Quantifying the impact of environmental contamination using *Drosophila melanogaster* across biological sex and genetic background

by

Alexandra Sutherland-Hutchings

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science (MSc) in Chemical Sciences

Faculty of Graduate Studies
Laurentian University
Sudbury, Ontario, Canada

THESIS DEFENCE COMMITTEE/COMITÉ DE SOUTENANCE DE THÈSE
Laurentian Université/Université Laurentienne
Faculty of Graduate Studies/Faculté des études supérieures

Title of Thesis Titre de la thèse	Quantifying the impact of environmental contamination using Drosophila melanogaster across biological sex and genetic background
Name of Candidate Nom du candidat	Sutherland-Hutchings, Alexandra
Degree Diplôme	Master of Science
Program Programme	Chemical Sciences
Date of Defence Date de la soutenance	February 26, 2025

APPROVED/APPROUVÉ

Thesis Examiners/Examinateurs de thèse:

Dr. Thomas Merritt
(Supervisor/Directeur(trice) de thèse)

Dr. Joy Gray-Munro
(Committee member/Membre du comité)

Dr. Krista Currie
(Committee member/Membre du comité)

Approved for the Faculty of Graduate Studies
Approuvé pour la Faculté des études supérieures
Dr. Alain Simard
Dean of Graduate Studies and International
Doyen des Études supérieures et de la stratégie internationale
Laurentian University / Université Laurentienne

Dr. Jason Tennensen
(External Examiner/Examinateur externe)

ACCESSIBILITY CLAUSE AND PERMISSION TO USE

I, **Alexandra Sutherland-Hutchings**, hereby grant to Laurentian University and/or its agents the non-exclusive license to archive and make accessible my thesis, dissertation, or project report in whole or in part in all forms of media, now or for the duration of my copyright ownership. I retain all other ownership rights to the copyright of the thesis, dissertation or project report. I also reserve the right to use in future works (such as articles or books) all or part of this thesis, dissertation, or project report. I further agree that permission for copying of this thesis in any manner, in whole or in part, for scholarly purposes may be granted by the professor or professors who supervised my thesis work or, in their absence, by the Head of the Department in which my thesis work was done. It is understood that any copying or publication or use of this thesis or parts thereof for financial gain shall not be allowed without my written permission. It is also understood that this copy is being made available in this form by the authority of the copyright owner solely for the purpose of private study and research and may not be copied or reproduced except as permitted by the copyright laws without written authority from the copyright owner.

Abstract

The world is a stressful space. There is a strikingly large knowledge gap in the genetics of stress responses. Stressors are present in nature, such as to exposures to extreme pH, temperatures, and toxicants. We used the response of *Drosophila melanogaster* to environmental nickel (Ni) as a model to better understand stress, where stress is defined by dysregulation homeostasis. To broadly characterize stress responses, we assayed both males and females of three different types of genetic backgrounds – isogenic, isogenic constructed heterozygotes, and genetically diverse wild-caught lines – of *D. melanogaster* across a series of phenotypes that range in specificity. We measured Ni response broadly using mortality and more specifically using metabolites. Not surprisingly, Ni negatively impacted every phenotype. Lifespan, feeding, and total lipids were reduced when exposed to Ni. Surprisingly, the Ni effects were substantially different between the sexes, types of genetic backgrounds, and lines. For example, the differences between males and females themselves varied substantially across lines. Similarly, the types of genetic backgrounds and lines differed in response, highlighting genetic complexities. In sum, my results clearly demonstrate that to truly understand the biology of a system, both sexes and multiple lines must be used.

Keywords: genetic background, sexual dimorphism, nickel biology, *Drosophila melanogaster*

Acknowledgements

I would like to begin by thanking my supervisor, Thomas. I am incredibly thankful for all the support that you have given me over the last few years through my undergraduate and master's projects. Your continuous guidance, encouragement, and terrible jokes have gotten me through the most stressful times of my degree. I would not have become the scientist I am today without you as a supervisor. Thank you for believing in me, even when I didn't.

Next, I would like to thank my committee members Joy and Krista. You both are awesome, and I am so thankful to have had such amazing and inspiring scientists supporting me through my master's. Your insights and support on my research have been so appreciated.

Additionally, I would like to thank past and present Merritt Lab members for all their help with experiments. I could not have gotten through my master's without all the help, laughs, and games.

Miigwetch ☺

Table of Contents

List of Tables

List of Figures

List of Appendices

List of Abbreviations

ANOVA	Analysis of variance
ANCOVA	Analysis of covariance
CI	Confidence interval
CSY	Cornmeal-sugar-yeast diet
DGRP	*Drosophila* Genetic Reference Panel
F	Female
G6PD	Glucose-6-phosphate dehydrogenase
ICP-MS	Inductively coupled plasma mass spectrometry
IDH	Isocitrate dehydrogenase
LC	Lethal concentration
M	Male
MEN	Malic enzyme
NADPH	Nicotinamide adenine dinucleotide phosphate
Ni	Nickel
$NiSO_4$	Nickel sulfate
HSD	Honestly Significant Difference

CHAPTER 1 INTRODUCTION

Introduction

Living organisms are constantly exposed to a variety of environmental stressors (Genchi et al., 2020). As a result, organisms elicit stress responses that are a complex web of interacting biochemical mechanisms. Both biological sex and genetic background are modifying factors of susceptibility, but the magnitude of either and the bidirectional interaction is poorly understood. We explored the genetic and phenotypic variation of stress responses by utilizing nickel (Ni) as a model for environmental stress and *Drosophila melanogaster* as a model for biological response. Furthermore, we investigated how both biological sex and genetic background influence the stress response. My study has broader implications for xenobiotic exposures, contributing to our understanding of environmental toxicity and its effects on biology.

Stress responses and xenobiotics

As organisms are constantly exposed to stress, many biochemical mechanisms are triggered, mitigating cellular damage. Stress responses involve many interacting pathways and do not function in isolation. Stress responses are broadly defined as exposure to an environmental factor that reduces the fitness of an organism and disrupts homeostasis (Klepsatel et al., 2016; Rion & Kawecki, 2007). Many stress response mechanisms are highly conserved across species and can vary substantially depending on the age, sex, and genetic background of the organism.

The type of stressor largely influences the mechanisms that are triggered. Xenobiotics are a specific category of biological stressors, defined as a chemical substance foreign to an organism's biological system (Patterson et al., 2010). These include environmental pollutants,

pesticides, and metals that are not required for proper biological function. Although xenobiotics are not biologically necessary, many organisms have adapted detoxification mechanisms that minimize biological dysregulation (Gao et al., 2022). The detoxification of xenobiotics includes both specific and non-specific mechanisms. Pathways, such as CncC/Keap1 and HR96, among many others, are commonly triggered in response to xenobiotic exposures. The CncC/Keap1 controls the defence response to oxidative stress, a common product of xenobiotics, which is present in both insects and mammals (Gao et al., 2022). Xenobiotics directly bind to HR96 as ligands to activate the pathway. It is unknown as to whether the expression of the HR96 pathway has positive or negative impact on xenobiotic tolerance. While these mechanisms, among others, are involved in the detoxification of xenobiotics, the interactions these pathways and xenobiotic specificity are poorly understood (Gao et al., 2022).

Biology of metal response

Organisms respond differently to different metals, depending on the specific characteristics of the metals and their natural roles in the biology of those organisms. From a biological perspective, metals can be classified as either essential or non-essential. Metals that are required for proper biological function are classified as essential metals. To be considered essential, a metal must be present in tissues, cause severe damage in deficiency, and decrease deficiency damage through proper supplementation (Nielsen, 1985; Zoroddu et al., 2019).

Essential metals, such as copper, iron, and zinc, have specific biochemical mechanisms to regulate them, as they are required for 47% of enzymes (Martinez-Finley et al., 2012). In excess or deficiency, essential metals can cause adverse health effects. For

example, copper deficiency can cause anemia, neurological abnormalities, and impaired immune function, among others (Altarelli et al., 2019). In contrast, excess copper can result in the production of reactive oxygen species and cellular damage (Tchounwou et al., 2012).

In contrast, non-essential metals have no established biological function and are toxic in trace amounts (Tchounwou et al., 2012). Given their lack of biological role, there are no metal-specific mechanisms to metabolize non-essential metals in the same way that essential metals are metabolized. For instance, iron has a suite of mechanisms to maintain homeostasis (Calap-Quintana et al., 2017; Slobodian et al., 2021), but that is not the case for non-essential metals. Non-essential metals can interact with essential metal mechanisms, causing dysregulation. The displacement or interference of non-essential metals to essential metal mechanisms can lead to downstream effects, such as the production of reactive oxygen species, causing toxicity. Furthermore, non-essential metals can displace essential metal ions in metallothioneins and metalloproteins, resulting in a non-functional protein (Martinez-Finley et al., 2012).

The interaction mechanisms of some non-essential metals, such as aluminum, arsenic, cadmium, lead, and mercury, are far more understood than some others, such as Ni (Martinez-Finley et al., 2012; Slobodian et al., 2021). Although it is unclear how exactly Ni induces toxicity in an organism, a few mechanisms have been proposed. Ni has been shown to inhibit enzyme activity through various binding mechanisms (Macomber & Hausinger, 2011). For example, this has been studied in superoxide dismutase 1, where Ni displaces the natural cofactor, copper, producing a non-functional enzyme (Klein Couto et al., 2020). Additionally, Ni can indirectly cause oxidative stress. For example, Ni has been shown to deplete

glutathione activity, a marker of oxidative stress (Macomber & Hausinger, 2011; Misra et al., 1991; Valko et al., 2005).

Is Ni an essential metal?

Ni's classification as an essential or non-essential metal in animals is contentious. Some reports state that Ni is a non-essential metal (Denkhaus & Salnikow, 2002), whereas others state that Ni is an essential metal for animals (Barceloux & Barceloux, 1999). Some sources report that Ni as an essential metal for higher organisms is probable (World Health Organization et al., 1996), as eight Ni-dependent enzymes have been identified in various bacteria, archaea, and fungi species (Ragsdale, 2009). To date, there have been no Ni metalloenzymes found in any higher organism (Denkhaus & Salnikow, 2002).

As mentioned, there are a few conditions for a metal to be considered essential. Briefly, a metal is essential if deficiency consistently results in reversible biological dysregulation and is naturally present in tissues (Nielsen, 1985; Zoroddu et al., 2019).

Animals are constantly exposed to Ni through diet and its high abundance in nature (Genchi et al., 2020). Due to these natural exposures, the presence of Ni in tissues is on the order of ng/g (Dudek-Adamska et al., 2021). Thus, Ni's high abundance in foods, such as legumes, nuts, and oilseeds, makes it difficult to observe Ni deficiency (2015; Genchi et al., 2020). The daily intake of Ni is, on average, more than three times the "daily requirement." However, the Food and Drug Administration has not set a daily requirement for Ni (Trumbo et al., 2001). These contradicting reports arise from suggested Ni intake and potential beneficial concentrations of Ni, which have been extrapolated from animal studies (Nielsen, 2012).

Ni deficiency has been observed in several animal species, including the chick, cow, goat, minipig, rat, and sheep. However, due to incorrect dietary supplementation, signs of Ni deficiency may have been misinterpreted rather than truly demonstrating Ni as an essential metal (Nielsen, 1985; World Health Organization et al., 1996). Overall, there is not sufficient evidence to suggest Ni is an essential metal to animals.

Sexual dimorphism

Species with sexual distinction consist of males and females that are biologically distinct. Given that many animals exhibit sexual dimorphism, it is highly important to consider biological research to provide a more thorough understanding of biological responses. Sexual dimorphism refers to male-female phenotypic differences (Poissant et al., 2010). Historically, biological sex as an experimental factor has been overlooked. As such, female biology is far less understood than male biology (Ah-King, 2022).

Sexual dimorphism in *D. melanogaster* has been studied extensively. Males and females differ visually in abdominal size and pigmentation as well as physiologically in stress responses and lifespan (Millington & Rideout, 2018). Often, sexual dimorphism has been attributed to hormonal differences between the sexes. *D. melanogaster* sex determination does not involve sex hormones. Therefore, sexual dimorphic differences are purely driven by genetic differences (Asahina, 2018; Yamamoto & Koganezawa, 2013). When multiple genetic backgrounds are considered, the sexually dimorphic effects vary (Jang & Lee, 2015).

Genetic background and genetic diversity

Genetic background refers to all genes in a genome (Yoshiki & Moriwaki, 2006). A genetic background can be classified in reference to a population, including isogenic, heterozygous, or wild. Isogenic refers to each individual within a population having the same genetic background. Commonly, populations are made isogenic through approximately 20 generations of full-sibling inbreeding, resulting in complete genetic homozygosity (Mackay et al., 2012). Heterozygous refers to the genetic background with two different alleles, which we constructed by crossing two isogenic backgrounds. Wild refers to a population of genetically diverse individuals representing a subset of a natural population. A line is a population of individuals with a type of genetic background which is distinct from another. Wild lines are distinct by having different parental populations, whereas isogenic and heterozygous lines are distinct by differing genetic backgrounds across lines but not within.

Genetic background is a substantial driver of the complexity and diversity of metal responses (Leem et al., 2015). Genetic background effects are often considered inconsequential. However, failure to control for genetic background may lead to conflicting results in the replication of a study (Chandler et al., 2013). Furthermore, failure to account for multiple genetic backgrounds in a study limits the generalizability of obtained results (Sittig et al., 2016). As many studies work in one genetic background, the results apply to that genetic background being studied. Here, we look at the impact of multiple genetic backgrounds and their response to metal toxicity across both sexes.

Thesis overview

In my study, we utilized *D. melanogaster* as a model to represent the biological response to environmental contamination. Further, to model environmental contamination, we looked at Ni toxicity, as Ni is a common environmental contaminant in Sudbury, ON. Utilizing both sexes and multiple genetic backgrounds, we quantified the impact of Ni across four phenotypes while accounting for starvation stress, a fifth phenotype, as a potential source of mortality. We measured Ni exposure and starvation mortality and the change in food consumption, enzyme activity, and total lipid concentration post-Ni exposure. We measured mortality as a dose-response on Ni as a metric to score resistance or sensitivity, using lethal concentration (LC_x) values, where x is the percent mortality. We then looked at starvation resistance and changes in food consumption, as the Ni response may be confounded by food avoidance, resulting in starvation. We then looked at changes in enzyme activity and total lipid concentration as a metric for overall fly health. Across each phenotype, we expected the baseline responses of types of genetic backgrounds, in order of resistance to sensitivity, to be heterozygous, wild, and isogenic. Further, we expected that males and females would have different responses, which would largely be modulated by genetic background (Jang & Lee, 2015).

CHAPTER 2 QUANTIFYING THE IMPACT OF ENVIRONMENTAL CONTAMINATION USING *DROSOPHILA MELANOGASTER* ACROSS BIOLOGICAL SEX AND GENETIC BACKGROUND

Introduction

The constant exposure to environmental stressors highlights the importance of understanding stress biology. Toxicity from environmental metal contamination is of interest due to its widespread impact. However, many molecular and biochemical mechanisms underlying metal toxicity remain poorly understood. Adding complexity, both biological sex and genetic background are modifying factors of susceptibility, but the magnitude of either and the bidirectional interaction is poorly understood. To better characterize stress biology, we use the *Drosophila melanogaster* model system and nickel (Ni) exposure.

Toxicity can result from exposure to both essential and non-essential metals. Essential metals in excess lead to the dysregulation of biological mechanisms, causing toxicity. In contrast, non-essential metals cause toxicity in trace amounts. Essential metal toxicity is generally understood, as there are metal-specific mechanisms that regulate them. In contrast, non-essential metal toxicity is far less understood, especially for metals such as nickel Ni (Slobodian et al., 2021).

Although it is unclear how exactly Ni induces toxicity in an organism, a few mechanisms have been proposed. Ni has been shown to inhibit enzyme activity through various binding mechanisms (Macomber & Hausinger, 2011). For example, Ni inhibition has been studied in superoxide dismutase 1, where Ni displaces the natural cofactor, copper, producing a non-functional enzyme (Klein Couto et al., 2020). Additionally, Ni can indirectly cause oxidative stress. For example, Ni has been shown to deplete glutathione activity, a marker of oxidative stress (Macomber & Hausinger, 2011; Misra et al., 1991; Valko et al., 2005).

There are no specific mechanisms that are responsible for the efflux of non-essential metals. The general metal response pathway is a highly conserved, non-specific metal regarding the homeostasis mechanism, which responds to toxic concentrations of both essential and non-essential metals (Calap-Quintana et al., 2017; Slobodian et al., 2021). Briefly, the metal binds to activate metal transcription factor 1, which is then translocated to the nucleus, where it binds to a metal response gene and triggers metal efflux. One example of efflux is the activation of metallothioneins, a non-specific metalloprotein (Günther et al., 2012; LaRochelle et al., 2001; Saydam et al., 2002; Slobodian et al., 2021). Interestingly, metallothioneins are differentially expressed across biological sex (Egli et al., 2006).

We investigated Ni toxicity across three types of *Drosophila melanogaster* lines, each with distinct genetic background characteristics: wild-caught, isogenic, and constructed heterozygotes. Our wild-caught flies represent a snapshot of the natural population at the time of collection and serves as our baseline for comparison. Maintaining genetic diversity in wild-caught lines is highly unstable. It is likely that genetic diversity will reduce in each subsequent generation. Isogenic flies, as the name suggests, are sets of genetically identical flies that we use as a uniform population. There is little to no genetic diversity within an isogenic line, but each line is genetically distinct. We can, therefore, observe the effects of genetic diversity by comparing multiple lines. Utilizing these various backgrounds of isogenic flies, we constructed heterozygotes by performing an F1 cross between two lines. We used heterozygous flies as a synthetic way to mimic a natural population in the laboratory. Additionally, while heterozygous flies contain no genetic diversity within a line, we can further explore the impact of increased heterozygosity in and across populations that are stable

and reproducible. We expected that isogenic flies would have the most level of variation across lines while wild flies would have the most variation within lines.

In my study, we quantified the biological effects of Ni exposure, with specific interest in how that impact differs between the sexes and across genetic backgrounds. As part of this exploration of the effects of Ni exposure, we compared responses across a series of life history, behavioural, and biochemical phenotypes chosen to broadly characterize the biological impact of Ni. We used Ni and starvation mortality as a broad metric to quantify the differing responses across both sex and genetic backgrounds. To better understand Ni response, we quantified Ni avoidance and changes in enzyme activity and total lipid concentration. Given these five phenotypes, we can look for consistencies across each and provide a broad-scale response to stress. Overall, our results demonstrate that both biological sex and genetic background play a crucial role in stress responses, highlighting the importance of considering both in experimental design.

Materials and Methods

Fly rearing and fly lines

To determine the impact of sex and genetic background on phenotypic response, we assayed both sexes and four lines of each type of genetic background: isogenic, heterozygous, and wild *Drosophila melanogaster*. All flies used for experiments were reared to adulthood on a standard cornmeal-sugar yeast diet (CSY; 9.4 g/L agar, 33.2 g/L each cornmeal and yeast extract, 73.5 mL/L corn syrup, 2.1 g/L methylparaben in 95% ethanol, and 0.053 g/L penicillin), incubated at 25ºC in a 12-hour light-dark cycle. We reared flies by sorting groups of 8 males and 10 females on standard CSY that were allowed to mate and lay eggs for five days and then discarded. Larvae then grew for four days, at which point all early emerged flies were discarded. Flies were allowed to emerge for three days, were transferred to new vials, and aged for two days. We used these 2 to 5-day-old flies for experiments.

We used a subset of four lines from the *Drosophila* Genetic Reference Panel, DGRP, a panel of over 200 inbred fly lines (Mackay et al., 2012). With their respective Bloomington Stock Center stock numbers, we used DGRP 189 (28152), DGRP 437 (25194), DGRP 584 (28212), and DGRP 852 (25209). For simplicity, we refer to the DGRP lines as 189, 437, 584, and 852.

We constructed F1 heterozygous crosses by rearing virgin females and males of differing isogenic lines, creating the following crosses: 189x437, 189x852, 437x584, and 584x852, along with their reciprocal crosses. To mitigate maternal bias, one vial each from a cross and its reciprocal cross were mixed and randomly sorted into groups to be used in experiments.

We collected wild flies between July and August 2023 from a compost bin in Sudbury, Ontario, Canada (46.48718°N, 80.98286°S). We sorted *D. melanogaster* out from other and more abundant species in our sampling populations, which were not particularly large. Therefore, it would not have been possible to obtain enough flies after one generation removed from the wild. If our sampling populations were large enough from flies taken directly from the wild, our results would largely be confounded by a combination of age variability and lab adaptation. To mitigate the opportunity for lab adaptation, we used second-generation wild flies for experiments, while other studies utilized flies three generations removed from the wild (Everman et al., 2023). In each of the four fly bottles, we placed groups of 90 wild *D. melanogaster*, consisting of 40 males and 50 females, on standard CSY. To obtain replicate bottles, parents were transferred to fresh fly bottles every three days. Each group of 90 wild flies were treated as producing distinct lines named W1, W2, W3, and W4. After 14 days, the first lab generation of flies emerged and was placed into vials for fly rearing, producing second-generation flies that were used for experiments. We mitigated sampling bias as much as possible by combining replicate bottles or replicate vials, as well as utilizing as many flies as we had available. Our process unavoidably selected genetic backgrounds that survived the transition to a lab environment.

Quantification of Ni response

We measured mortality as a baseline dose response to Ni. We used $NiSO_4$ as a toxicant with the primary goal of investigating the toxicity of Ni. While SO_4^{2-} is toxic (Cao et al., 2022), the concentrations of Ni used are high enough that the anion toxicity is not resolvable (Appendix A – Supplemental Figure 1), as demonstrated in a previous study (Slobodian,

2024). We placed adult flies on concentrations of 0, 5, 10, 15, 20, 25, 30, 35, and 40 mM of Ni. We prepared Ni-contaminated food by incorporating an appropriate volume of either 0.5 M or 1 M stock solutions of $NiSO_4 \bullet 6H_2O$ (Nickel sulphate hexahydrate, BioShop, NIC700) to liquid CSY. We then dispensed approximately 5 mL of prepared food into fly vials. We refrigerated the food vials overnight to solidify. For each line, sex, and concentration, we used six replicate vials of 20 flies per vial. We transferred the flies to fresh vials of the same concentrations after 48 hours and recorded their mortality after 96 hours.

Measurement of starvation resistance

We measured starvation resistance to compare it to Ni resistance. We starved flies in vials containing ~5 mL of agar (7.4 g/L) as a non-nutritive water source to prevent desiccation. In addition, we had a non-starved control on standard CSY. Six replicate vials of 20 flies per vial were used for each sex, line, and condition. We recorded mortality every 12 hours until all the starved flies were dead. We transferred the flies to fresh vials every 48 hours.

Measurement of food intake

We used a dye-based method to quantify the change in food intake between clean and Ni-contaminated food, adapted from (Wong et al., 2009). We preconditioned adult flies to 10 mM Ni for 24 hours alongside a standard CSY control. We then transferred flies to vials containing 2.5% (w/v) blue food dye (Erioglaucine disodium salt, Sigma-Aldrich, 861146) and either 0 mM control or 10 mM Ni. In addition, we used age-matched flies exposed to non-dyed food as the baseline. After 90 minutes, we directly transferred the flies to 2 mL screw

cap tubes and flash frozen in liquid nitrogen (Figure 2.1 A). We kept the flies at -80ºC until analysis.

We homogenized five groups of either 20 males or 10 females for each line in distilled water using a mixer mill (TissueLyser) and 2 mm stainless steel beads at 30 Hz for 2 minutes. To account for differences in size and gut volume, we homogenized male flies at a concentration of 2 flies/100 µL and female flies at a concentration of 1 fly/100 µL. We centrifuged the homogenates three times at 13 000 RPM for 5 minutes to pellet fly debris and lipids, transferring 80% of the volume into new 1.5 mL microfuge tubes between each centrifugation. We pipetted volumes of 100 µL of homogenate into a 96-well plate and measured the absorbances at 629 nm using a spectrophotometer (Figure 2.1 B; Molecular Devices SpectraMax Plus 384 microplate reader). We assayed each homogenate twice and used the means for analysis.

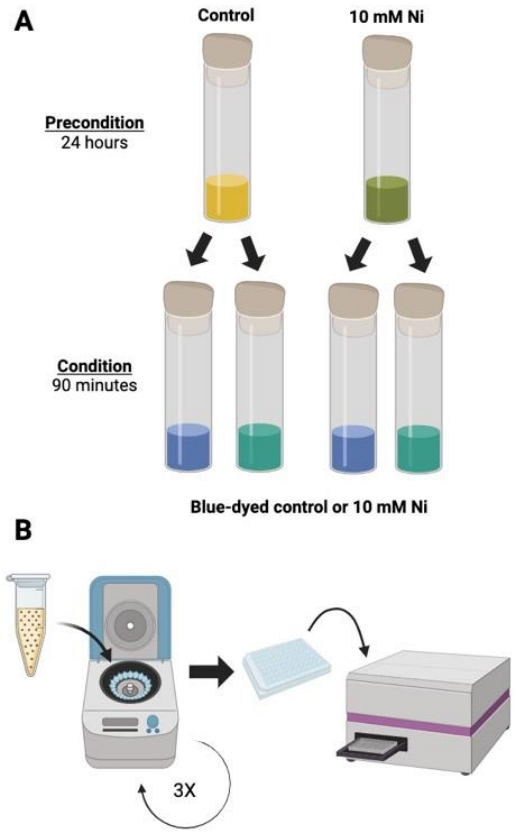


Figure 2.1 Schematic of the feeding assay protocol.
(A) We exposed the flies to a non-dyed medium for 24 hours, either control or ten mM Ni, acting as an acclimation period. To quantify the effect of Ni on feeding, we then exposed the flies to either a blue-dyed control or Ni medium. (B) The flies were then sacrificed, homogenized, and centrifuged three times, and the homogenate was read in a plate reader at 629 nm. Figure was created using BioRender,com.

Exposure protocol

For measurement of total lipid, enzyme activity, and protein, we exposed adult flies to either clean CSY as a control or 10 mM Ni for 24 hours. We then transferred the flies to 1.5 mL microfuge tubes and sacrificed them in liquid nitrogen. We sorted our fly samples into groups of five flies, using five replicates for each line, sex, and condition.

Total lipid measurement

We used an organic extraction to quantify total lipids in adult flies. We weighed each sample to the nearest 0.01 mg using an analytical scale (Wet weight; Mettler Toledo microbalance MX5). We then placed each sample into glass test tubes and allowed them to dry uncovered for approximately 12 hours at 50ºC. We reweighed the dehydrated fly samples (Dry weight 1). To extract the lipids, we added 1 mL of diethyl ether (Sigma) to the samples for 12 hours. We then removed the ether, and the samples were left to dry overnight to evaporate any residual ether. We weighed the remainder of the flies, which lacked both water and lipids (Dry weight 2). We calculated total lipid as the difference between dry weight 1 and dry weight 2. We standardized by initial fly weight by taking the ratio between total lipid and wet weight.

Fly wet weight

We determined the fly samples' wet mass to the nearest 0.01 mg using an analytical scale (Mettler Toledo microbalance MX5). We used weight as a covariate in analyses of covariance (ANCOVA) to standardize enzyme activities for differences in fly size.

Fly homogenization

For enzyme activity and soluble protein concentration assays, we homogenized whole flies using a Dremel tool and a nylon pestle at a concentration of 1 fly/100 µL in homogenizing buffer (0.1 M TRIS-HCl pH 7.4, 0.15 mM $NADP^+$) for approximately 5 seconds. We centrifuged the homogenates at 13 000 RPM for 5 minutes at 4ºC to pellet fly debris.

Following centrifugation, we transferred 250 µL of supernatant to a 96-well plate and used it as a master plate. Throughout the homogenization process, we kept the fly samples on ice.

Enzyme activity measurements

We assayed the activity of three nicotinamide adenine dinucleotide phosphate (NADPH) dependent enzymes associated with oxidative stress: malic enzyme (MEN), isocitrate dehydrogenase (IDH), and glucose-6-phosphate dehydrogenase (G6PD) following established protocols (Merritt et al., 2005). The activity of each enzyme is quantified by measuring the production of NADPH through time.

We transferred 10 µL, for MEN and IDH, or 20 µL, for G6PD, of homogenate from the master plate to a new 96-well plate. We then added 100 µL of each enzyme's respective assay solution, prepared as follows:

MEN: 100 mM TRIS-HCl pH 7.4, 0.34 mM $NADP^+$, 5 mM $MnCl_2$, 10 mM malate.

IDH: 100 mM TRIS-HCl pH 8.6, 0.1 mM $NADP^+$, 0.84 mM $MgSO_4$, 1.37 mM isocitrate.

G6PD: 20 mM TRIS-HCl pH 7.4, 0.2 mM $NADP^+$, 3.5 mM glucose-6-phosphate.

Soluble protein content

We quantified protein concentrations using the bicinchoninic acid (BCA) assay using a commercial assay kit (Pierce, Thermo Scientific, 23225). In a 96-well plate, we added 100 µL of BCA working reagent to 10 µL of fly homogenate. We compared each reading to a standard curve of bovine serum albumin (BSA). We covered and incubated each plate at 37ºC for 30 minutes. Once the plate reached room temperature, the plate was placed in the spectrophotometer and read at 562 nm. We assayed each sample twice, and the means were

used for analysis. We used total protein as a covariate in ANCOVA for enzyme activities to standardize by differences in fly size and homogenization.

Data analysis

We used JMP® 18.1 software (SAS Institute) for all statistical analysis and data-representative figure generation. We used the averages of all replicates for analysis. We generated Ni dose-response curves using a two-parameter probit model. We used these models to calculate the LC_{25} and LC_{50} with 95% confidence intervals. Significant differences between LC values are defined by the lack of overlap between the 95% confidence intervals. Each other phenotype has an error represented by ± standard error (SE). Where significant differences were present in the analysis of variance (ANOVA), we used a post hoc Tukey's honestly significant difference (HSD) to calculate differences between groups.

Results

Dose-response to Ni

We used mortality as a metric to score sensitivity or resistance to environmental Ni. Flies died at a faster rate when exposed to Ni and this effect was both statistically significant and dose-dependent ($F_{7,1102} = 364.5$, $P < .0001$). The increased mortality differed between the sexes ($F_{1,1102} = 290.6$, $P < .0001$), fly lines ($F_{11,1102} = 114.2$, $P < .0001$), and there was a significant interaction between these two modifiers ($F_{11,1102} = 14.0$, $P < 0.0001$). Overall, Ni response is biologically complex.

Biological sex strongly modified Ni response. In general, females were more resistant than males (Appendix A – Supplemental Figure 2), where resistance is defined as a higher LC_{25} or the LC_{50} value. Male-female differences varied by level of stress. At low levels of stress, or the LC_{25}, there was a 13% difference between the sexes, a marginal and insignificant difference, where significance is defined by the lack of overlap between 95% confidence intervals. At moderate stress levels or the LC_{50}, females were 41% more resistant than males, and that difference was statistically significant. The LC_{25} and LC_{50} values, as well as percent differences between the sexes, can be found in Appendix A - Supplemental Table 1.

As was the case in comparing the sexes, the response to Ni was distinct for each type of genetic background. Heterozygous flies were the most resistant, followed by wild, then isogenic (Appendix A – Supplemental Figure 3). Overall, wild flies were 10% different from isogenic and 64% different from heterozygous flies at the LC_{25}. Whereas at the LC_{50}, wild flies were 24% different from isogenic and 48% different from heterozygous flies. The trends in resistance across types of genetic backgrounds are consistent across the sexes and levels of stress. Across genetic backgrounds, the differences between males and females increases from

the LC_{25} to the LC_{50} by 24%-26%. The LC_{25} and LC_{50} values for each type of genetic background can be found in Appendix A - Supplemental Table 2.

There was an interaction effect between line and sex. The interaction effect between line and sex was only present in the isogenic and heterozygous genetic background ($F_{3,353}$ = 52.7, $P < .0001$ and $F_{3,369} = 8.3$, $P < .0001$, respectively) but not in the wild genetic background ($F_{3,366} = 0.467$, $P = .7062$). Each dose-response curve can be found in Figure 2.2-2.3.

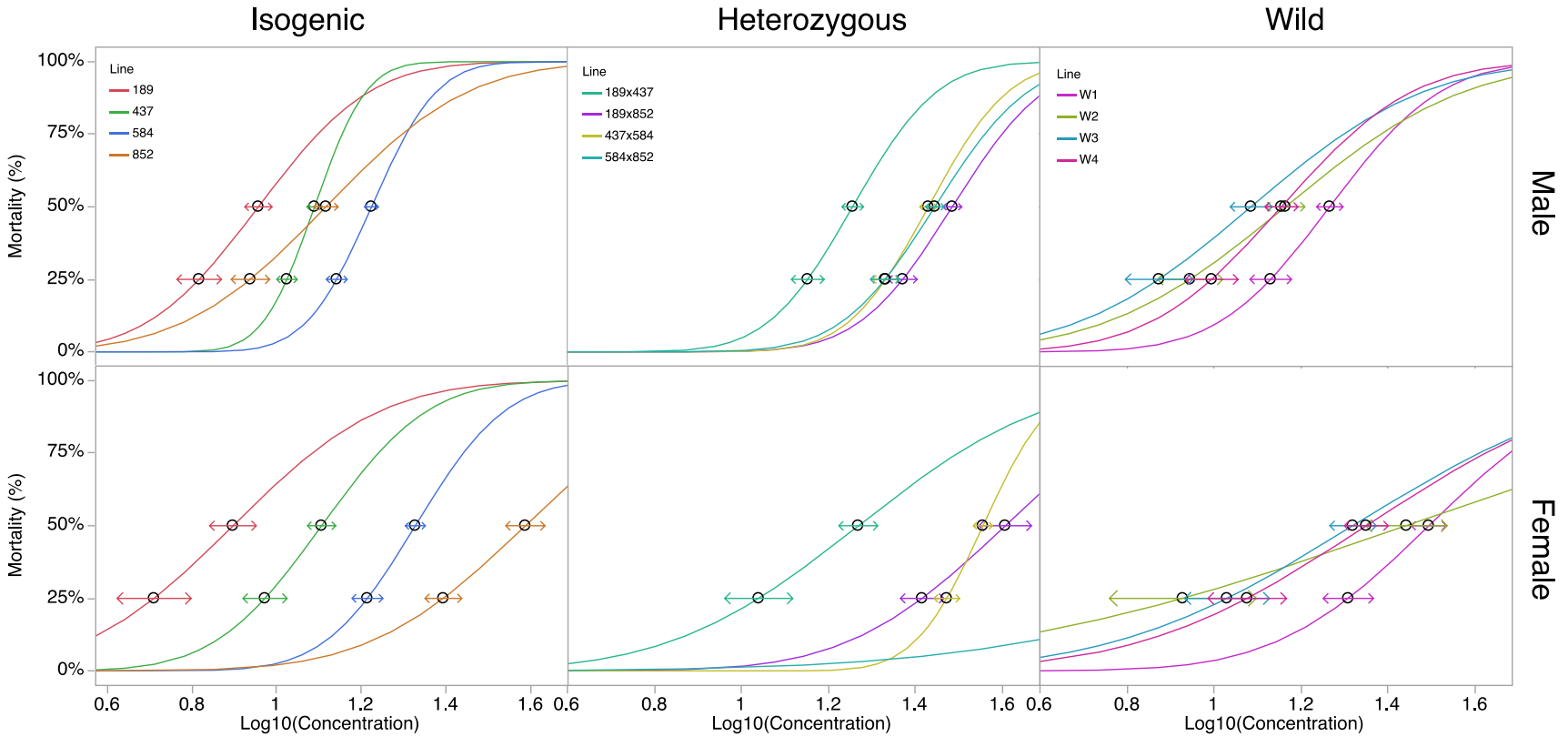


Figure 2.2 Dose-response curves for each line and sex by type of genetic background.
The 95% confidence intervals are present at the LC_{25} and LC_{50}. Intervals for line 584x852 females were removed from the graph for clarity. All values are found in Table 2.1.

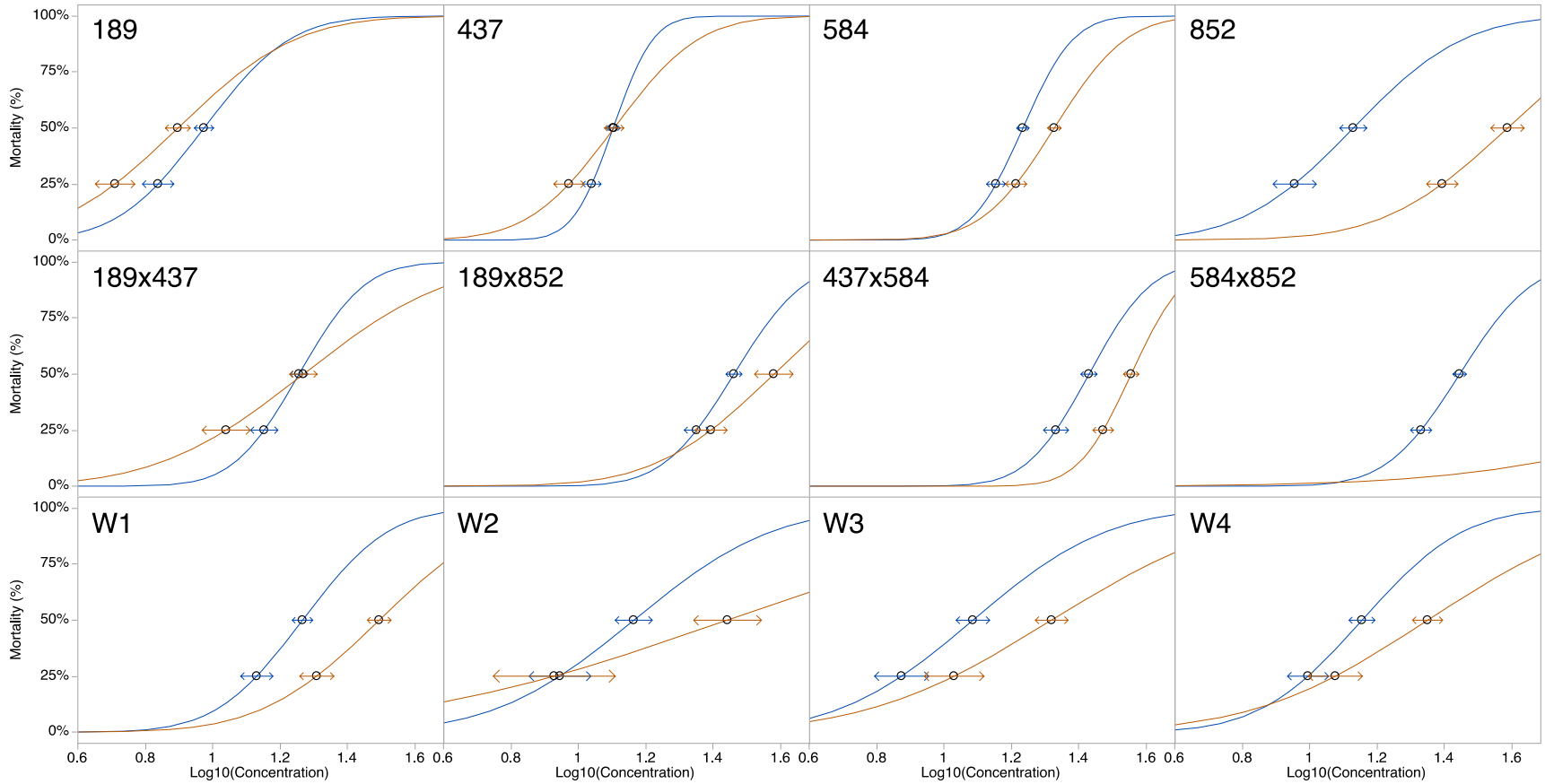


Figure 2.3 Dose-response curves for each sex by line and type of genetic background.
The 95% confidence intervals are present at the LC_{25} and LC_{50}. Intervals for line 584x852 females were removed from the graph for clarity. All values are found in Table 2.1.

We calculated line differences to explore the impact of type of genetic background on variation across lines. The variation across lines did not differ by level of stress. Therefore, we used LC_{50} values to quantify line differences. Variation across lines (Figure 2.4) differed significantly between types of genetic backgrounds and sex.

The variation across lines was significantly modified by genetic background in females ($F_{2,15} = 5.7$, $P = .0143$), but not in males ($F_{2,15} = 0.47$, $P = .6326$). Variation across lines were significantly higher in the heterozygous genetic background compared to the wild genetic background ($P = .0124$, Tukey's HSD).

Variation across lines was 2.6x higher in females than males in the isogenic genetic background ($P = .0095$, one-way ANOVA) and 4.0x higher in the heterozygous genetic background ($P = .0178$, one-way ANOVA), but not significantly different in the wild genetic background.

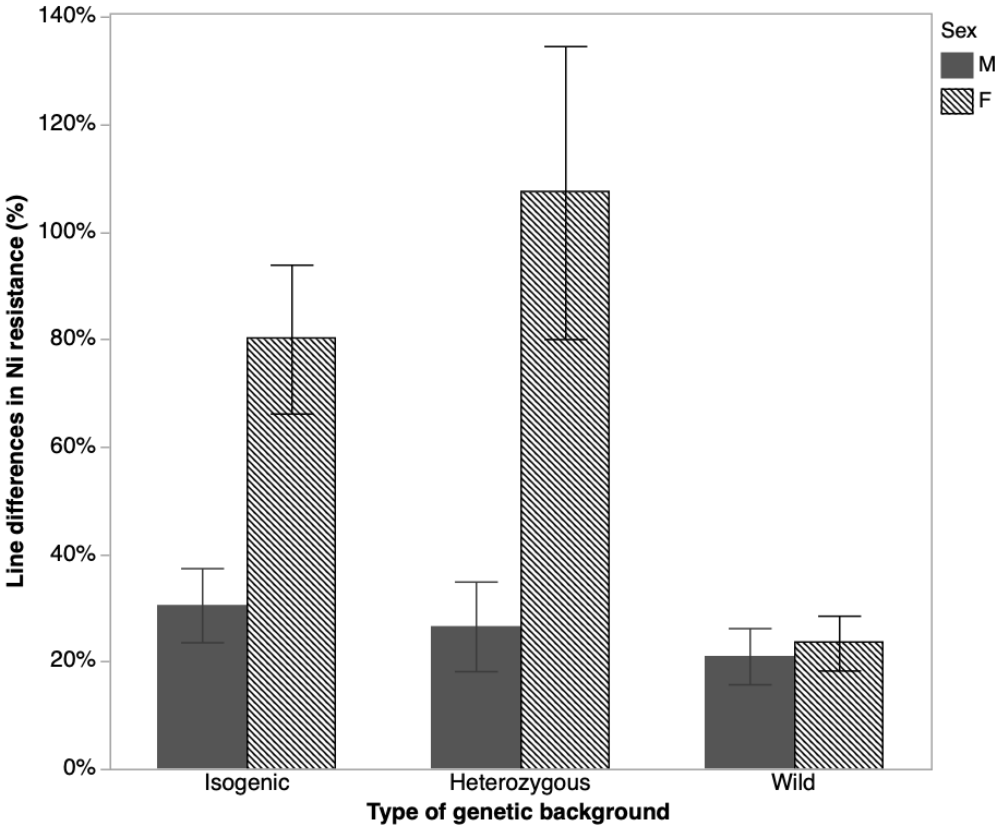


Figure 2.4 Average line differences in Ni resistance.

Average line differences were calculated from LC_{50} values, found in Table 2.1. Absolute differences among pairwise comparisons between lines were calculated (n = 6). Error bars represent ± SE. M = male, F = female.

Line	Type of Genetic Background	Male LC_{50} (mM)	Female LC_{50} (mM)	Percent Difference
189	Isogenic	9.43 [8.58, 10.37]	7.89 [6.81, 9.13]	-18%*
437		12.70 [12.03, 13.40]	12.78 [11.69, 13.96]	1%
584		17.17 [16.35, 18.02]	21.28 [19.99, 22.66]	21%*
852		13.49 [12.41, 14.67]	38.74 [34.26, 43.80]	97%*
189x437	Heterozygous	18.06 [17.11, 19.07]	18.59 [16.82, 20.55]	3%
189x852		30.62 [29.23, 32.08]	**40.51** [35.40, **46.36**]	28%*
437x584		26.97 [25.82, 28.16]	35.99 [34.26, 37.82]	29%*
584x852		27.92 [26.63, 29.26]	**326.0** [2.47, **43093**]	168%
W1	Wild	18.50 [17.39, 19.68]	31.24 [28.68, 34.04]	51%*
W2		14.62 [13.34, 16.01]	27.76 [22.73, 33.90]	62%*
W3		12.20 [11.06, 13.45]	20.91 [18.66, 23.43]	53%*
W4		14.32 [13.25, 15.47]	22.43 [20.14, 24.98]	44%*

Table 2.1 LC_{50} of each line and sex, with a percent difference between the sexes. LC_{50} values are presented as the value followed by [lower CI, upper CI]. Bolded values indicate extrapolation. * Indicates a significant difference between the sexes, defined by lack of overlap between 95% confidence intervals. Positive values indicate a female bias.

Sex differences varied by genetic line (Figure 2.5). By calculating the differences between the sexes by genetic line, there are no significant differences across types of genetic background (Tukey's HSD). Sex differences across lines is the most variable in the isogenic genetic background, which ranges from -18% to 97% difference, where a positive value indicates a female bias. In contrast, wild lines have the least level of variation, ranging from 44% to 62% differences between the sexes. Heterozygous has a range of 2% to 28% differences between the sexes. The variation in sex differences across lines is highly variable in the isogenic and heterozygous genetic background, compared to less variable in the wild genetic background, best represented by the error bars in Figure 2.5.

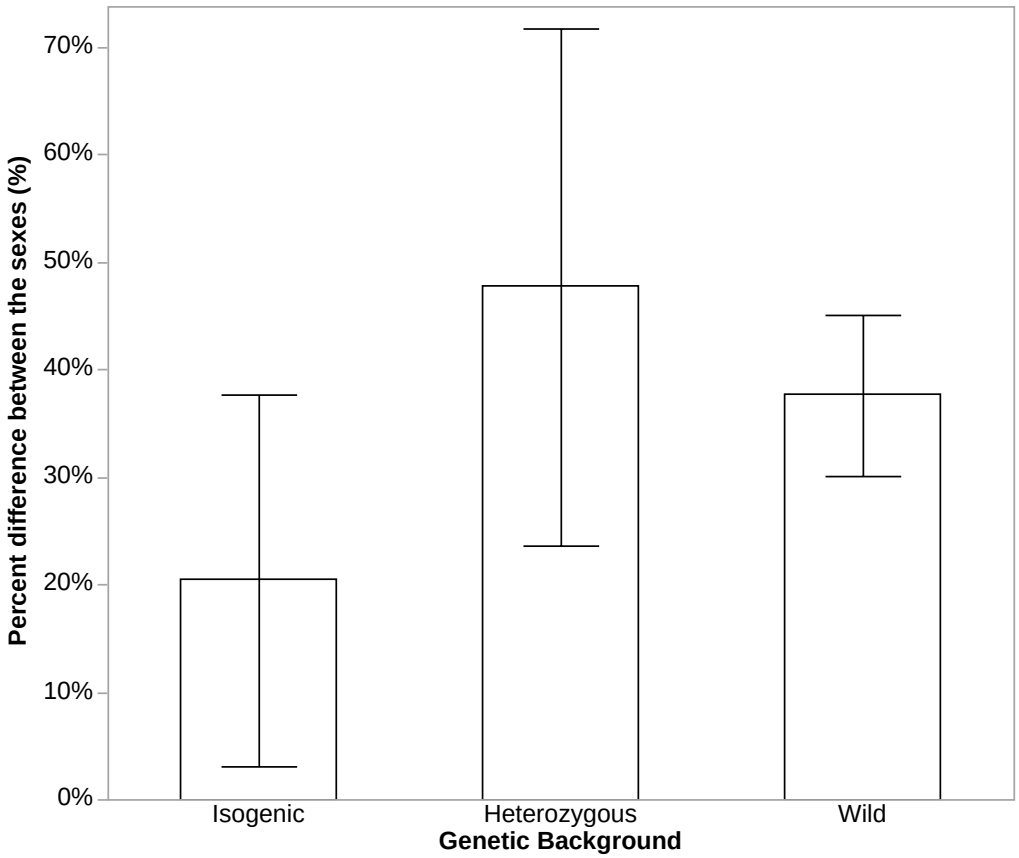


Figure 2.5 Average sex differences in Ni resistance.
Average differences between the sexes were calculated from LC_{50} values. Differences between the sexes were calculated for each line and separated by genetic background. Positive values indicate a female bias. Error bars represent ± SE.

Overall, we demonstrated that the type of genetic background, line, and sex significantly and substantially influence Ni mortality. Further, there are contradictory results based on which type of genetic background, line, or sex is being studied, highlighting the importance of considering both sexes and multiple genetic backgrounds.

Response to starvation

The differences in Ni response that we demonstrate above (Figures 2.2-2.3) may be complicated by a combination of toxicity and avoidance, resulting in starvation. In other words, different lines may differ in starvation resistance, in addition to or instead of differing in Ni response. To address this possibility, we quantified starvation resistance as a time-to-death response (Figure 2.6). Taking this data, we used average lifespan to score starvation resistance.

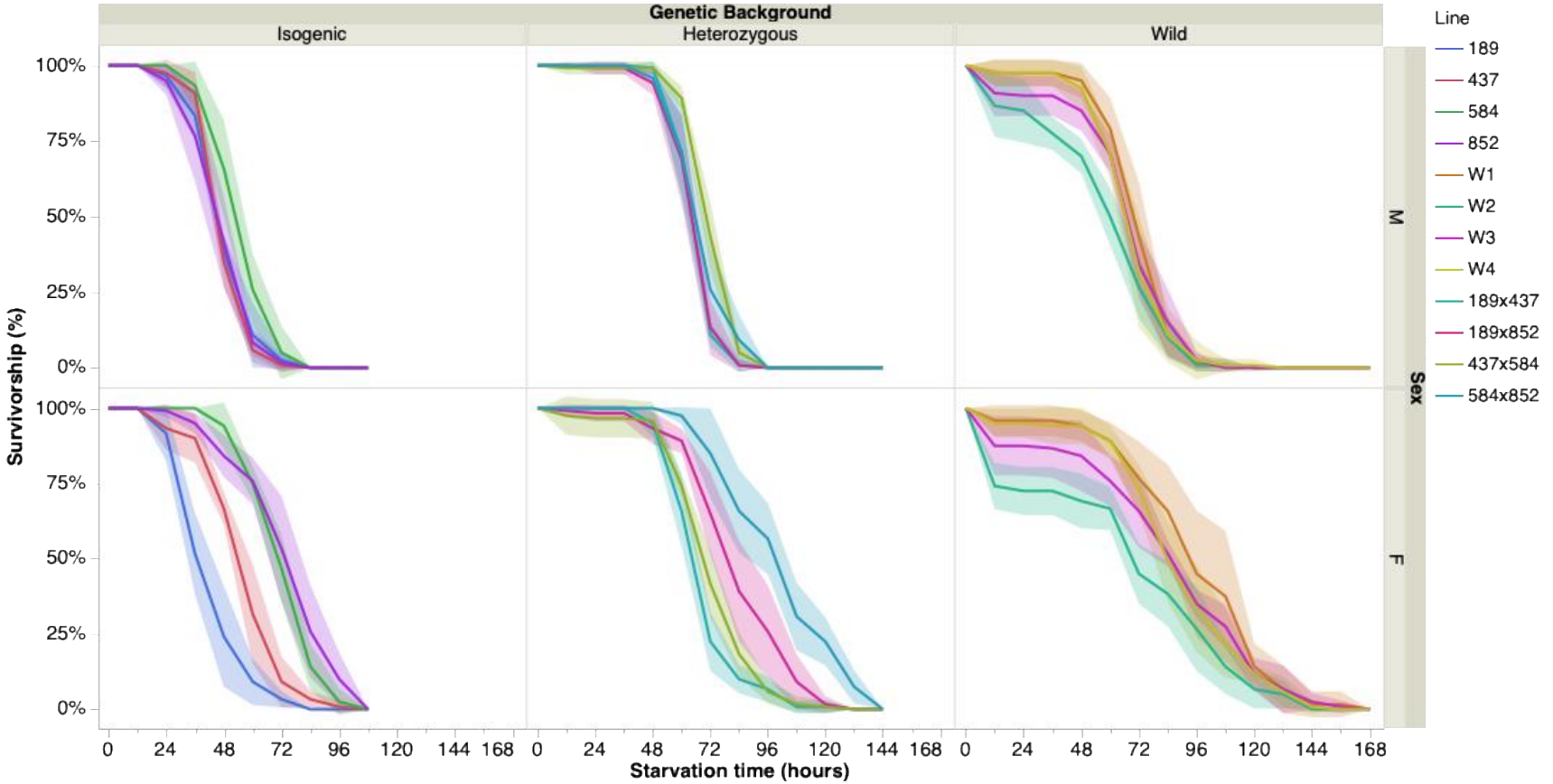


Figure 2.6 Time to death curves by each line, sex, and type of genetic background.

Females have higher line variation than males, further demonstrated in Figure 2.6. Shaded regions indicate 95% confidence. M = male, F = female.

The mortality of starved flies was 89%, compared to 46% for Ni-exposed flies, a 43% reduction in mortality. At our highest Ni concentration of 40 mM, there was 81% mortality. Overall, across types of genetic backgrounds, there was a 40% reduction in the isogenic, 59% in the heterozygous, and 32% in the wild genetic background. The reduction in mortality between starved and Ni-exposed flies was present in both sexes, having an overall 46% reduction in mortality in males and 41% reduction in females.

Flies died at a faster rate when starved, and this effect was statistically significant ($F_{14,1786} = 1183.4$, $P < .0001$). The impact of starvation on fly survivorship was significantly and substantially modified by sex ($F_{1,1786} = 204.3$, $P < .0001$) and fly line ($F_{11,1786} = 58.8$, $P < .0001$). In addition, there was an interaction effect between line and sex ($F_{11,1786} = 12.3$, $P < .0001$, ANOVA).

Starvation response differed by type of genetic background. Isogenic flies were the most sensitive, with the lowest average lifespan of 57.9 ± 0.8 hours, compared to 78.3 ± 0.8 and 78.1 ± 0.8 hours for heterozygous and wild, respectively. Female resistance to starvation is consistent across the type of genetic background (Figure 2.7) and generally across genetic lines (Figure 2.8).

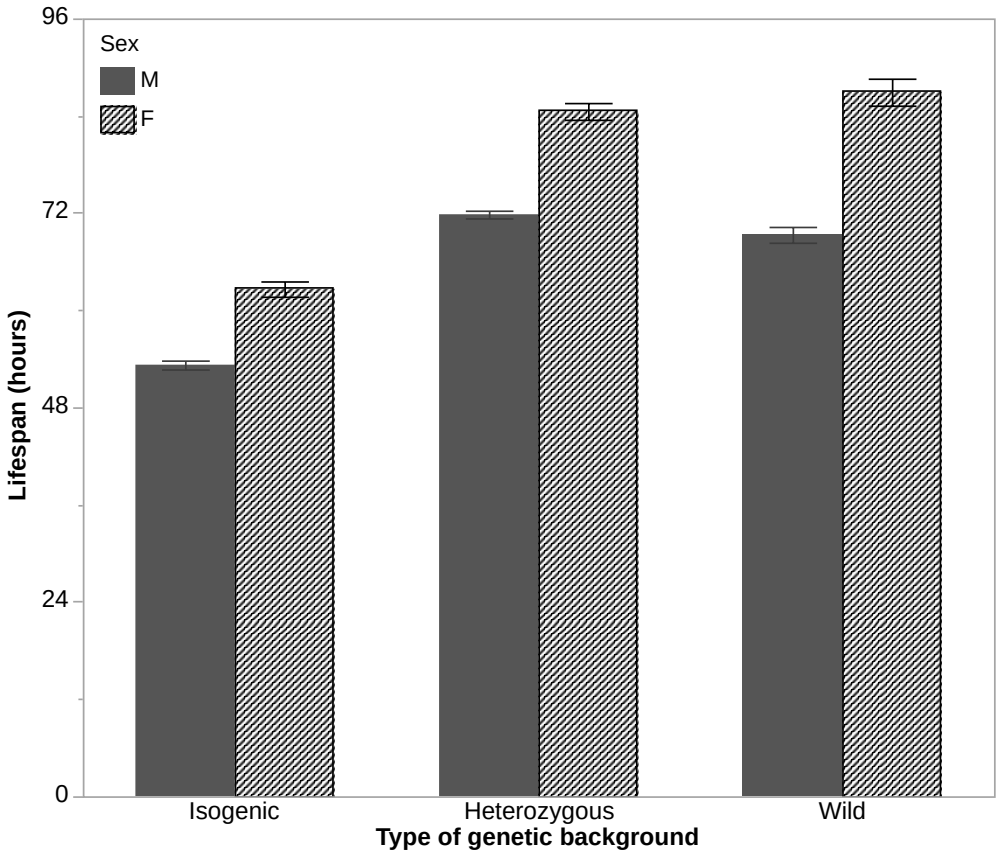


Figure 2.7 Average lifespan by type of genetic background and sex.
Females, on average, live longer than males, consistent across each type of genetic background. M = male, F = female. Error bars represent ± SE.

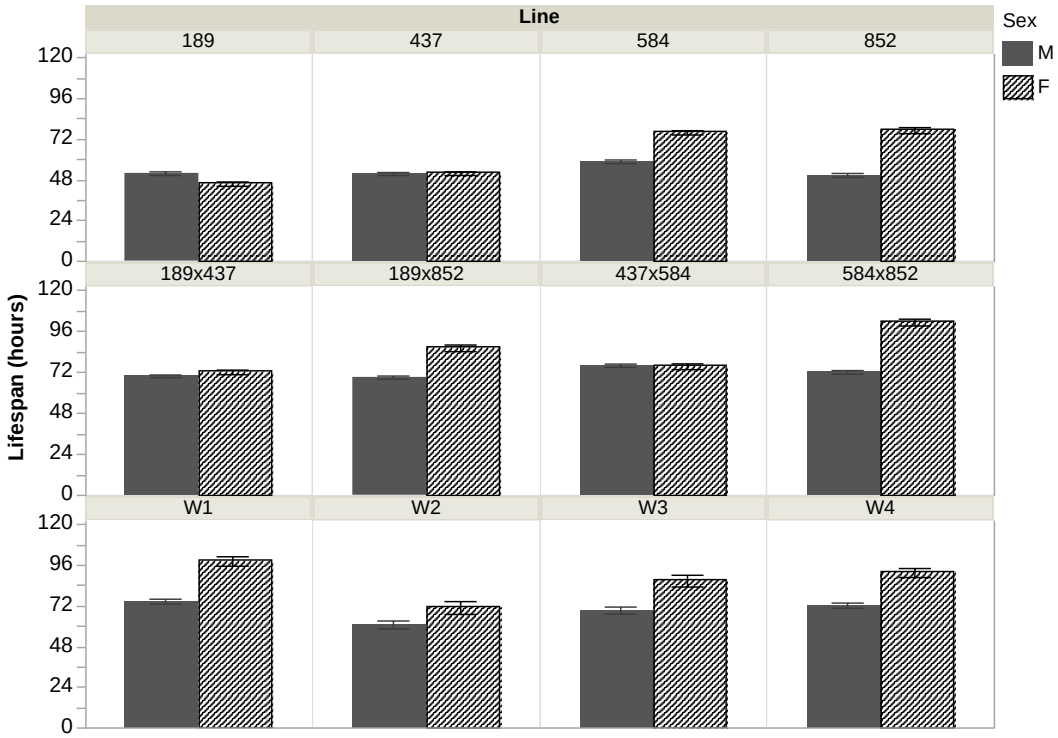


Figure 2.8 Average lifespan by hours starved for each line and sex.
Females generally live longer than males. M = male, F = female. Error bars represent ± S.E.

There was an interaction effect between line and sex. The interaction effect between line and sex was only present in the isogenic and heterozygous genetic background ($F_{3,952}$ = 74.4, P < .0001 and $F_{3,972}$ = 47.3, P < .0001, respectively) but not in the wild genetic background.

The variation across lines was higher in females than in males (Figure 2.9). Interestingly, the level of variation was consistent across type of genetic background. The variation of line differences was significantly higher by 4.4x in the isogenic (P = .0178, one-way ANOVA), and by 3.6x in the heterozygous genetic background (P = .0131, one-way ANOVA), but not in the wild genetic background. Across genetic backgrounds, isogenic lines were the most sensitive to starvation. Within genetic backgrounds, there are significant line

differences across both sexes. Heterozygous cross 584x852 and W1 females exhibited higher average lifespans which surpass the 96-hour threshold.

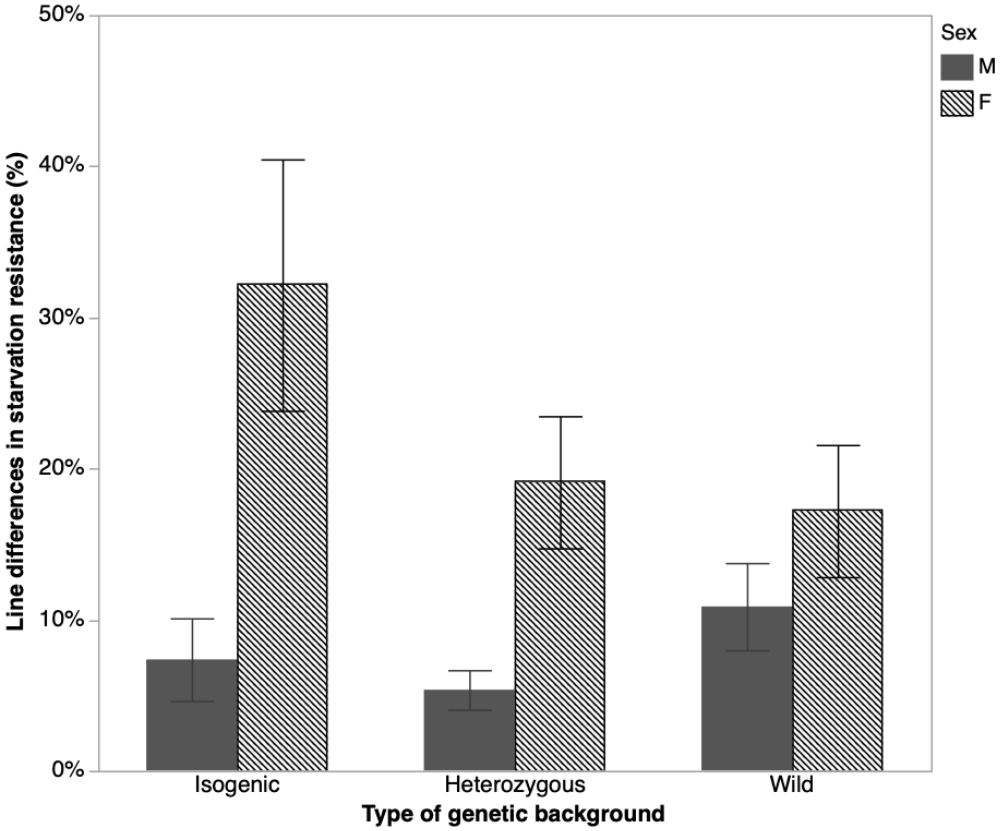


Figure 2.9 Average line differences in starvation resistance.
Average line differences were calculated from the mean lifespan on agar. Absolute differences among pairwise comparisons between lines were calculated (n = 6). Error bars represent ± SE. M = male, F = female.

Like Ni mortality, differences between the sexes varies by genetic line. The most variation in sex differences is across isogenic lines, ranging from -12% to 41% difference, where a positive value indicates a female bias. In contrast, variation in sex differences across wild lines range from 15% to 27%. Heterozygous has a range of 0% to 34% differences between the sexes. There is no significant difference in the sex differences across types of genetic background (Figure 2.10).

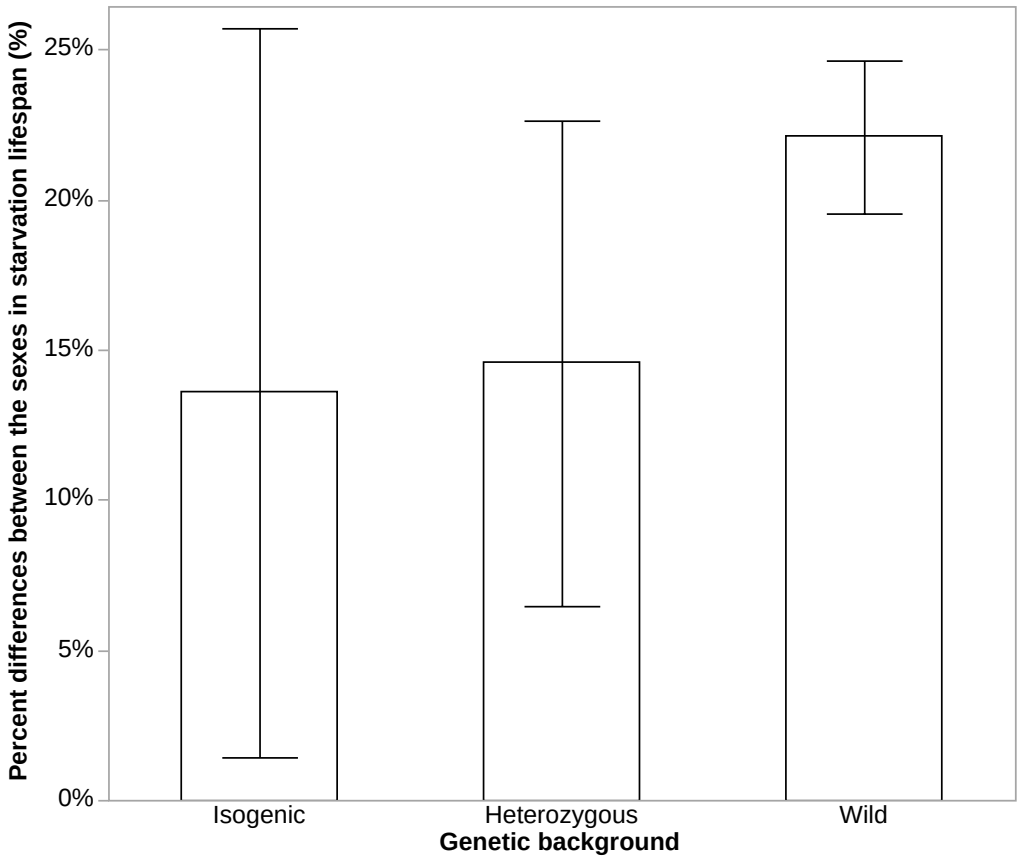


Figure 2.10 Average sex differences in starvation resistance.
Average sex differences were calculated from starvation lifespan. Differences between the sexes were calculated for each line and separated by genetic background. Positive values indicate a female bias. Error bars represent ± SE.

Overall, given that there is a reduction in mortality between Ni-exposed and starved flies, a dose-response relationship, and ~90% of flies are dead by 96 hours, flies are consuming the Ni food. However, the relative amount of food consumed may also differ by line and sex, further complicating analysis. The full extent of food avoidance is not characterized by starvation resistance. Therefore, to quantify food aversion, we measured the change in food consumption post-Ni exposure through a feeding assay.

Quantifying Ni avoidance

We quantified food aversion as a snapshot of how Ni influences feeding behaviour. In general, Ni exposure reduced food consumed compared to control by 4% in males ($F_{1,118}$ = 2.7, P = .1038) and 15% in females ($F_{1,116}$ = 38.1, P < .0001), and overall, by 9% ($F_{1,236}$ = 30.9, P < .0001). Food aversion significantly varied by line in the isogenic ($F_{3,69}$ = 27.9, P < .0001) and heterozygous ($F_{3,71}$ = 12.8, P < .0001) genetic background. The interaction effect between line and sex was present only in the isogenic ($F_{3,69}$ = 6.0, P = .0010) and heterozygous ($F_{3,71}$ = 9.1, P < .0001) genetic background.

There is no significant variation across lines between types of genetic background, consistent across both sexes (Figure 2.11). Variation across lines is 2.4x higher in females in the heterozygous genetic background ($F_{1,10}$ = 5.1, P = .0475).

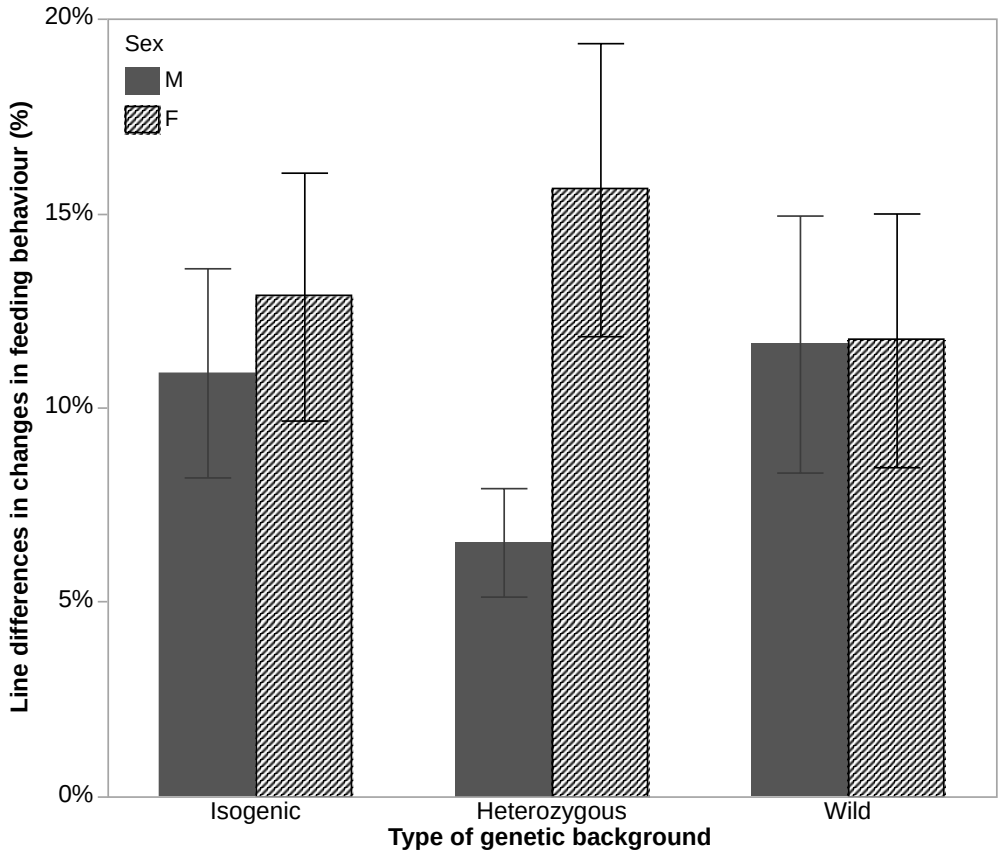


Figure 2.11 Average line differences in changes in feeding behaviour.

Average line differences were calculated from the ratio between control and Ni. Absolute differences among pairwise comparisons between lines were calculated (n = 6). Error bars represent ± SE. M = male, F = female.

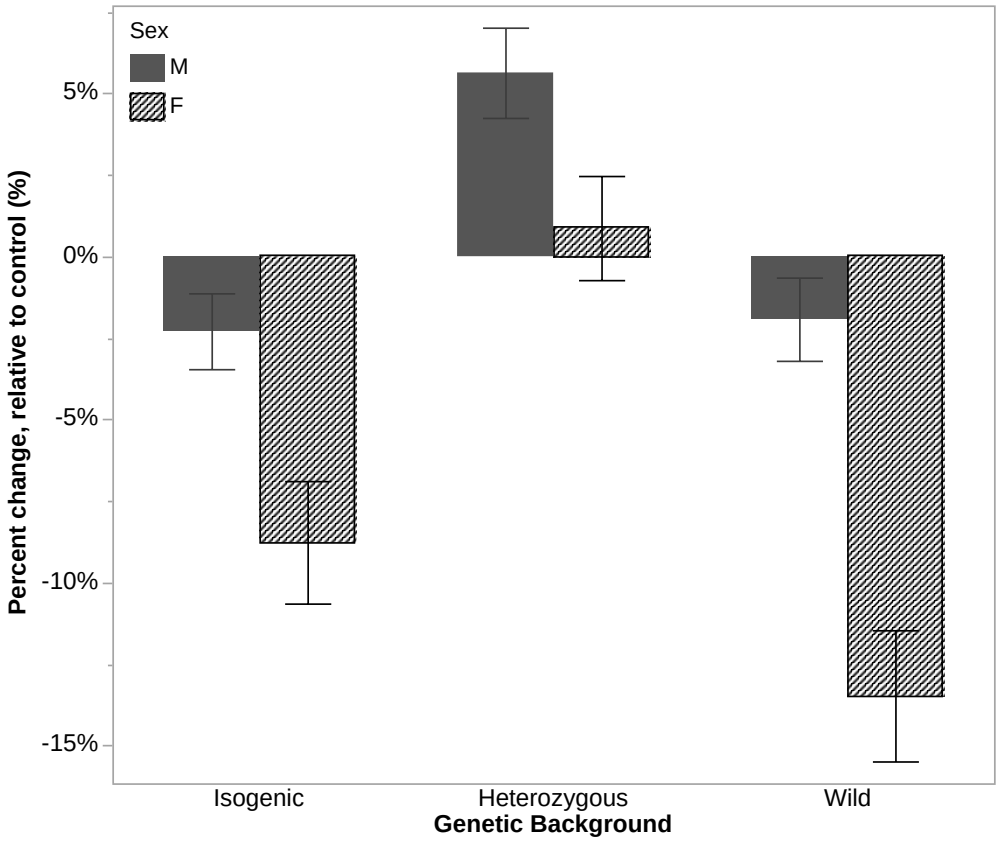


Figure 2.12 Average change in food consumed, relative to control by sex and type of genetic background.

The amount of food consumed is decreased in the isogenic and wild genetic background but does not significantly change in the heterozygous genetic background. M = male, F = female. Error bars represent ± SE.

Influence of Ni on enzyme activity

We quantified changes in enzymatic activity as a broad measure of the overall health of the organism. To explore the physiological response to Ni toxicity, we quantified the change following Ni exposure in the activity of three enzymes associated with oxidative stress: MEN, IDH, and G6PD. We conducted an analysis of covariance (ANCOVA) to evaluate the effect of Ni concentration on enzyme activity while controlling for fly weight and protein concentration.

After controlling for fly weight and protein concentration, there was no significant effect of Ni concentration on MEN activity in the isogenic ($F_{1,149}$ = 0.4488, P = .5039, η^2 = 0.0009), heterozygous ($F_{1,69}$ = 0.0857, P = .7706, η^2 = 0.0002), and wild ($F_{1,69}$ = 0.6826, P = .4115, η^2 = 0.0013) genetic background. In both the isogenic and wild genetic background, fly weight significantly influenced MEN activity ($F_{1,149}$ = 25.6677, P < .0001 and $F_{1,69}$ = 5.2944, P = .0244, respectively), while protein concentration did not ($F_{1,149}$ = 1.0352, P = .3106 and $F_{1,69}$ = 1.4633, P = .2305, respectively). In the heterozygous genetic background, both fly weight ($F_{1,69}$ = 3.0934, P = .0830) and protein concentration ($F_{1,69}$ = 0.0069, P = .9342) did not significantly influence MEN activity.

Ni concentration significantly affected IDH activity in the isogenic ($F_{1,149}$ = 22.3170, P < .0001, η^2 = 0.0271) and wild ($F_{1,69}$ = 8.3310, P = .0052, η^2 = 0.0097) genetic background, but not in the heterozygous genetic background ($F_{1,69}$ = 2.4394, P = .1229, η^2 = 0.0048). The covariates, fly weight and protein concentration, significantly influenced IDH activity in the isogenic ($F_{1,149}$ = 75.8468, P < .0001 and $F_{1,149}$ = 3.9789, P = .0479, respectively) and the wild ($F_{1,69}$ = 8.6998, P =.0043 and $F_{1,69}$ = 8.1868, P = .0056, respectively) genetic background. Fly weight and protein concentration did not significantly influence IDH activity in the heterozygous genetic background ($F_{1,69}$ = 2.3375, P = .1309 and $F_{1,69}$ = 2.4638, P = .1211, respectively).

Ni concentration significantly influenced G6PD activity in the isogenic ($F_{1,149}$ = 5.0542, P = .0260, η^2 = 0.0055) genetic background, but not the heterozygous ($F_{1,69}$ = 3.8376, P = .0542, η^2 = 0.0100) and wild ($F_{1,69}$ = 1.9550, P = .1665, η^2 = 0.0040) genetic background. Fly weight significantly influences G6PD activity in all types of genetic backgrounds, isogenic, heterozygous, and wild ($F_{1,149}$ = 67.0288, P < .0001; $F_{1,69}$ = 8.8934, P < .0001; $F_{1,69}$

= 16.6211, P = .0001, respectively). Protein concentration, however, does not significantly influence G6PD activity in isogenic, heterozygous, and wild genetic backgrounds ($F_{1,149}$ = 0.1546, P = .6947; $F_{1,69}$ = 0.8193, P = .3685; $F_{1,69}$ = 3.4654, P = .0669).

There is a correlation between enzyme activity and fly size. Therefore, we present our results as enzyme activity per unit mass of fly. Ni exposure differentially impacts enzyme activity between the sexes. For instance, MEN activity is significantly reduced by Ni exposure in males ($F_{1,158}$ = 4.6170, P = .0332) but is not significantly impacted in females. Additionally, IDH activity is significantly increased by Ni exposure in females ($F_{1,158}$ = 6.1242, P = .0144) but is not significantly impacted in males.

Taking the ratio between Ni and control for each line and sex, we calculated the percent differences between lines for each enzyme. Variation across lines is consistent across each enzyme for both males and females. Therefore, we combined line variation for all three enzymes for analysis. Variation across lines differs by type of genetic background and sex. Type of genetic background significantly influences variation across lines in both males and females ($F_{2,51}$ = 13.6610, P < .0001 and $F_{2,51}$ = 4.6945, P = .0134, respectively). The variation across lines is highest in wild males, compared to both isogenic and heterozygous (P < .0001 and P = .0002, respectively, Tukey's HSD). In females, wild had significantly higher line variation than in the isogenic genetic background (P = .0133, Tukey's HSD). Females have 1.7x higher variation across lines in the isogenic and 2.4x higher in the heterozygous genetic background ($F_{1,34}$ = 4.2852, P = .0461 and $F_{1,34}$ = 8.0497, P = .0076, respectively).

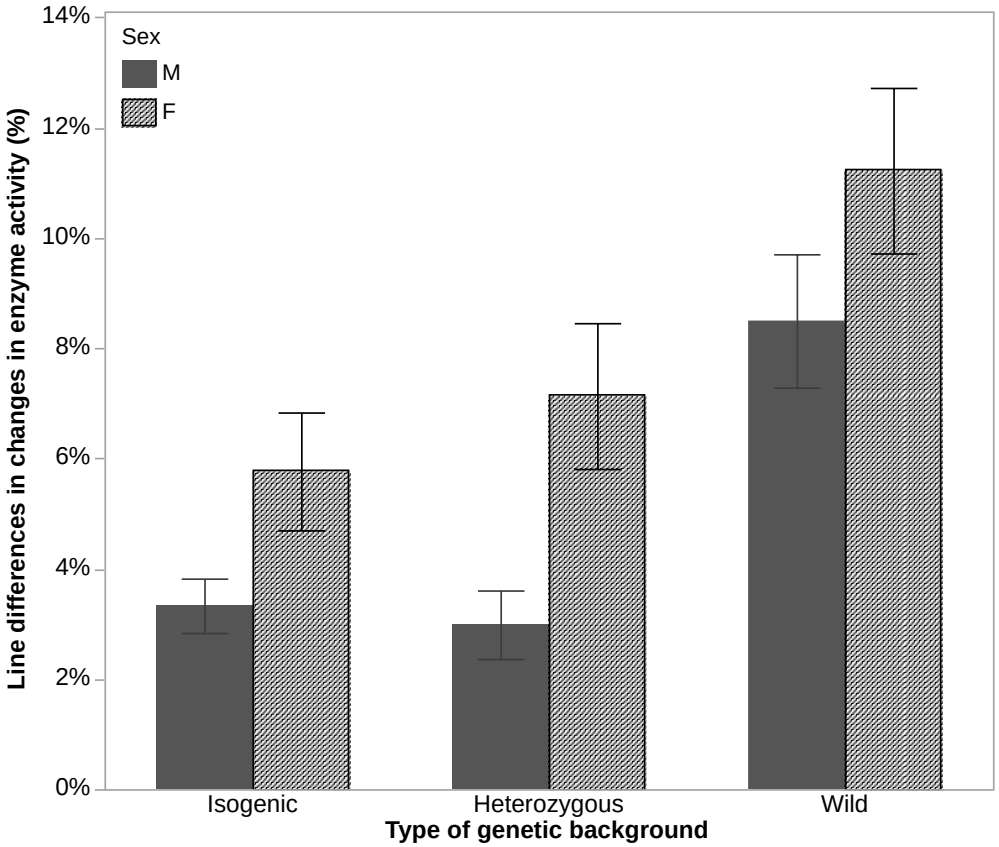


Figure 2.13 Average line differences in changes in enzyme activity.

Average line differences were calculated from the ratio of enzyme activities between control and Ni. There were no significant differences between enzymes. Therefore, values by enzyme were pooled. Absolute differences among pairwise comparisons between lines were calculated (n = 18). Error bars represent ± SE. M = male, F = female.

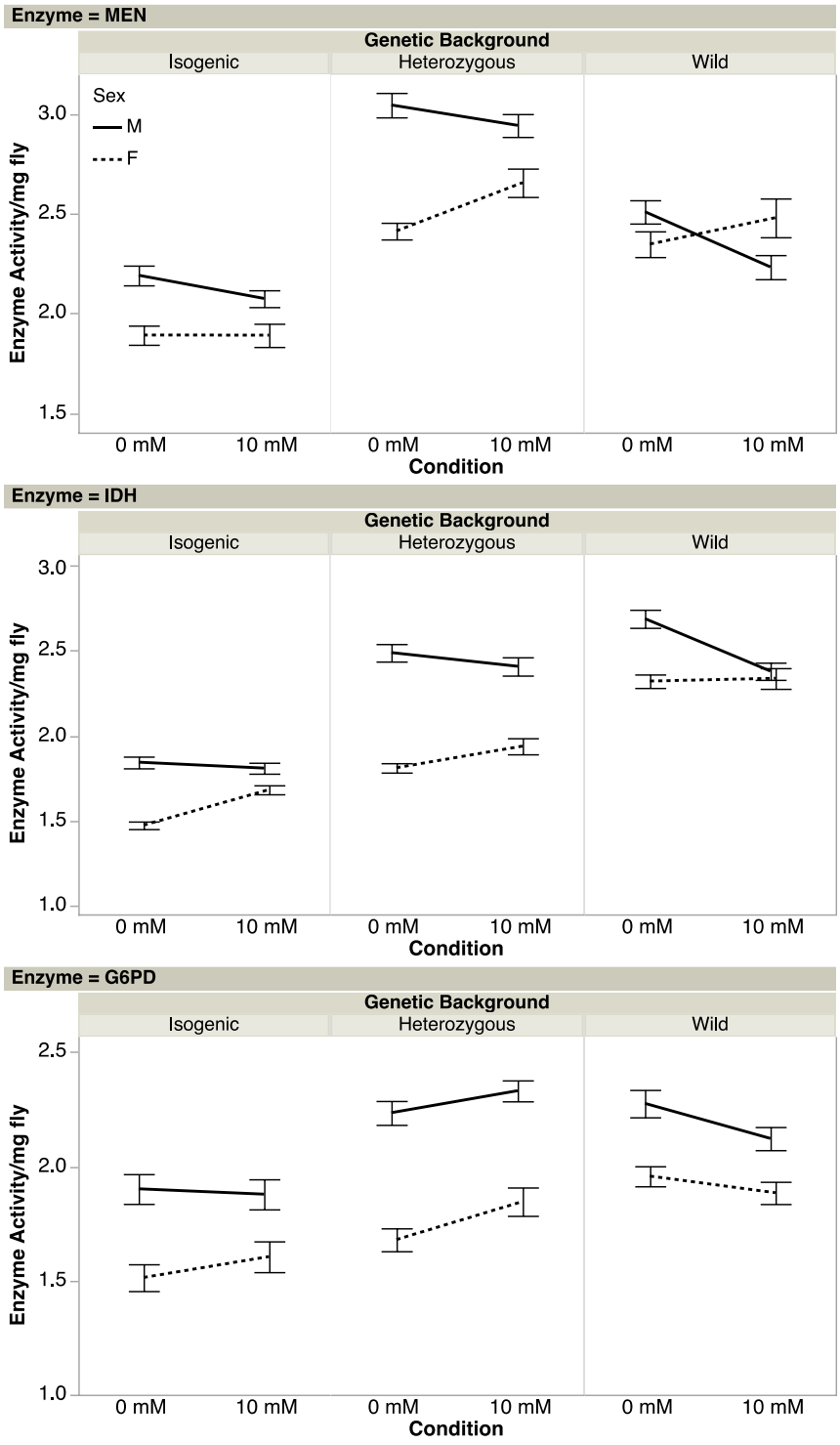


Figure 2.14 Average change in enzyme activity for each enzyme, sex, and type of genetic background.

Overall, Ni exposure does not substantially influence enzyme activity, with inconsistent responses across types of genetic backgrounds. MEN = malic enzyme, IDH = isocitrate dehydrogenase, G6PD = glucose-6-phosphate dehydrogenase. M = male, F = female. Error bars represent ± SE.

Change in total lipid concentration in response to Ni

Finally, we quantified changes in total lipid following Ni exposure as a metric for the overall health of the organism, providing insight into the impact of Ni on downstream processes, such as lipid peroxidation. Females have higher total body lipids, consistent across all types of genetic backgrounds (Figure 2.16).

The variation in Ni response largely differs by line, therefore we took the ratio of total lipids post Ni exposure to control to calculate the percent differences across each line and sex (Figure 2.15). The type of genetic background significantly influences line variation in females ($F_{2,15}$ = 5.8407, P = 0.0133) but not in males. Interestingly, line variation is 2.7x higher in males than females in the heterozygous genetic background ($F_{1,10}$ = 5.4903, P = 0.0411).

Ni impacted total lipid concentration differentially across lines. Several lines have minimal differences between the sexes, such as 189, 437, W2, and W4. At the same time, several lines have a large differences between the sexes, such as 189x852 and 584x852 (Figure 2.17). For instance, Ni reduces total lipid concentration by ~58% in line 189 ($F_{1,37}$ = 42.2354, P < .0001) but only by ~9% in line 437x584 ($F_{1,18}$ = 16.8674, P = .0007).

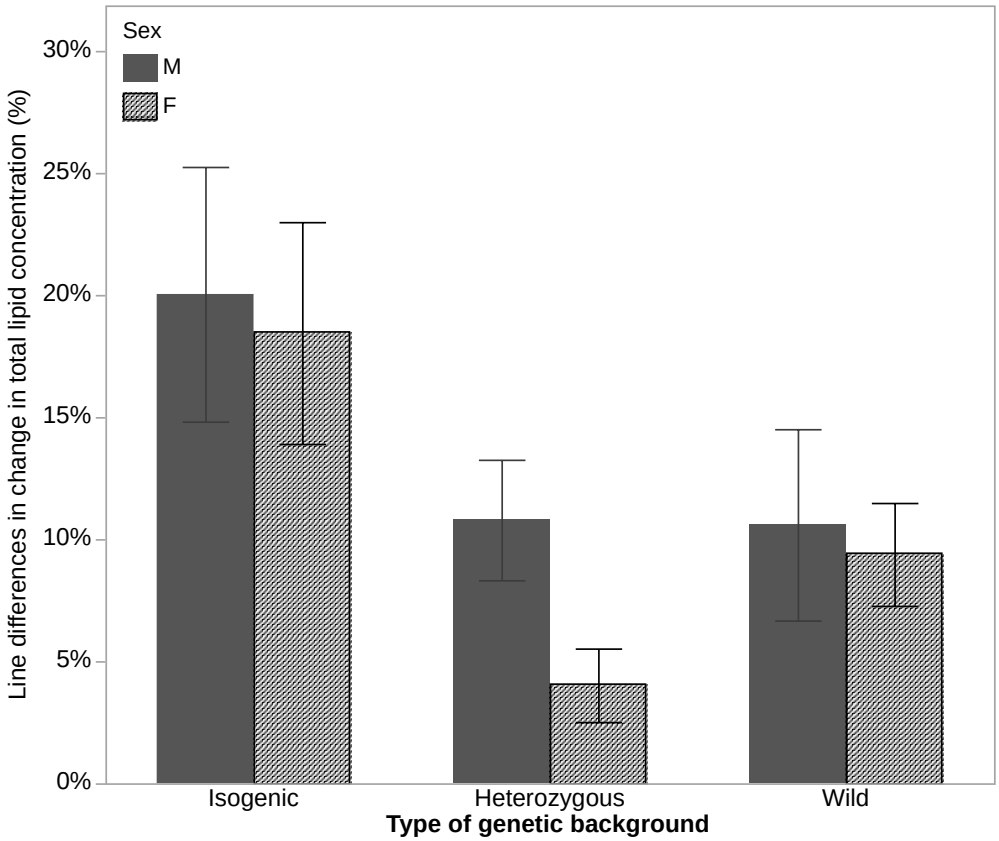


Figure 2.15 Average line differences in changes in total lipid concentration.
Average line differences were calculated from the ratio of total body lipid between control and Ni. Absolute differences among pairwise comparisons between lines were calculated (n = 6). Error bars represent ± standard error. M = male, F = female.

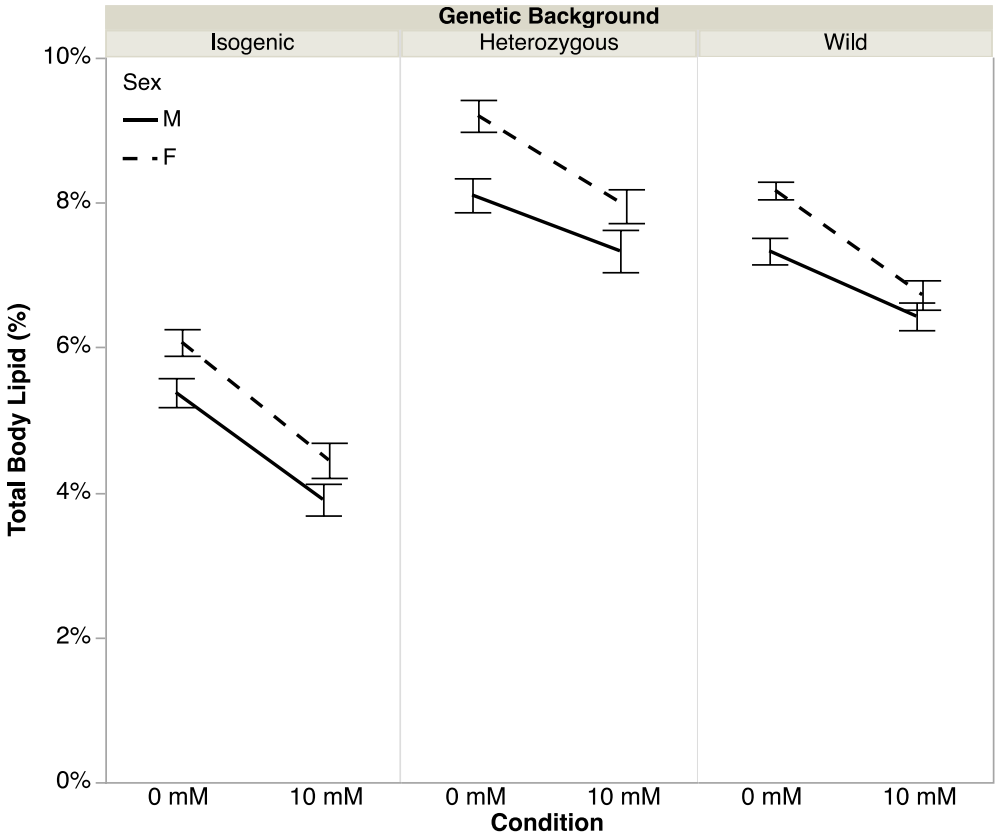


Figure 2.16 Average differences in total body lipid by sex and type of genetic background.

Females have higher proportions of their body weight being lipids. M = male, F = female.

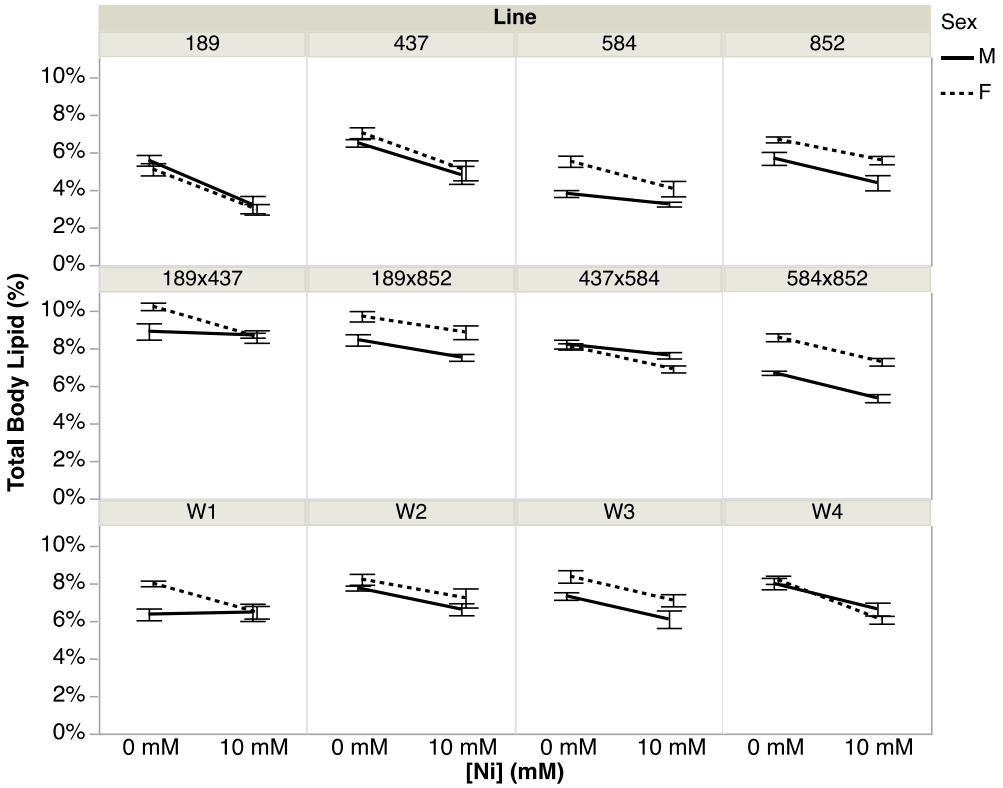


Figure 2.17 Total body lipid for each line and sex.

Ni exposure consistently reduces total body lipids in both sexes and across lines. M = male, F = female.

Discussion

Exposure to Ni results in significant and substantial changes across a suite of phenotypes, and this impact is dependent on the sex, genetic background, and line being studied. Strikingly, the magnitude of these differences is dependent on which phenotype is being investigated; different phenotypes are more sensitive to background than others. Overall, my study highlights that to truly understand the biology of a system, both sexes and multiple genetic backgrounds must be utilized.

Genetic background

Genetic background effects are often unaccounted for in biology, leading to contradictory results between studies (Chandler et al., 2013). We utilized multiple lines across isogenic, heterozygous, and wild backgrounds to obtain specific and broad-scale impacts of genetic background across multiple phenotypes. We expected that flies would vary in response to stress, with isogenic flies being the most sensitive, followed by wild and heterozygous.

Consistent with our expectations, isogenic flies were the most sensitive to Ni. Inbreeding results in a decrease in fitness, known as inbreeding depression of phenotypic traits (Charlesworth & Willis, 2009). Inbreeding depression can either be scored as a population average or on an individual basis.

Heterozygous flies were the most resistant to Ni, which was consistent with our expectations, as increasing heterozygosity in a population increases fitness, known as heterosis or hybrid vigour (Monson & Sadler, 2010).

Wild populations are expected to contain a moderate to high level of genetic diversity, shaped by factors such as population size and environmental conditions (Furlan et al., 2012). Typically, higher genetic diversity provides a broader range of alleles, contributing to high variance in susceptibility to various types of stress (Wang et al., 2020). Taken as an average, our wild fly lines reflected a response like that of the isogenic genetic background, indicating that our wild populations may contain a moderate level of homozygosity.

Female resistance across phenotypes

Overall, females were more resistant to Ni than males across all phenotypes we examined. Females generally had higher LC_{50} values (Figure 2.3, Table 2.1) and longer lifespans under starvation (Figure 2.6 and Figure 2.8). Female resistance can be attributed to differences in gene expression, body size, and biochemistry.

The mechanism of this sexual dimorphism in Ni resistance is unknown but could involve sex-specific differences in gene expression or simply differences in body size. Female resistance to Ni can potentially be explained by differences in metallothionein expression. In flies, the expression of metallothioneins has been shown to be sex-dependent. While not differing in internalized metal content, in one line of flies, males have 2x greater metallothionein expression than females, as males have a higher requirement for detoxification (Egli et al., 2006). Our results are consistent with the findings of Egli (2006); there is a substantial male response not seen in females at low concentrations. However, our results are dependent on genetic background and line. Future work can connect sexual dimorphism and gene interactions across lines.

A potential explanation for female resistance is due to sexual dimorphism in body size. As adults, female flies are larger than males by 10.5% and are consistently larger across various body parts, including the thorax, face width, and proboscis (Cowley & Atchley, 1988). Although females are generally larger than males, studies often use differences in body size as the explanation rather than mechanistic differences between the sexes (Gochfeld, 2017). Females are not just larger males, as sexual size dimorphism varies by line. There is a significant and strong correlation between body weight and Ni resistance in females and not males (Appendix A – Supplemental Figure 4). While interesting, resistance is not that simple. Therefore, future work can expand on exploring the mechanistic differences driving differential responses between the sexes.

While females are larger, they also generally have a larger portion of their body weight in lipids (Figure 2.14). Not unexpectedly, higher lipids are correlated with starvation resistance (Ballard et al., 2008). However, little is known if higher lipids correlate to resistance to other biological stressors. One study investigated the relationship between a high-fat diet and hypoxia in female flies. A high-fat diet results in a more resistant response that is dependent on the nature of hypoxic treatment (Heinrichsen & Haddad, 2012). Future work can utilize a combination of multi-omics approaches to establish if lipid metabolism plays a role in stress resistance, among establishing other markers of resistance.

Female variation across phenotypes

While females are generally more Ni-resistant than males, they are also more variable. Across most phenotypes, females demonstrate higher line variation than males (Appendix A – Supplemental Figure 5). In studies of vertebrates, females are often more variable than

males, a phenomenon attributed to the estrous cycle. This variability, or perception of greater variability, may underlie the dangerous exclusion of females from many biological studies (Zajitschek et al., 2017). Flies lack sex hormones, which attributes all sexual dimorphism to be driven by genetic differences (Gilbert, 2000). Autosomal genetic variation between males and females is consistent across isogenic and heterozygous lines. The phenotypic variation would then be explained by genetic variation on the X chromosome.

Across phenotypes, females consistently have greater variation between lines than males. Interestingly, change in total lipid concentration post-Ni exposure was an exception to higher female variability (Figure 2.15). Females may require higher concentrations to begin eliciting a biological response (Soldin & Mattison, 2009) but is not always true when considering different genetic backgrounds (Figure 2.3). We used the ratio of Ni to control to quantify line differences in total lipid. As a whole, 10 mM was likely a high enough concentration to elicit differential responses in males but remained relatively unchanged in females. Males have the potential to demonstrate high line variation which may have been clouded by a high level of stress, resulting in a similar overall response in other phenotypes, such as mortality and starvation.

Heterozygosity

We constructed heterozygous crosses based on resistance and sensitivity established in our isogenic mortality assay. We performed four crosses: a sensitive-by-sensitive (SS), two sensitive-by-resistant (SR), and a resistant-by-resistant (RR) cross. We expected the SS cross to be the most sensitive and RR to be the most resistant. The SS cross 189x437 was far more sensitive than each other cross for both sexes (Figure 2.2). Interestingly, the RR cross was

most resistant in females, where SR cross 189x852 was the most resistant cross in males. Taken as a whole, the RR cross suggests that, beyond expected heterosis, there are different mechanisms between the two isogenic parents which drive a resistant phenotype that is female biased. Though relatively understudied, molecular mechanisms of drug resistance vary with genetic background in parasites (Decuypere et al., 2012).

Starvation resistance influences Ni resistance

We quantified starvation resistance, as Ni mortality may be a combination of Ni and starvation stress. By 96 hours, nearly all starved flies were dead, whereas Ni-exposed flies demonstrated dose-response mortality. The caloric intake provided through the duration of Ni exposure significantly reduced the mortality of starved flies at 96 hours. Therefore, the flies were consuming the Ni-contaminated food. However, the rate at which flies were consuming the contaminated food or the amount was altered in some way, which may have contributed to Ni mortality.

By quantifying changes in feeding behaviour, it is a possibility that starvation resistance plays a more important role in Ni response. At an exposure of 10 mM for 24 hours, there is a significant reduction in food consumed (Figure 2.12). The more resistant lines' feeding behaviour is less impacted by Ni exposure. Those lines that had an increase in food consumed were among the most resistant males, 189x852 and 584x852. The most impacted reduction in feeding behaviour was W4 females, which were among the most resistant to starvation. There were no types of genetic background differences in changes in food consumption, suggesting that feeding behaviour is highly variable, regardless of genotype.

We then quantified changes in feeding behaviour for flies freshly introduced to either a control or Ni diet. We expected that flies previously exposed to Ni and freshly introduced to control would increase the amount of food consumed. In fact, regardless of when Ni is introduced to the flies, there is a reduction in food consumed. Taken together, this tells us that Ni can be detected throughout the duration of their exposure and that the toxic effects persist following exposure.

While quantifying food consumed broadly provides insight on Ni avoidance, it does not quantify metal absorbed by the digestive tract of the fly and therefore how much metal is being eliminated in fly feces. A study investigating metal localization of copper, iron, and zinc across the gastrointestinal tract in *D. melanogaster* highlights distinct roles of the gastrointestinal tract in metal absorption (Jones et al., 2015). Specific metal transporters are responsible for variation in metal absorption, however there are currently no known Ni transporters or proteins in animals (Denkhaus & Salnikow, 2002). The interaction of Ni specifically with essential metal transporters in the gastrointestinal tract would be an interesting avenue for future research. Additionally, future work can use Inductively Coupled Plasma Mass Spectrometry (ICP-MS) to determine Ni absorption from the GI tract to the fly body.

Our starvation data suggests that Ni resistance is likely correlated to starvation resistance. Taking our feeding data into account, starvation resistance likely plays a greater role in Ni resistance than originally thought. While feeding behaviour provides us with a snapshot of feeding behaviour post-Ni exposure, it does not tell us the relative amount of Ni within the system of the fly. Future work will quantify internalized metal within the system of the fly.

Ni and the NADPH enzyme network

Previous studies have demonstrated that inducing oxidative stress reduces MEN and IDH activity in males (Rzezniczak & Merritt, 2012). Overall, Ni exposure didn't significantly influence enzyme activity. Taking into consideration the fly weight and protein concentration as covariates, Ni differentially impacts enzyme activity depending on the enzyme and type of genetic background (Figure 2.14). Lack of overall Ni impact on enzyme activity can be explained by either (1) Ni does not interact with the NADPH network, (2) the Ni concentration wasn't high enough, or the exposure time wasn't long enough to induce oxidative stress.

There is a sex-specific response to Ni, where the trends between enzymes were not consistent. MEN activity was significantly reduced by Ni exposure in males but not in females. In contrast, IDH activity was significantly reduced by Ni exposure in females but not in males. Consistent with previous data reported by Rzezniczak & Merritt (2012), G6PD activity is not significantly impacted by oxidative stress, which applies to both sexes.

Sexual dimorphism

Sexual dimorphism has historically been overlooked in many toxicological studies (Gochfeld, 2017). Our results clearly demonstrate a sex-specific response across each phenotype. Strikingly, the level of sexual dimorphism present is genetic background and line dependent. Across lines, there is a spectrum of sexual dimorphism, ranging from minimal to high levels of sex differences, most abundantly present in the isogenic and heterozygous background.

It would be interesting to look at how sexual dimorphism in metallothionein expression compares to resistance. Our results demonstrate that metal response is genetically

driven. Given that, metallothionein expression has also been hypothesized to be genetic background dependent (Balinski & Woodruff, 2017). The variation in our results, especially in the isogenic and heterozygous genetic background, could potentially correlate to differences in metallothionein expression. However, our results tend to be dependent on the concentration of Ni that is being looked at. However, metallothionein expression has only been found to be dose-dependent *in vitro* (Chen et al., 2014). It would be interesting to look at metallothionein expression as a factor of line, both sexes and differing levels of stress.

Conclusion

Taken as a whole, my study highlights the complexity of Ni response across biological sex and genetic backgrounds. Overall, Ni has a negative impact on health, as measured by mortality, feeding behaviour, and total lipid concentration. Data across phenotypes demonstrates a negative Ni effect, which is likely to be a result of the impact of downstream processes and not directly on enzyme activity or total lipid concentration. In general, our data demonstrates the complexity of genetic background on phenotypic response within and across multiple phenotypes. Additionally, metal stress is a combinatorial stressed system that differs mechanistically across lines. Most importantly, to truly understand the biology of a system, both sexes and multiple genetic backgrounds must be considered.

CHAPTER 3 CONCLUSIONS AND FUTURE DIRECTIONS

Conclusions

The biological effects of Ni are substantial, but complicated. My study clearly demonstrates the negative effects of environmental Ni on fly health and lifespan: Ni exposed flies are poorer in health and die faster than their control counterparts. Furthermore, the magnitude of Ni impact was strongly modified by both sex and genetic background. Sexually dimorphic effects are present across each phenotype I studied but differ in magnitude between genetic backgrounds and lines. Each type of genetic background represents an example of the impact of genetic variation on phenotypic responses. Additionally, my study has highlighted how important genetic background is to consider, as the sexual dimorphic responses are largely genetic background specific. Line effects are often overlooked, leading to conflicting results between studies. My work, and that of others, clearly demonstrate large biological differences between lines. Therefore, to truly understand the biology of a system, line effects need to be considered.

Across phenotypes, there are similar trends in the magnitude of both line and sex effects. We used a mortality assay to establish a dose response to Ni, allowing us to quantify line and sex differences across a gradient of stress. Starvation and feeding data provide information about food avoidance and suggest that starvation is likely adding additional stress to the flies. Ni negatively impacts the overall health of the flies, quantified by changes in enzyme activity and total lipid concentration. Taken as a whole, my results highlight the complexity of Ni response across the sexes and genetic backgrounds and provide interesting avenues for future research.

Future Directions

The main results of my research suggest that Ni resistance is driven by differences across lines in biological mechanisms and metal consumed, in addition to their respective genetic differences. These results opens many potential avenues for future research. While considering the differential responses across sex and genetic background, potential areas of future research include the quantification of metal content within the flies, exposure to a combination of metal stressors, and quantification of change using multi-omics. Additionally, phenotyping more lines is essential in determining large-scale consistencies and conclusions in susceptibility to stressors.

Across phenotypes, my results have demonstrated differences in Ni susceptibility across sex and genetic background. However, differences in internalized metal content may correlate to differences in Ni response. My results suggest that quantifying metal content in the flies would be interesting because it would allow us to understand the connection between internalized metal content and phenotypic variation.

Exposure to Ni is likely a combinatorial stressed system, as demonstrated by the results of our starvation and feeding assay. Therefore, it would be interesting for future research to consider the biological effects of combinatorial metal stress, which would allow us to understand the biological response to stress present in natural populations.

The results from using heterozygous crosses suggest that Ni response mechanisms differ between resistant isogenic lines, producing an F1 generation with multiple mechanisms, driving a highly resistant phenotype. The use of a combination of multi-omics will allow us to determine if there are different mechanisms driving resistance, as opposed to differences in the regulation of the same pathways.

Metal quantification

Metal quantification allows for the determination of metals within an organism. Quantifying internalized metal content within our fly samples will allow us to answer the following questions: (1) Are the differential phenotypic responses across sex and genetic background simply a reflection of differences in metal concentration in the flies? (2) How does the influx of one non-essential metal, such as Ni, influence the concentration of other essential metals? We can use metal quantification techniques, such as inductively coupled plasma mass spectrometry or ICP-MS, to answer these questions.

Internalized metal content and phenotypic variation

The quantification of internalized metal content will allow for better characterization of resistance. Ni resistance, defined by the highest LC_{50} values determined from our mortality assay, may be confounded by starvation. As such, we quantified starvation and changes to feeding and starvation resistance plays more of a role in Ni mortality. By quantifying internalized metal content, we can connect our results and standardize them. My results suggest that quantifying metal content in the flies would be interesting because it would allow us to understand the connection between internalized metal content and phenotypic variation.

Ni interaction with essential metals

Quantifying a suite of metals will allow for the determination of how the influx of one non-essential metal influences the concentration of essential metals. Despite there being metal-specific mechanisms, there are interactions between essential metal mechanisms and non-essential metals. In particular, the way that Ni interacts with essential metals is not well

understood. My results have demonstrated that Ni negatively influences fly health. However, we are not certain that Ni is the sole source of stress. Ni may reduce the concentration of essential metals, resulting in deficiency and negative health impacts. Future work can explore these interactions to provide holistic insight into Ni, or other non-essential metal, toxicity.

Combinatorial metal stress

In nature, there is a combination of stressors that cause biological dysregulation simultaneously. For biological relevance, it is important to understand stress as a whole and not in isolation. My study highlighted various aspects of biology and biochemistry that vary in the magnitude of response to Ni exposures. Using Ni as a proxy for xenobiotic stress, in general, has allowed for the characterization of phenotypic impacts that may be confounded by additional stress, such as starvation. Addressing these confounding possibilities was the first step before we explored a combinatorial metal-stressed system.

Combinations of metals can have additive, enhancing, or mitigating effects on toxicity. For instance, one metal may disrupt cellular defences, resulting in higher susceptibility to the second metal. Conversely, another metal might induce protective mechanisms that help mitigate cellular damage. Understanding these interactions could reveal how organisms are impacted by complex metal exposures in ways that differ from isolated exposures. For example, this has been observed in *Bacillus cereus*, where a combination of metal stressors present in a natural environment resulted in iron depletion (Goff et al., 2023), an interaction that would not have been observed in a single metal-stressed environment. Furthermore, as metal mechanisms function as a web of interactions between pathways and metals,

combinatorial metal stress coupled with ICP-MS may uncover new interactions that can be further explored using multi-omics.

Multi-omics approaches

Multi-omics approaches, such as genomics, transcriptomics, proteomics, metabolomics, and epigenomics, will allow for the characterization of resistance and susceptibility mechanisms that may differ across genetic backgrounds and the sexes. For example, while metabolomics analyzes metabolites, transcriptomics identifies which genes are upregulated or downregulated in response to Ni stress. Both metabolomics and transcriptomics connect gene regulation and the consequences of changes in gene regulation (i.e., metabolite concentration), which will expand our understanding of the Ni stress response network. Taken together, multi-omics approaches can be used to gain a comprehensive understanding of the molecular mechanisms underlying sex and genetic background differences in response to xenobiotic stressors.

My results demonstrate a substantial genetic background effect in response to Ni exposure. By utilizing multiple lines across three types of genetic backgrounds, we have generated a spectrum of genetic complexity by using populations with differing levels of genetic diversity. Isogenic and heterozygous lines control for genetic variability, which demonstrated a range of sexual dimorphism and line effects. In our resistant lines, females demonstrate higher resistance than males. When two Ni-resistant genotypes are crossed, there is substantially higher female resistance, suggesting a possible additive effect; females have mechanisms from both genotypes that result in a highly Ni-resistant phenotype beyond expected heterosis. Multi-omics would allow for the elucidation of these mechanisms.

References

Ah-King, M. (2022). The history of sexual selection research provides insights as to why females are still understudied. *Nature Communications*, *13*(1), Article 1. https://doi.org/10.1038/s41467-022-34770-z

Altarelli, M., Ben-Hamouda, N., Schneider, A., & Berger, M. M. (2019). Copper Deficiency: Causes, Manifestations, and Treatment. *Nutrition in Clinical Practice*, *34*(4), 504–513. https://doi.org/10.1002/ncp.10328

Asahina, K. (2018). Sex differences in Drosophila behavior: Qualitative and quantitative dimorphism. *Current Opinion in Physiology*, *6*, 35–45. https://doi.org/10.1016/j.cophys.2018.04.004

Balinski, M. A., & Woodruff, R. C. (2017). Differential sexual survival of Drosophila melanogaster on copper sulfate. *Genetica*, *145*(2), 131–137. https://doi.org/10.1007/s10709-017-9951-4

Ballard, J. W. O., Melvin, R. G., & Simpson, S. J. (2008). Starvation resistance is positively correlated with body lipid proportion in five wild caught Drosophila simulans populations. *Journal of Insect Physiology*, *54*(9), 1371–1376. https://doi.org/10.1016/j.jinsphys.2008.07.009

Barceloux, D. G., & Barceloux, D. (1999). Nickel. *Journal of Toxicology: Clinical Toxicology*, *37*(2), 239–258. https://doi.org/10.1081/CLT-100102423

Calap-Quintana, P., González-Fernández, J., Sebastiá-Ortega, N., Llorens, J. V., & Moltó, M. D. (2017). Drosophila melanogaster Models of Metal-Related Human Diseases and Metal Toxicity. *International Journal of Molecular Sciences*, *18*(7), 1456. https://doi.org/10.3390/ijms18071456

Cao, G., Zhao, J., Zhao, G., Wan, D., Wu, Z., Li, R., & He, Q. (2022). Determination of the Acute and Chronic Toxicity of Sulfate from the Sulfur Autotrophic Denitrification Process to Juvenile Zebrafish (Danio rerio). *ACS Omega*, *7*(50), 47165–47173. https://doi.org/10.1021/acsomega.2c06320

Chandler, C. H., Chari, S., & Dworkin, I. (2013). Does your gene need a background check? How genetic background impacts the analysis of mutations, genes, and evolution. *Trends in Genetics*, *29*(6), 358–366. https://doi.org/10.1016/j.tig.2013.01.009

Charlesworth, D., & Willis, J. H. (2009). The genetics of inbreeding depression. *Nature Reviews Genetics*, *10*(11), Article 11. https://doi.org/10.1038/nrg2664

Chen, L., Ma, L., Bai, Q., Zhu, X., Zhang, J., Wei, Q., Li, D., Gao, C., Li, J., Zhang, Z., Liu, C., He, Z., Zeng, X., Zhang, A., Qu, W., Zhuang, Z., Chen, W., & Xiao, Y. (2014). Heavy Metal-induced Metallothionein Expression Is Regulated by Specific Protein Phosphatase 2A Complexes. *The Journal of Biological Chemistry*, *289*(32), 22413–22426. https://doi.org/10.1074/jbc.M114.548677

Cowley, D. E., & Atchley, W. R. (1988). Quantitative Genetics of Drosophila Melanogaster. II. Heritabilities and Genetic Correlations between Sexes for Head and Thorax Traits. *Genetics*, *119*(2), 421. https://doi.org/10.1093/genetics/119.2.421

Decuypere, S., Vanaerschot, M., Brunker, K., Imamura, H., Müller, S., Khanal, B., Rijal, S., Dujardin, J.-C., & Coombs, G. H. (2012). Molecular Mechanisms of Drug Resistance in Natural Leishmania Populations Vary with Genetic Background. *PLOS Neglected Tropical Diseases*, *6*(2), e1514. https://doi.org/10.1371/journal.pntd.0001514

Denkhaus, E., & Salnikow, K. (2002). Nickel essentiality, toxicity, and carcinogenicity. *Critical Reviews in Oncology/Hematology*, *42*(1), 35–56. https://doi.org/10.1016/S1040-8428(01)00214-1

Dudek-Adamska, D., Lech, T., Konopka, T., & Kościelniak, P. (2021). Nickel Content in Human Internal Organs. *Biological Trace Element Research*, *199*(6), 2138–2144. https://doi.org/10.1007/s12011-020-02347-w

EFSA CONTAM Panel. (2015). Scientific Opinion on the risks to public health related to the presence of nickel in food and drinking water. *EFSA Journal*, *13*(2), 4002. https://doi.org/10.2903/j.efsa.2015.4002

Egli, D., Yepiskoposyan, H., Selvaraj, A., Balamurugan, K., Rajaram, R., Simons, A., Multhaup, G., Mettler, S., Vardanyan, A., Georgiev, O., & Schaffner, W. (2006). A Family Knockout of All Four Drosophila Metallothioneins Reveals a Central Role in Copper Homeostasis and Detoxification. *Molecular and Cellular Biology*, *26*(6), 2286–2296. https://doi.org/10.1128/MCB.26.6.2286-2296.2006

Everman, E. R., Macdonald, S. J., & Kelly, J. K. (2023). The genetic basis of adaptation to copper pollution in Drosophila melanogaster. *Frontiers in Genetics*, *14*, 1144221. https://doi.org/10.3389/fgene.2023.1144221

Furlan, E., Stoklosa, J., Griffiths, J., Gust, N., Ellis, R., Huggins, R. M., & Weeks, A. R. (2012). Small population size and extremely low levels of genetic diversity in island populations of the platypus, Ornithorhynchus anatinus. *Ecology and Evolution*, *2*(4), 844. https://doi.org/10.1002/ece3.195

Gao, L., Qiao, H., Wei, P., Moussian, B., & Wang, Y. (2022). Xenobiotic responses in insects. *Archives of Insect Biochemistry and Physiology*, *109*(3), e21869. https://doi.org/10.1002/arch.21869

Genchi, G., Carocci, A., Lauria, G., Sinicropi, M. S., & Catalano, A. (2020). Nickel: Human Health and Environmental Toxicology. *International Journal of Environmental Research and Public Health*, *17*(3), 679. https://doi.org/10.3390/ijerph17030679

Gilbert, S. F. (2000). Chromosomal Sex Determination in Drosophila. In *Developmental Biology. 6th edition*. Sinauer Associates. https://www.ncbi.nlm.nih.gov/books/NBK10025/

Gochfeld, M. (2017). Sex Differences in Human and Animal Toxicology: Toxicokinetics. *Toxicologic Pathology*, *45*(1), 172–189. https://doi.org/10.1177/0192623316677327

Goff, J. L., Chen, Y., Thorgersen, M. P., Hoang, L. T., Poole, F. L., II, Szink, E. G., Siuzdak, G., Petzold, C. J., & Adams, M. W. W. (2023). Mixed heavy metal stress induces global iron starvation response. *The ISME Journal*, *17*(3), 382–392. https://doi.org/10.1038/s41396-022-01351-3

Günther, V., Lindert, U., & Schaffner, W. (2012). The taste of heavy metals: Gene regulation by MTF-1. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, *1823*(9), 1416–1425. https://doi.org/10.1016/j.bbamcr.2012.01.005

Heinrichsen, E. T., & Haddad, G. G. (2012). Role of High-Fat Diet in Stress Response of Drosophila. *PLOS ONE*, *7*(8), e42587. https://doi.org/10.1371/journal.pone.0042587

Jang, T., & Lee, K. P. (2015). The genetic basis for mating-induced sex differences in starvation resistance in Drosophila melanogaster. *Journal of Insect Physiology*, *82*, 56–65. https://doi.org/10.1016/j.jinsphys.2015.09.002

Jones, M. W. M., de Jonge, M. D., James, S. A., & Burke, R. (2015). Elemental mapping of the entire intact Drosophila gastrointestinal tract. *JBIC Journal of Biological Inorganic Chemistry*, *20*(6), 979–987. https://doi.org/10.1007/s00775-015-1281-3

Klein Couto, G., Rodrigues, J. C., Pacheco, B. S., Damé Simões, L., Paschoal, J. D., Seixas, F. K., Acunha, T. V., Iglesias, B. A., & Collares, T. (2020). Zinc(II), copper(II) and nickel(II) ions improve the selectivity of tetra-cationic platinum(II) porphyrins in photodynamic therapy and stimulate antioxidant defenses in the metastatic melanoma lineage (A375). *Photodiagnosis and Photodynamic Therapy*, *31*, 101942. https://doi.org/10.1016/j.pdpdt.2020.101942

Klepsatel, P., Gáliková, M., Xu, Y., & Kühnlein, R. P. (2016). Thermal stress depletes energy reserves in Drosophila. *Scientific Reports*, *6*(1), 33667. https://doi.org/10.1038/srep33667

LaRochelle, O., Gagné, V., Charron, J., Soh, J.-W., & Séguin, C. (2001). Phosphorylation Is Involved in the Activation of Metal-regulatory Transcription Factor 1 in Response to Metal Ions *. *Journal of Biological Chemistry*, *276*(45), 41879–41888. https://doi.org/10.1074/jbc.M108313200

Leem, A. Y., Kim, S. K., Chang, J., Kang, Y. A., Kim, Y. S., Park, M. S., Kim, S. Y., Kim, E. Y., Chung, K. S., & Jung, J. Y. (2015). Relationship between blood levels of heavy metals and lung function based on the Korean National Health and Nutrition Examination Survey IV–V. *International Journal of Chronic Obstructive Pulmonary Disease*, *10*, 1559–1570. https://doi.org/10.2147/COPD.S86182

Mackay, T. F. C., Richards, S., Stone, E. A., Barbadilla, A., Ayroles, J. F., Zhu, D., Casillas, S., Han, Y., Magwire, M. M., Cridland, J. M., Richardson, M. F., Anholt, R. R. H.,

Barrón, M., Bess, C., Blankenburg, K. P., Carbone, M. A., Castellano, D., Chaboub, L., Duncan, L., … Gibbs, R. A. (2012). The Drosophila melanogaster Genetic Reference Panel. *Nature*, *482*(7384), 173–178. https://doi.org/10.1038/nature10811

Macomber, L., & Hausinger, R. P. (2011). Mechanisms of nickel toxicity in microorganisms. *Metallomics*, *3*(11), 1153–1162. https://doi.org/10.1039/c1mt00063b

Martinez-Finley, E. J., Chakraborty, S., Fretham, S. J. B., & Aschner, M. (2012). Cellular transport and homeostasis of essential and nonessential metals. *Metallomics*, *4*(7), 593–605. https://doi.org/10.1039/c2mt00185c

Merritt, T. J. S., Duvernell, D., & Eanes, W. F. (2005). Natural and Synthetic Alleles Provide Complementary Insights Into the Nature of Selection Acting on the Men Polymorphism of Drosophila melanogaster. *Genetics*, *171*(4), 1707–1718. https://doi.org/10.1534/genetics.105.048249

Millington, J. W., & Rideout, E. J. (2018). Sex differences in Drosophila development and physiology. *Current Opinion in Physiology*, *6*, 46–56. https://doi.org/10.1016/j.cophys.2018.04.002

Misra, M., Rodriguez, R. E., North, S. L., & Kasprzak, K. S. (1991). Nickel-induced renal lipid peroxidation in different strains of mice: Concurrence with nickel effect on antioxidant defense systems. *Toxicology Letters*, *58*(2), 121–133. https://doi.org/10.1016/0378-4274(91)90166-4

Monson, C. A., & Sadler, K. C. (2010). Inbreeding Depression and Outbreeding Depression Are Evident in Wild-Type Zebrafish Lines. *Zebrafish*, *7*(2), 189–197. https://doi.org/10.1089/zeb.2009.0648

Nielsen, F. H. (1985). The Importance of Diet Composition in Ultratrace Element Research. *The Journal of Nutrition*, *115*(10), 1239–1247. https://doi.org/10.1093/jn/115.10.1239

Nielsen, F. H. (2012). Manganese, molybdenum, boron, chromium, and other trace elements. In J. W. Erdman & International Life Sciences Institute (Eds.), *Present knowledge in nutrition* (10. ed, pp. 586–607). Wiley-Blackwell.

Patterson, A. D., Gonzalez, F. J., & Idle, J. R. (2010). XENOBIOTIC METABOLISM – A VIEW THROUGH THE METABOLOMETER. *Chemical Research in Toxicology*, *23*(5), 851–860. https://doi.org/10.1021/tx100020p

Poissant, J., Wilson, A. J., & Coltman, D. W. (2010). SEX-SPECIFIC GENETIC VARIANCE AND THE EVOLUTION OF SEXUAL DIMORPHISM: A SYSTEMATIC REVIEW OF CROSS-SEX GENETIC CORRELATIONS. *Evolution*, *64*(1), 97–107. https://doi.org/10.1111/j.1558-5646.2009.00793.x

Ragsdale, S. W. (2009). Nickel-based Enzyme Systems*. *Journal of Biological Chemistry*, *284*(28), 18571–18575. https://doi.org/10.1074/jbc.R900020200

Rion, S., & Kawecki, T. J. (2007). Evolutionary biology of starvation resistance: What we have learned from Drosophila. *Journal of Evolutionary Biology*, *20*(5), 1655–1664. https://doi.org/10.1111/j.1420-9101.2007.01405.x

Rzezniczak, T. Z., & Merritt, T. J. S. (2012). Interactions of NADP-Reducing Enzymes Across Varying Environmental Conditions: A Model of Biological Complexity. *G3 Genes|Genomes|Genetics*, *2*(12), 1613–1623. https://doi.org/10.1534/g3.112.003715

Saydam, N., Adams, T. K., Steiner, F., Schaffner, W., & Freedman, J. H. (2002). Regulation of Metallothionein Transcription by the Metal-responsive Transcription Factor MTF-

1: IDENTIFICATION OF SIGNAL TRANSDUCTION CASCADES THAT CONTROL METAL-INDUCIBLE TRANSCRIPTION*. *Journal of Biological Chemistry*, *277*(23), 20438–20445. https://doi.org/10.1074/jbc.M110631200

Sittig, L. J., Carbonetto, P., Engel, K. A., Krauss, K. S., Barrios-Camacho, C. M., & Palmer, A. A. (2016). Genetic Background Limits Generalizability of Genotype-Phenotype Relationships. *Neuron*, *91*(6), 1253–1259. https://doi.org/10.1016/j.neuron.2016.08.013

Slobodian, M. R. (2024). *The impacts of sex and genetic background on the response of Drosophila melanogaster to essential and non-essential metal toxicity.* https://laurentian.scholaris.ca/handle/10219/4187

Slobodian, M. R., Petahtegoose, J. D., Wallis, A. L., Levesque, D. C., & Merritt, T. J. S. (2021). The Effects of Essential and Non-Essential Metal Toxicity in the Drosophila melanogaster Insect Model: A Review. *Toxics*, *9*(10), 269. https://doi.org/10.3390/toxics9100269

Soldin, O., & Mattison, D. (2009). Sex Differences in Pharmacokinetics and Pharmacodynamics. *Clinical Pharmacokinetics*, *48*(3), 143–157. https://doi.org/10.2165/00003088-200948030-00001

Tchounwou, P. B., Yedjou, C. G., Patlolla, A. K., & Sutton, D. J. (2012). Heavy Metal Toxicity and the Environment. In A. Luch (Ed.), *Molecular, Clinical and Environmental Toxicology: Volume 3: Environmental Toxicology* (pp. 133–164). Springer. https://doi.org/10.1007/978-3-7643-8340-4_6

Trumbo, P., Yates, A. A., Schlicker, S., & Poos, M. (2001). Dietary Reference Intakes: Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron,

Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. *Journal of the American Dietetic Association*, *101*(3), 294–301. https://doi.org/10.1016/S0002-8223(01)00078-5

Valko, M., Morris, H., & Cronin, M. T. D. (2005). Metals, Toxicity and Oxidative Stress. *Current Medicinal Chemistry*, *12*(10), 1161–1208.

Wang, J. B., Elya, C., & St. Leger, R. J. (2020). Genetic variation for resistance to the specific fly pathogen Entomophthora muscae. *Scientific Reports*, *10*(1), 14284. https://doi.org/10.1038/s41598-020-71262-w

Wong, R., Piper, M. D. W., Wertheim, B., & Partridge, L. (2009). Quantification of Food Intake in Drosophila. *PLOS ONE*, *4*(6), e6063. https://doi.org/10.1371/journal.pone.0006063

World Health Organization, Food and Agriculture Organization of the United Nations, & International Atomic Energy Agency (Eds.). (1996). *Trace elements in human nutrition and health*. World Health Organization.

Yamamoto, D., & Koganezawa, M. (2013). Genes and circuits of courtship behaviour in Drosophila males. *Nature Reviews Neuroscience*, *14*(10), Article 10. https://doi.org/10.1038/nrn3567

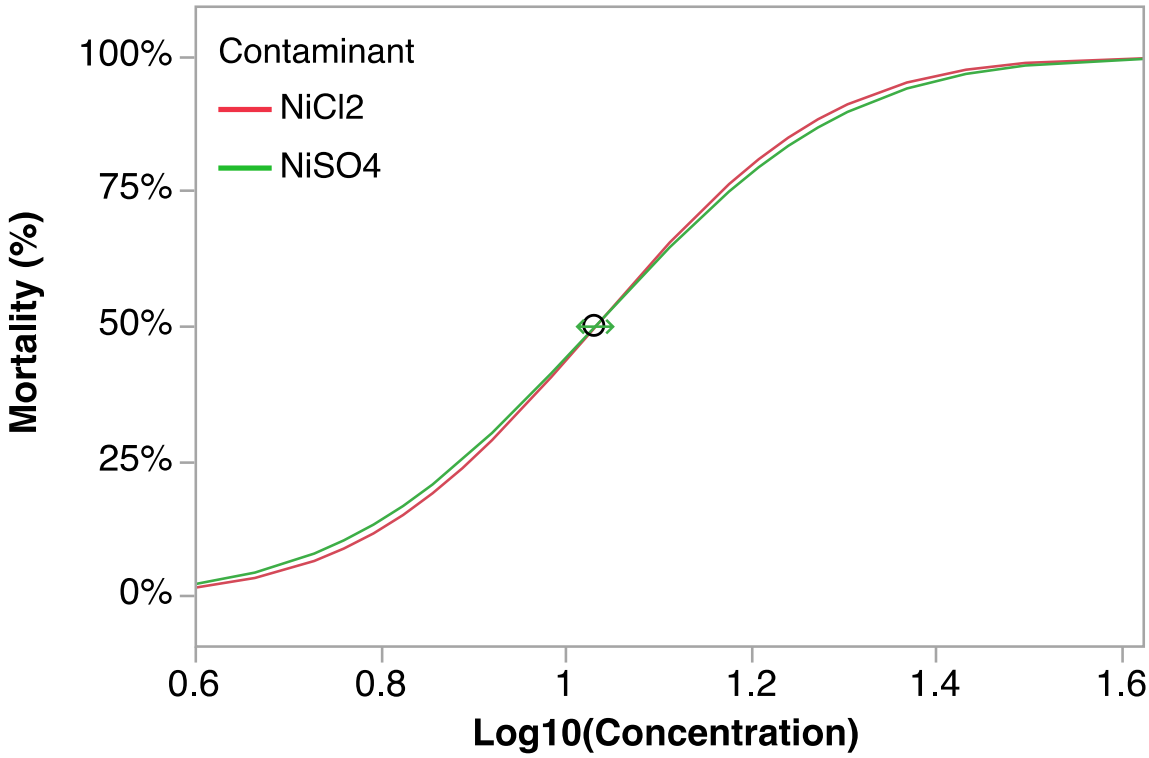
Yoshiki, A., & Moriwaki, K. (2006). Mouse phenome research: Implications of genetic background. *ILAR Journal*, *47*(2), 94–102. https://doi.org/10.1093/ilar.47.2.94

Zajitschek, S. R., Zajitschek, F., Bonduriansky, R., Brooks, R. C., Cornwell, W., Falster, D. S., Lagisz, M., Mason, J., Senior, A. M., Noble, D. W., & Nakagawa, S. (2017). Sexual dimorphism in trait variability and its eco-evolutionary and statistical implications. *eLife*, *9*, e63170. https://doi.org/10.7554/eLife.63170

Zoroddu, M. A., Aaseth, J., Crisponi, G., Medici, S., Peana, M., & Nurchi, V. M. (2019). The essential metals for humans: A brief overview. *Journal of Inorganic Biochemistry, 195*, 120–129. https://doi.org/10.1016/j.jinorgbio.2019.03.013

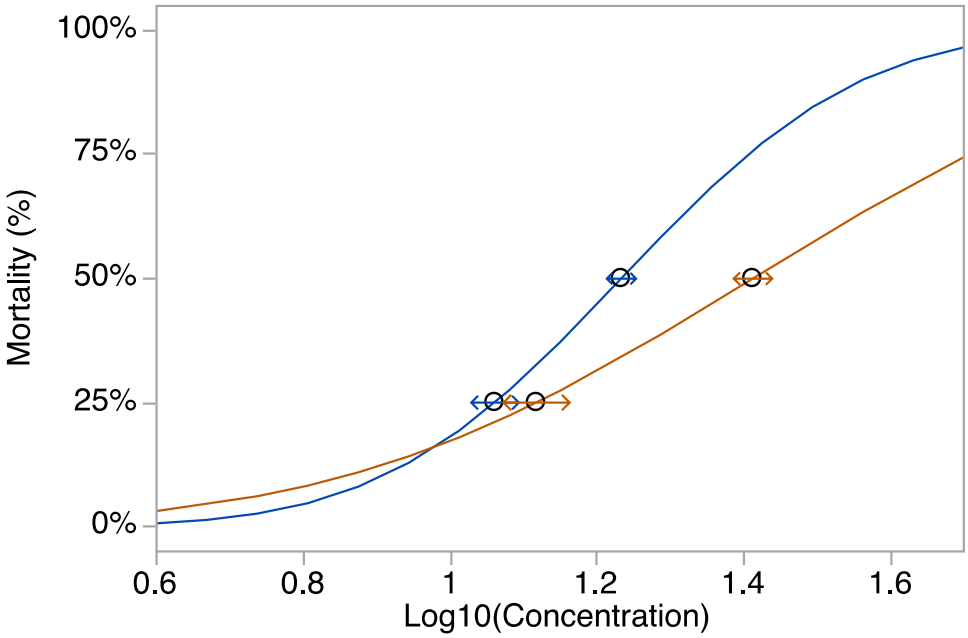
Appendix A

Supplementary Materials



Supplemental Figure 1 Dose-response curve of $NiCl_2$ and $NiSO_4$.

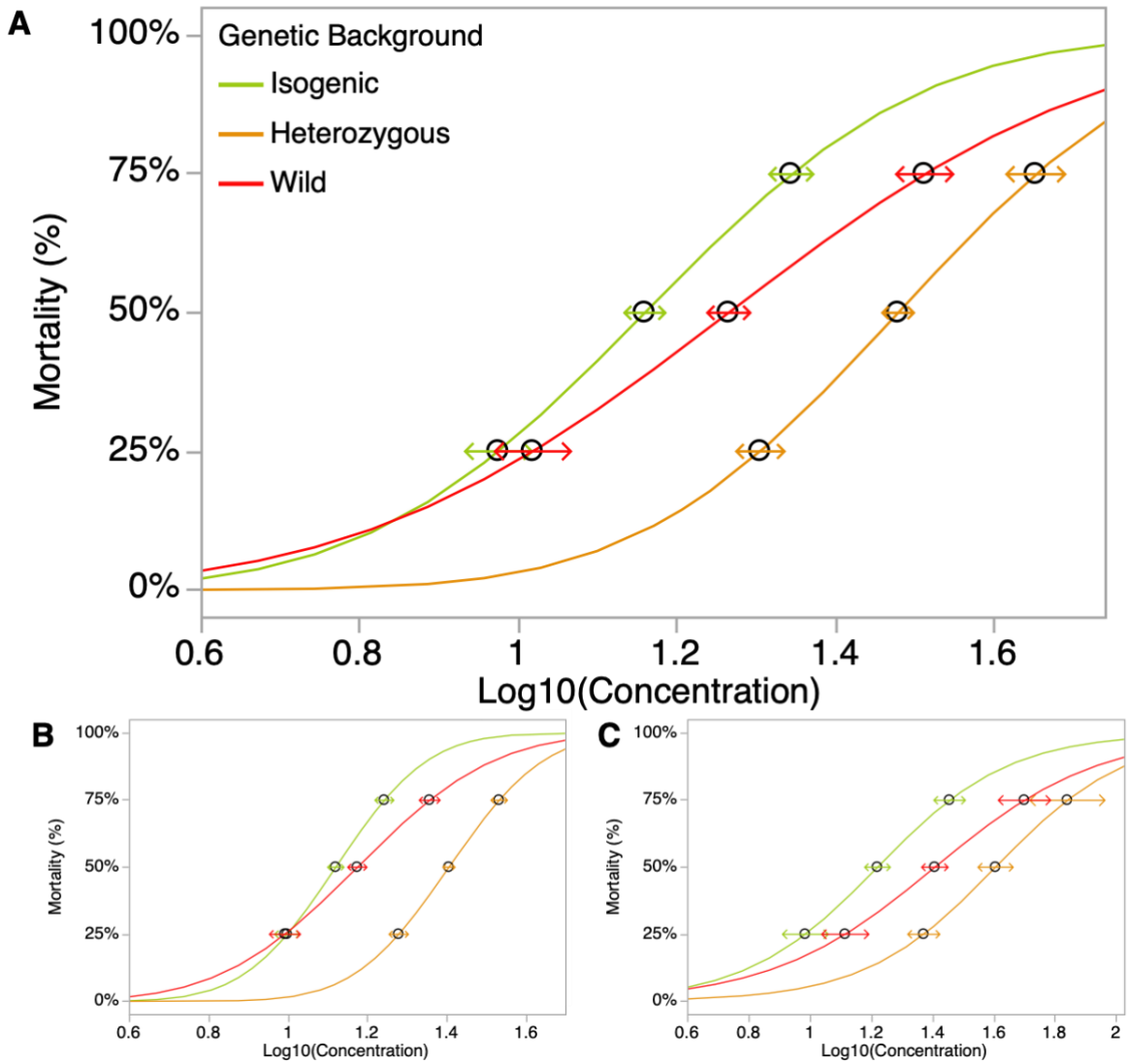
By combining four lines and both sexes, the toxic response of $NiCl_2$ and $NiSO_4$ are not significantly different. $NiCl_2$ and $NiSO_4$ are not significantly different across all lines and both sexes (data not shown, see Slobodian 2024). Error is indicated by the 95% confidence interval at the LC_{50}.



Supplemental Figure 2 Dose-response by sex.
Error is indicated by the 95% confidence intervals present at the LC_{25} and LC_{50}. Values are found in Supplementary Table 1.

	Male	Female	Percent difference
LC_{25} (mM)	11.50 [10.68, 12.40]	13.11 [11.82, 14.54]	13%
LC_{50} (mM)	17.10 [16.33, 17.91]	25.82 [24.31, 27.42]	41%*

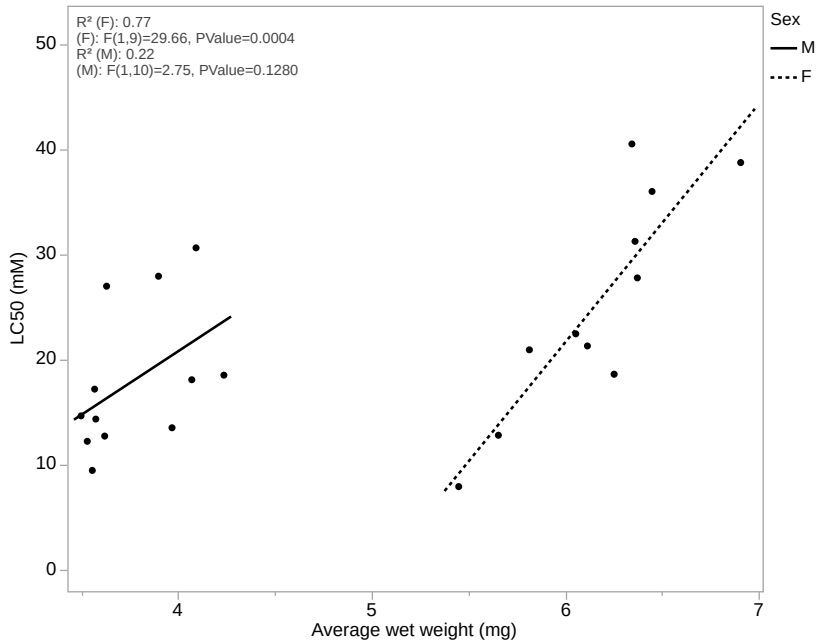
Supplemental Table 1 LC_{25} and LC_{50} values for males and females.
The error is indicated by [lower CI, upper CI], where CI is the 95% confidence interval. * Indicates significance, defined by the lack of overlap between confidence intervals.



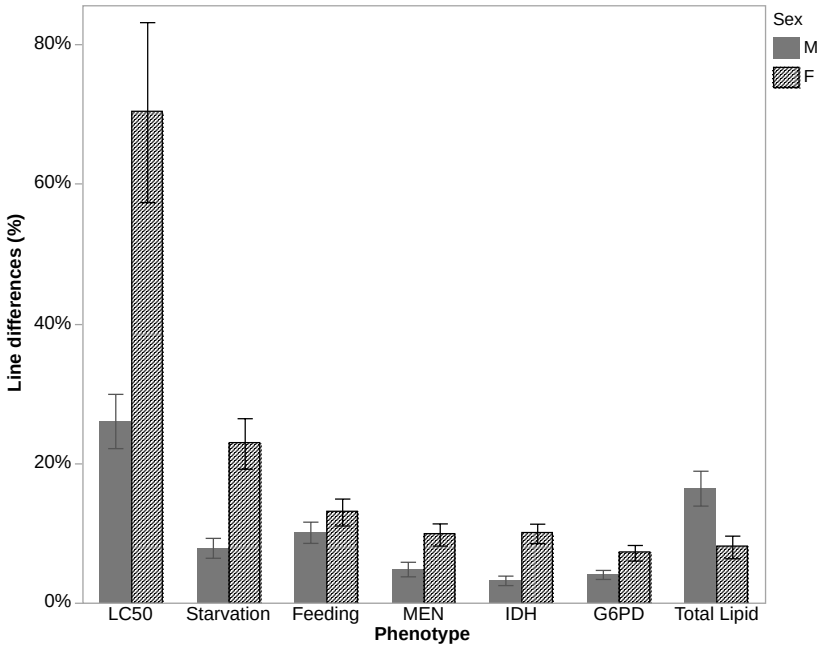
Supplemental Figure 3 Dose-response by genetic background.
(A) Overall dose-response, combining the sexes, (B) Male dose-response, (C) Female dose-response to Ni. Error is indicated by the 95% confidence intervals present at the LC_{25} and LC_{50}. All LC values are found in Supplemental Table 2.

		Isogenic	Heterozygous	Wild
LC_{25} (mM)	Overall	9.43 [8.56, 10.39]	20.20 [18.84, 21.66]	10.43 [9.34, 11.64]
	Male	9.93 [9.24, 10.68]	18.97 [17.94, 20.07]	9.81 [8.96, 10.73]
	Female	9.51 [8.02, 11.28]	23.13 [20.52, 26.07]	12.69 [10.64, 15.12]
LC_{50} (mM)	Overall	14.44 [13.61, 15.32]	30.15 [28.80, 31.55]	18.43 [17.32, 19.61]
	Male	13.17 [12.56, 13.80]	25.39 [24.52, 26.30]	14.92 [14.12, 15.77]
	Female	16.53 [15.01, 18.19]	40.79 [35.37, 47.04]	25.38 [22.92, 28.10]

Supplemental Table 2 LC_{25} and LC_{50} values for males and females by type of genetic background.
The error is indicated by [lower CI, upper CI], where CI is the 95% confidence interval.



Supplemental Figure 4 Comparison between average fly weight and Ni resistance. As fly weight increases, Ni resistance increases, significant only in females ($F_{1,9}$ = 29.66, P = 0.0004).



Supplemental Figure 5 Line differences across phenotypes. Generally, females have higher variation across lines, except in change in total lipid concentration, where males have higher variation.