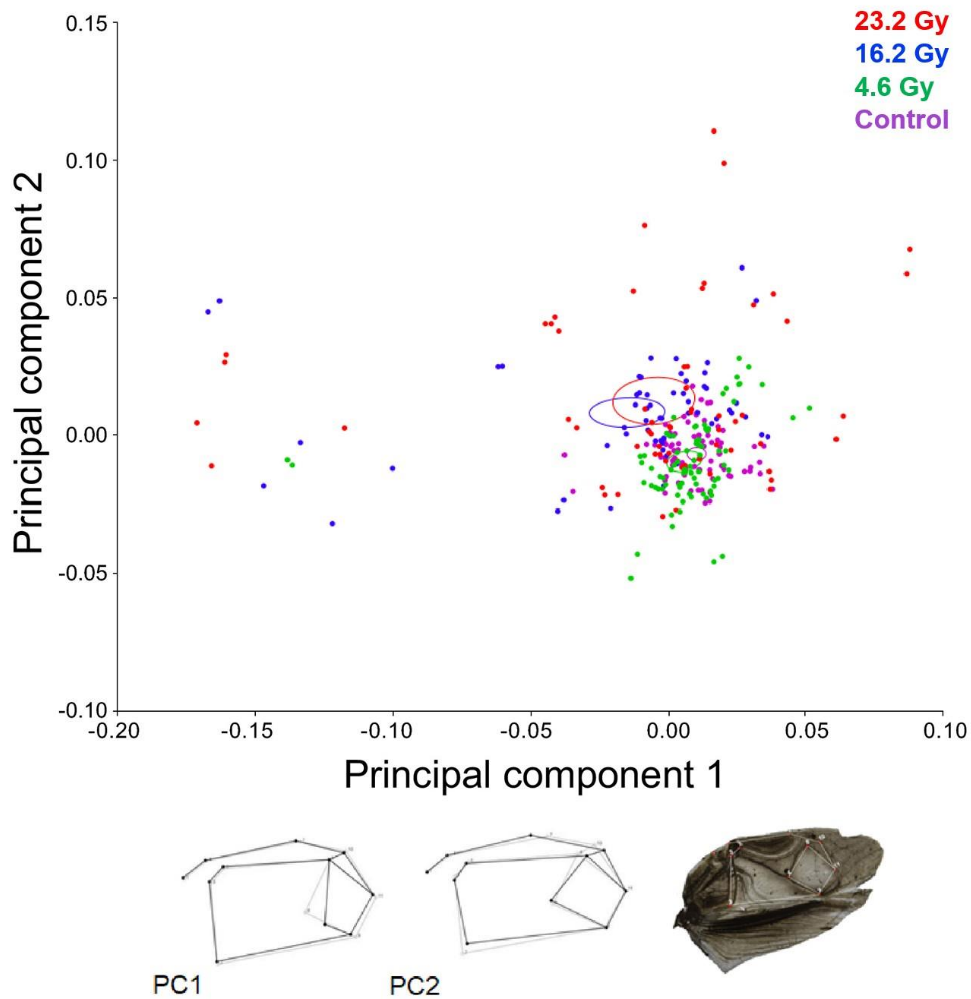


Figure 2. Principal component (PC) analysis of *Acheta domesticus* wing shape. Scatterplot displays the first two principal components which contribute to 56% of wing variation across the entire dataset. Confidence ellipses display means of each experimental group within a 90% probability. The most variant points are almost exclusively in either the 16.2 or 23.2 Gy radiation groups. Wire frame images illustrate the morphometric variation along the two principal component axes. The light gray wireframe is the average shape of all control individuals whereas the black wireframe displays in which areas variation is occurring.

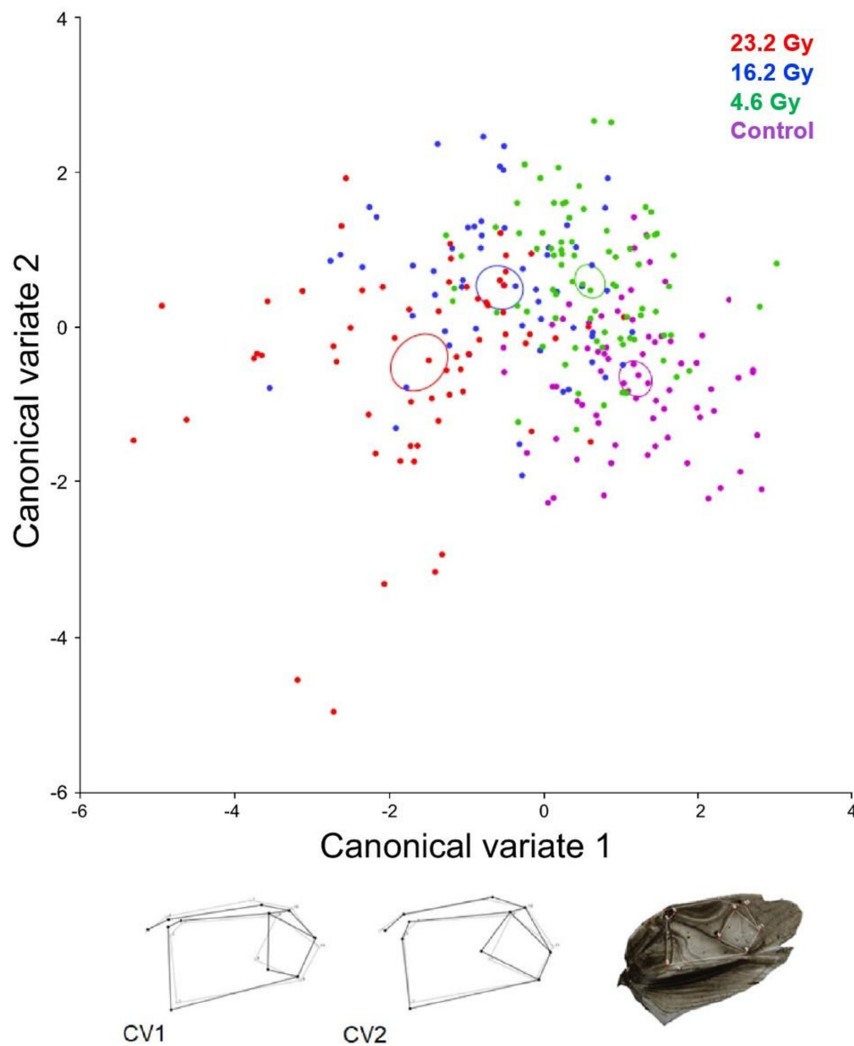


Figure 3. Canonical variate (CV) analysis of *Acheta domesticus* wing shape between control and radiation groups. Three CV axes described 100% of the variation between experimental groups CV1 (69.85%), CV2 (20%), and CV3 (10.15%). Confidence ellipses which display means of each experimental group within a 90% probability, show distinct variation between groups. Wireframe drawings represent the variation in wing shape, with the gray outline representing the average control shape and the black outline where group variation is occurring. A scale factor of 10 was used for both wireframe images to exaggerate the variation.

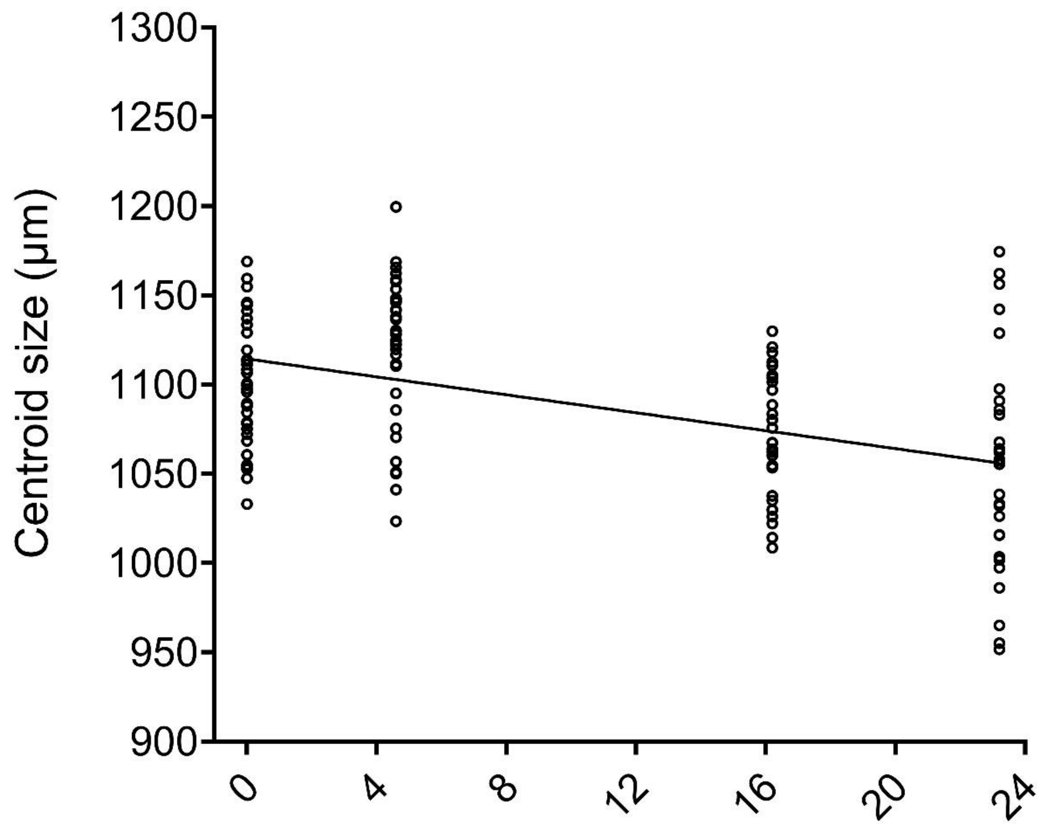


Figure 4. Centroid size (μm) of control and irradiated *Acheta domesticus* male wings. All males were irradiated (4.6, 16.2, or 23.2 Gy; control: 0 Gy) at 14 days of age and wings were detached at 2 weeks post maturation. Each data point represents the mean of two replicate wing images.

1.7 REFERENCES

Alexander RD (1967) Acoustical communication in arthropods. Annual Review of Entomology 12: 495–526.

Alibert P, Moureau B, Dommergues J-L & David B (2001) Differentiation at a microgeographical scale within two species of ground beetle, *Carabus auronitens* and *C. nemoralis* (Coleoptera, Carabidae): a geometrical morphometric approach. Zoologica Scripta 30: 299–311.

Bakri A, Mehta K & Lance DR (2005) *Sterilizing insects with ionizing radiation. Sterile Insect Technique – Principles and Practice in Area-Wide Integrated Pest Management* (ed. by VA Dyck, J Hendrichs & AS Robinson), pp. 233- 268. Springer, Dordrecht, The Netherlands

Balakrishnan R & Pollack GS (1996) Recognition of courtship song in the field cricket, *Teleogryllus oceanicus*. Animal Behaviour 51: 353–366.

Beasley DAE, Bonisoli-Alquati A, Welch SM, Møller AP & Mousseau TA (2012) Effects of parental radiation exposure on developmental instability in grasshoppers. Journal of Evolutionary Biology 25: 1149–1162.

Beasley DAE, Bonisoli-Alquati A & Mousseau TA (2013) The use of fluctuating asymmetry as a measure of environmentally induced developmental instability: a meta-analysis. Ecological Indicators 30: 218–226.

Bennet-Clark HC (2003) Wing resonances in the Australian field cricket *Teleogryllus oceanicus*. Journal of Experimental Biology 206: 1479–1496.

Blankers T, Block R & Hennig RM (2017) Codivergence but limited covariance of wing shape and calling song structure in field crickets (*Gryllus*). Evolutionary Biology 45: 144–155.

Bookstein FL (1991) Morphometric Tools Landmark Data: Geometry and Biology. Cambridge University Press, Cambridge, UK.

Dallas LJ, Keith-Roach M, Lyons BP & Jha AN (2012) Assessing the impact of ionizing radiation on aquatic invertebrates: a critical review. Radiation Research 177: 693–716.

Dambach M & Gras A (1994) Bioacoustics of a miniature cricket, *Cycloptiloides canariensis* (Orthoptera: Gryllidae: Mogoplistinae). Journal of Experimental Biology 198: 721-728.

Dyck VA, Hendrichs J & Robinson AS (2005) Sterile Insect Technique: Principles and Practice in Area-Wide Integrated Pest Management. Springer, Dordrecht, The Netherlands.

Gray DA & Eckhardt G (2001) Is cricket courtship song condition dependent? Animal Behaviour 62: 871-877.

Hedwig BG (2016) Sequential filtering processes shape feature detection in crickets: a framework for song pattern recognition. Frontiers in Physiology 7: 46.

Hiyama A, Nohara C, Kinjo S, Taira W, Gima S et al. (2012) The biological impacts of the Fukushima nuclear accident on the pale grass blue butterfly. Scientific Reports 2: 570.

Huber F & Thorson J (1985) Cricket auditory communication. Scientific American 253: 60-68.

ICRP (2008) Environmental protection: the concept and use of reference animals and plants. Annals of the ICRP 39(6): 1-242,

Imanaka T, Hayashi G & Endo S (2015) Comparison of the accident process, radioactivity release and ground contamination between Chernobyl and Fukushima-1. Journal of Radiation Research 56: i56–i61.

Ingleby F (2015) Insect cuticular hydrocarbons as dynamic traits in sexual communication. Insects 6: 732–742.

Jirakanjanakit N, Leemingsawat S, Thongrungkiat S, Apiwathnasorn C, Singhaniyom S et al. (2007) Influence of larval density or food variation on the geometry of the wing of *Aedes* (*Stegomyia*) *aegypti.* Tropical Medicine & International Health 12: 1354–1360.

Kashparov VA, Lundin SM, Zvarych SI, Yoshchenko VI, Levchuk SE et al. (2003) Territory contamination with the radionuclides representing the fuel component of Chernobyl fallout. *Science of the Total Environment* **317**: 105– 119.

Keuper A, Weidemann S, Kalmring K & Kaminski D (1988) Sound production and sound emission in seven species of European tettigoniids part II. Wing morphology and the frequency content of the song. Bioacoustics 1: 171–186.

Klassen W & Curtis CF (2005) History of the sterile insect technique. Sterile Insect Technique – Principles and Practice in Area-Wide Integrated Pest Management (ed. by VA Dyck, J Hendrichs & AS Robinson), pp. 3–36. Springer, Dordrecht, The Netherlands.

Klingenberg CP & McIntyre GS (1998) Geometric morphometrics of developmental instability: analyzing patterns of fluctuating asymmetry with Procrustes methods. Evolution 52: 1363–1375.

Klingenberg CP, Barluenga M & Meyer A (2002) Shape analysis of symmetric structures: quantifying variation among individuals and asymmetry. Evolution 56: 1909–1920.

Klingenberg CP, Debat V & Roff DA (2010) Quantitative genetics of shape in cricket wings: developmental integration in functional structure. Evolution 64: 2935-2951.

Klingenberg CP (2011) MorphoJ: an integrated software package for geometric morphometrics. Molecular Ecology Resources 11: 353-357.

Klingenberg CP (2016) Size, shape, and form: concepts of allometry in geometric morphometrics. Development Genes and Evolution 226: 113–137.

Lajus D, Katolikova M, Strelkov P & Hummel H (2015) Fluctuating and directional asymmetry of the blue mussel (Mytilus edulis): improving methods of morphological analysis to explore species

performance at the northern border of its range. Symmetry 7: 488–514.

Møller AP & Mousseau TA (2009) Reduced abundance of insects and spiders linked to radiation at Chernobyl 20 years after the accident. Biology Letters 5: 356–359.

Montealegre-Z F, Jonsson T & Robert D (2011) Sound radiation and wing mechanics in stridulating field crickets (Orthoptera: Gryllidae). Journal of Experimental Biology 214: 2105–2117.

Moradian NR & Walker SE (2008) Relationships between body size and sound-producing structures in crickets: do large males have large harps? Invertebrate Biology 127: 444–451.

World Nuclear Association (2020) Nuclear Power Today – Nuclear Energy. https://world-nuclear.org/information-library/current-and-future-generation/nuclear-power-in-the-world-today.aspx (accessed 28 May 2020

Otte D (1992) Evolution of cricket songs. Journal of Orthoptera Research 1: 25-49.

Rebar D, Bailey NW & Zuk M (2009) Courtship songs role during female mate choice in the field cricket *Teleogryllus oceanicus*. Behavioral Ecology 20: 1307-1314.

Rost R & Honegger HW (1987) The timing of premating and mating behavior in a field population of the cricket *Gryllus campestris* L. Behavioral Ecology and Sociobiology 21: 279-289.

Simmons LW & Ritchie MG (1996) Symmetry in the songs of crickets. Proceedings of the Royal Society of London 263: 305–311.

Simmons LW, Tinghitella RM & 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**: 1330– 1336.

Thomson IR, Darveau C-A & Bertram SM (2014) Body morphology, energy stores, and muscle enzyme activity explain cricket acoustic mate attraction signaling variation. PLoS ONE 9(3): e90409.

Wagner WE & Reiser MG (2000) The importance of calling song and courtship song in female mate choice in the variable field cricket. Animal Behaviour 59: 1219-1226.

Walker TJ (1957) Specificity in the response of female tree crickets (Orthoptera, *Gryllidae, Oecanthinae*) to calling songs of the males. Annals of the Entomological Society of America 50: 626–636.

CHAPTER 2

TITLE

Impacts of ionizing radiation on the cuticular hydrocarbon profile and mating success of male house crickets (*Acheta domesticus*).

AUTHORS

Tamara M. Fuciarelli* & C. David Rollo

Department of Biology, McMaster University, 1280 Main Street West, Hamilton ON, L8S 4L8, Canada Accepted: 8 January 2021. *Corresponding author: Email fuciartm@mcmaster.ca

Published online: 11 February 2021
International Journal of Radiation Biology 2021©

CITATION

Fuciarelli, T. M., & Rollo, C. D. (2021). Impacts of ionization radiation on the cuticular hydrocarbon profile and mating success of male House Crickets (*Acheta domesticus*). *International Journal of Radiation Biology*, 1–17.

2.1 INTRODUCTION

Radiation can inflict damage through the direct ionization of biological molecules, including DNA, or indirectly through excess free radical production associated with the ionization of cellular water (Einor et al., 2016). A large amount of research has been conducted on radiation impacts to insect reproduction as it pertains to sterilization. This knowledge is

largely associated with the use of the sterile insect technique (SIT), a method which uses radiation to sterilize males and reduce insect populations (Dyck et al., 2005). However, much less research has focused on the more subtle radiation impacts such as to sexual signaling, which may have a large effect on mating success and reproduction as a whole (Dyck et al., 2005).

In *Acheta*, copulation requires females to mount males to accept spermatophores (Balakrishnan & Pollack, 1997). Thus, males cannot coerce females into copulation, making their ability to elicit mounting through sexual signaling vital (Balakrishnan & Pollack, 1997). In most insects, species-specific chemical communication is highly developed (Botha et al., 2017). For *A. domesticus* and many other insects, key male chemical signals include cuticular hydrocarbons (Tregenza & Wedell, 1997; Thomas & Simmons, 2010; Pavkovic-Lucic et al., 2012; Botha et al., 2017). Hydrocarbons and to a lesser extent lipids are the predominant components of insect cuticular extracts and have been documented in over 100 species (Assis et al., 2017). These compounds are synthesized in the epidermal cells associated with the cuticle and have been shown to have a sex specific hydrocarbon profile in *Acheta* (Warthen & Uebel, 1980; Assis et al., 2017). Other then playing a role in sex and species recognition, stress induced alterations to male sexual traits may be employed by females to detect males of lower fitness. Indeed, females can avoid 'damaged' males in a variety of species, including flies, spiders, rats, deer, and birds if one or more secondary sexual characteristics are altered (Kotiaho et al., 1996; Kavaliers et al., 2004; Mays & Hill, 2004; Surinov, 2007; Velando et al., 2008).

Understanding the impacts of radiation stress on cuticular hydrocarbon profiles is of relevance to the economically important sterile insect technique (SIT). The SIT refers to the release of radiation sterilized males into the breeding population, resulting in a reduction of female fertility and thus the target population as a whole (Dyck et al., 2005). However, the SIT

functions under the paradigm that males are exposed to doses of radiation that sterilize them but allow them to remain competitive amongst their non-irradiated counterparts (Dyck et al., 2005). As female insects have been shown to have preference for a particular cuticular profile in males, any subtle changes to the profile can result in devastating impacts on male competitiveness (Peschke, 1987; Kortet & Hedrick, 2005; Thomas & Simmons, 2009). Several studies have indicated that other stressors such as dietary condition can alter the cuticle profile and alter conspecific responses (Henneken et al., 2017). Other studies have shown stress responses to the cuticular profile alterations are stress specific. A study conducted by Engl et al. (2018) described alterations to male Tsetse fly cuticular hydrocarbon profiles when exposed to antibiotic treatment but not to ionizing radiation (Engl et al., 2018).

Here we aimed to analyze the impact of ionizing radiation on the cuticular hydrocarbon profile of our model, the House Cricket (*Acheta domesticus*). Furthermore, we aimed to assess the impact of ionizing radiation on mating success on irradiated male crickets paired with normal females.

2.2 METHODS

Breeding colony: *Acheta domesticus* were generated in a large breeding colony housed in an acrylic terrarium (93 x 64.2 x46.6 cm), insulated with 1.5 cm thick Durofoam insulation. Fans provided air circulation. The colony was maintained at 29°C ± 2°C on a 12 h day-12h night photoperiod. Food consisted of *ad libitum* 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 distilled water and

replaced weekly. The colony was provided with oviposition medium (Vigoro Organic Garden Soil®) in small plastic containers (7 x 7 x7 cm). These were collected after 24 h and incubated at 29°C ± 2°C until hatching, providing cohorts of nymphs of known age.

Experimental groups: Experimental animals were generated from the breeding colony by leaving an oviposition container filled with Vigoro Organic Garden Soil®. To ensure experimental animals were of the same age, the oviposition container was removed from the breeding colony after 24 h and incubated at 29°C ± 2°C. After individuals hatched from the oviposition containers (~14 days), approximately 1000 nymphs were separated after 24 h of hatching, to again ensure the same aged individuals were used. Experimental individuals were housed in the same conditions as the breeding colony. At 14 days of age (4th instar nymphs), approximately 600 individuals (100 per group) were randomly selected, separated, and irradiated for specific durations using a Cs-137 source at a dose rate of 0.58 Gy/min totaling (0, 4.6 Gy, 9.3 Gy, 16.2 Gy, 23.2 Gy, 27.8 Gy) at the Taylor Radiobiology Source at McMaster University. A maximum dose of 27.8 Gy was chosen as previous work has shown sterilization to occur around this dose. All individuals from each experimental group were irradiated during a single exposure. 100 individuals were chosen to ensure enough individuals survived to maturity for later analysis. All groups were then immediately brought to McMaster's Life Sciences Building (LSB) where they were maintained for life. Adult males are known to fight other males for access to females, often causing bodily damage, so at approximately 30 days of age, before maturation, females were removed from all experimental groups. Prior to this males and females are not distinguishable, therefore it was not until later life stages, when females develop an ovipositor,

that females were removed. All experimental individuals used for cuticular hydrocarbon analysis were males.

Cuticular hydrocarbon isolation: Cuticular hydrocarbons were collected from randomly selected unmated/virgin male crickets from each experimental group 0Gy (n = 6), 4.6Gy (n = 6), 9.3Gy (n = 5), 16.2Gy (n = 5), 23.2 Gy (n = 6), and 27.8 Gy (n = 6). Individuals one week post-maturation (50 days of age) were removed and immobilized with CO_2. They were then individually weighed and swirled in hexane (dissolving the cuticular hydrocarbons) for 5 min at a concentration of 5ml per 0.5 g of cricket mass (to control for cricket size) in a sterilized glass container. After 5 min in hexane, crickets were removed and disposed of.
Glass containers with the collected hexane and cuticular hydrocarbons were then sealed with an airtight lid and wrapped in elastic tape to ensure no outside contamination. Samples were stored at 4 °C until processing.

Cuticular hydrocarbon analysis: Samples were analyzed at McMaster's regional center for mass spectrometry. Samples were first vortexed and then evaporated using nitrogen gas to remove the hexane solvent. Once evaporated, samples were reconstituted in 40ul of pure hexane and 10ul of internal standard (Napthalene-d8), making each sample volume a total of 50ul. Samples were then processed using an Agilent 5973/6890 for gas chromatography and mass spectrometry analysis. Output was analyzed with Bruker Compass DataAnalysis 4.0.® . Significant peaks were identified using the DataAnalysis program and the area/concentration under each peak was recorded. Each significant peak was shown to have a very similar 'staircase' like mass/charge pattern, indicating its hydrocarbon composition. Peaks which were

not identified as hydrocarbons were excluded from analysis. The average intensity of each peak in the control group was then compared to average intensity in each irradiated group.

Mating Success: At midlife, approximately 56–67 days post-hatching, experimental adult males from each group Control (n = 55), 4.6 Gy (n = 60), 9.3Gy (n = 59), 16.2Gy (n = 53), 23.2 Gy (n = 30), 27.8 Gy (n = 43) were individually paired with a non irradiated female for a 15 min period to assess mating success. During this period each pair had access to food and distilled water. Mating was considered successful if females mounted a male for more then 5 s. This period was chosen as males occasionally force themselves underneath females, in which females, if rejecting the male will jump off. All males were given a second 15 min trial with a new female to ensure that female inability to mate was not the cause of a failed mating trial.

Statistics: A two-way ANOVA followed by a Dunnett's multiple comparisons test was employed to determine significant differences in the average intensity of individual peaks in each irradiated group compared to the control group. The impact of dose on sample variation was also analyzed. For mating success significant differences were analyzed using a one-way ANOVA followed by a Dunnett's multiple comparisons test to distinguish differences between groups compared to controls. All statistical analyses were carried out using Prism Graph Pad 8.

2.3 RESULTS

Cuticular hydrocarbons: The typical ion chromatogram for control male crickets revealed 26 significant peaks/hydrocarbons used for our analysis (Figure 1). Results of the two-

way ANOVA indicated significant contributions of dose on total variance ($F(5,728) = 8.153$, $p<.0001$). Results of the two-way ANOVA are summarized in Table 1. A Dunnett's multiple comparisons test indicated significant differences in peak 22; 4.6Gy ($p = .0014$), 9.3Gy ($p = .008$), 16.2Gy ($p<.0001$), 27.8 Gy (p¼.0124) and peak 23; 4.6Gy ($p<.0001$), 9.3Gy ($p<.0001$), 16.2Gy ($p<.0001$), 23.2 Gy (p¼.0303) and 27.8 Gy ($p<.0001$) compared to controls (Figure 2). Increased concentrations of hydrocarbons, although not significant, were evident in most irradiated groups. Specifically, doses between 4.6-27.8Gy showed increased concentration compared to controls in 16 of the peaks analyzed. Percent differences between controls and all irradiated groups are summarized in Table 2. Likely hydrocarbon candidates for each identified peak are summarized in Table 3.

Mating Success: Male mating success were recorded as successful when females mounted the male for more then 5 s. An observable linear decline in mating success with increase dose was observed (Figure 3). A one-way ANOVA indicated significant differences between groups ($F(5,294) = 2.386$, $p<.0001$). A Dunnett's multiple comparisons test indicated significant differences in mating success in 27.8 Gy ($p<.0001$), 23.2 Gy (0.0001), and 16.2Gy (0.0060) groups compared to non-irradiated controls.

2.4 DICUSSION

The importance of male cuticular hydrocarbons in insect sex and species recognition, as well as mating behavior has been well studied in literature. von Hormann-Heck (2010) was one

of the first researchers to show that male crickets required contact with the female cuticle to differentiate between sexes. Later, Tregenza and Wedell (1997) showing that without these female chemical's males would not display mating behavior. Further studies using *Acheta domesticus* showed that males touched with the antenna of another male will display aggressive behavior but when touched with a female antenna would produce mating behavior i.e. courtship songs (Tregenza & Wedell, 1997). Further studies have indicated that males touched with specifically the cuticle extract from a male cricket showed aggressive and avoidance behavior and with female extract showed mating behavior (Iwasaki & Katagiri, 2008). In regard to mating, there are several studies, many in cockroaches, which indicate that chemical signals allow females to detect aspects of male fitness, including male dominance, immunocompetence, status, and genetic relatability (Moore et al., 1995; Moore et al., 1997; Rantala & Kortet, 2003; Thomas & Simmons, 2009; Thomas & Simmons, 2011).

In our study, we focused on chemo-signaling, as they are vital to communication in the vast majority of species (Lockey, 1985; Johansson & Jones, 2007). The production and radiation alterations of cuticular hydrocarbons were analyzed in both irradiated and control male crickets. Previous studies using cricket species found that volatile hydrocarbon pheromones were distinct between sex's and among species (Warthen & Uebel, 1980). Results indicated a species-specific signature associated with male cuticular hydrocarbons. Both irradiated and control *Acheta* males showed identical 26 hydrocarbon peaks in their chromatogram (Figure 1). Although the same 26 peaks were present in all groups and that male size was controlled for, chromatograph 'intensity', which refers to concentration in the sample, was altered between control and irradiated males. Two of the larger peaks (22 and 23), were significantly altered in intensity in almost all males exposed to radiation doses spanning 4.6-27.8Gy (Figure 2). In these two peaks intensity was

significantly increased, in some cases almost doubling in concentration compared to controls. Furthermore, all irradiated groups tended toward increased concentration in all irradiated groups for many hydrocarbons (16 of 26 peaks) (Table 2).

Our results also indicated an observed dose-dependent decline in mating success as dose increased. Significant differences were evident in the 27.8 Gy ($p<.0001$), 23.2 Gy (0.0001), and 16.2Gy (0.0060) groups compared to non-irradiated controls. Although this decline may not be entirely due to the cuticular hydrocarbon alterations, we postulate that hydrocarbon alterations are likely a contributing factor. This is due to the plethora of literature on how vital male cuticular profiles are to mating success and recognition. However, we acknowledge that male *Acheta domesticus* produce multiple signal modalities to attract and mate females (including acoustics), it is possible that signals interact or synergize (Wagner & Reiser, 2000).

Our results also indicate the importance of further research focusing on the impacts of radiation stress on the sexual signals of insects, particularly in relation to the SIT and in environmental contamination zones. The objective of the SIT is to release sterilized males to compete for mates with normal males resulting in a reduction of the insect populations (Dyck et al., 2005). Understanding radiation impacts to sexual signals is therefore vital as if males are sterilized but are unable to attract mates the desired outcome of the SIT will be diminished. As well, in areas of contamination sites such as Chernobyl, Fukushima, and waste sites associated with the normal operating procedures of nuclear reactors, determining the impacts to species communication is essential (Imanaka et al., 2015).

Further research should focus more on understanding the subtle impacts of radiation exposure such as to chemical cues but also other modes in which organisms communicate. As well, future experiments to test the specific influence of hydrocarbon alterations, whether the

changes to specific peaks correspond to mating success or whether the entire signature is required would be most interesting. As well, the influence of chemical signaling irrespective of other sexual signals would also be intriguing.

2.5 ACKNOWLEDGMENTS

The authors thank Dr. Fan Fei and Dr. M. Kirk Green for assistance with performing and interpreting the GC-MS analyses.

2.6 TABLES AND FIGURES

Table 1: Two-way ANOVA results representing the relative impact of error, dose, and peak on *Acheta domesticus* cuticular hydrocarbon analysis.

	SS	**DF**	**MS**	**F (DFn, DFd)**	**P value**
Interaction	2.405e+016	125	1.924e+014	F (125, 728) = 1.552	P=0.003
Peak	4.238e+017	25	1.924e+014	F (25, 728) = 136.8	P<0.0001
Dose	5.053e+015	5	1.011e+015	F (5, 728) = 8.153	P<0.0001
Residual	9.024e+016	728	1.240e+014		

Table 2: Summary of GC results for cuticular hydrocarbon peaks. Concentration for Control males, calculated as area under each peak, with % change from control (4.6-27.8Gy).

Peak #	Control (Peak Area)	4.6Gy	9.3Gy	16.2Gy	23.2Gy	27.8Gy
1	17409157.5	0.21	-7.96	37.51	7.63	24.24
2	6698274.5	24.37	-1.91	13.50	-8.75	8.32
3	7716919	5.08	14.91	193.25	13.69	55.18
4	10946526.3	-16.14	-18.96	-24.92	24.63	23.59
5	4219188.33	-20.69	-15.71	-32.85	46.81	18.07
6	34076790.7	34.64	10.91	48.39	1.75	27.14
7	5214956	66.70	45.23	61.29	4.23	48.56
8	25207099.5	37.57	20.89	22.47	18.48	29.99
9	4336674.5	138.65	97.12	225.87	59.36	100.78
10	5276627.67	-29.75	-29.07	-42.84	9.56	-5.33
11	3303594.17	47.67	27.77	69.15	11.53	53.69
12	1298574.67	98.53	129.77	212.14	38.94	119.50
13	26059292.2	14.32	0.27	-15.97	20.48	24.23
14	9826848.33	4.31	-12.39	-22.15	0.23	-4.09
15	5900219.17	-13.12	-18.49	-25.03	14.43	15.18
16	5189231	-20.47	-33.20	-38.82	-0.41	-24.72
17	5501772.33	125.30	111.63	93.97	22.21	79.37
18	23853652.5	30.01	6.23	-8.85	17.67	25.76
19	29395787.7	6.47	-14.30	-32.85	0.74	-3.77
20	3789247.33	87.39	96.08	75.38	6.63	68.59
21	5358352.83	2.22	34.43	76.41	36.71	34.30
22	24211545.2	96.79 **	87.53 *	132.81 ****	17.17	79.84 *
23	74548407.7	79.90 ****	69.97 ****	85.05 ****	23.33 *	53.06 ****
24	5917652.17	149.49	175.96	218.35	61.62	99.56
25	6846318	161.47	124.19	118.86	95.44	163.73
26	11032329.3	107.51	75.631	81.94	39.25	92.05

Table 3. Summary of GC/MS results for male cuticle profile with likely compound candidates using a pooled sample including individuals from 0–27.8Gy.

Peak #	RT (min)	Hydrocarbon	Compound	Match	Mol. Weight	Branched
1	25.857	$C_{34:0}$	Hexacosane, 9-octyl	855	478	Y
2	26.217	$C_{29:0}$	Nonacosane	922	408	N
3	26.355	$C_{29:1}$	Z-14-Nonacosane	852	406	N
4	26.427	$C_{30:0}$	11-Methylnonacosane	855	422	Y
5	27.213		Unresolved			
6	27.55	$C_{36:o}$	Hexatriacontane	855	506	N
7	28.00	$C_{35:1}$	Tetratriacontane	745	490	N
8	28.03	$C_{34:0}$	13-Methylnonacosane	747	478	Y
9	28.13		Unresolved			
10	28.77	$C_{36:0}$	Hexatriacontane	811	506	N
11	29.01	$C_{34:0}$	Tetratriacontane	710	478	N
12	29.06	$C_{35:1}$	17-Pentatriacontene	626	490	N
13	29.55	$C_{39:0}$	11,15-Dimethylheptatriacontane	722	548	Y
14	29.62	$C_{43:0}$	Tritetracontane	734	604	N
15	30.21	$C_{40:0}$	Tetracontane	792	562	N
16	30.32	$C_{44:0}$	Tetratetracontane	685	618	N
17	30.8	$C_{35:1}$	17-Pentatriacontene	833	490	N
18	30.94	$C_{40:0}$	Tetracontane	768	562	N
19	31.05	$C_{37:0}$	15,19-Dimethylpentatriacontane	857	520	Y
20	31.49	$C_{35:1}$	17-Pentatriacontene	755	490	N
21	31.68	$C_{38:2}$	1,37-Octatriacontadiene	693	530	N
22	31.97	$C_{35:1}$	17-Pentatriacontene	821	490	N
23	32.61	$C_{38:2}$	1,37-Octatriacontadiene	736	530	N
24	32.74		Unresolved			
25	34.36	$C_{38:2}$	1,37-Octatriacontadiene	760	530	N
26	34.45	$C_{38:2}$	1,37-Octatriacontadiene	660	530	N

Potential candidates were identified using NIST library. Match probability, and compound characteristics are shown. Cn:x where n is the number of carbons and x is the number of double bonds.

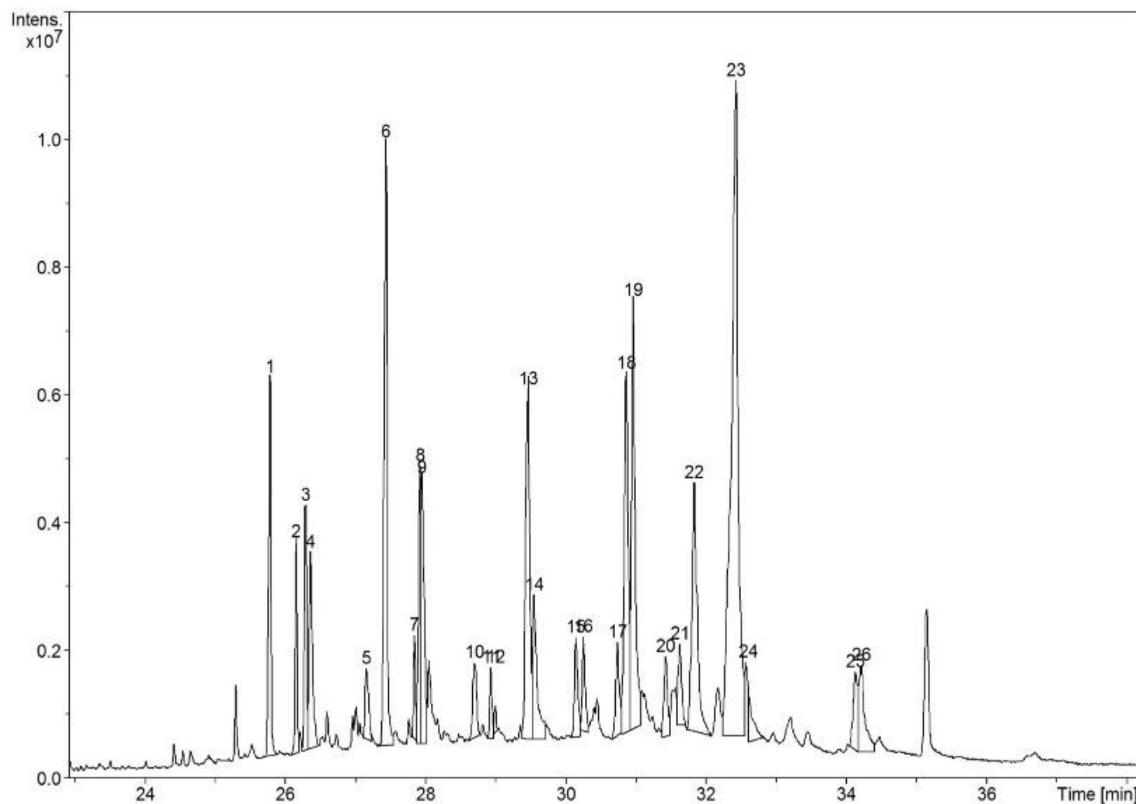


Figure 1. A typical total ion chromatogram of a control male *Acheta domesticus* extracted using a hexane solvent at 1 week post maturation (~50 days). All individuals were immobilized with CO_2 and swirled in hexane for 5 min at a concentration of 5ml per 0.5 g of cricket mass. Samples were analyzed at McMaster's regional center for mass spectrometry using an Agilent 5973/6890 gas chromatography instrument and Bruker Compass DataAnalysis 4® software for analysis. All 26 hydrocarbon peaks analyzed are labeled. Each peak corresponds to a specific hydrocarbon compound.

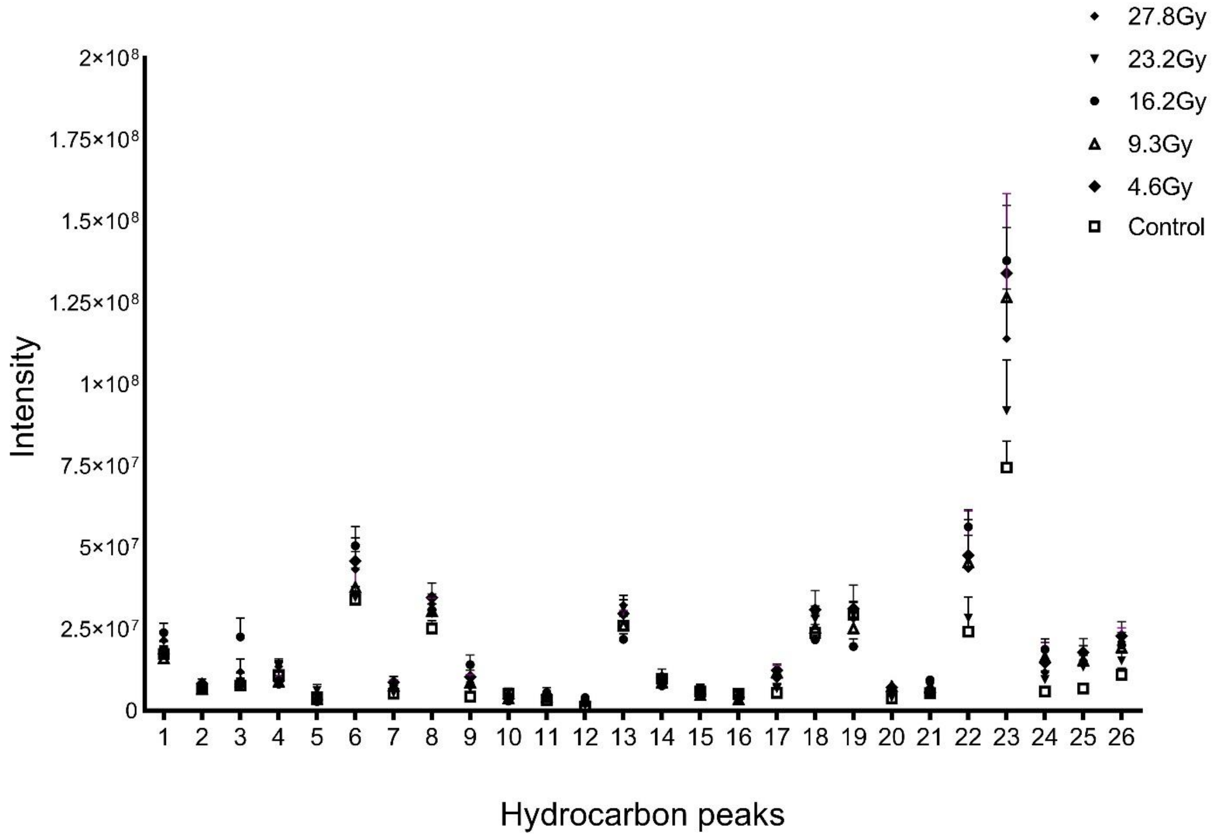


Figure 2. Dose-response effects of early life radiation on cuticular hydrocarbons, specifically the effect on each of the 26 significant hydrocarbon peaks identified using gas-liquid chromatography. All individuals were irradiated at 14 days of age at 0.58 Gy/min with hydrocarbon extraction occurring 1-week post maturation. Values are shown as the mean area under each peak/concentration at each dose ± SEM; 0 Gy (n = 6), 4.6Gy (n = 6), 9.3 Gy (n = 5), 16.2 Gy (n = 5), 23.2 Gy (n = 6), and 27.8 Gy (n = 6). Significant impacts were identified for peak 22; 4.6Gy (p = .0014), 9.3Gy (p = .0080), 16.2Gy (p<.0001), and 27.8Gy (p = .0124) and peak 23; 4.6Gy (p<.0001), 9.3Gy (p<.0001), 16.2Gy (p<.0001), 23.2Gy (p = .0303) and 27.8Gy (p<.0001) compared to control values. All significant differences were analyzed compared to control values using a 2-way ANOVA followed by Dunnett's multiple comparison test. Mass of the cricket was controled for when extracting hydrocarbons.

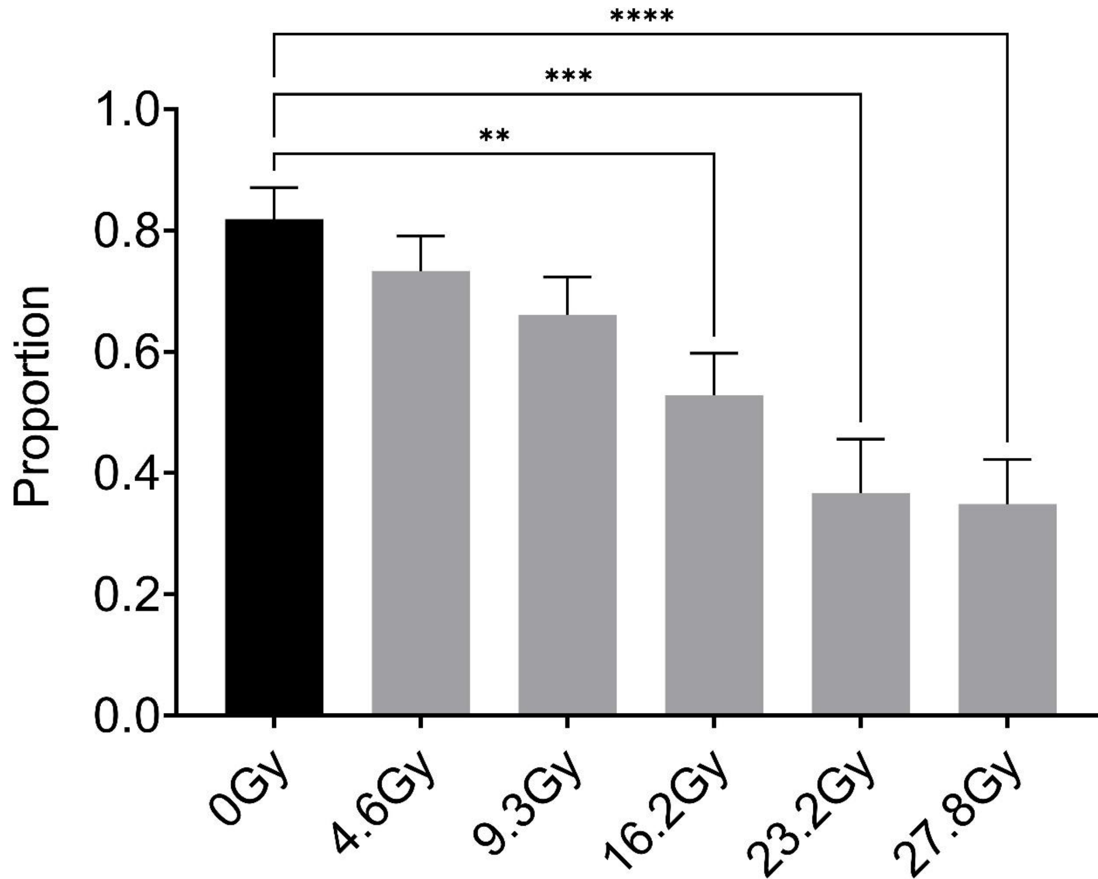


Figure 3. Effects of early life radiation on male *Acheta domesticus* mating success. Males were irradiated at 14 days of age (0–27.8Gy) at a dose rate of 0.58 Gy/min and paired with normal unirradiated females 2–3 weeks post maturation. Success was indicated as female mounting of males. A one-way ANOVA indicated significant differences between groups ($F(5,294) = 2.386$, $p<.0001$). A Dunnett's multiple comparisons test indicated significant differences in mating success in 27.8 Gy ($p<.0001$), 23.2 Gy (0.0001), and 16.2 Gy (0.0060) groups compared to non-irradiated controls.

2.7 REFERENCES

Assis, B. A., Trietsch, C., & Foellmer, M. W. (2017). Male mate choice based on chemical cues in the cricket *Acheta domesticus* (O rthoptera: G ryllidae). *Ecological Entomology*, *42*(1), 11–17.

Balakrishnan, R., & Pollack, G. S. (1997). The role of antennal sensory cues in female responses to courting males in the cricket *Teleogryllus oceanicus*. *Journal of Experimental Biology*, *200*(3), 511–522.

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.

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

Einor, D., Bonisoli-Alquati, A., Costantini, D., Mousseau, T. A., & Møller, A. P. (2016). Ionizing radiation, antioxidant response and oxidative damage: A meta-analysis. *Science of the Total Environment*, *548*, 463–471.

Engl, T., Michalkova, V., Weiss, B. L., Uzel, G. D., Takac, P., Miller, W. J., Abd-Alla, A. M., Aksoy, S., & Kaltenpoth, M. (2018). Effect of antibiotic treatment and gamma-irradiation on cuticular hydrocarbon profiles and mate choice in tsetse flies (*Glossina m. Morsitans*). *BMC Microbiology*, *18*(1), 155–167.

Henneken, J., Goodger, J. Q., Jones, T. M., & Elgar, M. A. (2017). Diet-mediated pheromones and signature mixtures can enforce signal reliability. *Frontiers in Ecology and Evolution*, *4*, 145.

Hörmann-Heck, S. (2010). Untersuchungen über den Erbgang einiger Verhaltensweisen bei Grillenbastarden (*Gryllus campestris* L." *Gryllus bimaculatus* De Geer) 1. *Zeitschrift Für Tierpsychologie*, *14*(2), 137–183.

Imanaka, T., Hayashi, G., & Endo, S. (2015). Comparison of the accident process, radioactivity release and ground contamination between Chernobyl and Fukushima-1. *Journal of Radiation Research*, *56*(suppl_1), i56–i61.

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.

Johansson, B. G., & Jones, T. M. (2007). The role of chemical communication in mate choice. *Biological Reviews*, *82*(2), 265–289.

Kavaliers, M., Choleris, E., Ågmo, A., & Pfaff, D. W. (2004). Olfactory-mediated parasite recognition and avoidance: Linking genes to behavior. *Hormones and Behavior*, *46*(3), 272–283.

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*, 77–83.

Kotiaho, J., Alatalo, R. V., Mappes, J., & Parri, S. (1996). Sexual selection in a wolf spider: Male drumming activity, body size, and viability. *Evolution*, *50*(5), 1977–1981.

Lockey, K. H. (1985). Insect cuticular lipids. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry*, *81*(2), 263–273.

Moore, A. J., Reagan, N. L., & Haynes, K. F. (1995). Conditional signalling strategies: Effects of ontogeny, social experience and social status on the pheromonal signal of male cockroaches. *Animal Behaviour*, *50*(1), 191–202.

Moore, P. J., Reagan-Wallin, N. L., Haynes, K. F., & Moore, A. J. (1997). Odour conveys status on cockroaches. *Nature*, *389*(6646), 25–25.

Pavković-Lučić, S., Miličić, D., & Lučić, L. (2012). *Insect Hydrocarbons. Biology, Biochemistry, and Chemical Ecology edited by Gary J. Blomquist, Anne-Geneviève Bagnères*.

Peschke, K. (1987). Cuticular hydrocarbons regulate mate recognition, male aggression, and female choice of the rove beetle, *Aleochara curtula*. *Journal of Chemical Ecology*, *13*, 1993–2008.

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.

Surinov, B. P. (2007). Mice with radiation or toxic damage or malignant tumors produce aversive chemosignals repelling intact animals. *Doklady Biological Sciences*, *414*(1), 199–201.

Thomas, M. L., & Simmons, L. W. (2009). Male dominance influences pheromone expression, ejaculate quality, and fertilization success in the Australian field cricket, *Teleogryllus oceanicus*. *Behavioral Ecology*, *20*(5), 1118–1124.

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.

Thomas, M. L., & Simmons, L. W. (2011). Crickets detect the genetic similarity of mating partners via cuticular hydrocarbons. *Journal of Evolutionary Biology*, *24*(8), 1793–1800.

Tregenza, T. O. M., & WEDELL, N. (1997). Definitive evidence for cuticular pheromones in a cricket. *Animal Behaviour*, *54*(4), 979–984.

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.

Wagner Jr, 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.

Warthen Jr, J. D., & Uebel, E. C. (1980). Comparison of the unsaturated cuticular hydrocarbons of male and female house crickets, *Acheta domesticus* (L.)(Orthoptera: Gryllidae). *Insect Biochemistry*, *10*(4), 435–439.

CHAPTER 3

TITLE

Ionizing radiation alters male *Acheta domesticus* courtship songs that are critical for mating success

AUTHORS

Tamara M. Fuciarelli* & C. David Rollo

Department of Biology, McMaster University, 1280 Main Street West, Hamilton ON, L8S 4L8, Canada Accepted: 26 April 2021. *Corresponding author: Email fuciartm@mcmaster.ca

Published online: 10 July 2021
Animal Behaviour 2021©

CITATION

Fuciarelli, T. M., & Rollo, C. D. (2021). Ionizing radiation alters male (*Acheta domesticus)* courtship songs that are critical for mating success. *Animal Behaviour,* 176, 209-216. https://doi.org/10.1016/j.anbehav.2021.06.018

3.1 INTRODUCTION

Ionizing radiation has in recent times become a focus of research relating to the impacts of environmental stress. This is due to both the increase in environmental contamination zones from disasters such as in Chernobyl (in 1986) and more recently Fukushima (in 2011), as well as zones associated with normal operating waste from nuclear reactors (Imanaka et al., 2015). Research in this area has been conducted mainly on vertebrate species with invertebrates having

been largely underrepresented (ICRP, 2008). Invertebrates, and more specifically insects, however, provide vital ecosystem services including pollination, predation and decomposition (Tanaka et al., 2016). Studies conducted in areas surrounding Chernobyl, 20 years post disaster, indicate an unprecedented reduction in the abundance of insect pollinators, which has resulted in negative impacts to ecosystem functioning, i.e. lower fruit production and reductions in the number of frugivores (Møller et al., 2012). More generally, the Fukushima and Chernobyl contamination zones have exhibited widespread reductions in the abundance and diversity of many invertebrate species (Møller et al., 2009; Mousseau & Møller, 2014; Tanaka et al., 2016). Most remarkably, impacts to invertebrates have been shown in areas in which the dose rate ranges from 0.01 to 1 uGy/h, indicating that, even at these relatively low dose rates, invertebrates are being impacted (Møller et al., 2009). Reductions in invertebrate diversity and abundance may have drastic effects on ecosystem functioning and are therefore of importance to understanding the full impacts of radiation contamination zones (Jankielsohn, 2018; Møller et al., 2009). Nevertheless, due to their general perception as ‘pests’ to human agriculture, their ecological importance is often overlooked, and the research that is conducted often focuses on their eradication, i.e. the sterile insect technique (SIT) (Jankielsohn, 2018).

The majority of cellular radiation damage occurs as a result of the excess production of reactive oxygen species (ROS) (Einor et al., 2016). ROSs at concentrations that exceed cell tolerance can damage macromolecules including DNA. At high doses of exposure this damage can manifest as reduced function, growth, fertility and longevity (Einor et al., 2016; Ma, 2013). Radiation impacts may also extend to evolutionary consequences. As outlined by Bickham (2011), environmental contamination can produce genome-wide changes to genetic diversity within populations, changes to genotypic frequencies due to selection acting on survivorship,

changes in dispersal or gene flow and changes in genotypic frequencies caused by increased mutation rates. Mutations rates, in particular, have been shown in the Chernobyl region to be highly correlated with radiation exposure (Møller et al., 2015). As well, germ-line mutation rates have been shown to have remained elevated in populations found within Chernobyl, even after 20 years (Møller & Mousseau, 2003, 2006).

Sexual selection theory states that individuals in poor condition should be less able to obtain mates if there are individuals in better condition in the population (Bertram & Rook, 2012). Individuals may use several modalities to signal their quality to potential mates. Insects, like many other species in the animal kingdom, require the use of sexual signals for successful reproduction, where a choosy sex uses signal(s) to determine the 'best' mate (Steiger & Stökl, 2014). Sexual signals can therefore provide honest information about mate quality when signalling to potential mates (Harrison et al., 2013). In Chernobyl, the syllable rate of cuckoo calls has been shown to be positively correlated with radiation levels and habitat (Møller et al., 2016). As well, in Fukushima, physical abnormalities in lycaenid butterflies and in gall-forming aphids have been discovered to persist and compound through multiple generations (Tanaka et al., 2016). Physical abnormalities that occur in structures associated with sexual signalling may have drastic impacts to an individual's ability to mate and overall fitness. In the case of the cuckoos, where calls are radiation dependent, the impact occurs in the individuals' ability to compete with other individuals that do not produce altered calls.

In the case of cricket species in which females are the 'choosy sex', males use a combination of acoustic sexual signalling and chemical pheromones to mediate female preference (Balakrishnan & Pollack, 1996; Crankshaw, 1979; Holzer, 2003; Libersat et al., 1994; Nelson & Nolen, 1997). Male crickets are known to produce three distinct acoustic signals: a

calling song, which is used to attract females from a distance; an aggressive song, which is used when males encounter other males; and a courtship song, which is used to elicit a mating response from females (Alexander, 1961). Mating consists of females mounting males to accept spermatophores. In *Acheta domesticus,* courtship songs have also been shown to be necessary components to induce the female mounting response (Crankshaw, 1979). Male acoustic traits should therefore be modulated to enhance reproductive success, and female preferences for these traits should result in minimal signal variation persisting in the population (Thomson et al., 2014). Several studies have indicated the condition dependence of acoustic signals in crickets and how structural changes to courtship songs alter male mating success (Bailey & Gwynne, 1988; Bertram & Rook, 2012; Brown et al., 1996; Gray & Eckhardt, 2001). Females have been shown to preferentially orient towards conspecific males using fundamental frequency and temporal parameters (Doherty & Hoy, 1985; Eisner & Popov, 1978). Parameters shown to impact female preference have included chirp rate and chirp duration (Wagner & Reiser, 2000). Radiation-induced alterations to vital components of mating (acoustic signalling) may therefore impact male mating success.

The importance of crickets as a model, although not as well known as the premier insect genetic model (*Drosophila melanogaster*)*,* has been outlined in several articles, but most notably in the book entitled *The Cricket as a Model Organism* (Horch et al., 2017). Here, crickets are described as an ideal model as they are not only found in all the worlds ecosystems, with the exception of the Subarctic and Arctic regions, but they are also an ideal model for the study of sexual signalling and mating success. Indeed, over the last century cricket mating behaviour has been studied extensively in a behavioural, acoustic and neurophysiological context (Horch et al., 2017). Studies in cricket species have aimed to tease apart the relative contribution of the two

main sexual signals used by male crickets to attract females: chemical pheromones and acoustic signalling (Horch et al., 2017). As mentioned, studies have indicated that acoustic signalling is vital to mating and that these signals are condition dependent, relaying important information about male quality (Balakrishnan & Pollack, 1996; Bennet-Clark, 2003; Gray & Eckhardt, 2001; Simmons & Ritchie, 1996; Thomson et al., 2014). Cuticular hydrocarbons and pheromones have been shown to be species and sex specific, as well as vital for mating success in males (Tregenza & Wedell, 1997). Crickets have also been studied in relation to radiation impacts to various life history traits including life expectancy and reproduction (Hunter & Krithayakiern, 1971; Menhinick & Crossley, 1968).

Here, we aimed to determine the impacts of early life ionizing radiation exposure on male crickets' (*A. domesticus*) acoustic signalling, specifically, courtship songs. We predicted that, due to radiation-induced damage to male wing structures, acoustic signalling would be altered in a dose-dependent manner and this would subsequently translate to a reduction in male mating success. We analysed temporal and spectral acoustic parameters of courtship songs from irradiated and control males (0–27.8 Gy). We further analysed the impacts of radiation exposure on mating success of both control and irradiated males paired with normal females to determine the impacts of potential acoustic signalling on male mating success.

3.2 METHODS

Breeding Colony: *Acheta domesticus* were generated in a large breeding colony housed in an acrylic terrarium (93 × 64.2 × 46.6 cm), insulated with 1.5 cm thick Durofoam insulation. Fans provided air circulation. The colony was maintained at 29 ± 2 ºC on a 12:12 h light:dark

cycle. Food consisted of *ad libitum* chick feed (Country Range MultiFowl Grower, 17% protein, Quick Feeds Feed Mill, Copetown, Canada) and *ad libitum* distilled water (soaked cellulose sponges) replaced daily. Crickets were provided with egg carton shelters and paper towels sprayed daily with distilled water and replaced weekly. The colony was provided with oviposition medium (Vigoro Organic Garden Soil, The Mosaic Co., Lake Forest, IL, U.S.A.) in small plastic containers (7 × 7 × 7 cm).

Experimental Groups: Experimental animals were generated from the breeding colony by leaving an oviposition container filled with Vigoro Organic Garden Soil. To ensure experimental animals were of the same age, the oviposition container was removed from the breeding colony after 24 h and incubated at 29 ± 2 ºC. After individuals hatched from the oviposition containers (after 14 days), approximately 1000 nymphs were separated after 24 h of hatching, to again ensure the same-aged individuals were used. Experimental individuals were housed in the same conditions as the breeding colony. At 14 days of age, fourth-instar nymphs were randomly selected, separated and irradiated for specific durations using a Cs-137 source at a dose rate of 0.58 Gy/min totalling 0 Gy, 4.6 Gy, 9.3 Gy, 16.2 Gy, 23.2 Gy and 27.8 Gy at the Taylor Radiobiology Source at McMaster University (Hamilton, ON, Canada). A maximum dose of 27.8 Gy was chosen as previous work has shown sterilization to occur around this dose. All individuals from each experimental group were irradiated during a single exposure. All groups were then immediately brought to McMaster's Life Sciences Building (LSB) where they were maintained for life. Adult males are known to fight other males for access to females, often causing bodily damage, so at approximately 30 days of age, before adult maturation, females were removed from all experimental groups. Prior to 30 days of age, males and females are

indistinguishable from one another.

Mating Success: At midlife, approximately 56–67 days post hatching, experimental adult males from each group (control: $N = 55$; 4.6Gy: $N = 60$; 9.3Gy: $N = 59$; 16.2Gy: $N = 53$; 23.2Gy: $N = 30$; 27.8Gy: $N = 43$) were individually paired with a nonirradiated female for a 15 min period to assess various mating parameters. During this period, each pair had access to food and distilled water. Ambient temperature of the room was maintained at 26–28 ºC. Mating was considered successful if females mounted a male for more than 5 s. This period was chosen as males occasionally force themselves underneath females, whereby females, if rejecting the male, will jump off. If males successfully mated, the time to mate was recorded. We also recorded any courtship songs produced during the mating period. If males were unsuccessful in their first trial, they were given a second 15 min trial with a new female to ensure that female inability to mate was not the cause of a failed mating trial. If a male was successful in mating, forewings were removed at the tegmina. Males were then given a 15 min rest period and re-paired with the same female to assess the obligatory nature of courtship songs as it relates to mating success. Data regarding mating success was originally submitted in a sister article regarding male cuticular hydrocarbons. Although data for both experiments were collected in unison, new data regarding wing removal, proportion of singing males, the proportion of singing males that successfully mated and the time to mate are only reported here.

Courtship Recordings: Courtship songs were recorded from adult male *A. domesticus* during the above-described mating trials. Recordings were made using a Zoom H5 recorder (24-bit/44.1 kHz sample rate). Because of extreme variances in amplitude (dB) modulation of control

versus irradiated males, we used variable distances from the microphone in order to reduce sound clipping. The size of the container, which allowed the male and female the freedom to move and turn in any direction, also made controlling for distance difficult. All recordings were taken 10–50 cm away from the centre of each container. We recorded the males, then saved and stored the samples for subsequent spectral and temporal analysis.

Recording Analysis: All song analysed were completed using Raven Pro (v.1.6.1; Center for Conservation Bioacoustics, NewYork, U.S.A., http://ravensoundsoftware.com/). High-frequency chirps were analysed separately from low-frequency trills in order to distinguish exactly where variation was occurring (Fig 1). For each recording, a maximum of 11 high-frequency chirps and 20 low-frequency trills were taken from each individual male to ensure a large number of males were analysed in each group. Because of the reasons mentioned above, as well as the importance of frequency (Hz) and not amplitude (dB) modulation influencing male mating success (Balakrishnan & Pollack, 1996), we aimed to analyse only frequency-related spectral parameters. Temporal patterns were analysed by manually measuring syllable durations (delta time) using the selection tool in Raven Pro. Similarly, spectral patterns were analysed by manually measuring peak and mean/centre frequency and average entropy for each syllable. Sample sizes for high-frequency chirps included 0Gy ($N = 275$), 4.6Gy ($N = 294$), 9.3Gy ($N = 243$), 16.2Gy ($N = 139$), 23.2Gy ($N = 40$) and 27.8Gy ($N = 57$) and those for low-frequency trills included 0Gy ($N = 482$), 4.6Gy ($N = 521$), 9.3Gy ($N = 434$), 16.2Gy ($N = 245$), 23.2Gy ($N = 72$) and 27.8Gy ($N = 98$). All spectral and temporal parameters are reported as mean ± SE.

Statistics: All courtship parameters were analysed using a one-way ANOVA followed by

a Dunnett's multiple comparisons test to determine significant differences in irradiated males compared to control males. Song parameter analysis was completed on each spectral and temporal parameter, and separately for both the high-frequency chirps and low-frequency trills. All mating parameters were also analysed using a one-way ANOVA followed by a Dunnett's multiple comparisons test to determine significant differences in irradiated groups compared to control males. All statistical analyses were completed using Prism GraphPad 9.0.0 (https://www.graphpad.com).

Ethical Note: To avoid unnecessary harm to our animals, groups were maintained daily, ensuring that they were provided with adequate food and water. We also ensured that containers were kept at relatively low densities to reduce stress. Containers were cleaned weekly. We also provided egg carton containers for stimulation. House crickets do not require any licences or permits. Our study was completed following guidelines prescribed by McMaster University for the care of invertebrate species. Approximately 500 males and 500 females were used in this study. This large number was used as we needed adults for this study. This species like many insects have high mortality rates before adulthood, so large numbers were required. Individuals were disposed of after completion of the experiment by placing housing containers in a -20 °C freezer, which killed the crickets within 10 min. Crickets were irradiated as part of the study and kept unharmed until adulthood. The only manipulation was to remove wings after mating. This was done by anaesthetizing the crickets using CO_2 gas, removing their wings and allowing them a recovery period (15 min) before the experiment continued.

3.3 RESULTS

The average courtship song of a control male *A. domesticus* is illustrated in Fig. 1. Both the waveform and spectrogram displays are shown. All singing males demonstrated this pattern of intermittent high-frequency chirps followed by many lower-frequency trills. Both high-frequency chirps and low-frequency trills were analysed individually.

High-frequency Chirps: *Mean frequency:* There were significant differences in mean frequency between groups ($F_{5,1041} = 4.075$, $P = 0.0011$), with significant decreases in mean frequency in 16.2Gy ($P = 0.0340$) and 27.8Gy ($P = 0.0112$) males (Fig. 2). *Peak frequency:* There was no significance difference in peak frequency between groups ($F_{5,1042} = 1.980$, $P = 0.0791$). However, 16.2Gy males showed a significant decline ($P = 0.0457$) after Dunnett's multiple comparisons test (Fig. 2). *Delta time:* Significant differences in delta time among groups were evident ($F_{5,1042} = 32.63$, $P < 0.0001$) with a significant increase in 4.6Gy ($P = 0.0027$) and a significant decrease in 27.8Gy ($P < 0.0001$) males (Fig. 2). *Average entropy:* Significant differences in average entropy among groups were apparent ($F_{5,1041} = 29.96$, $P < 0.0001$) with significant increases detected in 4.6Gy males ($P = 0.0001$) and in 9.3Gy, 16.2Gy, 23.2Gy and 27.8Gy males ($P < 0.0001$) compared to controls (Fig. 2).

Low-frequency Trills: *Mean frequency:* Significant differences in mean frequency were found among groups ($F_{5,1842} = 181.7$, $P < 0.0001$) with significant increases in mean frequency in 16.2Gy, 23.2Gy and 27.8Gy males ($P < 0.0001$; Fig. 3). *Peak frequency:* Significant differences in peak frequency were also found among groups ($F_{5,1846} = 94.61$, $P < 0.0001$) with

significant increases ($P < 0.0001$) in 16.2Gy, 23.2Gy and 27.8Gy males (Fig. 3). *Delta time:* Significant differences in delta time among groups were revealed ($F_{5,1846} = 42.04$, $P < 0.0001$) with significant decreases detected in 16.2Gy ($P < 0.0001$), 23.2Gy ($P = 0.0202$) and 27.8Gy ($P < 0.0001$) males (Fig. 3). A*verage entropy:* Significant differences in average entropy among groups were also detected ($F_{5,1842} = 127.9$, $P < 0.0001$) with significant increases in 4.6Gy males ($P = 0.0228$) and in 16.2Gy, 23.2Gy and 27.8Gy males ($P < 0.0001$; Fig. 3).

Mating: *Mating success:* Significant declines in mating success were found between 16.2Gy males ($\chi^2_1 = 10.35$, $N = 53$, $P = 0.0013$), 23.2Gy males ($\chi^2_1 = 17.61$, $N = 30$, $P < 0.0001$) and 27.8Gy males ($\chi^2_1 = 22.39$, $N = 47$, $P < 0.0001$) compared to controls. *Proportion of males singing:* Significant decreases in the proportion of males singing were evident in 16.2Gy males ($\chi^2_1 = 5.336$, $N = 53$, $P = 0.0209$), 23.2Gy males ($\chi^2_1 = 24.45$, $N = 30$, $P < 0.0001$) and 27.8Gy males ($\chi^2_1 = 33.43$, $N = 43$, $P < 0.0001$) compared to controls (Fig. 4). *Proportion of singing males that mated:* Significant declines in the proportion of singing males that mated were evident in 16.2Gy males ($\chi^2_1 = 4.629$, $N = 35$, $P = 0.0314$), 23.2Gy males ($\chi^2_1 = 7.01$, $N = 17$, $P = 0.0081$) and 27.8Gy males ($\chi^2_1 = 19.90$, $N = 49$, $P < 0.0001$) compared to nonirradiated controls (Fig. 4b). No significant differences were observed in the time it took for mating to occur in any irradiated groups compared to unirradiated control males (Fig. 4c). Wing removal completely negated male mating behaviour. No males from either control or irradiated groups successfully mated following wing removal. Observational results showed males failing to display typical mating behaviour, i.e. following females around container and aggressively 'backing up' under females to transfer spermatophores.

3.4 DISCUSSION

Here we describe alterations to male *A. domesticus* courtship song parameters with corresponding impacts to male mating parameters. Specifically, for low-frequency trills, we found a dose-dependent increase in both peak and mean frequency, with significant differences in 16.2Gy, 23.2Gy and 27.8Gy males compared to controls. We also observed an increase in average entropy, with significant increases in 4.6Gy, 16.2Gy, 23.2Gy and 27.8Gy males compared to controls. For low-frequency temporal parameters, we saw dose-dependent decreases in the length of each chirp. For high-frequency chirps, dose-dependent increases were evident only in average entropy, with other spectral and temporal parameters showing little change. These alterations in low-frequency trills corresponded to dose-dependent reductions in mating success, with a significant reduction in mating success in 16.2Gy, 23.2Gy and 27.8Gy males compared to controls. Other mating parameters such as the proportion of males who sang as well as the proportion of males who sang and mated were reduced significantly in males that received 16.2–27.8 Gy doses. As high-frequency chirps showed very little spectral or temporal alterations (except in the highest doses), we suggest that low-frequency trills must be a larger contributor to the reduction of mating success.

It has been hypothesized that male song characteristics, i.e. spectral (frequency) and temporal structure convey information about male quality and are vital to male mating success in cricket species (Brown et al., 1996; Libersat et al., 1994; Nelson & Nolen, 1997; Rebar et al., 2009; Tregenza et al., 2006; Zuk et al., 2008). Frequency has been described in the literature as being a likely identifier of male size, with larger males producing lower-frequency calls (Bailey & Gwynne, 1988; Brown et al., 1996). Female crickets prefer males that produce lower-

frequency calls (Brown et al., 1996). Female crickets have also been shown to be tuned to respond to particular frequency ranges, with altered frequencies therefore being disadvantageous to male mating success (Bunting & Hedrick, 2018; Pollack & Faulkes, 1998). Our results support both the condition dependence of courtship songs, i.e. radiation modulates song parameters and that females preferentially choose males of better quality, i.e. mating success of irradiated males (with poorer song quality) was significantly lower than that of controls.

As crickets use signalling modalities other than song for mating, impacts of radiation on these other signalling modalities may also have contributed to the reduced mating success we observed. Although a majority of males that successfully mated produced sound, this was not always the case. This indicates that there were males that were able to successfully mate without courtship song production. However, males were unable to successfully mate with females after wing removal, even if they were successful prior to wing removal (with or without song production). This may indicate that other signalling modalities such as chemical signals (cuticular hydrocarbons) contribute to female preference and male mating success (Botha et al., 2017; Rebar et al., 2009; Thomas & Simmons 2010; Tregenza & Wedell, 1997). It may also suggest the importance of intact wings on male mating behaviour. This is consistent with similar work indicating complete cessation of male mating behaviour upon wing removal even though winged but silent males are still able to mate (Balakrishnan & Pollack, 1996; Crankshaw, 1979; Libersat et al., 1994; Rebar et al., 2009). Recently published work has also indicated that cuticular hydrocarbons are significantly altered by radiation exposure (Fuciarelli & Rollo, 2021b).

The cause of the observed spectral and temporal changes is likely associated with morphological damage to important wing components. Previous studies have indicated

significant dose-dependent declines in centroid size (wing size) and significant morphological alterations to irradiated male wings (Fuciarelli & Rollo, 2021a). Wing deformities were mainly associated with the wings main resonating structures (harp, mirror) (Fuciarelli & Rollo, 2021a). These resonance structures act as regulators of song frequency (Bennet-Clark, 2003) and may explain alterations in frequency that we observed. Wing morphological changes also may explain the increase in average entropy seen in both high- and low-frequency song components, as an increase in average entropy indicates there are more frequencies present in each selection (Bunting & Hedrick, 2018).

Note, however, that the doses applied to male crickets in this experiment were significantly higher than what would be found in even the most highly contaminated areas surrounding Chernobyl and Fukushima. In a laboratory setting it is not always practical to administer chronic low doses through the number of generations needed to achieve the environment in the ecosystems surrounding nuclear disaster sites. We however postulate that damage seen at these relatively high doses may reflect what may be observed in contamination zones after many generations of low-dose chronic exposure. In Chernobyl, mutation rates and the level of genetic damage have compounded over many generations and have even been shown to be correlated with phenotypic effects (Mousseau & Møller, 2014). In both Chernobyl and Fukushima, there has been an increasing number of studies showing a wide range of physiological, developmental, morphological and behavioural consequences of generational chronic exposures (Mousseau & Møller, 2014). It has been suggested that the cause of such impacts is of a genetic basis, specifically of multigenerational mutation accumulation of recessive deleterious mutations. Such genetic alterations include not only germ-line mutations and an increase in mutation frequency, but also changes to gene regulation (e.g. antioxidants). In

birds living in areas surrounding Chernobyl, phenotypic consequences of generational exposure include albinism, abnormal sperm behaviour, increased tumour formation, cataracts and most notably alterations to the shape and size of wings (Mousseau & Møller, 2014). Radiation-induced generational morphological abnormalities to insect wings have also been identified in butterflies in areas surrounding Fukushima over 5 years post-accident (Sakauchi et al., 2020).

These results and the doses administered here, however, have direct relevance to the SIT (sterile insect technique) as they often administer extremely high (i.e. 200 Gy) doses (Dyck et al., 2005). SIT involves sterilization of males using radiation and subsequent release, allowing the sterilized males to mate with normal females and thus reduce the insect population. However, this strategy hinges on these sterilized males being able to compete with normal nonirradiated males for access to females (Dyck et al., 2005). If the dose required to sterilize males reduces their ability to produce the correct sexual signals, they may be unable to mate successfully. Research in our laboratory has shown sterilization as well as severe reproductive decline in males at 23.2 Gy and 27.8 Gy (Fuciarelli & Rollo, 2020). This reproductive decline was observed without severe impacts to longevity or survivorship. This is therefore of importance as males irradiated at these doses may be ideal for release via the SIT but may not be able to mate efficiently due to issues associated with their ability to produce correct sexual signalling.

Our results are significant in understanding the radiation impacts of both environmental contamination sites as well as the impacts associated with the SIT. Radiation exposure may have drastic impacts on the ability of males to use acoustics as the main sexual signal to attract mates. Impacted species also include more than just cricket species. Other acoustic-producing species include lacewings and cicadas as well as other non insect species such as frogs (Fitzpatrick & Gray, 2001). More research should focus on the impact of radiation on other acoustic-producing

species as well as the impact that multiple stressors may have on male mating success and female preference.

3.5 ACKNOWLEDGMENTS

This work was funding by a Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Grant (RGPIN-05693-2015).

3.6 TABLES AND FIGURES

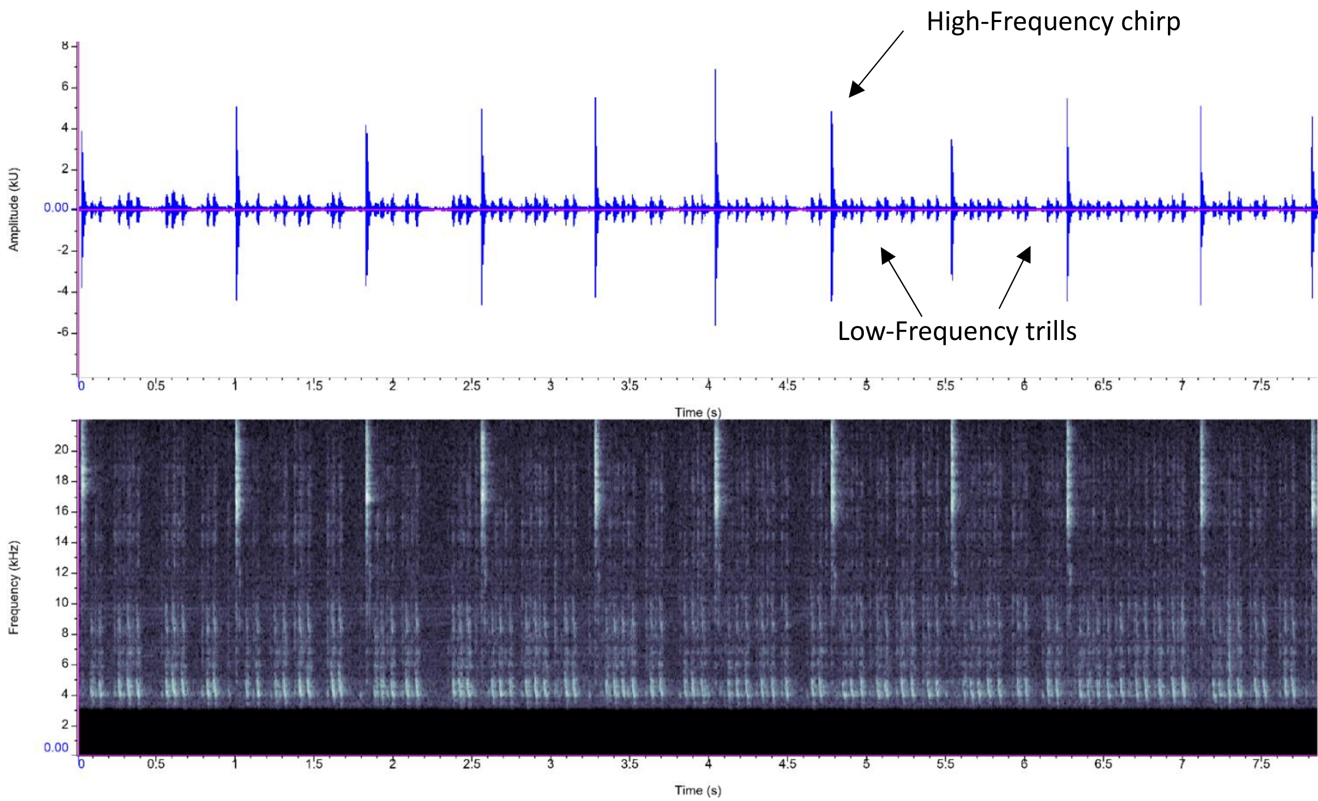


Figure 1. The courtship song of a control male house cricket, *Acheta domesticus*, displayed using Raven Pro software. Courtship song spectral and temporal components analysed included the change in time, peak and mean frequency and average entropy for each selected chip or trill. High-frequency and low-frequency chips were analysed separately and are highlighted above.

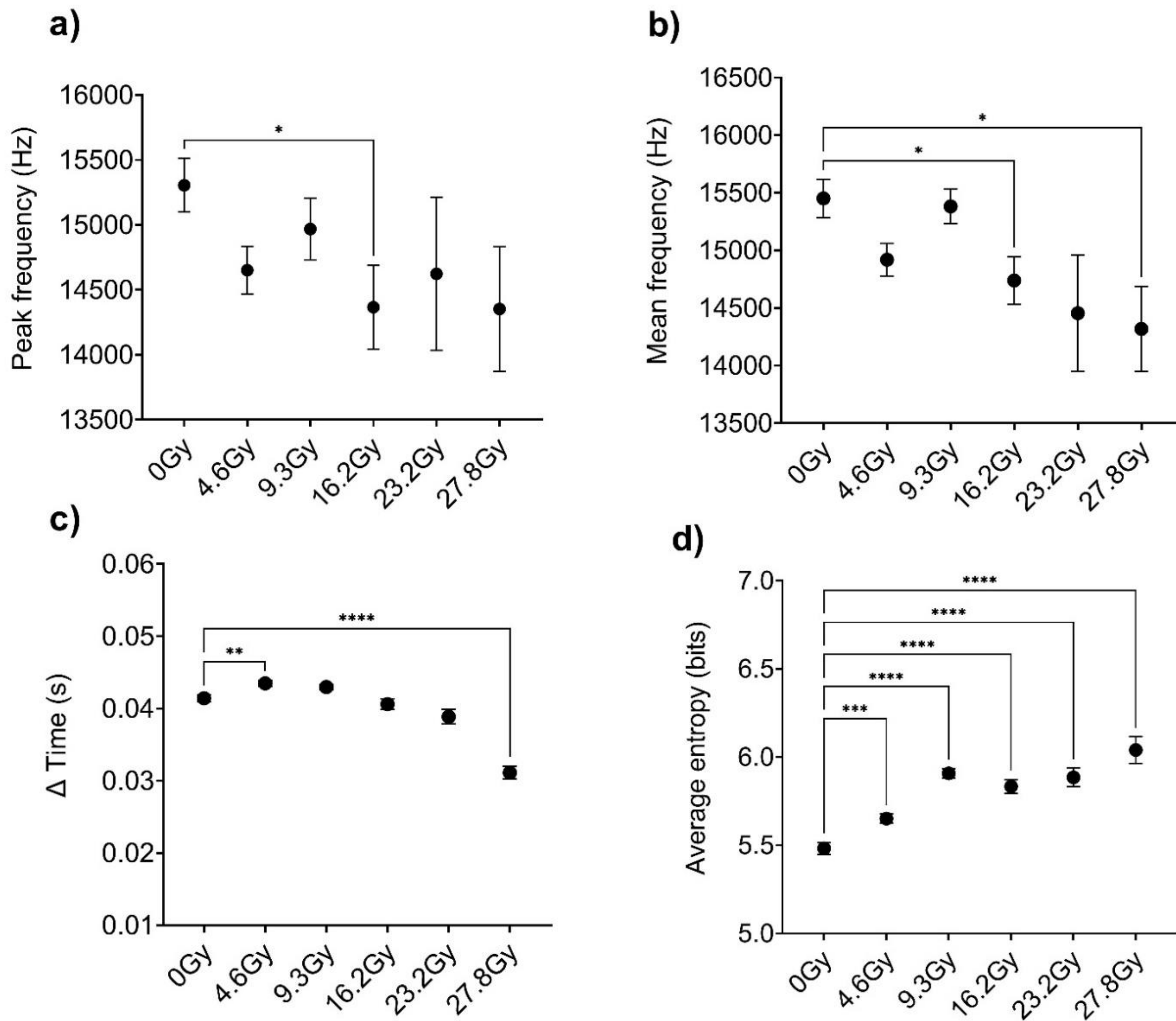


Figure 2. Impacts to courtship song parameters of high-frequency chirps of male *Acheta domesticus* exposed to ionizing radiation (0–27.8 Gy). All males were irradiated at 14 days of age with recordings occurring 2–3 weeks post adult moult. Samples sizes for high-frequency chirps for all parameters: 0Gy ($N = 275$), 4.6Gy ($N = 294$), 9.3Gy ($N = 243$), 16.2Gy ($N = 139$), 23.2Gy ($N = 40$), 27.8Gy ($N = 57$). A maximum of 10 ticks per male was recorded to ensure a range of males were used. Data for all parameters are described as mean ± SE. Parameters included (a) peak frequency, (b) centre/mean frequency, (c) delta time and (d) average entropy. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$.

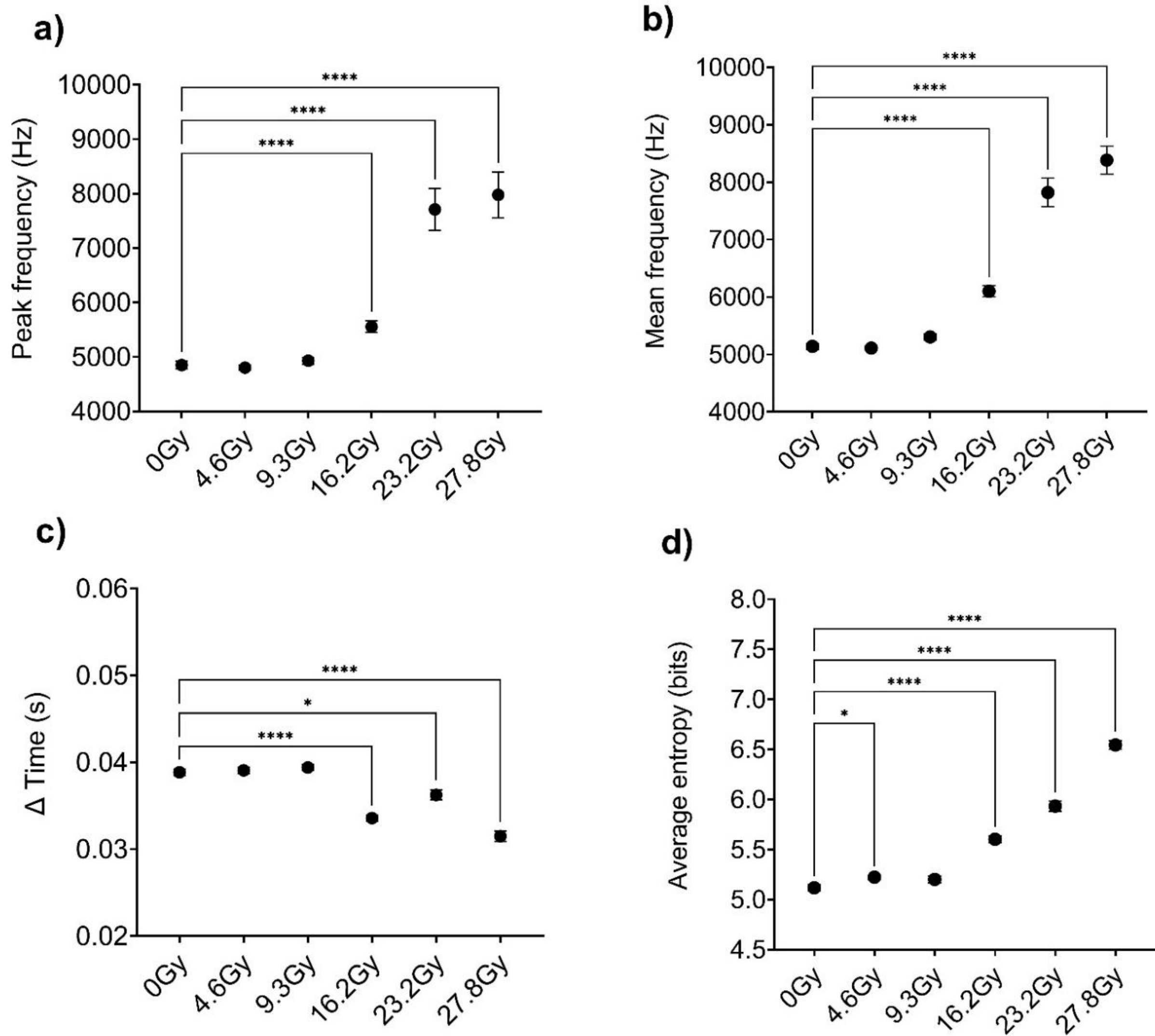


Figure 3. Impacts to courtship song parameters of low-frequency trills of male *Acheta domesticus* exposed to ionizing radiation (0–27.8 Gy). All males were irradiated at 14 days of age with recordings occurring 2–3 weeks post adult moult. Sample sizes for lower-frequency trills for all parameters: 0Gy ($N = 482$), 4.6Gy ($N = 521$), 9.3Gy ($N = 434$), 16.2Gy ($N = 245$), 23.2Gy ($N = 72$), 27.8Gy ($N = 98$). A maximum of 20 ticks per male was recorded to ensure a range of males were used. Data for all parameters are described as mean ± SE. Parameters included (a) peak frequency, (b) centre/mean frequency, (c) delta time and (d) average entropy. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$; $****P < 0.0001$.

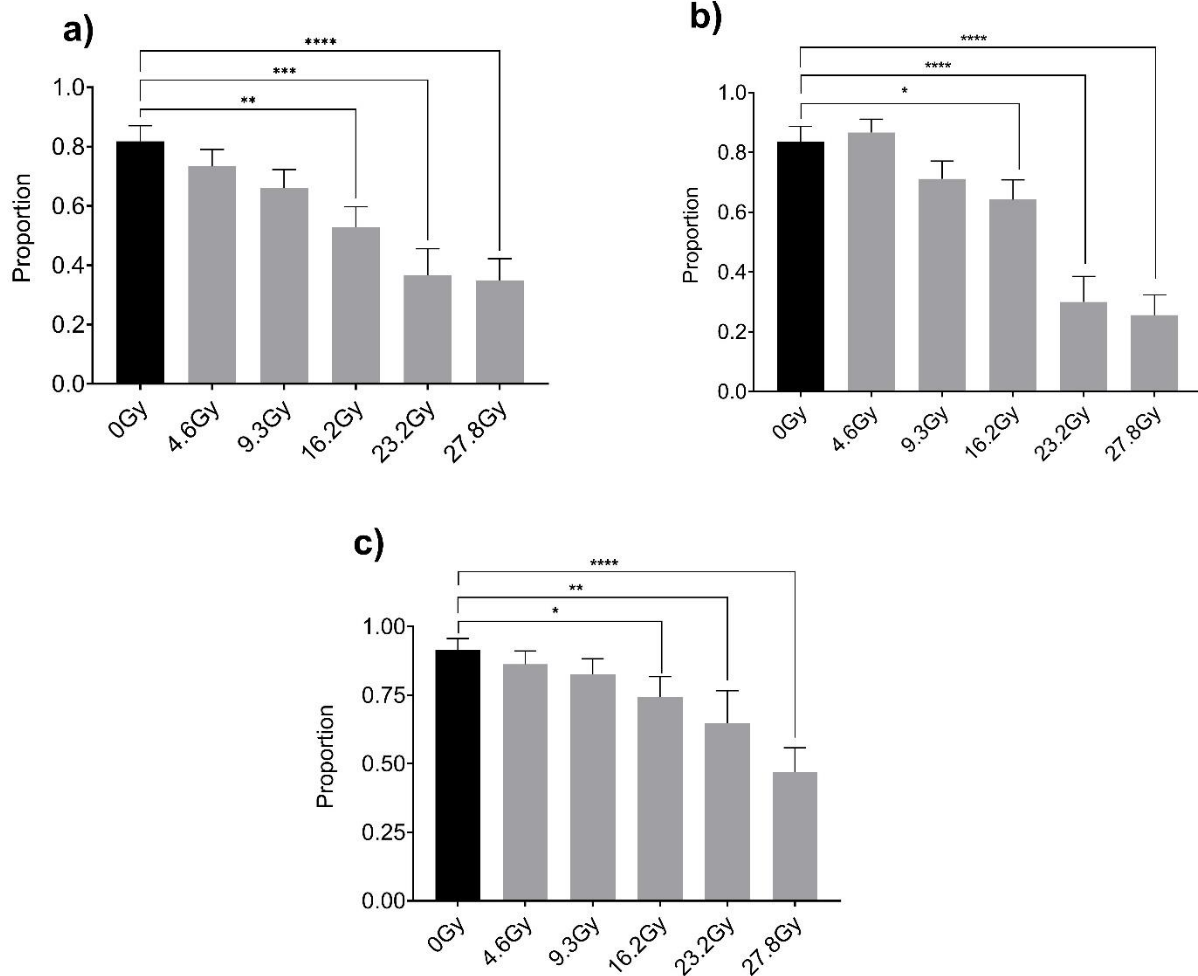


Figure 4. Impacts of radiation (0–27.8 Gy) on male *Acheta domesticus* mating parameters. All males were irradiated at 14 days of age with recordings occurring 2–3 weeks post adult moult. Each male was individually paired with a nonirradiated female for 15 min, during which all data were collected. Mating was considered successful if females mounted a male for a minimum of 5s. Data for all parameters are described as mean ± SE. Parameters included (a) proportion of males that mated, (b) proportion of males who sang and (c) proportion of males who sang and mated. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$.

3.7 REFERENCES

Alexander, R. D. (1961). Aggressiveness, territoriality, and sexual behavior in field crickets (Orthoptera: Gryllidae). *Behaviour*, *17*(2–3), 130–223.

Bailey, W. J., & Gwynne, D. T. (1988). Mating system, mate choice and ultrasonic calling in a zaprochiline katydid (Orthoptera: Tettigoniidae). *Behaviour*, *105*(3–4), 202–223.

Balakrishnan, R., & Pollack, G.S. (1996). Recognition of courtship song in the field cricket, *Teleogryllus oceanicus*. *Animal Behaviour, 51*, 353–366.

Bennet-Clark, H. C. (2003). Wing resonances in the Australian field cricket *Teleogryllus oceanicus*. *Journal of Experimental Biology*, *206*(9), 1479–1496.

Bertram, S. M., & Rook, V. (2012). Relationship between condition, aggression, signaling, courtship, and egg laying in the field cricket, *Gryllus assimilis*: Relationship between condition, aggression, signaling, courtship, and egg laying. *Ethology*, *118*(4), 360–372.

Bickham, J. W. (2011). The four cornerstones of evolutionary toxicology. *Ecotoxicology*, *20*(3), 497–502.

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.

Brown, W. D., Wideman, J., Andrade, M. C. B., Mason, A. C., & Gwynne, D. T. (1996). Female choice for an indicator of male size in the song of the black-horned tree cricket, *Oecanthus nigricornis* (Orthoptera: Gryllidae: Oecanthinae). *Evolution*, *50*(6), 2400–2411.

Bunting, J. E., & Hedrick, A. V. (2018). Male field cricket songs are altered after aggressive interactions. *Frontiers in Ecology and Evolution*, *6*, 164. doi:10.3389/fevo.2018.00164

Crankshaw, O. S. (1979). Female choice in relation to calling and courtship songs in *Acheta domesticus*. *Animal Behaviour*, *27*, 1274–1275.

Doherty, J., & Hoy, R. (1985). The auditory behavior of crickets: Some views of genetic coupling, song recognition, and predator detection. *Quarterly Review of Biology*, *60*(4), 457–472.

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

Einor, D., Bonisoli-Alquati, A., Costantini, D., Mousseau, T. A., & Møller, A. P. (2016). Ionizing radiation, antioxidant response and oxidative damage: A meta-analysis. *Science of the Total Environment*, *548–549*, 463–471.

Eisner, N., & Popov, A. V. (1978). Neuroethology of acoustic communication. *Advances in Insect Physiology, 13* , 229–355.

Fitzpatrick, M. J., & Gray, D. A. (2001). Divergence between the courtship songs of the field crickets

Gryllus texensis and *Gryllus rubens* (Orthoptera, Gryllidae). *Ethology*, *107*(12), 1075–1085.

Fuciarelli, T. M., & Rollo, C. D. (2020). Trans-generational impacts of paternal irradiation in a cricket: Damage, life-history features and hormesis in F1 offspring. *Dose-Response*, *18*(4), 1559325820983214.

Fuciarelli, T. M., & Rollo, C. D. (2021a). Radiation exposure causes developmental alterations in size and shape of wings and structures associated with song production in male crickets (*Acheta domesticus*). *Entomologia Experimentalis et Applicata, 169*(2), 227–234. https://doi.org/10.1111/eea.12999

Fuciarelli, T. M., & Rollo, C. D. (2021b). Impacts of ionization radiation on the cuticular hydrocarbon profile and mating success of male house crickets (*Acheta domesticus*). *International Journal of Radiation Biology, 97*(4), 564–570.

Gray, D. A., & Eckhardt, G. (2001). Is cricket courtship song condition dependent? *Animal Behaviour*, *62*(5), 871–877.

Harrison, S. J., Thomson, I. R., Grant, C. M., & Bertram, S. M. (2013). Calling, courtship, and condition in the fall field cricket*, Gryllus pennsylvanicus*. *PLoS One*, *8*(3), e60356.

Holzer, B. (2003). Condition-dependent signaling affects male sexual attractiveness in field crickets, *Gryllus campestris*. *Behavioral Ecology*, *14*(3), 353–359.

Horch, H. W., Mito, T., Popadić, A., Ohuchi, H., & Noji, S. (Eds.). (2017). *The cricket as a model organism: Development, regeneration, and behavior* (1st ed.). Tokyo, Japan: Springer.

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

ICRP. (2008). Environmental protection: The concept and use of reference animals and plants. *Annals of the ICRP, 39*(6), 1–242.

Imanaka, T., Hayashi, G., & Endo, S. (2015). Comparison of the accident process, radioactivity release and ground contamination between Chernobyl and Fukushima-1. *Journal of Radiation Research*, *56*(Suppl. 1), i56–i61.

Jankielsohn, A. (2018). The importance of insects in agricultural ecosystems. *Advances in Entomology*, *6*(2), 62–73.

Libersat, F., Murray, J. A., & Hoy, R. R. (1994). Frequency as a releaser in the courtship song of two crickets, *Gryllus bimaculatus* (de Geer) and *Teleogryllus oceanicus*: A neuroethological analysis. *Journal of Comparative Physiology A: Sensory, Neural, and Behavioral Physiology*, *174*(4), 485–494.

Ma, Q. (2013). Role of Nrf2 in oxidative stress and toxicity. *Annual Review of Pharmacology and*

Toxicology, *53*, 401–426.

Menhinick, E. F., & Crossley, D. A., Jr. (1968). Comparison of radiation profiles of *Acheta domesticus* and *Tenebrio molitor*. *Annals of the Entomological Society of America*, *61*(6), 1359–1365.

Møller, A.P., Barnier, F., & Mousseau, T. A. (2012). Ecosystems effects 25 years after Chernobyl: Pollinators, fruit set and recruitment. *Oecologia*, *170*(4), 1155–1165.

Møller, A.P., Morelli, F., Mousseau, T. A., & Tryjanowski, P. (2016). The number of syllables in Chernobyl cuckoo calls reliably indicate habitat, soil and radiation levels. *Ecological Indicators*, *66*, 592–597.

Møller, A. P., & Mousseau, T. A. (2003). Mutation and sexual selection: A test using barn swallows from Chernobyl. *Evolution*, *57*(9), 2139–2146.

Møller, A.P., & Mousseau, T. A. (2006). Biological consequences of Chernobyl: 20 years on. *Trends in Ecology & Evolution*, *21*(4), 200–207.

Møller, A.P., & Mousseau, T. A. (2009). Reduced abundance of insects and spiders linked to radiation at Chernobyl 20 years after the accident. *Biology Letters*, *5*(3), 356–359.

Møller, A.P., & Mousseau, T. A. (2015). Strong effects of ionizing radiation from Chernobyl on mutation rates. *Scientific Reports*, *5*(1), 8363.

Mousseau, T. A., & Møller, A. P. (2014). Genetic and ecological studies of animals in Chernobyl and Fukushima. *Journal of Heredity*, *105*(5), 704–709.

Nelson, C. M., & Nolen, T. G. (1997). Courtship song, male agonistic encounters, and female mate choice in the house cricket, *Acheta domesticus* (Orthoptera: Gryllidae). *Journal of Insect Behavior*, *10*(4), 557–570.

Pollack, G., & Faulkes, Z. (1998). Representation of behaviorally relevant sound frequencies by auditory receptors in the cricket *Teleogryllus oceanicus*. *Journal of Experimental Biology*, *201*(1), 155–163.

Rebar, D., Bailey, N. W., & Zuk, M. (2009). Courtship song's role during female mate choice in the field cricket *Teleogryllus oceanicus*. *Behavioral Ecology*, *20*(6), 1307–1314.

Sakauchi, K., Taira, W., Hiyama, A., Imanaka, T., & Otaki, J. M. (2020). The pale grass blue butterfly in ex-evacuation zones 5.5 years after the Fukushima nuclear accident: Contributions of initial high-dose exposure to transgenerational effects. *Journal of Asia-Pacific Entomology*, *23*(1), 242–252.

Simmons, L. W., & Ritchie, M. G. (1996). Symmetry in the songs of crickets. *Proceedings of the Royal Society B: Biological Sciences*, *263*(1375), 1305–1311.

Steiger, S., & Stökl, J. (2014). The role of sexual selection in the evolution of chemical signals in insects. *Insects*, *5*(2), 423–438.

Tanaka, S., Hatakeyama, K., Takahashi, S., & Adati, T. (2016). Radioactive contamination of arthropods

from different trophic levels in hilly and mountainous areas after the Fukushima Daiichi nuclear power plant accident. *Journal of Environmental Radioactivity*, *164*, 104–112.

Thomson, I. R., Darveau, C.-A., & Bertram, S. M. (2014). Body morphology, energy stores, and muscle enzyme activity explain cricket acoustic mate attraction signaling variation. *PLoS One*, *9*(3), e90409.

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.

Tregenza, T., Simmons, L. W., Wedell, N., & Zuk, M. (2006). Female preference for male courtship song and its role as a signal of immune function and condition. *Animal Behaviour*, *72*(4), 809–818.

Tregenza, T., & Wedell, N. (1997). Definitive evidence for cuticular pheromones in a cricket. *Animal Behaviour*, *54*(4), 979–984.

Wagner, W. E., Jr, & 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.

Zuk, M., Rebar, D., & Scott, S. P. (2008). Courtship song is more variable than calling song in the field cricket *Teleogryllus oceanicus*. *Animal Behaviour*, *76*(3), 1065–1071.

CHAPTER 4

TITLE

Differential impacts of ionizing radiation on a sexually dimorphic trait in male and female *Acheta domesticus.*

AUTHORS

Tamara M. Fuciarelli*, Selvi Patel, & C. David Rollo

Department of Biology, McMaster University, 1280 Main Street West, Hamilton ON, L8S 4L8, Canada Accepted: 26 April 2021. *Corresponding author: Email fuciartm@mcmaster.ca

Published online: June 14 2023
International Journal of Radiation Biology 2023©

CITATION

Fuciarelli, T. M., Patel, S., & Rollo, C. D. (2023). Differential impacts of ionizing radiation on a sexually dimorphic trait in male and female *Acheta domesticus*. *International Journal of Radiation Biology*, *just-accepted*, 1–16.

4.1 INTRODUCTION

Sexual dimorphism in a species can encompass development, metabolism, gene expression, anatomy, behaviour, physiology, stress responses, and lifespan (Stillwell et al., 2010; Tower et al., 2020). These sexual differences can result in drastic variation in an individual's ability to respond and tolerate environmental stress. As well, it may affect our understanding of

the severity of anthropological impacts and the sensitivity of species previously thought of as resistant or tolerant.

Ionizing radiation (IR) is an ideal stressor for investigating sex differences in stress responses as dose and exposure can be precisely applied. As well, IR is a relevant environmental stressor given nuclear disasters, and application in the Sterile Insect Technique (SIT) where radiation is applied to control pest species and disease vectors (Seth & Reynolds, 1993; Krafsur, 1998; Souza et al., 2014). The sensitivity between sexes seems to be species specific with some species illustrating females to be more sensitive and others males. For example, a study in screwworm flies found that 50Gy was required to sterilize females but 25Gy sterilized males (Bushland, 1960). In *Plodia interpunctella* however, females were more sensitive, with 100% sterility at 300Gy whereas males at that dose showed only 48% sterility (Ayvaz et al., 2008). In codling moths 300-400Gy completely sterilized females and reduced male fertility to less than 10% (ICRP, 2008). Sensitivity can also vary greatly depending on the age at which stress occurs. The LD50 values for adult insects vary widely from 20-3000Gy and are generally lower in juveniles (ICRP, 2008). Increased juvenile sensitivity likely reflects higher numbers of cells undergoing division compared to adults (Nation et al., 1999). In insects with a nymphal stage for example, immature forms are highly sensitive at stages in which wings and internal reproductive tissues develop (Nation et al., 1999).

In many Orthopteran species (Insecta) including crickets, wings are sexually dimorphic (Itoh & Murakami, 2001; Duncan et al., 2021). This stark sex difference in wing morphology reflects sexual selection in which female choice has selected male forewings that produce courtship calls attractive to females (Gwynne, 1977; Klingenberg et al., 2010). These acoustic signals originate from highly complex forewings which contain specialized structures that

produce species-specific acoustic signals (Montealegre-Z et al., 2011; Duncan et al., 2021). These signals are used to attract and court females and can be used by said females to assess male quality such as body size (Andersson, 1994; Gray, 1997; Stoffer & Walker, 2012). Along with size and shape, wing symmetry has also been indicated in the cricket *G. campestris* as a factor in female preference for pure tones with low carrier frequency (Simmons & Ritchie, 1996). Studies of female preference indicate that male acoustic cues provide females with an honest indicator of male phenotypic quality (Gray, 1997). This highlights the importance of wing morphology in males. Unlike male crickets, where acoustic signals produced by highly specialized wings are critical for mating success, female cricket's wings have no known function, and their wings lack courtship call-producing structures (Itoh & Murakami, 2001). As well, the presence or absence of wings in female *Gryllus bimaculatus* does not impact mating success nor species or sex recognition (Itoh & Murakami, 2001).

Geometric morphometrics can be utilized to understand the implications of environmental condition on the size and shape of phenotypically variable traits (Lemic et al., 2020). Within geometric morphometrics, calculating fluctuating asymmetry (FA) is a commonly used method to specifically detect levels of environmental stress and predict individual fitness through subtle changes in the symmetry of a physical trait (Dongen, 2006; Ivankovic Tatalovic et al., 2020). The application of such techniques on insect wing shape and size can be analysed to understand biological patterns associated with environmental sensitivity for stressors (Klingenberg 2010; Lemic et al., 2020).

High levels of variability in FA scores have been described between even closely related species (Sukhodolskaya, 2013). Although it is often overlooked in many FA studies, understanding the difference in FA scores between sex can be important in understanding the

impact of a stressor on a species (Ivankovic Tatalovic et al., 2020). This may be especially true in species where the trait being analyzed varies between the sexes, such as in the case of cricket wings where each sex has vastly different wing structure and function, and therefore will likely have variable sensitivities. Several studies have described variation in FA between sexes as it pertains to environmental stressors such as water pollution, insecticide, as well as soil and weather condition (Hardersen, 2001; Ribeiro et al., 2007; Lemic et al., 2020).

Here, we analyze the variability of morphological changes between male and female *Acheta domesticus* wings exposed to early life IR (0-27.8Gy). We applied geometric morphometric techniques, including fluctuating asymmetry, to examine IR impacts through shape and size alterations to the wings of both male and female crickets. We also aimed to compare the sex specific alterations in wing morphology to compare relative sensitivity to IR exposure. Our results seek to expand our knowledge on sex specific impacts due to a stressor in a sexually dimorphic trait. We predict that IR will negatively impact wing morphology in both males and females. As well, males, due to the relative importance of wing shape, size, and symmetry on successful reproduction will be more developmentally stable but overall, more sensitive to damage from IR exposure than females, indicated by increased variability in shape, decreased size, and decreased bilateral symmetry.

4.2 METHODS

Breeding Colony*:* Male and female *Acheta domesticus* were generated from a large inbred (>50 generations) breeding colony. Breeding colony individuals were housed in an acrylic terrarium (93 x 64.2 x 46.6 cm), insulated with 1.5 cm thick Durofoam insulation and provided

with air circulation via fans. The colony is maintained at 29 ± 2 °C with a L12:D12 photoperiod. Chick feed (17% protein, Country Range MultiFowl Grower Quick Feeds Feed Mill, Copetown, Canada) and distilled water via soaked cellulose sponges were provided ad libitum. To generate experimental crickets for both the male and female wing analysis, the breeding colony was provided with oviposition medium (Vigoro Organic Garden soil, Swiss Farms Products, Marysville, OH, USA) in small plastic containers (7 x 7 x 7 cm). Experimental groups for the male and female wing analysis were generated from two different oviposition containers collected from the same breeding colony 2 days apart. One container was utilized for male irradiation/analysis and one for female irradiation/analysis. All described methods below were consistent for both oviposition containers for both male and female analysis. After 24 hours, oviposition containers are removed from the colony and incubated until hatch.

Experimental Groups: Individuals for both the male and female wing analysis hatched from individual oviposition containers approximately 14 days post-oviposition. 24 hours after hatch, to ensure that experimental individuals were the same age, the oviposition container, and any unhatched individuals were removed. Experimental and control individuals were then housed in the same conditions as the breeding colony. At 14 days post-hatch, in which individuals are at the 4th instar, approximately 150 individuals were separated per experimental group. Individuals from each group were then irradiated for specific durations using a ^{137}Cs source (Taylor Radiobiology Source at McMaster University, Hamilton, ON, Canada). Distance from the source was maintained to allow for a consistent dose-rate of 0.58Gy/min. For both the male and female experiment individuals were given a dose totalling 0, 0.58, 2.3, 4.6, 16.2, 23.2, or 27.8Gy. The 0 dose group is designated as our control and experienced the transfer and

preparation for irradiation without actually being irradiated. The unit Gray (Gy) was used to measure IR exposure as it describes the absorbed dose and correlates with biological effect (Fisher & Fahey, 2018). All individuals were irradiated during a single acute exposure. All groups were then immediately brought to McMaster's Life Sciences Building where they were maintained for life. At approximately 30 days of age, once sex is easily distinguishable, males and females were separated to ensure that male-male conflict for females would not result in bodily damage. At approximately 40-60 days of age, surviving male and female *Acheta* were used for morphometric analysis.

Dosimetry: The radiation fields of the Taylor Source are accurately known and therefore exact doses can be obtained by exposing subjects to a specific placement for a specific period. The radiation field was determined as follows: Using LiF chips, that you find inside a TLD badge, were arranged on a board in a x axis and y axis pattern with a few outliers. The board was placed at varying heights and irradiated for 30 seconds. After each test at a specific height, the chips were exchanged for new ones. Then the chips were sent to Mirion and measured. Here, position and orientations of the subjects was facilitated by placing specimens in a tube apparatus contained 7 individual tubes tied into a circle (with the center tube remaining empty). These were placed equidistant from the circular opening beneath the source (16.4 cm). Crickets were confined in each tube, allowing some freedom of movement to reduce stress while ensuring sufficient restriction to calculate the received dose with confidence.

Specimen Preparation and Data collection: Left and right forewings were removed at their base from anesthetized male and female *Acheta* (0-27.8Gy). Wing removal occurred at

approximately 2 weeks post-maturation (40-60 days post hatch). The period required for removal reflects the delayed maturation of groups that were highly irradiated. Once removed each pair of wings were mounted between two microscope slides that were then permanently attached for later imaging and analysis. Photographs of each male and female wing from all experimental groups were taken at 0.75x magnification using a Nikon 16.25 Megapixel camera mounted on a SMZ18 stereoscope (Nikon, Melville, NY, USA). Photos were then digitized using tpsUtil32 (v.1.78) and tspDig2 (v.2.31; both available at ttp://life.bio.sunysb.edu/morph/, courtesy Dr. FJ Rohlf) **(Figure 1)**. For both male and female experiments images were taken of all survived individuals with intact forewings from each experimental and control group. Sample size for both left and right wings, as well as replicate images to control for measurement error included for the males 499 images and for females 654 images. For the males: Control (n = 72), 0.58Gy (n = 103), 2.3Gy (n = 133), 4.6Gy (n = 80), 16.2Gy (n = 57), and 23.2Gy (n = 54). For the females: Control (n = 96), 0.58Gy (n = 92), 2.3Gy (n = 88), 4.6Gy (n = 98), 16.2Gy (n = 96), 23.2Gy (n = 90), and 27.8Gy (n = 94). The 27.8Gy male experimental group was excluded from analysis as wings did not contain enough viable landmarks due to IR damage.

Wing Shape: As male and female wings have different shape, size, and therefore landmarks, each dataset was analyzed separately as described below. To determine differences in shape variation all landmarked coordinates were superimposed using a Procrustes fit. The Procrustes fit superimposes/projects each individual landmark for each image into tangent space. Each set of landmarks are then scaled (to control for size), aligned, and rotated. This procedure, as described by Klingenberg & McIntyre (1998), simultaneously removes the influence of orientation, location, and size from the image so that shape can be accurately compared. Prior to

analysis, images of opposite wings were flipped using tpsDig so each image had the same orientation for easier landmarking.

Variation in wing shape was then analyzed using two separate statistical tests. First a principal component analysis (PCA) was applied which highlights major sources of variation across the entire dataset regardless of experimental group, and then a Canonical variates analysis (CVA) which highlights shape variation that is associated with each experimental group and its significance (Blankers et al., 2017). CVA results are reported as P-values after 10 000 permutations. This statistical test determines if shape variation is significant, i.e., that the variance between groups is higher than could be produced through random permutations.

Fluctuating Asymmetry: Fluctuating asymmetry was measured as a biomarker for environmental stress (Parsons, 1991; Beasley et al., 2013). To determine levels of fluctuating asymmetry a Procrustes ANOVA was completed separately for each control and radiation group for both the male and female dataset. To determine the affect of sex on FA, a 2-way ANOVA was conducted using a full model which included dose, sex, and interaction effects. A follow up Šídák's multiple comparisons test was also used to determine significant differences in FA between sex and dose across each experimental group.

Centroid Size: Centroid size (CS) is a commonly used measure or proxy for object size within geometric morphometrics (Klingenberg, 2016). CS is calculated as the square root of the sum of squared distances from each assigned landmark within an image to their centroid. The centroid refers to the average x and y coordinates of all landmarks (Bookstein, 1991; Jirakanjanakit et al., 2007). Average centroid size of all individual wings in each control and

radiation group for both datasets was analyzed using a one-way ANOVA. A Dunnett's multiple comparisons test was also utilized to determine significant differences between radiation group means compared to their respective control. Replicate images were averaged in order to increase accuracy of each wings centroid size.

Measurement Error: To minimize error due to both image acquisition and landmarking, a procedure adapted from Alibert et al. (2001) was applied as follows. After each wing from both the male and female dataset was photographed and digitized, approximately two weeks later wings were re-positioned, re-photographed, and re-digitized by the same researcher. This procedure reduces measurement error associated with both image acquisition and landmarking. The statistical significance of measurement error was determined through a Procrustes ANOVA (Table 1; Klingenberg et al., 2002). The Procrustes ANOVA is a commonly used method for quantifying the amount of variation accruing within the dataset due to error and experimental conditions (Klingenberg & McIntyre, 1998; Klingenberg et al., 2002). All analyses associated with measurement error were performed in MorphoJ v.1.07a (Klingenberg, 2011).

4.3 RESULTS

Within Dataset Variation: PCA analysis was completed for both male and female datasets to measure shape variation within the dataset. *Male:* For the male dataset, the PCA revealed that the first five principal components described ~75% of total wing variation (PC1, 35.8%; PC2, 16.3%; PC3, 10.2%; PC4,6.5%; PC5, 5.9%). 90% confidence ellipses indicated shape variation between control and experimental groups **(Figure 2a)**. Two of the higher

radiation groups (16.2Gy and 23.2Gy) displayed the most morphologically variable wing shape as is indicated by the size of the ellipse. *Female:* For the female data set the first 4 principal components described ~82% of total wing variation (PC1, 31%; PC2, 29.5%, PC3, 13.8%, PC4, 7.7%). The 90% confidence ellipses of radiation groups show close association with the control group, with the two highest doses, 27.8Gy and 23.2Gy showing the most morphological variability **(Figure 2b)**.

Variation Between Radiation Groups*:* CVA analysis was conducted to determine the significance of shape changes between control and radiation groups. *Male:* For the male dataset five canonical variates described 100% of variation between groups, with CVA 1 and CVA 2 representing ~89% of this variation **(Figure 3a)**. Procrustes distances among groups using 10,000 permutations showed significant differences in all groups; 0.58Gy ($P < 0.0001$), 2.3Gy ($P < 0.0001$), 4.6Gy ($P = 0.0015$), 16.2Gy ($P < 0.0001$), and 23.2Gy ($P < 0.0001$) compared to the control. *Female:* For the female dataset six canonical variates described 100% of variation between groups, with CVA 1 and CVA 2 representing ~80% of this variation **(Figure 3b).** Results from the Procrustes distances using 10,000 permutations indicated that highly significant variation in shape change were evident in the highest doses, 27.8Gy ($P < 0.0001$) and 23.2Gy ($P < 0.0001$) groups. Significant differences were also detected in the 0.58Gy ($P = 0.0052$) and 16.2Gy ($P = 0.0031$) group compared to the control group.

Centroid Size: *Male:* A One-way ANOVA on male centroid size indicated significant differences between groups ($F_{5,233} = 13.82$, $P < 0.0001$). A follow up Dunnett's multiple comparisons test which corrects for multiple comparisons indicated significant declines between

the 23.2Gy P = 0.0012), 16.2Gy (P = 0.0279), and a significant increase between the 0.58Gy (P = 0.0419) group compared to control males **(Figure 4a)**. This represents a decline of 3.4% (23.2Gy), 2.4% (16.2Gy), and increase of 1.9% (0.58Gy). A linear regression indicated a significant negative correlation between centroid size and increased IR exposure:($R^2 = 0.1767$; $F_{1,237} = 50.87$, $P < 0.0001$). *Female:* A One-way ANOVA on female centroid size indicated significant differences between groups ($F_{6,320} = 12.07$, $P < 0.0001$). A Dunnett's multiple comparisons test then indicated significant differences in centroid size between the 27.8Gy ($P < 0.0001$) and 4.6Gy ($P = 0.0479$) groups compared to control females **(Figure 4b)**. This represents a decline of 5.5% and increase of 2.5% respectively. A linear regression indicated a significant negative correlation between centroid size and IR exposure: ($R^2 = 0.09671$; $F_{1,325} = 34.80$, $P < 0.0001$).

Fluctuating Asymmetry*:* A Procrustes ANOVA was applied to all control and experimental groups for both datasets to determine levels of fluctuating asymmetry (individual*side interaction) **(Table 1).** For males, FA remained relatively stable at mid-low doses but increased >4.6Gy. For females, similar to the males, female FA increased >4.6Gy but to a much lesser extent than males. Results of the two-way ANOVA indicated significant contributions of dose ($F_{(5,244)} = 8.89$, $p < 0.0001$) sex ($F_{(1,244)} = 142.7$, $p < 0.0001$), and to a lesser extent the interaction between the two ($F_{(5, 244)} = 2.502$, $p = 0.0312$) on total variance. The results of the two-way ANOVA are summarized in **Table 3.** A follow up Šídák's multiple comparisons test indicated significant differences between male and female FA at doses between 0-4.6Gy ($P < 0.0001$). Significant differences in FA were not indicated for female radiation groups

compared to the control. For male's significant differences in FA were indicated for the 23.2Gy group (P = 0.0003) compared to the control.

Measurement Error: The relative impact of measurement error was determined to be low compared to the variation between experimental groups. A Procrustes ANOVA was executed on both centroid size and shape to determine the contribution of error on total variation for both the male and female datasets. For the male dataset, the variation attributed to error for wing shape and size was calculated as 5.26% and 0.004% respectively. For the female dataset the variation attributed to error for wing shape and size was determined to be 1.48% and 0.24% respectively. Results of the Procrustes ANOVA for measurement error are described in **Table 2.** It was concluded that measurement error did not significantly bias results.

4.4 DISCUSSION:

Crickets, like many other insect species possess sexually dimorphic traits that derive from sexual selection. In *Acheta domesticus,* forewing morphology is a sexually dimorphic trait that differs in structure and function (Itoh & Murakami, 2001). In males, forewings are highly complex with acoustic producing structures that are vital for mating success (Gwynne, 1977) **(Figure 1).** In females, wings lack specialized morphology and serve no currently apparent purpose (Itoh & Murakami, 2001) **(Figure 1)**. Stress related impacts and sensitivities to this dimorphic trait may differ given their varying importance and developmental complexity. Here, we show through morphometric analysis that early life ionizing radiation significantly altered both size and shape in both male and female forewings. As well, through FA analysis, male FA

was generally lower, indicating developmental stability but also were shown to be more sensitive to IR exposure than females. Overall, our results confirm our hypothesis that female forewing morphology was indeed less sensitive to radiation exposure across the difference metrics applied in our study.

PCA analysis indicated morphological variation across the entire dataset for both males and females. Morphological variation was highest in doses ≥16.2Gy in males and to a lesser extent females as indicated by the size and position of confidence ellipses compared to controls **(Figure 2)**. The CVA analysis, which determines significant shape variation between experimental groups also indicated increased sensitivity in the male wing. Shape variation was shown to be significantly altered at all doses (2.3Gy – 23.2Gy) in males. The highest dose, 27.8Gy was so severely damaged it could not be successfully landmarked and was excluded from analysis **(Figure 3a)**. In females, significant variation in shape change was only evident in the highest doses, 27.8Gy and 23.2Gy groups. The 0.58Gy and 16.2Gy groups also indicated less significant shape changes **(Figure 3b)**. Results in wing size are also consistent with males being more affected to early life exposures than females. Linear regression analysis showed both male and female wing size decreased in a dose-dependent manner. **(Figure 4)**.

Our results are consistent with laboratory and field studies in various invertebrate species describing stress induced morphological alterations to wing shape and size. In field studies conducted in the aftermath of the Fukushima Dai-ichi NPP disaster, morphological abnormalities were evident in *Zizeeria maha* exposed to relatively lower, but chronic doses of IR (Hiyama et al., 2012). The researchers of this study found similar results including malformations and forewing reductions in corresponding laboratory experiments (Hiyama et al., 2012). More generally, other stressors, such as temperature, nutrition, density, and insecticides have been

shown to impact wing morphology in insect species (Bitner-Mathe & Klaczko, 1999; Debat et al., 2003; Ribeiro et al., 2007; Pellegroms et al., 2009).

Increased male sensitivity to stress induced wing alterations is likely to be species specific as literature has shown that some species display increased male sensitivity to shape and size alterations and others female. Research on the fly *Bactrocera zonata* showed that sterilization doses of 90Gy resulted in abnormalities in several morphological parameters. In this study, wings of both males and females showed abnormalities associated with intravein swelling. However, only males showed variation in wing shape associated with vein angles (El-Akhdar et al., 2009). In *Culex quinquefasciatus* insecticide exposure dose dependently altered the wing shape of males but not females (Mpho et al., 2001). However, a study in the butterfly *Pararge aegeria* showed that larval food stress impacted wing morphology and size in both sexes but showed that females were more severely impacted than males (Pellegroms et al., 2009). Given this variation in the literature it is therefore likely that future research should consider sex, stress, and species in the study of stress induced impacts.

Fluctuating asymmetry (FA) was measured in both male and female *Acheta* as a measure of developmental instability. FA has been suggested as a monitoring tool for measuring environmental stress with implications for fitness (Moller, 1997; Klingenberg, 2003; Leamy & Klingenberg, 2005; Dongen, 2006; Ribeiro et al., 2007). Although fitness is often difficult to define, here we relate fitness to an individual's ability to pass on their genes i.e., reproduce (Barker, 2009). This is because FA, which measures diversion from perfect bilateral symmetry is a measure of an organism's ability to buffer or resist against environmental stressors that may impede normal developmental processes (Mpho et al., 2000). Indeed, literature suggests that

symmetrical individuals have faster growth, higher fecundity, and better survival than individuals that are more asymmetrical (Moller, 1997).

2-way ANOVA results indicated that both sex and dose had significant effects on FA in *A. domesticus* forewings. Regarding sex, our results indicate that females have a higher baseline FA then males and that FA is significantly higher in females than males at doses ≤4.6Gy. Higher baseline FA values in females compared to males indicates that male wings show higher developmental stability than the female wing, indicated by higher bilateral symmetry (Hosken et al., 2000). It is likely that this is due to the fact that male wings having functionality i.e., for acoustic signaling/mating while females wings are more vestigial in nature (Moller & Pomiankowski, 1993, Crespi & Vanderkist, 1997). The effects of functionality on FA has been shown in the little brown bat (*Myotis lucifugus*) in which FA was reported to be higher in the legs than the wings of individuals (Gummer & Brigham, 1995). Most notably, a study on the thrips *Oncothrips tepper* showed higher FA in morphs in which wings were vestigial (non functional) when compared to those where wings were functional and used for dispersion (Crespi & Vanderkist, 1997). Regarding dose, unlike female FA which remains relatively constant with dose, male FA increases by 90.6% (16.2Gy) and 160% (23.2Gy) compared to male controls **(Figure 5)**. The increase in FA is an indication that at these doses males are experiencing environmental stress which has led to the development of less bilaterally symmetrical wings. The increase in FA of a trait in response to environmental stress has been shown across multiple stressors including food restriction and heat stress (Hosken et al., 2000). These results therefore suggest both that FA is significantly affected by both sex and dose and that male FA is more sensitive to IR exposure at doses ≥16.2Gy.

There are few studies analyzing sex differences in FA using environmental stressors, however, similar to sensitivity to shape and size alterations described above, environmental sensitivity as shown by FA scores are likely to be species and stress specific (Hosken et al., 2000). In *Triatoma infestans* exposure to pyrethroid insecticides for example, showed increased FA (increased sensitivity) in males compared to females in both shape and size (Nattero et al., 2019). Similarly, in *Culex quinquefasciatus* FA increased with increasing doses of organophosphate insecticides, but only in males and not females (Mpho et al., 2001). Interestingly, when normalized to the control group doses ≤4.6 Gy showed negative FA values. Although not observed in radiation studies, research using insecticides have shown that insecticide resistant populations of *Sitophilus zeamais* have lower FA than non-resistant or susceptible strains (Ribeiro et al., 2007). This suggests that lower FA i.e., increased symmetry in females at these doses, may indicate increased resistance or fitness after exposure to these doses. More research should be conducted on sex differences in stress responses using FA values as meta-analyses have confirmed the use of FA is a sensitive environmental biomarker (Beasley et al., 2013)

These results highlight the importance of understanding stress impacts in both sexes. In the case of *Acheta domesticus* wings, IR induced wing damage may result in the reduced ability to mate in males, while in females it has no currently known consequence, highlights the stark differences in stress induced impacts of alterations and variable sensitivities to this dimorphic trait. These results may also have implications for the sterile insect technique which relies on sterilizing males while maintaining their propensity to mate with females (Nguyen et al., 2021). Doses used in this experiment are sub-sterile but have been observed to reduce reproductive success. Here, the increased sensitivity of the male forewing shape and size has been linked to

reduced mating success in males via. altered acoustic signals (Fuciarelli & Rollo, 2021a; Fuciarelli & Rollo, 2021b). It is therefore suggested that sex differences and endpoints other then sterilization such as morphology and sexual signaling be more intensely studied. However, it is important to note that this experiment was conduced under normoxic conditions unlike the hypoxic condition used in the SIT. As insects typically become more resistant to morphological and developmental damage with age, it is suggested to avoid male reductions in mating success, later irradiation should be considered (Seth & Reynolds, 1993). These alterations may also persist through several generations as was observed in F2 offspring of *Z. maha* exposed to IR from the Fukushima Dai-ichi NPP and should be further studied (Hiyama et al., 2012). Finally, as the dose rate used in this experiment, 0.58Gy, is quite low, it is suggested that future studies investigate higher dose rates as well as long-term chronic exposures as this may impact the severity of impacted observed (ICRP, 2008).

4.5 ACKNOWLEDGMENTS:

This work was funding by the NSERC Discovery Grant (RGPIN-05693-2015).

4.6 TABLES AND FIGURES

Table 1: Procrustes ANOVA on shape for fluctuating asymmetry in male and female *Acheta domesticus* wings exposed to 0 (control), 0.58Gy, 2.3Gy, 4.6Gy, 16.2Gy, 23.2Gy, and 27.8Gy ionizing radiation. Each male and female wing image contained 11 or 12 landmarks respectively. Analysis included left and right wings along with replicate images for each group. The individual*side term provides an estimate for fluctuating asymmetry which is used as a biomarker of environmental stress.

	Sex	SS	**MS**	d.f.	F	P
Control	Male	0.019	**0.00006**	306	6.49	<.0001
	Female	0.223	**0.00051**	440	57.93	<.0001
0.58Gy	Male	0.023	**0.00005**	450	11.11	<.0001
	Female	0.136	**0.00034**	400	49.16	<.0001
2.3Gy	Male	0.0365	**0.00006**	612	7.71	<.0001
	Female	0.154	**0.00039**	400	48.97	<.0001
4.6Gy	Male	.0215	**0.00006**	342	3.84	<.0001
	Female	0.220	**0.00048**	460	80.76	<.0001
16.2Gy	Male	0.0561	**0.00026**	216	20.72	<.0001
	Female	0.235	**0.00054**	440	120.37	<.0001
23.2Gy	Male	0.116	**0.00059**	198	19.34	<.0001
	Female	0.244	**0.00064**	380	19.82	<.0001
27.8Gy	Female	0.321	**0.00076**	420	72.52	<.0001

Table 2: Procrustes ANOVA values for centroid size (μm) and shape (Procrustes distance) representing the relative impact of measurement error and experimental group on *Acheta domesticus* wing analysis for both male and female wings.

Male	**Effect**	**SS**	**MS**	**d.f.**	**F**	**P**
Centroid Size	Experimental Group	6455.326	1291.065	5	106.55	<0.0001
	Error	0.293	0.00125	235	0.01	
Shape	Experimental Group	0.12269	0.00136	90	6.66	<0.0001
	Error	0.04928	0.00001	4230	0.20	
Female	**Effect**	**SS**	**MS**	**d.f.**	**F**	**P**
Centroid Size	Experimental Group	153.647	25.608	6	5.55	<0.0001
	Error	2.125	0.0069	308		
Shape	Experimental Group	0.2028	0.00169	2940	1.94	<0.0001
	Error	0.0653	0.00001	6160		

Table 3: Two-way ANOVA results representing the relative impact of error, dose, and sex on *Acheta domesticus* wing fluctuating asymmetry**.**

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	0.01107	5	0.002214	F (5, 244) = 2.502	P=0.0312
Dose	0.03932	5	0.007864	F (5, 244) = 8.890	P<0.0001
Sex	0.1262	1	0.1262	F (1, 244) = 142.7	P<0.0001
Residual	0.2158	244	0.000885		

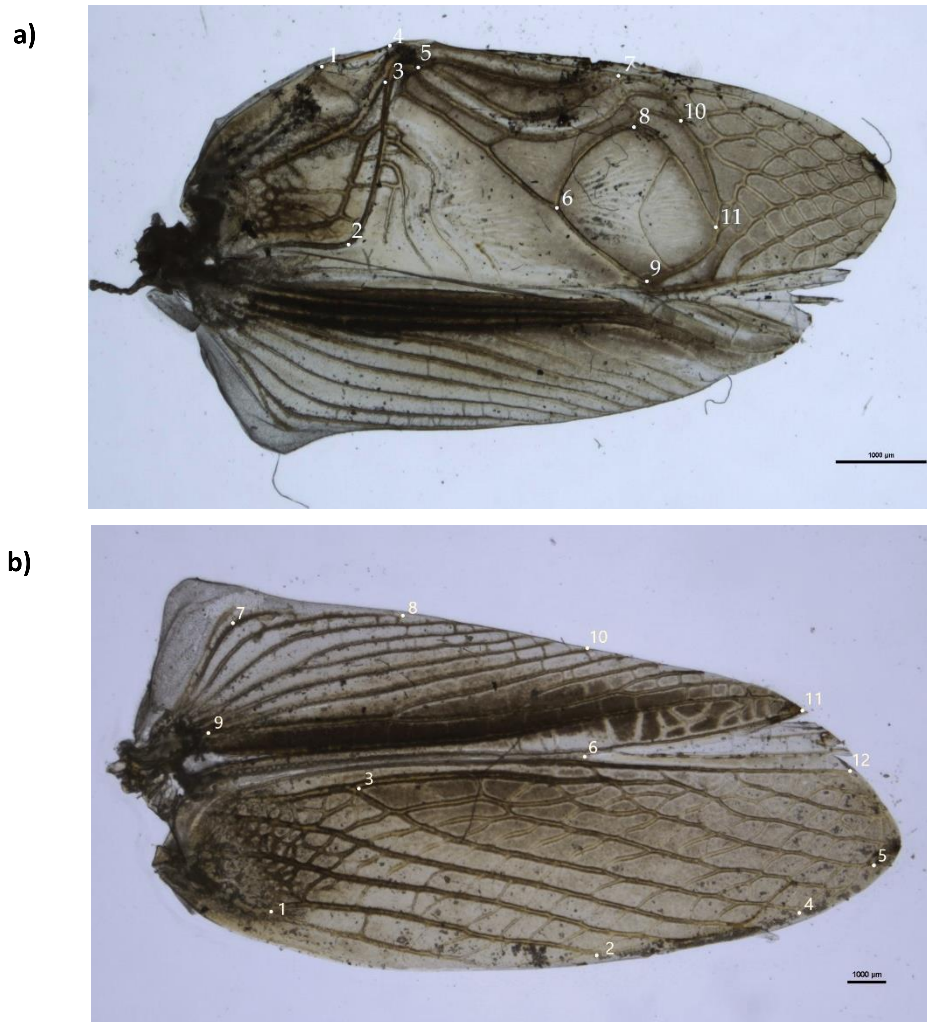


Figure 1: Position of landmarks (white dots) superimposed on a photograph of a control male (a) and female (b) *Acheta domesticus* wing. Images were taken using a Nikon 16.25-megapixel camera mounted on a SMZ18 stereoscope. All photographed wings were landmarked using the same 11 landmarks (male) and 12 landmarks (female) which were present in all wings analyzed. Images of both forewings were analyzed for each individual, with the opposing wing being digitally flipped to allow for same orientation landmarking. The scale bar for both images measures 1 mm.

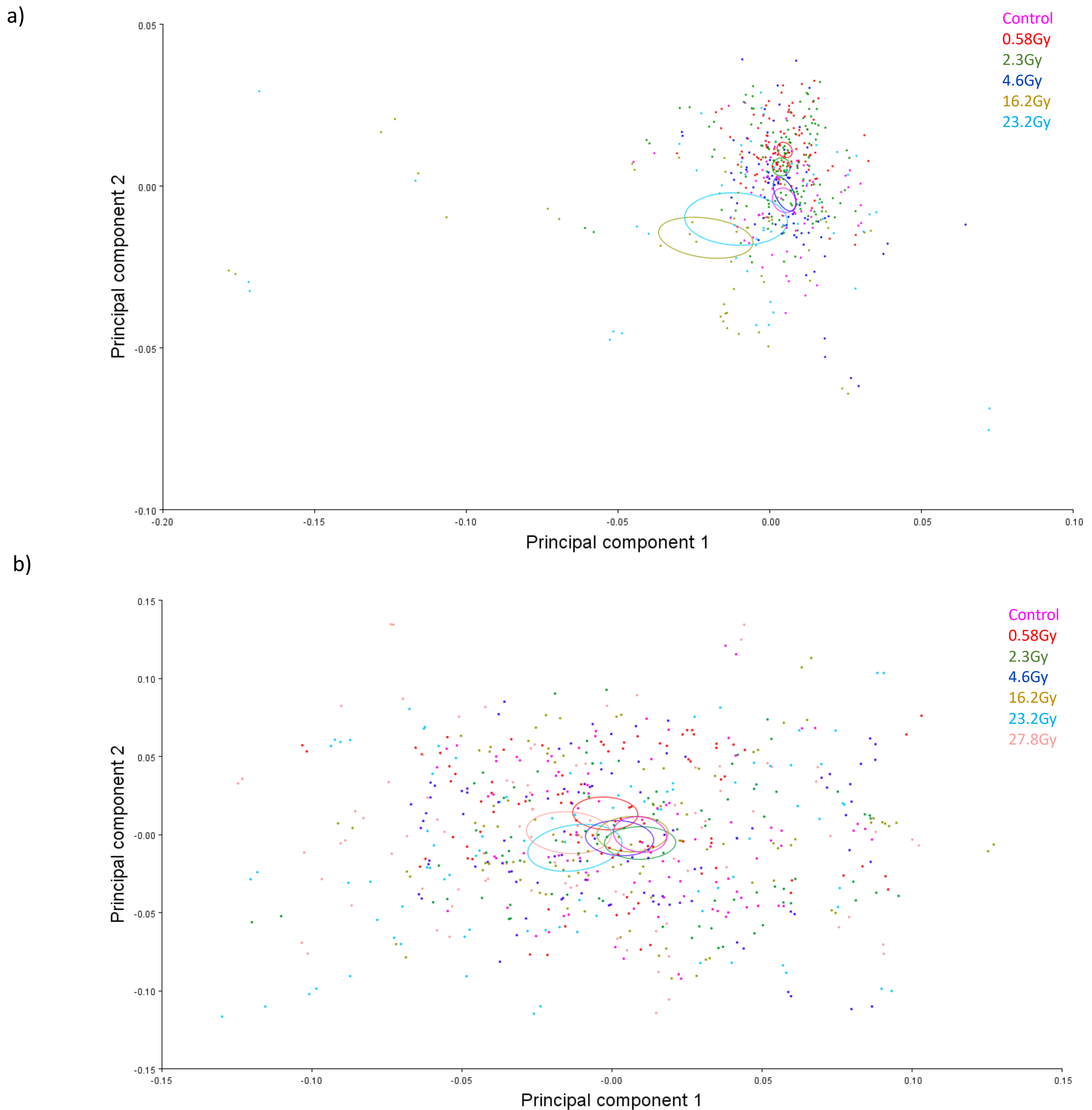


Figure 2: Principal component (PC) analysis of male and female *Acheta domesticus* wing shape. Scatterplots for both male (a) and female (b) wing shape display the first two principal components which contribute to 36% and 16% of variation for males and 31% and 39.5% of variation for females. Confidence ellipses illustrate mean shape with 90% probability for each control and radiation group. Much of the apparent variation in both male and female wing shape are evident at doses >16.2Gy.

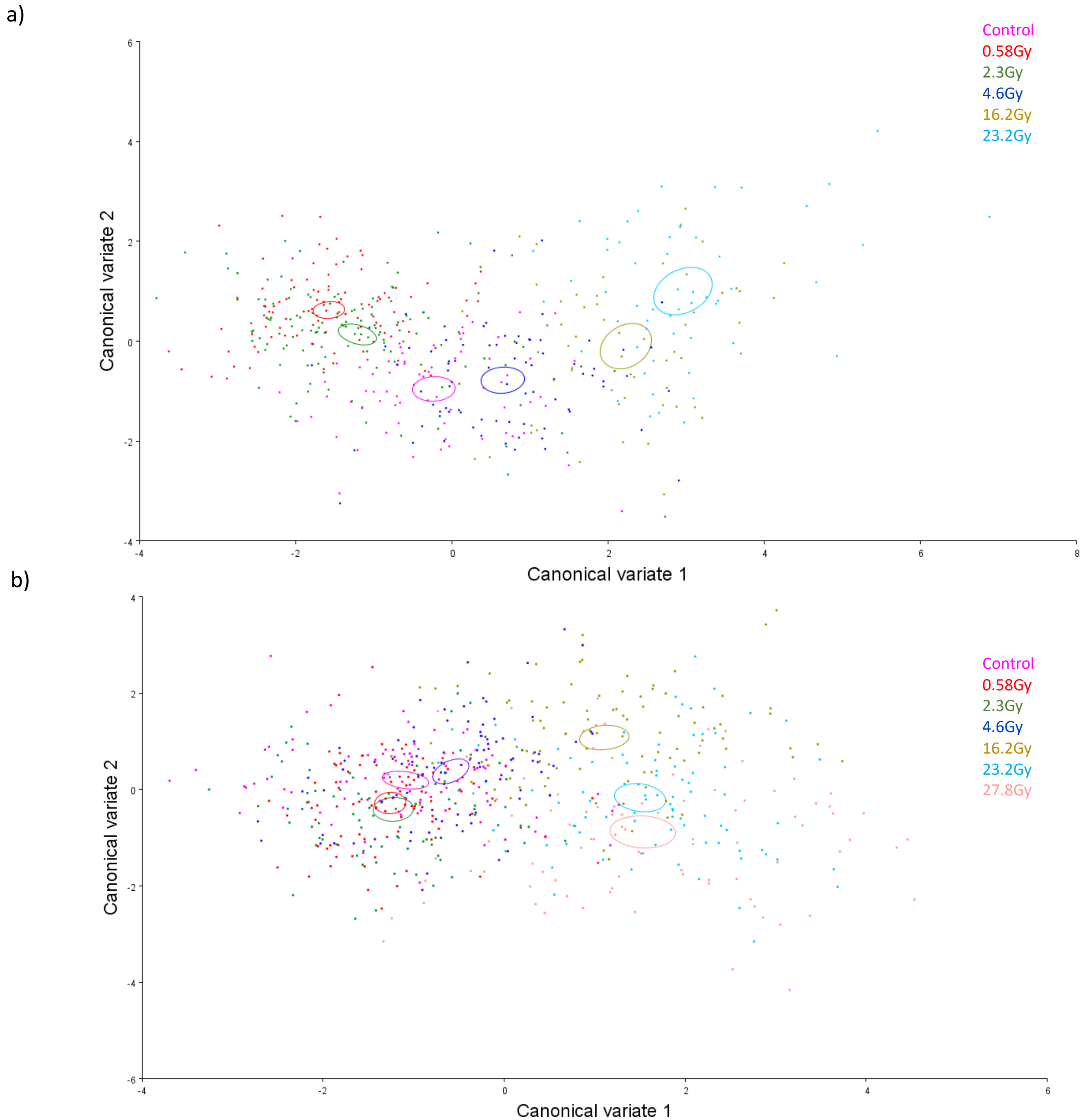


Figure 3: Canonical variate (CV) analysis of wing shape variation between control and radiation groups in male (a) and female (b) *Acheta domesticus*. For the male dataset 5 CV axes described all shape variation with the first three representing ~95% (CV1 = 76%, CV2 = 13%, and CV3 = 6%) of dataset variation. For the female dataset 6 CV axes described all variation with the first three being responsible for ~90% (CV1 = 65%, CV2 = 15%, and CV3 = 10%) of dataset variation. Confidence ellipses display the mean shape of each experimental group within a 90% probability and indicate distinct shape variation between control and radiation groups.

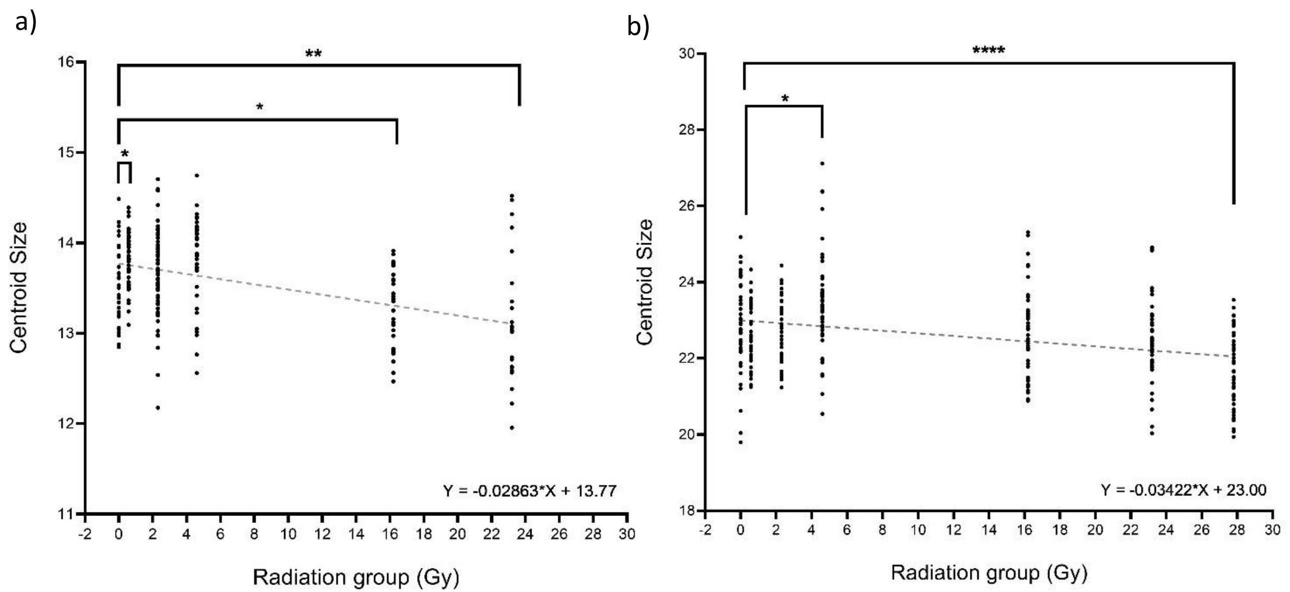


Figure 4: Centroid size of control and irradiated male (a) and female (b) *Acheta domesticus* wings. All individuals were irradiated between 0-27.8Gy at 14 days of age. Each datapoint represents the average of two replicates for each left- and right-wing image. A linear regression was applied to both data sets which indicated a negative correlation between centroid size and dose for both male and female crickets. P-value asterisks are defined as (ns) $p > 0.05$, (*) $p \leq 0.05$, (**) $p \leq 0.01$, (***) $p \leq 0.001$, (****) $p \leq 0.0001$.

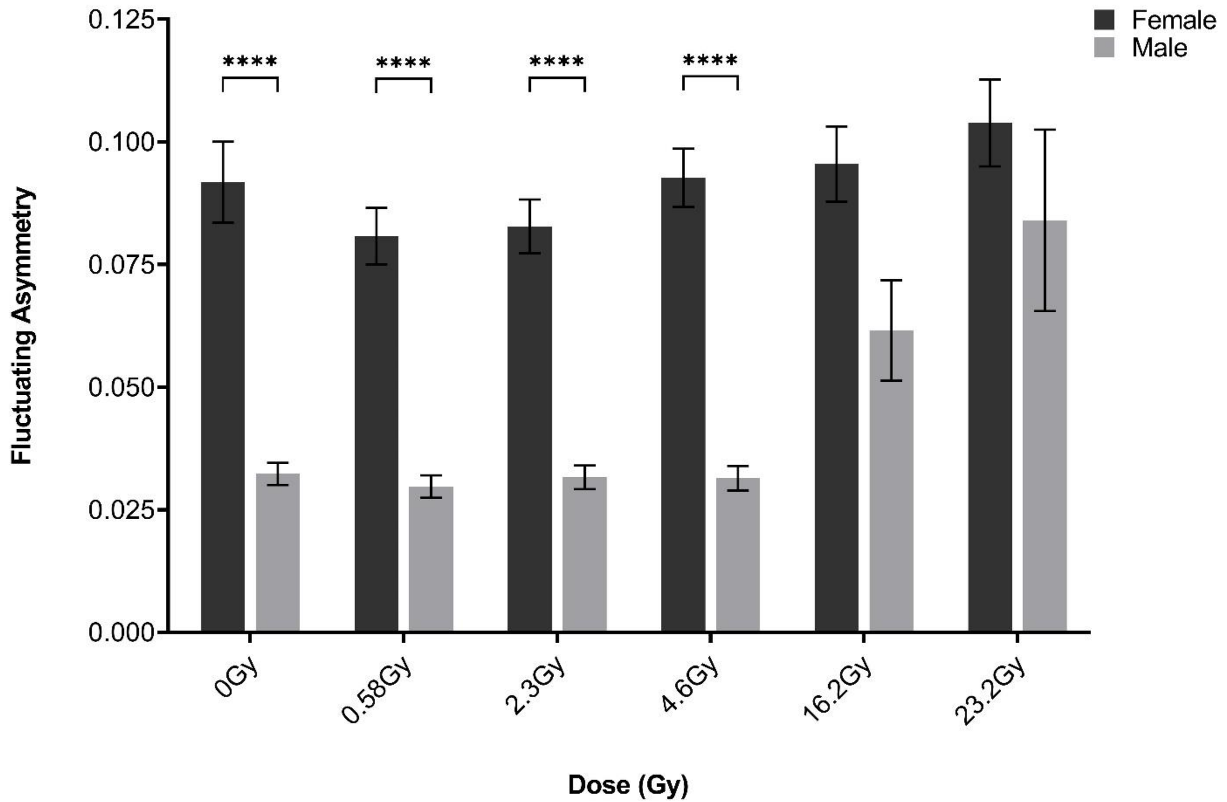


Figure 5: Fluctuating asymmetry for male and female *Acheta domesticus* exposed to IR 0-27.8Gy at 14 days of age. Data is represented as the mean FA scores for each experimental group ± SEM. A 2-way ANOVA with a Šídák's multiple comparisons test determined significant differences between male and female FA at dose between 0-4.6Gy. Fluctuating asymmetry refers to the individual*side term of the Procrustes ANOVA's conducted on each individual experimental group. Each group for both datasets included both left- and right-wing images and two replicates. P-value asterisks are defined as (ns) $p > 0.05$, (*) $p \leq 0.05$, (**) $p \leq 0.01$, (***) $p \leq 0.001$, (****) $p \leq 0.0001$.

4.7 REFERENCES

Andersson, M. (2019). Sexual selection. In *Sexual Selection*. Princeton University Press.

Ayvaz, A., Albayrak, S., & Karaborklu, S. (2008). Gamma radiation sensitivity of the eggs, larvae and pupae of Indian meal moth *Plodia interpunctella* (Hübner)(Lepidoptera: Pyralidae). *Pest Management Science: Formerly Pesticide Science*, *64*(5), 505–512.

Barker, J. S. (2009). Defining fitness in natural and domesticated populations. *Adaptation and Fitness in Animal Populations: Evolutionary and Breeding Perspectives on Genetic Resource Management*, 3–14.

Beasley, D. A. E., Bonisoli-Alquati, A., & Mousseau, T. A. (2013). The use of fluctuating asymmetry as a measure of environmentally induced developmental instability: A meta-analysis. *Ecological Indicators*, *30*, 218–226. https://doi.org/10.1016/j.ecolind.2013.02.024

Bitner-Mathé, B. C., & Klaczko, L. B. (1999). Plasticity of *Drosophila melanogaster* wing morphology: Effects of sex, temperature and density. *Genetica*, *105*(2), 203–210.

Crespi, B. J., & Vanderkist, B. A. (1997). Fluctuating asymmetry in vestigial and functional traits of a haplodiploid insect. *Heredity*, *79*(6), 624–630.

Bushland, R. C. (1960). Male sterilization for the control of insects. *Advances in Pest Control Research*, *3*, 1–25.

Debat, V., Béagin, M., Legout, H., & David, J. R. (2003). Allometric and nonallometric components of *Drosophila* wing shape respond differently to developmental temperature. *Evolution*, *57*(12), 2773–2784.

Dongen, S. V. (2006). Fluctuating asymmetry and developmental instability in evolutionary biology: Past, present and future. *Journal of Evolutionary Biology*, *19*(6), 1727–1743.

Duncan, J., Soulsbury, C. D., & Montealegre-Z, F. (2021). Differentiation between left and right wing stridulatory files in the field cricket *Gryllus bimaculatus* (Orthoptera: Gryllidae). *Arthropod Structure & Development*, *65*, 101076. https://doi.org/10.1016/j.asd.2021.101076

El-Akhdar, E. A. H., & Afia, Y. E. (2009). Functional ultrastructure of antennae, wings and their associated sensory receptors of peach fruit fly, *Bactrocera zonata* (Saunders) as influenced by the sterilizing dose of gamma irradiation. *J. Rad. Res. Appl. Sci*, *2*(4), 797–817.

Fisher, D. R., & Fahey, F. H. (2017). Appropriate Use of Effective Dose in Radiation Protection and Risk Assessment. *Health Physics*, *113*(2), 102–109. https://doi.org/10.1097/HP.0000000000000674

Fuciarelli, T. M., & Rollo, C. D. (2021a). Radiation exposure causes developmental alterations in size and shape of wings and structures associated with song production in male crickets (*Acheta domesticus*). *Entomologia Experimentalis et Applicata*, *169*(2), 227–234.

Fuciarelli, T. M., & Rollo, C. D. (2021b). Ionizing radiation alters male *Acheta domesticus* courtship songs that are critical for mating success. *Animal Behaviour*, *178*, 209–216.

Gray, D. A. (1997). Female house crickets *Acheta domesticus*, prefer the chirps of large males. *Animal Behaviour*, *54*(6), 1553–1562. https://doi.org/10.1006/anbe.1997.0584

Gummer, D. L., & Brigham, R. M. (1995). Does fluctuating asymmetry reflect the importance of traits in little brown bats (Myotis lucifugus)? *Canadian Journal of Zoology*, *73*(5), 990–992.

Gwynne, D. T. (1977). Mating behavior of *Neoconocephalus ensiger* (Orthoptera: Tettigoniidae) with notes on the calling song. *The Canadian Entomologist*, *109*(2), 237–242.

Hardersen, S. (2000). The role of behavioural ecology of damselflies in the use of fluctuating asymmetry as a bioindicator of water pollution. *Ecological Entomology*, *25*(1), 45–53. https://doi.org/10.1046/j.1365-2311.2000.00204.x

Hiyama, A., Nohara, C., Kinjo, S., Taira, W., Gima, S., Tanahara, A., & Otaki, J. M. (2012). The biological impacts of the Fukushima nuclear accident on the pale grass blue butterfly. *Scientific Reports*, *2*(1), 570. https://doi.org/10.1038/srep00570

Hosken, D. J., Blanckenhorn, W. U., & Ward, P. I. (2000). Developmental stability in yellow dung flies (Scathophaga stercoraria): Fluctuating asymmetry, heterozygosity and environmental stress. *Journal of Evolutionary Biology*, *13*(6), 919–926.

ICRP. (2008). ICRP publication 108: Environmental protection- the concept and use of reference animals and plants. *Ann ICRP*, *37*, 1–242.

Itoh, M. T., & Murakami, S. (2002). The effect of female wings on male courtship behavior in the cricket *Gryllus bimaculatus*. *Naturwissenschaften*, *89*(5), 230–232.

Ivanković Tatalović, L., Anđelić, B., Jelić, M., Kos, T., A. Benítez, H., & Šerić Jelaska, L. (2020). Fluctuating Asymmetry as a Method of Assessing Environmental Stress in Two Predatory Carabid Species within Mediterranean Agroecosystems. *Symmetry*, *12*(11), 1890. https://doi.org/10.3390/sym12111890

Klingenberg, C. P. (2003). A developmental perspective on developmental instability: Theory. *Developmental Instability: Causes and Consequences; Polak, M., Ed.; Oxford University Press: New York, NY, USA*, 14–34.

Klingenberg, C. P., Debat, V., & Roff, D. A. (2010). Quantitative genetics of shape in cricket wings: Developmental integration in a functional structure. *Evolution: International Journal of Organic Evolution*, *64*(10), 2935–2951.

Klingenberg, C. P. (2010). Evolution and development of shape: Integrating quantitative approaches. *Nature Reviews Genetics*, *11*(9), 623–635. https://doi.org/10.1038/nrg2829

Krafsur, E. S. (1998). Sterile insect technique for suppressing and eradicating insect population: 55 years and counting. *Journal of Agricultural Entomology*, *15*(4), 303–317.

Lemic, D., Benítez, H. A., Bjeliš, M., Órdenes-Claveria, R., Ninčević, P., Mikac, K. M., & Živković, I. P. (2020). Agroecological effect and sexual shape dimorphism in medfly *Ceratitis capitata* (Diptera: Tephritidae) an example in Croatian populations. *Zoologischer Anzeiger*, *288*, 118–124. https://doi.org/10.1016/j.jcz.2020.08.005

Leamy, L. J., & Klingenberg, C. P. (2005). The genetics and evolution of fluctuating asymmetry. *Annual Review of Ecology, Evolution, and Systematics*, 1–21.

Møller, A. P. (1997). Developmental stability and fitness: A review. *The American Naturalist*, *149*(5), 916–932.

Møller, A. P., Pomiankowski, & A. (1993). Fluctuating asymmetry and sexual selection. *Genetica*, *89*(1), 267–279.

Montealegre-Z, F., Jonsson, T., & Robert, D. (2011). Sound radiation and wing mechanics in stridulating field crickets (Orthoptera: Gryllidae). *Journal of Experimental Biology*, *214*(12), 2105–2117.

Mpho, M., Holloway, G. J., & Callaghan, A. (2000). Fluctuating wing asymmetry and larval density stress in *Culex quinquefasciatus* (Diptera: Culicidae). *Bulletin of Entomological Research*, *90*(3), 279–283.

Mpho, M., Holloway, G. J., & Callaghan, A. (2001). A comparison of the effects of organophosphate insecticide exposure and temperature stress on fluctuating asymmetry and life history traits in *Culex quinquefasciatus*. *Chemosphere*, *45*(6), 713–720. https://doi.org/10.1016/S0045-6535(01)00140-0

Nation, J. L., Smittle, B. J., & Milne, K. R. (1999). *Physiological markers in insects indicating treatment with ionizing radiation*.

Nattero, J., Piccinali, R. V., Gaspe, M. S., & Gürtler, R. E. (2019). Fluctuating asymmetry and exposure to pyrethroid insecticides in *Triatoma infestans* populations in northeastern Argentina. *Infection, Genetics and Evolution*, *74*, 103925. https://doi.org/10.1016/j.meegid.2019.103925

Nguyen, T. N., Choo, A., & Baxter, S. W. (2021). Lessons from *Drosophila*: Engineering genetic sexing strains with temperature-sensitive lethality for sterile insect technique applications. *Insects*, *12*(3), 243.

Parsons, P. A. (1992). Fluctuating asymmetry: A biological monitor of environmental and genomic stress. *Heredity*, *68*(4), 361–364.

Pellegroms, B., Van Dongen, S., Van Dyck, H., & Lens, L. (2009). Larval food stress differentially affects flight morphology in male and female speckled woods (*Pararge aegeria*). *Ecological Entomology*, *34*(3), 387–393.

Ribeiro, B., Guedes, R. N. C., Corrêa, A. S., & Santos, C. T. (2007). Fluctuating Asymmetry in Insecticide-Resistant and Insecticide-Susceptible Strains of the Maize Weevil, *Sitophilus zeamais* (Coleoptera: Curculionidae). *Archives of Environmental Contamination and Toxicology*, *53*(1), 77–83. https://doi.org/10.1007/s00244-006-0162-8

Seth, R. K., & Reynolds, S. E. (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.

Simmons, L. W., & Ritchie, M. G. (1996). Symmetry in the songs of crickets. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *263*(1375), 1305–1311.

Stillwell, R. C., Blanckenhorn, W. U., Teder, T., Davidowitz, G., & Fox, C. W. (2010). Sex differences in phenotypic plasticity affect variation in sexual size dimorphism in insects: From physiology to evolution. *Annual Review of Entomology*, *55*, 227–245. https://doi.org/10.1146/annurev-ento-112408-085500

Souza, J. M. G. A. de, Lima-Filho, P. A. de, Molina, W. F., Almeida, L. M. de, Gouveia, M. B. de, Macêdo, F. P. de, Laumann, R. A., & Paranhos, B. A. J. (2015). Wing morphometry and acoustic signals in sterile and wild males: Implications for mating success in *Ceratitis capitata*. *The Scientific World Journal*, *2015*.

Stoffer, B., & Walker, S. E. (2012). The use of multimodal communication in mate choice decisions by female house crickets, *Acheta domesticus*. *Animal Behaviour*, *83*(4), 1131–1138. https://doi.org/10.1016/j.anbehav.2012.02.006

Sukhodolskaya, R. (2013). Intraspecific body size variation in ground beetles (Coleoptera, Carabidae) in urban-suburban-rural-natural gradient. *Acta Biologica Universitatis Daugavpiliensis*, *13*(1), 121–128.

Tower, J., Pomatto, L. C. D., & Davies, K. J. A. (2020). Sex differences in the response to oxidative and proteolytic stress. *Redox Biology*, *31*, 101488. https://doi.org/10.1016/j.redox.2020.101488

CHAPTER 5

TITLE

Cuticular hydrocarbons of female house crickets (*Acheta domesticus*): Identification, sexual dimorphism, and stress induced impacts

AUTHORS

Tamara M. Fuciarelli*, Selvi Patel, & C. David Rollo
Department of Biology, McMaster University, 1280 Main Street West, Hamilton ON, L8S 4L8, Canada Accepted: 26 April 2021. *Corresponding author: Email fuciartm@mcmaster.ca

5.1 INTRODUCTION

For most animals chemical signalling is of vital importance in communication, foraging, avoiding danger and reproduction (Ali & Morgan, 1990; Wyatt, 2014; Stökl & Steiger, 2017). Chemical signals can be classified as either allelochemicals, that communicate information between species, or pheromones, that communicate information within species (Ali & Morgan, 1990; Wyatt, 2014; El-Ghany, 2019). In insects, use of allelochemicals and pheromones are widespread and mediate functions including defence against predators and pathogens, species and gender recognition, foraging and social interactions (Ali & Morgan, 1990; Witzgall et al., 2010; Stökl & Steiger, 2017).

A key function of pheromones is attracting mates and sexual reproduction (Wyatt, 2014). Pheromones promote defined sexual behaviours and responses among conspecifics, and convey

important information about individuals, sex, age, fecundity, mating status, and fitness (Gomez-Diaz & Benton, 2013). There is enormous diversity in the quantity and identity of pheromones associated with mating, selection pressure, and species (Wyatt, 2014). In insects, pheromones involved in sexual signalling are typically long-chain hydrocarbons. Nematodes on the other hand utilize ascaroside glycolipids, and vertebrates employ peptides or small proteins (Gomez-Diaz & Benton, 2013). In some insect species including crickets, chemical substances on the insect body function as cuticular pheromones as well as sex pheromones (Nagamoto et al., 2005).

Sex pheromones may encompass one or several compounds depending on the species (Wyatt, 2014). The sex pheromone of female silkworm moths, *Bombyx mori,* is a single compound called bombykol (Butenandt et al., 1961; Ando et al., 1988), whereas in *D. melanogaster* female sex pheromones consist of a set of hydrocarbon-based compounds (Ferveur, 2005). Among species, differences are conveyed mostly through variation in the pheromone structure or the component blend, ratio, or intensity among compounds (Carde & Haynes, 2004; de Bruyne & Baker, 2008; Wyatt, 2014; Gomez-Diaz & Benton, 2013).

Pheromones also play a role in mate selection as “honest” or reliable signals of an individual’s ability or resources (Zahavi, 1975; Guilford et al., 1987; Wyatt, 2014; Ingleby, 2015). Aspects of individual diet, age, mating status, and vicinity of conspecifics can all produce variability in pheromone production (De Pasqual et al., 2021). Sub-optimal diets have been shown to affect sex pheromones in larval and adult insects (De Pasqual et al., 2021). In larval *D. melanogaster* suboptimal diets alter pheromone profiles, body size, and female attractiveness (Schultzhaus et al., 2018). However, environmental impacts on pheromones varies with species

and prevailing stress. In *Heliconius melpomene* for example, diet altered the pheromone composition in larvae, but not in adults (Darragh et al., 2019).

The impact of altered pheromone production due to environmental factors can vary based on the species sexual behavior, such as which sex is being selective regarding mate choice. In insects, females are typically the choosy sex and can therefore use pheromone signals to gain information on key traits to gauge male quality. In female tiger moths *Utetheisa ornatrix,* females choose mates based on the concentration of courtship pheromone which conveys information about male size and quality (Dussourd et al., 1991; Iyengar & Eisner, 1999). In the tobacco moth (*Ephestia elutella*) females are twice as likely to mate with larger males producing more than twice as much pheromone as smaller ones (Phelan & Baker, 1986). Similarly, in *Nauphoeta cinerea* males have increased pheromones with better body condition with make them more attractive to females (South et al., 2011). Despite females being the choosey sex, the female pheromone is still vital for successful reproduction. Females of the desert spider *Agenelopsis aperta* emit volatile pheromones that attract males (Riechert & Singer, 1995). In the female cricket, *Gryllus bimaculatus,* lack of cuticular hydrocarbons results in loss of courtship behaviour from males (Tregenza & Wedell, 1997). Similarly, in the firefly, *Ellychnia corrusca*, females lacking CHC pheromones failed to elicit copulation attempts from conspecific males (Ming & Lewis, 2010). It is therefore highly detrimental to an individual of either sex if pheromone production is disrupted or altered.

In *A. domesticus,* sex pheromones for both males and females include CHCs identified as important for species and sex recognition (Warthen & Uebel, 1980; Ingleby, 2015). Male *A. domesticus* that experienced stress-induced impacts on pheromone production and acoustic signaling expressed reduced reproductive success (Fuciarelli & Rollo, 2021a, 2021b). Here, we

aim to elucidate the female cuticular pheromone profile in *Acheta domesticus*. We also aim to understand how environmental stress impacts pheromone production. An early-life ionizing radiation stress spanning 0.0 - 27.8 Gy was applied to female nymphs (4th instar) *Acheta domesticus*. Ionizing radiation is a stressor of particular interest due to its relevance to contamination zones such as Chernobyl and Fukushima (Imanaka et al., 2015). We hypothesize that females and males will have unique cuticular profiles, and as in males, this will reflect alterations following ionizing radiation exposure.

5.2 METHODS

Breeding Colony: Experimental *Acheta domesticus* were generated from a large, highly inbred (>50 generations) breeding colony. The colony was housed in a large acrylic terrarium (93 x 64.2 x 46.6 cm) insulated with 1.5 thick Durofoam insulation. The colony was maintained at a constant temperature of 29ºC ± 2ºC and a 12h day-12h night photoperiod. Chick feed (Country Range MultiFowl Grower®, 17% protein) was provided as food *ad libitum.* Distilled water via soaked cellulose sponges were provided *ad libitum* and replaced daily. Several egg carton shelters were also provided and replaced as needed. To produce experimental groups colony crickets were provided with oviposition medium (Vigoro Organic Garden Soil®) in small plastic containers (7 x 7 x 7 cm). Soils were collected after a 24h period to provide nymphs of known age. Soils were then incubated until hatching, approximately 14d post oviposition.

Experimental Groups: All experimental animals were generated from a single oviposition container provided to the colony. To ensure experimental individuals were of the

same age, 24 hours after oviposition, oviposition containers were removed from the colony. Once hatched, experimental individuals were housed and maintained in the same conditions as the breeding colony. At 14d post-hatch (4th instar nymphs) approximately 100 individuals per group were randomly separated and irradiated using a Cs-137 source (Taylor Radiobiology Source at McMaster University). Groups were irradiated for a specific duration at 0.58Gy/min totalling (0, 0.58Gy, 2.3Gy, 4.6Gy, 9.3Gy, 16.2Gy, 23.2Gy, and 27.8Gy). To ensure the stress of transfer did not affect results, a control group labeled Sham was also generated which was brought to the source but not irradiated. These doses were chosen as doses above 30Gy have been previously observed to induce severe reproductive decline and sterility in this species. All experimental groups were irradiated on the same day during a single exposure. Although only a few individuals were used for GC/MS analysis, 100 individuals per group were irradiated to ensure enough individuals reached maturity for later analysis. Post-irradiation, all individuals were immediately brought to McMaster's Life Sciences Building (LSB) where they were maintained for life. Prior to analysis, to ensure mating and male/male fighting for female copulation would not alter or damage the female cuticle, prior to maturation males were removed from experimental containers. All experimental individuals analyzed for cuticular profiles were female.

Cuticular profile isolation*:* Cuticular compounds were extracted from randomly selected unmated/virgin female crickets from each experimental group: Control (n= 6), Sham (n = 6), 0.58Gy (n= 6), 2.3Gy (n= 7), 4.6Gy (n= 7), 9.3Gy (n= 7), 16.2Gy (n= 7), 23.2Gy (n= 7), and 27.8Gy (n= 7). Approximately 2-3 weeks post-maturation individuals from each group were removed and anesthetized with CO_2. Each individual was then weighed using a Accuris

analytical balance with a readability of 0.001g ± 0.002 g. Individuals were then swirled in pure hexane to dissolve cuticular compounds for 5 minutes at a concentration of 5ml per 0.5g of cricket mass (to control for variable cricket size). After collection, crickets were removed and disposed of. Hexane extracts were collected into sterilized amber glass containers. Containers were sealed using an airtight lid with elastic tape wrapping to ensure no outside contamination occurred. Samples were stored at 4°C until processing.

Cuticular profile analysis: All experimental samples were analyzed at McMaster's regional center for mass spectrometry. Samples were first vortexed for 5 seconds to ensure compounds had not settled within the container. 1ml of each sample was then placed into a new smaller sterilized container and evaporated using nitrogen gas to remove the hexane solvent. Once evaporated, samples were reconstituted in 40µl of pure hexane with 10ul of internal standard (Napthalene-d8), making each sample a total of 50µl. Samples were then processed using an Agilent 5973/6890 for gas chromatography and mass spectrometry analysis. Data output was then analyzed using Bruker Compass DataAnalysis 4.0 ®. Peaks of interest were determined using the DataAnalysis program. The concentration or area under each peak was recorded for all samples. The average intensity of each peak in the control and experimental groups were compared. ADMIS V2.71 and NIST Mass Spectral Search Program V2.0 software was utilized to identify probable compounds associated with each peak.

Statistics: A two-way ANOVA followed by a Dunnett's multiple comparison test was applied to determine significant differences in the mean concentration of each cuticular compound in each experimental group compared to the control group. The effects of dose, peak,

and interaction between the two on dataset variation was also analyzed. Concentration was measured as the area under each peak. All statistical analysis was carried out using Prism GraphPad 9.

5.3 RESULTS

Cuticular Hydrocarbons*:* The typical ion chromatogram for a control female *A. domesticus* indicated 20 significant hydrocarbon peaks **(Figure 1).** Results of the 2-way ANOVA indicated significant contributions of both dose ($F_{(8, 1019)}$ = 103.5, p<0.0001), peak ($F_{(19, 1019)}$ = 228.9, p<0.0001) , and interaction effects ($F_{(152, 1019)}$ = 8.235, p<0.0001) on total variance (**Table 1)**. A Dunnett's multiple comparisons test to analyze differences between peak concentration compared to the control identified significant differences in most peaks including 2,4-10, and 13-19. Most notably, of the most prominent peaks (2,4,6, and 17), significant increases were seen in most cases at doses greater than 9.3Gy. As well, other then the Sham group, all irradiated groups showed increased concentration in pheromone production compared to the control **(Figure 2)**. The unirradiated but transported to the source, Sham group only showed significant differences to the control in one peak p = 0.0043. Results of the Dunnett's post hoc test and % change from the control are summarized in **Table 2**. Likely compounds identified for each peak included branched and unbranched saturated alcohols and alkanes and are summarized in **Table 3.**

5.4 DISCUSSION

Cuticular profile composition and sexual dimorphism

The vast majority of insect species utilize sex pheromones to facilitate reproduction (Landolt, 1997). Most species utilize a multicomponent pheromone where a particular combination of compounds result in a unique pheromone signature (Wyatt, 2014). Here, we illustrate the cuticular pheromone for the female cricket *Acheta domesticus* as containing 20 unique compounds **(Figure 1),** mainly composing of hydrocarbons of between 24-44 carbons in length including both branched and unbranched saturated alcohols and alkanes **(Table 3).** These results are similar to others analyzing cricket pheromone profiles. A study analyzing the pheromone profile in male *Teleogryllus oceanicus* identified compounds as being hydrocarbons, specifically alkanes and alkenes between 31-35 carbons long (Thomas & Simmons, 2009). However, the presence of alcohols within the cuticular profile of female crickets to our knowledge has not been shown in other cricket species. However, the presence of hydrocarbon chains with alcohols, aldehydes, and acetate esters are quite common in other female insects including in many Lepidopteran and Dipteran species. In female moths for example, sex pheromones usually consist of a blend of between 5-6 hydrocarbons (unbranched fatty acids, alcohols, acetates, or aldehydes) of 10-18 carbons in length (Carde & Haynes, 2004; de Bruyne & Baker, 2008). In two other Lepidopteran species, *Helothis virescens* and *H. zea,* C14 saturated and C16 monounsaturated primary alcohols have been identified within the female sex pheromone gland (Teal & Tumlinson, 1987).

Interestingly our results also indicate sex differences between the male and female *Acheta domesticus* pheromone. The male pheromone, similar to that of the female has been shown to

consist of branched and unbranched hydrocarbons of between 29-44 carbons in length. However, unlike the female pheromone, male compounds did not include alcohols and instead consisted of only alkanes and alkenes (Fuciarelli & Rollo, 2021a). These results are similar to that of other closely related cricket species *G. bimaculatus* and T.*oceanicus* which indicated significant differences between the concentration of compounds in the male and female pheromone signature (Tregenza & Wedell, 1996; Thomas & Simmons, 2008). As well, sexual dimorphism in cuticular hydrocarbons has been identified in many different species including those within the Orthoptera, Lepidoptera, and Diptera orders (Thomas & Simmons, 2008). To our knowledge this is the first study to identify sexual dimorphism in the cuticular hydrocarbon structure in *Acheta domesticus* with likely hydrocarbon compounds.

The results identifying the male and female sex pheromone of *Acheta* is important in informing compounds used for pheromone pest management. Insect pheromones can be used as an effective and safe alternative to pesticides in pest management, in which pest populations can be managed without harm to the environment (Carde & Minks, 1995). Pheromones can be utilized in monitoring, mating disruption, and mass trapping, as well as other modes to disrupt insect behaviour (Wyatt, 2014). Although *Acheta domesticus* is not a known agricultural pest, understanding the sex pheromone profile of both sexes is important to ensure that pheromones used to control pest populations do not have unintended consequences on non-target species. The sex pheromone of two species may contain similar or identical compounds and are only altogether unique based on their relative concentration or ratio to other compounds (Wyatt, 2014; Gomez-Diaz & Benton, 2013). This implies that the potential lack of specificity on the level of compound concentration in the use of sex pheromones in pest control may disrupt mating behaviour in non-target species.

Stress induced alterations to cuticular profile

Pheromone production is a plastic trait and may change in relation to biotic and abiotic conditions. Our results indicated significant alterations to the female cuticular profile due to ionizing radiation exposure (0.58Gy-27.8Gy) **(Figure 2)**. A two-way ANOVA indicated that peak, dose, and interaction between the two had a significant impact on peak concentration **(Table 1)**. All peaks across all doses, with the exception of 23.2Gy peak 3, showed increases in hydrocarbon production compared to the control **(Table 2)**. This increase in concentration was significant for 2 (0.58Gy), 6 (2.3Gy), 4 (4.6Gy), 7 (9.3Gy), 14 (16.2Gy), 4 (23.2Gy), and 9 (27.8gy) peaks. The sham group, which was brought to the irradiation chamber but not irradiated only had one peak significantly different to that of the control. These results are similar to results using IR stress on male *Acheta domesticus,* where males showed general increases in pheromone concentration across radiation dose (Fuciarelli & Rollo, 2020). However, unlike what was observed in males, radiation impacts in females were not concentrated on a few specific peaks but instead impacted a variety of different compounds. As females are the choosy sex, female *Acheta* likely utilize pheromones not to indicate their quality but specifically to signal to males that they are of the opposite sex of the same species once in close proximity (Wyatt, 2014; Allison & Cardé, 2016). In other cricket species such as in T.*oceanicus* and *G. bimaculatus* sex discrimination is understood to be determined by a particular blend of cuticular compounds (Tregenza & Wedell, 1996; Thomas & Simmons, 2008). Therefore, impacts to cuticular hydrocarbon profiles i.e., in the relative concentration and ratio of compounds, may reduce the female's ability to elicit sexual responses from males via their pheromone signature. It has been shown that when female *Gryllus bimaculatus* and *Ellychnia corrusca* lack cuticular pheromones,

conspecific males will fail to produce mating behaviour (Tregenza & Wedell, 1997; Ming & Lewis, 2010). However, the potential impacts on female attractiveness from radiation exposure has not yet been tested.

These results have important implications to insects inhabiting areas which have been contaminated with high levels of ionizing radiation i.e., Chernobyl (1986) and Fukushima (2011). In the Chernobyl region in particular, insects have been shown to have significantly reduced abundance with increasing dose (Moller & Mousseau, 2009). The specific causes of this decline however are poorly understood. Here we show that ionizing radiation exposure can affect female chemical signaling and hypothesize that this may further impact female reproductive success via. signaling to male conspecifics. It is important to note however that this work uses doses much higher than that found in these contamination areas. However, we postulate that high acute doses may shed light to what may be seen over several generations of low chronic exposure. Studies in the Chernobyl region have indicated that the level of genetic damage and mutation rates have accumulated over many generations and are also correlated with phenotypic outcomes (Mousseau & Moller, 2014).

Our work displays the cuticular hydrocarbon profile or pheromone of the female cricket *Acheta domesticus.* We have identified the likely compounds for this signature as branched and unbranched saturated alkanes and alcohols which are unique from the male profile which contains mainly branched and unbranched alkanes and alkenes. We also show that similar to the male, female's cuticular profiles are sensitive to environmental conditions with ionizing radiation exposure generally increasing the concentration of these compounds. Although we aimed to control for all outside variables there is still likely individual variation in pheromone quantity and quantity. Furthermore, our GC analysis had a limited sample size so definitive conclusions

on dose-response trends or which areas of the profile are most sensitive could not be fully elucidated. Instead, we hope this preliminary work aids in future studies where a larger sample size is possible. As well, future work on the impacts of IR exposure on the cuticular profile of female *Acheta* on both sex and species recognition would further elucidate how pheromone stress induced alterations are impacting reproduction in this species.

5.5 ACKNOWLEDGEMENTS

The authors thank Dr. M. Kirk Green for assistance with performing and interpreting the GC-MS analyses. This work was supported by the NSERC Discovery Grant [RGPIN-05693-2015]

5.6 TABLES AND FIGURES

Table 1: Two-way ANOVA results representing the relative impact of dose, peak, and interaction effects on female *Acheta domesticus* cuticular profile.

	SS	DF	MS	F (DFn, DFd)	P value
Interaction	8.923e+016	152	5.870e+014	F (152, 1019) = 8.235	P<0.0001
Peak	3.101e+017	19	1.632e+016	F (19, 1019) = 228.9	P<0.0001
Dose	5.904e+016	8	7.380e+015	F (8, 1019) = 103.5	P<0.0001
Residual	7.264e+016	1019	71287467492340		

Table 2: Summary of GC results for female cuticular profile (0-27.8Gy). Significant differences from control are shown with % change from the control.

Peak #	Sham	0.58Gy	2.3Gy	4.6Gy	9.3Gy	16.2Gy	23.2Gy	27.8Gy
1	-13.18	11.92	41.00	35.52	95.40	120.83	3.84	46.32
2	-14.88	46.13	76.32	76.36	**95.53****	**243.64******	**144.43******	**124.51*****
3	-2.65	41.83	47.83	37.67	74.40	138.09	-2.55	49.26
4	-54.70	3.09	10.04	28.53	**143.52*****	**171.05******	39.43	39.17
5	91.66	112.49	156.23	119.74	159.95	229.15	64.81	**268.69***
6	12.43	58.99	**90.86***	**94.48***	**132.48******	**214.91******	49.88	**141.82******
7	1.94	87.76	79.30	96.65	74.20	**181.41*****	67.45	117.25
8	56.90	96.32	**118.20***	**112.66***	104.68	**199.80******	19.73	**160.75*****
9	-2.18	50.12	47.56	48.53	120.46	**184.53***	67.96	146.33
10	148.44	**188.90***	**215.91****	**163.26***	126.68	**269.39*****	39.47	**306.88******
11	111.09	152.98	164.50	125.60	173.58	258.15	78.71	269.66
12	174.15	211.29	240.92	206.54	222.11	300.74	133.07	412.77
13	-37.69	25.56	80.20	83.99	128.32	**304.06******	60.64	55.63
14	141.60	190.38	**217.83***	157.57	151.25	**319.37******	71.95	**330.74******
15	182.24	**259.67***	**258.90***	206.36	**229.03***	**329.09****	45.88	**404.21******
16	-50.99	3.36	58.30	105.75	**158.03****	**277.02******	**111.30***	109.35
17	**-36.64****	20.87	**72.06******	**102.60******	**99.03******	**284.78******	**74.94******	**91.97******
18	-53.20	18.52	63.10	91.41	**226.97******	**350.58******	**198.23******	**173.77*****
19	-35.81	27.39	39.67	67.09	175.06	**254.17***	109.96	145.55
20	-26.16	49.74	90.41	144.41	173.78	302.65	94.61	169.06

P-value asterisks are defined as (ns) $p > 0.05$, (*) $p \leq 0.05$, (**) $p \leq 0.01$, (***) $p \leq 0.001$, (****) $p \leq 0.0001$.

Table 3: Summary of GC/MS results for the female cuticle profile with likely compound candidates.

Compound Number	Retention Time	Chemical Formula	Compound Name	Match Probability	Branched
1	24.86	$C_{28}H_{58}$	Octacosane	909	N
2	25.35	$C_{34}H_{70}$	Hexacosane, 9-octyl-	875	Y
3	25.73	$C_{29}H_{60}$	Nonacosane	919	N
4	25.86	$C_{28}H_{58}O$	1-Octacosanol	888	N
5	25.93	$C_{30}H_{62}$	11-Methylnonacosane	837	Y
6	27	$C_{36}H_{74}$	Hexatriacontane	853	N
7	27.42	$C_{28}H_{58}O$	1-Octacosanol	854	N
8	27.48	$C_{24}H_{50}O$	n-Tetracosanol-1	861	N
9	27.56	$C_{32}H_{66}$	11-Methylhentriacontane	833	Y
10	29.04	$C_{44}H_{90}$	Tetratetracontane	812	N
11	29.14	$C_{44}H_{90}$	Tetratetracontane	753	N
12	29.73	$C_{40}H_{82}$	Tetracontane	820	N
13	30.32	$C_{28}H_{58}O$	1-Octacosanol	887	N
14	30.43	$C_{28}H_{58}O$	1-Octacosanol	852	N
15	30.53	$C_{37}H_{76}$	15,19-Dimethylpentatriacontane	849	Y
16	31.38	$C_{28}H_{58}O$	1-Octacosanol	825	N
17	31.92	$C_{30}H_{62}O_2$	1,30-Triacontanediol	796	N
18	32.06		Unresolved		
19	33.48	$C_{30}H_{62}O_2$	1,30-Triacontanediol	758	N
20	33.57	$C_{40}H_{82}O_2$	Tetracontane-1,40-diol	655	N

Potential candidates were identified using ADMIS software and NIST library. Match probability and compound characteristics are shown.

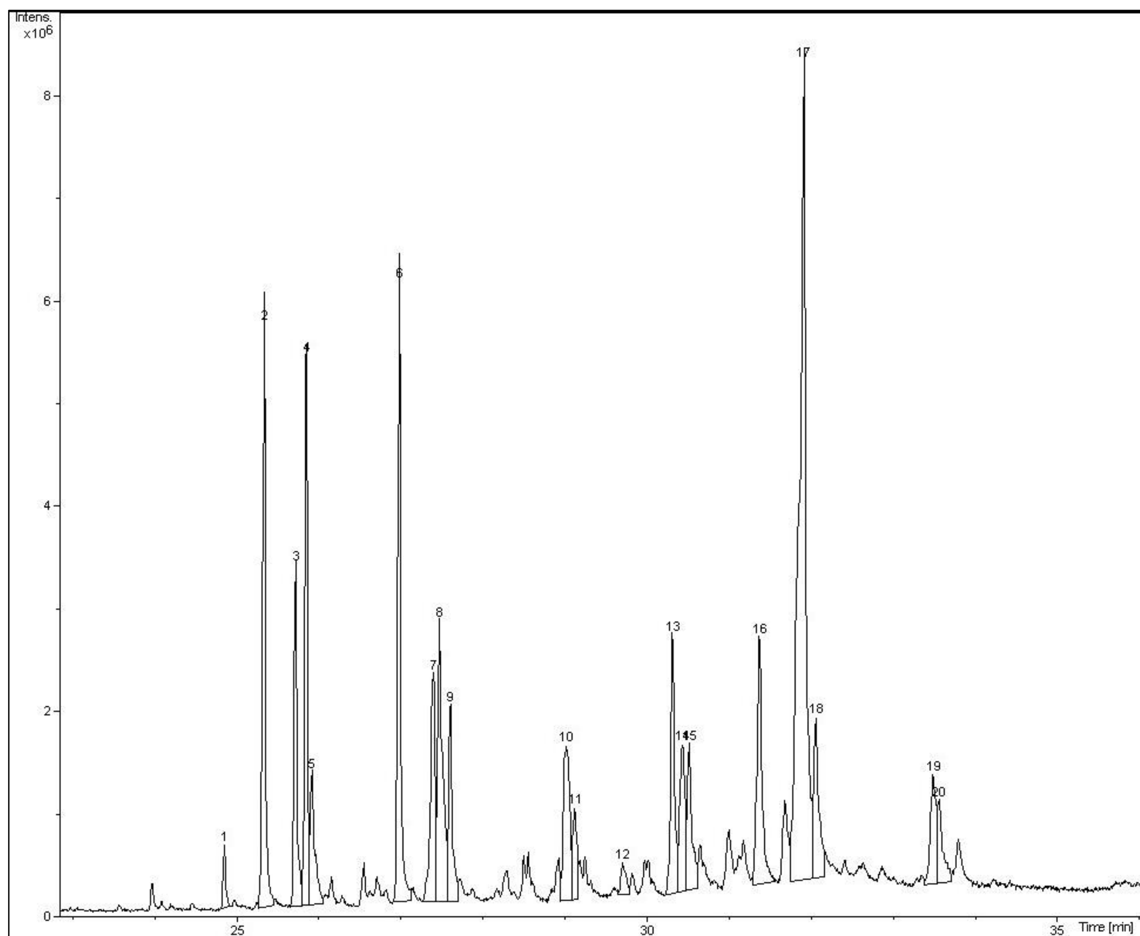


Figure 1: A typical total ion chromatogram of a control female *Acheta domesticus* extracted using a hexane solvent at a 5ml:0.5g hexane to cricket ratio. Samples were extracted approximately 2-3 weeks post maturation. Samples were analyzed at McMaster's regional center for mass spectrometry using an Agilent 5973/6890 gas chromatogram system and Bruker Compass DataAnalysis 4.0.VR software. All 20 peaks identified were at distinguishable concentrations across all samples analyzed in all groups. Each peak corresponds to a particular compound.

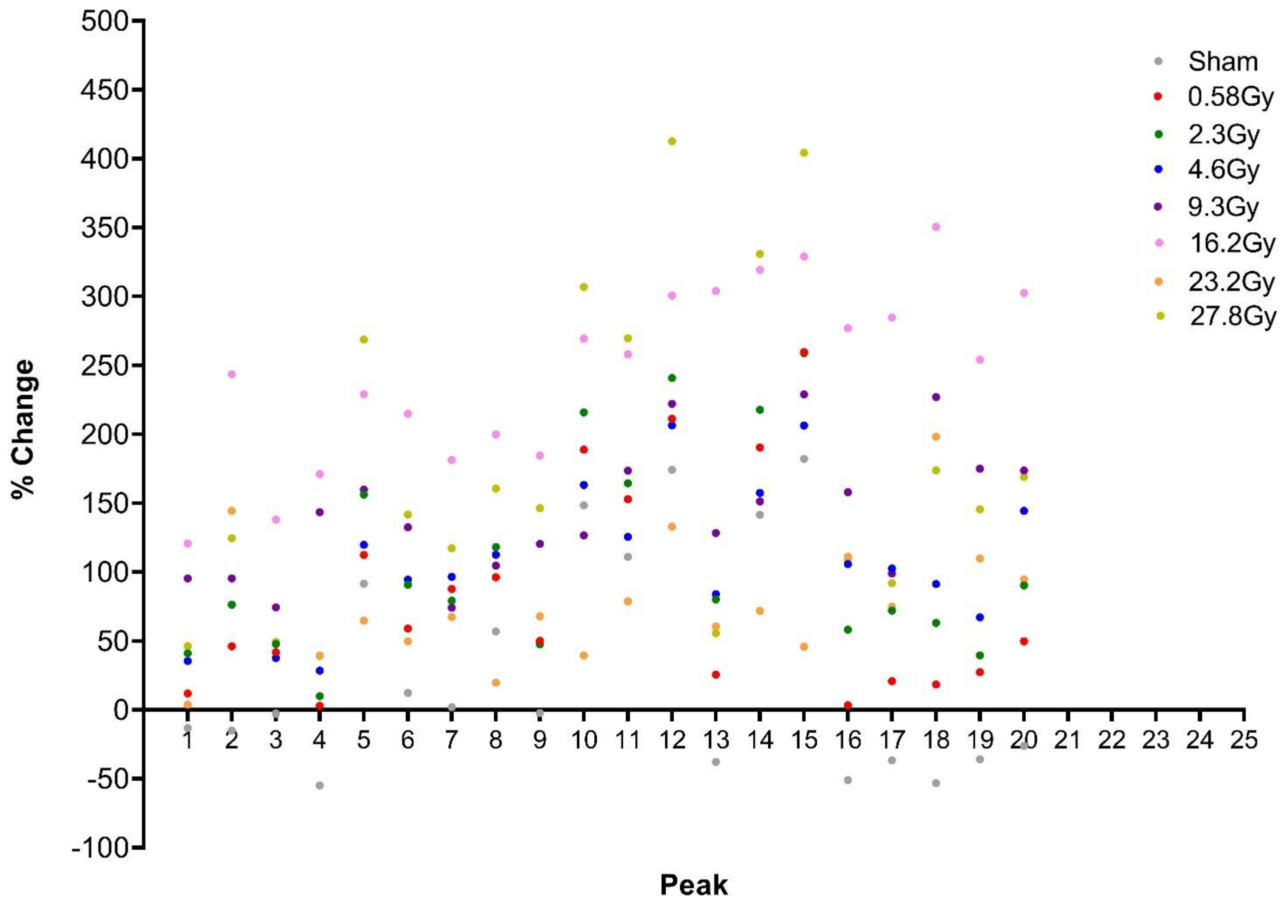


Figure 2: Dose-response effects of early life radiation on the cuticular profile on the identified 20 peaks for female *Acheta domesticus*. Results are displayed as % change from the control for the mean of each peak within each experimental group. The sham group was not irradiated but underwent the transfer stress of the irradiation process. All irradiations took place at 14 days of age at 0.58Gy/min with cuticular extraction occurring approximately 2-3 weeks post maturation. All significant differences were analyzed compared to control values using a 2-way ANOVA followed by Dunnett's multiple comparison test. A summary of ANOVA and multiple comparison results are shown in Table 1 and 2.

5.7 REFERENCES

Ali, M. F., & Morgan, E. D. (1990). Chemical communication in insect communities: A guide to insect pheromones with special emphasis on social insects. *Biological Reviews*, *65*(3), 227–247.

Allison, J. D., & CardŽ, R. T. (2016). *Pheromone communication in moths: Evolution, behavior, and application*. Univ of California Press.

Ando, T., Hase, T., Arima, R., & Uchiyama, M. (1988). Biosynthetic pathway of bombykol, the sex pheromone of the female silkworm moth. *Agricultural and Biological Chemistry*, *52*(2), 473–478.

Butenandt, A., Beckmann, R., & Stamm, D. (1961). *Über den Sexuallockstoff des Seidenspinners, II. Konstitution und konfiguration des Bombykols*.

Cardé, R. T., & Haynes, K. F. (2004). Structure of the pheromone communication. *Advances in Insect Chemical Ecology*, *283*.

Carde, R. T., & Minks, A. K. (1995). Control of moth pests by mating disruption: Successes and constraints. *Annual Review of Entomology*, *40*(1), 559–585.

Darragh, K., Byers, K. J., Merrill, R. M., McMillan, W. O., Schulz, S., & Jiggins, C. D. (2019). Male pheromone composition depends on larval but not adult diet in *Heliconius melpomene*. *Ecological Entomology*, *44*(3), 397–405.

De Bruyne, M., & Baker, T. C. (2008). Odor detection in insects: Volatile codes. *Journal of Chemical Ecology*, *34*(7), 882–897.

De Pasqual, C., Groot, A. T., Mappes, J., & Burdfield-Steel, E. (2021). Evolutionary importance of intraspecific variation in sex pheromones. *Trends in Ecology & Evolution*, *36*(9), 848–859. https://doi.org/10.1016/j.tree.2021.05.005

Dussourd, D. E., Harvis, C. A., Meinwald, J., & Eisner, T. (1991). Pheromonal advertisement of a nuptial gift by a male moth (*Utetheisa ornatrix*). *Proceedings of the National Academy of Sciences*, *88*(20), 9224–9227.

El-Ghany, N. M. A. (2019). Semiochemicals for controlling insect pests. *Journal of Plant Protection Research*, *59*(1).

Ferveur, J.-F. (2005). Cuticular hydrocarbons: Their evolution and roles in Drosophila pheromonal communication. *Behavior Genetics*, *35*(3), 279–295.

Fuciarelli, T. M., & Rollo, C. D. (2021a). Impacts of ionization radiation on the cuticular hydrocarbon profile and mating success of male house crickets (*Acheta domesticus*). *International Journal of Radiation Biology*, *97*(4), 564–570.

Fuciarelli, T. M., & Rollo, C. D. (2021b). Ionizing radiation alters male *Acheta domesticus* courtship songs that are critical for mating success. *Animal Behaviour*, *178*, 209–216.

Gomez-Diaz, C., & Benton, R. (2013). The joy of sex pheromones. *EMBO Reports*, *14*(10), 874–883.

Guilford, T. I. M., Nicol, C., Rothschild, M., & Moore, B. P. (1987). The biological roles of pyrazines: Evidence for a warning odour function. *Biological Journal of the Linnean Society*, *31*(2), 113–128.

Imanaka, T., Hayashi, G., & Endo, S. (2015). Comparison of the accident process, radioactivity release and ground contamination between Chernobyl and Fukushima-1. *Journal of Radiation Research*, *56*(suppl_1), i56–i61.

Ingleby, F. C. (2015). Insect cuticular hydrocarbons as dynamic traits in sexual communication. *Insects*, *6*(3), 732–742.

Landolt, P. J. (1997). Sex attractant and aggregation pheromones of male phytophagous insects. *American Entomologist*, *43*(1), 12–22.

Ming, Q.-L., & Lewis, S. M. (2010). Mate recognition and sex differences in cuticular hydrocarbons of the diurnal firefly *Ellychnia corrusca* (Coleoptera: Lampyridae). *Annals of the Entomological Society of America*, *103*(1), 128–133.

Møller, A. P., & Mousseau, T. A. (2009). Reduced abundance of insects and spiders linked to radiation at Chernobyl 20 years after the accident. *Biology Letters*, *5*(3), 356–359.

Mousseau, T. A., & Møller, A. P. (2014). Genetic and ecological studies of animals in Chernobyl and Fukushima. *Journal of Heredity*, *105*(5), 704–709.

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.

Phelan, P. L., & Baker, T. C. (1986). Male-size-related courtship success and intersexual selection in the tobacco moth, *Ephestia elutella*. *Experientia*, *42*(11), 1291–1293.

Schultzhaus, J. N., Bennett, C. J., Iftikhar, H., Yew, J. Y., Mallett, J., & Carney, G. E. (2018). High fat diet alters Drosophila melanogaster sexual behavior and traits: Decreased attractiveness and changes in pheromone profiles. *Scientific Reports*, *8*(1), 1–13.

South, S. H., House, C. M., Moore, A. J., Simpson, S. J., & Hunt, J. (2011). Male cockroaches prefer a high carbohydrate diet that makes them more attractive to females: Implications for the study of condition dependence. *Evolution: International Journal of Organic Evolution*, *65*(6), 1594–1606.

Stökl, J., & Steiger, S. (2017). Evolutionary origin of insect pheromones. *Current Opinion in Insect Science*, *24*, 36–42. https://doi.org/10.1016/j.cois.2017.09.004

Teal, P. E. A., & Tumlinson, J. H. (1987). The role of alcohols in pheromone biosynthesis by two noctuid moths that use acetate pheromone components. *Archives of Insect Biochemistry and Physiology*, *4*(4), 261–269.

Thomas, M. L., & Simmons, L. W. (2008). Sexual dimorphism in cuticular hydrocarbons of the Australian field cricket *Teleogryllus oceanicus* (Orthoptera: Gryllidae). *Journal of Insect Physiology*, *54*(6), 1081–1089. https://doi.org/10.1016/j.jinsphys.2008.04.012

Thomas, M. L., & Simmons, L. W. (2009). Sexual selection on cuticular hydrocarbons in the Australian field cricket, *Teleogryllus oceanicus*. *BMC Evolutionary Biology*, *9*(1), 162. https://doi.org/10.1186/1471-2148-9-162

Tregenza, T. O. M., & Wedell, N. (1997). Definitive evidence for cuticular pheromones in a cricket. *Animal Behaviour*, *54*(4), 979–984.

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

Witzgall, P., Kirsch, P., & Cork, A. (2010). Sex Pheromones and Their Impact on Pest Management. *Journal of Chemical Ecology*, *36*(1), 80–100. https://doi.org/10.1007/s10886-009-9737-y

Wyatt, T. D. (2014). *Pheromones and Animal Behavior: Chemical Signals and Signatures* (2nd ed.). Cambridge University Press. https://doi.org/10.1017/CBO9781139030748

Zahavi, A. (1975). Mate selection—A selection for a handicap. *Journal of Theoretical Biology*, *53*(1), 205–214.

CHAPTER 6

TITLE

Trans-generational paternal and maternal effects of ionizing radiation exposure on life-history features of *Acheta domesticus*

AUTHORS

Tamara M. Fuciarelli*, & C. David Rollo

Department of Biology, McMaster University, 1280 Main Street West, Hamilton ON, L8S 4L8, Canada Accepted: 26 April 2021. *Corresponding author: Email: fuciartm@mcmaster.ca

6.1 INTRODUCTION

Stress, although difficult to describe, can be defined as an organism's response to an aversive or harmful agent (Even et al., 2012). As environments are unlikely to be constant and benign, it is likely that organisms will encounter various forms of stress throughout their lifetime, with the frequency and amplitude varying between various environmental conditions (Boggs, 2009). Organisms however have evolved to manage a variety of internal and external disturbances through the use of compensatory responses (Mothersill & Seymour, 2013; Chaby, 2016). These compensatory responses can occur on various levels of biological organisation; molecular, cellular, physiological, and even social (Even et al., 2012). However, if stress is

prolonged, compensatory mechanisms may be exceeded and the result may be damage and dysfunction.

Mounting a coordinated stress response to increase survival is an energy intensive process and may result in trade-offs with other key life-history features; growth, survival, and reproduction (Isaksson et al., 2011). There are many stressors that have been shown in insects to stimulate trade-offs between key life history features including temperature (Lu et al., 2014), food stress (Boggs & Freeman, 2005); predators (Stevens et al., 1999), and density (Jones et al., 2018; Mahavidanage et al., 2023). However, these responses tend to be both stress and species specific. In the butterfly *S. mormonia* for example*,* food stress in the larval stage resulted in reduced adult survival, but when applied to adults, food stress reduced only adult reproduction (Boggs & Freeman, 2005). However, in other butterfly species *Bicylus anynana* and *P. napi* food restriction in adults reduced adult male survival but had variable affects on spermatophore production (Ferkau & Fischer, 2006).

Sex has also been shown to play a vital role in the stress responses observed between species (Boggs, 2009). In the alfalfa leaf cutting bee *Megachile rotundata*, heat stress decreased bee activity, starvation resistance, and longevity, with the affect being more pronounced in males than females (Hayes & Lopez-martinez, 2021). Similarly, differences in stress responses between sexes has been indicated in insects for a variety of stressors including radiation (Weisman et al., 2014), starvation (Hoffmann et al., 2005), pesticides (Yao et al., 2023), heavy metals (Wilczek et al., 2008), and temperature (Hayes & Lopez-martinez, 2021). It is therefore vital to assess stress responses and trade-offs using a variety of stressors as well as incorporating aspects of age, sex, and species.

In recent years due to the nuclear accidents in Chernobyl (1986) and Fukushima (2011) as well as other forms of anthropogenic contamination, the use of ionizing radiation as a stressor has become highly relevant in determining how this particular stress may impact individuals, populations, and ultimately ecosystem function (Koch & Hill, 2017). Ionization radiation exposure is well studied in its effects on human health with the effects of high dose exposure, mechanisms, and cellular pathways being well characterized (Koch & Hill, 2017). At high doses, ionizing radiation exposure causes cellular damage through two main routes; directly, through the direct interaction between ionizing particles and cellular macromolecules, and indirectly, through the ionization of water which creates reactive oxygen species (ROS's) (Koch & Hill, 2017). ROS molecules can react with and damage various cellular components including DNA, proteins, and lipids, and can therefore be highly toxic (Speakman & Garratt, 2013). As well, as animal cells predominately contain water, indirect damage is the main cause of radiotoxicity at high dose exposure (Koch & Hill, 2017). However, ROS's are naturally produced by the cell and are important signaling molecules that aid in maintaining homeostasis. These molecules are tightly regulated through antioxidant mechanisms (i.e. ascorbic acid, catalase, superoxide dismutase ect.) and repair mechanisms (DNA dlycosylase, DNA polymerase, and DNA ligase). It is therefore only when ROS production exceeds cellular tolerance that lasting damage, dysfunction and mortality occurs (Isaksson et al., 2011; Speakman & Garratt, 2013; Koch & Hill, 2017). On an organismal scale, although high dose radiation exposure has conclusively shown to result in sterilization or reduced reproduction, other life-history impacts tend to be more species specific (Bakri et al., 2005). For example, in the Mexican fruit fly, doses that decreased fertility in both males and females did not reduce development time or longevity (Stone, 1963). In the Marsh Mosquito however, sterilization doses applied to pupal stage result in high mortality post-

emergence (Stone, 1963). In hornfly's, males and females exposed to sterilization doses showed no impacts to longevity (Stone, 1963) A recent meta-analysis on the impacts of radiation exposure on wildlife has indicated that responses are highly variable among species (Einor et al., 2016). It is important to note however, that much of the work conducted on the impacts of ionizing radiation on insects has focused on species associated with the SIT due to their role as agricultural pests or disease vectors.

Although a variety of stressors have been shown to negatively impact organismal life-history traits, there is also evidence that low levels of stress can be radioprotective or hormetic (Koch & Hill, 2017). This model postulates that radiation exposure is characterised by low-dose benefits and high-dose harm. However, hormetic responses tend to be highly species, and sex specific. For example, increased longevity by low-dose radiation has been shown in a variety of insect species including in *Tribolium confusum* (Davey, 1919)*, Drosophila subobscura,*(lamb, 1964), *D. imagoes* (Sacher, 1963)*, Musca domestica* (Dauer et al., 1965)*, Acheta domesticus* (Menhinick & Crossley, 1968; Hunter & Krithayakiern, 1971)*,* and *Bracon hebetir* (Grosch, 1956). However, several studies have indicated that these responses are evident in males and not females, or to a lesser extent (Bhatnagar et al., 1965; Calabrese, 2013). Our study aims to investigate both lower-dose <1Gy and high dose exposures >1Gy in the cricket *Acheta domesticus*.

One endpoint of particular interest in the field of radiation biology is the impacts of ionizing radiation exposure across generations. Transgenerational affects can be genomic i.e., mutations, or non-genomic i.e., through induced alternations in gene expression through epigenetic modification (Curley et al., 2010; Matthews & Phillips, 2012). These impacts can potentially influence the physiology and outcomes of future generations as well as provide a

means for parents to transfer information to their offspring about their environmental conditions (Rando, 2012; Matthews & Phillips, 2012). This is highly relevant as organisms inhabiting environments in and around contamination zones as these individuals are receiving chronic, generational exposure. In Chernobyl, studies have indicated variable generational responses in invertebrate species within the Chernobyl Exclusion zone, with some species showing impacts and others not (Cannon & Kiang, 2022). A study on aquatic macroinvertebrates indicated no correlation with diversity and abundance and radiation dose (Murphy et al., 2011). However, in terrestrial invertebrates, several different insect and arachnid species were shown to have decreased abundance and diversity withing the area (Moller & Mousseau, 2009). As well, species that in Chernobyl show generational impacts i.e. genetic damage, have not been found in Fukushima (Cannon & Kiang, 2022). This may be a result of different dose rates or length of exposure, but ultimately more research must be done. However, recent reviews do indicate that despite insects being considered generally radio-resistance, they have been shown to be significantly affected by radiation in both contamination sites (Cannon & Kiang, 2022). As well, it is expected that increased and accumulative genetic damage will result in some form of physiological, developmental, and behavioural effects in exposed individuals (Mousseau & Moller, 2014).

Another interesting facet of generational effects are paternal and maternal effects, in which the maternal or paternal environment can influence offspring phenotype (Guillaume et al., 2016). These affects are not always detrimental to offspring but can sometimes be adaptive, allowing offspring to shift life-history strategies, mate choice, or behavior to better suite their environment (Mitchell & Read, 2005). Much of what we know about parental affects stems from research into maternal affects, although recently it has become apparent that paternal effects also

play a role other than just in the provision of genes alone (Guillaume et al., 2016). In insects this is also the case due to male insects generally lacking contributions to juvenile care. However, much of what we do know about paternal generational impacts is a result of the sterile insect technique (SIT), which aims to reduce insect populations through the release of sterilized or sub sterile males into insect populations. SIT studies in the tobacco hornworm where irradiated males mating with normal females showed that the longevity and fertility of F1 males and females were reduced (Seth & Reynolds, 1993). In a multigeneration study on the offspring of irradiated *Musca domestica* males found that fecundity and emergence was significantly reduced in F2 and F3 offspring (Hasaballah, 2021). In general, SIT studies have shown that impacts to F1 offspring from paternal irradiation include decreased fecundity, fertility, increased mortality, and increased development time (Dyck et al., 2005).

Unlike paternal effects, maternal effects on offspring have been well studied as mothers directly influence offspring phenotype via nuclear genes, extra-nuclear genetic elements, hormones (Bateson, 2001; Breuner, 2008; Guillaume et al., 2016). Maternal effects have been widely reported across taxa and in response to various factors including nutritional stress, mate quality, and the presence of predators (Mitchell & Read, 2005). In *Daphnia magna* for example, poor maternal environment (low food, crowding) increased offspring ability to resist bacterial infection when compared to mothers raised in optimal conditions (Mitchell & Read, 2005). Similarly, in *D.manga*, mothers in poor conditions have been shown to produce larger eggs and better quality offspring with increased survivorship (Lynch & Ennis, 1983). In the insect *Lymantria dispar* maternal diet was shown to influence offspring pupal weights, dispersal period, and development time (Mousseau & Dinge, 2003). In the fly *drosophila suzukii* heat-shock

treatment of females prior to egg laying increased offspring egg-to-adult viability (Green et al., 2019).

Both paternal and maternal experiences have been shown to impact offspring phenotype, however, what has been sparsely studied in insects is the relative contribution of each (Guillaume et al., 2016). A recent study in the marine tubeworm *Galeolaria caespitosa* discovered that paternal and maternal experiences of environmental stress (temperature) impacted F1 performance (fertilization and larval development), and most interestingly that paternal effects were often stronger than maternal effects (Guillaume et al., 2016).

Here we explored the life history impacts of males and females exposed to ionization radiation (0-27.8Gy) as well as the offspring of males mated with normal females and females mated with normal males, to generate a paternal and maternal F1 and F2 line respectively. We assessed the survivorship, growth, and longevity of all groups across all generations F0-F2 through daily monitoring. Our aim was two-fold: first to determine any potential generational impacts derived from paternal and maternal lines, and secondly to compare both paternal and maternal lines to detect potential differences. As radiation impacts are highly species and sex specific, this work aims to enhance our knowledge on the potential presence and difference in paternally and maternally inherited stress impacts in the House Cricket.

6.2 METHODS

Breeding colony: All F0 experimental crickets were generated from an inbred breeding colony. The breeding colony is housed in an acrylic terrarium (93 x 64.2 x 46.6 cm) insulated with 1.5 cm tick Durofoam insulation. Air circulation is improved using several small fans. The

colony is maintained on a 12 hour day – 12 hour night cycle at a consistent temperature of 29°C ± 2°C. 17% protein MultiFowl Grower chick feed (Quick Feeds Feed Mill, Copetown, Canada) is provided to the colony for food *ad libitum*. Distilled water is provided through soaked cellulose sponges *ad libitum*. Egg cartons are also provided to the colony as shelter. To generate the F0 experimental groups, oviposition medium, Organic Garden Soil (Swiss Farms Products Inc., Marysville, USA) was provided to the colony for a 24h period. Soils were then removed after the 24h period and incubated until hatching (~14 days). Once hatching begins nymphs were removed after a 24-hour period to ensure all experimental crickets were of known age.

F0 experimental group and irradiation: At 14 days post-hatch (~4th instar nymphs) individuals were randomly separated into 7 experimental groups; Control (0Gy), 0.58Gy, 2.3Gy, 9.3Gy, 16.2Gy, 23.2Gy, and 27.8Gy. Specific doses were achieved using a single exposure of Cs-137 source at a dose rate of 0.58Gy/min at the Taylor Radiobiology Source at McMaster University. The control group was brought and prepared for irradiation but not irradiated. This was done to ensure that all groups experienced the stress of transfer and preparation. Once irradiation was complete all groups were brought back to McMaster's Life Sciences Building (LSB) where they were monitored for life in conditions similar to that of the breeding colony. Group sample size for survival were: *Males:* 0Gy (n = 60), 0.58Gy (n = 64), 2.3Gy (n = 66) , 9.3Gy (n = 64), 16.2Gy (n = 78), 23.2Gy (n = 52), and 27.8Gy (n = 56); *Females*: 0Gy (n = 56), 0.58Gy (n = 53), 2.3Gy (n = 51) , 9.3Gy (n = 50), 16.2Gy (n = 41), 23.2Gy (n = 50), and 27.8Gy (n = 55). For growth rate, sample sizes included: *Males:* 0Gy (n = 59), 0.58Gy (n = 66), 2.3Gy (n = 63) , 9.3Gy (n = 67), 16.2Gy (n = 72), 23.2Gy (n = 48), and 27.8Gy (n = 50); *Females*: 0Gy (n = 58), 0.58Gy (n = 53), 2.3Gy (n = 55) , 9.3Gy (n = 52), 16.2Gy (n = 46), 23.2Gy (n = 55), and

27.8Gy (n = 58). ROUT method (Q= 1%) was used to identify potential outliers for growth and survival data. Outliers were identified for survival and longevity data: 0Gy male (n=1), 0Gy female (n=1), 2.3Gy male (n=2), 2.3Gy female (n=2), 9.3Gy male (n=2), 16.2Gy (n=1), 23.2Gy (n=1), 27.8Gy (n=2). For growth no outliers were detected. Outliers were removed from analysis.

Generation of Paternal and Maternal F1 Groups: To determine the impacts of paternal and maternal irradiation on F1 offspring, males and females from F0 irradiated and control groups were mated with unirradiated virgin conspecifics. F0 males and females were mated approximately 2 weeks post maturation with 20-30 non-irradiated conspecifics. Non-irradiated individuals were collected pre-maturation to ensure they had not mated previously. Oviposition medium was provided for a 48-hour period. Following this 48-hour period non-experimental individuals were removed, and oviposition medium was then incubated until hatch (~14d). 24 hours after hatch, oviposition medium was removed, and individuals were housed in the same conditions as the F0 groups. 14 days post hatch, daily monitoring for mortality and maturation data occurred.

For the Paternal F1 line survival and longevity sample sizes included for *males:* 0Gy (n = 77), 0.58Gy (n = 73), 2.3Gy (n = 77) , 9.3Gy (n = 72), 16.2Gy (n = 87), 23.2Gy (n = 78), and 27.8Gy (n = 86); *Females*: 0Gy (n = 67), 0.58Gy (n = 68), 2.3Gy (n = 66) , 9.3Gy (n = 74), 16.2Gy (n = 62), 23.2Gy (n = 57), and 27.8Gy (n = 62). For Paternal F1 growth parameters sample sizes were for *males:* 0Gy (n = 77), 0.58Gy (n = 74), 2.3Gy (n = 77) , 9.3Gy (n = 71), 16.2Gy (n = 84), 23.2Gy (n = 78), and 27.8Gy (n = 86); *females*: 0Gy (n = 68), 0.58Gy (n = 70), 2.3Gy (n = 69) , 9.3Gy (n = 74), 16.2Gy (n = 65), 23.2Gy (n = 61), and 27.8Gy (n = 60). The

ROUT method (Q= 1%) was used to identify potential outliers in survival, longevity, and growth data. For growth no outliers were detected. For survival and longevity, outliners included: 9.3Gy male (n=1), 9.3Gy female (n=3), 23.2Gy male (n=1). All identified outliers were removed from analysis.

For the maternal F1 line survival and longevity sample sizes included for *males:* 0Gy (n = 84), 0.58Gy (n =64), 2.3Gy (n = 66) , 9.3Gy (n = 56), 16.2Gy (n = 64), *females*: 0Gy (n = 59), 0.58Gy (n = 76), 2.3Gy (n = 74) , 9.3Gy (n = 91), 16.2Gy (n = 76). For maternal F1 growth parameters sample sizes were for *males:* 0Gy (n = 81), 0.58Gy (n = 63), 2.3Gy (n = 66), 9.3Gy (n = 57), 16.2Gy (n = 67), *females*: 0Gy (n = 64), 0.58Gy (n = 75), 2.3Gy (n = 75), 9.3Gy (n = 91), 16.2Gy (n = 77). Offspring from 23.2Gy and 27.8Gy irradiated F0 females were unable to produce offspring for the F1 groups. ROUT method (Q= 1%) was used to identify outliers. For survival and longevity one outlier was identified for the 0.58Gy males (n=1). For growth rate a single outlier was detected in the 16.2Gy female groups (n=1). All outliers were removed from analysis.

Generation of Paternal and Maternal F2 Generation: To generate F2 experimental and control groups individuals from each F1 paternally and maternally derived groups were provided oviposition medium approximately 2-weeks post maturation. This delay is necessary as females require time to produce viable eggs and males viable spermatophores. Oviposition medium was provided to each group for a 48-hour period after which they were removed and incubated until hatch (~14d). 24 hours after hatch oviposition medium was removed and individuals were housed in the same conditions as the F0 groups. 14 days post hatch, daily

monitoring for mortality and maturation data occurred. A summary of how F0-F2 groups were generated can be seen in Appendix **A1**.

For the Paternal F2 line survival and longevity sample sizes included for *males:* 0Gy (n = 74), 0.58Gy (n = 78), 2.3Gy (n = 79) , 9.3Gy (n = 82), 16.2Gy (n = 78), 23.2Gy (n = 79), and 27.8Gy (n = 64); *females*: 0Gy (n = 56), 0.58Gy (n = 62), 2.3Gy (n = 57) , 9.3Gy (n = 48), 16.2Gy (n = 52), 23.2Gy (n = 63), and 27.8Gy (n = 81). For Paternal F2 growth parameter sample sizes were for *males:* 0Gy (n = 76), 0.58Gy (n = 75), 2.3Gy (n = 77) , 9.3Gy (n = 78), 16.2Gy (n = 79), 23.2Gy (n = 80), and 27.8Gy (n = 60); *females*: 0Gy (n = 61), 0.58Gy (n = 67), 2.3Gy (n = 68) , 9.3Gy (n = 54), 16.2Gy (n = 58), 23.2Gy (n = 63), and 27.8Gy (n = 85). The ROUT method (Q= 1%) was used to identify potential outliers in survival, longevity, and growth data. For survival/longevity outliers were detected in the 2.3Gy female (n=2), 23.2Gy male (n=1) groups. For growth parameters, outliers were identified in the 0Gy male (n=1) 2.3Gy female (n=1), 9.3Gy female (n=1), 16.2Gy male (n=1), and the 23.2 male (n=1) group. All identified outliers were removed from analysis.

For the maternal F2 line, survival and longevity sample sizes included for *males:* 0Gy (n = 93), 0.58Gy (n = 69), 2.3Gy (n = 82) , 9.3Gy (n = 81), 16.2Gy (n = 69), f*emales*: 0Gy (n = 44), 0.58Gy (n = 71), 2.3Gy (n = 49) , 9.3Gy (n = 53), 16.2Gy (n = 58). For maternal F2 growth parameters sample sizes were for *males:* 0Gy (n = 94), 0.58Gy (n = 71), 2.3Gy (n = 82), 9.3Gy (n = 83), 16.2Gy (n = 72), *females*: 0Gy (n = 48), 0.58Gy (n = 72), 2.3Gy (n = 55), 9.3Gy (n = 59), and 16.2Gy (n = 61). The ROUT method (Q= 1%) was used to identify potential outliers in survival, longevity, and growth data. For survival/longevity, outliers were detected in only the 0Gy female (n=1) group. For growth rate several outliers were identified in the 9.3Gy male

(n=1), 16.2Gy male (n=2), and 16.2Gy female (n=2) groups. All identified outliers were removed from analysis.

Life-History Traits: Three endpoints were investigated in the F0 – F2 groups; survivorship, longevity, and growth rate. Data collection for all individuals in all groups began at 14 d post hatch. This is due to individuals being too small to accurately count or observe for mortality. At 14d until death, individuals were monitored for mortality as well as maturation, which is denoted by the development of wings. Mortality data was utilized for survivorship curves as well as to measure longevity. Growth rates were determined by measuring the mass at maturation (mg) divided by the development time to maturation (days). This allowed us to calculate mean growth rate in mg/day.

Statistics: As described above the data collected was utilized to determine growth rate, survivorship, and longevity. Growth rate was calculated for all groups as maturation mass (mg) / development time (days) and displayed as boxplots with all datapoints. Differences between groups for F0-F2 were determined by applying a one-way ANOVA followed by a Tukey's HSD test to determine significant differences between specific groups compared to the control. Similarly, differences in longevity between groups was determined through a one-way ANOVA followed by a Tukey's HSD test to determine significant differences between specific groups compared to the control. For survivorship, significant differences between experimental and control groups were detected using a Gehan-Breslow-Wilcoxon test. All statistical analysis was conducted using Prism Graph Pad 9. Significance values are displayed as follows ns $p > 0.05$, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$.

6.3 RESULTS

F0 Generation: *Survival:* Survival data was collected through the daily monitoring of all groups for mortality (**Figure 1**). In males, a Gehan-Breslow-Wilcoxon test only detected significant differences between the 23.2Gy ($p < 0.0001$) males compared to male controls. In females, significant differences were observed in the 9.3Gy ($p = 0.0009$), the 23.2Gy ($p = 0.0028$), and 27.8Gy ($p < 0.0001$) group compared to female controls. *Growth:* Growth rates for paternal F2 offspring are reported as min/max box plots with all datapoints shown (**Figure 2**). Growth rates were collected by dividing mass at maturation (mg) by development time (days). A one-way ANOVA indicated significant differences between groups $F(13, 788) = 199.1$, $p < 0.0001$. A follow up Tukey's multiple comparisons test detected significant differences between irradiated males in the 2.3Gy ($p = 0.011$), 9.3Gy, 16.2Gy, 23.2Gy, and 27.8Gy ($p < 0.0001$) groups compared to unirradiated male controls. In females, a multiple comparisons test detected significant differences in the 9.3Gy ($p = 0.0027$), 16.2Gy, 23.2Gy, and 27.8Gy ($p<0.0001$) groups compared to female controls. *Longevity:* Although a one-way ANOVA indicated significant differences between groups $F(13, 770) = 4.715$, $p < 0.0001$, a follow up multiple comparisons test only indicated slight significant differences between the 27.8Gy ($p = 0.0005$) females compared to female controls **(Figure 3)**. A summary of growth, longevity, and survival data for all groups from F0 through F2 can be found in **Table 1**.

Paternal F1: Paternal F1 groups were generated by mating males (0-27.8Gy) with virgin unirradiated control females. *Survival:* Survival data for Paternal F1 offspring were generated through the daily monitoring of all groups for mortality **(Figure 4a)**. In male F1 offspring a

Gehan-Breslow-Wilcoxon test indicated significant differences only between the 16.2Gy ($p = 0.0318$) males compared to non irradiated controls as well as 0.58Gy ($p = 0.0192$), 9.3Gy ($p<0.0001$), and 16.2Gy ($p=0.0002$) females compared to unirradiated females. *Growth:* Growth rates for paternal F2 offspring are reported as min/max box plots with all datapoints shown **(Figure 5a)**. Growth rates were collected by dividing mass at maturation (mg) by development time (days). A one-way ANOVA indicated significant differences between groups $F(13, 999) = 19.67$, $p < 0.0001$. A Tukey's multiple comparisons test indicated significant differences between the 23.2Gy ($p = 0.0136$) males compared to F1 control males. Significant differences were also observed between the 9.3Gy ($p=0.0403$) and 23.2Gy ($p = 0.0022$) females compared to F1 control females. *Longevity:* A one-way ANOVA detected significant differences between groups $F (13, 987) = 13.19$, $p < 0.0001$. However, a Tukey's HSD follow up test only indicated significant differences between 9.3Gy ($p =0.0144$) and 16.2Gy ($p=0.0382$) females compared to F1 female controls.

Maternal F1: Maternal F1 groups were generated by mating males (0-27.8Gy) with virgin nonirradiated control females. *Survival:* Survival data for Maternal F1 offspring were generated through the daily monitoring of all groups for mortality **(Figure 4b)**. For maternal F1 offspring a Gehan-Breslow-Wilcoxon test indicated significant differences only between the 0.58Gy ($p = 0.0132$) males compared to unirradiated F1 males and between 0.58Gy ($p=0.0004$) females compared to unirradiated F1 females. *Growth:* Growth rates for maternal F1 offspring are reported as min/max box plots with all datapoints shown **(Figure 5b)**. Growth rates were collected by dividing mass at maturation (mg) by development time (days). A one-way ANOVA detected significant differences between groups $F (9,705) = 22.41$, $p <0.0001$. However, a follow

up Tukey's HSD test determined that there were no significant differences between male or female Maternal F1 offspring compared to their respective F1 controls. *Longevity:* A one-way ANOVA detected significant differences between groups $F(9, 699) = 17.88$, $p < 0.0001$, however a follow up Tukey's multiple comparisons test only detected significant differences between the 0.58Gy ($p = 0.0490$) females compared to unirradiated F1 control females **(Figure 6b)**.

Paternal F2: Paternal F2 groups were generated by mating males and females from Paternal F1 groups (0-27.8Gy). *Survival*: Survival data for Paternal F2 offspring were generated through the daily monitoring of all groups for mortality **(Figure 7a).** Significant differences in survivorship were only detected between the 2.3Gy males ($p = 0.0267$) compared to F2 control males. In the Paternal F2 female's, significant differences were observed between the 2.3Gy ($p < 0.0001$), 9.2Gy ($p<0.0001$), 23.2Gy ($p<0.0001$), and 27.8Gy ($p < 0.0001$) females compared to F2 female controls. *Growth:* Growth rates for paternal F2 offspring are reported as min/max box plots with all datapoints shown **(Figure 8a)**. Growth rates were collected by dividing mass at maturation (mg) by development time (days). A one-way ANOVA detected significant differences between groups $F(13,962) = 27.12$, $p < 0.0001$. Follow up analysis indicated significant differences only between F2 female offspring; 2.3Gy ($p<0.0001$), and 23.2Gy ($p=0.0008$) compared to control F2 females. *Longevity:* A one-way ANOVA detected significant differences between groups $F(13,936) = 13.54$, $p < 0.0001$ **(Figure 9a)**. Follow up analysis indicated significant differences between the 2.3Gy ($p=0.0021$), 9.3Gy ($p=0.0054$), 23.2Gy ($p=0.0027$), and 27.8Gy ($p=0.0047$) females compared to F2 female controls. No significant differences in males were observed.

Maternal F2: Maternal F2 groups were generated by mating males and females from each maternal F1 group (0-16.2Gy). *Survival:* Survival data for maternal F2 groups were obtained through daily mortality monitoring **(Figure 7b).** A Gehan-Breslow Wilcoxon test detected significant differences between 0.58Gy ($p=0.0022$) and 16.2Gy ($p<0.0001$) males compared to F2 control males. In females, significant differences were evident in the 0.58Gy ($p=0.0016$), 9.3Gy ($p=0.0374$), and 16.2Gy ($p<0.0001$) groups compared to F2 female controls. *Growth:* Growth rates for paternal F2 offspring are reported as min/max boxplots with all datapoints shown **(Figure 8b)**. Growth rates were collected by dividing mass at maturation (mg) by development time (days). A one-way ANOVA detected significant differences between groups $F(9,682) = 25.07$, $p<0.0001$, however a follow up Tukey's multiple comparisons test only detected a significant difference between the 9.3Gy ($p=0.0386$) males compared to F2 male controls. *Longevity:* Significant differences were also detected by a one-way ANOVA for longevity $F(9,658) = 25.52$, $p <0.0001$ **(Figure 9b)**. A Tukey's multiple comparisons test detected significant differences between the 16.2Gy ($p=0.0001$) males compared to F2 control males as well as in the 16.2Gy ($p=0.0468$) females compared to F2 female controls.

6.4 DISCUSSION

Identification of generational impacts of ionizing radiation as well as potential sex differences is of vital importance to understanding the lasting impacts of populations living within environmental contamination sites. Here, we investigated such IR impacts on F1 and F2 *Acheta domesticus* from both F0 irradiated maternal and paternal exposures. Our results revealed variable effects in both the maternal and paternal lines as well as across endpoints.

Within the F0 irradiated groups, increases in survival were observed in 23.2Gy males as well as in the 9.3, 23.2, and 27.8Gy females when compared to unirradiated controls **(Figure 1)**. As well, noticeable reductions in growth rate were observed at doses ≥ 9.3Gy in both F0 irradiated males and females **(Figure 2)**. Longevity however, remained largely unaffected with only the 27.8Gy female group showing increased longevity of about 14% compared to controls **(Figure 3)**. Similar results were observed in our previous research where higher dose groups, 16.2Gy and 23.2Gy, exhibited increased survivorship compared to controls (Fuciarelli & Rollo, 2020). Increased longevity at higher doses as well as a dose-dependent reductions in growth rate were also evident in this previous research. However, it is important to note that this previous work displays results using combined male/female data and is therefore not directly comparable to this current work (Fuciarelli & Rollo, 2020).

Differential impacts to life-history features have been shown in other radiation studies. In *Drosophila suzukii,* juvenile irradiation resulted in negative impacts to reproduction but not to growth or survival (Lanouette et al., 2017). However, in *Plodia interpunctella* (Abbas et al., 2011) and *Agrotis ipsilon* (Salem et al., 2014) early life exposure to IR dose-dependently increased developmental periods with growth being significantly decreased in the *Plodia interpunctella* population. In the mosquito *Culex pipiens* irradiated as pupae, individuals showed that gamma dose significantly increased pupal mortality, decreased adult emergence percentages, and greatly reducing fertility in irradiated adults (Hasaballah, 2018). However, in *Anopheles arabiensis* early life IR exposure in males had no effect on adult emergence or survival but did show decreases in reproductive ability (Helinski et al., 2006). Current literature therefore highlights the need for species specific data across a variety of endpoints, doses, exposure rates, and sexes.

Although a key aspect of potential life-history trade-offs between growth, survival, and reproduction, the reproductive ability of the F0-F2 generation was not directly investigated here. However, similar to the variable effects described above, reproductive effects are highly dependant on dose, with higher doses significantly reducing reproductive ability. The sterile insect technique (SIT), which aims to reduce insect populations through the release of sterile or sub sterile males to mate with normal females, has shown that IR can be used to successfully reduce insect populations through impaired reproduction (Dyck et al., 2005). As well, even sub-sterile irradiated males can produce offspring that are themselves sterile or have reduced reproductive capacity (Bloem et al., 2005). IR at high doses has been conclusively shown to reduce or abolish reproductive ability in many insect species including in *Epiphyas posvittana* (Soopaya et al., 2011), *Culex pipiens* (Hasaballah, 2018), *Ephestia cautella* (Al-Taweel et al., 1990), *Pectinophora gossypiella* (Qureshi et al., 1995), *Conopomorpha sinensis* (Fu et al., 2016), *Aedes aegypti* (Shetty et al., 2016) and *Glossina brevipalpis* (de Beer et al., 2017). Reproductive declines in future generations from irradiated parents has also been shown to occur from sub-sterile parents including in *Spodoptera littoralis* (Sallam & Ibrahim, 1993), *Conopomorpha sinensis* (Fu et al., 2016), and *Aedes aegypti* (Shetty et al., 2016). At lower doses however, reproductive IR exposure has been shown to have hormetic or stimulatory effects in several insect species. In *Acheta domesticus,* low dose (<1Gy) juvenile exposure increased female fecundity, offspring size, and offspring performance (Shephard et al., 2018). In males, increased mating success and sperm activity was described in *Spodoptera litura* when exposed as juveniles to 0.75Gy and 1Gy doses of IR (Vimal et al., 2023).

Reproductive endpoints are a key factor in life-history trade-offs, especially at lower doses where hormetic responses may be evident. Our results, although not directly testing

potential sterility, detected possible sterility in females, as we were unable to generate maternal lines for F1 and F2 groups at doses ≥ 16.2Gy. In males however, all doses up to 27.8Gy were able to be successfully generated. Interestingly however, previous work has indicated that doses ≥ 27.8Gy would cause sterility in male *Acheta domesticus* (Fuciarelli & Rollo, 2020). However, in this previous study it is likely that the limited mating time (24h) and the described reduced mating success of these males resulted in the lack of an F1 generation and not necessarily sterility (Fuciarelli & Rollo, 2020; Fuciarelli & Rollo 2021). However, female insects being more radiosensitive as compared to males has been documented in several species. In *Epiphyas posvittana* declines in fertility and fecundity were more pronounced in females than in irradiated males (Soopaya et al., 2011). As well, in the Codling moth *Cydia pomonella* females were considerably more radiosensitive to sterility than males (Blomefield et al., 2010).

Results for the F1 and F2 life-history impacts from maternal and paternal irradiated *Acheta* were varied. In the F1 paternal line, 16.2Gy males and females showed increased survivorship as well as the 0.58Gy and 9.3Gy females when compared to controls **(Figure 4a)**. The 9.3Gy and 16.2Gy female increases in survivorship also translated to a slight increase in longevity **(Figure 6a)**. In the F1 maternal line however, only the 0.58Gy male and female offspring showed decreased survivorship and longevity **(Figure 4b, 6b)**. F1 growth in both maternal and paternal lines remained largely unaffected, with only a few groups in the paternal lines showing very slight decreases in growth rate **(Figure 5)**.

For F2 paternal and maternal offspring similar trends were observed with paternal offspring being more impacted than maternal offspring. Survivorship of F2 paternal offspring was significantly decreased in the 2.3Gy males and 2.3, 9.3, 23.2, and 27.8Gy females compared to controls **(Figure 7a)**. For the female groups this also resulted in reduced longevity **(Figure**

9a). In F2 maternal offspring, 0.58Gy males and females as well as 9.3Gy females displayed decreased survival **(Figure 7b)**. However, the 16.2Gy group in both males and females showed increased survival and longevity **(Figure 9b)**. Growth rates for both F2 maternal and paternal lines were largely unaffected **(Figure 8)**.

Similar to the impacts of IR on F0 irradiated insects, generational studies also indicate variable responses that tend to be species specific. A generational study of F1 offspring of irradiated male and female *Epiphyas posvittana* indicated deleterious effects in the F1 generation including reduction in F1 female survival, decreased adult emergence, increased mortality, and reduced F1 and F2 reproductive output (Soopaya et al., 2011). Several studies investigating generational impacts of parental irradiation have shown that F1 progeny develop more slowly and have reduced emergence than control individuals (North, 1975). In F0 IR exposed *Aedes aegypti* for example, a stark dose-dependent decline in adult emergence in F1-F3 generation was observed (Shetty et al., 2016). As well, in *Manduca sexa* F1 offspring of irradiated males mated with normal females grew and developed more slowly when compared to controls, and displayed increased mortality (Seth & Reynolds, 1993). Similarly in *Drosophila melanogaster,* F1 offspring of irradiated parents had decreased adult body weight (Vaiserman et al., 2004). This was not evident in our study here, growth rate parameters seemed to recover from parental exposure with very little change observed between maternal or paternal F1 and F2 groups compared to the controls.

Although some groups in the F1 and F2 paternal and maternal offspring had decreases in longevity and survivorship, some groups exhibited increases in longevity compared to controls. This is contradictory to many of the studies in the literature which find that F1 and F2 offspring of irradiated parents have either neutral responses to survival or longevity or negative responses.

In the literature, many of the declines associated with survival and longevity are also paired with reductions in reproduction. In the gypsy moth *Porthetria dispar,* F1 offspring of irradiated parents exhibited increased mortality in larval stages as well as partial sterility (North, 1975). In the fly *Drosophila suzukii, w*hen irradiated males were mated with normal females, egg hatchability was greatly reduced, with surviving F1 offspring showing increased egg-adult morality (Lanouette et al., 2017). Similar experiments in the false codling moth *Cryptophlebia leucotreta* showed that F1 offspring of irradiated parents exhibited decreased fecundity and fertility, and increased mortality (Bloem et al., 2003).

Another interesting aspect of our results is that more alterations to life-history endpoints in F1 and F2 offspring occurred on the paternal line when compared to the maternal line. Although there is sparce literature on the topic of distinguishing between paternal and maternal effects with radiation, the increased sensitivity of the paternal line is supported in the literature. In the fall armyworm *Spodoptera frugiperda* F0 deleterious affects were shown to be more pronounced in F0 females than for males (Carpenter et al., 1986). However, in offspring, inheritance of deleterious affects was greater for paternal lines than maternal lines (Carpenter et al., 1986). There is current literature outlining paternal effects (largely associated with the SIT) and maternal effects with radiation in several different species. What is currently lacking however is an understanding of the various modes of stress inheritance and how they differ between maternal and paternal irradiation. It is therefore vital more research be conducted in single experiments where both parental affects are described across various endpoints.

A vast majority of studies discussed here are directly related to various insect eradication methods, which largely limits the scope of species studied to two orders: Lepidoptera and Diptera. Very little data has been conducted on non pest or disease vector species. To better

understand and compare the results here it is vital more work be conducted on a variety of species, as many radiation induced responses tend to be highly species specific. As it is generally understood that insects increase in radiation tolerance with age and developmental stage, it is important to test a variety of ages for how IR exposure impacts various life-history features (North, 1975; Soopaya et al., 2011). This work aimed to better elucidate the impacts of ionizing radiation exposure on future generations which may be relevant in releases of sterile or sub-sterile insects for the SIT as well as populations inhabiting various radiation contamination zones.

6.5 ACKNOWLEDGEMENTS

This work was supported by the Mitacs Elevate Grant (IT05851), and the NSERC Discovery Grant (RGPIN-05693-2015).

6.6 TABLES AND FIGURES

Table 1: Summary of survival and growth data of F0 control and irradiated *Acheta domesticus* as well as F1 and F2 male and female offspring. Maternal F1 and F2 lines are highlighted in green whereas the F1 and F2 Paternal Lines are highlighted in blue.

	Group	Median longevity (days)	% of controls	Mean longevity (days)	Maximal longevity (days)	Avg. Mass at Maturation (mg)	Avg. Development Time (days)	Avg. Growth rate (mg/day)	% of control
Control	F0 Male	148		146.4	187	399.6	41.9	9.52	
	F0 Female	136		135.1	174	439.6	41.2	10.64	
	Paternal F1 Male	142		142.2	180	340.4	44.4	7.68	
	Paternal F1 Female	128		126.2	163	361.1	41.8	8.64	
	Maternal F1 Male	143.5		145.2	204	354.6	47.7	7.45	
	Maternal F1 Female	129.0		129.3	176	377.8	45.3	8.36	
	Paternal F2 Male	151		150.4	215	371.9	49.4	7.6	
	Paternal F2 Female	146.5		144.7	184	406.9	46.2	8.46	
	Maternal F2 Male	150		148	188	374.5	48.3	7.75	
	Maternal F2 Female	132		133.2	156	404.8	47.1	8.59	
0.58 Gy	F0 Male	148	0%	150.6	221	398.4	41.4	9.6	0.84
	F0 Female	142	4.4%	140	185	462.6	40.8	11.32	6.4
	Paternal F1 Male	137	-3.5%	142.3	195	335	45.1	7.44	-3.1
	Paternal F1 Female	133	3.9%	133.9	181	346.6	42.5	8.16	-5.6

	Maternal F1 Male	137	-4.5	137.2	170	357.7	46.6	7.66	2.8
	Maternal F1 Female	120	-7.0	177.6	143	383.3	44.1	8.68	3.8
	Paternal F2 Male	147	-2.7%	152	222	337.5	46.2	7.31	-3.8
	Paternal F2 Female	140	-4.4%	140.4	211	376.2	45	8.37	-1.1
	Maternal F2 Male	136	-9.3%	137.2	197	391.4	47.6	8.21	5.9
	Maternal F2 Female	126	-4.6%	123.1	156	413.6	45.4	9.09	5.8
2.3 Gy	F0 Male	139.5	-5.7%	143.8	187	380	43.2	8.76	-8.0
	F0 Female	141	3.7%	142.7	191	445.8	42.2	10.56	-0.75
	Paternal F1 Male	142	0%	138.7	189	351.6	45.1	7.8	1.6
	Paternal F1 Female	121	-5.5%	122.1	175	370.5	42.9	8.64	0
	Maternal F1 Male	141	-1.7	142.9	183	336.6	44.6	7.55	1.3
	Maternal F1 Female	129.5	0.4	129.9	171	367.4	42.4	8.67	3.7
	Paternal F2 Male	141	-6.6	142	207	388.9	48.6	8.0	5.2
	Paternal F2 Female	127	-13.3	126.2	149	429.8	46.4	9.4	11
	Paternal F2 Male	149	-0.7%	150.3	212	380.8	47.7	7.98	3.0
	Paternal F2 Female	125	-5.3%	121.7	163	409.4	46	8.89	3.5

9.3 Gy	F0 Male	151	2.0%	150.1	191	362.6	43.3	8.33	-12.5
	F0 Female	151	11.0%	149.3	208	407.2	41.6	9.78	-8.1
	Paternal F1 Male	148	4.2%	149.2	202	318.6	43.5	7.32	-4.7
	Paternal F1 Female	139	8.6%	139.7	180	337.8	41.7	8.1	-6.25
	Maternal F1 Male	147	2.4%	146.8	200	345.4	44.8	7.70	3.4
	Maternal F1 Female	132	2.3%	130.9	173	361.8	42.9	8.42	0.7
	Paternal F2 Male	151.5	0.33%	151.3	205	342.5	45.3	7.57	-0.4
	Paternal F2 Female	128	-12.6%	126.6	174	384.6	45.4	8.4	-0.7
	Maternal F2 Male	150	0%	145.9	202	337.6	47.1	7.24	-6.6
	Maternal F2 Female	126	-4.6%	126.3	190	363	44.7	8.13	-5.4
16.2 Gy	F0 Male	152	2.7%	148.5	189	329.8	47.1	6.99	-26.6
	F0 Female	146	7.4%	138.8	178	366.1	45.2	8.1	-23.9
	Paternal F1 Male	149	4.9%	150.3	197	324.2	44.1	7.35	-4.3
	Paternal F1 Female	137	7.0%	139.1	174	344.2	42.1	8.19	-5.2
	Maternal F1 Male	145.5	1.4%	153.8	215	330.7	44.7	7.4	-0.7
	Maternal F1 Female	134.5	4.3%	131.4	180	355.7	43.6	8.11	-3.0
	Paternal F2 Male	154.5	2.3%	157	208	348.5	49.4	7.12	-6.3
	Paternal F2 Female	145.5	0.7%	141.3	177	381.6	47.2	8.09	-4.4

	Maternal F2 Male	161	7.3%	164.4	209	333.7	47	7.27	-6.2
	Maternal F2 Female	147	11.4%	147.3	178	354.3	46	7.98	-7.1
23.2 Gy	F0 Male	159	7.4%	159.9	198	271	48.3	5.64	-40.8
	F0 Female	156.5	15.1%	148.2	205	311.3	47.5	6.65	-37.5
	Paternal F1 Male	144	1.4%	145.1	181	345.5	48.5	7.12	-7.3
	Paternal F1 Female	128	0%	125.4	170	367.6	46.3	7.94	-8.1
	Paternal F2 Male	146	-3.3%	146.3	193	375.6	52.5	7.22	-5.0
	Paternal F2 Female	126	-14%	127	158	390.2	51	7.66	-9.5
27.8 Gy	F0 Male	145	-2.0%	141.7	197	255.3	51.6	4.99	-47.6
	F0 Female	155	14.0%	154.4	198	274.5	52.3	5.37	-49.5
	Paternal F1 Male	141.5	-0.4	139.1	177	332.7	46.3	7.85	2.2
	Paternal F1 Female	126.5	-1.2	124.3	171	347.2	40.9	8.5	-1.6
	Paternal F2 Male	140.5	-7.0%	142.3	211	362.7	47.6	7.62	0.3
	Paternal F2 Female	128	-12.6%	128.5	163	386	46.3	8.36	-1.2

All radiation groups are compared to their respective sex from that generations control group. All growth parameters (development time, mass at maturation, growth rate) are shown as mean values for each group.

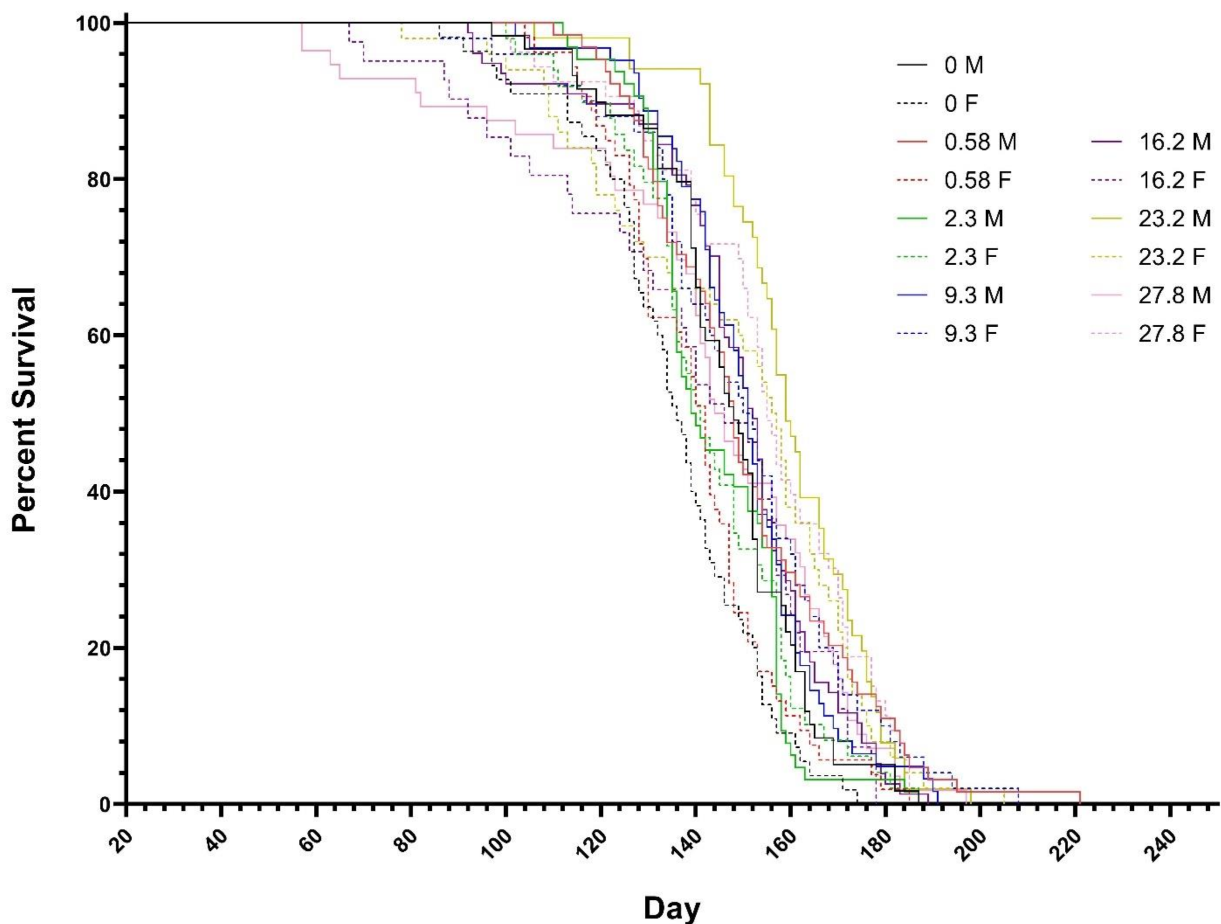


Figure 1: Kaplan-Meier survival curves for the F0 population of control and irradiated (0.58 – 27.8Gy) male and female *Acheta domesticus.* A Gehan-Breslow-Wilcoxon test indicated significant differences between only the 23.2Gy ($p < 0.0001$) males compared to the male controls. In females, significant differences were seen in the 9.3Gy ($p = 0.0009$), the 23.2Gy ($p = 0.0028$), and 27.8Gy ($p < 0.0001$) compared to female controls.

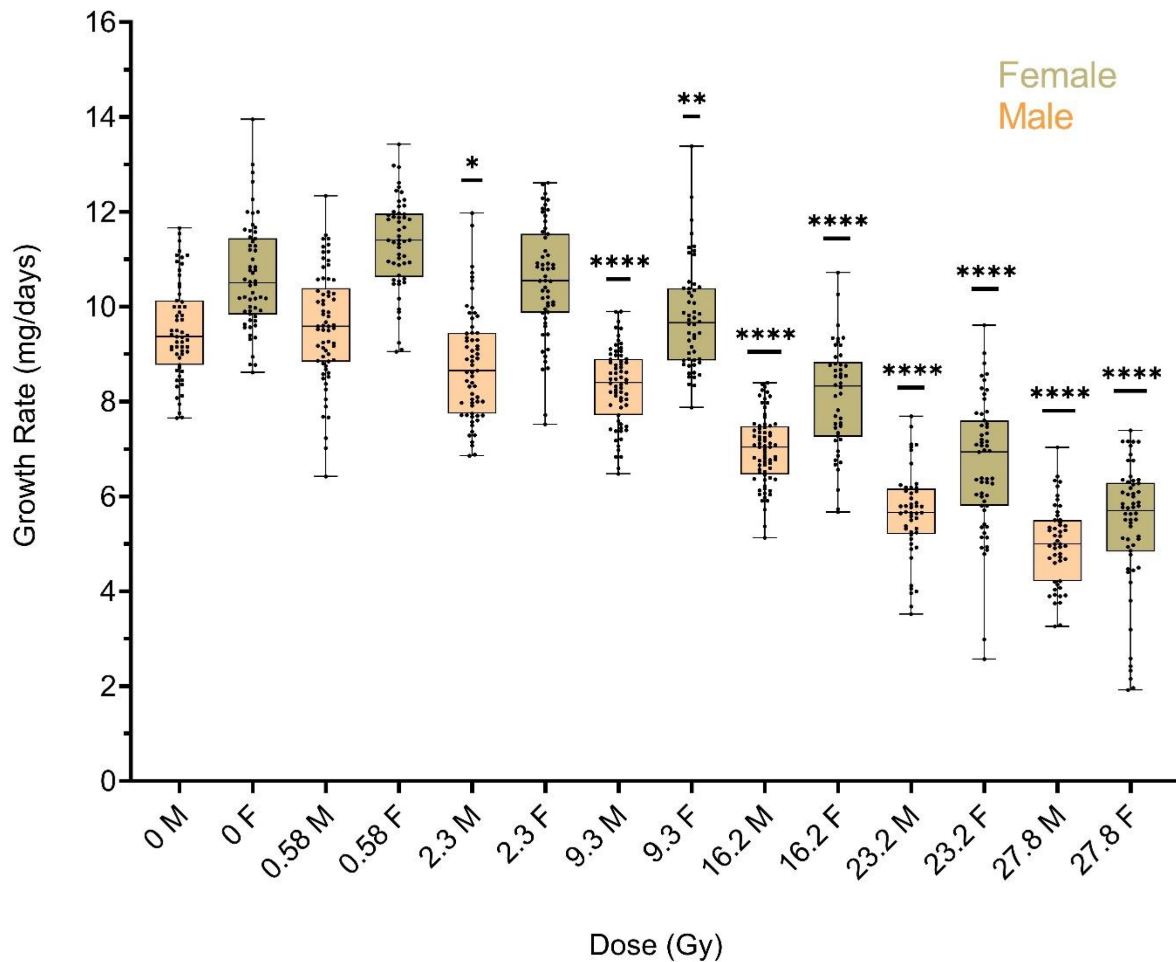


Figure 2: Impacts of ionizing radiation exposure (0-27.8Gy) on the growth rate of male and female *Acheta domesticus.* Individual values as well as the group mean, 25th, and 75th percentile are shown for each group. A one-way ANOVA indicated significant differences between groups $F(13, 788) = 199.1$, $p < 0.0001$. A Tukey's multiple comparisons test indicated significant differences in most irradiated males 2.3-27.8 and irradiated females 9.3-27.8Gy compared to their respective controls.

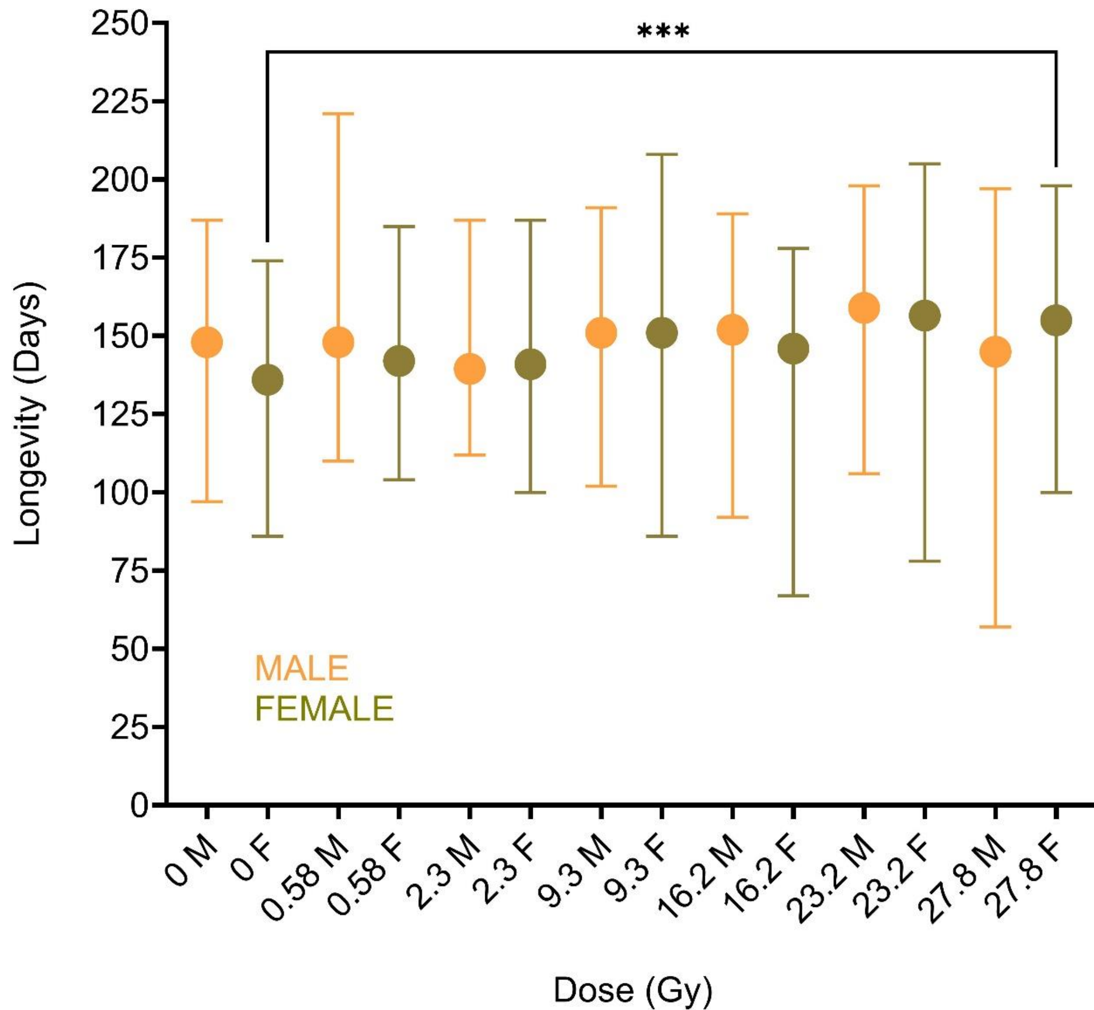


Figure 3: Impacts of ionizing radiation exposure (0.58 – 27.8Gy) on male and female *Acheta domesticus* longevity. All values are represented as median longevity with bars indicating the range of values observed. A one-way ANOVA detected significant differences between groups $F_{(13, 770)} = 4.715$, $p < 0.0001$, however, a follow up test determined that significant differences were only present between the 27.8Gy ($p = 0.0005$) females compared to the unirradiated female controls.

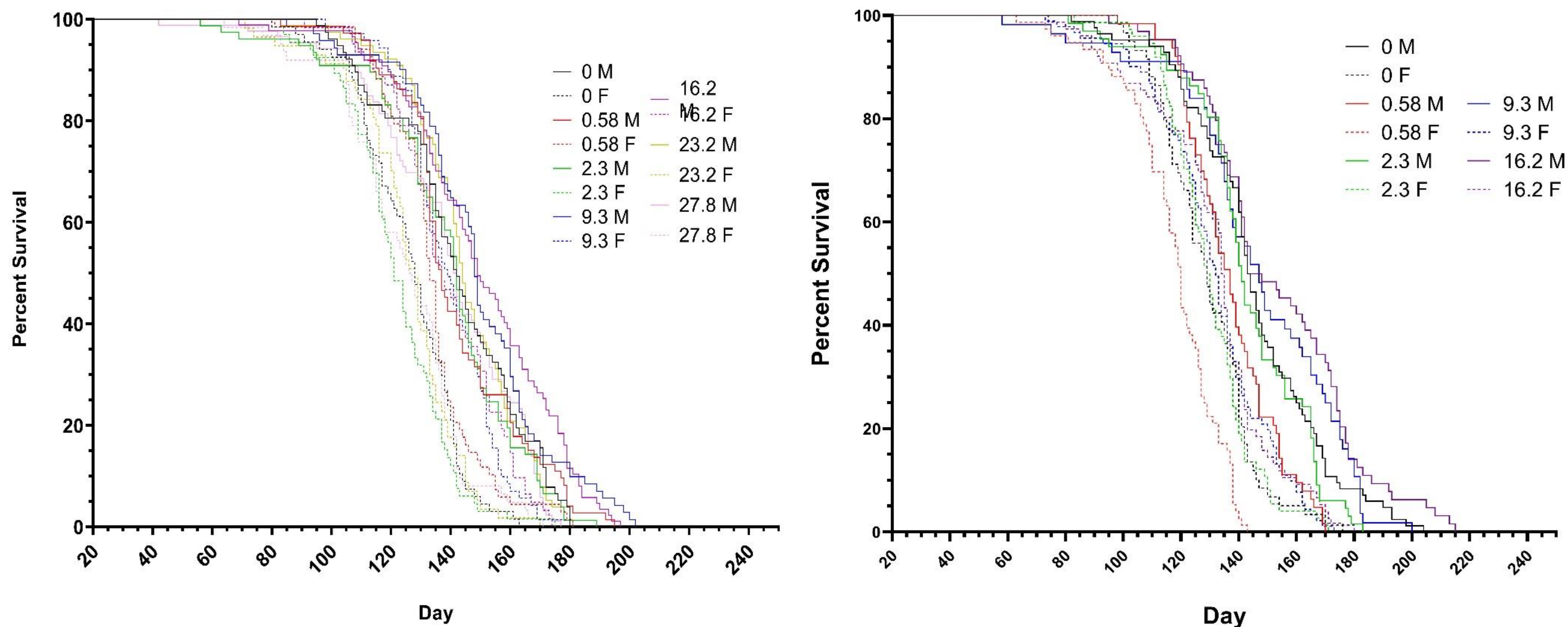


Figure 4: Kaplan-Meier survival curves for the F1 population of offspring from paternal (a) and maternal (b) control and irradiated (0.58 – 27.8Gy) lines of *Acheta domesticus.* Each survival curve represents the male and female offspring of control or irradiated fathers (a) or mothers (b) mated with non-irradiated conspecifics. For paternal F1 offspring a Gehan-Breslow-Wilcoxon test indicated significant differences only between the 16.2Gy ($p = 0.0318$) males compared to non irradiated controls as well as 0.58Gy ($p = 0.0192$), 9.3Gy ($p<0.0001$), and 16.2Gy ($p=0.0002$) females compared to non-irradiated females (a). For maternal F1 offspring a Gehan-Breslow-Wilcoxon test indicated significant differences only between the 0.58Gy ($p = 0.0132$) males compared to F1 male controls and between 0.58Gy ($p=0.0004$) females compared to F1 female controls(b). Offspring of F0 females at doses >16.2Gy were not able to be generated.

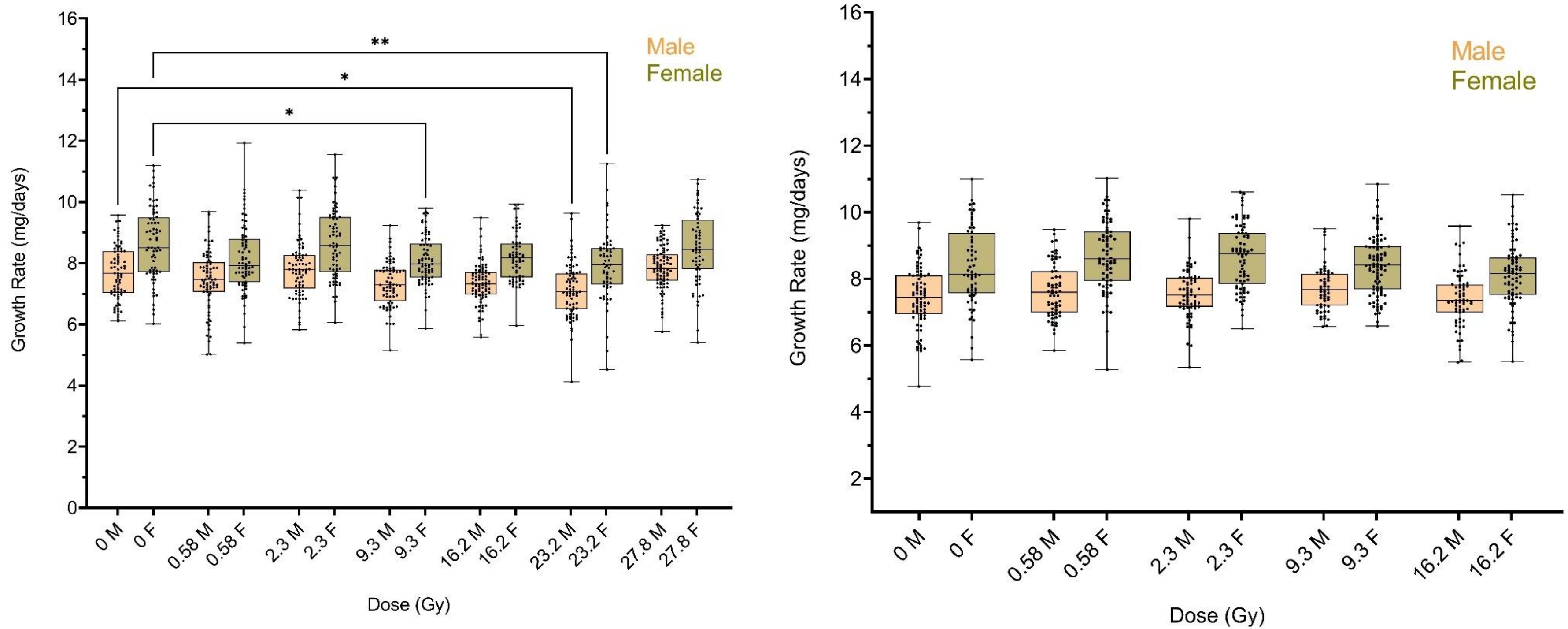


Figure 5: Growth rates (mg/day) of *Acheta domesticus* for both paternally (a) and maternally (b) derived F1 male and female offspring. Individual values as well as the group mean, 25th, and 75th percentile are shown for each group. For F1 paternal offspring a one-way ANOVA indicated significant differences between groups $F(13, 999) = 19.67$, $p < 0.0001$. A Tukey's multiple comparisons test indicated significant differences between the 23.2Gy ($p = 0.0136$) males compared to F1 control males. Significant differences were also observed between the 9.3Gy ($p=0.0403$) and 23.2Gy ($p = 0.0022$) females compared to F1 control females. (a). For maternal F1 offspring significant differences were also observed between groups $F (9, 705) = 22.41$, $p < 0.0001$. However, the follow up test detected no significant differences between maternally derived groups in males or females when compared to F1 control groups.

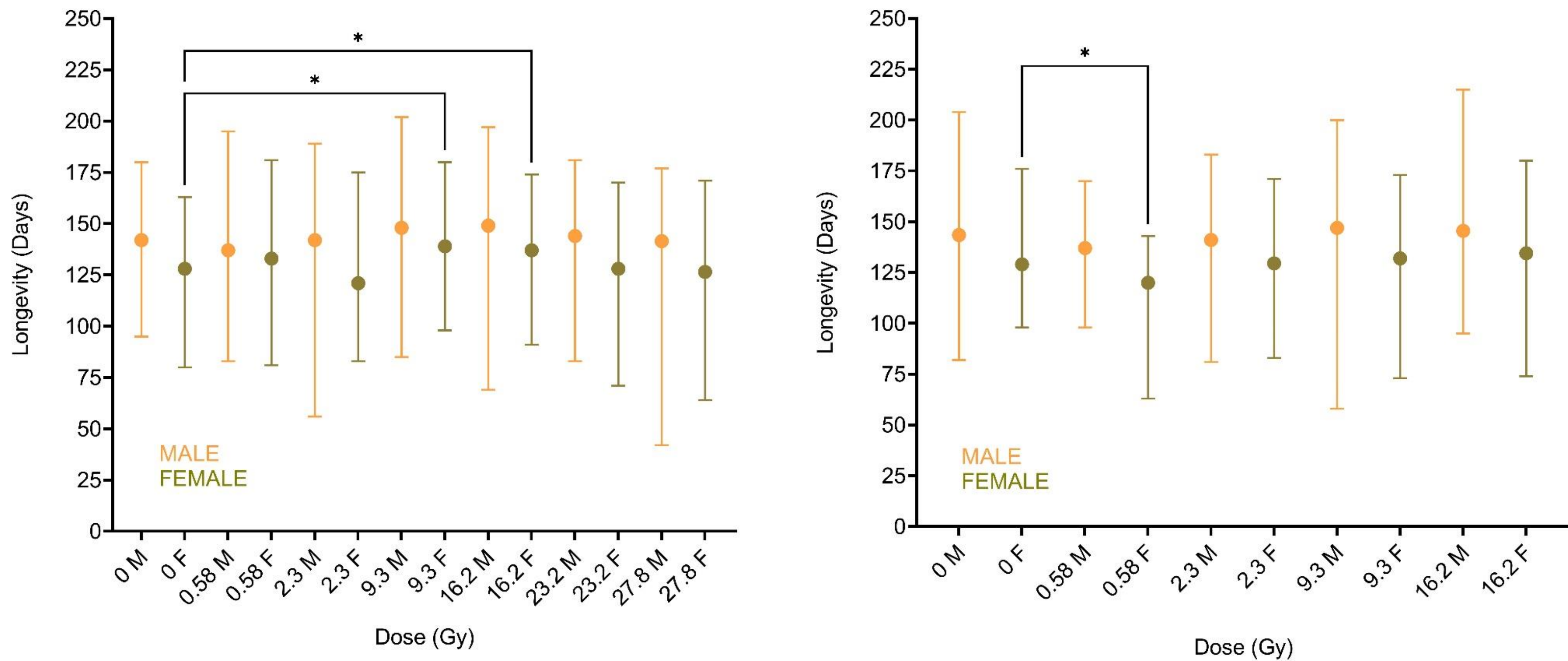


Figure 6: Impacts of ionizing radiation exposure (0.58 – 27.8Gy) on F1 male and female *Acheta domesticus* derived from paternal (a) and maternal (b) F0 parental lines. All values are represented as median longevity with bars indicating the range of values observed. For paternal F1 offspring, a one-way ANOVA detected significant differences between groups $F(13, 987) = 13.19$, $p < 0.0001$. A Tukey's HSD follow up test only indicated significant differences between 9.3Gy ($p = 0.0144$) and 16.2Gy ($p = 0.0382$) females compared to F1 female controls (a). For F1 maternal offspring a one-way ANOVA detected significant differences between groups $F(9, 699) = 17.88$, $p < 0.0001$, however follow up statistical analysis showed significant differences only between the 0.58Gy ($p = 0.0490$) females compared to F1 female controls (b).

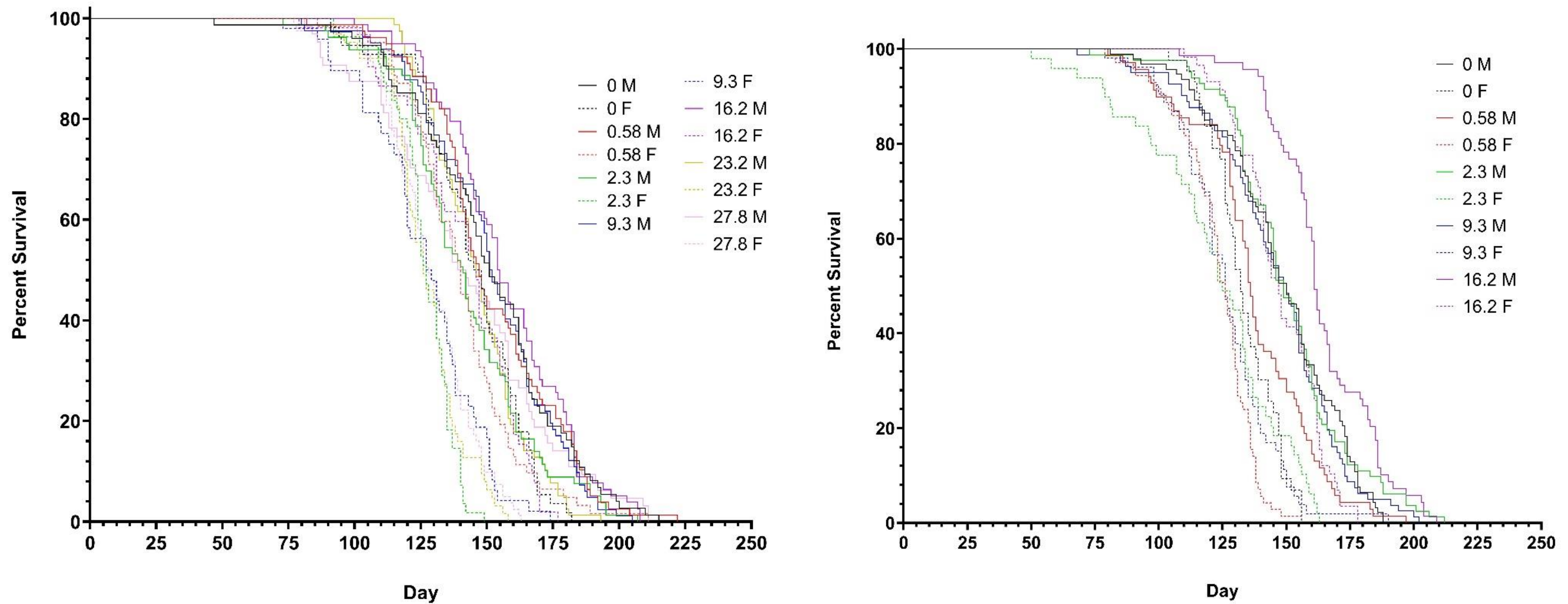


Figure 7: Kaplan-Meier survival curves for the F2 populations of offspring from paternal (a) and maternal (b) F1 groups of *Acheta domesticus.* Each survival curve represents the male and female offspring of each paternal or maternally derived F1 group. For F2 paternal groups significant differences in survivorship were only detected between the 2.3Gy males ($p = 0.0267$) compared to F2 control males. In female F2 paternal offspring significant differences were observed between the 2.3Gy ($p < 0.0001$), 9.3Gy ($p<0.0001$), 23.2Gy ($p<0.0001$), and 27.8Gy ($p <0.0001$) females compared to F2 female controls (a). For F2 maternal groups statistical analysis detected significant differences between 0.58Gy ($p=0.0022$) and 16.2Gy ($p<0.0001$) males compared to F2 control males. In females, significant differences were evident in the 0.58Gy ($p=0.0016$), 9.3Gy ($p=0.0374$), and 16.2Gy ($p<0.0001$) groups compared to F2 female controls (b).

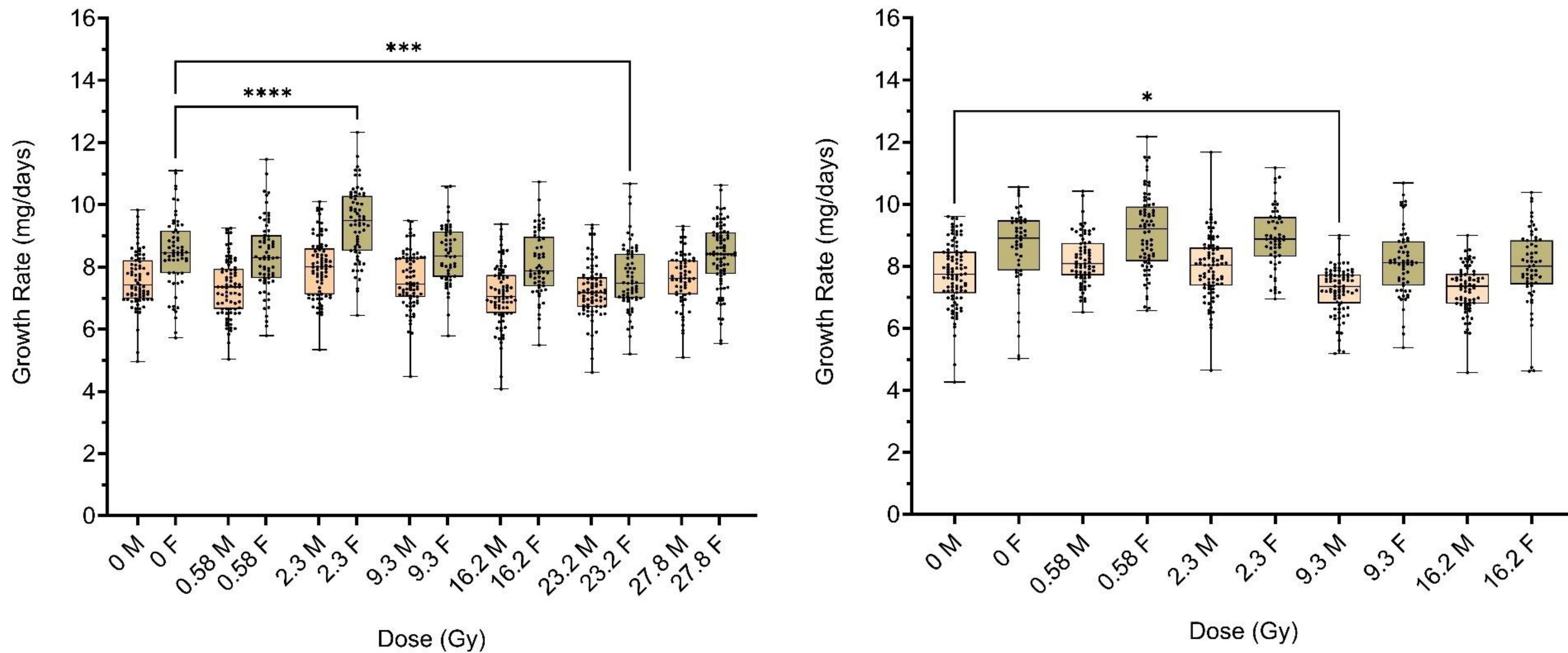


Figure 8: Growth rates (mg/day) of *Acheta domesticus* for both paternally (a) and maternally (b) derived F2 male and female offspring. Individual values as well as the group mean, 25th, and 75th percentile are shown for each group. For F2 paternal offspring, a one-way ANOVA indicated significant differences between groups $F(13, 962) = 27.12$, $p < 0.0001$. A follow up Tukey's HSD test determined significant differences between the 2.3Gy ($p<0.0001$) and 23.2Gy ($p=0.0008$) males compared to controls (a). For maternal F2 offspring significant differences were also observed between $F(9,682) = 25.07$, $p<0.0001$, however a follow up Tukey's multiple comparisons test only detected a significant difference between the 9.3Gy ($p=0.0386$) males compared to F2 male controls (b).

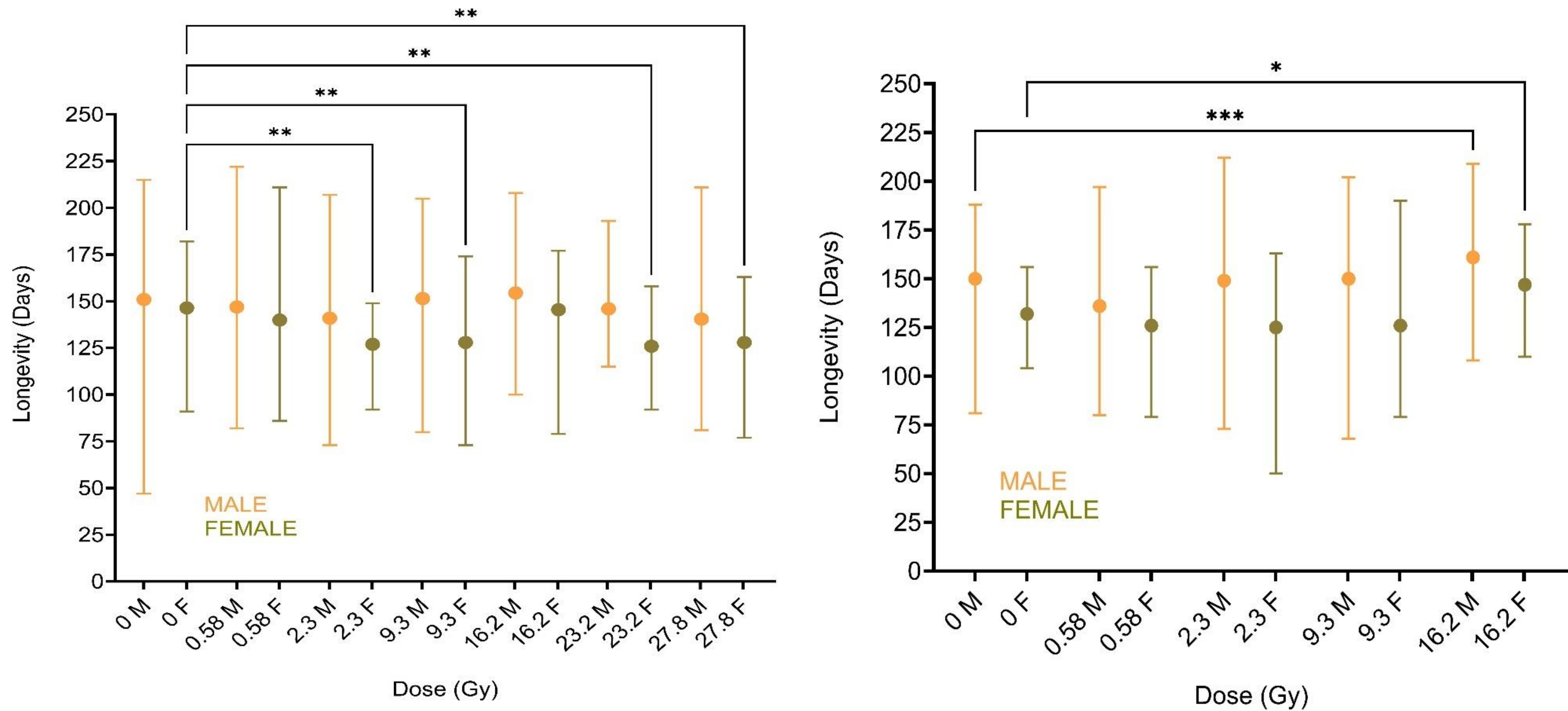


Figure 9: Longevity (days) of *Acheta domesticus* for both paternally (a) and maternally (b) derived F2 male and female offspring. For F2 paternal offspring a one-way ANOVA detected significant differences between groups F (13,936) = 13.54, p <0.0001. Follow up analysis indicated significant differences between the 2.3Gy (p=0.0021), 9.3Gy (p=0.0054), 23.2Gy (p=0.0027), and 27.8Gy (p=0.0047) females compared to F2 female controls (a). For F2 maternal offspring, significant differences were also detected by a one-way ANOVA for longevity F(9,658) = 25.52, p <0.0001. A Tukey's multiple comparisons test determined significant differences between the 16.2Gy (p=0.0001) males compared to F2 control males as well as in the 16.2Gy (p=0.0468) females compared to F2 female controls. (b)

6.7 REFERENCES

Abbas, H., Nouraddin, S., Reza, Z. H., Iraj, B., Mohammad, B., Hasan, Z., Hossein, A. M., & Hadi, F. (2011). Effect of gamma radiation on different stages of Indian meal moth *Plodia interpunctella* Hübner (Lepidoptera: Pyralidae). *African Journal of Biotechnology*, *10*(20), 4259–4264.

Al-Taweel, A. A., Ahmed, M. S., Kadhum, S. S., & Hameed, A. A. (1990). Effects of gamma radiation on the progeny of irradiated *Ephestia cautella* (Walker)(Lepidoptera: Pyralidae) males. *Journal of Stored Products Research*, *26*(4), 233–236.

Bakri, A., Mehta, K., & Lance, D. R. (2005). Sterilizing insects with ionizing radiation. *Sterile Insect Technique: Principles and Practice in Area-Wide Integrated Pest Management*, 233–268.

Bateson, P. (2001). Fetal experience and good adult designa. *International Journal of Epidemiology*, *30*(5), 928–934. https://doi.org/10.1093/ije/30.5.928

Bhatnagar, P. L., Rockstein, M., & Dauer, M. (1965). X-irradiation of pupae of the house fly *Musca domestica* L. and adult survival. *Experimental Gerontology*, *1*(2), 149–159.

Bloem, K. A., Bloem, S., & Carpenter, J. E. (2005). Impact of moth suppression/eradication programmes using the sterile insect technique or inherited sterility. *Sterile Insect Technique: Principles and Practice in Area-Wide Integrated Pest Management*, 677–700.

Bloem, S., Carpenter, J. E., & Hofmeyr, J. H. (2003). Radiation biology and inherited sterility in false codling moth (Lepidoptera: Tortricidae). *Journal of Economic Entomology*, *96*(6), 1724–1731.

Blomefield, T. L., Bloem, S., & Carpenter, J. E. (2010). Effect of radiation on fecundity and fertility of codling moth *Cydia pomonella* (Linnaeus) (Lepidoptera: Tortricidae) from South Africa. *Journal of Applied Entomology*, *134*(3), 216–220. https://doi.org/10.1111/j.1439-0418.2008.01377.x

Boggs, C. L. (2009). Understanding insect life histories and senescence through a resource allocation lens. *Functional Ecology*, *23*(1), 27–37.

Boggs, C. L., & Freeman, K. D. (2005). Larval food limitation in butterflies: Effects on adult resource allocation and fitness. *Oecologia*, *144*, 353–361.

Breuner, C. (2008). Maternal stress, glucocorticoids, and the maternal/fetal match hypothesis. *Hormones and Behavior*, *54*(4), 485.

Cannon, G., & Kiang, J. G. (2022). A review of the impact on the ecosystem after ionizing irradiation: Wildlife population. *International Journal of Radiation Biology*, *98*(6), 1054–1062.

Carpenter, J. E., Young, J. R., & Sparks, A. N. (1986). Fall armyworm (Lepidoptera: Noctuidae): comparison of inherited deleterious effects in progeny from irradiated males and females. *Journal of Economic Entomology*, *79*(1), 46–49.

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.

Curley, J. P., Mashoodh, R., & Champagne, F. A. (2011). Epigenetics and the Origins of Paternal Effects. *Hormones and Behavior*, *59*(3), 306–314. https://doi.org/10.1016/j.yhbeh.2010.06.018

Dauer, M., Bhatnagar, P. L., & Rockstein, M. (1965). X-Irradiation of Pupae of the House Fly, *Musca Domestica* L., and Male Survival1. *Journal of Gerontology*, *20*(2), 219–223. https://doi.org/10.1093/geronj/20.2.219

Davey, W. P. (1919). Prolongation of life of *Tribolium confusum* apparently due to small doses of X-rays. *Journal of Experimental Zoology*, *28*(3), 447–458.

De Beer, C. J., Moyaba, P., Boikanyo, S. N., Majatladi, D., Yamada, H., Venter, G. J., & Vreysen, M. J. (2017). Evaluation of radiation sensitivity and mating performance of *Glossina brevipalpis* males. *PLoS Neglected Tropical Diseases*, *11*(3), e0005473.

Dyck, V. A., Hendrichs, J., & Robinson, A. S. (2021). *Sterile insect technique: Principles and practice in area-wide integrated pest management*. Taylor & Francis.

Einor, D., Bonisoli-Alquati, A., Costantini, D., Mousseau, T. A., & Møller, A. P. (2016). Ionizing radiation, antioxidant response and oxidative damage: A meta-analysis. *Science of the Total Environment*, *548*, 463–471.

Even, N., Devaud, J.-M., & Barron, A. B. (2012). General stress responses in the honey bee. *Insects*, *3*(4), 1271–1298.

Ferkau, C., & Fischer, K. (2006). Costs of reproduction in male *Bicyclus anynana* and *Pieris napi* butterflies: Effects of mating history and food limitation. *Ethology*, *112*(11), 1117–1127.

Fu, H., Zhu, F., Deng, Y., Weng, Q., Hu, M., & Zhang, T. (2016). Development, reproduction and sexual competitiveness of *Conopomorpha sinensis* (Lepidoptera: Gracillariidae) gamma-irradiated as pupae and adults. *Florida Entomologist*, *99*(sp1), 66–72.

Fuciarelli, T. M., & Rollo, C. D. (2020). Trans-generational impacts of paternal irradiation in a cricket: Damage, life-history features and hormesis in F1 offspring. *Dose-Response*, *18*(4), 1559325820983214.

Fuciarelli, T. M., & Rollo, C. D. (2021). Ionizing radiation alters male *Acheta domesticus* courtship songs that are critical for mating success. *Animal Behaviour*, *178*, 209–216. https://doi.org/10.1016/j.anbehav.2021.06.018

Green, C. K., Moore, P. J., & Sial, A. A. (2019). Impact of heat stress on development and fertility of *Drosophila suzukii* Matsumura (Diptera: Drosophilidae). *Journal of Insect Physiology*, *114*, 45–52.\

Grosch, D. S. (1956). Induced lethargy and the radiation control of insects. *Journal of Economic Entomology*, *49*(5), 629–631.

Guillaume, A. S., Monro, K., & Marshall, D. J. (2016). Transgenerational plasticity and environmental stress: Do paternal effects act as a conduit or a buffer? *Functional Ecology*, *30*(7), 1175–1184.

Hasaballah, A. I. (2018). Impact of gamma irradiation on the development and reproduction of *Culex pipiens* (Diptera; Culicidae). *International Journal of Radiation Biology*, *94*(9), 844–849.

Hasaballah, A. I. (2021). Impact of paternal transmission of gamma radiation on reproduction, oogenesis, and spermatogenesis of the housefly, *Musca domestica* L.(Diptera: Muscidae). *International Journal of Radiation Biology*, *97*(3), 376–385.

Hayes, T., & López-Martínez, G. (2021). Resistance and survival to extreme heat shows circadian and sex-specific patterns in A cavity nesting bee. *Current Research in Insect Science*, *1*, 100020.

Hoffmann, A. A., Hallas, R., Anderson, A. R., & Telonis-Scott, M. (2005). Evidence for a robust sex-specific trade-off between cold resistance and starvation resistance in *Drosophila melanogaster*. *Journal of Evolutionary Biology*, *18*(4), 804–810.

Helinski, M. E., Parker, A. G., & Knols, B. G. (2006). Radiation-induced sterility for pupal and adult stages of the malaria mosquito *Anopheles arabiensis*. *Malaria Journal*, *5*, 1–10.

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

Isaksson, C., Sheldon, B. C., & Uller, T. (2011). The challenges of integrating oxidative stress into life-history biology. *Bioscience*, *61*(3), 194–202.

Jones, B., Shipley, E., & Arnold, K. E. (2018). Social immunity in honeybees—Density dependence, diet, and body mass trade-offs. *Ecology and Evolution*, *8*(10), 4852–4859.

Koch, R. E., & Hill, G. E. (2017). An assessment of techniques to manipulate oxidative stress in animals. *Functional Ecology*, *31*(1), 9–21.

Lanouette, G., Brodeur, J., Fournier, F., Martel, V., Vreysen, M., Cáceres, C., & Firlej, A. (2017). The sterile insect technique for the management of the spotted wing drosophila, *Drosophila suzukii*: Establishing the optimum irradiation dose. *PLOS ONE*, *12*(9), e0180821. https://doi.org/10.1371/journal.pone.0180821

Lamb, M. J. (1964). The effects of radiation on the longevity of female *Drosophila subobscura*. *Journal of Insect Physiology*, *10*(3), 487–497.

Lü, Z.-C., Wang, Y.-M., Zhu, S.-G., Yu, H., Guo, J.-Y., & Wan, F.-H. (2014). Trade-offs between survival, longevity, and reproduction, and variation of survival tolerance in Mediterranean *Bemisia tabaci* after temperature stress. *Journal of Insect Science*, *14*(1), 124.

Lynch, M., & Ennis, R. (1983). Resource availability, maternal effects, and longevity. *Experimental Gerontology*, *18*(2), 147–165.

Mahavidanage, S., Fuciarelli, T. M., Li, X., & Rollo, C. D. (2023). The effects of rearing density on growth, survival, and starvation resistance of the house cricket *Acheta domesticus*. *Journal of Orthoptera Research*, *32*(1), 25–31.

Matthews, S. G., & Phillips, D. I. (2012). Transgenerational inheritance of stress pathology. *Experimental Neurology*, *233*(1), 95–101. https://doi.org/10.1016/j.expneurol.2011.01.009

Menhinick, E. F., & Crossley Jr, D. A. (1968). A comparison of radiation profiles of *Acheta domesticus* and *Tenebrio molitor*. *Annals of the Entomological Society of America*, *61*(6), 1359–1365.

Mitchell, S. E., & Read, A. F. (2005). Poor maternal environment enhances offspring disease resistance in an invertebrate. *Proceedings of the Royal Society B: Biological Sciences*, *272*(1581), 2601–2607. https://doi.org/10.1098/rspb.2005.3253

Møller, A. P., & Mousseau, T. A. (2009). Reduced abundance of insects and spiders linked to radiation at Chernobyl 20 years after the accident. *Biology Letters*, *5*(3), 356–359

Mothersill, C., & Seymour, C. (2013). Uncomfortable issues in radiation protection posed by low-dose radiobiology. *Radiation and Environmental Biophysics*, *52*, 293–298.

Mousseau, T. A., & Dingle, H. (1991). Maternal effects in insect life histories. *Annual Review of Entomology*, *36*(1), 511–534.

Mousseau, T. A., & Møller, A. P. (2014). Genetic and ecological studies of animals in Chernobyl and Fukushima. *Journal of Heredity*, *105*(5), 704–709.

Murphy, J. F., Nagorskaya, L. L., & Smith, J. T. (2011). Abundance and diversity of aquatic macroinvertebrate communities in lakes exposed to Chernobyl-derived ionising radiation. *Journal of Environmental Radioactivity*, *102*(7), 688–694. https://doi.org/10.1016/j.jenvrad.2011.04.007

North, D. T. (1975). Inherited sterility in Lepidoptera. *Annual Review of Entomology*, *20*(1), 167–182.

Qureshi, Z. A., Ahmad, N., Hussain, T., & Ali, S. S. (1995). Effects of gamma radiation on one-day old adults of pink bollworm and their F∼ 1 progeny. *Pakistan Journal of Zoology*, *27*, 21–26.

Rando, O. J. (2012). Daddy Issues: Paternal Effects on Phenotype. *Cell*, *151*(4), 702–708. https://doi.org/10.1016/j.cell.2012.10.020

Sacher, G. A. (1963). Effects of X-rays on the survival of *Drosophila imagoes*. *Physiological Zoology*, *36*(4), 295–311.

Salem, H. M., Fouda, M. A., Abas, A. A., Ali, W. M., & Gabarty, A. (2014). Effects of gamma irradiation on the development and reproduction of the greasy cutworm, *Agrotis ipsilon* (Hufn.). *Journal of Radiation Research and Applied Sciences*, *7*(1), 110–115.

Sallam, H. A., & Ibrahim, S. M. (1993). Inherited sterility in progeny of gamma irradiated male cotton leafworm, *Spodoptera littoralis* (Boisd.). In *Radiation induced F 1 sterility in Lepidoptera for area-wide control*.

Seth, R. K., & Reynolds, S. E. (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.

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), 1559325818797499.

Shetty, V., Shetty, N. J., Harini, B. P., Ananthanarayana, S. R., Jha, S. K., & Chaubey, R. C. (2016). Effect of gamma radiation on life history traits of *Aedes aegypti* (L.). *Parasite Epidemiology and Control*, *1*(2), 26–35.

Soopaya, R., Stringer, L. D., Woods, B., Stephens, A. E., Butler, R. C., Lacey, I., Kaur, A., & Suckling, D. M. (2011). Radiation biology and inherited sterility of light brown apple moth (Lepidoptera: Tortricidae): developing a sterile insect release program. *Journal of Economic Entomology*, *104*(6), 1999–2008.

Speakman, J. R., & Garratt, M. (2014). Oxidative stress as a cost of reproduction: Beyond the simplistic trade-off model. *BioEssays*, *36*(1), 93–106.

Stevens, D. J., Hansell, M. H., Freel, J. A., & Monaghan, P. (1999). Developmental trade–offs in caddis flies: Increased investment in larval defence alters adult resource allocation. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *266*(1423), 1049–1054.

Stone, W. E. (1963). Effects of ionizing radiation on insects and other arthropods. *Radiation and Radioisotopes Applied to Insects of Agricultural Importance. Proceedings of the Symposium on the Use and Application of Radioisotopes and Radiation in the Control of Plant and Animal Insect Pests*.

Vaiserman, A. M., Koshel, N. M., Mechova, L. V., & Voitenko, V. P. (2004). Cross-life stage and cross-generational effects of γ irradiations at the egg stage on *Drosophila melanogaster* life histories. *Biogerontology*, *5*, 327–337.

Vimal, N., Sengupta, M., Angmo, N., & Seth, R. K. (2023). Assessing the radiation hormesis on the reproductive behaviour of male *Spodoptera litura* (F.) treated with low dose ionizing radiation in the pre-imaginal stages. *Indian Journal of Entomology*, 103–108.

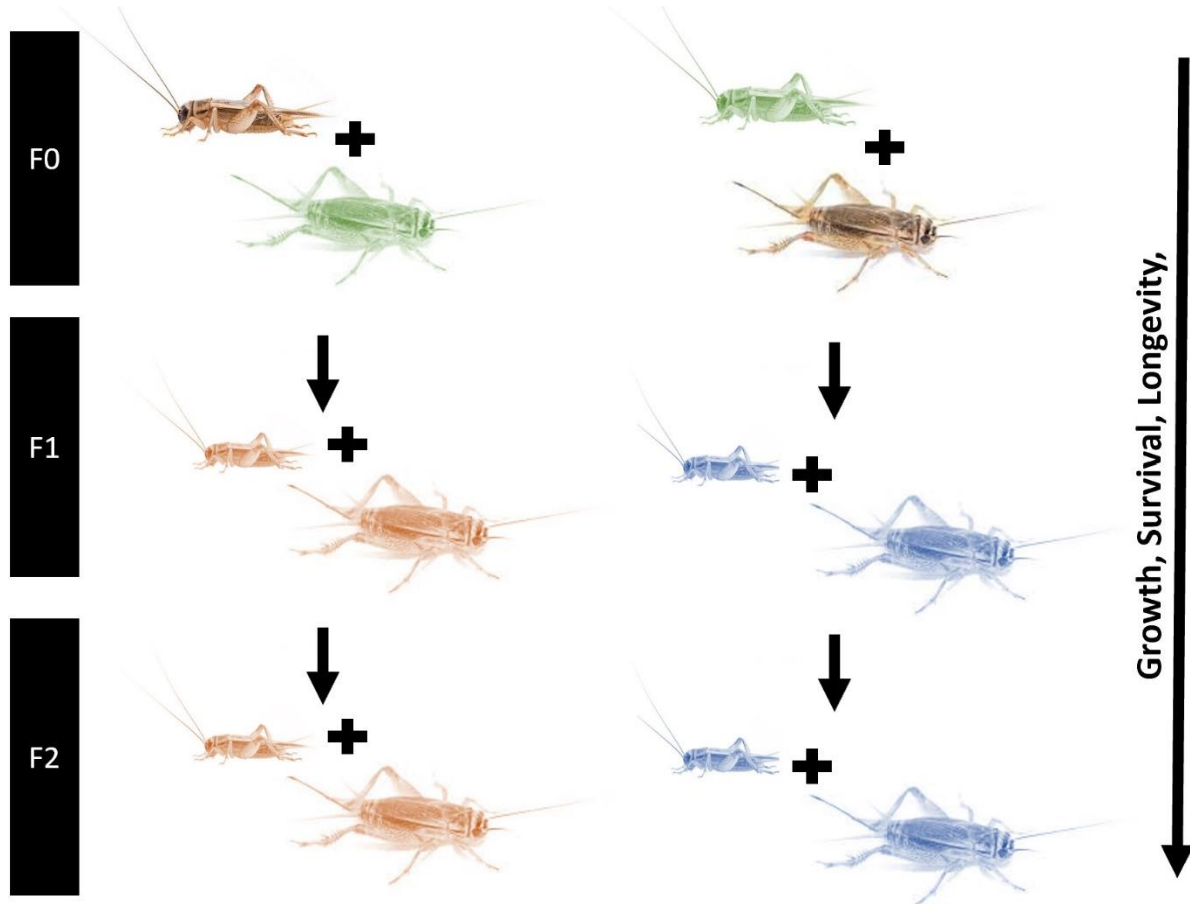
Weisman, N. Y., Fedorov, V. I., Nemova, E. F., & Nikolaev, N. A. (2014). Survival and life span of *Drosophila melanogaster* in response to terahertz radiation. *Advances in Gerontology*, *4*, 187–192.

Wilczek, G., Babczyńska, A., Wilczek, P., Doleżych, B., Migula, P., & Młyńska, H. (2008). Cellular stress reactions assessed by gender and species in spiders from areas variously polluted with heavy

metals. *Ecotoxicology and Environmental Safety*, *70*(1), 127–137. https://doi.org/10.1016/j.ecoenv.2007.03.005

Yao, Q., Quan, L., Wang, S., Xing, D., Chen, B., & Lu, K. (2023). Predatory stink bug, *Eocanthecona furcellata* (Wolff) responses to oral exposure route of λ-cyhalothrin via sex-specific modulation manner. *Pesticide Biochemistry and Physiology*, *192*, 105381. https://doi.org/10.1016/j.pestbp.2023.105381

6.8 APPENDIX



A1: Visual representation of the methodology applied to generate the paternal and maternal F1 and F2 generations of *Acheta domesticus*. In the left column is the maternal line where irradiated females were mated with normal males to generate the F1. The F1 was then mated to generate the F2 generation. The right column which is the paternal line was generated in a similar manner except the F0 consisted of irradiated or control males that were mating with normal females. Growth, survival, and longevity were monitoring in both lines from F0-F2.

CHAPTER 7

TITLE

Radiation induced bystander effects on the life-history features of the House Cricket *Acheta domesticus*

AUTHORS

Tamara M. Fuciarelli*, & C. David Rollo

Department of Biology, McMaster University, 1280 Main Street West, Hamilton ON, L8S 4L8, Canada. *Corresponding author: Email fuciartm@mcmaster.ca

7.1 INTRODUCTION

The impacts of ionizing radiation (IR) on both humans and other organisms are well studied due to both its use as a treatment for various types of cancer, as well as for its propensity to be released through large scale contamination events i.e., Chernobyl (1986) and Fukushima (2011) (Ryan, 2012; Desouky et al., 2015). The generally accepted cellular effects of IR exposure include cell death, chromosomal aberrations, DNA damage, mutagenesis, and carcinogenesis (Little, 2003; Desouky et al., 2015). On an organismal level, effects at high doses have generally shown to include reduced survival, growth, and impaired reproduction (Sarapultseva & Dubrova,

2016; Fuciarelli & Rollo, 2020). The magnitude of damage observed however is highly dependent on several key factors. Firstly, the source used; β radiation penetrates only a few centimeters into skin/tissues where γ radiation can penetrate deep into tissues causing more severe damage (Ryan, 2012). As well, radiation damage is impacted by total dose, dose-rate, and acute versus chronic exposure (Ryan, 2012).

The historically accepted theory associated with IR damage is the target theory of cellular responses. This theory posits that targeted damage occurs when ionizing particles deposit on a specific cellular target such as DNA, and results in a negative biological effect (Little, 2003; Mothersill & Seymour, 2006; Desouky et al., 2015). However, as water molecules encompass 70% of cellular composition, a majority of "targets" will be water molecules, which in turn are ionized and go on to damage macromolecules (Desouky et al., 2015). As mentioned above, the result of targeted effects is the development of physiological, genetic, and epigenetic alterations that can impact not only the exposed cells but also the descendants of these cells (Koturbash et al., 2008). Underpinning target theory and its implications is that only the cells or tissues irradiated will show legacy impacts of exposure (Morgan, 2003).

Recent research however into non-targeted effects (NTE) has challenged the target theory framework of IR damage (Desouky et al., 2015). NTE's are phenomena defined by the ability for irradiated cells to covey manifestations of damage or responses in other cells that were not themselves irradiated (Morgan, 2003; Desouky et al., 2015). One of the most well studied NTE's include the radiation induced bystander effect (RIBE) (Seymour & Mothersill, 2001). RIBE is defined as the induction of biological effects in unirradiated cells from some signal mediated transfer from irradiated cells (Seymour & Mothersill, 2001). RIBE has been observed in cells along a variety of endpoints including DNA damage induction (Yang et al., 2005), cell death or

apoptosis (Belyakov et al., 2002), and chromosomal instability (Seymour & Mothersill, 2000; Limoli & Giedzinski, 2003). RIBE effects tend to dominate in low-dose exposures, with data suggesting that the effects plateau as dose increases (Seymour & Mothersill, 2000; Mothersill & Seymour, 2006).

There are several possible mechanisms that have been shown to be potential candidates for bystander signals, however the exact mechanism is yet to be fully understood (Lyng et al., 2000). It is however, generally understood that RIBE on a cellular level is signal mediated and has been shown to be induced through Gap Junction Intercellular Communication (GJIC), soluble factors, or physical signalling i.e., photon emissions (Azzam et al., 2001; Desouky et al., 2015; Li et al., 2023). One novel mechanism recently published by Matarese et al. (2022) described X-ray induced acoustic waves in two irradiated cell lines. They posit these acoustic waves may be potential bystander signal candidates in tissues and cell cultures (Matarese et al., 2022). As these mechanisms do not seem to be mutually exclusive it is likely a combination of bystander signals that result in the various bystander effects observed (Desouky et al., 2015).

Much of the literature on RIBE focuses on cellular studies, with much less investigation being conducted on RIBE on an organismal level. One of the first landmark studies in this area was conducted by Surinov et al. (2004) in mice, which indicated that chemical mediated (urine) signals from irradiated individuals resulted in immunosuppressant effects in non-irradiated animals. A more recent study in fish, indicated that irradiated fish exposed to <1Gy were releasing soluble factors into the water, ultimately causing increased cell death in non-irradiated fish tissue (Mothersill et al., 2006). In addition to organismal RIBE being generally understudied, what we do understand as organismal RIBE has primarily focused on vertebrates with invertebrates, such as insects being much less studied. However, in recent years there have

been a few key studies which have illustrated RIBE in invertebrates. A study in the earthworm's *Eisenia fetida* and *Enchytraeus albidus* discovered cross species bystander effects in DNA integrity to and from one exposed individual to a non-exposed individual (Fernandes et al., 2020). As well, in eutardigrades and daphnia, RIBE resulted in reduced survival in non-exposed individuals (Fernandez et al., 2016; Reis et al., 2018). Another recent study in the cricket *Acheta domesticus* described RIBE in the life-history features of individuals which were co-habitated with irradiated individuals (Li et al., 2023). These studies indicate that RIBE can occur in a variety of species and can be evident not only on a cellular level but also on an organismal level. Both cellular and full body mechanisms have important implications to potential bystander responses when IR is used in radiotherapy, diagnostics, and through environmental and occupational exposures (Howell et al., 2006; Li et al., 2023).

Here we investigated organismal RIBE in unirradiated *Acheta domesticus* exposed to individuals irradiated at various doses (0.58Gy, 2.3Gy, and 16Gy). Bystander individuals were put in close proximity to irradiated individuals to where acoustic and chemical signals could transfer between the two groups. Controls were isolated from both irradiated and bystander individuals and were used as a baseline. Several life-history features including growth rate, survival, and longevity, were investigated for possible bystander effects. This study aimed to elucidate organismal RIBE. The results here also have relevant implications to populations living in environmental contamination sites as well as in the use of radiation as a treatment.

7.2 METHODS

Breeding colony: All experimental crickets were generated from a highly inbred breeding colony. The breeding colony is housed in an acrylic terrarium (93 x 64.2 x 46.6 cm) insulated with 1.5 cm tick Durofoam insulation. Air circulation is improved through the use of several small fans. The colony is maintained on a 12 hour day – 12 hour night cycle at a consistent temperature of 29℃ ± 2℃. 17% protein MultiFowl Grower chick feed (Quick Feeds Feed Mill, Copetown, Canada) is provided to the colony *ad libitum*. Distilled water is provided through soaked cellulose sponges *ad libitum*. Egg cartons are also provided to the colony crickets as shelters.

Experimental Groups: All experimental groups were generated by a single oviposition container provided to the breeding colony for a 24-hour period. The colony was provided with oviposition containers (7x7x7cm) containing Organic Garden Soil (Swiss Farms Products Inc., Marysville, USA). Soils were then removed after the 24h period and incubated until hatching (~14 days). Once hatching began soils were removed after a 24-hour period to ensure that all experimental crickets were of known age.

Three separate experiments were simultaneously conducted to determine potential dose-dependent responses: Three trials; 0.58Gy, 2.3Gy, and 16.2Gy. At 14 days post-hatch (~4th instar), nymphs for each trail were separated into a total of 3 groups: irradiation, bystander, and control group. All experimental groups were housed in a 13.3 x 2.2 x 19.4 cm Mesh Net Fish Breeding Container (Lee's, Texas, USA). These containers were chosen to allow for bystander and irradiation groups to be within range of potential chemical and acoustic signals. All control

and bystander groups were brought to the irradiation chamber but not irradiated. Once irradiation was complete all groups were brough back to the lab. Bystander groups were placed in close proximity to their respective irradiation group. Both bystander and radiation group containers were placed in a large lidded acrylic container to isolate them from the control group. Each trial therefore had an irradiated group (0.58Gy, 2.3Gy, or 16.2Gy) in close proximity to a nonirradiated bystander group, with a control group nearby but chemically and visually isolated from either the radiation or bystander groups. All groups were provided the same food, shelter, and water *ad libitum* as the breeding colony. See diagram **A1** in the appendix for an illustration of the experimental set up.

Irradiation: Irradiation for the 3 radiation groups was conducted using a Cs-137 source at a dose rate of 0.58Gy/min. Radiation groups were exposed at 14d of age (4th instar) in a single acute exposure totalling 0.58Gy, 2.3Gy, and 16.2Gy. As mentioned above, all bystander and control groups were brought to the source and not irradiated. Irradiations took place at the Taylor Radiobiology Source at McMaster University. The source is calibrated by Health Physics and Facility Management. To determine the dose field LiF chips are used, that you find inside a TLD badge. They are arranged on a board in a x axis and y axis pattern with a few outliers. The board is then placed at varying heights and irradiated for 30 seconds. After each test at a specific height, the chips are exchanged for new ones.

Dosimetry: The procedure to irradiate groups were as follows. The position and orientation of the subjects were facilitated by placing specimens in a tube apparatus containing 7 individual tubes tied into a circle (with the center tube remaining empty). These were placed

equidistant from the circular opening beneath the source (16.4 cm). Crickets were confined in each tube, allowing some freedom of movement to reduce stress while ensuring sufficient restriction to calculate the received dose with confidence.

Life-history Traits: Three endpoints were investigated for each trial for the control, bystander, and irradiation group. Data collection for all individuals in all groups began at 14 d post hatch when irradiation groups were irradiated and placed in proximity of their bystander groups. This age was chosen for irradiation as before this, individuals are too small to accurately count or observe for mortality. Sample sizes for mortality and survivorship for each trial were as follows: **0.58Gy Trail**: 0.58Gy (n=44), bystander (n=43), control (n=48); **2.3Gy Trail**: 2.3Gy (n=41), bystander (n=39), control (n=41); **16.2Gy Trial**: 16.2Gy (n=40), bystander (n=40), control (n=41).

At 14d until death individuals were monitored daily for mortality as well as maturation, which is denoted by the development of wings. Once mature, individuals were marked with non-toxic, non-scented paint to ensure they would not be counted twice. Mortality data was utilized for survivorship curves as well as to measure median longevity. Growth rates were determined by measurements taken at maturation and calculated by measuring the mass at maturation (mg) divided by the development time to maturation (days). This allowed us to calculated mean growth rate (mg/days). Sample sizes for mortality and survivorship for each trial were as follows: **0.58Gy Trail**: 0.58Gy (n=43), bystander (n=43), control (n=47); **2.3Gy Trail**: 2.3Gy (n=41), bystander (n=39), control (n= 40); **16.2Gy Trial**: 16.2Gy (n=41), bystander (n=40), control (n=43).

Statistics: For survivorship curves, each bystander and irradiated group for each trail was compared to their respective control using a Gehan-Breslow-Wilcoxon test. For longevity and growth rate data, bystander and irradiated groups were compared to their respective control using a one-way ANOVA followed by a Tukey's multiple comparisons follow up test. All statistical analysis was performed using Prism Graph pad 9.

7.3 RESULTS

Growth Rate: Growth rate was measured through the daily monitoring of individuals for their adult molt (denoted by the development of wings). At adult molt individual growth rate (mg/day) was determined using development time (days) and mass at maturation (mg). A one-way ANOVA detected significant differences between groups $F_{(17,361)} = 29.34$, $p < 0.0001$ **(Figure 1)**. A follow up Tukey's multiple comparisons test detected significant differences between several groups compared to their respective controls. In the 0.58Gy trial both male and female irradiated groups and bystander groups had significantly higher growth rates ($p<0.0001$) than their respective controls. Similarly in the 2.3Gy trail, significantly increased growth rates were detected in the 2.3Gy males ($p=0.0012$) and female ($p<0.0001$) as well as in the 2.3Gy bystander male ($p=0.0046$) and females ($p=0.0006$) compared to their respective controls. For the 16Gy trail significant increases were only evident in bystander males ($p=0.0019$) and females ($p<0.0001$) compared to controls. A complete summary of life-history data can be viewed in **Table 1.**

Survival: Survival data was collected through daily monitoring of all groups for mortality (**Figure 2**). In the 0.58Gy trail, a Gehan-Breslow-Wilcoxon test only detected a slight significant difference between the 0.58Gy males (p=0.0335) compared to control males. In the 2.3Gy trial a Gehan-Breslow-Wilcoxon test only detected slight significant differences between the 2.3Gy bystander group males (p=0.0457) compared to control males. In the 16.2Gy trail a Gehan-Breslow-Wilcoxon test detected significant differences between 16.2Gy males (p<0.0001) compared to control males as well as bystander males (p = 0.0013) and females (p-0.0022) compared to their respective controls.

Longevity: Longevity data was collected through the daily monitoring of all groups for morality **(Figure 3).** Although a one-way ANOVA indicated slight significant differences between groups F (17, 359) = 2.032, p = 0.0093, a follow up Tukey's multiple comparisons test only indicated significant differences between the 16.2Gy males (p = 0.0167) compared to their respective male controls.

7.4 DISCUSSION

Radiation induced bystander effects (RIBE) is a growing area of research due to its far-reaching implications to not only the clinical use of radiation but also in contamination zones and use in the SIT (Li et al., 2023). RIBE is defined as the phenomena where individuals or cells exhibit a biological response due to signal mediated communications from irradiated individuals or cells (Mothersill & Seymour, 2001). Although much RIBE research has focused on cellular RIBE, organismal impacts have also been shown in several species. Organismal RIBE however

is largely understudied, specially in non-mammalian species. Here we investigated the propensity for RIBE's to impact the life-history features of non-irradiated bystander crickets *(Acheta domesticus)*. To accomplish this, we reared bystander crickets in visual, chemical, and auditory proximity (without physical contact) to irradiated individuals (0.58Gy, 2.3Gy, and 16.2Gy).

Our results indicated that life-history features were indeed impacted in bystander individuals compared to their respective controls. However, these impacts were concentrated in the growth and survivorship endpoints, with little being observed in mean longevity. For growth rate, several key findings were observed. Notably, in both the 0.58Gy and 2.3Gy trials, bystander males and females had significantly increased growth rates compared to their respective control **(Figure 1)**. This increase in the bystander groups reflects the significant alterations we observed in the irradiated groups. In the highest dose, 16.2Gy, the irradiated groups no longer experienced increased growth rate when compared to the control. However, in this group the bystander individuals maintained this increased growth rate compared to the control.

The results for survivorship were a bit more variable **(Figure 2).** For the most part, control males and females had increased survivorship compared to both bystander and irradiated groups. This difference was most apparently in the 16.2Gy group where both bystander males and females as well as irradiated males had significantly reduced survivorship compared to their respective controls. For longevity, bystander and irradiated groups were not significantly impacted **(Figure 3)**. Only one group (16.2Gy males), showed significant differences from controls**.**

An interesting facet of these results is that the growth rate of bystander individuals in the 0.58Gy and 2.3Gy groups were increased similar to the increase observed in their respective irradiated counterparts. Firstly, this beneficial response in the low-dose irradiated groups is

supported in literature on lowest-dose stress responses. Specifically, our results show hormetic effects in the 0.58Gy and 2.3Gy irradiated groups. Hormesis is a phenomenon defined by beneficial or stimulatory responses at low levels of stress with the expected detrimental and harmful effects occurring at higher doses (Tang & Loke, 2015). Although hormetic effects have been described in species spanning bacteria to vertebrates and for over 1000 stressors (including ionizing radiation), beneficial effects tend to be highly endpoint and species specific (Constantini et al., 2010; Vaiserman, 2010). Low doses of radiation have been shown to increase longevity in several insect species including *Musca domestica* (Dauer et al., 1965)*, Acheta domesticus* (Menhinick & Crossley, 1968; Hunter & Krithayakiern, 1971)*, Tribolium confusum* (Davey, 1919)*.* In other species, growth rate has shown hormetic responses to exposure including to insecticides in *Musca domestica* (Afifi & Knutson, 1956), *Aphis citricola* (Neubauer et al., 1983), and *Acheta domesticus* (Luckey, 1968). Although not investigated here, increased reproductive output is also a common hormetic effect in insects due to various stressors (Cutler, 2013).

The 0.58Gy and 2.3Gy bystander groups also exhibited hormetic responses, despite not having been irradiated. Recently published research in our lab has also indicated increases in growth rate to juveniles exposed for a short period of time to irradiated adults (Li et al., 2023). In these experiments, irradiated adults were allowed to cohabitate with unirradiated juveniles. Exposed juveniles had increases in their growth rate compared to the non-exposed juveniles (Li et al., 2023). In our work, despite juveniles having no physical contact with irradiated individuals, growth rates were still increased in bystander individuals. Also, similar to the work of Li et al. (2023), this increase in growth rate was largely due to faster development time.

The adjustment of life-history strategies in both irradiated and bystander groups due to signals of a "high-risk environment" i.e., ionizing radiation, is supported in the literature. Predation is among one of the most potent means of natural and sexual selection and have been shown to significantly alter the behavior and life-history trajectories of individuals and populations (Hamilton & Zuk, 1982). In *Manduca sexta*, the presence of predators resulted in decreased development time with individuals gaining the same mass as unthreatened individuals (Thaler et al., 2012). Along with predation risk, the presence of parasitism or disease can cause individual and population level alterations to life-history strategies of the target species (Valenzuela-Sanchez et al., 2021). Specifically, studies examining the impacts of parasitism have described, similar to the effects of predation, a decrease in development time to favor early reproduction (Hochberg et al., 1992; Stearns, 2000; Valenzuela-Sanchez et al., 2021). In the marine snail *Cerithidea californica,* in the presence of parasitic worms within the population, individuals mature significantly earlier then non-parasitized populations (Lafferty, 1993). We posit that radiation environments may signal a similar "high stress, high-risk" environment to non-exposed individuals. Our results are conducive to the predicted response to high-risk environments where individuals decrease development time, therefore having a shorter juvenile period and increasing an individual's probability of survival and reproduction (Roff, 1993; Stearns, 2000).

It is also likely that these beneficial responses in both the irradiated and bystander groups are associated with trade-offs along other life-history features (Isaksson et al., 2011). Here, we did not observe any irradiation or bystander impacts to longevity, however, we did observe reduced survivorship in several groups. As well, although not investigated here, an important aspect of potential trade-offs is associated with reproductive endpoints. Studies in insects have

indicated various life history trade-offs in key life history features including temperature (Lu et al., 2014), food stress (Boggs & Freeman, 2005); predators (Stevens et al., 1999), and density (Jones et al., 2018; Mahavidanage et al., 2023).

Our results also support the hypothesis of saturability in regard to RIBE as growth benefits do not seem to improve with increasing dose. Although literature on RIBE saturability is sparse, recent work in *Acheta* has indicated that growth benefits in juveniles exposed to irradiated adults treated with 23.2Gy and 69.6Gy showed no differences in any of the endpoints observed (Li et al., 2023). As well, ionizing radiation research in both daphnia and eutardigrades describe saturability of bystander signalling (Fernandez et al., 2016; Reis et al., 2018). Work in this area is highly lacking, and therefore I encourage future research into both organismal RIBE as well as saturability effects.

There are several plausible mechanisms for the transmission of RIBE to unirradiated bystander individuals in *Acheta.* Firstly, a well studied mechanism is through chemical pheromones. *Acheta* like many other insects utilize chemical signaling for both sex and species recognition (Tregenza & Wedell, 1997; Thomas & Simmons, 2010). These compounds have been identified as hydrocarbons, and unlike lipids are volatile (Tregenza & Wedell, 1997). In crickets, exposure to radiation has been shown to alter the hydrocarbon profile of both male and female *Acheta (*Fuciarelli & Rollo, 2021a). The altered chemical pheromones of irradiated individuals can act as stress signals in unirradiated bystanders. Chemical signals have been shown to be RIBE signals in at least two other species. In mice, volatile secretion from irradiated individuals resulted in immune and behavioral effects in bystander individuals (Surinov et al., 2004). As well, in fish, bystander signals were shown to be communicated through soluble chemicals in the water resulting in increased cell death in various tissues from unirradiated

individuals (Mothersill et al., 2006). Another potential bystander signal more specific to *Acheta*, are acoustic signaling. There is much less support for this potential theory although we do know that acoustic signalling is vital for male reproduction and that females discriminate against males with poor signalling (Balakrishnan & Pollack, 1996; Crankshaw, 1979; Holzer, 2003; Fuciarelli & Rollo, 2021b). Recent work in our lab has indicated that altered acoustic signaling in male *Acheta* resulted in reduced mating success (Fuciarelli & Rollo, 2021b). I posit that at least in males, acoustic signaling may be a potential bystander signal candidate. It is important to note neither theory has been tested empirically but may serve as an avenue for future work on the subject.

Here our work aims to highlight the presence of RIBE's in a large-bodied insect *Acheta domesticus.* Our work indicates that despite lack of contact RIBE signaling has the potential to impact bystander individuals' life-history via adjustments and trade-offs. Although our work in this area is preliminary, understanding RIBE on a cellular and organismal level has important ecological and medical implications and should therefore continue to be studied. Future studies on *Acheta* as well as other species should focus on additional endpoints, potential trade-offs, as well as the mechanism for organismal RIBE.

7.5 ACKNOWLEDGEMENTS

This work was supported by the Mitacs Elevate Grant (IT05851), and the NSERC Discovery Grant (RGPIN-05693-2015).

7.6 TABLES AND FIGURES

Table 1: Summary of life-history (growth, longevity, survival) for *Acheta domesticus* controls, irradiated (0.58Gy, 2.3Gy, 16.2Gy), and bystander groups.

Group	N	Median Longevity (days)	Mean Longevity (days)	Maximal Longevity (days)	Growth Rate (mg/day)	Percentage of Controls	Development Time (days)	Mass at Maturation (mg)
Control Male	17	192	187.7	251	7.1		53.12	377.2
Control Female	29	175	172.8	222	7.3		52.95	383.4
0.58Gy Male	23	172	164.4	218	8.6	21.1	48.09	414.2
0.58Gy Female	21	179	174.4	216	9.2	26.0	46.59	429.3
0.58Gy Bystander Male	26	186	174.8	227	8.7	22.5	49.54	430.3
0.58Gy Bystander Female	17	179	180.3	215	9.6	31.5	47.29	453.9
Control Male	15	213	197.7	238	6.6		57.71	379.4
Control Female	26	193	176.4	241	7.2		55.46	400.1
2.3Gy Male	22	184	183.6	216	7.9	19.7	52.86	416.3
2.3Gy Female	19	189	187.2	233	8.5	18.1	51.15	436.6
2.3Gy Bystander Male	23	177	172.8	225	7.8	18.2	53.17	412.8
2.3Gy Bystander Female	16	183.5	175.9	219	8.5	18.1	52.93	448.7
Control Male	22	213	207.5	241	6.4		59.57	381.9
Control Female	19	192	193.4	240	7.1		57.68	409.2
16.2Gy Male	19	176	163.3	217	6.0	-6.3	54.20	324.2
16.2Gy Female	21	189	175.4	238	6.7	-5.6	53.95	361.9
16.2Gy Bystander Male	20	188.5	172	225	7.6	18.75	52.67	399.8
16.2Gy Bystander Female	20	178	163.6	205	8.6	21.1	50	431.8

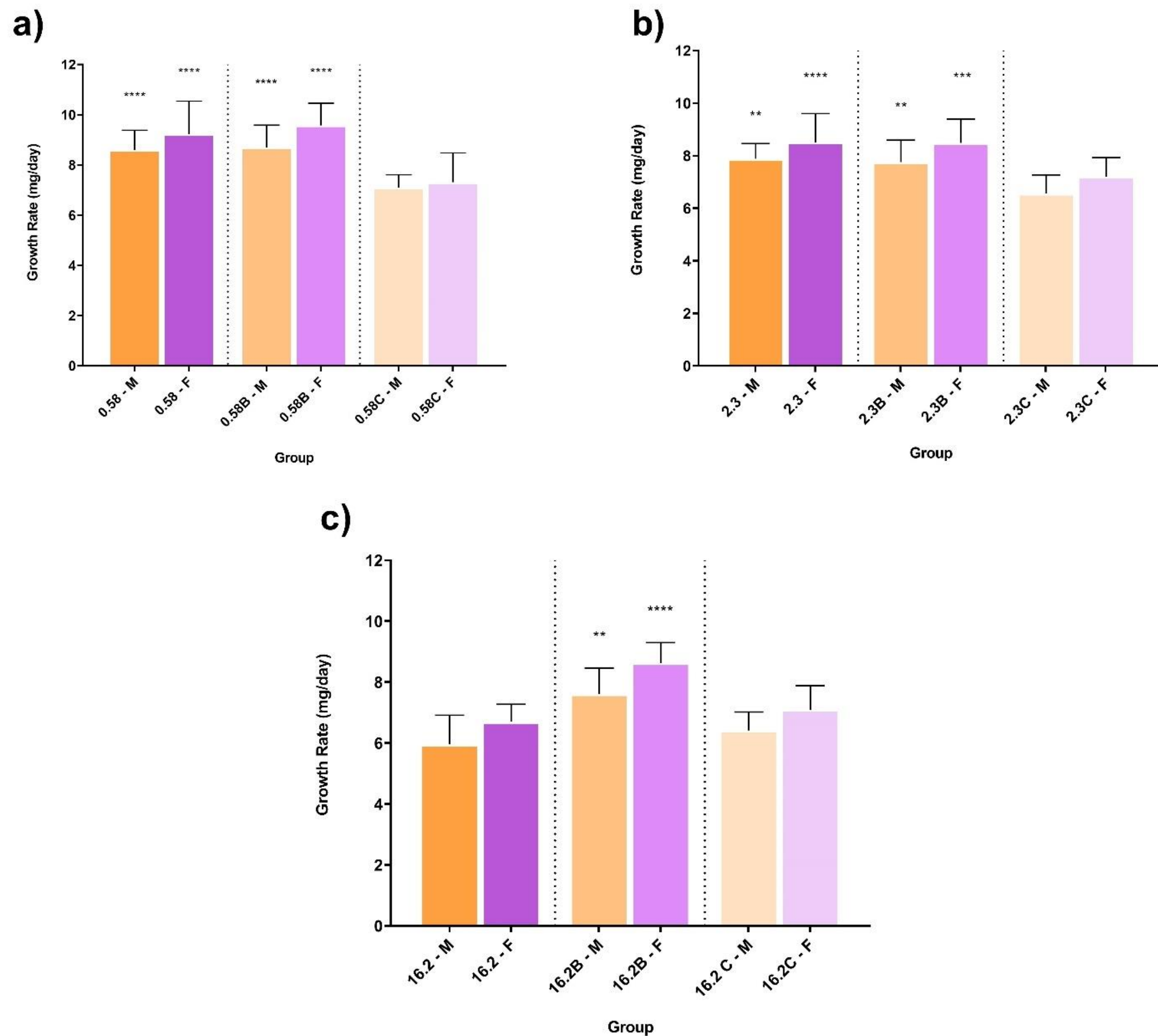


Figure 1: Impacts of ionizing radiation and RIBE on the growth rate of male and female *Acheta domesticus.* Group rates are displayed as mean growth rate (mg/day) ± SD. Graphs represent each experimental trial of individuals irradiated at 0.58Gy (a), 2.3Gy (b), 16.2Gy (c) with their respective unirradiated bystander and control group. A one-way ANOVA indicated significant differences between groups $F_{(17,361)} = 29.34$, $p < 0.0001$. A Tukey's multiple comparisons test indicated significant differences in most irradiated and bystander males and females compared to their respective controls.

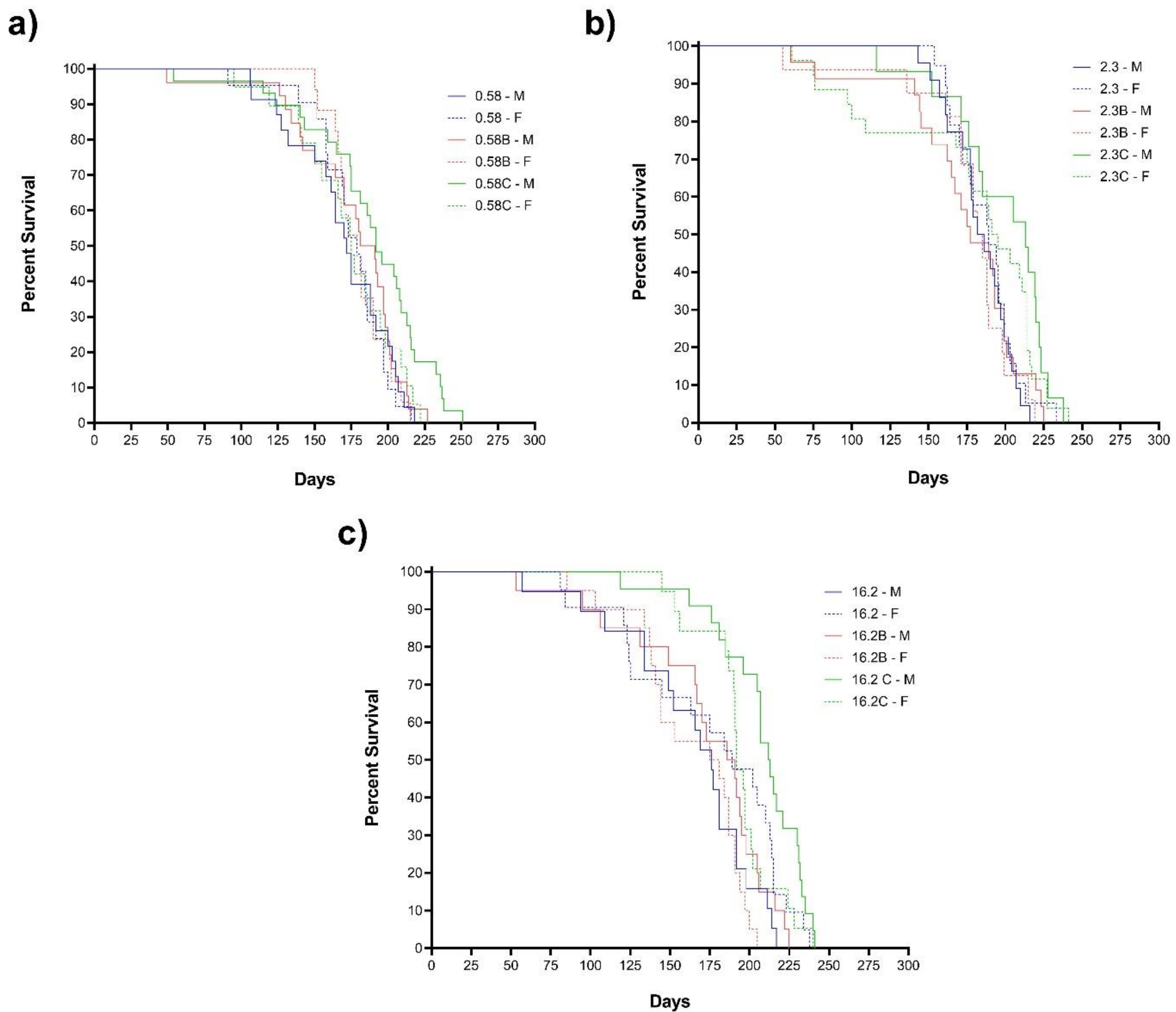


Figure 2: Impacts of ionizing radiation and RIBE on male and female *Acheta domesticus* survivorship. Three trials were conducted with individuals being irradiated with 0.58Gy (a), 2.3Gy (b), or 16.2Gy (c). Each irradiated group was paired with unirradiated bystander and control individuals. Survivorship is displayed as percent survival for each group. Impacts to survivorship were detecting using Gehan-Breslow-Wilcoxon tests. Impacts to survival were shown in 0.58Gy males (p=0.0335), 2.3Gy bystander males (p=0.0457), 16.2Gy males (p<0.0001), and 16.2Gy bystander males (p = 0.0013) and females (p-0.0022) when compared to their respective controls.

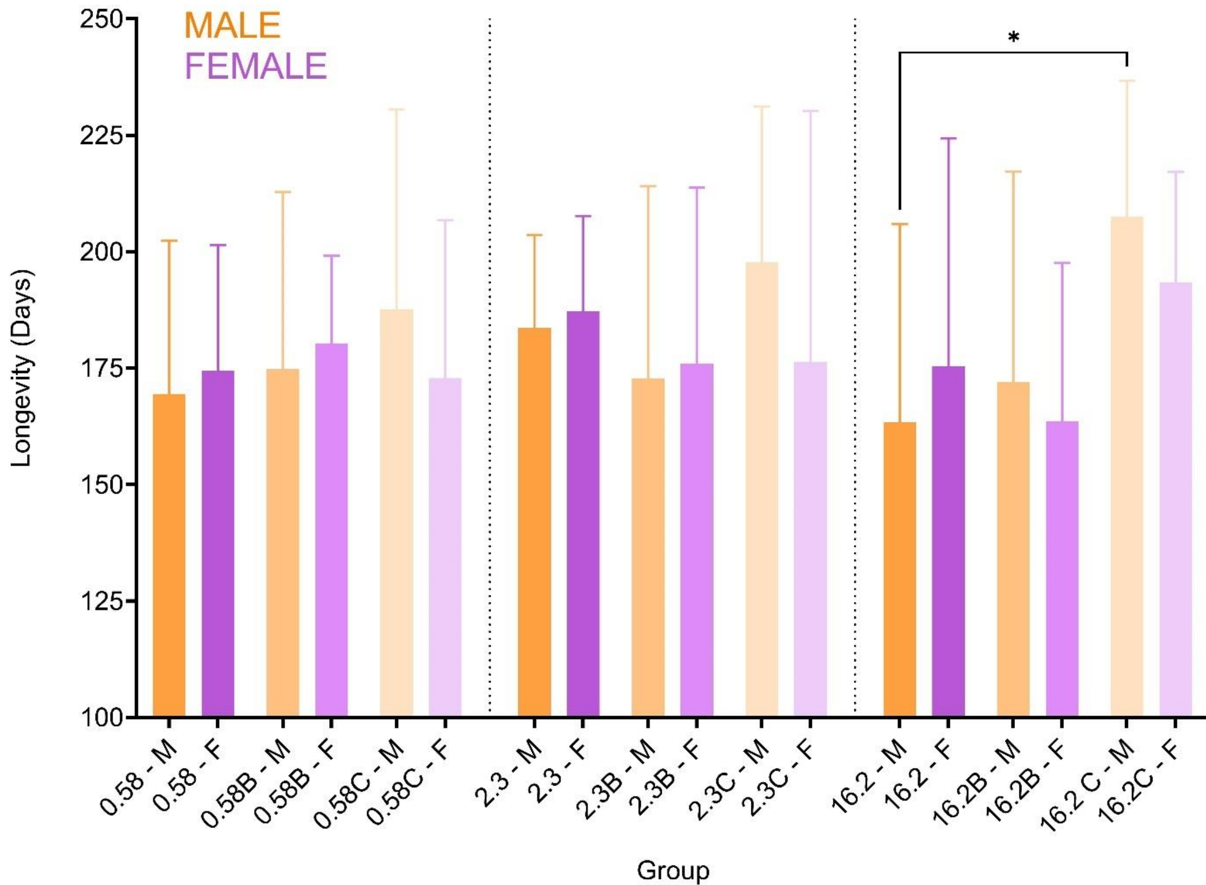


Figure 3: Impacts of ionizing radiation and RIBE on *Acheta domesticus* longevity. Three trials were conducted with individuals being irradiated with either 0.58Gy, 2.3Gy, or 16.2Gy and paired with unirradiated bystander and control individuals. Longevity data is displaced here as median longevity ± SD. A one-way ANOVA indicated significant differences between groups F (17, 359) = 2.032, p = 0.0093. However, follow up tests only indicated significant differences between the 16.2Gy males (p = 0.0167) compared to their respective male controls.

7.7 REFERENCES

Afifi, S. E., & Knutson, H. (1956). Reproductive Potential, Longevity, and Weight of House Flies which survived one insecticidal Treatment. *Journal of Economic Entomology*, *49*(3).

Balakrishnan., & Pollack, G. S. (1996). Recognition of courtship song in the field cricket*, Teleogryllus oceanicus. Animal Behaviour*, *51*(2), 353–366.

Boggs, C. L., & Freeman, K. D. (2005). Larval food limitation in butterflies: Effects on adult resource allocation and fitness. *Oecologia*, *144*, 353–361.

Boots, M., & Begon, M. (1993). Trade-offs with resistance to a granulosis virus in the Indian meal moth, examined by a laboratory evolution experiment. *Functional Ecology*, *7*(5), 528.

Costantini, D., Metcalfe, N. B., & Monaghan, P. (2010). Ecological processes in a hormetic framework. *Ecology Letters*, *13*(11), 1435–1447.

Crankshaw, O. S. (1979). *Female choice in relation to calling and courtship songs in Acheta domesticus*.

Cutler, G. C. (2013). Insects, insecticides and hormesis: Evidence and considerations for study. *Dose-Response*, *11*(2), dose-response.

Dauer, M., Bhatnagar, P. L., & Rockstein, M. (1965). X-Irradiation of Pupae of the House Fly, *Musca Domestica* L., and Male Survival1. *Journal of Gerontology*, *20*(2), 219–223. https://doi.org/10.1093/geronj/20.2.219

Davey, W. P. (1919). Prolongation of life of Tribolium confusum apparently due to small doses of X-rays. *Journal of Experimental Zoology*, *28*(3), 447–458.

Fernandez, C., Vasanthan, T., Kissoon, N., Karam, G., Duquette, N., Seymour, C., & Stone, J. R. (2016). Radiation tolerance and bystander effects in the eutardigrade species *Hypsibius dujardini* (Parachaela: Hypsibiidae). *Zoological Journal of the Linnean Society*, *178*(4), 919–923.

Fuciarelli, T. M., & Rollo, C. D. (2021a). Impacts of ionization radiation on the cuticular hydrocarbon profile and mating success of male house crickets (*Acheta domesticus*). *International Journal of Radiation Biology*, *97*(4), 564–570.

Fuciarelli, T. M., & Rollo, C. D. (2021b). Ionizing radiation alters male *Acheta domesticus* courtship songs that are critical for mating success. *Animal Behaviour*, *178*, 209–216.

Hamilton, W. D., & Zuk, M. (1982). Heritable true fitness and bright birds: a role for parasites? *Science (New York, N.Y.)*, *218*(4570), 384–387.

Hochberg, M. E., Michalakis, Y., & de Meeus, T. (1992). Parasitism as a constraint on the rate of life-history evolution. *Journal of Evolutionary Biology*, *5*(3), 491–504

Holzer, B., Jacot, A., & Brinkhof, M. W. (2003). Condition-dependent signaling affects male sexual attractiveness in field crickets, *Gryllus campestris*. *Behavioral Ecology*, *14*(3), 353–359.

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

Isaksson, C., Sheldon, B. C., & Uller, T. (2011). The challenges of integrating oxidative stress into life-history biology. *Bioscience*, *61*(3), 194–202.

Jones, B., Shipley, E., & Arnold, K. E. (2018). Social immunity in honeybees—Density dependence, diet, and body mass trade-offs. *Ecology and Evolution*, *8*(10), 4852–4859.

Lafferty, K. D. (1993). The marine snail, *Cerithidea californica*, matures at smaller sizes where parasitism is high. *Oikos (Copenhagen, Denmark)*, *68*(1), 3.

Li, X., Seymour, C. B., Mothersill, C., & Rollo, C. D. (2023). Investigation of presence and impact of radiation-induced bystander effect in *Acheta domesticus*. *International Journal of Radiation Biology*, 1–12.

Lü, Z.-C., Wang, Y.-M., Zhu, S.-G., Yu, H., Guo, J.-Y., & Wan, F.-H. (2014). Trade-offs between survival, longevity, and reproduction, and variation of survival tolerance in Mediterranean *Bemisia tabaci* after temperature stress. *Journal of Insect Science*, *14*(1), 124.

Luckey, T. D. (1968). Insecticide hormoligosis. *Journal of Economic Entomology*, *61*(1), 7–12.

Mahavidanage, S., Fuciarelli, T. M., Li, X., & Rollo, C. D. (2023). The effects of rearing density on growth, survival, and starvation resistance of the house cricket *Acheta domesticus*. *Journal of Orthoptera Research*, *32*(1), 25–31.

Menhinick, E. F., & Crossley Jr, D. A. (1968). A comparison of radiation profiles of *Acheta domesticus* and *Tenebrio molitor*. *Annals of the Entomological Society of America*, *61*(6), 1359–1365.

Mothersill, C., Bucking, C., Smith, R. W., Agnihotri, N., O'Neill, A., Kilemade, M., & Seymour, C. B. (2006). Communication of radiation-induced stress or bystander signals between fish in vivo. *Environmental Science & Technology*, *40*(21), 6859–6864.

Mothersill, C., & Seymour, C. (2001). Radiation-induced bystander effects: Past history and future directions. *Radiation Research*, *155*(6), 759–767.

Neubauer, I., Raccah, B., Aharonson, N., Swirski, E., & Ishaaya, I. (1983). Systemic effect of aldicarb, dimethoate and ethiofencarb on mortality and population dynamics of the spirea aphid, *Aphis citricola* Van der Goot. *Crop Protection*, *2*(2), 211–218.

Reis, P., Lourenço, J., Carvalho, F. P., Oliveira, J., Malta, M., Mendo, S., & Pereira, R. (2018). RIBE at an inter-organismic level: A study on genotoxic effects in *Daphnia magna* exposed to waterborne uranium and a uranium mine effluent. *Aquatic Toxicology*, *198*, 206–214.

Roff, D. (1993). *Evolution of life histories: Theory and analysis*. Springer Science & Business Media.

Stearns, S. C. (2000). Life history evolution: successes, limitations, and prospects. *The Science of Nature*, *87*(11), 476–486.

Stevens, D. J., Hansell, M. H., Freel, J. A., & Monaghan, P. (1999). Developmental trade–offs in caddis flies: Increased investment in larval defence alters adult resource allocation. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *266*(1423), 1049–1054.

Surinov, B. P., Isaeva, V. G., & Dukhova, N. N. (2004). Postirradiation volatile secretions of mice: Syngeneic and allogeneic immune and behavioral effects. *Bulletin of Experimental Biology and Medicine*, *138*(10), 384–386.

Tang, F. R., & Loke, W. K. (2015). Molecular mechanisms of low dose ionizing radiation-induced hormesis, adaptive responses, radioresistance, bystander effects, and genomic instability. *International Journal of Radiation Biology*, *91*(1), 13–27.

Thaler, J. S., McArt, S. H., & Kaplan, I. (2012). Compensatory mechanisms for ameliorating the fundamental trade-off between predator avoidance and foraging. *Proceedings of the National Academy of Sciences*, *109*(30), 12075–12080.

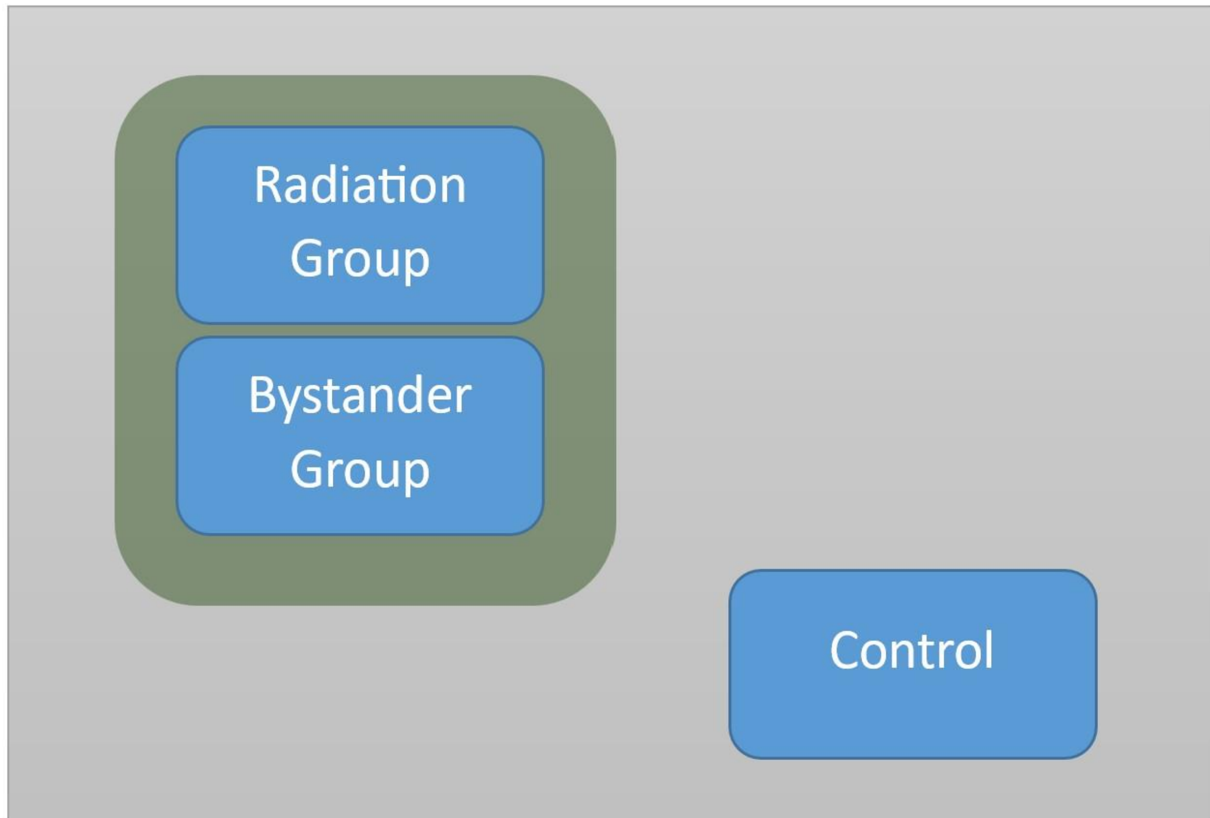
Thomas, M. L., & Simmons, L. W. (2010). Cuticular hydrocarbons influence female attractiveness to males in the Australian field cricket, *Teleogryllus oceanicus*. *Journal of Evolutionary Biology*, *23*(4), 707–714.

Tregenza, T. O. M., & Wedell, N. (1997). Definitive evidence for cuticular pheromones in a cricket. *Animal Behaviour*, *54*(4), 979–984.

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

Valenzuela-Sánchez, A., Wilber, M. Q., Canessa, S., Bacigalupe, L. D., Muths, E., Schmidt, B. R., … Cayuela, H. (2021). Why disease ecology needs life-history theory: a host perspective. *Ecology Letters*, *24*(4), 876–890.

7.8 APPENDIX



A1: Diagram of experimental setup for each RIBE trial. Three trials were completed using a different radiation group; 0.58Gy, 2.3Gy, and 16.2Gy. Each radiation group was paired with an unirradiated bystander group. All groups were housed in mesh containers which contained food, water, and shelter. Bystander and radiation groups were isolated within a lidded acrylic container (green) which allowed for only visual, auditory, and chemical cues to be transferred between radiation and bystander groups. The control group was housed nearby within the same larger acrylic container (grey).

CHAPTER 8

TITLE

The effects of rearing density on growth, survival, and starvation resistance of the house cricket *Acheta domesticus*

AUTHORS

Siyumi Mahavidanage, Tamara M. Fuciarelli*, Xiaobing Li, & C. David Rollo

Department of Biology, McMaster University, 1280 Main Street West, Hamilton ON, L8S 4L8, Canada Accepted: 23 August 2022. *Corresponding author: Email fuciartm@mcmaster.ca

Published online: 21 February 2023
Journal of Orthoptera Research 2023©

CITATION

Mahavidanage S, Fuciarelli TM, Li X, Rollo CD (2023) The effects of rearing density on growth, survival, and starvation resistance of the house cricket *Acheta domesticus.* Journal of Orthoptera Research 32(1): 25–31. https://doi.org/10.3897/jor.32.8649

8.1 INTRODUCTION

Accelerating global population growth and consumption has put increasing strain on the world's agricultural system (Godfrayet al., 2010; Sorjonen et al., 2019). Our ability to

produce enough food for the world's growing population is also diminishing due to factors such as urban expansion, land degradation, climate change, and water scarcity (Sorjonen et al., 2019). As a result, levels of global undernourishment have risen to an all-time high of 9.9%, with between 720 and 811 million people facing food insecurity (United Nations, 2020). Food demand worldwide is predicted to increase, with demand rising by 70% by 2070. As traditional food production methods are unlikely to fulfil current and future global food demands, alternative sources of food have been posited (Sorjonen et al., 2019).

One key area of food production that has received much attention due to its current unsustainability is traditional protein sources. Currently, global protein requirements are fulfilled largely by red meat, poultry, and seafood (Thavamani et al., 2020). These foods are a key source of several micronutrients, including iron, zinc, phosphorus, vitamin B6, and B12 (Ajwalia, 2020). However, meat production is highly unsustainable, with major concerns surrounding environmental degradation, animal welfare, and the negative effects of excessive meat consumption (Thavamani et al., 2020). Along with plant-based foods, cultured meats, and mycoprotein-based foods, insect-based protein sources are growing in popularity as meat/protein alternatives (Vandeweyer, 2018, Thavamani et al., 2020). Insects, like traditional protein sources, provide high volumes of fat, protein, zinc, iron, and several key vitamins (DeFoliart, 1991; Schabel, 2010; Alexander et al., 2017; Thavamani et al., 2020). The rearing of insects for this purpose is also more efficient and sustainable than for traditional livestock, consuming orders of magnitude less water and producing significantly less greenhouse gas emissions (GHGs) (Lundy & Parrella, 2015). Insects are also superior sources of protein when compared to traditional protein products, containing a protein content of 50–82% of the dry weight (Thavamani et al., 2020). In addition to producing significantly less GHGs, insects can utilize organic waste

products (low-value diets) as food sources, which provides further prospects for sustainable insect rearing (Sorjonen et al., 2020; Thavamani et al., 2020). Currently, the insect-as-food industry is expected to grow by 47% from 2019 to 2026, with 730,000 tons being produced by 2030 (Savio et al., 2022).

Despite the increasing global production of insect food sources, there is a paucity of information and research on best farming practices, including optimal densities for farmed species (Hanboonsong et al., 2013). Optimizing rearing density is one of the main considerations when seeking to increase production while maintaining food safety. Currently, it is generally understood that the effects of overcrowding include alterations in insect behavior, physiology, and, most importantly, life history (growth and survival) (Maciel-Vergara et al., 2021). However, industrial mass rearing densities are often unnaturally high, and the extent to which these densities affect insect behavior, performance, and well-being is not well understood (Francuski & Beukeboom, 2020). In industrial insect rearing, mortality is a common negative effect of increased density (Peters & Barbosa, 1997). In the Mediterranean fruit fly, for example, densities of 1–15 eggs/cm^2 show increased mortality as density increases due to increased food competition (Peters & Barbosa, 1997). Insect overcrowding also increases mortality as a result of increased pathogen susceptibility caused by higher temperatures, reduced immune response, and nutrient deficiencies (Vergara et al., 2021; Savio et al., 2022). Viral, fungal, bacterial, and microsporidian pathogens are all frequently found to infect mass-reared insects (Vergara et al., 2021). Viruses such as *Acheta domesticus* densovirus (AdDV) can result in mass mortality and have even been behind the bankruptcy of a few cricket-rearing companies (Weissman et al., 2012). Increases in disease transmission ultimately result in the increased mortality of mass reared insects. Insect growth rates are highly dependent on environmental conditions including

temperature, predators, humidity, and resource availability (Barragan Fonseca et al., 2018). Although most of these stimuli can be easily controlled, nutrient deficiencies due to often-overcrowded conditions in mass-rearing facilities can result in delayed maturation, survival, and reduced body size. These are highly negative outcomes when mass rearing insects where high survival and increased body size are desired (Averill & Prokopy, 1987; Agnew et al., 2002; Reiskind et al., 2004; Rivers & Dahlem, 2014). In *Anopheles gambiae* (Jannat & Roitberg, 2013), larval mortality increased by 36% in high-density groups due to waste products decreasing substrate quality. In both the fruit fly *Bactrocera tryoni* (Morimoto et al., 2019) and Western tarnished plant bugs [*Lygus hesperus* (Brent, 2010)], significant reductions in adult emergence, body weight, fecundity, and energetic reserves were evident in crowded treatments. Declines in growth and prolongation of larval developmental periods have also been shown in several species, including *Culex quinquefasciatus* (Ikeshoji & Mullai, 1970), *Trogoderma glabrum* (Beck, 1973), *Tipula oleracea* (Laughlin, 1960), *Endrosis sarcitrella* (Andersen, 1956), and *Ptinustectus* (Peters & Barbosa, 1997). Increased population density can also create greater social contact and irritation, increasing competition, aggressive encounters between individuals, fighting, and habitat destruction (Southwick, 1971; Weaver & Mcfarlane; 1990). Mating can also be affected by overcrowded conditions (Gavrilets, 2000). Martin and Hosken (2003) observed that females of the dung fly *Sepsis cynipsea* became less interested in remating when the population density increased. Interestingly, however, some species have shown a shortening of developmental time in response to increased density (Cohen, 1968; Hodjat, 1969). In addition, shifts in development time and size have often been shown in both high-density (overcrowding) and low-density (group effects) treatments (Peters & Barbosa, 1997). As it is often difficult to determine what rearing densities are indicative of overcrowding in any particular insect, as well

as between-species behavioral and physiological differences, research on optimal densities in a variety of species is necessary. Investigating the effects of rearing densities not only on survival and growth but also on resistance to starvation due to these overcrowded conditions (i.e., resource competition) is also of interest.

Although 1900 different species of insects are consumed globally, crickets are currently one of the most highly cultivated insect species, being utilized for both human consumption and as food sources for other species (Lundy & Parrella, 2015; Francuski & Beukeboom, 2020; Savio et al., 2022). In this study, we investigated optimal rearing densities in the cricket *Acheta domesticus*, considering the negative outcomes of overcrowding on growth, survival, and starvation resistance in groups at densities relevant to mass insect rearing. Densities of 0.09, 0.19, 0.47, 0.93, and 1.86 cricket/cm^2 were reared starting at 14 days of age (4th instar) and maintained until death. Resistance to adult starvation was also investigated at these various densities to understand the impacts of various rearing densities on food competition, as this is likely to occur in high-density mass-rearing facilities.

8.2 METHODS

Breeding colony: House crickets *A. domesticus* were housed in a 93 × 64.2 × 46.6 cm acrylic terrarium covered with 1.5-cm thick Durofoam insulation. The colony crickets were from a homogenous stock inbred for > 60 generations. Fans on top of the enclosed structure provided air circulation. Crickets were sustained at a 12 h-day–12 h-night photoperiod and at a constant temperature of 29°C ± 2°C using 60-volt UV heat lamps. *Ad libitum* food (Country Range MultiFowl Grower, 17% protein, Quick Feeds Feed Mill, Copetown, Canada) was provided. *Ad*

libitum distilled water was made available in soaked cellulose sponges, and egg cartons were provided for shelter. Oviposition medium (Vigoro Organic Garden Soil, The Mosaic Co., Lake Forest, IL, U.S.A.) was present in small plastic containers (7 × 7 × 7 cm). Oviposition containers were collected after a 24-hour period and incubated at 29°C ± 2°C until eggs hatched (~ 14 days).

Density variation: *A. domesticus* were taken two weeks (14 days, 4th instar) post-hatch from a single colony oviposition container and randomly separated into four experimental groups. Crickets at this age/molt are approximately 0.25 mm in length. The experimental groups consisted of 50, 100, 250, and 500 crickets, creating densities of approximately 0.09, 0.19, 0.47, and 0.93 cricket/cm^2. respectively. Densities given are an approximation. A range of densities below 0.93 cricket/cm^2 was chosen to allow for density-dependent observations, as densities above this had been observed in the lab to result in mass die-offs. All groups were given a 2 × 2 egg carton, which lowered the density slightly. The 0.09 cricket/cm^2 group was chosen as the control, as the lowest-density group is expected to maximize resources per cricket and thus have fewer negative interactions. Experimental housing containers were 29 × 18.5 × 12 cm, and the crickets were housed for life and in the same conditions as the colony. Cricket containers were continuously monitored for cleanliness, as extremely dirty rearing environments may negatively impact growth and development. Containers were cleaned (crickets were moved into fresh containers) at a minimum of once a week; however, in the higher-density groups, cleaning occurred approximately every other day due to increased mortality and excrement production. Food and water were replaced daily to ensure the same resource availability in each group.

Life-history measurements: Maturation in *A. domesticus* is denoted by the adult molt in which wings develop and individuals become sexually mature. Females are easily identifiable by their fully-developed ovipositor. Crickets reach adulthood approximately 5–60 days post-hatch. Sex, maturation mass, and development time (number of days post-hatch) were recorded for all density groups. Sample sizes for growth rate were 0.09 cricket/cm^2 (n = 41), 0.19 cricket/cm^2 (n = 68), 0.47 cricket/cm^2 (n = 142), and 0.93 cricket/ cm^2 (n = 224). Sample sizes for the proportion of individuals surviving maturation were 0.09 cricket/cm^2 (n = 50), 0.19 cricket/ cm^2 (n = 100), 0.47 cricket/cm^2 (n = 250), and 0.93 cricket/cm^2 (n = 500). Mass was measured using an Accuris analytical balance with a readability of 0.001 g ± 0.002 g. Maturation mass (g) and development time were employed to calculate the growth rate. The number of crickets that matured was used to determine the proportion of individuals that successfully matured.

Starvation resistance: After determining the impacts of various rearing densities on growth and survival parameters, a second experiment was conducted to determine the potential responses to adult starvation at various rearing densities. Individuals from a second oviposition container were arranged as described above. Group densities were slightly altered based on our initial results that had suggested that although the proportion matured was reduced at densities ≥ 0.47 cricket/cm^2, growth rate was not affected. The experimental groups were separated and maintained as described above. Experimental groups included 50, 100, 500, and 1000 individuals, representing densities of approximately 0.09, 0.19, 0.93, and 1.86 cricket/cm^2, respectively. Three to four weeks post-maturation, 10 females and 10 males from each density group were weighed and placed in individual containers to prevent cannibalism. The mass of each individual was recorded to determine whether increased body mass is related to starvation

resistance. The number of surviving individuals in the 1.86 cricket/cm^2 group consisted of 7 males and 10 females. For the starvation treatment, each individual cricket from each group was placed into a small cylindrical container and covered with plastic wrap secured by a rubber band. Holes were added to the plastic wrap to provide ventilation, and the sex and group of each individual was noted on the container. *Ad libitum* water was provided by placing a water-soaked cellulose sponge in the container; the sponges were re-soaked daily. Although the crickets consumed the cellulose sponges, they provided insufficient nutrients. Mortality was noted daily and used to determine longevity.

Statistics: To determine the effect of sex, density, and possible interaction (sex*density) on both growth and starvation resistance, a multiple linear regression was conducted using the most appropriate model. The best-fit model was determined using a step-wise AICc comparison. A D'Agostino–Pearson omnibus normality test was conducted to ensure data was normally distributed. Survival curves were analyzed using a Gehan–Breslow–Wilcoxon survival analysis to determine differences in survivorship among density groups. To analyze differences in the proportion that survived to maturation, chi-square tests were applied. To determine significant differences between the various rearing densities and the control, a Fisher's exact test was applied to each rearing density compared to the control. Finally, significant differences in the mass of starvation groups were determined using a one-way ANOVA followed by Tukey's multiple comparisons test. All statistical analyses were carried out using Prism Graph Pad 9.

8.3 RESULTS

Survival to maturation: Chi-square tests indicated significant differences in proportion matured among the different rearing density groups ($p < 0.0001$). Fisher's exact tests indicated significant differences between the 0.47 cricket/cm^2 ($p = 0.0008$) and 0.93 cricket/cm^2 ($p < 0.0001$) groups compared to the 0.09 cricket/cm^2 density group. This constituted a 31% and 45% decrease in the proportion that matured, respectively **(Figure 1)**. Growth rates were collected for all rearing density groups and are reported as mean growth rate ± SD **(Figure 2)**. Prior to analysis, a D'Agostino-Pearson omnibus normality test was performed and confirmed normal distribution. We used AICc model selection to determine the best model for describing the relationship between sex, density, and growth. The best-fit model carried 77.46% of the cumulative model weight and included two predictors (sex and density) with interaction effects $F(3, 471) = 70.79$, $p < 0.0001$, $R2 = 0.3108$; $y = 8.277 + 1.186\beta 1 - 1.094\beta 2 - 0.5763\beta 3$. Results suggest that sex ($p < 0.0001$), density ($p < 0.0001$), and, to a lesser extent, sex*density ($p = 0.0345$) are significant predictors of growth rate. The multiple regression results are outlined in **Table 2**. A summary of growth and development measurements is outlined in **Table 1**.

Mass of starvation groups: The mass (g) of each male and female *A. domesticus* used for starvation-resistant treatment was recorded immediately prior to experimentation and are represented as mean mass ± SD **(Figure 3)**. A one-way ANOVA indicated significant differences between groups $F(7, 69) = 58.47$, $p < 0.0001$, with a Tukey's multiple comparison test indicating significantly reduced masses ($p < 0.0001$) in both the 0.93 and 1.87 cricket/cm^2 females compared to the 0.09 cricket/cm^2 female controls. Significant reductions in mass were also

detected in 0.93 ($p = 0.0018$) and 1.87 ($p < 0.0001$) cricket/cm^2 males compared to the 0.09 cricket/cm^2 male controls. Between-sex differences ($p < 0.0001$) were also detected in the 0.09, 0.19, and 0.93 cricket/cm^2 groups.

Density–starvation: Starvation resistance was measured as days survived after the removal of sufficient food for all density groups and is reported as mean survival ± SD **(Figure 4)**. Prior to analysis, a D'Agostino–Pearson omnibus normality test confirmed normal distribution. We used AICc model selection to determine the best model to describe the relationship between sex, density, and starvation resistance. The best-fit model carried 72.65% of the cumulative model weight and included two predictor values: sex and density (F (2, 74) = 28.05, $p < 0.0001$, $R2 = 0.4312$; $y = 10.46 – 3.661\ \beta 1–2.701\beta 2$). The results suggest that both sex ($p = 0.0005$) and density ($p < 0.0001$) are significant predictors of starvation resistance. Results of the multiple regression are summarized in **Table 3.** The Gehan–Breslow–Wilcoxon test showed significant differences in survivorship between the 0.93 cricket/cm^2 ($p = 0.0030$) and 1.86 cricket/cm^2 ($p = 0.0008$) groups compared to the lowest density (0.09 cricket/cm^2) female group. Variation in survivorship was also evident in the 0.19 ($p = 0.0328$), 0.93 ($p = 0.0063$), and 1.86 ($p = 0.0001$) cricket/cm^2 groups compared to the males in the lowest-density groups.

8.4 DISCUSSION

Insects have been proven to be a valuable alternative source of protein, fat, and essential vitamins and minerals (DeFoliart, 1991; Schabel, 2010; Zaelor & Kitthawee, 2018). Utilizing insects as food may fill the gaps in our ability to meet current and future food demands. Crickets

such as *A. domesticus* are one of the most cultivated insect species globally (Lundy & Parrella, 2015, Zaelor & Kitthawee, 2018, Francuski & Beukeboom, 2020). *Acheta domesticus* is considered an excellent candidate for mass-rearing endeavors due to its low food requirements and beneficial nutritional profile (Fernandez-Cassi et al., 2019). To mass rear insects for these purposes, it is vital to understand optimal densities for increasing individual size and survival (output) while reducing disease and stress. In this study, we investigated the impacts of various rearing densities on *A. domesticus* life-history features as well as their ability to survive prolonged starvation. We reared experimental groups for life-history analysis (growth rate and survival to maturation) at 0.09, 0.19, 0.47, and 0.93 cricket/cm^2. We determined that the number of individuals reaching maturation was significantly reduced in the 0.47 and 0.93 cricket/cm^2 density groups **(Figure 1)**. This represented a decline of 31% and 45% compared to the lowest density 0.09 cricket/cm^2 group but still resulted in 142 and 224 individuals maturing, respectively. For growth rate, multiple regression analysis found that both sex ($p < 0.0001$) and density ($p < 0.0001$) have highly significant predictive power for growth rate. Interaction between sex and density ($p = 0.0345$) also had a slightly significant impact on growth rate **(Figure 2)**. A decline in growth rate was evident between the highest density males and females compared to their lowest density conspecifics, constituting a 5.18% and 12.45% decline, respectively.

Although our study did not indicate large declines in growth due to increased density, our results suggest that density is a strong predictor of growth rate. Prior studies have indicated that both increased mortality and decreased growth are expected due to overcrowding (Peters & Barbosa, 1997; Zaelor & Kitthawee, 2018). These impacts on survival and growth are likely due to increasing population density, which has been shown to increase competition, physical injury,

and stress (Parry et al., 2017). In an early study on the rearing densities of larval American cockroach *Periplaneta Americana* (Wharton et al., 1967), increasing density reduced both survival and growth. Other studies show similar trends, although the magnitude of survival and growth reductions seem to vary among even closely related species. A study by Parry et al. (2017) found that both survival and growth were significantly affected by density; the relationship was not linear and was significantly different between the five blow fly species used. Studies on the English grain aphid *Sitobion avenae* (Xing et al., 2021) revealed decreases in both the growth and survival of early-instar nymphs with increased population density. The effects of density also seem to be age dependent, with some species being more resistant to density in later life stages. For example, adult density does not significantly affect survival or reproductive traits in *C. homnivorax* (Berkebile et al., 2006). Varying results in the literature indicate that population density effects are highly complex and species-specific, with some species being more resistant to the negative effects of overcrowding than others (Xing et al., 2021). Synergistic effects, such as temperature and other environmental factors, should also be considered, as they may influence the effects of density, as was shown in a study using *Sitobion avenae* (Xing et al., 2021). This variability highlights the need for species-specific data on density impacts. It is also likely that a more substantial reduction in growth may have appeared at higher densities than used here. In addition, as shown in **Figure 1**, it is likely that the use of even higher densities may not only further impact growth but also survival. The second key aspect of this study was to investigate the impact of rearing density on starvation resistance. Intermittent food shortages are common in both the wild and in high-density mass rearing environments where competition for food is high (Zhang et al., 2019). Lack of food over extended periods of time can affect the growth, survival, and reproduction of individuals within the population (Zhang et al., 2019). Our

chosen regression model suggested that sex ($p = 0.0005$) and to a larger extent density ($p < 0.0001$) but not the interaction between the two are significant predictors of starvation resistance (**Figure 4)**. Analysis of survivorship curves indicated that most groups showed significant differences in survivorship compared to their same-sex lowest density group. The survival rate of the males in the 1.86 cricket/cm^2 density group was less than the males in the 0.09 cricket/cm^2 control group. It is important to note, however, that the mass of individuals used in the starvation resistance experiments was significantly lower in the 1.83 cricket/cm^2 and 0.93 cricket/cm^2 groups for both males and females **(Figure 3)**. The 1.83 cricket/cm^2 density group was not measured for growth rate, but it is likely that at this extremely high density the growth rate would be reduced given the significant reduction in mass observed before starvation. Our results are in line with to the manner in which insects are able to resist starvation in conditions in which migration is not feasible. Under starvation conditions, insects will undergo physiological modifications that alter their metabolism to help them cope (Zhang et al., 2019). They will first metabolize blood sugar (trehalose) and then lipids (triglycerides) to improve hunger resistance (Zhang et al., 2019). It is therefore likely that the reduction in mass observed in the 1.83 cricket/cm^2 and 0.93 cricket/cm^2 disadvantaged these individuals in terms of their ability to break down sugar and fat stores as effectively as larger individuals.

Although not always considered, sex often plays a key role in stress-related impacts on life-history features. Our results for both growth and starvation resistance showed significant contributions of sex on both variables **(Table 2,3)**. Increased density has been shown to not only affect life-history traits but also alter interactions between different sexes (Rull et al., 2012; Parry et al., 2017). This may lead to alterations in fecundity and fertility (Rull et al., 2012). Sex differences are expected, as females of this species are typically larger than males (Lyn et al.,

2012). For starvation resistance, sex differences were evident in the 1.86 and 0.93 cricket/cm^2 groups, with females being more sensitive to starvation than males **(Figure 4)**. However, most studies have found female insects to be generally more resistant to food-related stress, as females are typically larger and therefore have more nutrient stores. For example, Gaskin et al. (2002) found male *Ceratitis capitata* to be more negatively impacted by increases in density than females. The researchers postulated that this was due to increased aggression and behavioral costs to mate successfully in males (Gaskin et al., 2002). A study conducted on five species of blow flies reared at various densities indicated that females survived longer than males across all species used (Parry et al., 2017). Higher mortality in males versus females due to density variation has been recorded in *Lucilia sericata* (Parry et al., 2017) and *Ceratitis capitata* (Gaskin et al., 2002). Males of *Cochliomyia homnivorax* (Berkebile et al., 2006; Pitti et al., 2011) tend to show increased mortality under several rearing conditions, including high density, protein rich diets, and high temperature. While it is unclear why females in our study were more sensitive to starvation, trade-offs between cell maintenance and repair, resulting in aging and death, and the energetic costs of egg production have been documented in female insects (De Loof, 2011). The females used in this study were sexually mature adults, potentially making them less able to mitigate the impacts of starvation. These results have profound implications for insect farming in which productivity is often deterred by the increased competition and stress associated with high density (Zaelor & Kitthawee, 2018). Our results indicate that optimal densities for the mass rearing of *A. domesticus* are likely to be < 0.93 cricket/cm^2, as this minimizes reductions in growth and maintains adequate resistance to starvation in adulthood. In addition, although survival to maturation is significantly reduced at this density, the number of individuals that do survive, in this case 224, is significantly greater than at densities with < 250 individuals. Thus, as

the goal of mass rearing is to produce the largest number of individuals at the maximum body size while reducing stress, we believe that < 0.93 cricket/cm^2 is optimal. These results should guide future mass-rearing endeavors to optimize production while reducing mortality and other negative effects of overcrowding. It is also recommended that future research focus on a diversity of endpoints, such as how density influences reproductive output, immune responses, and survivorship in this species to further inform optimal rearing densities. Synergistic effects between density and other environmental factors such as temperature, humidity, etc., should also be investigated.

8.5 ACKNOWLEDGEMENTS

This work was funded by the NSERC Discovery Grant (RG-PIN-05693-2015).

8.6 TABLES AND FIGURES

Table 1. Summary of growth parameters of each *Acheta domesticus* rearing density group.

Experimental Group	N	Growth Rate (mg/day)	Percentage of controls	Upper 95% CI	Maximal Growth rate	Developme nt time (Days)	Mass at maturation (g)
0.09 Male (control)	14	7.629		8.080	8.98	50.79	0.388
0.09 Female (control)	27	8.941		9.280	10.91	48.15	0.431
0.19 Male	34	8.239	8.00%	8.518	10.00	50.00	0.413
0.19 Female	34	9.109	1.88%	9.475	12.04	48.97	0.446
0.47 Male	71	7.830	2.63%	8.026	9.68	49.51	0.387
0.47 Female	71	8.963	0.25%	9.219	11.24	47.32	0.424
0.93 Male	112	7.234	- 5.18%	7.392	9.87	48.21	0.349
0.93 Female	112	7.829	- 12.45%	8.017	10.96	46.71	0.366

Table 2. Multiple linear regression analysis with AICc comparison was used to determine the most correct model for predicting growth rate based on sex and density group. AICc comparison was utilized to select the best model. Our model includes sex, density, and sex*density interactions, which carried 77.46% of the cumulative model weight. Each predictor value had a significant correlation with growth rate: sex ($p < 0.0001$), density ($p < 0.0001$), and sex*density ($p = 0.0345$)

Variable	Coefficient (β)	SE	95% CI	P Value
Intercept	8.277	0.1398	8.002 to 8.552	<0.0001
Sex	1.186	0.1885	0.815 to 1.556	<0.0001
Density	- 1.094	0.1991	- 1.485 to - 0.703	<0.0001
Sex*Density	- 0.576	0.2718	- 1.110 to - 0.042	0.0345

Table 3. Multiple linear regression analysis with AICc comparison was used to determine the most correct model for predicting survival from sex and density group. Our model includes both sex and density, with no sex*density interactions which carried 72.65% of the cumulative model weight. Each predictor value had a significant correlation with growth rate; Sex ($p = 0.0005$), density ($p < 0.0001$).

Variable	Coefficient (β)	SE	95% CI	P Value
Intercept	10.46	0.6495	9.164 to 11.750	<0.0001
Sex	2.70	0.7451	1.217 to 4.186	0.0005
Density	- 3.66	0.5405	- 4.738 to - 2.584	<0.0001

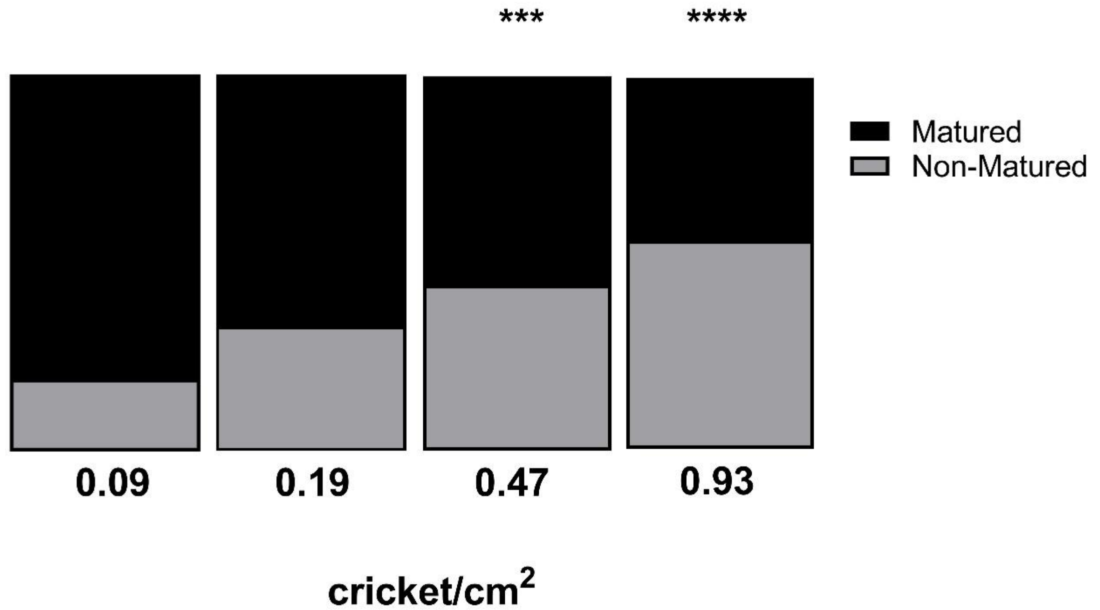


Figure 1: The proportion of *Acheta domesticus* reaching maturation in each rearing density (0.09, 0.19, 0.47, and 0.93 cricket/cm^2). A chi-square test indicated significant differences in survival among rearing densities ($p < 0.0001$). A Fisher's exact test showed significant differences in proportion matured between 0.19 cricket/cm^2 ($p = 0.0008$) and 0.93 cricket/cm^2 ($p < 0.0001$) compared to the 0.09 cricket/cm^2 density group.

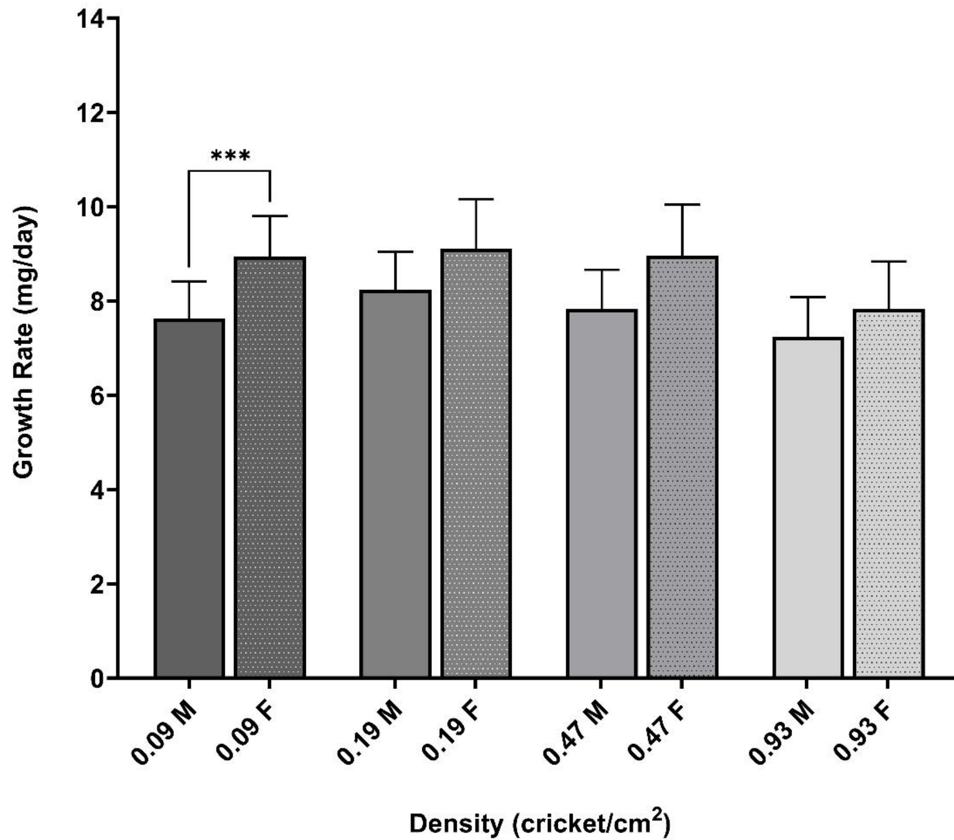


Figure 2: Density-dependent effects of various rearing densities on the growth rate of male and female *Acheta domesticus*. Values are represented as mean growth rate of each group ± SD. Growth rates were calculated by dividing the mass at maturation (mg) by the time taken to reach maturation (days) of each individual. Multiple regression analysis determined that sex ($p < 0.0001$), density ($p < 0.0001$), and to a lesser extent sex*density ($p = 0.0345$) interaction were strong predictors of growth rate.

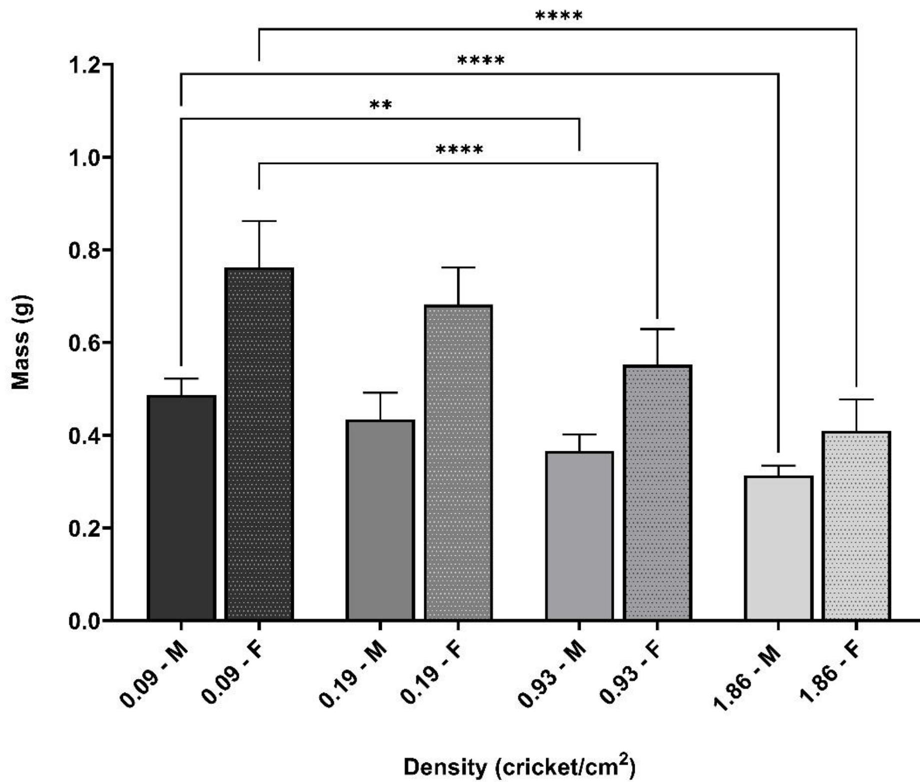


Figure 3: Mass of adult *Acheta domesticus* used for starvation treatment from each density group. Values represent the mean mass of each group ± SD. The mass of each individual was recorded prior to starvation treatment. A one-way ANOVA indicated significant differences between groups ($F(7, 69) = 58.47$, $p < 0.0001$), with a Tukey's multiple comparison test indicating significantly reduced masses ($p < 0.0001$) in both the 0.93 and 1.87 cricket/cm^2 females compared to the 0.09 cricket/cm^2 female controls. Significant reductions in mass were also detected in the 0.93 ($p = 0.0018$) and 1.87 ($p < 0.0001$) cricket/cm^2 males compared to the 0.09 cricket/cm^2 male controls. Although not represented on the graph, between-sex differences ($p < 0.0001$) were also detected between males and females in the 0.09, 0.19, and 0.93 cricket/cm^2 groups.

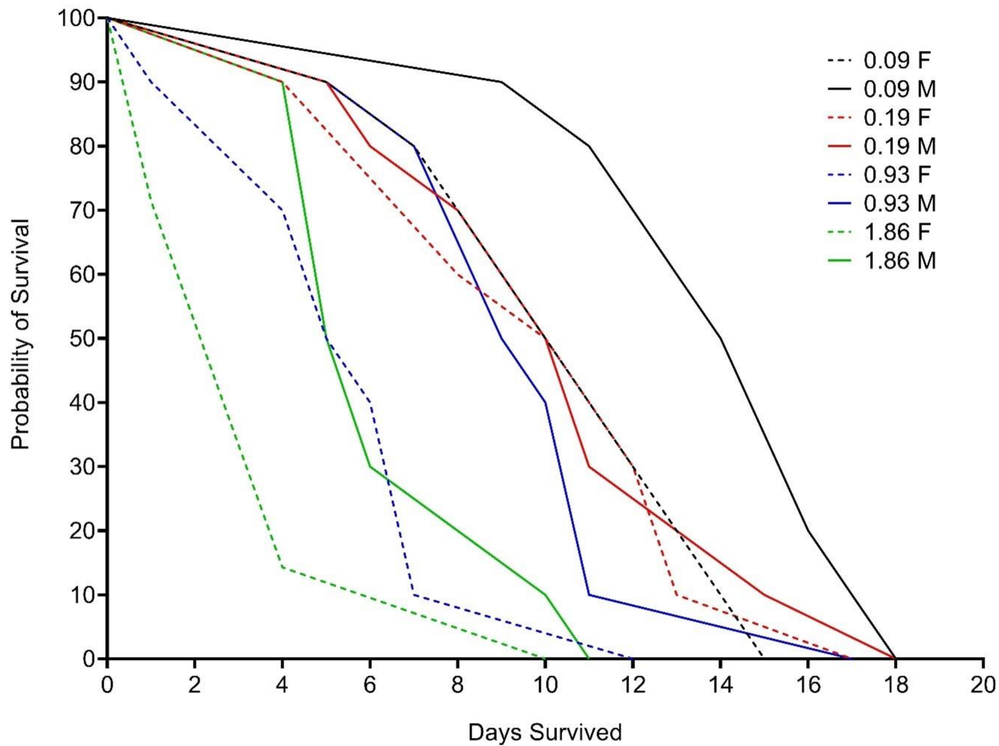


Figure 4: Starvation resistance of adult *Acheta domesticus* reared at various densities. All density and control groups were separated at 14 days of age (4th instar) and maintained until adulthood. Three to four weeks post-maturation individuals were separated and deprived of sufficient food. Multiple regression analysis suggests both sex ($p = 0.0005$) and density ($p < 0.0001$) to be significant predictors of starvation resistance. The Gehan–Breslow–Wilcoxon test showed significant differences in survivorship between the 0.93 cricket/cm^2 ($p = 0.0030$) and 1.86 cricket/cm^2 ($p = 0.0008$) groups compared to the lowest density (0.09 cricket/cm^2) female group. Variation in survivorship was also evident in the 0.19 ($p = 0.0328$), 0.93 ($p = 0.0063$), and 1.86 ($p = 0.0001$) cricket/cm^2 groups compared to the lowest-density males.

8.7 REFERENCES

Agnew, P., Hide, M., Sidobre, C., & Michalakis, Y. (2002). A minimalist approach to the effects of density-dependent competition on insect life-history traits. *Ecological Entomology*, *27*(4), 396–402.

Alexander, P., Brown, C., Arneth, A., Dias, C., Finnigan, J., Moran, D., & Rounsevell, M. D. (2017). Could consumption of insects, cultured meat or imitation meat reduce global agricultural land use? *Global Food Security*, *15*, 22–32.

Andersen, F. S. (1956). Effects of crowding *in Endrosis sarcitrella*. *Oikos*, *7*(2), 215–226.

Averill, A. L., & Prokopy, R. J. (1987). Intraspecific competition in the tephritid fruit fly *Rhagoletis pomonella*. *Ecology*, *68*(4), 878–886.

Barragan-Fonseca, K. B., Dicke, M., & van Loon, J. J. (2018). Influence of larval density and dietary nutrient concentration on performance, body protein, and fat contents of black soldier fly larvae (*Hermetia illucens*). *Entomologia Experimentalis et Applicata*, *166*(9), 761–770.

Beck, S. D. (1973). Growth and retrogression in larvae of *Trogoderma glabrum* (Coleoptera: Dermestidae). 4. Developmental characteristics and adaptive functions. *Annals of the Entomological Society of America*, *66*(4), 895–900.

Berkebile, D. R., Sagel, A., Skoda, S. R., & Foster, J. E. (2006). Laboratory environment effects on the reproduction and mortality of adult screwworm (Diptera: Calliphoridae). *Neotropical Entomology*, *35*, 781–786.

Brent, C. S. (2010). Stage-specific effects of population density on the development and fertility of the western tarnished plant bug, *Lygus hesperus*. *Journal of Insect Science*, *10*(1).

Cohen, B. (1968). The influence of larval crowding on the development time of populations of *Drosophila melanogaster* on chemically defined medium. *Canadian Journal of Zoology*, *46*(3), 493–497.

De Loof, A. (2011). Longevity and aging in insects: Is reproduction costly; cheap; beneficial or irrelevant? A critical evaluation of the “trade-off” concept. *Journal of Insect Physiology*, *57*(1), 1–11.

DeFoliart, G. R. (1991). Insect fatty acids: Similar to those of poultry and fish in their degree of unsaturation, but higher in the polyunsaturates. *The Food Insects Newsletter*, *4*(1), 1–4.

Fernandez-Cassi, X., Supeanu, A., Vaga, M., Jansson, A., Boqvist, S., & Vagsholm, I. (2019). The house cricket (*Acheta domesticus*) as a novel food: A risk profile. *Journal of Insects as Food and Feed*, *5*(2), 137–157.

Francuski, L., & Beukeboom, L. W. (2020). Insects in production–an introduction. *Entomologia Experimentalis et Applicata*, *168*(6–7), 422–431.

Gaskin, T., Futerman, P., & Chapman, T. (2002). Increased density and male–male interactions reduce male longevity in the medfly, *Ceratitis capitata*. *Animal Behaviour*, *63*(1), 121–129.

Gavrilets, S. (2000). Rapid evolution of reproductive barriers driven by sexual conflict. *Nature*, *403*(6772), 886–889.

Godfray, H. C. J., Beddington, J. R., Crute, I. R., Haddad, L., Lawrence, D., Muir, J. F., Pretty, J., Robinson, S., Thomas, S. M., & Toulmin, C. (2010). Food security: The challenge of feeding 9 billion people. *Science*, *327*(5967), 812–818.

Hanboonsong, Y., Jamjanya, T., & Durst, P. B. (2013). Six-legged livestock: Edible insect farming, collection and marketing in Thailand. *RAP Publication*, *3*, 8–21.

Hodjat, S. H. (1969). The effects of crowding on the survival, rate of development, size, colour and fecundity of *Dysdercus fasciatus* (Hem., Pyrrhocoridae) in the laboratory. *Bulletin of Entomological Research*, *58*(3), 487–504.

Ikeshoji, T., & Mullai, M. S. (1970). Overcrowding factors of mosquito larvae. *Journal of Economic Entomology*, *63*(1), 90–96.

Jannat, K. N.-E., & Roitberg, B. D. (2013). Effects of larval density and feeding rates on larval life history traits in *Anopheles gambiae* ss (Diptera: Culicidae). *Journal of Vector Ecology*, *38*(1), 120–126.

Laughlin, R. (1960). Biology of *Tipula oleracea* L.: Growth of the larva. *Entomologia Experimentalis et Applicata*, *3*(3), 185–197.

Lorenz, M. W. (2003). Adipokinetic hormone inhibits the formation of energy stores and egg production in the cricket *Gryllus bimaculatus*. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, *136*(2), 197–206.

Lundy, M. E., & Parrella, M. P. (2015). Crickets are not a free lunch: Protein capture from scalable organic side-streams via high-density populations of *Acheta domesticus. PloS One*, *10*(4), e0118785.

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*, 371–387.

Maciel-Vergara, G., Jensen, A. B., Lecocq, A., & Eilenberg, J. (2021). Diseases in edible insect rearing systems. *Journal of Insects as Food and Feed*, *7*(5), 621–638.

Martin, O. Y., & Hosken, D. J. (2003). The evolution of reproductive isolation through sexual conflict. *Nature*, *423*(6943), 979–982.

Morimoto, J., Nguyen, B., Dinh, H., Than, A. T., Taylor, P. W., & Ponton, F. (2019). Crowded developmental environment promotes adult sex-specific nutrient consumption in a polyphagous fly. *Frontiers in Zoology*, *16*, 1–11.

Parry, N. J., Pieterse, E., & Weldon, C. W. (2017). Longevity, fertility and fecundity of adult blow flies (Diptera: Calliphoridae) held at varying densities: Implications for use in bioconversion of waste. *Journal of Economic Entomology*, *110*(6), 2388–2396.

Peters, T. M., & Barbosa, P. (1977). Influence of population density on size, fecundity, and developmental rate of insects in culture. *Annual Review of Entomology*, *22*(1), 431–450.

Pitti, A., Skoda, S. R., Kneeland, K. M., Berkebile, D. R., Molina-Ochoa, J., Chaudhury, M. F., Youm, O., & Foster, J. E. (2011). Effect of adult screwworm male size on mating competence. *Southwestern Entomologist*, *36*(1), 47–60.

Reiskind, M. H., Walton, E. T., & Wilson, M. L. (2004). Nutrient-dependent reduced growth and survival of larval *Culex restuans* (Diptera: Culicidae): laboratory and field experiments in Michigan. *Journal of Medical Entomology*, *41*(4), 650–656

Rivers, D. B., & Dahlem, G. A. (2022). *The science of forensic entomology*. John Wiley & Sons.

Rull, J., Birke, A., Ortega, R., Montoya, P., & López, L. (2012). Quantity and safety vs. quality and performance: Conflicting interests during mass rearing and transport affect the efficiency of sterile insect technique programs. *Entomologia Experimentalis et Applicata*, *142*(1), 78–86.

Savio, C., Mugo-Kamiri, L., & Upfold, J. K. (2022). Bugs in bugs: The role of probiotics and prebiotics in maintenance of health in mass-reared insects. *Insects*, *13*(4), 376.

Schabel, H. G. (2010). Forest insects as food: A global review. Forest insects as food: Humans Bite Back. *Food & Agriculture Org*.

Sorjonen, J. M., Valtonen, A., Hirvisalo, E., Karhapää, M., Lehtovaara, V. J., Lindgren, J., Marnila, P., Mooney, P., Mäki, M., & Siljander-Rasi, H. (2019). The plant-based by-product diets for the mass-rearing of *Acheta domesticus* and *Gryllus bimaculatus*. *PLoS One*, *14*(6), e0218830

Southwick, C. H. (1971). *The biology and psychology of crowding in man and animals*.

Thavamani, A., Sferra, T. J., & Sankararaman, S. (2020). Meet the meat alternatives: The value of alternative protein sources. *Current Nutrition Reports*, *9*, 346–355.

Vandeweyer, D., Wynants, E., Crauwels, S., Verreth, C., Viaene, N., Claes, J., Lievens, B., & Van Campenhout, L. (2018). Microbial dynamics during industrial rearing, processing, and storage of tropical house crickets (*Gryllodes sigillatus*) for human consumption. *Applied and Environmental Microbiology*, *84*(12), e00255-18.

Weaver, D. K., & McFarlane, J. E. (1990). The effect of larval density on growth and development of *Tenebrio molitor*. *Journal of Insect Physiology*, *36*(7), 531–536.

Weissman, D. B., Gray, D. A., Pham, H. T., & Tijssen, P. (2012). Billions and billions sold: Pet-feeder crickets (Orthoptera: Gryllidae), commercial cricket farms, an epizootic densovirus, and government regulations make for a potential disaster. *Zootaxa*, *3504*(1), 67–88.

Wharton, D. R., Lola, J. E., & Wharton, M. L. (1967). Population density, survival, growth, and development of the American cockroach. *Journal of Insect Physiology*, *13*(5), 699–716.

Xing, K., Sun, D., Zhang, J., & Zhao, F. (2021). Wide diurnal temperature amplitude and high population density can positively affect the life history of *Sitobion avenae* (Hemiptera: Aphididae). *Journal of Insect Science*, *21*(2), 6.

Zaelor, J., & Kitthawee, S. (2018). Growth response to population density in larval stage of darkling beetles (Coleoptera; Tenebrionidae) *Tenebrio molitor* and *Zophobas atratus*. *Agriculture and Natural Resources*, *52*(6), 603–606.

Zhang, D.-W., Xiao, Z.-J., Zeng, B.-P., Li, K., & Tang, Y.-L. (2019). Insect behavior and physiological adaptation mechanisms under starvation stress. *Frontiers in Physiology*, *10*, 163.

CONCLUSIONS

All important conclusions are summarized within each chapter. Overall, I discussed the impacts of acute ionizing radiation exposure on *Acheta domesticus* along various endpoints. My focus was to investigate endpoints that were less studied in the literature in a less studied species. Namely, a species which is not considered a disease vector or agricultural pest, which is the focus of a vast majority of IR studies in insects. To keep my results comparable, I irradiated all groups from all chapters at 14 days of age and reared them at densities determined by my results from chapter 8.

The first part of my thesis highlights the impacts of IR on sexual signaling and mating success, which are subtle but important aspects to reproduction. *Acheta* utilizes both acoustic and chemical signaling to successfully court and mate with females. My results indicated that both wing morphology and associated acoustic signals, as well as chemical signals were altered due to IR exposure. As well, these changes ultimately resulted in reduced mating success in these males. An interesting aspect of these studies was when parallel studies were done in female *Acheta*, sexual dimorphism in the response to IR was observed. Female wings are much less symmetric than males. This makes sense as wings in males, unlike in females, are extremely important to successful mating. As well, the female pheromone tended to be more impacted by IR then the male pheromone, again, likely due to the importance of these signals for male courtship of females. These results indicate the importance of analyzing other endpoints associated with reproduction other then those associated with SIT (sterilization). When investigating the efficiency of SIT, these subtle traits associated with sexual signaling may greatly impact mating success. As well, the importance of sex specific data is highlighted as males and females may have variable responses to stress.

My next study focused on the potential for radiation stress to be transmitted to F1 and F2 offspring from both maternal and paternal lines. The potential for stress to be inherited and possibly accumulate is highly relevant for individuals living within contamination zones, where damage may accumulate over many generations. Although my results here are inconclusive, they do show possible consequences particularly to survivorship due to IR exposure. However, stress responses are multifaceted, and therefore more endpoints, including reproduction, should be investigated further to better understand these impacts.

I also investigated the possibility for RIBE to occur in non-irradiated crickets reared in close (non-contact) proximity to irradiated crickets. There are only a small number of studies which have looked at potential life-history affects of organismal RIBE in insects. My results here were quite interesting and show that bystander crickets did have altered growth and survival parameters when compared to the control. These results have interesting implications to both SIT and contamination sites, as the effect size of exposure may be larger then currently predicted.